

REGISTRATION REPORT
Part B
Section 9
Ecotoxicology
Detailed summary of the risk assessment

Product code: ADM.00150.I.2.A, MCW-2222
Product name(s): LEAXO
Chemical active substance:
Acetamiprid, 200 g/L

Central Zone
Zonal Rapporteur Member State: PL

CORE ASSESSMENT/
(Authorisation acc. to Art. 33)

Applicant: Country organisation / representative of ADAMA,
as given in Part A

Submission date: September 2023

MS Finalisation date: December 2023 (initial Core Assessment),
update July 2024, December 2024 (final Core Assessment)

Version history

When	What
March 2023	Original submission
January 2024	<p>Revision 1, based on a request by zRMS Poland</p> <ul style="list-style-type: none"> - Additional PEC_{gw} calculations for GAP refinements (considering only Tier 1 results), for other application timings and with surrogate crops for missing FOCUS scenarios. - Additional $PEC_{sw/sed}$ calculations for other application timing and with surrogate crops for missing FOCUS scenario. <p>Updated risk assessment for aquatic organism were provided. All changes are highlighted in yellow.</p>
July 2024	<p>Initial zRMS assessment</p> <p>The report in the dRR format has been prepared by the Applicant, therefore all comments, additional evaluations and conclusions of the zRMS are presented in grey commenting boxes. Minor changes are introduced directly in the text and highlighted in grey. Not agreed or not relevant information are struck through and shaded for transparency.</p> <p>Following the evaluation and before sending the document for commenting, all coloured highlighting was removed, from the parts updated by the Applicant, for better legibility</p>
December 2024	<p>Final report (Core Assessment updated following the commenting period)</p> <p>Additional information/assessments included by the zRMS in the report in response to comments received from the Applicant are highlighted in yellow. Not agreed or not relevant information are struck through and shaded for transparency.</p>

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9 Ecotoxicology (KCP 10)

9.1 Critical GAP and overall conclusions

Table 9.1-1: Table of critical GAPs

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
Use- No. *	Member state(s)	Crop and/or situation (crop destination / purpose of crop)	F, Fn, Fpn G, Gn, Gpn or I **	Pests or Group of pests controlled (additionally: developmental stages of the pest or pest group)	Application				Application rate			PHI (days)	Remarks: e.g. g saf- ener/ syner- gist per ha	Conclusion						
					Method / Kind	Timing / Growth stage of crop & season	Max. number a) per use b) per crop/ season	Min. interval between applications (days)	kg or L product/ha a) max. rate per appl. b) max. total rate per crop/season	g or kg as/ha a) max. rate per appl. b) max. total rate per crop/season	Water L/ha min/max			Birds	Mammals	Aquatic organisms	Bees	Non-target arthropods	Soil organisms	Non-target plants
Zonal uses (field or outdoor uses, certain types of protected crops)																				
I	Central	Corn	F	See below	foliar spraying, overall	Jun-Aug/ BBCH 51- 75	a) 1 b) 1	-	a) 0.3 b) 0.3	a) 60 b) 60	300-500	56	Umbrella GAP	A	A	A	A	R	A	A
1	Hungary	Corn	F	<i>Diabrotica virgifera</i> <i>virgifera</i> <i>Ostrinia nubilalis</i>	foliar spraying, overall	Jun-Aug/ BBCH 51-75	a) 1 b) 1	-	a) 0.3 b) 0.3	a) 60 b) 60	300-500	56	in label: 0.2-0.3 L/ha	A	A	A	A	R	A	A
2	Slovakia	Corn	F	<i>Diabrotica virgifera</i> <i>virgifera</i> <i>Ostrinia nubilalis</i>	foliar spraying, overall	Jun-Aug/ BBCH 51- 75	a) 1 b) 1	-	a) 0.3 b) 0.3	a) 60 b) 60	300-500	56	in label: 0.2-0.3 L/ha	A	A	A	A	R	A	A
3	Slovenia	Corn	F	<i>Diabrotica virgifera</i> <i>virgifera</i> <i>Ostrinia nubilalis</i>	foliar spraying, overall	Jun-Aug/ BBCH 51- 75	a) 1 b) 1	-	a) 0.3 b) 0.3	a) 60 b) 60	300-500	56	in label: 0.2-0.3 L/ha	A	A	A	A	R	A	A
IIa	Central	Apple	F	<i>Cydia pomonella</i> and other pests	foliar spraying, overall	June-Aug/ BBCH 71- PHI	a) 1 b) 1	-	a) 0.4 0.3 b) 0.4 0.3	a) 80 60 b) 80 60	500- 1000	14	Umbrella GAP	A	A	R	R	R	A	A
IIb	Central	Apple	F	<i>Aphids species</i> and others pests	foliar spraying, overall	May-Oct/ BBCH 70 62 PHI	a) 1-2 b) 1-2	8	a) 0.125 b) 0.25	a) 25 b) 50	500- 1000	14	Do not apply during flowering (application			R	R From BBCH 70	R	A	A

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
													from BBCH 70 Umbrella GAP; To protect bees and pollinating insects; application during flowering against pests is possible only out of honey-bee flight during late evening hours							
4	Czech Republic	Apple	F	<i>Cydia pomonella</i> , <i>Quadraspidiotus perniciosus</i>	foliar spraying, overall	June-Aug/ BBCH 71- PHI	a) 1 b) 1	-	a) 0.4 0.3 b) 0.4 0.3	a) 80 60 b) 80 60	500- 1000	14	0.25 L/10000-m² LWA 0.1875 L/10000 m² LWA	A	A	R	R	R	A	A
5	Czech Republic	Apple	F	<i>Aphis</i> spp.	foliar spraying, overall	Jun-Sep/ BBCH 70 62 PHI	a) 1-2 b) 1-2	8	a) 0.125 b) 0.25	a) 25 b) 50	500- 1000	14	0.078 L/10000 m² LWA Do not apply during flowering (application from BBCH 70 To protect bees and pollinating insects; application			R	R From BBCH 70	R	A	A

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
													during flowering against pests is possible only out of honey-bee flight during late evening hours							
6	Germany	Apple	F	<i>Cydia pomonella</i> , <i>Quadraspidiotus perniciosus</i>	foliar spraying, overall	June-Aug/ BBCH 71- PHI	a) 1 b) 1	-	a) 0.4 0.3 b) 0.4 0.3	a) 80 60 b) 80 60	500-1000	14	0.25 L/10000 m ² LWA 0.1875 L/10000 m ² LWA	A	A	R	R	R	A	A
7	Germany	Apple	F	<i>Aphis</i> spp.	foliar spraying, overall	Jun-Sep/ BBCH 70 62 PHI	a) 1-2 b) 1-2	8	a) 0.125 b) 0.25	a) 25 b) 50	500-1000	14	0.078 L/10000 m ² LWA Do not apply during flowering (application from BBCH 70 To protect bees and pollinating insects, application during flowering against pests is possible only out of honey-bee flight during late evening hours	A	A	R	R From BBCH 70	R	A	A
8	Netherlands	Apple	F	<i>Aphis</i> spp.	foliar spraying,	Jun-Aug/	a) 1-2	8	a) 0.125 b) 0.25	a) 25	500-1000	14	0.078 L/10000 m ²	A	A	R	R	R	A	A

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
					overall	BBCH 71- PHI	b) 1-2			b) 50			LWA							
9	Hungary	Apple	F	<i>Cydia pomonella</i> , <i>Quadraspidiotus perniciosus</i> , <i>Eriosoma lanigerum</i> ,	foliar spraying, overall	June-Oct/ BBCH 71- PHI	a) 1 b) 1	-	a) 0.4 0.3 b) 0.4 0.3	a) 80 60 b) 80 60	600- 1000	14	in-label: 0.2-0.4 L/ha in-label: 0.125- 0.25 L/10000-m² LWA in label: 0.15-0.3 L/ha in label: 0.09375- 0.225 L / 10000 m² LWA	A	A	R	R	R	A	A
10	Hungary	Apple	F	<i>Aphids</i> spp.	foliar spraying, overall	May-Oct/ BBCH 70 62-PHI	a) 1-2 b) 1-2	8	a) 0.125 b) 0.25	a) 25 b) 50	600- 1000	14	in label: 0.09-0.125 L/ha 0.056 – 0.078 L/10000 m² LWA; Do not apply during flowering (application from BBCH 70 To protect bees and pollinating insects; application during flowering against pests-is possible only out of honey-bee flight	A	A	R	R From BBCH 70	R	A	A

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
													during late evening hours							
11	Poland	Apple	F	<i>Cydia pomonella</i>	foliar spraying, overall	June-Aug/ BBCH 71- PHI	a) 1 b) 1	-	a) 0.4 0.3 b) 0.4 0.3	a) 80 60 b) 80 60	500-900	14	0.25 L/10000 m ² LWA 0.1875 L/10000 m ² LWA	A	A	R	R	R	A	A
12	Poland	Apple	F	<i>Aphis</i> spp.	foliar spraying, overall	May-Oct/ BBCH 70- PHI	a) 1-2 b) 1-2	8	a) 0.125 b) 0.25	a) 25 b) 50	500-900	14	0.078 L/10000 m ² LWA Do not apply during flowering (application from BBCH 70) To protect bees and pollinating insects;			R	R From BBCH 70	R		A

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
													application during flowering against pests is possible only out of honey-bee flight during late evening hours							
13	Slovakia	Apple	F	<i>Cydia pomonella</i> <i>Quadraspidiotus perniciosus</i> , <i>Eriosoma lanigerum</i>	foliar spraying, overall	June-Aug/ BBCH 71- PHI	a) 1 b) 1	-	a) 0.4 0.3 b) 0.4 0.3	a) 80 60 b) 80 60	500-1000	14	in-label: 0.2-0.4 L/ha in-label: 0.125- 0.25 L/10000 m² LWA in label: 0.15-0.3 L/ha in label: 0.09375- 0.225 L / 10000 m² LWA	A	A	R	R	R	A	A
14	Slovakia	Apple	F	<i>Aphis</i> spp.	foliar spraying, overall	May-Sep/ BBCH 70 62-PHI	a) 1-2 b) 1-2	8	a) 0.125 b) 0.25	a) 25 b) 50	500-1000	14	in label: 0.09-0.125 L/ha 0.056 – 0.078 L/10000 m² LWA Do not apply during flowering (application from BBCH 70 To protect bees and pollinating	A	A	R	R From BBCH 70	R	A	A

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
													insects; application during flowering against pests-is possible only-out-of honey-bee flight during-late evening hours							
15	Slovenia	Apple	F	<i>Cydia pomonella</i> <i>Quadraspidiotus perniciosus</i> , <i>Eriosoma lanigerum</i>	foliar spraying, overall	June-Aug/ BBCH 71- PHI	a) 1 b) 1	-	a) 0.4 0.3 b) 0.4 0.3	a) 80-60 b) 80 60	500- 1000	14	in-label: 0.2-0.4 L/ha in-label: 0.125— 0.25 L/10000-m² LWA in label: 0.15-0.3 L/ha in label: 0.09375- 0.225 L / 10000 m² LWA	A	A	R	R	R	A	A
16	Slovenia	Apple	F	<i>Aphids</i> spp.	foliar spraying, overall	May-Oct/ BBCH 70 62-PHI	a) 1-2 b) 1-2	8	a) 0.125 b) 0.25	a) 25 b) 50	500- 1000	14	in label: 0.09-0.125 L/ha To-protect bees-and pollinating insects; application during flowering against pests-is possible only-out-of honey-bee flight	A	A	R	R From BBCH 70	R	A	A

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
													during-late evening hours 0.056 – 0.078 L/10000 m ² LWA Do not apply during flowering (application from BBCH 70)							
III	Central	Potato	F	See below	foliar spraying, overall	May-Sep/ BBCH 12-79	a) 1 b) 1	-	a) 0.18 b) 0.18	a) 36 b) 36	100-500	7	Umbrella GAP To protect bees and pollinating insects; application during flowering against pests is possible only out of honey-bee flight during-late evening hours	A	A	A	A	R	R	A
17	Czech Republic	Potato	F	<i>Leptinotarsa decemlineata</i> <i>Myzus persicae</i> <i>Macrosiphum euphorbia</i>	foliar spraying, overall	May-Sep/ BBCH 12-79	a) 1 b) 1	-	a) 0.18 b) 0.18	a) 36 b) 36	200-500	7	To protect bees and pollinating insects; application during flowering against pests is possible only out of	A	A	A	A	R A	R	A

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
													honey-bee flight during late evening hours							
18	Netherlands	Potato	F	<i>Leptinotarsa decemlineata</i> <i>Myzus persicae</i> <i>Macrosiphum euphorbia</i>	foliar spraying, overall	May-Sep/ BBCH 12-79	a) 1 b) 1	-	a) 0.18 b) 0.18	a) 36 b) 36	200-400	7	To protect bees and pollinating insects; application during flowering against pests is possible only out of honey-bee flight during late evening hours	A	A	A	A	R	R	A
19	Poland	Potato	F	<i>Leptinotarsa decemlineata</i> <i>Myzus persicae</i> <i>Macrosiphum euphorbia</i>	foliar spraying, overall	May-Sep/ BBCH 12-79	a) 1 b) 1	-	a) 0.18 b) 0.18	a) 36 b) 36	200-400	7	To protect bees and pollinating insects; application during flowering against pests is possible only out of honey-bee flight during late evening hours	A	A	A	A	R	R	A

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
20	Slovenia	Potato	F	<i>Leptinotarsa decemlineata</i> <i>Myzus persicae</i>	foliar spraying, overall	May-Sep/ BBCH 12-79	a) 1 b) 1	-	a) 0.18 b) 0.18	a) 36 b) 36	200-400	7	in label: 0.12-0.18 L/ha To protect bees and pollinating insects, application during flowering against pests is possible only out of honey-bee flight during late evening hours	A	A	A	A	R	R	A
21	Slovakia	Potato	F	<i>Leptinotarsa decemlineata</i>	foliar spraying, overall	May-Sep/ BBCH 12-79	a) 1 b) 1	-	a) 0.18 b) 0.18	a) 36 b) 36	200-400	7	in label: 0.12-0.18 L/ha To protect bees and pollinating insects, application during flowering against pests is possible only out of honey-bee flight during late evening hours	A	A	A	A	R	R	A
22	Germany	Potato	F	<i>Leptinotarsa decemlineata</i> <i>Myzus persicae</i> <i>Macrosiphum euphorbia</i>	foliar spraying, overall	May-Sep/ BBCH 12-79	a) 1 b) 1	-	a) 0.18 b) 0.18	a) 36 b) 36	200-500	7	To protect bees and pollinating insects, application during flowering	A	A	A	A	R	R	A

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
													against pests is possible only out of honey-bee flight during late evening hours							
IVa	Central	Spring wheat Spring barley Spring oats Spring Durum wheat Spring triticale	F	See below	foliar spraying, overall	Mar-Jul/ BBCH 40-69 (spring)	a) 1-2 b) 1-2	10	a) 0.175 b) 0.35	a) 35 b) 70	100-400	follow crop BBCH	Umbrella GAP	A	A	R	A	R	R	A
IVb	Central	Spring wheat Spring barley Spring oats Spring Durum wheat Spring triticale	F	See below	foliar spraying, overall	Mar-Jul/ BBCH 12-69 (spring)	a) 1 b) 1-2	30	a) 0.175 b) 0.35	a) 35 b) 70	100-400	follow crop BBCH	Umbrella GAP 1 application at BBCH 12-29 followed by 1 application at BBCH 40-69.	A	A	R	A	R	A	A
23	Czech Republic	Spring barley Spring oat Spring wheat Spring triticale	F	Aphids	foliar spraying, overall	May-Jun/ BBCH 40 - 69 (Spring)	a) 1-2 b) 1-2	10	a) 0.175 b) 0.35	a) 35 b) 70	200-400	follow crop BBCH		A	A	R	A	R	R	A
24	Czech Republic	Spring barley Spring oat Spring wheat Spring triticale	F	Aphids Virus Control	foliar spraying, overall	May-Jun/ BBCH 12-29 BBCH 20 - 29 (Spring)	a) 1 b) 1-2 b) 1	a) - b) 30	a) 0.175 b) 0.35 0.175	a) 35 b) 70 35	200-400	follow crop BBCH	1 application at BBCH 12-29 followed by 1 application	A	A	A	A	R	A	A

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
													at-BBCH 40-69.							
25	Netherlands	Spring barley Spring oat Spring wheat Spring triticale	F	Aphids	foliar spraying, overall	May-Jul/ BBCH 40 - 69 (Spring)	a) 1-2 b) 1-2	10	a) 0.175 b) 0.35	a) 35 b) 70	200-400	follow crop BBCH		A	A	R	A	R	R	A
26	Netherlands	Spring barley Spring oat Spring wheat Spring triticale	F	Aphids Virus Control	foliar spraying, overall	Mar-Apr/ BBCH 12 - 29 (Spring)	a) 1 b) 1-2	30	a) 0.175 b) 0.35	a) 35 b) 70	200-400	follow crop BBCH	1 application at BBCH 12-29 followed by 1 application at BBCH 40-69.	A	A	A	A	R	A	A
27	Germany	Spring barley Spring oat Spring wheat Spring triticale	F	Aphids	foliar spraying, overall	Mar-Jul/ BBCH 40 - 69 (Spring)	a) 1-2 b) 1-2	10	a) 0.175 b) 0.35	a) 35 b) 70	200-400	follow crop BBCH				R	A	R	R	A
28	Germany	Spring barley Spring oat Spring wheat Spring triticale	F	Aphids Virus Control	foliar spraying, overall	May-Jun/ BBCH 12 - 29 BBCH 20 - 29 (Spring)	a) 1 b) 1-2 b) 1	a) - b) 30	a) 0.175 b) 0.35 0.175	a) 35 b) 70 35	200-400	follow crop BBCH	1 application at BBCH 12-29 followed by 1 application at BBCH 40-69.	A	A	R	A	R	A	A
29	Slovenia	Spring barley Spring oat Spring wheat Spring Durum wheat Spring triticale	F	Aphids	foliar spraying, overall	May-Jun/ BBCH 40 - 69 (Spring)	a) 1-2 b) 1-2	10	a) 0.175 b) 0.35	a) 35 b) 70	200-400	follow crop BBCH		A	A	R	A	R	R	A
30	Poland	Spring barley Spring oat	F	Aphids	foliar spraying,	Mar-Jul/	a) 1-2 b) 1-2	10	a) 0.175 b) 0.35	a) 35 b) 70	200-400	follow crop		A	A	R	A	R	R	A

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
		Spring wheat Spring triticale			overall	BBCH 40 - 69 (Spring)						BBCH								
31	Poland	Spring barley Spring oat Spring wheat Spring triticale	F	Aphids Virus Control	foliar spraying, overall	May-Jun/ BBCH 12 -29 BBCH 20 - 29 (Spring)	a) 1 b) 1-2 b) 1	a) - b) 30	a) 0.175 b) 0.35 0.175	a) 35 b) 70 35	200-400	follow crop BBCH	1 application at BBCH 12-29 followed by 1 application at BBCH 40-69.	A	A	R	A	R	A	A
Va	Central	Winter wheat, Winter barley, Winter rye, Winter triticale, Spelt	F	Aphids	foliar spraying, overall	May-Jul/ BBCH 40 - 69 (Spring)	a) 1-2 b) 1-2	10	a) 0.18 b) 0.36	a) 36 b) 72	100-400	follow crop BBCH	Umbrella GAP	A	A	R	A	R	A	A
Vb	Central	Winter wheat, Winter barley, Winter rye, Winter triticale, Spelt	F	Aphids Virus Control	foliar spraying, overall	Aug-Nov/ BBCH 12 - 29 (Autumn)	a) 1 b) 1	-	a) 0.15 b) 0.15	a) 30 b) 30	100-400	follow crop BBCH	Umbrella GAP	A	A	R	A	R	R	A
32	Czech Republic	Winter wheat Winter barley Winter triticale Winter rye Spelt	F	Aphids	foliar spraying, overall	May-Jul/ BBCH 40 - 69 (Spring)	a) 1-2 b) 1-2	10	a) 0.18 b) 0.36	a) 36 b) 72	200-400	follow crop BBCH		A	A	R	A	R	A	A
33	Czech Republic	Winter wheat Winter barley Winter triticale Winter rye Spelt	F	Aphids Virus Control	foliar spraying, overall	Aug-Nov/ BBCH 12 - 29 (Autumn)	a) 1 b) 1	-	a) 0.15 b) 0.15	a) 30 b) 30	200-400	follow crop BBCH		A	A	R	A	R	R	A

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
34	Netherlands	Winter wheat Winter oat Winter barley Winter triticale Winter rye Spelt	F	Aphids	foliar spraying, overall	May-Jul/ BBCH 40 – 69 (Spring)	a) 1-2 b) 1-2	10	a) 0.18 b) 0.36	a) 36 b) 72	200-400	follow crop BBCH		A	A	R	A	R	A	A
35	Netherlands	Winter wheat Winter oat Winter barley Winter triticale Winter rye Spelt	F	Aphids Virus Control	foliar spraying, overall	Aug-Nov/ BBCH 12 - 29 (Autumn)	a) 1 b) 1	-	a) 0.15 b) 0.15	a) 30 b) 30	200-400	follow crop BBCH		A	AA	R	A	R	R	A
36	Germany	Winter wheat Winter barley Winter triticale Winter rye Spelt	F	Aphids	foliar spraying, overall	May-Jul/ BBCH 40 - 69 (Spring)	a) 1-2 b) 1-2	10	a) 0.18 b) 0.36	a) 36 b) 72	200-400	follow crop BBCH		A	A	R	A	R	A	A
37	Germany	Winter wheat Winter barley Winter triticale Winter rye Spelt	F	Aphids Virus Control	foliar spraying, overall	Aug-Nov/ BBCH 12 - 29 (Autumn)	a) 1 b) 1	-	a) 0.15 b) 0.15	a) 30 b) 30	200-400	follow crop BBCH		A	A	R	A	R	R	
38	PL	Winter wheat Winter barley Winter triticale Winter rye	F	Aphids Virus Control	foliar spraying, overall	Aug-Nov/ BBCH 12 - 29 (Autumn)	a) 1 b) 1	-	a) 0.15 b) 0.15	a) 30 b) 30	200-400	follow crop BBCH		A	A	R	A	R	R	A
39	PL	Winter wheat Winter barley Winter triticale Winter rye	F	Aphids	foliar spraying, overall	May-Jul/ BBCH 40 - 69 (Spring)	a) 1-2 b) 1-2	10	a) 0.18 b) 0.36	a) 36 b) 72	200-400	follow crop BBCH		A	A	R	A	R	A	A

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
40	Slovenia	Winter wheat Winter barley Winter triticale Winter rye	F	Aphids Virus Control	foliar spraying, overall	Aug-Nov/ BBCH 12 - 29 (Autumn)	a) 1 b) 1	-	a) 0-15 0.145 b) 0-15 0.145	a) 30 29 b) 30 29	200-400	follow crop BBCH		A	A	R	A	R	R	A
VIa	Central	Winter OSR	F	See below	foliar spraying, overall	Mar-Jun/ BBCH 31- 71 (spring)	a) 1-2 b) 1-2	7	a) 0.3 b) 0.6	a) 60 b) 120	100-400	28	Umbrella GAP To protect bees and pollinating insects, application during flowering against pests is possible only out of honey-bee flight during late evening hours Application in the evening, after the bee flight. Only single application during flowering allowed.	A	A	R	R	R	A	
VIb	Central	Winter OSR	F	See below	foliar spraying, overall	Aug-Nov/ BBCH 11- 19 (autumn)	a) 1 b) 1	-	a) 0.3 0.240 b) 0.3 0.240	a) 60 48 b) 60 48	100-200	28	Umbrella GAP	A	A	A	A	R	A	A
41	Czech Republic	Winter OSR	F	<i>Ceutorhynchus napi</i> , <i>C. quadridens</i>	foliar spraying, overall	Mar-Jun/ BBCH 31- 59	a) 1-2 b) 1-2	7	a) 0.3 b) 0.6	a) 60 b) 120	200-400	28		A	A	R	R	R	R	A
42	Czech	Winter OSR	F	<i>Meligethes aeneus</i>	foliar	Apr-Jun/	a) 1-2	7	a) 0.3	a) 60	200-400	28		A	A	R	R	R	R	A

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
	Republic				spraying, overall	BBCH 50- 59	b) 1-2		b) 0.6	b) 120										
43	Czech Republic	Winter OSR	F	<i>Dasyneura brassicae</i> , <i>Ceutorhynchus obstrictus (syn assimilis)</i>	foliar spraying, overall	May-Jun/ BBCH 61- 71	a) 1-2 b) 1-2	7	a) 0.3 b) 0.6	a) 60 b) 120	200-400	28	To protect bees and pollinating insects; application during flowering against pests is possible only out of honey-bee flight during late evening hours Application in the even- ing, after the bee flight. Only single application during flowering allowed.	A	A	R	R	R	R	A
44	Czech Republic	Winter OSR	F	<i>Psylliodes chrysocephala</i> <i>Phyllotreta</i> Spp. (Flea beetle)	foliar spraying, overall	Sep-Oct/ BBCH 11- 19 (autumn)	a) 1 b) 1	-	a) 0.3 0.240 b) 0.3 0.240	a) 60 48 b) 60 48	200-400	28		A	A	A	A	R	A	A
45	Czech Republic	Winter OSR	F	Aphid vectors of Turnip yellow virus - <i>Myzus persicae</i>	foliar spraying, overall	Aug-Nov/ BBCH 11- 19 (autumn)	a) 1 b) 1	-	a) 0.2 b) 0.2	a) 40 b) 40	200-400	28		A	A	A	A	R	A	A
46	Hungary	Winter OSR	F	<i>Ceutorhynchus napi</i> , <i>C. quadriens</i>	foliar spraying, overall	Mar-May/ BBCH 31- 69	a) 1-2 b) 1-2	7	a) 0.3 b) 0.6	a) 60 b) 120	200-400	28	in label: 0.15-0.3 L/ha To protect bees and pollinating insects;	A	A	R	R	R	R	A

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
													application during flowering against pests-is possible only-out-of honey-bee flight during-late evening hours Application in the evening, after the bee flight. Only single application during flowering allowed-							
47	Hungary	Winter OSR	F	<i>Dasyneura brassicae</i> , <i>Ceutorhynchus obstrictus</i> (syn <i>assimilis</i>)	foliar spraying, overall	Mar-May/ BBCH 31-71	a) 1-2 b) 1-2	7	a) 0.3 b) 0.6	a) 60 b) 120	200-400	28	in label: <i>C. obstrictus</i> 0.15-0.3 L/ha <i>D. brassicae</i> 0.18-0.3 L/ha To protect bees and pollinating insects, application during flowering against pests-is possible only-out-of honey-bee flight during-late	A	A	R	R	R	R	A

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
													evening hours Application in the evening, after the bee flight Only single application during flowering allowed.							
48	Hungary	Winter OSR	F	<i>Meligethes aeneus</i>	foliar spraying, overall	Mar-May/ BBCH 50-59	a) 1-2 b) 1-2	7	a) 0.3 b) 0.6	a) 60 b) 120	200-400	28	in label: 0.18-0.3 L/ha	A	A	R	R	R	R	A
49	Poland	Winter OSR	F	<i>Meligethes aeneus</i>	foliar spraying, overall	May-Jun/ BBCH 50-59	a) 1-2 b) 1-2	7	a) 0.3 b) 0.6	a) 60 b) 120	200-400	28		A	A	R	R	R	R	A
50	Poland	Winter OSR	F	<i>Dasyneura brassicae</i> , <i>Ceutorhynchus obstrictus</i> (syn <i>assimilis</i>)	foliar spraying, overall	May-Jun/ BBCH 61-71	a) 1-2 b) 1-2	7	a) 0.3 b) 0.6	a) 60 b) 120	200-400	28	To protect bees and pollinating insects, application during flowering against pests is possible only out of honey-bee flight during late evening hours Application in the evening, after the bee flight. Only single application during flowering allowed	A	A	R	R	R	R	A

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
51	Poland	Winter OSR	F	<i>Ceutorhynchus napi</i> , <i>C. quadridens</i>	foliar spraying, overall	Mar-Jun/ BBCH 31- 59	a) 1-2 b) 1-2	7	a) 0.3 b) 0.6	a) 60 b) 120	200-400	28		A	A	R	R	R	R	A
52	Poland	Winter OSR	F	<i>Psylliodes chrysocephala</i>	foliar spraying, overall	Sep-Oct/ BBCH 11- 19 (autumn)	a) 1 b) 1	-	a) 0.3 0.240 b) 0.3 0.240	a) 60 48 b) 60 48	200-400	28		A	A	A	A	R	R	A
53	Slovakia	Winter OSR	F	<i>Ceutorhynchus napi</i> , <i>C. quadridens</i>	foliar spraying, overall	Mar-Jun/ BBCH 31- 69	a) 1-2 b) 1-2	7	a) 0.3 b) 0.6	a) 60 b) 120	200-400	28	in label: 0.15-0.3 L/ha To protect bees and pollinating insects; application during flowering against pests is possible only out of honey-bee flight during late evening hours Application in the evening, after the bee flight Only single application during flowering allowed.	A	A	R	R	R	A	
54	Slovakia	Winter OSR	F	<i>Meligethes aeneus</i>	foliar spraying, overall	Mar-Jun/ BBCH 50- 59	a) 1-2 b) 1-2	7	a) 0.3 b) 0.6	a) 60 b) 120	200-400	28	in label: 0.18-0.3 L/ha	A	A	R	A	R	A	A

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
55	Slovakia	Winter OSR	F	<i>Dasyneura brassicae</i> , <i>Ceutorhynchus obstrictus</i> (syn. <i>assimilis</i>)	foliar spraying, overall	May-Jun/ BBCH 61-71	a) 1-2 b) 1-2	7	a) 0.3 b) 0.6	a) 60 b) 120	200-400	28	in label: <i>C. obstrictus</i> 0.15-0.3 L/ha <i>D. brassicae</i> 0.18-0.3 L/ha To protect bees and pollinating insects, application during flowering against pests is possible only out of honey-bee flight during late evening hours Application in the evening, after the bee flight. Only single application during flowering allowed	A	A	RA	R	R	R	A
56	Germany	Winter OSR	F	<i>Ceutorhynchus napi</i> , <i>C. quadridens</i>	foliar spraying, overall	Mar-Jun/ BBCH 31-59	a) 1-2 b) 1-2	7	a) 0.3 b) 0.6	a) 60 b) 120	200-400	28		A	A	R	R	R	R	A
57	Germany	Winter OSR	F	<i>Meligethes aeneus</i>	foliar spraying, overall	Apr-Jun/ BBCH 50-59	a) 1-2 b) 1-2	7	a) 0.3 b) 0.6	a) 60 b) 120	200-400	28		A	A	R	R	R	R	A
58	Germany	Winter OSR	F	<i>Dasyneura brassicae</i> ,	foliar spraying,	May-Jun/	a) 1-2 b) 1-2	7	a) 0.3 b) 0.6	a) 60	200-400	28	To protect bees and	A	A	R	R	R	R	A

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
				<i>Ceutorhynchus obstrictus</i> (syn <i>assimilis</i>)	overall	BBCH 61-71				b) 120			pollinating insects; application during flowering against pests is possible only out of honey-bee flight during late evening hours Application in the evening, after the bee flight Only single application during flowering allowed.							
59	Germany	Winter OSR	F	<i>Psylliodes chrysocephala</i> <i>Phyllotreta</i> Spp. (Flea beetle)	foliar spraying, overall	Aug-Nov/ BBCH 11-19 (autumn)	a) 1 b) 1	-	a) 0.3 0.240 b) 0.3 0.240	a) 60 48 b) 60 48	200-400	28		A	A	A	A	R	A	A
60	Germany	Winter OSR	F	Aphid vectors of Turnip yellow virus - <i>Myzus persicae</i>	foliar spraying, overall	Aug-Nov/ BBCH 11-19 (autumn)	a) 1 b) 1	-	a) 0.2 b) 0.2	a) 40 b) 40	200-400	28		A	A	A	A	R	A	A
61	Germany	Winter OSR	F	<i>Ceutorhynchus picipitarsis</i> (Rape winter stem weevil)	foliar spraying, overall	Oct-Nov/ BBCH 13-17	a) 1 b) 1	-	a) 0.3 0.240 b) 0.3 0.240	a) 60 48 b) 60 48	200-400	28		A	A	A	A	R	A	A
62	Slovenia	Winter OSR	F	<i>Ceutorhynchus napi</i> , <i>C. quadridens</i>	foliar spraying, overall	Mar-Jun/ BBCH 31-59	a) 1-2 b) 1-2	7	a) 0.3 b) 0.6	a) 60 b) 120	200-400	28		A	A	R	R	R	R	A
63	Slovenia	Winter OSR	F	<i>Meligethes aeneus</i>	foliar spraying, overall	Apr-Jun/ BBCH 50-	a) 1-2 b) 1-2	7	a) 0.3 b) 0.6	a) 60 b) 120	200-400	28		A	A	R	R	R	R	A

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
						59														
64	Slovenia	Winter OSR	F	<i>Dasyneura brassicae</i> , <i>Ceutorhynchus obstrictus</i> (syn <i>assimilis</i>)	foliar spraying, overall	May-Jun/ BBCH 61- 71	a) 1-2 b) 1-2	7	a) 0.3 b) 0.6	a) 60 b) 120	200-400	28	To protect bees and pollinating insects; application during flowering against pests is possible only out of honey-bee flight during late evening hours Application in the evening, after the bee flight. Only single application during flowering allowed. in label: C. obstrictus 0.15-0.3 L/ha D. brassicae 0.18-0.3 L/ha	A	A	R	R	R	R	A
65	Slovenia	Winter OSR	F	<i>Psylliodes chrysocephala</i>	foliar spraying, overall	Sep-Oct/ BBCH 11- 19 (autumn)	a) 1 b) 1	-	a) 0.3 0.240 b) 0.3 0.240	a) 60 48 b) 60 48	200-400	28		A	A	A	A	R	A	A
VIIa	Central	Spring OSR	F	See below	foliar spraying, overall	Mar-Jun/ BBCH 31- 71	a) 1-2 b) 1-2	7	a) 0.3 b) 0.6	a) 60 b) 120	100-400	28	Umbrella GAP. To protect bees and pollinating insects;	A	A	R	R	R	A	A

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
													application during flowering against pests is possible only out of honey-bee flight during late evening hours Application in the evening, after the bee flight. Only single application during flowering allowed.							
66	Germany	Spring OSR	F	<i>Ceutorhynchus napi</i> , <i>C. quadridens</i>	foliar spraying, overall	Mar-Jun/ BBCH 31-59	a) 1-2 b) 1-2	7	a) 0.3 b) 0.6	a) 60 b) 120	200-400	28		A	A	R	R	R	A	A
67	Germany	Spring OSR	F	<i>Meligethes aeneus</i>	foliar spraying, overall	Apr-Jun/ BBCH 50-59	a) 1-2 b) 1-2	7	a) 0.3 b) 0.6	a) 60 b) 120	200-400	28		A	A	R	R	R	A	A
68	Germany	Spring OSR	F	<i>Dasyneura brassicae</i> , <i>Ceutorhynchus obstrictus</i> (syn <i>assimilis</i>)	foliar spraying, overall	May-Jun/ BBCH 61-71	a) 1-2 b) 1-2	7	a) 0.3 b) 0.6	a) 60 b) 120	200-400	28	To protect bees and pollinating insects, application during flowering against pests is possible only out of honey-bee flight during late evening hours	A	A	R	R	R	A	A

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
													Application in the evening, after the bee flight flight. Only single application during flowering allowed.							
69	Poland	Spring OSR	F	<i>Meligethes aeneus</i>	foliar spraying, overall	Apr-Jun/ BBCH 50-59	a) 1-2 b) 1-2	7	a) 0.3 b) 0.6	a) 60 b) 120	200-400	28		A	A	R	R	R	A	A
70	Poland	Spring OSR	F	<i>Dasyneura brassicae</i> , <i>Ceutorhynchus obstrictus</i> (syn <i>assimilis</i>)	foliar spraying, overall	May-Jun/ BBCH 61-71	a) 1-2 b) 1-2	7	a) 0.3 b) 0.6	a) 60 b) 120	200-400	28	To protect bees and pollinating insects, application during flowering against pests is possible only out of honey-bee flight during late evening hours Application in the evening, after the bee flight Only single application during flowering allowed.	A	A	R	R	R	A	A
71	Slovakia	Spring OSR	F	<i>Ceutorhynchus napi</i> , <i>C. quadridens</i>	foliar spraying, overall	Mar-Jun/ BBCH 31-59	a) 1-2 b) 1-2	7	a) 0.3 b) 0.6	a) 60 b) 120	200-400	28	in label: 0.15-0.3 L/ha	A	A	R	R	R	A	A

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
72	Slovakia	Spring OSR	F	<i>Meligethes aeneus</i>	foliar spraying, overall	Apr-Jun/ BBCH 50-59	a) 1-2 b) 1-2	7	a) 0.3 b) 0.6	a) 60 b) 120	200-400	28	in label: 0.18-0.3 L/ha	A	A	R	R	R	A	A
73	Slovakia	Spring OSR	F	<i>Dasyneura brassicae</i> , <i>Ceutorhynchus obstrictus</i> (syn <i>assimilis</i>)	foliar spraying, overall	May-Jun/ BBCH 61-71	a) 1-2 b) 1-2	7	a) 0.3 b) 0.6	a) 60 b) 120	200-400	28	in label: <i>C. obstrictus</i> 0.15-0.3 L/ha <i>D. brassicae</i> 0.18-0.3 L/ha To protect bees and pollinating insects, application during flowering against pests is possible only out of honey-bee flight during late evening hours Application in the evening, after the bee flight. Only single application during flowering allowed.	A	A	R	R	R	A	A

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
74	Hungary	Spring OSR	F	<i>Ceutorhynchus napi</i> , <i>C. quadridens</i>	foliar spraying, overall	Mar-Jun/ BBCH 31- 59	a) 1-2 b) 1-2	7	a) 0.3 b) 0.6	a) 60 b) 120	200-400	28	in label: 0.15-0.3 L/ha	A	A	R	R	R	A	A
75	Hungary	Spring OSR	F	<i>Meligethes aeneus</i>	foliar spraying, overall	Apr-Jun/ BBCH 50- 59	a) 1-2 b) 1-2	7	a) 0.3 b) 0.6	a) 60 b) 120	200-400	28	in label: 0.18-0.3 L/ha	A	A	R	R	R	A	A
76	Hungary	Spring OSR	F	<i>Dasyneura brassicae</i> , <i>Ceutorhynchus obstrictus (syn assimilis)</i>	foliar spraying, overall	May-Jun/ BBCH 61- 71	a) 1-2 b) 1-2	7	a) 0.3 b) 0.6	a) 60 b) 120	200-400	28	in label: 0.15-0.3 L/ha D. brassicae 0.18-0.3 L/ha To protect bees and pollinating insects, application during flowering against pests is possible only out of honey-bee flight during late evening hours Application in the evening, after the bee flight. Only single application during flowering allowed.	A	A	R	R	R	A	A
77	Czech	Spring OSR	F	<i>Ceutorhynchus napi</i> ,	foliar	Mar-Jun/	a) 1-2	7	a) 0.3	a) 60	200-400	28		A	A	R	R	R	A	A

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
	Republic			<i>C. quadridens</i>	spraying, overall	BBCH 31-59	b) 1-2		b) 0.6	b) 120										
78	Czech Republic	Spring OSR	F	<i>Meligethes aeneus</i>	foliar spraying, overall	Apr-Jun/ BBCH 50-59	a) 1-2 b) 1-2	7	a) 0.3 b) 0.6	a) 60 b) 120	200-400	28		A	A	R	R	R	A	A
79	Czech Republic	Spring OSR	F	<i>Dasyneura brassicae</i> , <i>Ceutorhynchus obstrictus</i> (syn <i>assimilis</i>)	foliar spraying, overall	May-Jun/ BBCH 61-71	a) 1-2 b) 1-2	7	a) 0.3 b) 0.6	a) 60 b) 120	200-400	28	To protect bees and pollinating insects; application during flowering against pests is possible only out of honey-bee flight during late evening hours Application in the evening, after the bee flight. Only single application during flowering allowed.	A	A	R	R	R	A	A
VIIIa	Central	Sugar beet	F	See below	foliar spraying, overall	Apr-Aug/ BBCH 12-39	a) 2 b) 2	7	a) 0.25 b) 0.5	a) 50 b) 100	200-400	35	Umbrella GAP Fall-back rate 1 applic @ 0.2 L/ha	A	A	R	A	R	R	A
80	Poland	Sugar beet	F	<i>Myzus persicae</i> <i>Aphis fabae</i> <i>Macrosiphum euphorbiae</i>	foliar spraying, overall	Apr-Aug/ BBCH 12-39	a) 2 b) 2 a) 1 b) 1	a) 7 b) 7 -	a) 0.25 b) 0.5 0.25	a) 50 b) 100 50	200-400	35	Do not apply in more than 1 out of 2 years!	A	A	R	A	R	R	A

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
81a	Germany	Sugar beet	F	<i>Myzus persicae</i> <i>Aphis fabae</i> <i>Macrosiphum euphorbiae</i>	foliar spraying, overall	Apr-Aug/ BBCH 12-39	a) 2 b) 2	a) 7 b) 7	a) 0.25 b) 0.5	a) 50 b) 100	200-400	35	Do not apply in more than 1 out of 3 years!	A	A	R	A	R	R	A
81b	Germany	Sugar beet	F	<i>Myzus persicae</i> <i>Aphis fabae</i> <i>Macrosiphum euphorbiae</i>	foliar spraying, overall	Apr-Aug/ BBCH 12-39	a) 1 b) 1	-	a) 0.25 b) 0.25	a) 50 b) 50	200-400	35	Do not apply in more than 1 out of 2 years!	A	A	R	A	R	R	A
82	Netherlands	Sugar beet	F	<i>Myzus persicae</i> <i>Aphis fabae</i> <i>Macrosiphum euphorbiae</i>	foliar spraying, overall	Apr-Aug/ BBCH 12-39	a) 2 b) 2	7	a) 0.25 b) 0.5	a) 50 b) 100	200-400	35		A	A	R	A	R	R	A
83a	Czech Republic	Sugar beet	F	<i>Myzus persicae</i> <i>Aphis fabae</i> <i>Macrosiphum euphorbiae</i>	foliar spraying, overall	Apr-Aug/ BBCH 12-39	a) 2 b) 2	a) 7 b) 7	a) 0.25 b) 0.5	a) 50 b) 100	200-400	35	Do not apply in more than 1 out of 3 years!	A	A	R	A	R	R	A
83b	Czech Republic	Sugar beet	F	<i>Myzus persicae</i> <i>Aphis fabae</i> <i>Macrosiphum euphorbiae</i>	foliar spraying, overall	Apr-Aug/ BBCH 12-39	a) 1 b) 1	-	a) 0.25 b) 0.25	a) 50 b) 50	200-400	35	Do not apply in more than 1 out of 2 years!	A	A	R	A	R	R	A
84	Slovenia	Sugar beet	F	<i>Myzus persicae</i> <i>Aphis fabae</i> <i>Macrosiphum euphorbiae</i>	foliar spraying, overall	Apr-Aug/ BBCH 12-39	a) 2 b) 2 a) 1 b) 1	a) 7 b) 7 -	a) 0.25 b) 0.5 0.25	a) 50 b) 100 50	200-400	35	Do not apply in more than 1 out of 2 years!	A	A	R	A	A	R	A
IXa	Central	Flower bulbs and flower tubers	F	<i>Aphids</i>	foliar spraying, overall	Mar-Jul/ BBCH 12-91	a) 1 b) 1	-	a) 0.23 b) 0.23	a) 46 b) 46	200-400	n.a.	Umbrella GAP Application in the evening, after the bee flight To protect bees and pollinating insects, application	A	A	R	R	R	R	A

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
													during flowering against pests is possible only out of honey-bee flight during late evening hours							
IXb	Central	Flower bulbs and flower tubers	F	Aphids	foliar spraying, overall	Mar-Jul/ BBCH 20-91	a) 2 b) 2	7	a) 0.17 b) 0.34	a) 34 b) 68	200-400	n.a.	Umbrella GAP Application in the evening, after the bee flight To protect bees and pollinating insects, application during flowering against pests is possible only out of honey-bee flight during late evening hours	A	A	R	R	R	R	A
85	Netherlands	Flower bulbs and flower tubers	F	<i>Aphids</i>	foliar spraying, overall	Mar-Jul/ BBCH 12-91	a) 1 b) 1	-	a) 0.23 b) 0.23	a) 46 b) 46	200-400	n.a.	Application in the evening, after the bee flight To protect bees and pollinating insects, application	A	A	A	R	R	R	A

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
													during flowering against pests is possible only out of honey-bee flight during late evening hours!							
86	Netherlands	Flower bulbs and flower tubers	F	<i>Aphids</i>	foliar spraying, overall	Mar-Jul/ BBCH 20-91	a) 2 b) 2	7	a) 0.17 b) 0.34	a) 34 b) 68	200-400	n.a.	Application in the evening, after the bee flight. To protect bees and pollinating insects, application during flowering against pests is possible only out of honey-bee flight during late evening hours	A	A	R	R	R	R	A
87	Slovenia	Flower bulbs and flower tubers	F	<i>Aphids</i>	foliar spraying, overall	Mar-Jul/ BBCH 12-91	a) 2 b) 2 a) 1 b) 1	a) 7 b) 7 -	a) 0.17 b) 0.34 0.17	a) 34 b) 68 34	200-400	n.a.	Application in the evening, after the bee flight. To protect bees and	A	A	R	R	R	A	A

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
													pollinating insects; application during flowering against pests is possible only out of honey-bee flight during late evening hours							
Xa	Central	Floriculture, Tree nursery & Perennial nursery crops	F	Aphids	foliar spraying, overall	Mar-Aug/ BBCH 12-91	a) 1 b) 1	-	a) 0.23 b) 0.23	a) 46 b) 46	200-1000	n.a.	Umbrella GAP To protect bees and pollinating insects; application during flowering against pests is possible only out of honey-bee flight during late evening hours	A	A	R	R See below	R	R	A
Xb	Central	Floriculture, Tree nursery & Perennial nursery crops	F	Aphids	foliar spraying, overall	Mar-Aug/ BBCH 12-91	a) 2 b) 2	7	a) 0.17 b) 0.34	a) 34 b) 68	200-1000	n.a.	Umbrella GAP To protect bees and pollinating insects; application during flowering against pests is possible	A	A	R	R See below	R	R	A

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
													only-out-of honey-bee flight during-late evening hours							
88	Netherlands	Floriculture crops, Tree nursery crops, Perennial nursery crops	F	<i>Aphids</i>	foliar spraying, overall	Mar-Aug/ BBCH 12- 91	a) 1 b) 1	-	a) 0.23 b) 0.23	a) 46 b) 46	200- 1000	n.a.	To-protect bees-and pollinating insects; application during flowering against pests-is possible only-out-of honey-bee flight during-late evening hours	A	A	R	R <u>See below</u>	R	R	A
88	Netherlands	Floriculture crops: - Flowering ornamental bushes	F	<i>Aphids</i>	foliar spraying, overall	Ma -Aug/ BBCH 70- 91	a) 1 b) 1	-	a) 0.23 b) 0.23	a) 46 b) 46	200- 1000	n.a.	Do not apply during flowering (application from BBCH 70	A	A	R	R From BBCH 70	R	R	A
		Floriculture crops: - Remaining ornamental plants and perennial nursery crops	F	<i>Aphids</i>	foliar spraying, overall	Mar-Aug/ BBCH 12- 91	a) 1 b) 1	-	a) 0.23 b) 0.23	a) 46 b) 46	200- 1000	n.a.	Application in the evening, after the bee flight	A	A	R	R	R	R	A
		Tree nursery crops -Flowering tree nursery	F	<i>Aphids</i>	foliar spraying, overall	Mar-Aug/ BBCH 70- 91	a) 1 b) 1	-	a) 0.23 b) 0.23	a) 46 b) 46	200- 1000	n.a.	Do not apply during flowering (application from BBCH 70	A	A	R	R From BBCH 70	R	R	A

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
		- Nurseries of non-flowering trees and bushes	F	<i>Aphids</i>	foliar spraying, overall	Mar-Aug/ BBCH 12-91	a) 1 b) 1	-	a) 0.23 b) 0.23	a) 46 b) 46	200-1000	n.a.		A	A	R	A	R	R	A
89	Netherlands	Floriculture crops: Flowering bushes Tree nursery crops Perennial nursery crops	F	<i>Aphids</i>	foliar spraying, overall	Mar-Aug/ BBCH 12-91	a) 2 b) 2	7	a) 0.17 b) 0.34	a) 34 b) 68	200-1000	n.a.		A	A	R	R <u>See below</u>	R	R	A
89	Netherlands	Floriculture crops: - Flowering ornamental bushes	F	<i>Aphids</i>	foliar spraying, overall	Mar-Aug/ BBCH 70 12 91	a) 2 b) 2	7	a) 0.17 b) 0.34	a) 34 b) 68	200-1000	n.a.	Do not apply during flowering (application from BBCH 70)	A	A	R	R From BBCH 70	R	R	A
		Floriculture crops: - Remaining ornamental plants and perennial nursery crops	F	<i>Aphids</i>	foliar spraying, overall	Mar-Aug/ BBCH 12-91	a) 2 b) 2	7	a) 0.17 b) 0.34	a) 34 b) 68	200-1000	n.a.	Application in the evening, after the bee	A	A	R	R	R	R	A
		Tree nursery crops: - Flowering tree nursery	F	<i>Aphids</i>	foliar spraying, overall	Mar-Aug/ BBCH 70 12 -91	a) 2 b) 2	7	a) 0.17 b) 0.34	a) 34 b) 68	200-1000	n.a.	Do not apply during flowering (application from BBCH 70)	A	A	R	R From BBCH 70	R	R	A
		- Nurseries of non-flowering trees and bushes	F	<i>Aphids</i>	foliar spraying, overall	Mar-Aug/ BBCH 12-91	a) 2 b) 2	7	a) 0.17 b) 0.34	a) 34 b) 68	200-1000	n.a.	-	A	A	R	A	R	R	A

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
90	Slovenia	Floriculture crops Tree nursery crops Perennial nursery crops	F	<i>Aphids</i>	foliar spraying, overall	Mar-Aug/ BBCH 12-91	a) 2 b) 2 a) 1 b) 1	a) 7 b) 7 -	a) 0.17 b) 0.34 0.17	a) 34 b) 68 34	200-1000	n.a.	To protect bees and pollinating insects, application during flowering against pests is possible only out of honey-bee flight during late evening hours	A	A	R	R See below	R	A	A
90	Slovenia	Floriculture crops: - Flowering ornamental bushes	F	<i>Aphids</i>	foliar spraying, overall	Mar-Aug/ BBCH 70 12-91	a) 2 b) 2 a) 1 b) 1	a) 7 b) 7 -	a) 0.17 b) 0.34 0.17	a) 34 b) 68 34	200-1000	n.a.	Do not apply during flowering (application from BBCH 70)	A	A	R	R From BBCH 70	R	A	A
		Floriculture crops: - Remaining ornamental plants and perennial nursery crops	F	<i>Aphids</i>	foliar spraying, overall	Mar-Aug/ BBCH 12-91	a) 2 b) 2 a) 1 b) 1	a) 7 b) 7 -	a) 0.17 b) 0.34 0.17	a) 34 b) 68 34	200-1000	n.a.	Application in the evening, after the bee flight	A	A	R	R	R	A	A
		Tree nursery crops: - Flowering tree nursery	F	<i>Aphids</i>	foliar spraying, overall	Mar-Aug/ BBCH 70 12-91	a) 1 b) 1	-	a) 0.17 b) 0.17	a) 34 b) 34	200-1000	n.a.	Do not apply during flowering (application from BBCH 70)	A	A	R	R From BBCH 70	R	A	A
		- Nurseries of non-flowering trees and bushes	F	<i>Aphids</i>	foliar spraying, overall	Mar-Aug/ BBCH 12-91	a) 1 b) 1	a) 7 b) 7 -	a) 0.17 b) 0.17	a) 34 b) 34	200-1000	n.a.		A	A	R	A	R	A	A

* Use number(s) in accordance with the list of all intended GAPs in Part B, Section 0 should be given in column 1

** F: professional field use, Fn: non-professional field use, Fpn: professional and non-professional field use, G: professional greenhouse use, Gn: non-professional greenhouse use, Gpn: professional

and non-professional greenhouse use, I: indoor application

Explanation for column 15 – 21 “Conclusion”

A	Acceptable, Safe use
R	Further refinement and/or risk mitigation measures required
C	To be confirmed by CMS
N	No safe use

Remarks table:

- (1) Numeration necessary to allow references
- (2) Use official codes/nomenclatures of EU
- (3) For crops, the EU and Codex classifications (both) should be used; where relevant, the use situation should be described (*e.g.* fumigation of a structure)
- (4) F: professional field use, Fn: non-professional field use, Fpn: professional and non-professional field use, G: professional greenhouse use, Gn: non-professional greenhouse use, Gpn: professional and non-professional greenhouse use, I: indoor application
- (5) Scientific names and EPPO-Codes of target pests/diseases/ weeds or when relevant the common names of the pest groups (*e.g.* biting and sucking insects, soil born insects, foliar fungi, weeds) and the developmental stages of the pests and pest groups at the moment of application must be named
- (6) Method, *e.g.* high volume spraying, low volume spraying, spreading, dusting, drench
Kind, *e.g.* overall, broadcast, aerial spraying, row, individual plant, between the plants - type of equipment used must be indicated
- (7) Growth stage at first and last treatment (BBCH Monograph, Growth Stages of Plants, 1997, Blackwell, ISBN 3-8263-3152-4), including where relevant, information on season at time of application
- (8) The maximum number of application possible under practical conditions of use must be provided
- (9) Minimum interval (in days) between applications of the same product.
- (10) For specific uses other specifications might be possible, *e.g.*: g/m³ in case of fumigation of empty rooms. See also EPPO-Guideline PP 1/239 Dose expression for plant protection products
- (11) The dimension (g, kg) must be clearly specified. (Maximum) dose of a.s. per treatment (usually g, kg or L product / ha).
- (12) If water volume range depends on application equipments (*e.g.* ULVA or LVA) it should be mentioned under “application: method/kind”.
- (13) PHI - minimum pre-harvest interval
- (14) Remarks may include: Extent of use/economic importance/restrictions

zRMS comments:

GAP table above has been amended by zRMS for Floriculture, Tree nursery and perennial nursery crops (Uses No: 88, 89 and 90) for clarity of the risk mitigation for bees for these uses.

9.1.1 Overall conclusions

9.1.1.1 Effects on birds (KCP 10.1.1), Effects on terrestrial vertebrates other than birds (KCP 10.1.2), Effects on other terrestrial vertebrate wildlife (reptiles and amphibians) (KCP 10.1.3)

Birds:

The acute and long-term risks of ADM.00150.I.2.A (containing 200 g/L acetamiprid) to birds were assessed from toxicity exposure ratios between toxicity endpoints, estimated from studies with acetamiprid, and maximum residues occurring on food items following applications according to the proposed use pattern.

The TER values for all uses, calculated for recommended scenarios, exceed the trigger values of 10 for acute risk and 5 for long-term risk, with the exception of the reproductive scenario small insectivorous bird “tit” in apple (1 x 80 g/ha), indicating that the risk to birds is acceptable following use of ADM.00150.I.2.A according to the proposed use pattern. The scenario small insectivorous bird “tit” was addressed in weight of evidence, presenting PT values for tits in orchards, demonstrating a low risk to representatives of this diet group.

The risk of secondary poisoning is not relevant.

The risk from drinking water was assessed demonstrating that the risk to birds is acceptable.

Mammals:

The acute and long-term risks of ADM.00150.I.2.A (containing 200 g/L acetamiprid) to mammals were assessed from toxicity exposure ratios between toxicity endpoints, estimated from studies with acetamiprid, and maximum residues occurring on food items following applications according to the proposed use pattern.

The acute TER values for all uses, calculated for recommended scenarios, exceed the trigger values of 10 for acute risk indicating an acceptable risk to mammals following use of ADM.00150.I.2.A according to the proposed use pattern.

Considering the long-term risk, several uses showed a potential risk in the tier 1 risk assessment to small and large herbivorous, and frugivorous mammals. The long-term risks to small and large herbivorous mammals and frugivorous mammals were addressed in higher tier risk assessments.

After refinement of the deposition factor (DF), refined RUD values for food items based on published literature, refinement of the diet and refinement of DT₅₀ with substance-specific residue decline data for monocotyledonous and dicotyledonous plants, a low risk to mammals could be demonstrated.

The risk of secondary poisoning is not relevant.

The risk from drinking water was assessed demonstrating that the risk to mammals is acceptable.

9.1.1.2 Effects on aquatic organisms (KCP 10.2)

The standard and refined risk assessment provided for the insecticidal product ADM.00150.I.2.A, the active substance acetamiprid and its major metabolites demonstrate that the application of ADM.00150.I.2.A as intended in the GAP according to good agricultural practice is of low risk to aquatic ecosystems if certain mitigation measures are considered.

Umbrella GAP number	Intended uses	Single GAP uses covered	Mitigating measures*
IIa	Apple, BBCH 71-PHI, 1 x 80 g a.s./ha, late	4, 6, 9, 11, 13, 15	<ul style="list-style-type: none"> - 20 m DBZ plus 20 m VFS or - 10 m DBZ plus 10 m VFS plus 50% DRN or - 10 m DBZ plus 10 m VFS

Umbrella GAP number	Intended uses	Single GAP uses covered	Mitigating measures*
Iib	Apple, BBCH 62-PHI, 2 x 25 g a.s./ha (8 days interval), early	5, 7, 8, 10, 12, 14, 16	- 15 m DBR or - 10 m DBZ plus 50% DRN or - 75% DRN
Iib	Apple, BBCH 62-PHI, 2 x 25 g a.s./ha (8 days interval), late	5, 7, 8, 10, 12, 14, 16	- 10 m DBZ or - 50% DRN
IVa	Spring cereals and winter cereals as surrogate for R1, R3, BBCH 40-69, 2 x 35 g a.s./ha (10 days interval)	23, 25, 27, 28, 29	- 10 m DBZ plus 10 m VFS
Va	Winter cereals, BBCH 40 – 69, 1-2 x 36 g a.s./ha (10 days interval)	32, 34, 36, 39	- 10 m DBZ plus 10 m VFS
Vb	Winter cereals, 12 – 29, 1 x 30 g a.s./ha	33, 35, 37, 38, 40	- 10 m DBZ plus 10 m VFS
VIa	Winter oilseed rape, BBCH 31 – 71, 1-2 x 60 g a.s./ha (7 days interval)	41, 42, 43, 46, 47, 48, 49, 50, 51, 53, 54, 55, 56, 57, 58, 62, 63, 64	- 10 m DBZ plus 10 m VFS
VIIa	Spring oilseed rape, BBCH 31 – 69, 1-2 x 60 g a.s./ha (7 days interval)	66 - 79	- 10 m DBZ plus 10 m VFS
VIIIa	Sugar beet, BBCH 12-39, 2 x 50 g a.s./ha (7 days interval)	80 – 84	- 15 m DBZ plus 10 m VFS
IXb	Flower bulbs and flower tubers, BBCH 20 – 91, 2 x 34 g a.s./ha (7 days interval)	86, 87	- 10 m DBZ plus 10 m VFS
Xa	Floriculture, Tree nursery and Perennial nursery crops, BBCH 12-91, 1 x 46 g a.s./ha (Pome and stonefruit early)	88	- 20 m DBZ or - 15 m DBZ plus 50% DRN or - 10 m DBZ plus 75% DRN or - 90% DRN
Xa	Floriculture, Tree nursery and Perennial nursery crops, BBCH 12-91, 1 x 46 g a.s./ha (Pome and stonefruit late)	88	- 10 m DBZ or - 75% DRN
Xb	Floriculture, Tree nursery and Perennial nursery crops, BBCH 20– 91, 2 x 34 g a.s./ha (Leafy vegetables)	89, 90	- 10 m DBZ plus 10 m VFS
Xb	Floriculture, Tree nursery and Perennial nursery crops, BBCH 20– 91, 2 x 34 g a.s./ha (Pome and stonefruit early)	89, 90	- 20 m DBZ or - 15 m DBZ plus 50% DRN or - 10 m DBZ plus 75% DRN or - 90% DRN
Xb	Floriculture, Tree nursery and Perennial nursery crops, BBCH 20– 91, 2 x 34 g a.s./ha (Pome and stonefruit late)	89, 90	- 10 m DBZ or - 50% DRN

DBZ: drift buffer zone; DRN: drift reducing nozzles; VFS: vegetated filter strip; VFS_{mod}: vegetated filter strip modified
* Mitigation reductions based on the EoP approach are also included

zRMS comments:

The risk mitigation measures based on the RAC of 0.56 µg/L provided in the Table above agreed by zRMS. As different scenarios are considered representative in various cMS and required risk mitigation measures varied among scenarios, the summary table presenting mitigation measures for each scenario separately has been prepared by the zRMS for convenience of the cMS and performed under Point 9.5.2.

Commenting period process:

We would like to stressed that if the other MSs are different opinion of endpoint used from mesocosm study by Hommen 2022, they should consider it further at National level.

9.1.1.3 Effects on bees (KCP 10.3.1)

The risk assessment performed in line with SANCO/1039/2002 demonstrated acceptable risk to bees following application of ADM.00150.I.2.A to all intended crops.

However, as acetamiprid is an insecticide with the specific mode of action, evaluation of the chronic risk to adult bees and bee larvae was also deemed necessary. In absence of the chronic and larvae risk assessment scheme, the zRMS concluded that the risk assessment as provided in EFSA (2013) will be most relevant to cover the risk to all bee stages and all exposure patterns, even though the guidance is not noted yet at the EU level.

Evaluation based on indications of EFSA (2013) demonstrated acceptable acute and chronic risk to adult bees and larvae exposed following intended uses of ADM.00150.I.2.A in maize, cereals and potatoes.

Based on the evaluation of higher tier studies for bees evaluated by zRMS, the following restriction is applied to protect bees and other pollinators:

Apple, application rate 1-2 x25 g a.s./ha:

- application from BBCH 70 (Don't apply during flowering)

Floriculture crops:

Flowering ornamental bushes:

- application from BBCH 70 (Don't apply during flowering)

Remaining ornamental plants and perennial nursery crops at BBCH 12-91:

- application during flowering in the evening after bee flight

Tree nursery crops:

Flowering tree nursery:

- application from BBCH 70 (Don't apply during flowering)

Nurseries of non-flowering trees and bushes:

No risk mitigation is required.

Flower bulb and flower tubers at rate 34 g a.s./ha and 46 g a.s./ha at BBCH 12-91:

Application during flowering period in the evening after bee flight

Oil seed rape: Only one application during flowering allowed, applied in the evening after bee flight

Sugar beet: no risk mitigation is required

All HQ values for the oral and contact exposure of adult honey bees were below the trigger of 50, based on the proposed maximum application rates of the respective umbrella GAPs. This demonstrated that no negative effects on honey bees are expected when ADM.00150.I.2.A (containing 200 g/L acetamiprid) is applied according to the intended application rates up to 80 g a.s./ha.

Furthermore, a comparison of the LD₅₀ values deriving from chronic adult and larvae laboratory studies indicate that toxicity is very similar compared to the acute oral endpoint, and no difference in sensitivity was observed. For bumble bees, the acute endpoints indicated that bumblebees are not more susceptible to acetamiprid than honey bees.

The outcome of the theoretical risk assessment is confirmed by the results of one semi-field bee brood study and three full-field studies with detailed bee brood assessments. The data showed that the application of ADM.00150.I.2.A to flowering, bee attractive crops after daily bee flight does not adversely affect the survival and fitness of adult and pupal honey bees, honey bee brood and their colonies after acute and chronic exposure when applied to flowering, bee attractive crops up to a rate of 100 g acetamiprid/ha. The

application in the evening after bee flight is regarded as a suitable risk mitigation measure providing a good margin of safety for applications in the flowering and bee attractive crops.

Overall, it can be concluded that ADM.00150.I.2.A is of low risk for bees, their brood and their colonies when used according to the proposed GAP.

9.1.1.4 Effects on arthropods other than bees (KCP 10.3.2)

Regarding non-target arthropods in in-field habitats, the available data from aged residue studies clearly demonstrate that recovery within an ecologically relevant timeframe can be expected, especially as the available field study demonstrates that recolonization from the off-field is not impaired.

Regarding non-target arthropods in off-field habitats, the data from the available field study show that no unacceptable risks are to be expected when ADM.00150.I.2.A is applied according to good agricultural practice, except for the intended application in pomefruit (1 x 60 80-g a.s./ha and 2 x 25 g a.s./ha) and floriculture, tree nursery & perennial nursery crops (2 x 34 g a.s./ha and 1 x 46 g a.s./ha), corn and winter/spring OSR (Use No. VIa, VII), based NOER of 1.4 g a.s/ha from field study.

When NOEAER of 3.4 g a.s/ha value from the field study were used an unacceptable risk is indicated only for pomefruit (1 x 60 g a.s./ha and 2 x 25 g a.s./ha) and floriculture, tree nursery & perennial nursery crops (2 x 34 g a.s./ha and 1 x 46 g a.s./ha).

The risk to off-field non-target arthropods based on NOEAER of 3.4 g a.s./ha is acceptable following use of ADM.00150.I.2.A in pome fruit (1 x 60 g a.s./ha), provided when the following risk mitigation measures are applied:

- 10 m or
- 5 m+ 50% DRN or
- 75% DRN

The risk to off-field non-target arthropods based on NOEAER of 3.4 g a.s./ha is acceptable following use of ADM.00150.I.2.A in pome fruit (2 x 25 g a.s./ha), provided the following risk mitigation measures are applied:

- 5 m buffer or
- 50% DRN

The risk to off-field non-target arthropods based on NOEAER of 3.4 g a.s./ha is acceptable following use of ADM.00150.I.2.A in floriculture, tree nursery & perennial nursery crops (1 x 46 g a.s./ha), provided the following risk mitigation measures are applied:

- 10 m or
- 5 m + 50% DRN or
- 75% DRN

The risk to off-field non-target arthropods based on NOEAER of 3.4 g a.s./ha is acceptable following use of ADM.00150.I.2.A in floriculture, tree nursery & perennial nursery crops (2 x 34 g a.s./ha), provided the following risk mitigation measures are applied

- 5 m or
- 50% DRN

The risk to off field non target arthropods is acceptable following use of ADM.00150.I.2.A in pomefruit (1 x 80 g a.s./ha), provided when the following risk mitigation measures are applied:

- 90% drift reduction or
- 5 m buffer and 75% drift reduction or
- 10 m buffer and 50% drift reduction or
- 15 m buffer

The risk to off field non target arthropods is acceptable following use of ADM.00150.I.2.A in pomefruit (2 x 25 g a.s./ha), provided the following risk mitigation measures are applied:

- 5 m buffer and 50% drift reduction or
- 10 m buffer

The risk to off field non target arthropods is acceptable following use of ADM.00150.I.2.A in winter/spring OSR (2 x 60 g a.s./ha), provided the following risk mitigation measures are applied:

- 50% drift reduction or
- 5 m buffer

The risk to off field non target arthropods is acceptable following use of ADM.00150.I.2.A in floriculture, tree nursery & perennial nursery crops (1 x 46 g a.s./ha and 2 x 34 g a.s./ha), provided the following risk mitigation measures are applied:

- 75% drift reduction or
- 5 m buffer and 50% drift reduction
- 0 m buffer

We would like to stressed that if the other MSs are different opinion of endpoint used by zRMS from field study, they should consider it further at National level.

9.1.1.5 Effects on non-target soil meso- and macrofauna (KCP 10.4)

In a one year filed study, no effects occurred at tested rates up to 2 x 80 g a.s./ha, indicating that the risk to soil meso- and macrofauna is acceptable following the use of ADM.00150.I.2.A according to the proposed use patterns. We would like to stressed that if the other MSs are different opinion of endpoint from field for Folsomia then presented by zRMS (NOER and NOEAR endpoints) , they should consider it further at National level.

9.1.1.6 Effects on soil microbial activity (KCP 10.5)

The risk of ADM.00150.I.2.A to soil microorganisms was evaluated by comparison of the maximum concentrations with effects <25% derived from laboratory tests, with maximum PEC_{soil}. For metabolite IM-1-5 the evaluation was performed with consideration of the maximum agreed accumulated PEC_{soil} and assumption that metabolite is 10 times more toxic for the parent.

No effects > 25% occurred at tested rates exceeding the relevant PEC_{soil} values, indicating that the risk to soil microorganisms is acceptable following the use of ADM.00150.I.2.A according to the proposed use patterns. Risk from metabolites IM-1-2, IM-1-4 and IC-0 is considered to be covered by evaluation performed for the parent.

9.1.1.7 Effects on non-target terrestrial plants (KCP 10.6)

The application of ADM.00150.I.2.A according to the proposed use pattern will pose an acceptable risk to non-target terrestrial plants.

9.1.1.8 Effects on other terrestrial organisms (flora and fauna) (KCP 10.7)

Not relevant.

9.1.2 Grouping of intended uses for risk assessment

The following table documents the grouping of the intended uses to support application of the risk envelope approach (according to SANCO/11244/2011).

Table 9.1-2: Critical use pattern of formulation grouped according to criterion

		Grouping according to criterion		
Umbrella GAP number	Crop group	Intended uses	relevant use parameters for grouping	relevant parameter or value for sorting
Effects on birds (9.2) and mammals (9.3)				
I	Maize	Corn, BBCH 51-75, 1 x 60 g a.s./ha	Crop groups and crop species according to EFSA/2009/1438	Number of applications, maximum application rate and BBCH stage
IIa	Orchard	Apple, BBCH 71-PHI, 1 x 60 g a.s./ha		
IIb		Apple, BBCH 62-PHI, 2 x 25 g a.s./ha (8 days interval)		

		Grouping according to criterion		
Umbrella GAP number	Crop group	Intended uses	relevant use parameters for grouping	relevant parameter or value for sorting
III	Potatoes	Potato, BBCH 12-79, 1 x 36 g a.s./ha		
IVa	Cereals	Spring cereals, BBCH 40 – 69, 1-2 x 35 g a.s./ha (10 days interval)		
IVb		Spring cereals, 12 – 69, 1-2 x 35 g a.s./ha (30 days interval)		
Va		Winter cereals, BBCH 40 – 69, 1-2 x 36 g a.s./ha (10 days interval)		
Vb		Winter cereals, 12 – 29, 1 x 30 g a.s./ha		
VIa	Oilseed rape	Winter oilseed rape, BBCH 31 – 71, 1-2 x 60 g a.s./ha (7 days interval)		
VIb		Winter oilseed rape, BBCH 11 – 19, 1 x 48 g a.s./ha 6-9 g a.s./ha		
VIIa		Spring oilseed rape, BBCH 31 – 69, 1-2 x 60 g a.s./ha (7 days interval)		
VIIIa	Sugar beet	Sugar beet, BBCH 12 – 39, 2 x 50 g a.s./ha (7 days interval)		
IXa	Bulbs and onion like crops	Flower bulbs and flower tubers, BBCH 12-91, 1 x 46 g a.s./ha		
IXb		Flower bulbs and flower tubers, BBCH 20 – 91, 2 x 34 g a.s./ha (7 days interval)		
Xa	Ornamentals/nursery	Floriculture, Tree nursery and Perennial nursery crops, BBCH 12-91, 1 x 46 g a.s./ha		
Xb		Floriculture, Tree nursery and Perennial nursery crops, BBCH 20– 91, 2 x 34 g a.s./ha		
Effects on aquatic organisms (9.5)				
I	Maize	Corn, BBCH 51-75, 1 x 60 g a.s./ha GAP use nr.: 1, 2, 3	Worst-case PEC	Number of applications, maximum application rate and BBCH stage
IIa	Orchards	Apple, BBCH 71-PHI, 1 x 80 g a.s./ha, late GAP use nr.: 4, 6, 9, 11, 13, 15		
IIb		Apple, BBCH 62-PHI, 2 x 25 g a.s./ha (8 days interval), early GAP use nr.: 5, 7, 8, 10, 12, 14, 1		
IIb		Apple, BBCH 62-PHI, 2 x 25 g a.s./ha (8 days interval), late GAP use nr.: 5, 7, 8, 10, 12, 14, 16		
III	Potatoes (maize as surrogate for D5 & R4)	Potato, BBCH 12-79, 1 x 36 g a.s./ha GAP use nr.: 17-22		
IVa	Cereals	Spring cereals, BBCH 40 – 69, 1-2 x 35 g a.s./ha (10 days interval) GAP use nr.: 23, 25, 27, 28, 29 (winter cereals as surrogate for R1,		

		Grouping according to criterion		
Umbrella GAP number	Crop group	Intended uses	relevant use parameters for grouping	relevant parameter or value for sorting
		R3)		
IVb		Spring cereals, 12 – 69, 1-2 × 35 g a.s./ha (30 days interval) GAP use nr.: 24, 26, 28, 31 (winter cereals as surrogate for R1, R3)		
Va		Winter cereals, BBCH 40 – 69, 1-2 × 36 g a.s./ha (10 days interval) GAP use nr.: 32, 34, 36, 39		
Vb		Winter cereals, 12 – 29, 1 × 30 g a.s./ha GAP use nr.: 33, 35, 37, 38, 40		
VIa	Oilseed rape	Winter oilseed rape, BBCH 31 – 71, 1-2 × 60 g a.s./ha (7 days interval) GAP use nr.: 41, 42, 43, 46, 47, 48, 49, 50, 51, 53, 54, 55, 56, 57, 58, 62, 63, 64 (winter cereals as surrogate for R4)		
VIb		Winter oilseed rape, BBCH 11 – 19, 1 × 60 g a.s./ha GAP use nr.: 44, 45, 52, 59, 60, 61, 65 (winter cereals as surrogate for R4)		
VIIa		Spring oilseed rape, BBCH 31 – 69, 1-2 × 60 g a.s./ha (7 days interval) GAP use nr.: 66 – 79 (legumes as surrogate for R3 & R4)		
VIIIa	Sugar beet	Sugar beet, BBCH 12 – 39, 2 × 50 g a.s./ha (7 days interval) GAP use nr.: 80 – 84 (maize as surrogate for D5 & R4)		
IXa	Bulbs and onion like crops (legumes as surrogate for D5)	Flower bulbs and flower tubers, BBCH 12-91, 1 x 46 g a.s./ha GAP use nr.: 85		
IXb		Flower bulbs and flower tubers, BBCH 20 – 91, 2 × 34 g a.s./ha (7 days interval) GAP use nr.: 86, 87		
Xa	Ornamentals/nursery (leafy vegetables) (legumes as surrogate for D5)	Floriculture, Tree nursery and Perennial nursery crops, BBCH 12-91, 1 x 46 g a.s./ha GAP use nr.: 88		
Xa	Ornamentals/nursery (pome/stone fruit, early) legumes as surrogate for D5)	Floriculture, Tree nursery and Perennial nursery crops, BBCH 12-91, 1 x 46 g a.s./ha GAP use nr.: 88		
Xa	Ornamentals/nursery (pome/stone fruit, late)	Floriculture, Tree nursery and Perennial nursery crops, BBCH 12-91, 1 x 46 g a.s./ha		

		Grouping according to criterion		
Umbrella GAP number	Crop group	Intended uses	relevant use parameters for grouping	relevant parameter or value for sorting
		GAP use nr.: 88		
Xb	Ornamentals/nursery (leafy vegetables)	Floriculture, Tree nursery and Perennial nursery crops, BBCH 20– 91, 2 x 34 g a.s./ha GAP use nr.: 89, 90		
Xb	Ornamentals/nursery (pome/stone fruit, early)	Floriculture, Tree nursery and Perennial nursery crops, BBCH 20– 91, 2 x 34 g a.s./ha GAP use nr.: 89, 90		
Xb	Ornamentals/nursery (pome/stone fruit, late)	Floriculture, Tree nursery and Perennial nursery crops, BBCH 20– 91, 2 x 34 g a.s./ha GAP use nr.: 89, 90		
Effects on bees (9.6)				
I	Maize	Corn, BBCH 51-75, 1 x 60 g a.s./ha	Crop groups and crop species	Number of applications and maximum application rate
IIa	Orchard	Apple, BBCH 71-PHI, 1 x 60 g a.s./ha		
IIb		Apple, BBCH 62-PHI, 2 x 25 g a.s./ha; to protect bees and pollinating insects, do not apply during daily bee flight if crop is flowering		
III	Potatoes	Potato, BBCH 12-79, 1 x 36 g a.s./ha; to protect bees and pollinating insects, do not apply during daily bee flight if crop is flowering		
IVa	Cereals	Spring wheat, spring barley, spring oats, spring durum, wheat, spring triticale, BBCH 40-69 (spring), 1-2 x 35 g a.s./ha		
IVb		Spring wheat, spring barley, spring oats, spring durum, wheat, spring triticale, BBCH 12-69 (spring), 1 x 35 g a.s./ha		
Va		Winter wheat, winter barley, winter rye, winter triticale, BBCH 40-69 (spring), 2 x 36 g a.s./ha		
Vb		Winter wheat, winter barley, winter rye, winter triticale, BBCH 12-29 (autumn), 1 x 30 g a.s./ha		
VIa	Oilseed rape	Winter OSR, BBCH 31-71 (spring), 1-2 x 60 g a.s./ha; to protect bees and pollinating insects, do not apply during daily bee flight if crop is flowering.		
VIb		Winter OSR, BBCH 11-19 (autumn), 1 x 48 g a.s./ha		
VIIa		Spring OSR, BBCH 31-71, 1-2 x 60 g a.s./ha; to protect bees and pollinating insects, do not apply during daily bee flight if crop is		

		Grouping according to criterion		
Umbrella GAP number	Crop group	Intended uses	relevant use parameters for grouping	relevant parameter or value for sorting
		flowering;		
VIII	Sugar beet	Sugar beet, BBCH 12-39, 2 x 50 g a.s./ha		
IXa	Bulbs and onion like crops	Flower bulbs and flower tubers, BBCH 12-91, 1 x 46 g a.s./ha; to protect bees and pollinating insects, do not apply during daily bee flight if crop is flowering;		
IXb		Flower bulbs and flower tubers, BBCH 20-91, 2 x 34 g a.s./ha; to protect bees and pollinating insects, do not apply during daily bee flight if crop is flowering;		
Xa	Ornamentals/nursery	Floriculture, Tree nursery & Perennial nursery crops, BBCH 12-91, 1 x 46 g a.s./ha; to protect bees and pollinating insects, do not apply during daily bee flight if crop is flowering;		
Xb		Floriculture, Tree nursery & Perennial nursery crops, BBCH 12-91, 2 x 34 g a.s./ha; to protect bees and pollinating insects, do not apply during daily bee flight if crop is flowering;		
Effects on arthropods other than bees (9.7)				
I	Maize	Corn, 1 x 60 g a.s./ha	Crop groups and crop species according to EFSA/2009/1438	Number of applications, maximum application rate
IIa	Orchard	Apple, 1 x 60 80 g a.s./ha		
IIb		Apple, 2 x 25 g a.s./ha		
III	Potatoes	Potato, 1 x 36 g a.s./ha		
IVa, VIb, Va	Cereals	Spring/winter cereals, 1-2 x35 g a.s./ha and 1-2 x 36 g a.s./ha		
Vb		Winter cereals, 1 x 30 g a.s./ha		
VIa, VII	Oilseed rape	Winter/spring oilseed rape, 1-2 x 60 g a.s./ha		
VIb		Winter oilseed rape, 1 x60 g a.s./ha		
VIII	Sugar beet	Sugar beet, 2 x 50 g a.s./ha		
IXa	Bulbs and onion like crops	Flower bulbs and flower tubers, 1 x 46 g a.s./ha		
IXb		Flower bulbs and flower tubers, 2 x 34 g a.s./ha		
Xa	Ornamentals/nursery	Floriculture, Tree nursery and Perennial nursery crops, 1 x 46 g a.s./ha		
Xb		Floriculture, Tree nursery and Perennial nursery crops, 2 x 34 g a.s./ha		
Effects on non-target soil meso- and macrofauna (9.8) *				
I	Maize	Corn, 1 x 60 g a.s./ha	Crop groups and	Number of applications,

		Grouping according to criterion		
Umbrella GAP number	Crop group	Intended uses	relevant use parameters for grouping	relevant parameter or value for sorting
IIa	Orchard	Apple, 1 x 60 80 g a.s./ha	crop species according to EFSA/2009/1438	maximum application rate
IIb		Apple, 2 x 25 g a.s./ha		
III	Potatoes	Potato, 1 x 36 g a.s./ha		
IVa	Cereals	Spring cereals, 1-2 ×35 g a.s./ha		
IVb		Spring cereals, 1-2 ×35 g a.s./ha		
Va		Winter cereals, 1-2 x 36 g a.s./ha		
Vb		Winter cereals, 1 × 30 g a.s./ha		
VIa, VII	Oilseed rape	Winter/spring oilseed rape, 1-2 × 60 g a.s./ha		
VIb		Winter oilseed rape, 1 ×60 g a.s./ha		
VIII	Sugar beet	Sugar beet, 2 × 50 g a.s./ha		
IXa	Bulbs and onion like crops	Flower bulbs and flower tubers, 1 x 46 g a.s./ha		
IXb		Flower bulbs and flower tubers, 2 × 34 g a.s./ha		
Xa	Ornamentals/nursery	Floriculture, Tree nursery and Perennial nursery crops, 1 x 46 g a.s./ha		
Xb		Floriculture, Tree nursery and Perennial nursery crops, 2 x 34 g a.s./ha		
Effects on soil microbial activity (9.9)				
VIII	Sugar beet	2 x 50 g a.s./ha, covering all intnded uses		Maximum PEC _{soil} to assess risk to soil microbial activity to demonstrate acceptable risk according to EFSA
Effects on non-target terrestrial plants (9.10)				
VIa, VII	Oilseed rape	2 x 60 g a.s./ha, covering all intnded uses		Number of applications, maximum application rate

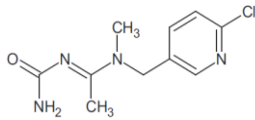
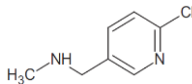
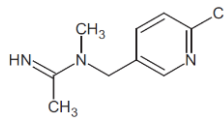
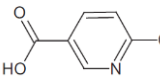
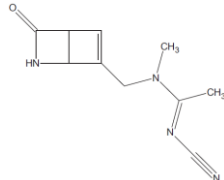
zRMS comments:

Generally, since the original submission, the GAP was changed by the Applicant due to issues with groundwater (see Part B section 8 chapter 8.8), i.e. the application rate or number of applications was lowered, or the initial BBCH increased in some cases. In the context of a risk envelope, the original application patterns were kept for the PEC_{SW/SED} calculations.

9.1.3 Consideration of metabolites

A list of metabolites found in environmental compartments is provided below. The need for conducting a metabolite-specific risk assessment in the context of the evaluation of ADM.00150.I.2.A is indicated in the table.

Table 9.1-3 Metabolites of acetamiprid

Metabolite	Chemical structure	Molar mass (g/mol)	Maximum occurrence in compartments	Risk assessment required?
IM-1-2		240.69	Maximum in soil: 55% Maximum in water/sediment: 13.4%	Soil: yes Water/sediment: yes
IM-1-4		156.61	Maximum in soil: 72% Maximum in water/sediment: 81.5% *	Soil: yes Water/sediment: yes
IM-1-5		197.66	Maximum in soil: 20% (calcareous soils only)	Soil: yes Water/sediment: yes
IC-0 6- Chloronicotinic Acid (IV-0)		157.55	Maximum in soil: 11.3% Maximum in water/sediment: 29.5%	Soil: yes Water/sediment: yes
IB-1-1		204.23	Maximum in water/sediment: 35% **	Soil: no Water/sediment: yes

* Observed in aerobic mineralisation study

** Formed only via aqueous photochemical degradation

zRMS comments:

Information regarding acetamiprid metabolites provided in Table 9.1-3 above is in line with EU agreed data reported in EFSA Journal 2016;14(11):4610.

9.2 Effects on birds (KCP 10.1.1)

9.2.1 Toxicity data

Avian toxicity studies have been carried out with acetamiprid and its relevant metabolites. Full details of these studies are provided in the respective EU DAR and related documents.

Effects on birds of ADM.00150.I.2.A were not evaluated as part of the EU assessment of acetamiprid. New data submitted with this application are listed in Appendix 1 and summarised in Appendix 2.

Table 9.2-1: Endpoints and effect values relevant for the risk assessment for birds

Species	Substance	Exposure System	Results	Endpoints used in risk assessment	Reference
<i>Anas platyrhynchos</i> (mallard duck)	Acetamiprid	Acute	LD ₅₀ = 98 mg/kg bw	Geometric mean LD₅₀ = 38.2 mg/kg bw	EFSA, 2016b
<i>Colinus virginianus</i> (bobwhite quail)	Acetamiprid	Acute	LD ₅₀ > 100 mg/kg bw		
<i>Poephila guttata</i> (zebra finch)	Acetamiprid	Acute	LD ₅₀ = 5.7 mg/kg bw		
<i>Anas platyrhynchos</i> (mallard duck)	Acetamiprid	Long-term	NOEL = 9.5 mg/kg bw/d (developmental neurotoxicity study)	LD₅₀/10 = 3.8 mg/kg bw	

Values shown in **bold** used for risk assessment

For reproductive risk assessments, the NOEL from chronic bird studies is compared to the acute oral LD₅₀ value used in the acute avian assessment (either the LD₅₀ for a single species, or the geometric mean for multiple species) and divided it by 10 to obtain LD₅₀/10. For acetamiprid, the ratio LD₅₀/10 is lower than the EU agreed NOEL from chronic bird testing and provides a more conservative endpoint. Therefore, the conservative ratio LD₅₀/10 = 3.8 mg/kg bw is used for risk assessments.

zRMS comments:

Avian toxicity data for acetamiprid in Table 9.2-1 above is in line with EU agreed data reported in EFSA Journal 2016;14(11):4610.

9.2.1.1 Justification for new endpoints

The selection of studies and endpoints for the risk assessment is in line with the results of the EU review process and therefore no new endpoints were selected.

9.2.2 Risk assessment for spray applications

The risk assessment is based on the methods presented in the Guidance Document on Risk Assessment for Birds and Mammals on request from EFSA (EFSA Journal 2009; 7(12): 1438; hereafter referred to as EFSA/2009/1438).

Metabolites:

Acute mammal toxicity studies with metabolites show that metabolites are either of much lower toxicity (IM-I-0, IM-1-3, IM-1-4, IM-2-1, IM-2-3, IM-1-2, IS-1-1, IS-2-1, IC-0, IB-1-1) or show a similar toxicity (IM-1-5) compared to the parent acetamiprid ($LD_{50} = 146 \text{ mg/kg bw}$, please refer to EFSA conclusion 2016 Appendix A LOE). Since acetamiprid was identified by far as the major component of the residues in almost all plant matrices (EFSA, 2016) and toxicity of metabolites is lower or within the same range, the toxicity of metabolites was concluded to be covered by the toxicity of the parent acetamiprid. It should also be noted that the metabolites are formed slowly and with a time delay from the parent substance and thus the amount of metabolites found at any one time will never reach that of the parent substance.

Further, it was concluded by RMS NL in 2016 (see Acetamiprid Volume 3, B9 (PPP, Draft Re-Assessment Report and Proposed decision of the Netherlands prepared in the context of the possible renewal of acetamiprid under Regulation (EC) 1107/2009), that even if no bird toxicity data are available, the risk assessment for the parent is considered to cover the risk of the metabolites if all available information is considered.

Risk to birds and mammals via secondary poisoning is not required, as the log Pow values of metabolites are below 3 (Vol.3 B.2 (AS)), indicating a low risk.

zRMS comments:

We agree that parent is considered to cover the risk of the metabolites.

9.2.2.1 First-tier assessment (screening/generic focal species)

The results of the acute and reproductive first-tier risk assessments are summarised in the following tables.

Table 9.2-2: First-tier assessment of the acute and long-term/reproductive risk for birds due to the use of ADM.00150.I.2.A in corn (BBCH 51-75, use no. I)

Intended use		Corn (BBCH 51-75)				
Active substance/product		Acetamiprid				
Application rate (g/ha)		1 × 60				
Acute toxicity (mg/kg bw)		38.2				
TER criterion		10				
Crop scenario Growth stage	Indicator/generic focal species	SV ₉₀	MAF ₉₀	DDD ₉₀ (mg/kg bw/d)	TER _a	
BBCH ≥ 40	Medium granivorous bird “gamebird”	1.6	1.0	0.10	397.9	
BBCH ≥ 40	Small omnivorous bird “lark”	6.0	1.0	0.36	106.1	
BBCH ≥ 40	Medium herbivorous/granivorous bird “pigeon”	13.9	1.0	0.83	45.8	
BBCH ≥ 20	Small insectivorous bird “wagtail”	12.6	1.0	0.76	50.5	
Reprod. toxicity (mg/kg bw/d)		3.8				
TER criterion		5				
Crop scenario Growth stage	Indicator/generic focal species	SV _m	MAF _m × TWA	DDD _m (mg/kg bw/d)	TER _{lt}	
BBCH ≥ 40	Medium granivorous bird “gamebird”	0.8	1.0 × 0.53	0.03	149.4	
BBCH ≥ 40	Small omnivorous bird “lark”	2.7	1.0 × 0.53	0.09	44.3	
BBCH ≥ 40	Medium herbivorous/granivorous bird “pigeon”	5.7	1.0 × 0.53	0.18	21.0	
BBCH ≥ 20	Small insectivorous bird “wagtail”	4.8	1.0 × 0.53	0.15	24.9	

SV: shortcut value; MAF: multiple application factor; TWA: time-weighted average factor; DDD: daily dietary dose; TER: toxicity to exposure ratio. TER values shown in bold fall below the relevant trigger.

Table 9.2-3: First-tier assessment of the acute and long-term/reproductive risk for birds due to the use of ADM.00150.I.2.A in apple (BBCH 71-PHI, use no. IIa)

Intended use		Apple (BBCH 71-PHI)				
Active substance/product		Acetamiprid				
Application rate (g/ha)		1 × 60 80				
Acute toxicity (mg/kg bw)		38.2				
TER criterion		10				
Crop scenario Growth stage	Indicator/generic focal species	SV ₉₀	MAF ₉₀	DDD ₉₀ (mg/kg bw/d)	TER _a	
Spring, summer	Small insectivorous bird “tit”	46.8	1.0	2.8 3.74	13.64 10.2	
BBCH ≥ 40	Small insectivorous/ worm feeding species “thrush”	2.2	1.0	0.13 0.18	293.85 217.0	
BBCH ≥ 40	Small granivorous bird "finch"	8.2	1.0	0.49 0.66	77.96 58.2	
Reprod. toxicity (mg/kg bw/d)		3.8				
TER criterion		5				
Crop scenario Growth stage	Indicator/generic focal species	SV _m	MAF _m × TWA	DDD _m (mg/kg bw/d)	TER _{lt}	
Spring, summer	Small insectivorous bird “tit”	18.2	1.0 × 0.53	0.57 0.77	6.66 4.9	
BBCH ≥ 40	Small insectivorous/ worm feeding species “thrush”	0.8	1.0 × 0.53	0.02 0.03	190 112.0	
BBCH ≥ 40	Small granivorous bird "finch"	3.8	1.0 × 0.53	0.12 0.16	31.66 23.6	

SV: shortcut value; MAF: multiple application factor; TWA: time-weighted average factor; DDD: daily dietary dose; TER: toxicity to exposure ratio. TER values shown in bold fall below the relevant trigger.

Table 9.2-4: First-tier assessment of the acute and long-term/reproductive risk for birds due to the use of ADM.00150.I.2.A in apple (BBCH 62-PHI, use no. IIb)

use of ADM-001304-22 in apple (BBCH 62-PH), use no.: 16)

Intended use		Apple (BBCH 62-PH)				
Active substance/product		Acetamiprid				
Application rate (g/ha)		1-2 × 25, 8 days interval				
Acute toxicity (mg/kg bw)		38.2				
TER criterion		10				
Crop scenario	Indicator/generic focal species	SV ₉₀	MAF ₉₀	DDD ₉₀ (mg/kg bw/d)	TER _a	
Growth stage						
Spring, summer	Small insectivorous bird “tit”	46.8	1.38	1.61	23.7	
BBCH ≥ 40	Small insectivorous/ worm feeding species “thrush”	2.2	1.38	0.08	503.3	
BBCH ≥ 40	Small granivorous bird "finch"	8.2	1.38	0.28	135.0	
Reprod. toxicity (mg/kg bw/d)		3.8				
TER criterion		5				
Crop scenario	Indicator/generic focal species	SV _m	MAF _m × TWA	DDD _m (mg/kg bw/d)	TER _{lt}	
Growth stage						
Spring, summer	Small insectivorous bird “tit”	18.2	1.57 × 0.53	0.38	10.0	
BBCH ≥ 40	Small insectivorous/ worm feeding species “thrush”	0.8	1.57 × 0.53	0.02	228.3	
BBCH ≥ 40	Small granivorous bird "finch"	3.8	1.57 × 0.53	0.08	48.1	

SV: shortcut value; MAF: multiple application factor; TWA: time-weighted average factor; DDD: daily dietary dose; TER: toxicity to exposure ratio. TER values shown in bold fall below the relevant trigger.

Table 9.2-5: First-tier assessment of the acute and long-term/reproductive risk for birds due to the use of ADM.00150.I.2.A in potato (BBCH 12-79, use no. III)

Intended use		Potato (BBCH 12-79)				
Active substance/product		Acetamiprid				
Application rate (g/ha)		1 × 36				
Acute toxicity (mg/kg bw)		38.2				
TER criterion		10				
Crop scenario	Indicator/generic focal species	SV ₉₀	MAF ₉₀	DDD ₉₀ (mg/kg bw/d)	TER _a	
Growth stage						
BBCH 10 - 39	Small omnivorous bird “lark”	24	1.0	0.86	44.2	
BBCH ≥ 40	Small omnivorous bird “lark”	7.2	1.0	0.26	147.4	
BBCH 10 - 19	Small insectivorous bird “wagtail”	26.8	1.0	0.96	39.6	
BBCH ≥ 20	Small insectivorous bird “wagtail”	25.2	1.0	0.91	42.1	
Reprod. toxicity (mg/kg bw/d)		3.8				
TER criterion		5				
Crop scenario	Indicator/generic focal species	SV _m	MAF _m × TWA	DDD _m (mg/kg bw/d)	TER _{lt}	
Growth stage						
BBCH 10 - 39	Small omnivorous bird “lark”	10.9	1.0 × 0.53	0.21	18.3	
BBCH ≥ 40	Small omnivorous bird “lark”	3.3	1.0 × 0.53	0.06	60.4	
BBCH 10 - 19	Small insectivorous bird “wagtail”	11.3	1.0 × 0.53	0.22	17.6	
BBCH ≥ 20	Small insectivorous bird “wagtail”	9.7	1.0 × 0.53	0.19	20.5	

SV: shortcut value; MAF: multiple application factor; TWA: time-weighted average factor; DDD: daily dietary dose; TER: toxicity to exposure ratio. TER values shown in bold fall below the relevant trigger.

Table 9.2-6: First-tier assessment of the acute and long-term/reproductive risk for birds due to the use of ADM.00150.I.2.A in spring cereals (BBCH 40-69, use no. IVa)

Intended use		Spring cereals (BBCH 40-69)				
Active substance/product		Acetamiprid				
Application rate (g/ha)		1-2 × 35, 10 days interval				
Acute toxicity (mg/kg bw)		38.2				
TER criterion		10				
Crop scenario	Indicator/generic focal species	SV ₉₀	MAF ₉₀	DDD ₉₀ (mg/kg bw/d)	TER _a	
Growth stage						
BBCH ≥ 40	Small omnivorous bird “lark”	7.2	1.3	0.33	116.6	
Reprod. toxicity (mg/kg bw/d)		3.8				
TER criterion		5				
Crop scenario	Indicator/generic focal species	SV _m	MAF _m × TWA	DDD _m (mg/kg bw/d)	TER _{lt}	
Growth stage						
BBCH ≥ 40	Small omnivorous bird “lark”	3.3	1.5 × 0.53	0.09	41.4	

SV: shortcut value; MAF: multiple application factor; TWA: time-weighted average factor; DDD: daily dietary dose; TER: toxicity to exposure ratio. TER values shown in bold fall below the relevant trigger.

Table 9.2-7: First-tier assessment of the acute and long-term/reproductive risk for birds due to the use of ADM.00150.I.2.A in spring cereals (BBCH 12-69, use no. IVb)

Intended use		Spring cereals (BBCH 12-69)			
Active substance/product		Acetamiprid			
Application rate (g/ha)		1-2 × 35, 30 days interval			
Acute toxicity (mg/kg bw)		38.2			
TER criterion		10			
Crop scenario	Indicator/generic focal species	SV ₉₀	MAF ₉₀	DDD ₉₀ (mg/kg bw/d)	TER _a
Growth stage					
BBCH 10 - 29	Small omnivorous bird “lark”	24	1.07	0.90	42.5
BBCH 30 -39	Small omnivorous bird “lark”	12	1.07	0.45	85.0

BBCH \geq 40	Small omnivorous bird “lark”	7.2	1.07	0.27	141.7
Reprod. toxicity (mg/kg bw/d)	3.8				
TER criterion	5				
Crop scenario	Indicator/generic focal species	SV_m	MAF_m × TWA	DDD_m (mg/kg bw/d)	TER_{lt}
Growth stage					
BBCH 10 - 29	Small omnivorous bird “lark”	10.9	1.13×0.53	0.23	16.6
BBCH 30 -39	Small omnivorous bird “lark”	5.4	1.13×0.53	0.11	33.6
BBCH \geq 40	Small omnivorous bird “lark”	3.3	1.13×0.53	0.07	54.9

SV: shortcut value; MAF: multiple application factor; TWA: time-weighted average factor; DDD: daily dietary dose; TER: toxicity to exposure ratio. TER values shown in bold fall below the relevant trigger.

Table 9.2-8: First-tier assessment of the acute and long-term/reproductive risk for birds due to the use of ADM.00150.I.2.A in winter cereals (BBCH 40-69, use no. Va)

Intended use	Winter cereals (BBCH 40-69)				
Active substance/product	Acetamiprid				
Application rate (g/ha)	1-2 × 36, 10 days interval				
Acute toxicity (mg/kg bw)	38.2				
TER criterion	10				
Crop scenario	Indicator/generic focal species	SV₉₀	MAF₉₀	DDD₉₀ (mg/kg bw/d)	TER_a
Growth stage					
BBCH \geq 40	Small omnivorous bird “lark”	7.2	1.3	0.34	113.4
Reprod. toxicity (mg/kg bw/d)	3.8				
TER criterion	5				
Crop scenario	Indicator/generic focal species	SV_m	MAF_m × TWA	DDD_m (mg/kg bw/d)	TER_{lt}
Growth stage					
BBCH \geq 40	Small omnivorous bird “lark”	3.3	1.5×0.53	0.09	40.2

SV: shortcut value; MAF: multiple application factor; TWA: time-weighted average factor; DDD: daily dietary dose; TER: toxicity to exposure ratio. TER values shown in bold fall below the relevant trigger.

Table 9.2-9: First-tier assessment of the acute and long-term/reproductive risk for birds due to the use of ADM.00150.I.2.A in winter cereals (BBCH 12-29, use no. Vb)

Intended use	Winter cereals (BBCH 12-29)				
Active substance/product	Acetamiprid				
Application rate (g/ha)	1 × 30				
Acute toxicity (mg/kg bw)	38.2				
TER criterion	10				
Crop scenario	Indicator/generic focal species	SV₉₀	MAF₉₀	DDD₉₀ (mg/kg bw/d)	TER_a
Growth stage					
Early (shoots) autumn-winter BBCH 10-29	Large herbivorous bird "goose"	30.5	1.0	0.92	41.7
BBCH 10 - 29	Small omnivorous bird “lark”	24	1.0	0.72	53.1
Reprod. toxicity (mg/kg bw/d)	3.8				
TER criterion	5				
Crop scenario	Indicator/generic focal species	SV_m	MAF_m × TWA	DDD_m (mg/kg bw/d)	TER_{lt}
Growth stage					
Early (shoots) autumn-winter BBCH 10-29	Large herbivorous bird "goose"	16.2	1.0×0.53	0.26	14.8
BBCH 10 - 29	Small omnivorous bird “lark”	10.9	1.0×0.53	0.17	21.9

SV: shortcut value; MAF: multiple application factor; TWA: time-weighted average factor; DDD: daily dietary dose; TER: toxicity to exposure ratio. TER values shown in bold fall below the relevant trigger.

Table 9.2-10: First-tier assessment of the acute and long-term/reproductive risk for birds due to the use of ADM.00150.I.2.A in winter oilseed rape (BBCH 31-71, use no. VIa) in spring oilseed rape (BBCH 31-69 spring, use no. VIIa)

Intended use		Spring oilseed rape (BBCH 31-69) and winter oilseed rape (BBCH 31-71)				
Active substance/product		Acetamiprid				
Application rate (g/ha)		1-2 × 60, 7 days interval				
Acute toxicity (mg/kg bw)		38.2				
TER criterion		10				
Crop scenario Growth stage	Indicator/generic focal species	SV ₉₀	MAF ₉₀	DDD ₉₀ (mg/kg bw/d)	TER _a	
late – late (with seeds) (BBCH 30- 99)	Small insectivorous bird "dunnock"	7.4	1.4	0.62	61.5	
BBCH 30 - 39	Small omnivorous bird “lark”	7.2	1.4	0.60	63.2	
BBCH ≥ 40	Small omnivorous bird “lark”	6	1.4	0.50	75.8	
BBCH 30 - 39	Medium herbivorous/granivorous bird "pigeon"	2.4	1.4	0.20	189.5	
BBCH ≥ 40	Medium herbivorous/granivorous bird "pigeon"	2	1.4	0.17	227.4	
Reprod. toxicity (mg/kg bw/d)		3.8				
TER criterion		5				
Crop scenario Growth stage	Indicator/generic focal species	SV _m	MAF _m × TWA	DDD _m (mg/kg bw/d)	TER _{lt}	
late – late (with seeds) (BBCH 30- 99)	Small insectivorous bird "dunnock"	2.7	1.6 × 0.53	0.14	27.7	
BBCH 30 - 39	Small omnivorous bird “lark”	3.3	1.6 × 0.53	0.17	22.6	
BBCH ≥ 40	Small omnivorous bird “lark”	2.7	1.6 × 0.53	0.14	27.7	
BBCH 30 - 39	Medium herbivorous/granivorous bird "pigeon"	1.1	1.6 × 0.53	0.06	67.9	
BBCH ≥ 40	Medium herbivorous/granivorous bird "pigeon"	0.9	1.6 × 0.53	0.05	83.0	

SV: shortcut value; MAF: multiple application factor; TWA: time-weighted average factor; DDD: daily dietary dose; TER: toxicity to exposure ratio. TER values shown in bold fall below the relevant trigger.

Table 9.2-11: First-tier assessment of the acute and long-term/reproductive risk for birds due to the use of ADM.00150.I.2.A in winter oilseed rape (in winter oilseed rape (BBCH 11-19 autumn, use no. VIb)

Intended use		Winter oilseed rape (BBCH 11-19)				
Active substance/product		Acetamiprid				
Application rate (g/ha)		1× 60				
Acute toxicity (mg/kg bw)		38.2				
TER criterion		10				
Crop scenario Growth stage	Indicator/generic focal species	SV ₉₀	MAF ₉₀	DDD ₉₀ (mg/kg bw/d)	TER _a	
early (shoots) (BBCH 10-19)	Large herbivorous bird "goose"	39	1.0	2.34	16.3	
BBCH 10 - 29	Small omnivorous bird “lark”	24	1.0	1.44	26.5	
BBCH 10 - 19	Medium herbivorous/granivorous bird "pigeon"	55.6	1.0	3.34	11.5	
BBCH 10 - 19	Small insectivorous bird “wagtail”	10.9	1.0	0.65	58.4	
Reprod. toxicity (mg/kg bw/d)		3.8				
TER criterion		5				
Crop scenario Growth stage	Indicator/generic focal species	SV _m	MAF _m × TWA	DDD _m (mg/kg bw/d)	TER _{lt}	
early (shoots) (BBCH 10-19)	Large herbivorous bird "goose"	15.9	1.0 × 0.53	0.51	7.5	
BBCH 10 - 29	Small omnivorous bird “lark”	10.9	1.0 × 0.53	0.35	11.0	

BBCH 10 - 19	Medium herbivorous/granivorous bird "pigeon"	22.7	1.0×0.53	0.72	5.3
BBCH 10 - 19	Small insectivorous bird "wagtail"	5.9	1.0×0.53	0.19	20.3

SV: shortcut value; MAF: multiple application factor; TWA: time-weighted average factor; DDD: daily dietary dose; TER: toxicity to exposure ratio. TER values shown in bold fall below the relevant trigger.

Table 9.2-12: First-tier assessment of the acute and long-term/reproductive risk for birds due to the use of ADM.00150.I.2.A in sugar beet (BBCH 12-39, use no. VIIIa)

Intended use		Sugar beet (BBCH 12-39)				
Active substance/product		Acetamiprid				
Application rate (g/ha)		2 × 50, 7 days interval				
Acute toxicity (mg/kg bw)		38.2				
TER criterion		10				
Crop scenario Growth stage	Indicator/generic focal species	SV ₉₀	MAF ₉₀	DDD ₉₀ (mg/kg bw/d)	TER _a	
Late (summer/ autumn) (BBCH 30-49)	Small granivorous bird "finch"	24.7	1.4	1.73	22.1	
Early (spring) (BBCH 10-19)	Small omnivorous bird "lark"	24	1.4	1.68	22.7	
BBCH 10-19	Small insectivorous bird "wagtail"	10.9	1.4	0.76	50.1	
BBCH 20 - 49	Small insectivorous bird "wagtail"	7.7	1.4	0.54	70.9	
BBCH 20 - 49	Small insectivorous bird "wagtail"	25.2	1.4	1.76	21.7	
Reprod. toxicity (mg/kg bw/d)		3.8				
TER criterion		5				
Crop scenario Growth stage	Indicator/generic focal species	SV _m	MAF _m × TWA	DDD _m (mg/kg bw/d)	TER _{lt}	
Late (summer/ autumn) (BBCH 30-49)	Small granivorous bird "finch"	11.4	1.6 × 0.53	0.48	7.9	
Early (spring) (BBCH 10-19)	Small omnivorous bird "lark"	10.9	1.6 × 0.53	0.46	8.2	
BBCH 10-19	Small insectivorous bird "wagtail"	5.9	1.6 × 0.53	0.25	15.2	
BBCH 20 - 49	Small insectivorous bird "wagtail"	2.8	1.6 × 0.53	0.12	32.0	
BBCH 20 - 49	Small insectivorous bird "wagtail"	9.7	1.6 × 0.53	0.41	9.2	

SV: shortcut value; MAF: multiple application factor; TWA: time-weighted average factor; DDD: daily dietary dose; TER: toxicity to exposure ratio. TER values shown in bold fall below the relevant trigger.

Table 9.2-13: First-tier assessment of the acute and long-term/reproductive risk for birds due to the use of ADM.00150.I.2.A in flower bulbs and flower tubers (BBCH 12-91, use no. IXa)

Intended use		Flower bulbs and flower tubers (BBCH 12-91)				
Active substance/product		Acetamiprid				
Application rate (g/ha)		1 × 46				
Acute toxicity (mg/kg bw)		38.2				
TER criterion		10				
Crop scenario Growth stage	Indicator/generic focal species	SV ₉₀	MAF ₉₀	DDD ₉₀ (mg/kg bw/d)	TER _a	
BBCH 10 - 39	Small granivorous bird “finch”	24.7	1.0	1.14	33.6	
BBCH ≥ 40	Small granivorous bird “finch”	14.8	1.0	0.68	56.1	
BBCH 10 - 39	Small omnivorous bird “lark”	24	1.0	1.10	34.6	
BBCH ≥ 40	Small omnivorous bird “lark”	14.4	1.0	0.66	57.7	
BBCH 10 - 19	Small insectivorous bird “wagtail”	26.8	1.0	1.23	31.0	
BBCH ≥ 20	Small insectivorous bird “wagtail”	25.2	1.0	1.16	33.0	
Reprod. toxicity (mg/kg bw/d)		3.8				
TER criterion		5				
Crop scenario Growth stage	Indicator/generic focal species	SV _m	MAF _m × TWA	DDD _m (mg/kg bw/d)	TER _{lt}	
BBCH 10 - 39	Small granivorous bird “finch”	11.4	1.0 × 0.53	0.28	13.7	

BBCH \geq 40	Small granivorous bird “finch”	6.9	1.0×0.53	0.17	22.6
BBCH 10 - 39	Small omnivorous bird “lark”	10.9	1.0×0.53	0.27	14.3
BBCH \geq 40	Small omnivorous bird “lark”	6.5	1.0×0.53	0.16	24.0
BBCH 10 - 19	Small insectivorous bird “wagtail”	11.3	1.0×0.53	0.28	13.8
BBCH \geq 20	Small insectivorous bird “wagtail”	9.7	1.0×0.53	0.24	16.1

SV: shortcut value; MAF: multiple application factor; TWA: time-weighted average factor; DDD: daily dietary dose; TER: toxicity to exposure ratio. TER values shown in bold fall below the relevant trigger.

Table 9.2-14: First-tier assessment of the acute and long-term/reproductive risk for birds due to the use of ADM.00150.I.2.A in flower bulbs and flower tubers (BBCH 20-91, use no. IXb)

Intended use		Flower bulbs and flower tubers (BBCH 20-91)				
Active substance/product		Acetamiprid				
Application rate (g/ha)		2 × 34, 7 days interval				
Acute toxicity (mg/kg bw)		38.2				
TER criterion		10				
Crop scenario	Indicator/generic focal species	SV ₉₀	MAF ₉₀	DDD ₉₀ (mg/kg bw/d)	TER _a	
Growth stage						
BBCH 10 - 39	Small granivorous bird “finch”	24.7	1.4	1.18	32.5	
BBCH ≥ 40	Small granivorous bird “finch”	14.8	1.4	0.70	54.2	
BBCH 10 - 39	Small omnivorous bird “lark”	24	1.4	1.14	33.4	
BBCH ≥ 40	Small omnivorous bird “lark”	14.4	1.4	0.69	55.7	
BBCH 10 - 19	Small insectivorous bird “wagtail”	26.8	1.4	1.28	29.9	
BBCH ≥ 20	Small insectivorous bird “wagtail”	25.2	1.4	1.20	31.8	
Reprod. toxicity (mg/kg bw/d)		3.8				
TER criterion		5				
Crop scenario	Indicator/generic focal species	SV _m	MAF _m × TWA	DDD _m (mg/kg bw/d)	TER _{It}	
Growth stage						
BBCH 10 - 39	Small granivorous bird “finch”	11.4	1.6 × 0.53	0.33	11.6	
BBCH ≥ 40	Small granivorous bird “finch”	6.9	1.6 × 0.53	0.20	19.1	
BBCH 10 - 39	Small omnivorous bird “lark”	10.9	1.6 × 0.53	0.31	12.1	
BBCH ≥ 40	Small omnivorous bird “lark”	6.5	1.6 × 0.53	0.19	20.3	
BBCH 10 - 19	Small insectivorous bird “wagtail”	11.3	1.6 × 0.53	0.33	11.7	
BBCH ≥ 20	Small insectivorous bird “wagtail”	9.7	1.6 × 0.53	0.28	13.6	

SV: shortcut value; MAF: multiple application factor; TWA: time-weighted average factor; DDD: daily dietary dose; TER: toxicity to exposure ratio. TER values shown in bold fall below the relevant trigger.

Tree nursery uses resembling orchard crops are covered by Tier 1 calculations for orchard uses.

Table 9.2-15: First-tier assessment of the acute and long-term/reproductive risk for birds due to the use of ADM.00150.I.2.A in floriculture, tree nursery and perennial nursery crops (BBCH 12-91, use no. Xa)

Intended use		Floriculture, tree nursery and perennial nursery crops (BBCH 12-91)				
Active substance/product		Acetamiprid				
Application rate (g/ha)		1 × 46				
Acute toxicity (mg/kg bw)		38.2				
TER criterion		10				
Crop scenario	Indicator/generic focal species	SV ₉₀	MAF ₉₀	DDD ₉₀ (mg/kg bw/d)	TER _a	
Growth stage						
Application to plant	Small insectivorous bird “tit”	46.8	1.0	2.15	17.7	
Application to plant – exposure to underlying ground	Small insectivorous/worm feeding species “thrush”	7.4	1.0	0.34	112.2	
Reprod. toxicity (mg/kg bw/d)		3.8				
TER criterion		5				
Crop scenario	Indicator/generic focal species	SV _m	MAF _m ×	DDD _m	TER _{It}	

Growth stage			TWA	(mg/kg bw/d)	
BBCH 10 - 39	Small granivorous bird “finch”	18.2	1.0×0.53	0.44	8.6
BBCH ≥ 40	Small granivorous bird “finch”	2.7	1.0×0.53	0.07	57.7

SV: shortcut value; MAF: multiple application factor; TWA: time-weighted average factor; DDD: daily dietary dose; TER: toxicity to exposure ratio. TER values shown in bold fall below the relevant trigger.

Table 9.2-16: First-tier assessment of the acute and long-term/reproductive risk for birds due to the use of ADM.00150.I.2.A in floriculture, tree nursery and perennial nursery crops (BBCH 20-91, use no. Xb)

Intended use		Floriculture, tree nursery and perennial nursery crops (BBCH 20-91)				
Active substance/product		Acetamiprid				
Application rate (g/ha)		2 × 34, 7 days interval				
Acute toxicity (mg/kg bw)		38.2				
TER criterion		10				
Crop scenario	Indicator/generic focal species	SV ₉₀	MAF ₉₀	DDD ₉₀ (mg/kg bw/d)	TER _a	
Growth stage						
Application to plant	Small insectivorous bird “tit”	46.8	1.4	2.23	17.1	
Application to plant – exposure to underlying ground	Small insectivorous/worm feeding species “thrush”	7.4	1.4	0.35	108.4	
Reprod. toxicity (mg/kg bw/d)		3.8				
TER criterion		5				
Crop scenario	Indicator/generic focal species	SV _m	MAF _m × TWA	DDD _m (mg/kg bw/d)	TER _{lt}	
Growth stage						
Application to plant	Small insectivorous bird “tit”	18.2	1.6 × 0.53	0.52	7.2	
Application to plant – exposure to underlying ground	Small insectivorous/worm feeding species “thrush”	2.7	1.6 × 0.53	0.08	48.8	

SV: shortcut value; MAF: multiple application factor; TWA: time-weighted average factor; DDD: daily dietary dose; TER: toxicity to exposure ratio. TER values shown in bold fall below the relevant trigger.

The acute tier 1 TER values exceed the relevant trigger values, indicating an acceptable risk following applications of ADM.00150.I.2.A according to the intended use pattern.

The reproductive tier 1 TER values exceed the relevant trigger values indicating an acceptable risk following applications of ADM.00150.I.2.A. according to the intended use pattern for all uses except for the intended use in orchards (1×80 g/ha) where the scenario small insectivorous bird “tit” is below the trigger value and a higher tier risk assessment is required.

zRMS comments:

The screening step and Tier 1 for acute and chronic risk assessment for proposed uses provided in Tables 9.2-2 to Tables 9.2-16 has been validated by zRMS with correction of application dose in apples at BBCH 71 from 80 g a.s./ha to 60 g a.s./ha according to the current GAP Table.

Based on the performed calculations, TER_A and TER_{LT} values are above trigger of 10 and 5, respectively. acceptable risk to birds from the active substance acetamiprid present in ADM.00150.I.2.A. can be concluded for all proposed all proposed uses.

No additional calculations using higher tier data of the risk assessment is required.

During the EU renewal it was concluded that based on the available data the dietary risk from metabolites is considered to be covered by evaluation performed for the parent. The same conclusion is applicable for the zonal assessment for ADM.00150.I.2.A.

Overall, acceptable acute and long-term dietary risk to birds may be concluded from all intended zonal uses of ADM.00150.I.2.A.

9.2.2.2 Higher-tier risk assessment

The EFSA/2009/1438 proposes the blue tit as representative small insectivorous focal species in orchards.

The UK's CSL has conducted radio-tracking studies on relevant species in fruit orchards (blackbird, chaffinch, blue tit and robin). The aim of the work conducted by Finch and Payne (2006; KCP 10.1.1/01) was to capture a representative day in the life of each tagged individual. Radio tags were mounted on the base of the tail feathers, with tag weight being no more than 5% in the orchard studies and the active time was recorded. (Finch and Payne, 2006).

This category included all instances of recorded foraging and excluded all instances where the animal was known to be performing some other activity (e.g. singing, nest building) or where it was considered to be inactive. Data were collected in orchards from April to September. In the study, 20 blue tits were radio-tracked to estimate the active time spent in this habitat. The proportion of time (PT) spent by individual birds in different crops has been estimated and are summarized in the table below.

Table 9.2 17: Percentage of active time spent by radio-tagged blue tits in orchards in the UK. Results are presented for the total sample of tracked birds ("all birds") as well as for the subsample of birds actually using the orchard ("consumers only")

Crop	Period	No. of birds	Mean (%)	90 th percentile	95 th percentile	Reference
All birds:						
Orchard	Apr–Sep	20	0.21	0.55	0.67	Finch and Payne, 2006
Consumers only:						
Orchard	Apr–Sep	16	0.27	0.58	0.68	Finch and Payne, 2006

It has been shown that blue tits do not spend their whole time in orchards and therefore a PT of 1 would overestimate their potential exposure. The recorded crop consumers (i.e. individuals foraging within an orchard, n = 16) spent on average 26.6% of their active time in orchards. The 90th percentile PT for this group is 0.58.

Since the TER value for the small insectivorous bird was already close to the trigger value of 5 (TER 4.9), the data from Finch and Payne (2006) are used in a weight of evidence for the representative species and no further identification of focal species is given, as a PT of 0.99 would already be sufficient to demonstrate a low risk.

Using a 90th percentile PT of 0.58 it is clearly demonstrated that the risk to small insectivorous birds feeding in orchards is above the trigger of 5 indicating an acceptable risk for this feeding guild.

In addition to the refined PT value provided above, a refined RUD value for arthropods could be applied. The draft Guidance for Birds and Mammals (EFSA, 2021) provides a revised residue dataset based on residue data collected by Lahr *et al.* (2018, KCP 10.1.1/02).

The revised dataset shows significant lower RUD values for foliage dwelling arthropods compared to the values reported in EFSA/2009/1438 (8.4 mg/kg compared to 21.0 mg/kg for foliage dwelling arthropods). This indicates that the exposure of insectivorous birds after the application of ADM.00150.I.2.A in apple orchards at a rate of 80 g a.s./ha is considerably lower and the risk assessment above utilizes conservative assumptions.

Based on the weight of evidence provided above, the risk to insectivorous birds after the application of ADM.00150.I.2.A in apple orchards at a rate of 80 g a.s./ha is considered acceptable.

zRMS comments:

As the risk assessment to birds has been resolved at Tier 1 and for this reason no additional calculations are required.

9.2.2.3 Drinking water exposure

When necessary, the assessment of the risk for birds due to uptake of contaminated drinking water is conducted for a small granivorous bird with a body weight of 15.3 g (*Carduelis cannabina*) and a drinking water uptake rate of 0.46 L/kg bw/d (cf. Appendix K of EFSA/2009/1438).

Leaf scenario

Since ADM.00150.I.2.A is not intended to be applied on leafy vegetables forming heads or crop plants with comparable water collecting structures at principal growth stage 4 or later, the leaf scenario does not have to be considered.

Puddle scenario

Due to the characteristics of the exposure scenario in connection with the standard assumptions for water uptake by animals, no specific calculations of exposure and TER are necessary when the ratio of effective application rate (in g/ha) to relevant endpoint (in mg/kg bw/d) does not exceed 50 in the case of less sorptive substances ($K_{oc} < 500$ L/kg) or 3000 in the case of more sorptive substances ($K_{oc} \geq 500$ L/kg).

With a $K(f)_{oc}$ of 106.2, acetamiprid belongs to the group of less sorptive substances. To achieve a concise risk assessment, the risk envelope approach is applied. Here, the assessment for the use in apples with 80 g a.s./ha also covers the risk for birds from all other intended uses (see 9.1.2).

Effective application rate (g/ha)	60 80	Ratio effective application rate to relevant endpoint (trigger: < 50)
Acute toxicity (mg/kg bw)	38.2	2.00 1.57
Reprod. toxicity (mg/kg bw/d)	3.8	21.1 15.78

zRMS comments:

The drinking water risk assessment for acetamiprid presented above is agreed by the zRMS. Evaluation was based on the maximum intended application rate, covering all uses of ADM.00150.I.2.A listed in GAP table.

As ADM.00150.I.2.A is not intended for use in crops with structures able to collect water, only puddle scenario is applicable.

Based on the screening evaluation no unacceptable risk via drinking water is anticipated for all intended zonal uses of ADM.00150.I.2.A.

It is noted that pertinent soil metabolites were not considered in this evaluation. Nevertheless, according to information available in the DRAR (August 2016), all relevant soil metabolites of acetamiprid (IM-1-2, IM-1-4, IC-0 and IM-1-5) are less toxic than the parent compound. Therefore, taking into account lower toxicity and lower exposure to metabolites, the ratios between metabolites rates and endpoints would be lower than these calculated for the parent substance and would not exceed the trigger of 50, applicable also for metabolites (all with $K_{foc} < 500$ mL/g).

Based on that no further drinking water evaluation is deemed necessary for metabolites and the risk is concluded to be acceptable.

9.2.2.4 Effects of secondary poisoning

The log P_{ow} of acetamiprid amounts to 0.8 and thus does not exceed the trigger value of 3. Identified metabolites also have log P_{ow} values below 3. A risk assessment for effects due to secondary poisoning is not required.

zRMS comments:

Log P_{ow} values for acetamiprid and relevant soil and aquatic metabolites (IM-1-2, IM-1-4, IM-1-5, IC-0 and IB-1-1) are all <3, hence the evaluation of the risk of secondary poisoning is not triggered.

9.2.2.5 Biomagnification in terrestrial food chains

Not relevant.

9.2.3 Risk assessment for baits, pellets, granules, prills or treated seed

Not relevant.

9.2.4 Overall conclusions

The acute and long-term risks of ADM.00150.I.2.A (containing 200 g/L acetamiprid) to birds were assessed from toxicity exposure ratios between toxicity endpoints, estimated from studies with acetamiprid, and maximum residues occurring on food items following applications according to the proposed use pattern. The TER values for all uses, calculated for recommended scenarios, exceed the trigger values of 10 for acute risk and 5 for long-term risk, with the exception of the reproductive scenario small insectivorous bird “tit” in apple (1 x 80 g/ha), indicating that the risk to birds is acceptable following use of ADM.00150.I.2.A according to the proposed use pattern. The scenario small insectivorous bird “tit” was addressed in weight of evidence, presenting PT values for tits in orchards, demonstrating a low risk to representatives of this diet group. The risk of secondary poisoning is not relevant.

The risk from drinking water was assessed demonstrating that the risk to birds is acceptable.

9.3 Effects on terrestrial vertebrates other than birds (KCP 10.1.2)

9.3.1 Toxicity data

Mammalian toxicity studies have been carried out with acetamiprid and its relevant metabolites. Full details of these studies are provided in the respective EU DAR and related documents as well as in Section 6 (Mammalian Toxicology).

Effects on mammals of ADM.00150.I.2.A were not evaluated as part of the EU assessment of acetamiprid. New data submitted with this application are listed in Appendix 1 and summarised in Section 6 (Mammalian Toxicology) of this report.

The selection of studies and endpoints for the risk assessment is in line with the results of the EU review process.

Table 9.3-1: Endpoints and effect values relevant for the risk assessment for mammals

Species	Substance	Exposure System	Results	Reference
Rat	Acetamiprid	Acute	LD₅₀ = 146 mg/kg bw	EFSA, 2016b
Rat	Formulation, calculation		795 mg/kg bw	Toxicity of formulation was calculated and details are provided in Part C
Rat	Acetamiprid	Long-term 90-d study	NOAEL = 12.4 mg/kg bw/d	EFSA, 2016b
Rat	Acetamiprid	Long-term Developmental neurotoxicity study	NOAEL = 2.5 mg/kg bw	

Values shown in **bold** used for risk assessment

zRMS comments:

Mammalian toxicity data for acetamiprid in Table 9.3-1 above is in line with EU agreed data reported in EFSA Journal 2016;14(11):4610.

9.3.1.1 Justification for new endpoints

The selection of studies and endpoints for the risk assessment is in line with the results of the EU review process and therefore no new endpoints were selected.

9.3.2 Risk assessment for spray applications

The risk assessment is based on the methods presented in the Guidance Document on Risk Assessment for Mammals and Mammals on request from EFSA (EFSA Journal 2009; 7(12): 1438; hereafter referred to as EFSA/2009/1438).

Metabolites:

Acute mammal toxicity studies with metabolites show, that metabolites are of much lower toxicity (IM-I-0, IM-1-3, IM-1-4, IM-2-1, IM-2-3, IM-1-2, IS-1-1, IS-2-1, IC-0, IB-1-1 or show a similar toxicity (IM-1-5 LD₅₀ = 132 mg/kg bw (f) and 141 mg/kg bw (m)) compared to the parent acetamiprid (LD₅₀ = 146 mg/kg bw, please refer to EFSA 2016b, Appendix A LOE). Since acetamiprid was identified by far as the major component of the residues in almost all plant matrices (EFSA 2016a) and toxicity of metabolites is lower or within the same range, the toxicity of metabolites was concluded to be covered by the toxicity of the parent acetamiprid. It should also be noted that the metabolites are formed slowly and with a time delay from the parent substance and thus the amount of metabolites found at any one time will never reach that of the parent substance.

Risk to birds and mammals via secondary poisoning is not required, as the log Pow values of metabolites are below 3 (Vol.3 B.2 (AS)), indicating a low risk.

zRMS comments:

We agree what parent is considered to cover the risk of the metabolites.

9.3.2.1 First-tier assessment (screening/generic focal species)

The results of the acute and reproductive first-tier risk assessments are summarised in the following tables.

Table 9.3-2: First-tier assessment of the acute and long-term/reproductive risk for mammals due to the use of ADM.00150.I.2.A in corn (BBCH 51-75, use no. I)

Intended use		Corn (BBCH 51-75)				
Active substance/product		Acetamiprid				
Application rate (g/ha)		1 × 60				
Acute toxicity (mg/kg bw)		146				
TER criterion		10				
Crop scenario	Indicator/generic focal species	SV ₉₀	MAF ₉₀	DDD ₉₀ (mg/kg bw/d)	TER _a	
Growth stage						
BBCH ≥ 20	Small insectivorous mammal “shrew”	5.4	1.0	0.32	450.6	
BBCH ≥ 40	Small herbivorous mammal "vole"	34.1	1.0	2.05	71.4	
BBCH ≥ 40	Small omnivorous mammal “mouse”	4.3	1.0	0.26	565.9	
Reprod. toxicity (mg/kg bw/d)		2.5				
TER criterion		5				
Crop scenario	Indicator/generic focal species	SV _m	MAF _m × TWA	DDD _m (mg/kg bw/d)	TER _{lt}	
Growth stage						
BBCH ≥ 20	Small insectivorous mammal “shrew”	1.9	1 × 0.53	0.06	41.4	
BBCH ≥ 40	Small herbivorous mammal "vole"	18.1	1 × 0.53	0.58	4.3	
BBCH ≥ 40	Small omnivorous mammal “mouse”	1.9	1 × 0.53	0.06	41.4	

SV: shortcut value; MAF: multiple application factor; TWA: time-weighted average factor; DDD: daily dietary dose; TER: toxicity to exposure ratio. TER values shown in bold fall below the relevant trigger.

Table 9.3-3: First-tier assessment of the acute and long-term/reproductive risk for mammals due to the use of ADM.00150.I.2.A in apple (BBCH 71-PHI, use no. IIa)

Intended use		Apple (BBCH 71-PHI)				
Active substance/product		Acetamiprid				
Application rate (g/ha)		1 × 60 80				
Acute toxicity (mg/kg bw)		146				
TER criterion		10				
Crop scenario	Indicator/generic focal species		SV ₉₀	MAF ₉₀	DDD ₉₀ (mg/kg bw/d)	TER _a
Growth stage						
BBCH ≥ 40	Small herbivorous mammal "vole"		40.9	1.0	2.45 3.27	59.60 44.6
BBCH 71-79	Frugivorous mammal "dormouse"		47.9	1.0	2.87 3.83	50.90 38.1
BBCH ≥ 40	Large herbivorous mammal “lagomorph”		10.5	1.0	0.63 0.84	231.75 173.8
BBCH ≥ 40	Small omnivorous mammal “mouse”		5.2	1.0	0.31 0.42	471 351.0
Reprod. toxicity (mg/kg bw/d)		2.5				
TER criterion		5				
Crop scenario	Indicator/generic focal species		SV _m	MAF _m × TWA	DDD _m (mg/kg bw/d)	TER _{lt}
Growth stage						
BBCH ≥ 40	Small herbivorous mammal "vole"		21.7	1 × 0.53	0.69 0.92	3.62 2.7
BBCH 71-79	Frugivorous mammal "dormouse"		22.7	1 × 0.53	0.72 0.96	3.47 2.6
BBCH ≥ 40	Large herbivorous mammal “lagomorph”		4.3	1 × 0.53	0.136 0.18	18.4 13.7
BBCH ≥ 40	Small omnivorous mammal “mouse”		2.3	1 × 0.53	0.073 0.10	34.24 25.6

SV: shortcut value; MAF: multiple application factor; TWA: time-weighted average factor; DDD: daily dietary dose; TER: toxicity to exposure ratio. TER values shown in bold fall below the relevant trigger.

Table 9.3-4: First-tier assessment of the acute and long-term/reproductive risk for mammals due to the use of ADM.00150.I.2.A in apple (BBCH 62-PHI, use no. IIb)

Intended use		Apple (BBCH 62-PHI)				
Active substance/product		Acetamiprid				
Application rate (g/ha)		1-2 × 25, interval 8 days				
Acute toxicity (mg/kg bw)		146				
TER criterion		10				
Crop scenario	Indicator/generic focal species	SV ₉₀	MAF ₉₀	DDD ₉₀ (mg/kg bw/d)	TER _a	
Growth stage						
BBCH ≥ 40	Small herbivorous mammal "vole"	40.9	1.38	1.41	103.5	
BBCH 71-79	Frugivorous mammal "dormouse"	47.9	1.38	1.65	88.3	
BBCH ≥ 40	Large herbivorous mammal “lagomorph”	10.5	1.38	0.36	403.0	
BBCH ≥ 40	Small omnivorous mammal “mouse”	5.2	1.38	0.18	813.8	
Reprod. toxicity (mg/kg bw/d)		2.5				
TER criterion		5				
Crop scenario	Indicator/generic focal species	SV _m	MAF _m × TWA	DDD _m (mg/kg bw/d)	TER _{lt}	
Growth stage						
BBCH ≥ 40	Small herbivorous mammal "vole"	21.7	1.57 × 0.53	0.45	5.5	
BBCH 71-79	Frugivorous mammal "dormouse"	22.7	1.57 × 0.53	0.47	5.3	
BBCH ≥ 40	Large herbivorous mammal “lagomorph”	4.3	1.57 × 0.53	0.09	27.9	
BBCH ≥ 40	Small omnivorous mammal “mouse”	2.3	1.57 × 0.53	0.05	52.3	

SV: shortcut value; MAF: multiple application factor; TWA: time-weighted average factor; DDD: daily dietary dose; TER: toxicity to exposure ratio. TER values shown in bold fall below the relevant trigger.

Table 9.3-5: First-tier assessment of the acute and long-term/reproductive risk for mammals due to the use of ADM.00150.I.2.A in potato (BBCH 12-79, use no. III)

Intended use		Potato (BBCH 12-79)				
Active substance/product		Acetamiprid				
Application rate (g/ha)		1 × 36				
Acute toxicity (mg/kg bw)		146				
TER criterion		10				
Crop scenario	Indicator/generic focal species	SV ₉₀	MAF ₉₀	DDD ₉₀ (mg/kg bw/d)	TER _a	
Growth stage						
BBCH 10 - 19	Small insectivorous mammal “shrew”	7.6	1.0	0.27	533.6	
BBCH ≥ 20	Small insectivorous mammal “shrew”	5.4	1.0	0.19	751.0	
BBCH ≥ 40	Small herbivorous mammal "vole	40.9	1.0	1.47	99.2	
BBCH 10 - 40	Large herbivorous mammal “lagomorph”	35.1	1.0	1.26	115.5	
BBCH ≥ 40	Large herbivorous mammal “lagomorph”	10.5	1.0	0.38	386.2	
BBCH 10 - 39	Small omnivorous mammal “mouse”	17.2	1.0	0.62	235.8	
BBCH ≥ 40	Small omnivorous mammal “mouse”	5.2	1.0	0.19	779.9	
Reprod. toxicity (mg/kg bw/d)		2.5				
TER criterion		5				
Crop scenario	Indicator/generic focal species	SV _m	MAF _m × TWA	DDD _m (mg/kg bw/d)	TER _{lt}	
Growth stage						
BBCH 10 - 19	Small insectivorous mammal “shrew”	4.2	1 × 0.53	0.08	31.2	
BBCH ≥ 20	Small insectivorous mammal “shrew”	1.9	1 × 0.53	0.04	69.0	
BBCH ≥ 40	Small herbivorous mammal "vole	21.7	1 × 0.53	0.41	6.0	
BBCH 10 - 40	Large herbivorous mammal “lagomorph”	14.3	1 × 0.53	0.27	9.2	
BBCH ≥ 40	Large herbivorous mammal “lagomorph”	4.3	1 × 0.53	0.08	30.5	
BBCH 10 - 39	Small omnivorous mammal “mouse”	7.8	1 × 0.53	0.15	16.8	
BBCH ≥ 40	Small omnivorous mammal “mouse”	2.3	1 × 0.53	0.04	57.0	

SV: shortcut value; MAF: multiple application factor; TWA: time-weighted average factor; DDD: daily dietary dose; TER: toxicity to exposure ratio. TER values shown in bold fall below the relevant trigger.

Table 9.3-6: First-tier assessment of the acute and long-term/reproductive risk for mammals due to the use of ADM.00150.I.2.A in spring cereals (BBCH 40-69, use no. IVa)

Intended use		Spring cereals (BBCH 40-69)				
Active substance/product		Acetamiprid				
Application rate (g/ha)		1-2 × 35, 10 days interval				
Acute toxicity (mg/kg bw)		146				
TER criterion		10				
Crop scenario	Indicator/generic focal species	SV ₉₀	MAF ₉₀	DDD ₉₀ (mg/kg bw/d)	TER _a	
Growth stage						
BBCH ≥ 20	Small insectivorous mammal "shrew"	5.4	1.3	0.25	594.2	
BBCH ≥ 40	Small herbivorous mammal "vole"	40.9	1.3	1.86	78.5	
BBCH ≥ 40	Small omnivorous mammal “mouse”	5.2	1.3	0.24	617.1	

Reprod. toxicity (mg/kg bw/d)		2.5				
TER criterion		5				
Crop scenario	Indicator/generic focal species	SV_m	MAF_m × TWA	DDD_m (mg/kg bw/d)	TER_{it}	
Growth stage						
BBCH ≥ 20	Small insectivorous mammal "shrew"	1.9	1.5 × 0.53	0.05	47.3	
BBCH ≥ 40	Small herbivorous mammal "vole"	21.7	1.5 × 0.53	0.60	4.1	
BBCH ≥ 40	Small omnivorous mammal "mouse"	2.3	1.5 × 0.53	0.06	39.1	

SV: shortcut value; MAF: multiple application factor; TWA: time-weighted average factor; DDD: daily dietary dose; TER: toxicity to exposure ratio. TER values shown in bold fall below the relevant trigger.

Table 9.3-7: First-tier assessment of the acute and long-term/reproductive risk for mammals due to the use of ADM.00150.I.2.A in spring cereals (BBCH 12-69, use no. IVb)

Intended use		Spring cereals (BBCH 12-69)				
Active substance/product		Acetamiprid				
Application rate (g/ha)		1-2 × 35, 30 days interval				
Acute toxicity (mg/kg bw)		146				
TER criterion		10				
Crop scenario	Indicator/generic focal species	SV ₉₀	MAF ₉₀	DDD ₉₀ (mg/kg bw/d)	TER _a	
Growth stage						
BBCH 10 - 19	Small insectivorous mammal "shrew"	7.6	1.07	0.28	513.0	
BBCH ≥ 20	Small insectivorous mammal "shrew"	5.4	1.07	0.20	722.0	
BBCH ≥ 40	Small herbivorous mammal "vole"	40.9	1.07	1.53	95.3	
Early (shoots)	Large herbivorous mammal “lagomorph”	42.1	1.07	1.58	92.6	
BBCH 10-29	Small omnivorous mammal “mouse”	17.2	1.07	0.64	226.7	
BBCH 30 - 39	Small omnivorous mammal “mouse”	8.6	1.07	0.32	453.3	
BBCH ≥ 40	Small omnivorous mammal “mouse”	5.2	1.07	0.19	749.7	
Reprod. toxicity (mg/kg bw/d)		2.5				
TER criterion		5				
Crop scenario	Indicator/generic focal species	SV _m	MAF _m × TWA	DDD _m (mg/kg bw/d)	TER _{it}	
Growth stage						
BBCH 10 - 19	Small insectivorous mammal "shrew"	4.2	1.13 × 0.53	0.09	28.4	
BBCH ≥ 20	Small insectivorous mammal "shrew"	1.9	1.13 × 0.53	0.04	62.8	
BBCH ≥ 40	Small herbivorous mammal "vole"	21.7	1.13 × 0.53	0.45	5.5	
Early (shoots)	Large herbivorous mammal “lagomorph”	22.3	1.13 × 0.53	0.47	5.3	
BBCH 10-29	Small omnivorous mammal “mouse”	7.8	1.13 × 0.53	0.16	15.3	
BBCH 30 - 39	Small omnivorous mammal “mouse”	3.9	1.13 × 0.53	0.08	30.6	
BBCH ≥ 40	Small omnivorous mammal “mouse”	2.3	1.13 × 0.53	0.05	51.9	

SV: shortcut value; MAF: multiple application factor; TWA: time-weighted average factor; DDD: daily dietary dose; TER: toxicity to exposure ratio. TER values shown in bold fall below the relevant trigger.

Table 9.3-8: First-tier assessment of the acute and long-term/reproductive risk for mammals due to the use of ADM.00150.I.2.A in winter cereals (BBCH 40-69, use no. Va)

Intended use		Winter cereals (BBCH 40-69)				
Active substance/product		Acetamiprid				
Application rate (g/ha)		1-2 × 36, interval 10 days				
Acute toxicity (mg/kg bw)		146				
TER criterion		10				
Crop scenario	Indicator/generic focal species	SV₉₀	MAF₉₀	DDD₉₀ (mg/kg bw/d)	TER_a	
Growth stage						

BBCH \geq 20	Small insectivorous mammal "shrew"	5.4	1.3	0.25	577.7
BBCH \geq 40	Small herbivorous mammal "vole"	40.9	1.3	1.91	76.3
BBCH \geq 40	Small omnivorous mammal "mouse"	5.2	1.3	0.24	599.9
Reprod. toxicity (mg/kg bw/d)	2.5				
TER criterion	5				
Crop scenario Growth stage	Indicator/generic focal species	SV_m	MAF_m × TWA	DDD_m (mg/kg bw/d)	TER_{it}
BBCH \geq 20	Small insectivorous mammal "shrew"	1.9	1.5 × 0.53	0.05	46.0
BBCH \geq 40	Small herbivorous mammal "vole"	21.7	1.5 × 0.53	0.62	4.0
BBCH \geq 40	Small omnivorous mammal "mouse"	2.3	1.5 × 0.53	0.07	38.0

SV: shortcut value; MAF: multiple application factor; TWA: time-weighted average factor; DDD: daily dietary dose; TER: toxicity to exposure ratio. TER values shown in bold fall below the relevant trigger.

Table 9.3-9: First-tier assessment of the acute and long-term/reproductive risk for mammals due to the use of ADM.00150.I.2.A in winter cereals (BBCH 12-29, use no. Vb)

Intended use	Winter cereals (BBCH 12-29)				
Active substance/product	Acetamiprid				
Application rate (g/ha)	1 × 30				
Acute toxicity (mg/kg bw)	146				
TER criterion	10				
Crop scenario Growth stage	Indicator/generic focal species	SV₉₀	MAF₉₀	DDD₉₀ (mg/kg bw/d)	TER_a
BBCH 10 - 19	Small insectivorous mammal "shrew"	7.6	1.0	0.23	640.4
BBCH \geq 20	Small insectivorous mammal "shrew"	5.4	1.0	0.16	901.2
Early (shoots)	Large herbivorous mammal "lagomorph"	42.1	1.0	1.26	115.6
BBCH 10-29	Small omnivorous mammal "mouse"	17.2	1.0	0.52	282.9
Reprod. toxicity (mg/kg bw/d)	2.5				
TER criterion	5				
Crop scenario Growth stage	Indicator/generic focal species	SV_m	MAF_m × TWA	DDD_m (mg/kg bw/d)	TER_{it}
BBCH 10 - 19	Small insectivorous mammal "shrew"	4.2	1.0 × 0.53	0.07	37.4
BBCH \geq 20	Small insectivorous mammal "shrew"	1.9	1.0 × 0.53	0.03	82.8
Early (shoots)	Large herbivorous mammal "lagomorph"	22.3	1.0 × 0.53	0.35	7.1
BBCH 10-29	Small omnivorous mammal "mouse"	7.8	1.0 × 0.53	0.12	20.2

SV: shortcut value; MAF: multiple application factor; TWA: time-weighted average factor; DDD: daily dietary dose; TER: toxicity to exposure ratio. TER values shown in bold fall below the relevant trigger.

Table 9.3-10: First-tier assessment of the acute and long-term/reproductive risk for mammals due to the use of ADM.00150.I.2.A A in in winter oilseed rape (BBCH 31-71, use no. VIa) in spring oilseed rape (BBCH 31-69 spring, use no. VIIa)

Intended use	Spring oilseed rape (BBCH 31-69) and winter oilseed rape (BBCH 31-71)				
Active substance/product	Acetamiprid				
Application rate (g/ha)	1-2 × 60, 7 days interval				
Acute toxicity (mg/kg bw)	146				
TER criterion	10				
Crop scenario Growth stage	Indicator/generic focal species	SV ₉₀	MAF ₉₀	DDD ₉₀ (mg/kg bw/d)	TER _a
BBCH ≥ 20	Small insectivorous mammal "shrew"	5.4	1.4	0.45	321.9
BBCH ≥ 40	Small herbivorous mammal "vole"	34.1	1.4	2.86	51.0
All season	Large herbivorous mammal "lagomorph"	35.1	1.4	2.95	49.5
BBCH 30 - 39	Small omnivorous mammal "mouse"	5.2	1.4	0.44	334.2
BBCH ≥ 40	Small omnivorous mammal "mouse"	4.3	1.4	0.36	404.2
Reprod. toxicity (mg/kg bw/d)	2.5				
TER criterion	5				
Crop scenario Growth stage	Indicator/generic focal species	SV _m	MAF _m × TWA	DDD _m (mg/kg bw/d)	TER _{lt}
BBCH ≥ 20	Small insectivorous mammal "shrew"	1.9	1.6 × 0.53	0.10	25.9
BBCH ≥ 40	Small herbivorous mammal "vole"	18.1	1.6 × 0.53	0.92	2.7
All season	Large herbivorous mammal "lagomorph"	14.3	1.6 × 0.53	0.73	3.4
BBCH 30 - 39	Small omnivorous mammal "mouse"	2.3	1.6 × 0.53	0.12	21.4
BBCH ≥ 40	Small omnivorous mammal "mouse"	1.9	1.6 × 0.53	0.10	25.9

SV: shortcut value; MAF: multiple application factor; TWA: time-weighted average factor; DDD: daily dietary dose; TER: toxicity to exposure ratio. TER values shown in bold fall below the relevant trigger.

Table 9.3-11: First-tier assessment of the acute and long-term/reproductive risk for mammals due to the use of ADM.00150.I.2.A in winter oilseed rape (BBCH 11-19 autumn, use no. VIb)

Intended use	Winter oilseed rape (BBCH 11-19)				
Active substance/product	Acetamiprid				
Application rate (g/ha)	1 × 48 60				
Acute toxicity (mg/kg bw)	146				
TER criterion	10				
Crop scenario Growth stage	Indicator/generic focal species	SV ₉₀	MAF ₉₀	DDD ₉₀ (mg/kg bw/d)	TER _a
BBCH 10 - 19	Small insectivorous mammal "shrew"	7.6	1.0	0.36 0.46	405.6 320.2
All season	Large herbivorous mammal "lagomorph"	35.1	1.0	1.68 2.11	86.90 60.3
BBCH 10-29	Small omnivorous mammal "mouse"	17.2	1.0	0.82 1.03	178.04 141.5
Reprod. toxicity (mg/kg bw/d)	2.5				
TER criterion	5				
Crop scenario Growth stage	Indicator/generic focal species	SV _m	MAF _m × TWA	DDD _m (mg/kg bw/d)	TER _{lt}
BBCH 10 - 19	Small insectivorous mammal "shrew"	4.2	1.0 × 0.53	0.10 0.13	25 18.7
All season	Large herbivorous mammal "lagomorph"	14.3	1.0 × 0.53	0.36 0.45	6.94 5.5
BBCH 10-29	Small omnivorous mammal "mouse"	7.8	1.0 × 0.53	0.19 0.25	13.15 10.1

SV: shortcut value; MAF: multiple application factor; TWA: time-weighted average factor; DDD: daily dietary dose; TER: toxicity to exposure ratio. TER values shown in bold fall below the relevant trigger.

Table 9.3-12: First-tier assessment of the acute and long-term/reproductive risk for mammals due to the use of ADM.00150.I.2.A in sugar beet (BBCH 12-39, use no. VIIa)

Intended use	Sugar beet (BBCH 12-39)				
Active substance/product	Acetamiprid				
Application rate (g/ha)	2 × 50, 7 days interval				
Acute toxicity (mg/kg bw)	146				
TER criterion	10				
Crop scenario Growth stage	Indicator/generic focal species	SV ₉₀	MAF ₉₀	DDD ₉₀ (mg/kg bw/d)	TER _a
BBCH 10 - 19	Small insectivorous mammal "shrew"	7.6	1.4	0.53	274.4
BBCH ≥ 20	Small insectivorous mammal "shrew"	5.4	1.4	0.38	386.2
BBCH 10-39	Large herbivorous mammal "lagomorph"	35.1	1.4	2.46	59.4
BBCH 10-39	Small omnivorous mammal "mouse"	17.2	1.4	1.20	121.3
Reprod. toxicity (mg/kg bw/d)	2.5				
TER criterion	5				
Crop scenario Growth stage	Indicator/generic focal species	SV _m	MAF _m × TWA	DDD _m (mg/kg bw/d)	TER _{lt}
BBCH 10 - 19	Small insectivorous mammal "shrew"	4.2	1.6 × 0.53	0.18	14.0
BBCH ≥ 20	Small insectivorous mammal "shrew"	1.9	1.6 × 0.53	0.08	31.0
BBCH 10-39	Large herbivorous mammal "lagomorph"	14.3	1.6 × 0.53	0.61	4.1
BBCH 10-39	Small omnivorous mammal "mouse"	7.8	1.6 × 0.53	0.33	7.6

SV: shortcut value; MAF: multiple application factor; TWA: time-weighted average factor; DDD: daily dietary dose; TER: toxicity to exposure ratio. TER values shown in bold fall below the relevant trigger.

Table 9.3-13: First-tier assessment of the acute and long-term/reproductive risk for mammals due to the use of ADM.00150.I.2.A in flower bulbs and flower tubers (BBCH 12-91, use no. IXa)

Intended use	Flower bulbs and flower tubers (BBCH 12-91)				
Active substance/product	Acetamiprid				
Application rate (g/ha)	1 × 46				
Acute toxicity (mg/kg bw)	146				
TER criterion	10				
Crop scenario Growth stage	Indicator/generic focal species	SV ₉₀	MAF ₉₀	DDD ₉₀ (mg/kg bw/d)	TER _a
BBCH 10 - 19	Small insectivorous mammal "shrew"	7.6	1.0	0.35	417.6
BBCH ≥ 20	Small insectivorous mammal "shrew"	5.4	1.0	0.25	587.8
BBCH ≥ 40	Small herbivorous mammal "vole"	81.9	1.0	3.77	38.8
BBCH 10 - 39	Small omnivorous mammal "mouse"	17.2	1.0	0.79	184.5
BBCH ≥ 40	Small omnivorous mammal "mouse"	10.3	1.0	0.47	308.1
Reprod. toxicity (mg/kg bw/d)	2.5				
TER criterion	5				
Crop scenario Growth stage	Indicator/generic focal species	SV _m	MAF _m × TWA	DDD _m (mg/kg bw/d)	TER _{lt}
BBCH 10 - 19	Small insectivorous mammal "shrew"	4.2	1.0 × 0.53	0.10	24.4

BBCH \geq 20	Small insectivorous mammal "shrew"	1.9	1.0×0.53	0.05	54.0
BBCH \geq 40	Small herbivorous mammal "vole"	43.4	1.0×0.53	1.06	2.4
BBCH 10 - 39	Small omnivorous mammal "mouse"	7.8	1.0×0.53	0.19	13.1
BBCH \geq 40	Small omnivorous mammal "mouse"	4.7	1.0×0.53	0.11	21.8

SV: shortcut value; MAF: multiple application factor; TWA: time-weighted average factor; DDD: daily dietary dose; TER: toxicity to exposure ratio. TER values shown in bold fall below the relevant trigger.

Table 9.3-14: First-tier assessment of the acute and long-term/reproductive risk for mammals due to the use of ADM.00150.I.2.A in flower bulbs and flower tubers (BBCH 20-91, use no. IXb)

Intended use	Flower bulbs and flower tubers (BBCH 20-91)				
Active substance/product	Acetamiprid				
Application rate (g/ha)	2×34 , 7 days interval				
Acute toxicity (mg/kg bw)	146				
TER criterion	10				
Crop scenario Growth stage	Indicator/generic focal species	SV ₉₀	MAF ₉₀	DDD ₉₀ (mg/kg bw/d)	TER _a
BBCH 10 - 19	Small insectivorous mammal "shrew"	7.6	1.4	0.36	403.6
BBCH \geq 20	Small insectivorous mammal "shrew"	5.4	1.4	0.26	568.0
BBCH \geq 40	Small herbivorous mammal "vole"	81.9	1.4	3.90	37.5
BBCH 10 - 39	Small omnivorous mammal "mouse"	17.2	1.4	0.82	178.3
BBCH \geq 40	Small omnivorous mammal "mouse"	10.3	1.4	0.49	297.8
Reprod. toxicity (mg/kg bw/d)	2.5				
TER criterion	5				
Crop scenario Growth stage	Indicator/generic focal species	SV _m	MAF _m \times TWA	DDD _m (mg/kg bw/d)	TER _{lt}
BBCH 10 - 19	Small insectivorous mammal "shrew"	4.2	1.6×0.53	0.12	20.6
BBCH \geq 20	Small insectivorous mammal "shrew"	1.9	1.6×0.53	0.05	45.6
BBCH \geq 40	Small herbivorous mammal "vole"	43.4	1.6×0.53	1.25	2.0
BBCH 10 - 39	Small omnivorous mammal "mouse"	7.8	1.6×0.53	0.22	11.1
BBCH \geq 40	Small omnivorous mammal "mouse"	4.7	1.6×0.53	0.14	18.4

SV: shortcut value; MAF: multiple application factor; TWA: time-weighted average factor; DDD: daily dietary dose; TER: toxicity to exposure ratio. TER values shown in bold fall below the relevant trigger.

Table 9.3-15: First-tier assessment of the acute and long-term/reproductive risk for mammals due to the use of ADM.00150.I.2.A in floriculture, tree nursery* and perennial nursery crops (BBCH 12-91, use no. Xa)

Intended use	Floriculture, tree nursery and perennial nursery crops (BBCH 12-91)				
Active substance/product	Acetamiprid				
Application rate (g/ha)	1×46				
Acute toxicity (mg/kg bw)	146				
TER criterion	10				
Crop scenario Growth stage	Indicator/generic focal species	SV ₉₀	MAF ₉₀	DDD ₉₀ (mg/kg bw/d)	TER _a
Application to plant – exposure to underlying ground	Small insectivorous mammal "shrew"	5.4	1.0	0.25	587.8

BBCH 40 - 49	Small herbivorous mammal "vole"	136.4	1.0	6.27	23.3
BBCH ≥ 50	Small herbivorous mammal "vole"	68.2	1.0	3.14	46.5
BBCH 10 - 49	Small omnivorous mammal "mouse"	17.2	1.0	0.79	184.5
BBCH ≥ 50	Small omnivorous mammal "mouse"	8.6	1.0	0.40	369.1
Reprod. toxicity (mg/kg bw/d)		2.5			
TER criterion		5			
Crop scenario Growth stage	Indicator/generic focal species	SV _m	MAF _m × TWA	DDD _m (mg/kg bw/d)	TER _{it}
Application to plant – exposure to underlying ground	Small insectivorous mammal "shrew"	1.9	1.0 × 0.53	0.05	54.0
BBCH 40 - 49	Small herbivorous mammal "vole"	72.3	1.0 × 0.53	1.76	1.4
BBCH ≥ 50	Small herbivorous mammal "vole"	36.1	1.0 × 0.53	0.88	2.8
BBCH 10 - 49	Small omnivorous mammal "mouse"	7.8	1.0 × 0.53	0.19	13.1
BBCH ≥ 50	Small omnivorous mammal "mouse"	3.9	1.0 × 0.53	0.10	26.3

SV: shortcut value; MAF: multiple application factor; TWA: time-weighted average factor; DDD: daily dietary dose; TER: toxicity to exposure ratio. TER values shown in bold fall below the relevant trigger.

*Tree nursery uses resembling orchard crops are covered by Tier 1 calculations for orchard uses.

Table 9.3-16: First-tier assessment of the acute and long-term/reproductive risk for mammals due to the use of ADM.00150.I.2. in floriculture, tree nursery and perennial nursery crops (BBCH 20-91, use no. Xb)

Intended use		Floriculture, tree nursery and perennial nursery crops (BBCH 20-91)			
Active substance/product		Acetamiprid			
Application rate (g/ha)		2 × 34, 7 days interval			
Acute toxicity (mg/kg bw)		146			
TER criterion		10			
Crop scenario Growth stage	Indicator/generic focal species	SV ₉₀	MAF ₉₀	DDD ₉₀ (mg/kg bw/d)	TER _a
Application to plant – exposure to underlying ground	Small insectivorous mammal "shrew"	5.4	1.4	0.26	568.0
BBCH 40 - 49	Small herbivorous mammal "vole"	136.4	1.4	6.49	22.5
BBCH ≥ 50	Small herbivorous mammal "vole"	68.2	1.4	3.25	45.0
BBCH 10 - 49	Small omnivorous mammal "mouse"	17.2	1.4	0.82	178.3
BBCH ≥ 50	Small omnivorous mammal "mouse"	8.6	1.4	0.41	356.7
Reprod. toxicity (mg/kg bw/d)		2.5			
TER criterion		5			
Crop scenario Growth stage	Indicator/generic focal species	SV _m	MAF _m × TWA	DDD _m (mg/kg bw/d)	TER _{it}
Application to plant – exposure to underlying ground	Small insectivorous mammal "shrew"	1.9	1.6 × 0.53	0.05	45.6
BBCH 40 - 49	Small herbivorous mammal "vole"	72.3	1.6 × 0.53	2.08	1.2
BBCH ≥ 50	Small herbivorous mammal "vole"	36.1	1.6 × 0.53	1.04	2.4
BBCH 10 - 49	Small omnivorous mammal "mouse"	7.8	1.6 × 0.53	0.22	11.1
BBCH ≥ 50	Small omnivorous mammal "mouse"	3.9	1.6 × 0.53	0.11	22.2

SV: shortcut value; MAF: multiple application factor; TWA: time-weighted average factor; DDD: daily dietary dose; TER: toxicity to exposure ratio. TER values shown in bold fall below the relevant trigger.

The acute tier 1 TER values exceed the relevant trigger values, indicating an acceptable risk following applications of ADM.00150.I.2.A according to the intended use pattern.

The following scenarios fall below the trigger of 5 for reproductive risk assessment and higher tier risk assessments are required:

Table 9.3-17: Summary of reproductive mammal scenarios below the trigger of 5

Crop	App rate	Interval	BBCH	Scenario	TER
Corn	1 x 60	-	≥ 40	Small herbivorous mammal 'vole'	4.3
Apple	1 x 80	-	≥ 40	Small herbivorous mammal 'vole'	2.7
			71-79	Frugivorous mammal 'dormouse'	2.6
Spring cereals	2 x 35	10	≥ 40	Small herbivorous mammal 'vole'	4.1
Winter cereals	2 x 36	10	≥ 40	Small herbivorous mammal 'vole'	4.0
Spring OSR/winter OSR	2 x 60	7	≥ 40	Small herbivorous mammal 'vole'	2.7
			All season	Large herbivorous mammal "lagomorph"	3.4
Sugar beet	2 x 50	7	10-39	Large herbivorous mammal "lagomorph"	4.1
Flower bulbs	1 x 46	-	≥ 40	Small herbivorous mammal 'vole'	2.4
Flower bulbs	2 x 34	7	≥ 40	Small herbivorous mammal 'vole'	2.0
Floriculture	1 x 46	-	40 - 49	Small herbivorous mammal 'vole'	1.4
			≥ 50	Small herbivorous mammal 'vole'	2.8
Floriculture	2 x 34	7	40 - 49	Small herbivorous mammal 'vole'	1.2
			≥ 50	Small herbivorous mammal 'vole'	2.4

zRMS comments:

Based on calculations performed in the Tables 9.3-2 to 9.3-16 the following scenarios needs further refinement:

Crop	App rate	Interval	BBCH	Scenario
Corn	1 x 60	-	≥ 40	Small herbivorous mammal 'vole'
Apple	1 x 60	-	≥ 40	Small herbivorous mammal 'vole'
			71-79	Frugivorous mammal 'dormouse'
Spring cereals	2 x 35	10	≥ 40	Small herbivorous mammal 'vole'
Winter cereals	2 x 36	10	≥ 40	Small herbivorous mammal 'vole'
Spring OSR/winter OSR	2 x 60	7	≥ 40	Small herbivorous mammal 'vole'
			All season	Large herbivorous mammal "lagomorph"
Sugar beet	2 x 50	7	10-39	Large herbivorous mammal "lagomorph"
Flower bulbs	1 x 46	-	≥ 40	Small herbivorous mammal 'vole'
Flower bulbs	2 x 34	7	≥ 40	Small herbivorous mammal 'vole'
Floriculture	1 x 46	-	40 - 49	Small herbivorous mammal 'vole'
			≥ 50	Small herbivorous mammal 'vole'
Floriculture	2 x 34	7	40 - 49	Small herbivorous mammal 'vole'
			≥ 50	Small herbivorous mammal 'vole'

Higher tier risk assessment for relevant species such as: vole, lagomorph and dormouse has been provided below Point 9.3.2.2.

9.3.2.2 Higher-tier risk assessment

Higher tier risk assessments for small and large herbivorous mammals and frugivorous mammals regarding

the exposure scenarios detailed above are conducted according to recommendations of EFSA/2009/1438 and presented below.

General refinement options (substance-/crop-specific) for various scenarios are presented below, followed by higher tier risk assessments for the scenarios identified as critical in the first-tier assessment.

The applied refinements are provided for each scenario.

Substance-/crop-specific refinements

1. Refinement of DT₅₀ for vegetation
2. Refinement of deposition values

The data for two refinements are presented in the chapters below.

1. Refinement of DT₅₀ for vegetation

Five residue decline studies were conducted in the Central and Southern zone to collect monocot and dicot plant species for analysis of residues and residue decline of acetamiprid. The studies can be used in higher tier risk assessment and are listed below:

Table 9.3-18: Residue studies for acetamiprid in plant material

GLP No.	Report No.	Adama report number	KCP	Author(s)	Date	Country	Study title
299	R1640039	R-37376	10.1.2/01	Weick, S. and Henkes, K.	2017	IT	Residues of acetamiprid in foliage-dwelling arthropods and ground vegetation after spray application of Acetamiprid 200 SL in a pome fruit orchard in Italy – magnitude of residues and time course of residue decline
513	R2040056	000106551	10.1.2/02	Staffel, J. and Brehm, C.	2021	ES	Residues of acetamiprid in monocotyledonous and dicotyledonous plants after spray application of Acetamiprid 200 SL in early vegetation stages in Spain – magnitude of residues and time course of residue decline
517	R2040057	000106552	10.1.2/03	Staffel, J. and Brehm, C.	2021	DE	Residues of acetamiprid in monocotyledonous and dicotyledonous plants after spray application of Acetamiprid 200 SL in early vegetation stages in Germany – magnitude of residues and time course of residue decline
519	R2040059	000106554	10.1.2/04	Staffel, J. and Brehm, C.	2022	DE	Residues of acetamiprid in monocotyledonous and dicotyledonous plants after spray application of Acetamiprid 200 SL in early vegetation stages in spring in Germany – magnitude of residues and time course of residue decline
520	R2040060	000106555	10.1.2/05	Gräf, K.	2022	DK	Residues of acetamiprid in monocotyledonous and dicotyledonous plants after spray application of Acetamiprid 200 SL in early vegetation stages in Northern Europe – magnitude of residues and time course of residue decline

In the studies conducted in 2021 and 2022 wheat and pea plants were harvested and analysed to represent monocotyledonous and dicotyledonous food items, respectively. During the study conducted in 2017, plant specimens collected in an apple orchard were not identified but the orchard undergrowth consisted mainly of grass (monocot) species, which can be seen in pictures (see Figure 1) of ground vegetation sampling and orchard pictures as given in the study report. Therefore, analysed decline data in this case represent monocotyledonous plants.



Figure 1: Ground vegetation sampling in grass understory in a pome fruit orchard in Italy in 2016 (photos: Rifcon GmbH)

The residue levels of the active substance acetamiprid were determined after spray application of Acetamiprid 200 SL in wheat (representing monocotyledonous plants) and pea (representing dicotyledonous plants) fields and for one study in a pome fruit orchard, where the undergrowth was analysed. Pea and wheat plants were collected at 3 study plots (=replicates) on DAT (Days After Treatment) -1 (before application), 0 (after application), 1, 2, 3, 5, 7, 10, 14. Ground vegetation were collected at 3 study plots (=replicates) on DAT -2 (before application), 0 (approx. 4 hours after the first application), 1, 2, 3, 7, 8 (approximately 4 hours after the second application), 9, 10, 11, 15, 22, and 29. The maximum concentrations of acetamiprid in ground vegetation were determined between the two applications (DAT 0-7) as well as after the second application until the end of the sampling period (DAT 8-29). The initial and maximum concentrations of acetamiprid on plants were calculated based on the arithmetic mean of three replicates (n=3). Residue concentrations of acetamiprid were used to calculate the RUDs (Residues per Unit Dose), based on an application rate of 1.0 kg a.s./ha. The DT₅₀ (dissipation time 50%) values of acetamiprid were calculated using single first order kinetics (SFO). The DT₅₀ values, Chi² error and p values are given in the table below for all studies.

A geometric mean is calculated based on all data combining Central and Southern zone data and for studies conducted in Germany and Denmark only representing the Central zone. Following EFSA (2019) the study conducted in Denmark can be considered being representative of the Central zone as it was conducted in close proximity to the German border (100 km distance) where similar climatic conditions as in the northern part of Germany can be expected.

Table 9.3-19: Acetamiprid DT₅₀ values and Chi² error values for plant material

Study number	Country	GLP No.	Matrix	DT ₅₀ (days)	Chi ² error [%]	t-test (p value)	DT ₅₀ Geometric mean (all trials) (days)	DT ₅₀ Geometric mean (Central zone) (days)
Monocotyledons represented by wheat and ground vegetation								
R1640039	IT	299	Ground vegetation	1.1*	15.93	<0.01	1.9	1.8
R1640039	IT	299	Ground vegetation	1.5**	15.54	<0.001		
2010056 R2040056	ES	513	Wheat	5.0	14.52	<0.001		
2010060 R2040060	DK	520	Wheat	1.3	8.25	<0.001		
2010057 R2040057	DE	517	Wheat	1.2	1.39	<0.001		
2010059 R2040059	DE	519	Wheat	3.8	21.54	<0.001		
Dicotyledons represented by peas								
2010056	ES	513	Peas	5.9	9.21	<0.001	3.5	2.9

R2040056								
2010060 R2040060	DK	520	Peas	2.8	11.84	<0.001		
2010057 R2040057	DE	517	Peas	3.6	17.99	<0.001		
2010059 R2040059	DE	519	Peas	2.4	5.78	<0.001		

*after first application from DAT 0 to DAT 8

** after second application from DAT 8 to DAT 29

The geomean values calculated for monocotyledonous and dicotyledonous plants for all studies show a slower decline than the decline seen in the geomean for the studies conducted in Germany and Denmark.

Since the study conducted in Spain shows a much slower decline, it is conservative to focus more on the spatial variability and include the trials conducted in Italy and Spain in order to get a larger database. The study in Italy was conducted in the province Cuneo, which is situated in the northern part of Italy and can therefore be considered broadening the spatial range of decline trials in a similar climatic area.

Since including all trials of the Central and Southern zone gives a higher DT₅₀ for both matrices, it seems to be justified to use the geomean DT₅₀ values for both matrices from all trials.

This is supported by the findings of Ebeling and Wang (2018, KCP 10.1.2/06), who re-assessed foliar dissipation based on a data set of 396 residue trials covering 30 compounds. They found that a comparison of the DT₅₀ values for the northern versus the southern residue zones (EU-N vs EU-S) showed that half-lives were very similar and that no significant differences in DT₅₀ values from EU-N versus EU-S were detected for any approach. The geometric mean DT₅₀ calculated from 30 currently used compounds was approximately 3 days (the 90th percentile was 7.9 days) which is in line with the DT₅₀ for acetamiprid measured in plant matrices.

Further, as given in the RAR for acetamiprid (RAR 19 Volume 3 CP B9, Acetamiprid 20 SC August 2016), it can be seen from residue decline trials in plant material (lettuce and alfalfa), that acetamiprid degrades fast. A geometric mean DT₅₀ was calculated from 10 trials evaluated as acceptable based on a visual assessment and statistics to assess the goodness of fit. No difference between datasets for Central and Southern zones could be detected, hence datasets of different zones were combined. A geometric DT₅₀ of 2.3 days was calculated for dicot plant material supporting the findings of the studies conducted in peas representing dicots as presented above.

In view of all arguments given above and following the approach presented in the RAR of combining residue decline datasets of different zones, a DT₅₀ of 1.9 days for monocotyledons and a DT₅₀ of 3.5 days for dicotyledons based on data from the Central and the Southern zone will be used in the risk assessment.

zRMS comments:

The geometric mean DT₅₀ of 1.9 days for monocotyledons and a geometric mean DT₅₀ of 3.5 days based on all data combining Central and Southern zone data and for studies conducted in Germany and Denmark representing only the Central zone have been accepted by zRMS. The decline study evaluation with kinetic analysis can be found in Appendix 2.

MAF x TWA refinement

If there is more than one application, MAF x TWA values should be re-calculated, applying the moving time window approach as recommended in EFSA/2009/1438. Re-calculations were carried out with the calculator tool provided by UBA, Germany (Bird and Mammal TER calculator, rev. 6j of 10.03.2014, Andreas Höllrigl-Rosta, Dessau, Germany). Re-calculations are done based on measured DT₅₀ values in monocotyledons (1.9 days) and dicotyledons (3.5 days) as detailed above.

Table 9.3-20: Re-calculated MAF_m and TWA values based on acetamiprid-specific residue data in plant material

Relevant crop use	Crop group	Matrix	Number of applications	Application interval (days)	DT ₅₀ (days)	MAF x TWA
Maize/corn	Monocots	Wheat and orchard ground vegetation	1	-	1.90	0.13
	Dicots	Peas	1	-	3.50	0.24
Oilseed rape/sugar beet/flower bulbs/floriculture	Monocots	Wheat and orchard ground vegetation	2	7	1.90	0.26
	Dicots	Peas	2	7	3.50	0.46
Spring cereals/winter cereals	Monocots	Wheat and orchard ground vegetation	2	10	1.90	0.26
	Dicots	Peas	2	10	3.50	0.45

zRMS comments:

zRMS confirmed that recalculation of MAF and twa are correct.

2. Refinement of deposition values

The Appendix E of the EFSA/2009/1438 provides interception / deposition factors for different crops and growth stages based on the FOCUS surface water report (FOCUS, 2001). Furthermore, it is stated that in the context of a higher-tier assessment the more detailed interception values of the FOCUS groundwater report (FOCUS, 2000) can be used. Meanwhile, an updated FOCUS Guidance Document for Groundwater assessment is applicable, providing updated crop specific information (FOCUS, 2014).

In the draft Guidance for Birds and Mammals (EFSA, 2021), deposition values based on this most recent FOCUS interception data were presented and their use for different crops specified in Appendix L and Annex B of the draft Birds and Mammals Guidance (EFSA, 2021). As this is the most up to date reference for the use of deposition values, all deposition values are taken from the draft Birds and Mammals Guidance (EFSA, 2021).

The deposition factors used in the refined risk assessment are summarised in the table below.

Table 9.3-21: Deposition factors for the relevant crops according to FOCUS (2014) and Draft Guidance Appendix L/Annex B (EFSA, 2021)

EFSA crop groups (FOCUS crop)	GAP relevant BBCH stage	Risk assessment relevant stage	Deposition values according to Annex B (EFSA 2021) and Focus (2014)	Location of food item	Deposition value
Corn	51-75	≥ 40	40-49	Weed	0.25
Apple	71-PHI	≥ 40	30-69	Weed	0.4
			70-89	Weed	0.35
Spring cereals, winter cereals	40-69	≥ 40	40-69	Weed	0.1
Spring OSR/winter OSR	31-71	≥ 40	30-89	Weed	0.2
		All season	31-89	Weed	0.2
Sugar beet	12-39	10-39	10-39	Weed	1
Flower bulbs	12-91	≥ 40	10-89	Weed	0.6
		≥ 40	90-99	Weed	0.4
Floriculture: Ornamental herbaceous plants, no persistent woody stem above	12-91	40 - 49	30 - 49	Weed	0.5
		≥ 50	50-89	Weed	0.25

ground					
Floriculture: Ornamental broad-leaved trees, shrubs, and climbing plants	12-91	40 - 49	10-69	Weed	0.4
		≥ 50	70-89	Weed	0.35

zRMS comments:

We agree with DF used by the Applicant for higher tier based on FOCUS Guidance Document for Groundwater assessment (FOCUS, 2014).

Higher tier risk assessment for generic and focal species

The following generic focal species and identified focal species are used for higher tier risk assessment:

1. Large herbivorous mammal “lagomorph”: European rabbit (*Oryctolagus cuniculus*)
2. Frugivorous mammal ‘dormouse’
3. Small herbivorous mammal ‘vole’: Common vole (*Microtus arvalis*)

1. Large herbivorous mammal “lagomorph”: European rabbit (*Oryctolagus cuniculus*)

According to EFSA/2009/1438, the rabbit represents the ‘generic focal species’ for the tier 1 risk assessment in oilseed rape and sugar beet.

The following refinement options were considered based on current scientific knowledge in line with EFSA/2009/1438:

- Focal species selection
- Refinement of deposition factors
- Refinement of substance specific residue decline data
- PT refinement

Focal species selection

The European rabbit, a medium sized herbivorous mammal, is a native in southwestern Europe and has been introduced to nearly all European countries as can be seen in the Figure below.

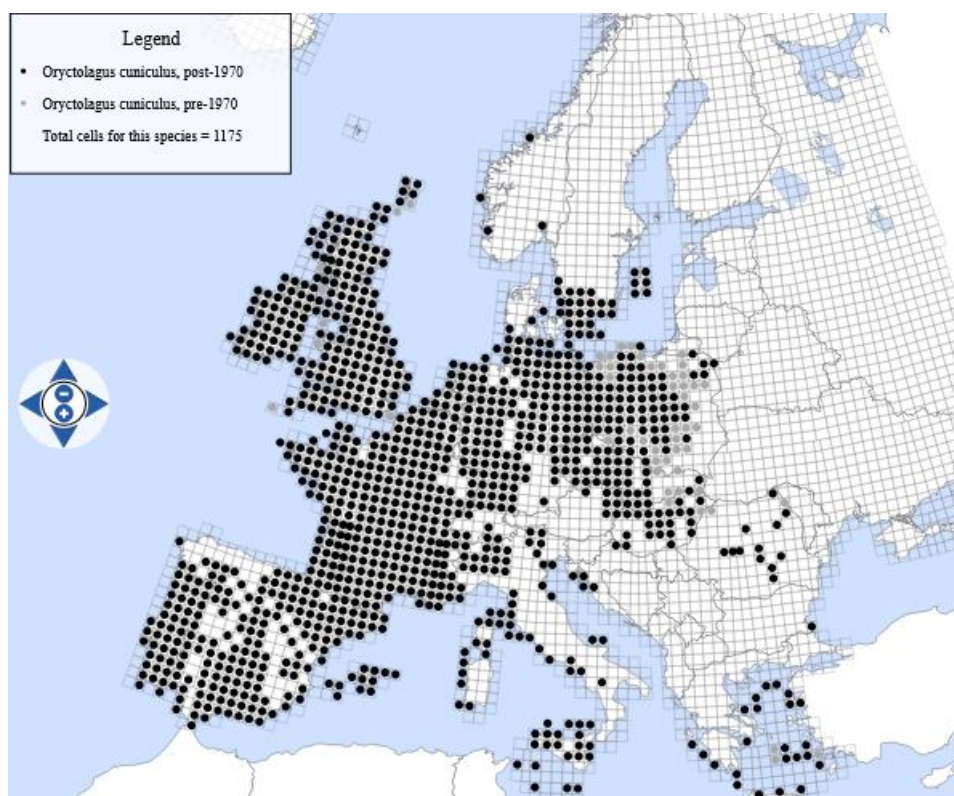


Figure 2: Distribution of the European rabbit in Europe. Distribution maps from: <https://www.european-mammals.org/php/mapmaker.php> (last access 11 April 2022)

Rabbits mainly inhabit open landscapes with low vegetation of bushes and scrubs on preferably sandy soils (Kaetzke *et al.*, 2003, KCP 10.1.2/07). Preferred habitats include thickets, forests, meadows, and woods on light soils where they can easily dig their warrens. Furthermore, pasture communities on soils with little human disturbance (e.g. ploughing) are preferred by rabbits (Gea-Izquierdo *et al.*, 2005, KCP 10.1.2/08). During the day, rabbits prefer to reside in warrens or vegetated patches, which they use for protection from predators. At night, they move into open fields to feed.

Field management practices such as ploughing, and harrowing (which destroy burrows) greatly disrupt the establishment of rabbits in agricultural landscapes. Rabbit populations seem to be greatest in ecotone habitats (a transition area between two different patches of the landscape) and less in agricultural fields and grasslands (Calvete *et al.* 2003, KCP 10.1.2/09). Since rabbits have the potential to be exposed to acetamiprid treated fields, this species is considered to be representative of other large herbivores.

Higher tier refinement in oilseed rape

Refinement of PT in oilseed rape

Katzschner *et al.* (2015, KCP 10.1.2/10, see study summary A 2.1.2.2.6) investigated the use of oilseed rape, sunflower and sugar beet fields as foraging habitat by European hares (*Lepus europaeus*) and rabbits (*Oryctolagus cuniculus*), and determined respective PT values (i.e. proportion of diet obtained in the treated area, calculated as proportion of foraging time spent potentially foraging in the crop of concern) via 24-hour radio-tracking of multiple individuals monthly over the entire growing season for each crop. In total, 25 individual rabbits at two study sites and 26 individual hares at three different study sites were radio-tracked during the entire crop development of oilseed rape, sugar beet and sunflower. Data recording lasted for 19 months, from April 2013 to October 2014, and in total 267 sessions of 24 h of telemetry were analysed (based on 228 sessions conducted). In oilseed rape, twelve different individuals in total were radio-tracked during the entire growing period (September to July) at the study site in Würzburg (Germany). For PT calculations 69 tracking sessions were analysed. The monthly mean PT values ranged from 0 to 0.57 and the 90th percentile ranged from 0 to 0.97. The highest PT values were calculated for April to July during

late crop development stages and before harvest (BBCH growth stages 57 to 85). Lowest values were calculated for early crop development stages (BBCH growth stages 09 to 24) from September to January and in March. The mean PT values and 90th percentile PT values are presented in the table below. The growing period of oilseed rape has been covered during data recordings of two different years. The given BBCH growth stages are summarised for species and sites.

Table 9.3-22: Summary of calculated PT values for oilseed rape for rabbits at different BBCH growth stages in different months

Crop	BBCH growth stage (month)	Rabbit PT	
		mean	90 th percentile
Oilseed rape	00-14 (Sep)	0.18	0.45
	12-17 (Oct)	0.00	0.00
	16-20 (Nov)	0.17	0.51
	16-21 (Dec)	0.16	0.46
	19-24 (Jan)	0.11	0.32
	19-29 (Feb)	0.38	0.87
	19-59 (March)	0.01	0.02
	24-65 (April)	0.42	0.82
	53-70 (May ^G /March ^H)	0.57	0.83
	63-80 (June ^G /April ^H)	0.55	0.87
	69-85 (July ^G /May ^H)	0.55	0.98
	86-99 (Aug ^G /June ^H)	no crop	no crop
	00-99 (Sep-Aug)	0.31	0.82

^G= Germany, ^H = Hungary

Since a risk to lagomorphs is indicated for the whole season in winter and spring oilseed rape, the 90th percentile value of 0.82 from all tracking sessions from September to July was taken to refine the risk to large herbivores.

Food intake rate (FIR/bw) of the European rabbit

According to Appendix A of EFSA/2009/1438, the diet of this species is composed of 100% crop leaves in oilseed rape and a FIR/bw of 0.5.

Residues in the food of European rabbit

The RUD values for non-grass herbs will be taken as given in Appendix A of EFSA/2009/1438.

Refined MAF x TWA

A refined MAF x TWA is taken from the Table 9.3-20.

Table 9.3-23: Higher-tier assessment of the long-term/reproductive risk for mammals due to the use of acetamiprid in oilseed rape – refined parameters (*) are further described and justified in the text

Intended use		Oilseed rape (BBCH 31-71)					
Active substance/product		Acetamiprid					
Application rate (g/ha)		2 × 60, 7 days interval					
Reprod. toxicity (mg/kg bw/d)		2.5					
TER criterion		5					
Focal species	Food category, % in diet	FIR/bw	RUD _m (mg/kg food)	MAF _m × TWA	PT	DDD _m (mg/kg bw/d)	TER _{it}
European rabbit	Non-grass herbs, 100 %	0.5	28.7	0.46	0.82	0.32	7.7
Hare	Non-grass herbs, 100 %	0.5	28.7	0.46	0.60	0.23	10.9

FIR/bw: Food intake rate per body weight; RUD: residue unit dose; DF: deposition factor (considering possible interception by the crop); MAF: multiple application factor; DDD: daily dietary dose; TER: toxicity to exposure ratio

zRMS comments:

zRMS agrees with refined risk assessment for oilseed rape provided for medium herbivorous species European rabbit with consideration of the PT of 0.82 (90th percentile) value based on field study - and refined MAFx ftwa values.

The TER_{LT} is above trigger of 5 indicated an acceptable long-term risk for medium sized herbivorous mammal-European rabbit.

Higher tier refinement in sugar beet

Refinement of PT in in sugar beet

The data presented in Katschnner et al. (2015, for details see above and study summary A 2.1.2.2.6) present PT data for rabbits (*Oryctolagus cuniculus*) for sugar beet fields. At the study site in Würzburg (Germany) a total of 13 individuals were radio-tracked during the growing period of sugar beet (April to October). Forty-two tracking sessions were analysed. The mean PT value per month ranged from 0 to 0.27 and the 90th percentile from 0 to 0.67. During early crop development in April (BBCH growth stages 00 to 10) no tagged animal spent potentially foraging time in the crop. During the remaining time of crop development from May to October (BBCH growth stages 14 to 49) mean PT values remained low except for July (see below). The PT in sugar beet during this growing period generally ranged from 0.02 to 0.06 (90th percentile 0.04 to 0.18). During the month of July (BBCH growth stages 37 to > 39) rabbits spent more time potentially foraging in sugar beet (mean PT = 0.27, 90th percentile = 0.67).

Table 9.3-24: Summary of calculated PT values for ~~oilseed rape~~ for rabbits at different BBCH growth stages in different months

Crop	BBCH growth stage (month)	Rabbit PT	
		mean	90 th percentile
Sugar beet	00-10 (April)	0.00	0.00
	14-18 (May)	0.02	0.04
	18-36 (June)	0.04	0.11
	37- >39 (July)	0.27	0.66
	>39 (Aug)	0.06	0.18
	<49 (Sep)	0.03	0.10
	49 (Oct)	0.03	0.08
	00-49 (April-Oct)	0.06	0.18

Since a risk to lagomorphs is indicated for early growth stages (BBCH 10-39), the highest 90th percentile value of 0.66 from the tracking sessions from April to July (BBCH 0-39) was taken to refine the risk to large herbivores.

Food intake rate (FIR/bw) of the European rabbit

According to Appendix A of EFSA/2009/1438, the diet of this species is composed of 100% non-grass herbs in sugar beet and a FIR/bw of 0.5.

Residues in the food of European rabbit

The RUD values for non-grass herbs will be taken as given in Appendix A of EFSA/2009/1438.

Refined MAF x TWA

A refined MAF x TWA is taken from the Table 9.3-20.

Table 9.3-25: Higher-tier assessment of the long-term/reproductive risk for mammals due to the use of acetamiprid in sugar beet – refined parameters (*) are further described and justified in the text

Intended use		Sugar beet (BBCH 12-39)					
Active substance/product		Acetamiprid					
Application rate (g/ha)		2 × 50, 7 days interval					
Reprod. toxicity (mg/kg bw/d)		2.5					
TER criterion		5					
Focal species	Food category, % in diet	FIR/bw	RUD_m (mg/kg food)	MAF_m × TWA	PT	DDD_m (mg/kg bw/d)	TER_{it}
European rabbit	Non-grass herbs, 100 %	0.5	28.7	0.46	0.66	0.22	11.5
Hare	Non-grass herbs, 100 %	0.417*	28.7	0.46	0.82	0.32	7.2

FIR/bw: Food intake rate per body weight; RUD: residue unit dose; DF: deposition factor (considering possible interception by the crop); MAF: multiple application factor; DDD: daily dietary dose; TER: toxicity to exposure ratio.
bw=2900 g bw , FIR/bw calculated according to EFSA GD for B&M, 2009.

zRMS comments:

zRMS agrees with refined risk assessment for sugar beet provided for medium European rabbit with consideration of refined parameters such as: PT of 0.66 (90th percentile) based on sugar beet field study by Katschnner et al., 2015 and refined MAF x ftwa parameters.

Commenting period process:

Some of MSs requested for additional risk assessment for hare. For this reason the risk in the Table 9.3-25 has been updated. has been updated by zRMS using FIR/BW calculated according to Appendix G in EFSA GD for B&M, 22009, based on the lowest bw in 2900 g for hare taken from Mammal Bible nad 100 % non-grass herbs in diet. In addition PT was used from the study by Katschnner et al., 2015 and refined MAF x ftwa parameters

The TER_{LT} is above trigger of 5 indicated an acceptable long-term risk for medium sized herbivorous mammal from exposure of acetamiprid in ADM.00150.I.2.A.

2. Frugivorous mammal ‘dormouse’

According to EFSA/2009/1438, the dormouse represents the ‘generic focal species’ for the tier 1 risk assessment in orchards.

The following refinement options were considered based on current scientific knowledge in line with EFSA/2009/1438:

- Refinement of RUD based on published literature

Focal species selection

As no ecological refinements are made, the selection of a focal species is not considered necessary as specified by EFSA. This approach is also reflected in the new draft of the EFSA Bird and Mammal Guidance (EFSA, 2021), where a tiered approach is described. Before selecting a “focal species”, substance-specific refinements like residue data can be applied to the Tier 1 species. Therefore, the refined RUD for fruits is substance-specific and the identification of a focal species is not considered necessary.

Residues on fruits

EFSA/2009/1438 (Appendix F) provides default and initial residue values after application for bird and mammal food items to be used in wildlife risk assessments. Most of these values are based on large numbers of registration relevant residue decline studies evaluated prior to the finalisation of the current guidance on environmental risk assessment.

ADM.00150.I.2.A is intended for application in apples. The most recent RUD value evaluation for fruits is provided in EFSA (2021). The document reveals a geometric mean RUD of 0.97 for pome fruits (e.g. apple, pear) which was used in the higher tier risk assessment for the frugivorous mammal.

zRMS would like to point out that the literature data by Schabacker et al. (2020) were evaluated by zRMS-PL previously for authorization of the product CA3573 (formally MCW-222) in 2021 and considered reliable for the risk assessment. zRMS evaluation is presented in the Appendix 2.

Residue decline trials were performed to measure the magnitude of residues of acetamiprid on apples at four different sites in the Central zone: two trials in Northern France, and one each in Germany and Poland (for details please see dRR Part B7). Either one or two applications at each site was done using airblast sprayer and sampling was made at days after last application (DALA) 0, 3, 7, 14 and 21. Four of the six trials were made with two applications and sampling also took place after the last treatment. Residues in treated specimens were analysed and using the actual application rate, normalised for an application rate of 1 kg active ingredient/ha (RUD). The results of the residue trials are presented in the table below.

Report-No	Trial No	Trial ID	Location	EU zone	Year	Application rate (kg a.s./ha)	Growth stage	Portion analyzed	Application pattern (kg/ha)	Sampling at DA LA	Measured residues (mg/kg)	RUD (mg/kg)
R-33599	DMC-13-16134 FR01		Northern France	NEU	2014	0.098	BBCH 85	Apple fruits	1 x 0.1	0	0.11	1.08
						0.099	BBCH 85	Apple fruits	2 x 0.1	0	0.17	1.65
R-34915	ChR 14 17311	FR01	Northern France	NEU	2014	0.104	BBCH 85	Apple fruits	1 x 0.1	0	0.08	0.83
						0.105	BBCH 86	Apple fruits	2 x 0.1	0	0.11	1.14
R-34915	ChR 14 17311	DE02	Germany	NEU	2014	0.103	BBCH87	Apple fruits	2 x 0.1	0	0.2	2.04
R-34915	ChR 14 17311	PL03	Poland	NEU	2014	0.101	BBCH 85	Apple fruits	2 x 0.1	0	0.09	0.91
											Geomean	1.21

The measured RUD value for acetamiprid in apples including 2 trials with 1 application and 4 trials with 2 applications is 1.21 mg/kg. This value is well within the range of the values presented by Schabacker *et al.* (2020) and the RUD value for pome fruits given in EFSA (2021). Since 4 of the trials were performed with two applications and the sample was taken after the last application, residues measured in these trials are on average higher than the ones performed with one application. Since a risk was identified for frugivorous mammal in apples with an application rate 1 x 80 g a.s./ha, the RUD value given in EFSA (2021) will be used as it is based on a much broader database and reflects the application pattern better.

The higher tier long-term reproductive risk for frugivorous mammals is calculated according to the refinements detailed above as presented in the tables below.

zRMS comments:

First of all, the zRMS would like to point out that the studies above in the Table 9.3.26 were evaluated by zRMS-PL previously for authorisation of the product CA3573 (formally MCW-222) in 2021. The conclusion can be found in Appendix 2.

For this reason, the conclusion of zRMS based on respective data together with RUD values calculated specifically for acetamiprid is still valid and has been presented below:

Please note that in order to cover worst case, the RUD values were calculated based on maximum residue level, regardless of the DALA.

Trial	Variety	BBCH at last treatment	No of applications ¹⁾	Rate [kg/ha]	Sampling day	Matrix	Residue level [mg/kg]	RUD [mg/kg] ¹⁾
ChR 14 17311 FR01 Nord Pas de Calais 59400 Fontaine Notre Dame, Northern France	Idared	85	1	104	0 DALA 3 DALA 7 DALA 14 DALA 21 DALA	Whole fruit	0.08 <u>0.09</u> 0.07 0.03 0.03	1.06
N-EU 2014		85	2	104 105	0 DALA 3 DALA 7 DALA 14 DALA 21 DALA	Whole fruit	<u>0.11</u> 0.11 0.11 0.06 0.07	1.06
ChR 14 17311 DE02 Rheinland-Pfalz 67551 Worms Pfeddersheim Germany	Braeburn	87	2	102 103	0 DALA 3 DALA 7 DALA 14 DALA 21 DALA	Whole fruit	0.20 0.18 0.16 <u>0.21</u> 0.20	2.06
N-EU 2014								
ChR 14 17311 PL03 Lodzkie 99307 Strzelce Poland	Topaz	85	2	101 101	0 DALA 3 DALA 7 DALA 14 DALA 21 DALA	Whole fruit	0.09 <u>0.10</u> 0.08 0.08 0.06	0.99
N-EU 2014								
DMC-13-16134 FR01 Centre 37110 Dame Marie les Bois Northern France	Antares	85	1	98	0 DALA 3 DALA 7 DALA 14 DALA 21 DALA	Whole fruit	<u>0.11</u> 0.09 0.07 0.06 0.06	1.12
N-EU 2014		85	2	97 102	0 DALA 3 DALA 7 DALA 14 DALA 21 DALA	Whole fruit	0.17 0.15 <u>0.18</u> 0.11 0.12	1.86

¹⁾ Interval between applications not given as not relevant for the intended GAP with only single application intended in orchards

²⁾ RUD based on maximum residues and lowest application rate in the trial (maximum residue underlined)

Although the range of the RUD values calculated on the basis of results of the residue trials performed with CA3573 (formally MCW-222), (0.99 to 2.06 mg/kg) is well within the range obtained by Schabacker et al. (2020), values obtained for acetamiprid are in general higher than the proposed refined generic RUD of 0.9.

For this reason, the zRMS in the previous evaluation, preferred to use the RUD calculated specifically for acetamiprid, but the number of trials (only 6 with measurements at 0 DALA) is not sufficient for RUD refinement. Nevertheless, all residue data available from the regulatory studies indicate that mean RUD value of 19.5 mg/kg based on Baril et al. (2005) and indicated in EFSA (2009) is highly overestimated.

Taking all available information into account, the zRMS was of the opinion that the risk refinement based on the maximum RUD of 4.8 mg/kg obtained by Schabacker et al. (2020) will be sufficiently protective, as this is the maximum value obtained in 127 trials performed in orchards and is two times higher than maximum RUD calculated specifically for acetamiprid.

It should be noted that for the current evaluation the Applicant referred to the most recent RUD value evaluation for fruits is provided in EFSA (2021). The document reveals a geometric mean RUD of 0.97 for pome fruits (e.g. apple, pear) which was used in the higher tier risk assessment for the frugivorous mammal.

According to the last harmonization meeting on December 2023 it was agreed that with regard to refinement of the exposure, it was proposed to use the new GD only for parameters that were indicated in EFSA (2009) as provisional or based on the limited data. For other parameters the indications of EFSA (2009) should be followed.

zRMS agrees with the refined risk assessment based on the most recent RUD of 0.97 mg a.s./kg value for fruits provided in EFSA (2021) for pome fruits (e.g. apple).

Higher tier calculation for frugivorous mammals:

Table 9.3-27: Higher-tier assessment of the long-term/reproductive risk for frugivorous mammals due to the use of ADM.00150.I.2.A in pome fruit – refined parameters (*) are further described and justified in the text

Intended use		Apple (BBCH 71-PHI)					
Active substance/product		Acetamiprid					
Application rate (g/ha)		1 × 60 80					
Reprod. Toxicity (mg/kg bw/d)		2.5					
TER criterion		5					
Growth stage	Generic focal species	FIR/bw	RUD _m *	MAF _m × TWA	PT	DDD _m (mg/kg bw/d)	TER _{it}
BBCH 71-79	Frugivorous mammal “dormouse”	1.16	0.97 4.8*	1.0 × 0.53	1	0.05 0.17	52.4 14.7

SV: shortcut value; MAF: multiple application factor; TWA: time-weighted average factor; DDD: daily dietary dose; TER: toxicity to exposure ratio.

*based on Schabacker et al. (2020)

zRMS comments:

zRMS agrees with the refined risk assessment based on the most recent RUD of 0.97 mg a.s./kg value for fruits provided in EFSA (2021) for pome fruits (e.g. apple).

According to the last harmonisation meeting in December 2023 it was agreed that with regard to refinement of the exposure, it was proposed to use the new GD for B&M for only for parameters that were indicated in EFSA (2009) as provisional or based on the limited data.

In addition, the risk assessment based on data Schabacker et al. (2020) has been added by zRMS.

3. Small herbivorous mammal ‘vole’: Common vole (*Microtus arvalis*)

According to EFSA/2009/1438, the common vole represents the worst-case ‘generic focal species’ for the tier 1 risk assessment in maize (corn), orchards (apple), cereals, oilseed rape, sugar beet, and ornamentals/nurseries.

The following refinement options were considered based on current scientific knowledge in line with EFSA/2009/1438:

- Focal species selection
- PD refinement
- Refinement of deposition factors
- Refinement of substance specific residue decline data

By definition, the common vole is as such representative and protective for all other small herbivorous mammals potentially exposed to ADM.00150.I.2.A in these crops.

The common vole is distributed homogeneously in large parts of Europe, from the Atlantic coast of France to Central Russia. It is absent from the British Isles, most of Mediterranean and Fennoscandia. The vole occupies open habitats in which primary and secondary habitats can be distinguished. Primary habitats of common voles are open, dry, uniformly grassy and largely undisturbed areas (such as meadows, set-asides or flower strips) with mixed grassland, herbs and weeds (i.e. a large number of different plant species available as potential food source) that provide appropriate cover to avoid predation (Jacob et al. 2013, KCP 10.1.2/12). However, the species also occurs in sub-optimal habitats and many intensively managed agricultural areas can be considered as secondary habitat.

Proportion of food items in the diet (PD)

In the diet of the common voles inhabiting a meadow in central Germany, dicotyledonous plant species predominated in spring and summer, while in autumn the proportion of monocotyledons increased. The average number of different plant species was 4.3 per stomach (range: 1-9). Comparing the biomass available (roughly 70% monocotyledons and 30% dicotyledons) with the biomass consumed by common voles (roughly 36% monocotyledons and 64% dicotyledons) during the study period it was evident that the common vole has a selective food intake and prefers dicotyledons (Rinke 1991, KCP 10.1.2/13). The proportions of monocotyledons and dicotyledons in the diet of common voles varies by season, with 24-25% monocotyledons and 75-76% dicotyledons in spring and summer, compared to 48% monocotyledons and 52% dicotyledons in autumn.

Table 9.3-28: Diet of common voles (% volume) in a meadow in central Germany (Rinke 1991)

Season	Monocotyledons (% volume)	Dicotyledons (% volume)	No. of voles
Spring	24	76	23
Summer	25	75	152
Autumn	48	52	188

Similar proportions of diet were found by Leutert (1983, KCP 10.1.2/14) in fertilised or unfertilised meadows on 20 study sites located in Northern Switzerland, where voles consumed on average 43% monocotyledons and 57% dicotyledons in spring and summer. In conclusion, voles prefer dicotyledonous over monocotyledonous plants and in a conservative approach and based on the data presented above, a mixed diet of 50% monocotyledons and 50% dicotyledons seems appropriate for a conservative higher tier risk assessment in arable crops.

This is in accordance with the Ctgb Evaluation Manual (2017) where it is stated that based on studies by Rinke (1991) and Lüthi *et al.* (2010, KCP 10.1.2/15), in dicotyledon dominated fields (agricultural crops etc.) a diet consisting of 50% monocotyledons and 50% of dicotyledons should be used in the higher tier risk assessment. In crops with monocotyledonous dominated underground (e.g. cereals and maize/corn) the proportions of the voles diet in the chronic risk assessment should be 75% monocotyledons and 25% dicotyledons.

A diet consisting of 50% monocotyledons and 50% of dicotyledons has been agreed for voles exposed in orchards during the peer-review of acetamiprid (please refer to RAR 19 Volume 3 CP B9, Acetamiprid 20 SC August 2016) and will be used in a higher tier risk assessment for consistency reason.

Food intake rate of the common vole (mixed diet, 50% monocots and 50% dicots) in orchards, oilseed rape, flower bulbs and tubers and floriculture

Assuming a mixed diet of 50% monocotyledons and 50% dicotyledons in orchards, oilseed rape, flower bulbs and tubers and floriculture, the full range of dietary components must be considered in the exposure assessment. Therefore, the food intake rate is not simply achieved by applying the respective fraction as a factor to the respective FIR for a ‘pure’ diet. In accordance with EFSA/2009/1438, the FIR has to be adjusted to reflect the actual contribution of each food item to the daily energy expenditure of the indicator species.

The relationship between body weight (b.w. in g) and daily energy expenditure (DEE in kJ) can be described by the equation: $\log DEE = \log a + b \times \log b.w.$, using the relevant constants for the species group (mammals) from Appendix G of EFSA/2009/1438. The energy expenditure of the common vole of 25 g b.w. results in a DEE of 65.1 kJ/day.

Step 1: Considering the fractions (PD_i) of individual food items in a mixed diet together with data on their respective moisture and energy content, the specific energy content of the mixed diet is calculated. Calculation of food energy of total mixed diet for the common vole is presented in the table below.

Table 9.3-29: Calculation of food energy of total mixed diet for common vole (mixed diet, 50% monocots and 50% dicots) in orchards, oilseed rape, flower bulbs and tubers and floriculture

Parameter	Unit	Grass cereal shoots	Non-grass herbs
Fraction of food item in mixed diet	PD _i fresh (%)	50.0	50.0
Food energy of food item [i] in mixed diet	FE (kJ/dry g)	17.6	17.8
Moisture content of food item [i] in mixed diet	MC (%)	76.4	88.1
Assimilation efficiency of food item [i] in mixed diet	AE (%)	47	76
Food energy of food item in diet	FE _{item,fresh} (kJ/g fr. weight)	0.98	0.81
Food energy of total mixed diet	FE _{total,fresh} (kJ/g fr. weight)	1.78	

Step 2: The food energy of total mixed diet is used to estimate the required amount of the mixed diet to satisfy the energy expenditure of common voles. The calculation of food intake rate (FIR) per body weight regarding the DEE of the common vole is given in the table below.

Table 9.3-30: Calculation of food intake rate per body weight for common voles (mixed diet, 50% monocots and 50% dicots) in orchards, oilseed rape, flower bulbs and tubers and floriculture

Parameter	Unit	Value
Daily energy expenditure	DEE (kJ/day)	65.1
Food energy of total mixed diet	FE _{total,fresh} (kJ/g fresh weight)	1.78
Food intake rate of total mixed diet	FIR _{total, fresh} (g fresh weight/d)	36.55
	FIR/bw (g fresh weight/d)	1.46

Food intake rate of the common vole (mixed diet, 75% monocots and 25% dicots) in maize/corn and cereals

Assuming a mixed diet of 75% monocots and 25% dicots in maize/corn and cereals, the FIR has to be adjusted to reflect the actual contribution of each food item to the daily energy expenditure of the indicator species.

The relationship between body weight (b.w. in g) and daily energy expenditure (DEE in kJ) can be described by the equation: $\log DEE = \log a + b \times \log b.w.$, using the relevant constants for the species group (mammals) from Appendix G of EFSA/2009/1438. The energy expenditure of the common vole of 25 g b.w. results in a DEE of 65.1 kJ/day.

Step 1: Considering the fractions (PD_i) of individual food items in a mixed diet together with data on their respective moisture and energy content, the specific energy content of the mixed diet is calculated. Calculation of food energy of total mixed diet for the common vole is presented in the table below.

Table 9.3-31: Calculation of food energy of total mixed diet for common vole (mixed diet, 75% monocots and 25% dicots) in maize/corn and cereals

Parameter	Unit	Grass cereal shoots	Non-grass herbs
Fraction of food item in mixed diet	PD_i fresh (%)	75.0	25.0
Food energy of food item [i] in mixed diet	FE (kJ/dry g)	17.6	17.8
Moisture content of food item [i] in mixed diet	MC (%)	76.4	88.1
Assimilation efficiency of food item [i] in mixed diet	AE (%)	47	76
Food energy of food item in diet	$FE_{item, fresh}$ (kJ/g fr. weight)	1.46	0.40
Food energy of total mixed diet	$FE_{total, fresh}$ (kJ/g fr. weight)	1.87	

Step 2: The food energy of total mixed diet is used to estimate the required amount of the mixed diet to satisfy the energy expenditure of common voles. The calculation of food intake rate (FIR) per body weight regarding the DEE of the common vole is given in the table below.

Table 9.3-32: Calculation of food intake rate per body weight for common voles (mixed diet, 75% monocots and 25% dicots) in maize/corn and cereals

Parameter	Unit	Value
Daily energy expenditure	DEE (kJ/day)	65.1
Food energy of total mixed diet	$FE_{total, fresh}$ (kJ/g fresh weight)	1.87
Food intake rate of total mixed diet	$FIR_{total, fresh}$ (g fresh weight/d)	34.87
	FIR/bw (g fresh weight/d)	1.39

Higher tier calculation for the common vole in maize/corn

Food intake rate (FIR/bw) of the common vole

The diet in maize/corn consists of 75% monocots and 25% dicots as explained above resulting in a FIR/bw of d a FIR/bw of 1.39.

Residues in the food of the common vole

The RUD values for non-grass herbs and grass will be taken as given in Appendix A of EFSA/2009/1438.

Deposition factor

Deposition factors are taken as given in the Table 9.3-21.

Refined MAF x TWA

A refined MAF x TWA is taken from the Table 9.3-20.

Table 9.3-33: Higher-tier assessment of the long-term/reproductive risk for small herbivorous mammals due to the use of ADM.00150.I.2.A in corn/maize – refined parameters (*) are further described and justified in the text

Intended use	Corn/maize (BBCH 51-75)						
Active substance/product	Acetamiprid						
Application rate (g/ha)	1 × 60						
Reprod. toxicity (mg/kg bw/d)	2.5						
TER criterion	5						
Focal species	Food category, % in diet*	FIR/bw*	$RUD_m \times DF$ (mg/kg food)	$MAF_m \times TWA$	PT	DDD_m (mg/kg bw/d)	TER_{it}
Common vole	Monocot plants, 75 %	1.39	54.2×0.25	0.13	1	0.11	

(Microtus arvalis) Mixed diet BBCH ≥ 40	Dicot plants, 25 %	1.39	28.7 × 0.25	0.24	1	0.04	
	Whole diet					0.15	17.1

FIR/bw: Food intake rate per body weight per percentage diet; RUD: residue unit dose; DF: deposition factor (considering possible interception by the crop); MAF: multiple application factor; DDD: daily dietary dose; TER: toxicity to exposure ratio.

Higher tier calculation for the common vole in orchards

Food intake rate (FIR/bw) of the common vole

The diet in orchards consists of 50% monocots and 50% dicots as explained above resulting in a FIR/bw of d a FIR/bw of 1.46.

Residues in the food of the common vole

The RUD values for non-grass herbs and grass will be taken as given in Appendix A of EFSA/2009/1438.

Deposition factor

Deposition factors are taken as given in the Table 9.3-21.

Refined MAF x TWA

A refined MAF x TWA is taken from the Table 9.3-20.

Table 9.3-34: Higher-tier assessment of the long-term/reproductive risk for small herbivorous mammals due to the use of ADM.00150.I.2.A in orchards – refined parameters (*) are further described and justified in the text

Intended use		Orchards (BBCH 71 – PHI)					
Active substance/product		Acetamiprid					
Application rate (g/ha)		1 × 60 80					
Reprod. toxicity (mg/kg bw/d)		2.5					
TER criterion		5					
Focal species	Food category, % in diet*	FIR/bw*	RUD _m × DF (mg/kg food)	MAF _m × TWA	PT	DDD _m (mg/kg bw/d)	TER _{it}
Common vole (Microtus arvalis) Mixed diet BBCH ≥ 40	Monocot plants, 75 50 %	1.39 1.46	54.2 × 0.4	0.13	1	0.176 0.16	
	Dicot plants, 25 50 %	1.39 1.46	28.7 × 0.4	0.24	1	0.06 0.16	
	Whole diet					0.236 0.33	10.6 7.7

FIR/bw: Food intake rate per body weight per percentage diet; RUD: residue unit dose; DF: deposition factor (considering possible interception by the crop); MAF: multiple application factor; DDD: daily dietary dose; TER: toxicity to exposure ratio.

Higher tier calculation for the common vole in cereals

Food intake rate (FIR/bw) of the common vole

The diet in maize/corn consists of 75% monocots and 25% dicots as explained above resulting in a FIR/bw of d a FIR/bw of 1.39.

Residues in the food of the common vole

The RUD values for non-grass herbs and grass will be taken as given in Appendix A of EFSA/2009/1438.

Deposition factor

Deposition factors are taken as given in the Table 9.3-21.

Refined MAF x TWA

A refined MAF x TWA is taken from the Table 9.3-20.

Table 9.3-35: Higher-tier assessment of the long-term/reproductive risk for small herbivorous mammals due to the use of ADM.00150.I.2.A in cereals – refined parameters (*) are further described and justified in the text

Intended use		Winter cereals covering spring cereals (2 × 35, 10 days interval) (BBCH 40-69)					
Active substance/product		Acetamiprid					
Application rate (g/ha)		2 × 36, 10 days interval					
Reprod. toxicity (mg/kg bw/d)		2.5					
TER criterion		5					
Focal species	Food category, % in diet*	FIR/bw*	RUD_m × DF (mg/kg food)	MAF_m × TWA	PT	DDD_m (mg/kg bw/d)	TER_{It}
Common vole (<i>Microtus arvalis</i>) Mixed diet BBCH ≥ 40	Monocot plants, 75 %	1.39	54.2 × 0.1	0.26	1	0.05	
	Dicot plants, 25 %	1.39	28.7 × 0.1	0.45	1	0.02	
	Whole diet					0.07	36.2

FIR/bw: Food intake rate per body weight per percentage diet; RUD: residue unit dose; DF: deposition factor (considering possible interception by the crop); MAF: multiple application factor; DDD: daily dietary dose; TER: toxicity to exposure ratio.

Higher tier calculation for the common vole oilseed rape

Food intake rate (FIR/bw) of the common vole

The diet in arable crops consists of 50% monocots and 50% dicots as explained above resulting in a FIR/bw of d a FIR/bw of 1.46.

Residues in the food of the common vole

The RUD values for non-grass herbs and grass will be taken as given in Appendix A of EFSA/2009/1438.

Deposition factor

Deposition factors are taken as given in the Table 9.3-21.

Refined MAF x TWA

A refined MAF x TWA is taken from the Table 9.3-20.

Table 9.3-36: Higher-tier assessment of the long-term/reproductive risk for small herbivorous mammals due to the use of ADM.00150.I.2.A in oilseed rape – refined parameters (*) are further described and justified in the text

Intended use		Oilseed rape (BBCH 31-71)					
Active substance/product		Acetamiprid					
Application rate (g/ha)		1-2 × 60, 7 days interval					
Reprod. toxicity (mg/kg bw/d)		2.5					
TER criterion		5					
Focal species	Food category, % in diet*	FIR/bw*	RUD_m × DF (mg/kg food)	MAF_m × TWA	PT	DDD_m (mg/kg bw/d)	TER_{It}
Common vole (<i>Microtus arvalis</i>) Mixed diet BBCH ≥ 40	Monocot plants, 50 %	1.46	54.2 × 0.2	0.26	1	0.12	
	Dicot plants, 50 %	1.46	28.7 × 0.2	0.46	1	0.12	
	Whole diet					0.24	10.5

FIR/bw: Food intake rate per body weight per percentage diet; RUD: residue unit dose; DF: deposition factor (considering possible interception by the crop); MAF: multiple application factor; DDD: daily dietary dose; TER: toxicity to exposure ratio.

Higher tier calculation for the common vole in flower bulbs

Food intake rate (FIR/bw) of the common vole

The diet in arable crops consists of 50% monocots and 50% dicots as explained above resulting in a FIR/bw of d a FIR/bw of 1.46.

Residues in the food of the common vole

The RUD values for non-grass herbs and grass will be taken as given in Appendix A of EFSA/2009/1438.

Deposition factor

Deposition factors are taken as given in the Table 9.3-21. The deposition factor for BBCH 10-89 covers BBCH stages with a lower DF.

Refined MAF x TWA

A refined MAF x TWA is taken from the Table 9.3-20.

Table 9.3-37: Higher-tier assessment of the long-term/reproductive risk for small herbivorous mammals due to the use of ADM.00150.I.2.A in flower bulbs and flower tubers – refined parameters (*) are further described and justified in the text

Intended use		Flower bulbs and flower tubers (BBCH 12-91)					
Active substance/product		Acetamiprid					
Application rate (g/ha)		1 × 46					
Reprod. toxicity (mg/kg bw/d)		2.5					
TER criterion		5					
Focal species	Food category, % in diet*	FIR/bw*	RUD_m × DF (mg/kg food)	MAF_m × TWA	PT	DDD_m (mg/kg bw/d)	TER_{lt}
Common vole (<i>Microtus arvalis</i>) Mixed diet BBCH ≥ 40	Monocot plants, 50 %	1.46	54.2 × 0.6	0.13	1	0.14	
	Dicot plants, 50 %	1.46	28.7 × 0.6	0.24	1	0.14	
	Whole diet					0.28	8.9

FIR/bw: Food intake rate per body weight per percentage diet; RUD: residue unit dose; DF: deposition factor (considering possible interception by the crop); MAF: multiple application factor; DDD: daily dietary dose; TER: toxicity to exposure ratio.

Table 9.3-38: Higher-tier assessment of the long-term/reproductive risk for small herbivorous mammals due to the use of ADM.00150.I.2.A in flower bulbs and flower tubers – refined parameters (*) are further described and justified in the text

Intended use		Flower bulbs and flower tubers (BBCH 12-91)					
Active substance/product		Acetamiprid					
Application rate (g/ha)		2 × 34, 7 days interval					
Reprod. toxicity (mg/kg bw/d)		2.5					
TER criterion		5					
Focal species	Food category, % in diet*	FIR/bw*	RUD_m × DF (mg/kg food)	MAF_m × TWA	PT	DDD_m (mg/kg bw/d)	TER_{lt}
Common vole (<i>Microtus arvalis</i>) Mixed diet BBCH ≥ 40	Monocot plants, 50 %	1.46	54.2 × 0.6	0.26	1	0.21	
	Dicot plants, 50 %	1.46	28.7 × 0.6	0.46	1	0.20	
	Whole diet					0.41	6.2

FIR/bw: Food intake rate per body weight per percentage diet; RUD: residue unit dose; DF: deposition factor (considering possible interception by the crop); MAF: multiple application factor; DDD: daily dietary dose; TER: toxicity to exposure ratio.

Floriculture, tree nursery and perennial nursery crop uses represented by crop group “Ornamentals/nurseries”

In EFSA/2009/1438 the crop group ornamentals/nurseries is described as flowers and plants for transplanting. In EFSA (2021) a note on ornamentals is given, since ornamental plants are a diverse group of plants, grown in a variety of ways, which can vary from small herbaceous plants to large ornamentals trees and the selection of appropriate parameters for environmental risk assessment has often been problematic. For this reason, more detailed risk assessment scenarios have been developed for birds and mammals using ornamentals/nurseries. Following the definitions given in the draft EFSA Guidance for Birds and Mammals (2021), the use in floriculture, tree nursery and perennial nursery crops will be split

in two scenarios, one representing ornamental broad-leaved trees, shrubs, and climbing plants and the other group describes ornamental herbaceous plants with no persistent woody stem above ground.

Food intake rate (FIR/bw) of the common vole

The diet in arable crops consists of 50% monocots and 50% dicots as explained above resulting in a FIR/bw of d a FIR/bw of 1.46.

Residues in the food of the common vole

The RUD values for non-grass herbs and grass will be taken as given in Appendix A of EFSA/2009/1438.

Deposition factor

Deposition factors are taken as given in the Table 9.3-21. The deposition factor for BBCH 10-89 covers BBCH stages with a lower DF.

Refined MAF x TWA

A refined MAF x TWA is taken from the Table 9.3-20.

Higher tier calculation for the common vole in floriculture representing ornamental broad-leaved trees, shrubs, and climbing plants

Table 9.3-39: Higher-tier assessment of the long-term/reproductive risk for small herbivorous mammals due to the use of ADM.00150.I.2.A in floriculture representing ornamental broad-leaved trees, shrubs, and climbing plants – refined parameters (*) are further described and justified in the text

Intended use		Floriculture representing ornamental broad-leaved trees, shrubs, and climbing plants (BBCH 12-91)					
Active substance/product		Acetamiprid					
Application rate (g/ha)		1 × 46					
Reprod. toxicity (mg/kg bw/d)		2.5					
TER criterion		5					
Focal species	Food category, % in diet*	FIR/bw*	RUD _m × DF (mg/kg food)	MAF _m × TWA	PT	DDD _m (mg/kg bw/d)	TER _{it}
Common vole (<i>Microtus arvalis</i>) Mixed diet BBCH 40-49	Monocot plants, 50 %	1.46	54.2 × 0.4	0.13	1	0.09	
	Dicot plants, 50 %	1.46	28.7 × 0.4	0.24	1	0.09	
	whole diet					0.187	13.4
Common vole (<i>Microtus arvalis</i>) Mixed diet BBCH ≥ 50	Monocot plants, 50 %	1.46	54.2 × 0.35	0.13	1	0.08	
	Dicot plants, 50 %	1.46	28.7 × 0.35	0.24	1	0.08	
	Whole diet					0.164	15.3

FIR/bw: Food intake rate per body weight per percentage diet; RUD: residue unit dose; DF: deposition factor (considering possible interception by the crop); MAF: multiple application factor; DDD: daily dietary dose; TER: toxicity to exposure ratio.

Table 9.3-40: Higher-tier assessment of the long-term/reproductive risk for small herbivorous mammals due to the use of ADM.00150.I.2.A in floriculture representing ornamental broad-leaved trees, shrubs, and climbing plants – refined parameters (*) are further described and justified in the text

Intended use		Floriculture representing ornamental broad-leaved trees, shrubs, and climbing plants (BBCH 12-91)					
Active substance/product		Acetamiprid					
Application rate (g/ha)		2 × 34, 7 days interval					
Reprod. toxicity (mg/kg bw/d)		2.5					
TER criterion		5					
Focal species	Food category, % in diet*	FIR/bw*	RUD _m × DF (mg/kg food)	MAF _m × TWA	PT	DDD _m (mg/kg bw/d)	TER _{it}

Common vole (<i>Microtus arvalis</i>) Mixed diet BBCH 40-49	Monocot plants, 50 %	1.46	54.2 × 0.4	0.26	1	0.14	9.2
	Dicot plants, 50 %	1.46	28.7 × 0.4	0.46	1	0.13	
	whole diet					0.271	
Common vole (<i>Microtus arvalis</i>) Mixed diet BBCH ≥ 50	Monocot plants, 50 %	1.46	54.2 × 0.35	0.26	1	0.12	10.5
	Dicot plants, 50 %	1.46	28.7 × 0.35	0.46	1	0.11	
	Whole diet					0.237	

FIR/bw: Food intake rate per body weight per percentage diet; RUD: residue unit dose; DF: deposition factor (considering possible interception by the crop); MAF: multiple application factor; DDD: daily dietary dose; TER: toxicity to exposure ratio.

Higher tier calculation for the common vole in floriculture representing ornamental herbaceous plants

Table 9.3-41: Higher-tier assessment of the long-term/reproductive risk for small herbivorous mammals due to the use of ADM.00150.I.2.A in floriculture representing ornamental herbaceous plants– refined parameters (*) are further described and justified in the text

Intended use		Floriculture representing ornamental herbaceous plants (BBCH 12-91)					
Active substance/product		Acetamiprid					
Application rate (g/ha)		1 × 46					
Reprod. toxicity (mg/kg bw/d)		2.5					
TER criterion		5					
Focal species	Food category, % in diet*	FIR/bw*	RUD _m × DF (mg/kg food)	MAF _m × TWA	PT	DDD _m (mg/kg bw/d)	TER _{it}
Common vole (<i>Microtus arvalis</i>) Mixed diet BBCH 40-49	Monocot plants, 50 %	1.46	54.2 × 0.5	0.13	1	0.12	10.7
	Dicot plants, 50 %	1.46	28.7 × 0.5	0.24	1	0.12	
	whole diet					0.23	
Common vole (<i>Microtus arvalis</i>) Mixed diet BBCH ≥ 50	Monocot plants, 50 %	1.46	54.2 × 0.25	0.13	1	0.06	21.4
	Dicot plants, 50 %	1.46	28.7 × 0.25	0.24	1	0.06	
	Whole diet					0.12	

FIR/bw: Food intake rate per body weight per percentage diet; RUD: residue unit dose; DF: deposition factor (considering possible interception by the crop); MAF: multiple application factor; DDD: daily dietary dose; TER: toxicity to exposure ratio.

Table 9.3-42: Higher-tier assessment of the long-term/reproductive risk for small herbivorous mammals due to the use of ADM.00150.I.2.A in floriculture representing ornamental herbaceous plants – refined parameters (*) are further described and justified in the text

Intended use		Floriculture representing ornamental herbaceous plants (BBCH 12-91) plants (BBCH 12-91)					
Active substance/product		Acetamiprid					
Application rate (g/ha)		2 × 34, 7 days interval					
Reprod. toxicity (mg/kg bw/d)		2.5					
TER criterion		5					
Focal species	Food category, % in diet*	FIR/bw*	RUD _m × DF (mg/kg food)	MAF _m × TWA	PT	DDD _m (mg/kg bw/d)	TER _{it}
Common vole (<i>Microtus arvalis</i>) Mixed diet BBCH 40-49	Monocot plants, 50 %	1.46	54.2 × 0.5	0.26	1	0.17	7.4
	Dicot plants, 50 %	1.46	28.7 × 0.5	0.46	1	0.16	
	whole diet					0.34	
Common vole (<i>Microtus arvalis</i>) Mixed diet BBCH ≥ 50	Monocot plants, 50 %	1.46	54.2 × 0.25	0.26	1	0.09	14.8
	Dicot plants, 50 %	1.46	28.7 × 0.25	0.46	1	0.08	
	Whole diet					0.17	

FIR/bw: Food intake rate per body weight per percentage diet; RUD: residue unit dose; DF: deposition factor (considering possible interception by the crop); MAF: multiple application factor; DDD: daily dietary dose; TER: toxicity to exposure ratio.

zRMS comments:

The risk refinement for species vole performed in Tables 9.3-35 to 9.3-42 has been accepted by the zRMS with consideration of the agreed refined parameters such as PD DF and MAF x twa .
Acceptable long-term risk for vole could be concluded for acetamiprid in ADM.00150.I.2.A.

9.3.2.3 Drinking water exposure

When necessary, the assessment of the risk for mammals due to uptake of contaminated drinking water is conducted for a small granivorous mammal with a body weight of 21.7 g (*Apodemus sylvaticus*) and a drinking water uptake rate of 0.24 L/kg bw/d (cf. Appendix K of EFSA/2009/1438).

Puddle scenario

Due to the characteristics of the exposure scenario in connection with the standard assumptions for water uptake by animals, no specific calculations of exposure and TER are necessary when the ratio of effective application rate (in g/ha) to relevant endpoint (in mg/kg bw/d) does not exceed 50 in the case of less sorptive substances ($K_{oc} < 500$ L/kg) or 3000 in the case of more sorptive substances ($K_{oc} \geq 500$ L/kg).

With a $K(f)_{oc}$ of 106.2, acetamiprid belongs to the group of less sorptive substances. To achieve a concise risk assessment, the risk envelope approach is applied. Here, the assessment for the use in apples with 60 80-g a.s./ha also covers the risk for mammals from all other intended uses (see 9.1.2).

Effective application rate (g/ha)	60 80	Ratio effective application rate to relevant endpoint (trigger: < 50)
Acute toxicity (mg/kg bw)	146	0.41 0.55
Reprod. toxicity (mg/kg bw/d)	2.5	24 32

zRMS comments:

The drinking water risk assessment for acetamiprid presented above is agreed by the zRMS. Evaluation was based on the maximum intended application rate, covering all uses of listed in GAP table.

As ADM.00150.I.2.A is not intended for use in crops with structures able to collect water, only puddle scenario is applicable.

Based on the screening evaluation no unacceptable risk via drinking water is anticipated for all intended zonal uses of ADM.00150.I.2.A.

It is noted that pertinent soil metabolites were not considered in this evaluation. Nevertheless, according to information available in the DRAR (August 2016), all relevant soil metabolites of acetamiprid (IM-1-2, IM-1-4, IC-0 and IM-1-5) are less toxic than the parent compound. Therefore, taking into account lower toxicity and lower exposure to metabolites, the ratios between metabolites rates and endpoints would be lower than these calculated for the parent substance and would not exceed the trigger of 50, applicable also for metabolites (all with $K_{foc} < 500$ mL/g).

Based on that no further drinking water evaluation is deemed necessary for metabolites and the risk is concluded to be acceptable.

9.3.2.4 Effects of secondary poisoning

The log P_{ow} of acetamiprid amounts to 0.8 and thus does not exceed the trigger value of 3. Identified metabolites also have log P_{ow} values below 3. A risk assessment for effects due to secondary poisoning is not required.

zRMS comments:

Log Pow values for acetamiprid and relevant soil and aquatic metabolites (IM-1-2, IM-1-4, IM-1-5, IC-0 and IB-1-1) are all <3, hence the evaluation of the risk of secondary poisoning is not triggered.

9.3.2.5 Biomagnification in terrestrial food chains

Not relevant.

9.3.3 Risk assessment for baits, pellets, granules, prills or treated seed

Not relevant.

9.3.4 Overall conclusions

The acute and long-term risks of ADM.00150.I.2.A (containing 200 g/L acetamiprid) to mammals were assessed from toxicity exposure ratios between toxicity endpoints, estimated from studies with acetamiprid, and maximum residues occurring on food items following applications according to the proposed use pattern.

The acute TER values for all uses, calculated for recommended scenarios, exceed the trigger values of 10 for acute risk indicating an acceptable risk to mammals following use of ADM.00150.I.2.A according to the proposed use pattern.

Considering the long-term risk, several uses showed a potential risk in the tier 1 risk assessment to the small and the large herbivorous mammal and the frugivorous mammal. The long-term risks to small and large herbivorous mammals and frugivorous mammals were addressed in higher tier risk assessments.

After refinement of the deposition factor (DF), refined RUD values for food items based on published literature, refinement of the diet and refinement of DT₅₀ with substance-specific residue decline data for monocotyledonous and dicotyledonous plants, a low risk to mammals could be demonstrated.

The risk of secondary poisoning is not relevant.

The risk from drinking water was assessed demonstrating that the risk to mammals is acceptable.

zRMS comments:

After refinement of the deposition factor (DF), RUD values, refinement of the diet and MAFxTWA parameters with substance-specific residue decline data for monocotyledonous and dicotyledonous plants, a low risk to mammals could be demonstrated.

9.4 Effects on other terrestrial vertebrate wildlife (reptiles and amphibians) (KCP 10.1.3)

According to the revised data requirements under regulation 1107/2009 (Commission Regulations (EU) 283/2013 and 284/2013 for the active ingredient and the plant protection products, respectively), the risk to amphibians and reptiles shall be addressed. Nevertheless, unlike birds and mammals, toxicity tests for amphibian and reptile species are not requested. In the EU, there is no guidance or validated regulatory protocols yet available. Neither on the type of regulatory testing necessary, nor how to conduct a risk assessment for amphibian and reptiles. In the case of acetamiprid, there are no studies in the literature on the toxicity of this active ingredient on amphibians and reptiles.

At the time being, no final conclusion could be drawn regarding to the risk for amphibians (especially terrestrial life stages) and reptiles.

zRMS comments:

As currently there are no agreed rules or criteria for evaluation of the risk to other terrestrial vertebrates like reptiles and amphibians, this issue should be addressed once respective guidance is available and EU agreed endpoints concluded.

9.5 Effects on aquatic organisms (KCP 10.2)

9.5.1 Toxicity data

Studies on the toxicity to aquatic organisms have been carried out with the formulated product ADM.00150.I.2.A as well as the active substance acetamiprid and its relevant metabolites. Full details of these studies are provided in the respective EU DAR and related documents.

The selection of studies and endpoints for the risk assessment is in line with the results of the respective EU review process with minor deviations. Justifications are presented below. New data / information considered in this submission are listed in Appendix 1 and summarized in Appendix 2.

Appropriate risk assessments are provided for the active substance and its metabolites and the formulated product ADM.00150.I.2.A for the proposed use pattern and are considered adequate.

Full references to cited literature are given at the end of this document.

Acetamiprid and its metabolites

In Table 9.5-1 all endpoints relevant for the aquatic risk assessment of Acetamiprid and its metabolites are listed.

Table 9.5-1: Endpoints and effect values relevant for the risk assessment for aquatic organisms - Acetamiprid and its metabolites.

Species	Test item	Exposure system	Results	Reference
<i>Cyprinodon variegatus</i>	Acetamiprid	96 h, f	LC ₅₀ = 100 mg/L nom	EFSA 2016
<i>Lepomis macrochirus</i>	Acetamiprid	96 h, f	LC ₅₀ > 119.3 mg/L mm	EFSA 2016
<i>Oncorhynchus mykiss</i>	Acetamiprid	96 h, s	LC₅₀ > 100 mg/L nom	EFSA 2016
<i>Pimephales promelas</i>	Acetamiprid	35 d, f	EC₁₀ = 9.4 mg/L mm	EFSA 2016
<i>Xenopus laevis</i>	Acetamiprid	21 d, f	NOEC = 2.6 mg/L mm ¹	EFSA 2016
<i>Americamysis bahia</i>	Acetamiprid	48 h, f	EC ₅₀ = 0.066 mg/L mm	EFSA 2016
<i>Chironomus riparius</i>	Acetamiprid	48 h, s	EC ₅₀ = 0.0207 mg/L mm	EFSA 2016
<i>Daphnia magna</i>	Acetamiprid	48 h, s	EC ₅₀ = 49.8 mg/L mm	EFSA 2016
<i>Gammarus fasciatus</i>	Acetamiprid	96 h, s	EC ₅₀ = 0.1 mg/L mm	EFSA 2016
<i>Gammarus pulex</i>	Acetamiprid	48 h, s	EC ₅₀ = 0.05 mg/L mm	EFSA 2016
<i>Simulium latigonium</i>	Acetamiprid	48 h, s	EC ₅₀ = 0.0037 mg/L mm	EFSA 2016
<i>Geomean, Invertebrate acute</i>	Acetamiprid	48 h, s	EC₅₀ = 0.0085 mg/L mm	EFSA 2016
<i>Daphnia magna</i>	Acetamiprid	21 d, ss	EC₁₀ = 2.96 mg/L mm NOEC = 5 mg/L mm	EFSA 2016
<i>Chironomus riparius</i>	Acetamiprid	28 d, s	EC₁₀ = 0.000235 mg/L mm	EFSA 2016

Species	Test item	Exposure system	Results	Reference
<i>Anabaena flos-aquae</i>	Acetamiprid	120 h, s	E_rC₅₀ > 1.3 mg/L mm	EFSA 2016
<i>Scenedesmus subspicatus</i>	Acetamiprid	72 h, s	E _r C ₅₀ > 98.3 mg/L mm E _b C ₅₀ > 98.3 mg/L mm	EFSA 2016
<i>Lemna gibba</i>	Acetamiprid	14 d, s	E _r C ₅₀ > 1 mg/L mm ⁴	EFSA 2016
<i>Chironomus riparius</i>	IM-1-2	48 h, s	EC₅₀ = 15 mg/L mm	EFSA 2016
<i>Daphnia magna</i>	IM-1-2	48 h, ss	EC₅₀ > 99.8 mg/L mm	EFSA 2016
<i>Oncorhynchus mykiss</i>	IM-1-4	96 h, ss	LC₅₀ = 98.1 mg/L mm	EFSA 2016
<i>Americamysis bahia</i>	IM-1-4	48 h, s	EC₅₀ = 19 mg/L mm	EFSA 2016
<i>Chironomus riparius</i>	IM-1-4	48 h, s	EC₅₀ = 76 mg/L mm	EFSA 2016
<i>Daphnia magna</i>	IM-1-4	48 h, ss	EC₅₀ = 43.9 mg/L mm	EFSA 2016
<i>Chironomus riparius</i>	IC-0	48 h, ss	EC₅₀ > 100 mg/L mm	EFSA 2016
<i>Daphnia magna</i>	IC-0	48 h, s	EC₅₀ > 95.1 mg/L mm	EFSA 2016
<i>Chironomus riparius</i>	IM-1-5	48 h, s	EC₅₀ = 68 mg/L mm	EFSA 2016
<i>Daphnia magna</i>	IM-1-5	48 h, s	EC₅₀ = 25 mg/L mm	EFSA 2016
<i>Daphnia magna</i>	IM-1-5	21 d, ss	NOEC = 26 mg/L mm	EFSA 2016
<i>Chironomus riparius</i>	IB-1-1	48 h, s	EC₅₀ > 100 mg/L mm	EFSA 2016
<i>Daphnia magna</i>	IB-1-1	48 h, ss	EC₅₀ > 100.8 mg/L mm	EFSA 2016
<i>Mesocosm</i> (Adama)	Acetamiprid	86 d, pe	NOEC = 0.00112 mg/L mm²	New study; Hommen, U., 2022; 000106190; ADM-025/7-52
<i>Mesocosm</i> (Nisso)	Acetamiprid	82 d, s	NOEC = 0.0011 mg/L mm³	EFSA 2016
Higher-tier studies (micro- or mesocosm studies)⁵				
<p>Outdoor mesocosm study: Effect assessment on macroinvertebrates, zooplankton, phytoplankton, periphyton and macrophytes in outdoor mesocosms. Test substance: Acetamiprid 20 SG (Mospilan 20 SG). 2 applications with a 14 day interval. Study duration: 82 days. Treatment rates: 0.5, 1.1, 2.6 and 6.0 µg a.s./L.</p> <p>Endpoints: NOEC and NOEAEC <0.5 µg/L based on class 5B effects on Naididae at 0.5-6.0 µg/L. Considering however the uncertainty associated with the findings for Naididae (not expected to be more sensitive than insects based on mode of action; relatively low numbers in control, although MDD was low) the reported conclusion by the study author NOEC based on class 2 effects to derive the ETO-RAC 1.1 µg/L; NOEAEC to derive ERO-RAC 1.1 µg/L based on class 5B effects on <i>Cloeon dipterum</i> at 2.6 µg/L) could be acceptable in case the findings for Naididae in the present study are negated by prolonged toxicity laboratory studies (e.g. at least 28 days duration) with representative taxa of Naididae.</p>				
Higher-tier studies (micro- or mesocosm studies)⁶				
<p>86-day outdoor mesocosm study (Hommen, U.; 2022; KCP 10.2.3/01): Effect assessment of acetamiprid on macroinvertebrates, zooplankton, phytoplankton, periphyton and macrophytes in outdoor mesocosms with a focus on the effects on aquatic insects, benthic macroinvertebrates and zooplankton. Test substance: Acetamiprid 200 SL (Code: ADM.00150.1.2.A); 2 applications with a 7-day interval; three replicates per treatment, 5 replicates for the control; study duration: 86 days; nominal treatment rates: 0.30, 0.51, 0.87, 1.5, 2.5 µg a.s./L corresponding to max. measured concentrations (day 7) of 0.55, 0.88, 1.4, 2.5, 4.5 µg a.s./L.</p> <p>The mesocosm study provides reliable (MDD Category 1) data for for 22 discreet taxa (i.e. those not appearing more than once in different sampling apparatus and those for which sum values and higher taxonomic levels are excluded). Of these, 6 originate from the most sensitive group of insects and while excluding community responses 9 provided reliable endpoints with NOECs below the highest test concentration.</p> <p>The maximum measured concentration of 1.4 µg a.s./L (geometric mean of two applications = 1.12 µg a.s./L, 0.87 µg a.s./L nominal) is the overall Class 2 concentration used to derive an ETO-RAC since no pronounced effects on potentially sensitive taxa were found and the uncertainty related to this concentration is considered small.</p>				

f: flow-through; s: static; ss: semi-static; pe: pulse exposure, nom: nominal; mm: mean measured

1: No data requirement; growth based endpoint is presented as additional data and not relied upon

2: Tested as ADM.00150.1.2.A containing 200.1 g a.s./L, density = 1.1374 g/ml and w/w = 17.59

3: Tested as Acetamiprid 20 SG

4: Based on fronds number

5: EFSA, 2016: Peer review of the pesticide risk assessment of the active substance acetamiprid, EFSA Journal 2016;14(11):4610

6: New study, Hommen, U.; 2022; KCP 10.2.3/01

ADM.00150.I.2.A

In Table 9.5-2 all endpoints relevant for the acetamiprid formulation ADM.00150.I.2.A are listed.

Table 9.5-2: Endpoints and effect values relevant for the risk assessment for aquatic organisms - ADM.00150.I.2.A.

Species	Test item	Exposure system	Results	Reference
<i>Aeshna</i> sp.	ADM.00150.I.2.A	48 h, s	EC ₅₀ > 69.36 mg/L nom ¹	New study; Taylor, S. & Joyce, F., D., 2015; R-35057; KCP 10.2.3/02
<i>Asellus aquaticus</i>	ADM.00150.I.2.A	48 h, s	EC ₅₀ = 1.28 mg/L nom ¹	
<i>Chaoborus crystallinus</i>	ADM.00150.I.2.A	48 h, s	EC ₅₀ = 0.47 mg/L nom ¹	
<i>Cloeon dipterum</i>	ADM.00150.I.2.A	48 h, s	EC ₅₀ = 64.81 mg/L nom ¹	
<i>Corixinae</i>	ADM.00150.I.2.A	48 h, s	EC ₅₀ = 0.99 mg/L nom ¹	
<i>Crangonyx pseudogracilis</i>	ADM.00150.I.2.A	48 h, s	EC ₅₀ = 3.74 mg/L nom ¹	
<i>Gammarus pulex</i>	ADM.00150.I.2.A	48 h, s	EC ₅₀ = 43.89 mg/L nom ¹	
<i>Ischnura elegans</i>	ADM.00150.I.2.A	48 h, s	EC ₅₀ = 0.54 mg/L nom ¹	
<i>Notonecta marmorea viridis</i>	ADM.00150.I.2.A	48 h, s	EC ₅₀ = 42.64 mg/L nom ¹	
<i>Phryganea bipunctata</i>	ADM.00150.I.2.A	48 h, s	EC ₅₀ = 0.48 mg/L nom ¹	
SSD, invertebrates acute	ADM.00150.I.2.A	48 h, s	HC ₅ = 5.55 µg a.s./L RAC = 1.11 µg a.s./L ⁴	New study; Koerner, O., 2015 R-36040 KCP 10.2.3/03
<i>Oncorhynchus mykiss</i>	ADM.00150.I.2.A	96 h, s	LC ₅₀ = 85.8 mg/L nom ¹	New study; R-33831 KCP 10.2.1/01
<i>Daphnia magna</i>	ADM.00150.I.2.A	48 h, s	EC ₅₀ = 100.2 mg/L nom ¹	New study; Juckeland, D., 2014b R-33832 KCP 10.2.1/02
<i>Chironomus riparius</i>	ADM.00150.I.2.A	48 h, s	EC ₅₀ = 0.0521 mg/L nom ¹	New study; Juckeland, D., 2015a 34873 KCP 10.2.1/03
<i>Desmodesmus subspicatus</i>	ADM.00150.I.2.A	72 h, s	EC ₅₀ = 3111 mg/L nom ¹	New study; Juckeland, D., 2014c; R-33833; KCP 10.2.1/04
Mesocosm (ETO) mean measured	ADM.00150.I.2.A	86 d, pe	NOEC = 0.006367 mg t.i./L mm ³ corresponding to NOEC = 1.12 µg a.s./L mm	New study; Hommen, U., 2022; 000106190; ADM-025/7-52 KCP 10.2.3/01

Higher-tier studies (micro- or mesocosm studies)

86-day outdoor mesocosm study (Hommen, U.; 2022; KCP 10.2.3/01): Effect assessment of acetamiprid on macroinvertebrates, zooplankton, phytoplankton, periphyton and macrophytes in outdoor mesocosms with a focus on the effects on aquatic insects, benthic macroinvertebrates and zooplankton. Test substance: Acetamiprid 200 SL (Code: ADM.00150.I.2.A); 2 applications with a 7-day interval; three replicates per treatment, 5 replicates for the control; study duration: 86 days; nominal treatment rates: 0.30, 0.51, 0.87, 1.5, 2.5 µg a.s./L corresponding to max. measured concentrations (day 7) of 0.55, 0.88, 1.4, 2.5, 4.5 µg a.s./L.

The mesocosm study provides reliable (MDD Category 1) data for 22 discreet taxa (i.e. those not appearing more than once in different sampling apparatus and those for which sum values and higher taxonomic levels are excluded). Of these, 6 originate from the most sensitive group of insects and while excluding community responses 9 provided reliable endpoints with NOECs below the highest test concentration.

The maximum measured concentration of 1.4 µg a.s./L (geometric mean of two applications = 1.12 µg a.s./L, 0.87 µg a.s./L nominal) is the overall Class 2 concentration used to derive an ETO-RAC since no pronounced effects on potentially sensitive taxa were found and the uncertainty related to this concentration is considered small.

Species	Test item	Exposure system	Results	Reference
The result's reliability is further supported by considering findings of a previous mesocosm study with acetamiprid (EFSA 2016) and the multi-species study conducted by Taylor & Joyce, 2015 KCP 10.2.3/02.				

s: static; pe: pulse exposure, nom: nominal; mm: mean measured; t.i.: test item

1: Tested as MCW-2222 equivalent to ADM.00150.I.2.A

2: Tested as MCW-2222 equivalent to ADM.00150.I.2.A. Shown for completeness since study is of lower tier and higher RAC as the mesocosm study

3: Tested as ADM.00150.I.2.A containing 200.1 g a.s./L, density = 1.1374 g/ml and w/w = 17.59

4: RAC Tier 2B (most sensitive species), based on AF = 5.

zRMS comments:

Studies on toxicity of the formulated product to fish, *Daphnia magna*, *Chironomus riparius* and algae were already evaluated in the course of the first zonal authorisation of MCW-2222 (equivalent to ADM.00150.I.2.A) in April 2018 and considered acceptable. The guidelines against which the studies were validated have not changed since that time, so re-evaluation of the studies was not necessary. Provided endpoints are confirmed to be correct.

It is also noted that in the course of the first zonal assessment also study on acute toxicity of the formulation to 10 additional aquatic invertebrate species was submitted and accepted by the zRMS-PL, although not used in the risk assessment. As results of this study were provided by the Applicant in Table 9.5-2 above, they are presented in table below for consistency, together with results obtained for standard species (Taylor, S. & Joyce, F., D., 2015) to calculate the geometric mean endpoints.

Summary of the study has been copied from the Core Assessment, Part B, Section 6 of April 2018 and presented in Appendix 2.

Toxicity of MCW-2222 to additional aquatic invertebrate species

Organism ¹⁾	Test substance	Endpoint	Value ¹⁾	Reference			
<i>Daphnia magna</i>	MCW-2222	48h EC ₅₀	22800 µg a.s./L	See Table 9.5-2			
<i>Chironomus riparius</i>	MCW-2222	48h EC ₅₀	9.29 µg a.s./L	See Table 9.5-2			
<i>Aeshna sp.</i>	MCW-2222	48h EC ₅₀	>2130 µg a.s./L	New study; Taylor, S. & Joyce, F., D., 2015; R-35057; KCP 10.2.3/02			
<i>Asellus aquaticus</i>			39.4 µg a.s./L				
<i>Chaoborus crystallinus</i>			1998 µg a.s./L				
<i>Cloeon dipterum</i>			14.4 µg a.s./L				
<i>Ischnura elegans</i>			16.6 µg a.s./L				
<i>Corixidae</i>			30.7 µg a.s./L				
<i>Crangonyx pseudogracilis</i>			115 µg a.s./L				
<i>Gammarus pulex</i>			1351 µg a.s./L				
<i>Phryganea bipunctata</i>			14.8 µg a.s./L				
<i>Notonecta marmorea viridis</i>			1314 µg a.s./L				
			Geometric mean EC ₅₀ (all species)		174.3 µg a.s./L		
			Geometric mean EC ₅₀ (all insect species)		93.1 µg a.s./L		
		Geometric mean EC ₅₀ (insect species, less sensitive species excluded)	15.9 µg a.s./L				

¹⁾ Insets and insect endpoints are highlighted in **bold**

In the previous evaluation for the MCW-2222 by zRMS it was agreed that the geometric mean EC₅₀ for all invertebrates listed in table above would be 0.174 mg a.s./L, while the geometric mean for aquatic insects was calculated to be 0.093 mg a.s./L when all insects are taken into account. When species clearly less sensitive comparing to other insects are excluded (*Aeshna sp.*, *C. dipterum* and *N. marmorea viridis*), the geometric mean EC₅₀ of 0.0159 is calculated. All these geometric mean values are higher comparing to EU agreed geometric mean EC₅₀ for insects of 0.0085 mg a.s./L, based on studies performed with the active compound. All this indicates that formulation is not more acutely toxic to aquatic insects comparing to active substance and EU agreed geometric mean EC₅₀ of 0.0085 mg a.s./L is relevant for the risk assessment purposes as representing worst case.

The summary of Applicant's approach is presented below:

Geometric mean - Applicant's approach (Tier 2A)

To obtain a geometric mean for the taxonomic groups *Insecta* as well as *Crustacea*, the available data were compiled and the respective geometric means were calculated. In total, data on 8 insect species and 4 crustacean species were available.

Applying an AF of 100 to the geometric means of 149 mg a.s./L for insects and 225 µg a.s./L for crustaceans, the acute Tier 2A RAC values are 1.49 µg a.s./L and 2.25 µg a.s./L, respectively (see table below).

According to the EFSA Aquatic GD (2013), the outcome of the geometric mean approach was tested for a biased data set, as the sensitivity of species differed in minimum of 2 orders of magnitude. The calculated difference between the lowest endpoint of insects (*Chironomus riparius*; EC₅₀ = 9.3 µg a.s./L) and Crustacea (*Crangonyx pseudogracilis*; EC₅₀ = 30.7 µg a.s./L) were less than a factor of 100 below the respective geometric means (16 and 7.3, respectively). Therefore, both geometric mean values are considered reliable and can be used in the risk assessment.

Group	Species	48h EC ₅₀ (µg a.s./L)	Geometric mean (µg a.s./L)	AF ^A	Tier 2A RAC (µg a.s./L)	Ratio ^B
Insecta	<i>Chironomus riparius</i>	9.3	149	100	1.49	16 (=149/9.3)
	<i>Cloeon dipterum</i>	14.4				
	<i>Corixinae</i>	16.6				
	<i>Phryganeae bipunctata</i>	14.8				
	<i>Notonecta marmoreal</i>	1314				
	<i>Ischnura elegans</i>	1351				
	<i>Chaoborus crystallinus</i>	1998				
	<i>Aeschna sp.</i>	> 2130				
Crustacea	<i>Crangonyx pseudogracilis</i>	30.7	225	100	2.25	7.3 (=225/30.7)
	<i>Asellus aquaticus</i>	39.4				
	<i>Gammarus pulex</i>	115				
	<i>Daphnia magna</i>	17 900				

^AAccording to the EFSA Aquatic GD (2013)

^B geomean divided by lowest endpoint

Based on the Applicants' calculations presented above all these geometric mean values (including all insects) are higher comparing to EU agreed geometric mean EC₅₀ for insects of 0.0085 mg a.s./L.

Species Sensitivity Distribution (SSD) approach (Tier 2B)

First SSD approach:

As *Chironomus riparius* is about four orders of magnitude more sensitive than *Daphnia magna*, a Species Sensitivity Distribution (SSD) based on the available insect data was constructed in a first step. For hawkers (*Aeschna sp.*), where no clear EC₅₀ value could be obtained in the study by Taylor and Joyce (2015), the EC₅₀ value >2130 µg a.s./L was included. Since hawkers are less sensitive compared to the 7 remaining species, it is considered appropriate according to EFSA Aquatic GD (2013) to include this data point in the construction of an insect SSD. The toxicity data did not fit the curve very well and the Anderson-Darling goodness of fit test was not accepted at the 5% level (p = 0.05). Hence, the data set was considered as not suitable for an SSD.

Second SSD approach:

To increase the number of sensitive arthropod species, the EC₅₀ values of *Crangonyx pseudogracilis*, *Gammarus pulex* and *Asellus aquaticus* were included in the construction of the arthropod SSD.

Since *Daphnia magna* appeared to be extremely insensitive to Acetamiprid compared to the other crustaceans, this species was excluded from further consideration. Still, the toxicity data did not fit the curve very well and the Anderson-Darling goodness-of-fit test was not accepted at the 5% level (p = 0.05). Hence, also this data set was not considered suitable for an SSD.

Final SSD approach:

As the relatively high EC₅₀ values for *Notonecta marmoreal*, *Ischnura elegans*, *Chaoborus crystallinus* and *Aeschna sp.* (EC₅₀ values of 1314, 1351, 1998 and >2130 µg a.s./L, respectively) were considered by the Applicant to be the

reason for non-normal distribution of the data set, these species were excluded from further analysis. Therefore, in total 7 different species (representing the most sensitive species) were available to calculate a species sensitivity distribution. The available arthropod data were analyzed according to the recommendations provided in the EFSA Aquatic GD (2013). Details of evaluation can be found in Appendix 2.

The estimated Applicant's RAC values based on laboratory studies are as follows:

RAC Tier 1: 0.093 µg a.s./L (based on an AF = 100)

RAC Tier 2A (geomean Insecta): 1.49 µg a.s./L (based on an AF = 100)

RAC Tier 2A (geomean Crustacea): 2.25 µg a.s./L (based on an AF = 100)

RAC Tier 2B (SSD of most sensitive species): 1.11 µg a.s./L (based on an AF = 5) based on data for the most sensitive species: 4 insects and 3 crustacea species.

Higher tier studies -Mesocosm study

It should be noted that submitted new mesocosm study by Hommen, U.; 2022; KCP 10.2.3/01 has been evaluated by the zRMS and considered reliable and acceptable with NOEC of 1.12 µg a.s./L based on effects class 2.

The study was performed in line with current requirements regarding this type of studies. The most sensitive taxa identified at the EU level during acetamiprid renewal were included in the study.

The study design with 2 applications of the test item dosed via separating funnels.

Obtained results were in good agreement with results of the mesocosm study by Hommen (2015) evaluated in the course of the renewal process. However, in the new study higher abundance of Naididae family was achieved enabling more robust statistical analysis of observed effects comparing to the EU agreed study.

Taking into account the good quality of the study, MDD values relevant to detect medium and small effects for sufficient number of taxa and populations (including those identified as sensitive), sufficiently reliable results for Naididae family and the fact that results of this study are in good agreement with results of the EU agreed study, the zRMS is of the opinion that AF of 2 is relevant for ETO-RAC derivation.

For detailed study summary and its evaluation by the zRMS, please refer to Appendix 2.

9.5.1.1 Justification for new endpoints

New studies are available for the formulated product ADM.00150.I.2.A which are required to fulfil the data requirements for plant protection products in accordance with Regulation (EC) 284/2013. These studies are summarized in Appendix 2 and the related endpoints are pointed out in Table 9.5-2.

- Fish acute toxicity: Based on a.s. content, the LC₅₀ from the study with ADM.00150.I.2.A is lower when compared with the study with the technical active substance.: 96-h LC₅₀ = 15.3 mg a.s./L.
- ~~Algae, 72h: Based on a.s. content, the E_rC₅₀ from the study with ADM.00150.I.2.A is lower than from the study with the technical active substance. 72 h E_rC₅₀ = 554.5 mg a.s./L.~~
- Mesocosm study: Although a NOEC using ecological threshold option (ETO) could be derived from the first mesocosm study (see Table 9.5-1 and EFSA, 2016) some shortcomings were identified and a new mesocosm study was performed. It was conducted in a similar way, but especially addressed several critical points of the study conducted on the active substance level. The main difference that for the new mesocosm study were a lowering of the application interval from 14 to 7 days, consideration of a fifth test concentration and additional procedures to monitor detrimental effects on Naididae.

Since acetamiprid dissipates slowly from the water phase, the exposure regime resulted in a mean measured concentration of 174% of nominal concentrations after the second dosing. Please see also the exposure overlay profiles presented under Appendix 3. Nonetheless, in terms of the most sensitive species (*Cloeon dipterum*), the results of the first study were confirmed. Naididae were also found to be sensitive, but not more sensitive than the other affected taxa. While 22 taxa were found to yield reliable endpoints, at least 9 taxa provided reliable endpoints from MDD category 1 data where the NOEC was below the maximum tested concentration. The study is robust and valid and due to the exposure regime applied, the geometric mean concentration between nominal and mean measured concentrations at t = 7 days is most suitable to derive the actual test concentrations.

This results in a mean measured concentration of 1.12 µg a.s./L (6.4 µg test item/L) used to derive an ETO-RAC. This is considered a conservative approach as it could be justified that the overall peak measured concentration (1.44 µg a.s./L) at the NOEC should be used to derive the ETO-RAC. Considering the conservatism of using the geometric mean measured concentration in addition to the high comparability of recent results with findings of the mesocosm study conducted with acetamiprid during the active substance renewal process, an assessment factor of 2 is justified according to EFSA (2013).

An **ETO-RAC of 0.56 µg a.s./L (3.18 µg test item/L)** is used in the risk assessment. For further information on ecological relevance and RAC derivation see the expert statement below.

- Multi-species study on invertebrates: Since invertebrates and especially insects are considered the most sensitive group, a further multi-species study with acetamiprid is summarized to further support the conclusions from the two mesocosm studies.

The mode of action and all available ecotoxicological data clearly indicate that invertebrates and particularly insects are by far the most sensitive group for detrimental effects caused by acetamiprid. Since ADM.00150.I.2.A is a solo-formulation and due to the higher-tier mesocosm study being considered in the active substance risk assessment, no separate risk assessment for the formulated product is conducted.

9.5.1.2 Mesocosm study with ADM.00150.I.2.A - Ecological Relevance and RAC derivation

Introduction:

Following the aquatic guidance document (AGD; EFSA 2013), a refinement option for the risk to aquatic organisms is conducting a higher-tier mesocosm study including realistic to worst-case exposure conditions such as pulsed (peak) exposure. An 86-day outdoor mesocosm study was performed to investigate potential effects of two applications of the insecticidal active substance acetamiprid on freshwater ecosystems in outdoor mesocosms. The main focus of the study was to investigate the direct effects of acetamiprid on aquatic insects, benthic macroinvertebrates and zooplankton. Nevertheless, algae and plants were also monitored to detect indirect effects.

The maximum measured concentration of 1.44 µg a.s./L (geometric mean of two applications 1.12 µg a.s./L, nominally 0.87 µg a.s./L) is the overall Class 2 concentration which can be used to derive an ETO-RAC.

The study is considered to be robust and valid for use in risk assessment. Due to the exposure regime applied, the geometric mean concentration between nominal and mean measured concentrations at t = 7 days is most suitable to derive the actual test concentrations. This results in a mean measured concentration of 1.12 µg a.s./L (6.4 µg test item/L) used to derive an ETO-RAC. This is considered a conservative approach as it could be justified that the overall peak measured concentration (1.44 µg a.s./L) at the NOEC should be used. Considering the conservatism of using the geometric mean measured concentration in addition to the high comparability of recent results with findings of the mesocosm study conducted with acetamiprid during the active substance renewal process, an assessment factor of 2 is justified according to EFSA (2013). This results in a **final ETO-RAC of 0.56 µg a.s./L.**

Sensitive Taxa:

The present mesocosm study provides reliable data (MDD Category 1) for 22 discrete taxa. Within this number, those taxa appearing more than once in different sampling apparatus as well as taxa for which sum values and higher taxonomic levels are available were excluded; where multiple data were available for a specific taxon, the lower endpoint was taken as a discrete value. Six of these 22 taxa are insects, including the mayfly *Cloeon dipterum*. Insects in general, and mayflies in particular, are known to be very sensitive to neonicotinoids.

When biologically different life stages of insects, e.g. larval and adult stages of the same taxon, are taken into account as discrete taxa, reliable data can be determined for 27 taxa (MDD category 1). If community responses and functional parameters (such as chlorophyll a, community metabolism, etc.) are considered,

the dataset comprises 34 reliable endpoints. Moreover, the organisms present in the mesocosm for which MDD category 1 data were determined include many different taxonomic groups as well as different life stages:

1. Insecta (larvae, pupae and adults)
2. Isopoda (juveniles and adults)
3. Clitellata (juveniles and adults)
4. Gastropoda (juveniles and adults)
5. Malacostraca (juveniles and adults)
6. Cladocera (juveniles and adults)
7. Copepoda (juveniles and adults)
8. Rotifera (cysts, juveniles and adults)
9. Mollusca (juveniles and adults)

Consequently, the reliable dataset obtained from the model ecosystems is clearly representative of communities associated with natural water bodies.

The AGD (EFSA, 2013) states: “besides representatives of different trophic levels, at least 8 different populations of the sensitive taxonomic group need to be present in the micro-/mesocosm test systems and for which a concentration–response relationship can be derived”.

In the present mesocosm study, 9 of the 34 potentially sensitive taxa fulfilled the MDD category 1 criterion and had NOEC values below the maximum tested concentration, demonstrating the sensitivity to acetamiprid exposure. In addition, NOEC values for community responses of macroinvertebrates and emerging insects were determined below the maximum tested concentration.

For the risk assessment, a total of 11 NOEC values were found to be relevant, of which 10 were assigned to Effect Class 1 (see table below). This exceeds the suggestion by Brock et al. (2015), demonstrating that a robust endpoint can be derived from the present study and used in the subsequent risk assessment.

Table 9.5-3: Sensitive taxa with determined NOEC values, Effect class categories and respective MDD categories from a mesocosm study by Hommen (2022)

Taxa name	Taxonomic class	Sampling method	MDD Cat	Life stage	NOEC (nom)	Effect class
<i>Cloeon dipterum</i>	Insecta	Macroinvertebrates	1	Larval	1.5	2
<i>Asellus aquaticus</i>	Isopoda	Macroinvertebrates	1	All	1.5	1
Naididae	Clitellata	Macroinvertebrates	1	All	0.87	1
<i>Cloeon dipterum</i>	Insecta	Emergence	1	Adult	0.87	1
Chironomidae	Insecta	Emergence	1	Adult	1.5	1
Coenagrionidae	Insecta	Emergence	1	Adult	1.5	1
Chydorus	Cladocera	Zooplankton	1	All	0.87	1
Cyclopidae	Copepoda	Zooplankton	1	Adult/Nauplia	1.5	1
Ostracoda	Mollusca	Zooplankton	1	All	1.5	1
Community	N/A	Macroinvertebrates	N/A	N/A	0.87	1
Community	N/A	Emergence	N/A	N/A	0.87	1

In a conservative approach, community responses are excluded. Consequently, 9 taxa are considered sensitive and relevant for RAC derivation.

Within the 9 taxa considered, *Cloeon dipterum* (Ephemeroptera) is an overlapping taxon, as aquatic and terrestrial life stages occur. Although unmistakably linked, effects on larval stages and emerging insects need to be considered as two discrete datapoints. Not only habitus and habitat differ between the two life stages, but also foraging strategies and biochemical processes undergo significant changes during

metamorphosis. These changes indicate that there are also differences in exposure to, and uptake of chemicals as well as in the magnitude of potential effects of chemicals between different life stages of holometabolous insects.

Examples emphasising survival of larvae and successful emergence of adults as separate endpoints are the need of two test guidelines for the pollinator *Apis mellifera*: OECD TG 245 (2017) for the testing of potential effects on adult chronic survival as well as OECD Guidance Document 245 (2016) for detecting possible effects on developmental stages.

In an ideal world, even more than the present 9 sensitive taxa would be present in such a dataset, but it has to be considered that some taxa (at least partly) will be dominating such well-established test systems and this reflects realistic conditions of natural edge-to-field surface waters and according to EFSA Journal (2013) should not be a reason to reject the study, especially since key and sensitive species are present in the study. Since summer generations that are known to be more sensitive than winter generations (Van den Brink, P.; 2016) the whole dataset can be considered a realistic worst-case with regards to the application timing.

Consistency of results with previous studies and assessment factor derivation:

Results of the present mesocosm study (Hommen, 2022) should be reviewed in context of already evaluated data on acetamiprid such as SSD data on aquatic invertebrates (including 7 insect species, as tested in the acute toxicity study by Taylor & Joyce, 2015) with a HC₅ RAC of 1.11 µg a.s./L (Koerner, 2015), and more specific, the previously EU agreed mesocosm (see EFSA, 2016). At the time of evaluation of the active ingredient, an assessment factor of 3 was proposed due to several shortcomings in the test design, resulting in a RAC value of 0.367 µg a.s./L. These shortcomings, i.e. application interval, number of concentrations tested, uncertain effects on Naididae, were successfully addressed in the present mesocosm study. Hence, the application of an AF of 2 is fully justified.

Sediment dwellers:

The requirement for a higher tier risk refinement via the present mesocosm study is based on a potential unacceptable risk to sediment dwellers. During evaluation, endpoints from a chronic *Chironomus riparius* study (Stäbler, 1999) conducted according to OECD TG 219 were recalculated based on measured test concentrations. As a result, an unreasonable low endpoint of 0.235 µg a.s./L was suggested by RMS NL during active ingredient renewal. Noteworthy, the original study did not include analytical verification of acetamiprid in the sediment and the used DT₅₀ value for re-calculation was a worst-case lab value of 97.6 h.

Based on available information (EFSA 2016), also some sediment-dwelling Oligochaetes (i.e. Naididae) might be sensitive to acetamiprid. To account for the potentially high sensitivity of this group, sediment dwellers were present and closely monitored in the present study. Additionally, the acetamiprid content in all enclosures was constantly measured under realistic worst-case conditions.

For Chironomidae, Class 1 effects were found at 2.5 µg a.s./L with a maximum sediment concentration of 2.3 µg a.s./kg dw. This is about an order of magnitude higher than the endpoint recalculated from the *C. riparius* study by Stäbler (EC₁₀ = 0.235 µg a.s./L). Adequately designed and conducted mesocosm studies represent a realistic scenario of the behaviour and potential effects of an active substance in edge-of-field water bodies. Acetamiprid was found to dissipate slowly from the water phase, resulting in a mean measured concentration of 174% of nominal concentrations after the second dosing. Consequently, the DT₅₀ value in this mesocosm study was 19 days, and thus significantly higher than the one derived by RMS NL based on the laboratory study on *C. riparius* (DT₅₀ = 97.6 h in Stäbler, 1999).

Integrated geomean concentration as realistic worst-case RAC and exposure overlay analysis:

Considering the short application interval of 7 days and a DT₅₀ of 19 days, the use of nominal test concentrations would result in an unrealistic worst-case scenario, leading to an overly conservative endpoint.

Due to the applied exposure regime, the geometric mean concentration between nominal and mean measured concentrations at t = 7 days is used to derive the actual test concentrations. This results in a mean

measured concentration of 1.12 µg a.s./L as the overall Class 2 concentration which is used to derive an ETO-RAC. Taking the high comparability of recent results with findings of the mesocosm study conducted with acetamiprid during the active ingredient renewal process justifies an assessment factor of 2 according to EFSA (2013). This is considered more conservative than using the overall peak measured concentration.

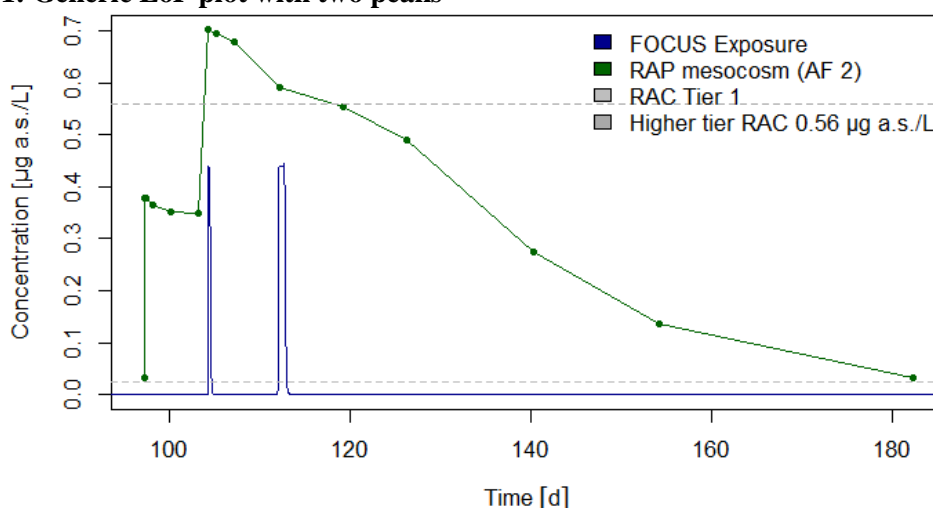
A RAC of 0.56 µg a.s./L (3.18 µg test item/L) is used in the risk assessment.

In order to demonstrate whether the exposure from the present mesocosm study covers the predicted exposure profiles (i.e. relevant FOCUS scenarios), a comparison analysis of the exposure profile in the higher-tier study to the predicted exposure profiles for acetamiprid was conducted. Graphical analysis of relevant FOCUS scenarios with tools such as the Exposure Pattern Analysis Tool (EPAT) are considered helpful and often requested by authorities. Based on the method of the EPAT analysis, an Exposure overlay Profile (EoP) analysis has been conducted and is presented in this submission (see Appendix 3).

EOP is a visual comparison of PEC_{SW} values for each relevant scenario and exposure tested in the mesocosm study. This allows the illustration and direct link of the modelled exposure of any relevant scenario (including mitigations) to the NOEC concentration. Additionally, the Regulatory Acceptable Profile (RAP) is included in the graphs, including an AF of 2 (green line). This RAP was derived from actual measured concentrations in the mesocosm study.

All presented graphs include the PEC_{SW} timeseries derived from the FOCUS models in blue, the tier 1 RAC (0.0235 µg a.s./L; *C. riparius*; EFSA, 2016) in light gray and the higher-tier RAC (0.56 µg a.s./L, mesocosm study by Hommen, 2022) in dark gray, as presented in the generic EoP plot below (Figure 9.5-1)

Figure 9.5-1: Generic EoP plot with two peaks



Usually, one graph per FOCUS scenario is shown presenting an overlay of the exposure in the mesocosm and the peak(s) calculated for surface water PECs in the respective FOCUS scenario. For this purpose, usually the maximum calculated peak was aligned with the peak of the mesocosm exposure curve. All relevant peaks from a focus profile were covered by the RAP. Multiple comparisons between exposure profiles and RAPs are presented in Appendix 3 and clearly indicate an acceptable risk for aquatic organisms if respective mitigation measures are considered.

Conclusion

- The mesocosm conducted is fully compliant with the respective requirements of the AGD 2013 and representative for an aquatic community. Taxa tested are sufficient in sensitivity and total numbers as well as statistical validity.
- The mesocosm study is valid and comparable to several other study results indicating consistency. Potential data gaps previously identified were addressed with the new study, hence, an assessment

factor of 2 is justified and should be used to derive a regulatory acceptable concentration (RAC).

- Sediment dwellers were intensely observed under realistic long-term exposure conditions.
- The usage of a geomean measured from the nominal and the maximum measured concentration is much more appropriate than the nominal test concentration. Hence, the use of a RAC of 0.560 µg a.s./L is justified.
- Integrating modelled and measured exposures using EoP indicates an acceptable risk for aquatic organisms.

zRMS comments:

As endpoints from studies on acute toxicity of ADM.001512.2.A (to fish, *D. magna* and *C. riparius*) were lower comparing to endpoints derived from studies performed with the active substance, consideration of lower values in the risk assessment is justified as representing worst case and addressing potentially higher toxicity of the formulated product.

With regard to aquatic insects, at the EU level the geometric mean acute endpoint of 0.0085 mg a.s./L has been calculated from the available studies and was significantly lower than geometric mean EC₅₀ value of 0.0159 mg a.s./L derived from studies performed with ADM.001512.2.A (clearly less sensitive insect species excluded). For this reason, it is justified to use the EU agreed endpoint in the acute risk assessment for insects.

It is noted that the risk assessment for algae was actually performed with consideration of endpoint derived from study carried out with the active substance, which is correct as this was lower comparing to formulation endpoint. Information provided in point b) above has been thus struck through as being not correct.

The mesocosm study has been agreed by the zRMS with NOEC of 1.12 µg a.s./L and AF of 2 proposed for ETO-RAC derivation. For justification of the AF, please refer to point 9.5.1 above, while for details of the study evaluation and derived endpoint, please refer to Appendix 2.

9.5.2 Risk assessment

The evaluation of the risk for aquatic and sediment-dwelling organisms was performed in accordance with the recommendations of the “Guidance document on tiered risk assessment for plant protection products for aquatic organisms in edge-of-field surface waters (EFSA AGD, 2013) in the context of Regulation (EC) No 1107/2009”, as provided by the Commission Services (SANTE-2015-00080, 15 January 2015). In accordance with the EFSA AGD (2013), risk assessment for algae and higher aquatic plants was performed considering only the more relevant endpoint “growth rate” (ErC₅₀).

Acetamiprid

In the following, the exposure-toxicity ratios (ETR) between predicted environmental concentrations in surface water bodies (PEC_{sw/sed}) and regulatory acceptable concentrations (RAC) for aquatic organisms are given per proposed use pattern for each organism group for the active substance acetamiprid. For details on the PEC calculations please refer to Part B, Section 8.9

Table 9.5-4: Aquatic organisms - acceptability of risk (PEC/RAC < 1) for acetamiprid for each organism group based on FOCUS Step 1 - 3 calculations for the proposed application (60 g a.s./ha) of ADM.00150.I.2.A in ‘corn (umbrella use I; Jun-Aug / BBCH 51-75)’

Group		Fish, acute	Fish, prolonged	Invertebrate, acute	Invertebrate, prolonged	Invertebrates incl. sed. dwellers, acute and prolonged	Sed. dwell. organisms, prolonged	Algae
Test Species		<i>C. variegatus</i>	<i>P. promelas</i>	Geomean, Invertebrate acute	<i>D. magna</i>	Mesocosm (ETO); mean measured	<i>C. riparius</i>	<i>A. flos-aquae</i>
Endpoint (µg/L)		LC ₅₀ 100000	EC ₁₀ 9400	EC ₅₀ 8.5	EC ₁₀ 2960	NOEC 1.10	EC ₁₀ 0.235	E _r C ₅₀ >1300
AF		100	10	100	10	2	10	10
RAC (µg/L)		1000	940	0.085	296	0.56	0.024	>130
FOCUS Scenario	PEC _{sw,max} (µg/L)	ETR (PEC/RAC)						
Step 1								
	18.065	0.02	0.02	213	0.06	32	769	<0.1
Step 2								
N-Europe	0.778	0.0008	0.0008	9.2	0.003	1.4	33	<0.006
S-Europe	0.714	0.0007	0.0008	8.4	0.002	1.3	30	<0.005
Step 3								
D3 ditch	0.315	0.0003	0.0003	3.7	0.001	0.6	13	<0.002
D4 pond	0.013	0.00001	0.00001	0.2	0.00004	0.02	0.6	<0.0001
D4 stream	0.282	0.0003	0.0003	3.3	0.001	0.5	12	<0.002
D5 pond	0.013	0.00001	0.00001	0.2	0.00004	0.02	0.6	<0.0001
D5 stream	0.308	0.0003	0.0003	3.6	0.001	0.6	13	<0.002
R1 pond	0.033	0.00003	0.00004	0.4	0.0001	0.06	1.4	<0.0003
R1 stream	0.535	0.0005	0.0006	6.3	0.002	0.955	23	<0.004
R3 stream	0.308	0.0003	0.0003	3.6	0.001	0.6	13	<0.002
R4 stream	0.213	0.0002	0.0002	2.5	0.0007	0.4	9.1	<0.002

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in **bold**

For the intended application of ADM.00150.I.2.A in “corn” at 60 g a.s./ha (umbrella use I; Jun-Aug / BBCH 51-75), the calculated PEC/RAC ratios indicate an acceptable risk from exposure to acetamiprid for all groups of aquatic organisms based on the higher tier mesocosm endpoint also covering the lower tier invertebrate endpoints and FOCUS Step 1-3 PEC values. Therefore, no further assessment is necessary.

Table 9.5-5: Aquatic organisms - acceptability of risk (PEC/RAC < 1) for acetamiprid for each organism group based on FOCUS Step 1 - 3 calculations for the proposed application (80 g a.s./ha, covering 60 g a.s./ha) of , .00150.I.2.A in ‘pome/stone fruit, late applications (umbrella use IIa single Appl.; Jun-Aug / BBCH 71-PHI)’

Application (60 g a.i.s./ha, covering 60 g a.i.s./ha) of 100/50/12/1 in peime/stone fruit, late applications (umbrella use in single appln, sun rug/ BBCH 77-100)								
Group		Fish, acute	Fish, prolonged	Invertebrate, acute	Invertebrate, prolonged	Invertebrates incl. sed. dwellers, acute and prolonged	Sed. dwell. organisms, prolonged	Algae
Test Species		<i>C. variegatus</i>	<i>P. promelas</i>	Geomean, Invertebrate acute	<i>D. magna</i>	Mesocosm (ETO); mean measured	<i>C. riparius</i>	<i>A. flos-aquae</i>
Endpoint (µg/L)		LC ₅₀ 100000	EC ₁₀ 9400	EC ₅₀ 8.5	EC ₁₀ 2960	NOEC 1.12	EC ₁₀ 0.235	E _r C ₅₀ >1300
AF		100	10	100	10	2	10	10
RAC (µg/L)		1000	940	0.085	296	0.56	0.024	>130
FOCUS Scenario	PEC _{sw,max} (µg/L)	ETR (PEC/RAC)						
Step 1								
	27.544	0.03	0.03	324	0.09	49	1172	<0.2
Step 2								
N-Europe	4.193	0.004	0.004	49	0.01	7.5	178	<0.03
S-Europe	4.193	0.004	0.004	49	0.01	7.5	178	<0.03
Step 3								
D3 ditch	2.941	0.003	0.003	35	0.01	5.3	125	<0.02
D4 pond	0.132	0.0001	0.0001	1.6	0.0004	0.2	5.6	<0.001
D4 stream	2.951	0.003	0.003	35	0.01	5.3	126	<0.02
D5 pond	0.132	0.0001	0.0001	1.6	0.0004	0.2	5.6	<0.001
D5 stream	3.184	0.003	0.003	37	0.01	5.7	135	<0.02
R1 pond	0.132	0.0001	0.0001	1.6	0.0004	0.2	5.6	<0.001
R1 stream	2.213	0.002	0.002	26	0.007	4.0	94	<0.02
R2 stream	3.026	0.003	0.003	36	0.01	5.4	129	<0.02
R3 stream	3.182	0.003	0.003	37	0.01	5.7	135	<0.02
R4 stream	2.257	0.002	0.002	27	0.008	4.0	96	<0.02

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in **bold**

For the intended application of ADM.00150.I.2.A in “pome/stone fruits” at 80 g a.s./ha (umbrella use IIa; Jun-Aug / 71-PHI), the calculated PEC/RAC ratios indicate an acceptable risk from exposure to acetamiprid for all groups of aquatic organisms based on tier 1 endpoints and FOCUS Step 1-3 PEC values, except the higher tier mesocosm endpoint also covering the lower tier invertebrate endpoints. Additional PEC/RAC ratios were calculated considering FOCUS Step 4 PEC values.

Table 9.5-6: Aquatic organisms - acceptability of risk (PEC/RAC < 1) for acetamiprid for invertebrates incl. sed. dwellers, acute and prolonged based on FOCUS Step 4 calculations for the proposed application (80 g a.s./ha, covering 60 g a.s./ha) of ADM.00150.I.2.A in ‘pome/stone fruit, late applications (umbrella use IIa; Jun-Aug / 71-PHI)’

Intended use		Pome/stone fruit, late applications, umbrella use IIa; Jun-Aug / 71-PHI							
Active substance		Acetamiprid							
Application rate (g/ha)		80							
FOCUS Step 4 PEC _{sw, max} (µg/L)									
Nozzle reduction	Non-sprayed buffer (m)	-	5	10	10	15	15	20	20
	Vegetated filter strip (m)	-	-	-	10	-	10 (VFSmod)	-	20
-	D3 ditch	--	1.985	0.887	0.887	0.448	0.448	0.274	0.274
50%		1.471	0.993	0.444	0.444	0.224	0.224	0.137	0.137
75%		0.735	0.496	0.222	0.222	0.112	0.112	0.068	0.068
90%		0.294	0.199	0.089	0.089	0.045	0.045	0.027	0.027
-	D4 pond	--	0.151	0.084	0.084	0.053	0.053	0.038	0.038
50%		0.066	0.075	0.042	0.042	0.027	0.027	0.019	0.019
75%		0.033	0.038	0.021	0.021	0.013	0.013	0.010	0.010
90%		0.013	0.015	0.008	0.008	0.005	0.005	0.004	0.004
-	D4 stream	--	2.304	1.029	1.029	0.520	0.520	0.318	0.318
50%		1.476	1.152	0.515	0.515	0.260	0.260	0.159	0.159
75%		0.738	0.576	0.257	0.257	0.130	0.130	0.079	0.079
90%		0.295	0.230	0.103	0.103	0.052	0.052	0.032	0.032
-	D5 pond	--	0.151	0.084	0.084	0.053	0.053	0.038	0.038
50%		0.066	0.075	0.042	0.042	0.027	0.027	0.019	0.019
75%		0.033	0.038	0.021	0.021	0.013	0.013	0.010	0.010
90%		0.013	0.015	0.008	0.008	0.005	0.005	0.004	0.004
-	D5 stream	--	2.485	1.111	1.111	0.561	0.561	0.343	0.343
50%		1.592	1.243	0.555	0.555	0.280	0.280	0.171	0.171
75%		0.796	0.621	0.278	0.278	0.140	0.140	0.086	0.086
90%		0.318	0.249	0.111	0.111	0.056	0.056	0.034	0.034
-	R1 pond	--	0.151	0.083	0.083	0.053	0.053	0.038	0.038
50%		0.066	0.075	0.042	0.042	0.027	0.027	0.019	0.019
75%		0.033	0.038	0.021	0.021	0.013	0.013	0.009	0.009
90%		0.013	0.015	0.008	0.008	0.006	0.005	0.005	0.004
-	R1 stream	--	1.727	0.772	0.772	0.390	0.390	0.317	0.238
50%		1.106	0.864	0.386	0.386	0.317	0.195	0.317	0.119
75%		0.553	0.432	0.317	0.193	0.317	0.133	0.317	0.067
90%		0.317	0.317	0.317	0.133	0.317	0.133	0.317	0.067
-	R2 stream	--	2.362	1.055	1.055	0.533	0.533	0.326	0.326
50%		1.513	1.181	0.528	0.528	0.266	0.266	0.163	0.163
75%		0.757	0.590	0.264	0.264	0.133	0.133	0.081	0.081
90%		0.303	0.236	0.106	0.106	0.053	0.053	0.033	0.033

Intended use		Pome/stone fruit, late applications, umbrella use IIa; Jun-Aug / 71-PHI							
Active substance		Acetamiprid							
Application rate (g/ha)		80							
FOCUS Step 4 PEC _{sw, max} (µg/L)									
Nozzle reduction	Non-sprayed buffer (m)	-	5	10	10	15	15	20	20
	Vegetated filter strip (m)	-	-	-	10	-	10 (VFSmod)	-	20
-	R3 stream	--	2.484	1.110	1.110	0.560	0.560	0.343	0.343
50%		1.591	1.242	0.555	0.555	0.280	0.280	0.171	0.171
75%		0.796	0.621	0.277	0.277	0.140	0.140	0.086	0.086
90%		0.318	0.248	0.111	0.111	0.056	0.056	0.034	0.034
-	R4 stream	--	1.762	1.113	0.787	1.113	0.487	1.113	0.251
50%		1.129	1.113	1.113	0.487	1.113	0.487	1.113	0.251
75%		1.113	1.113	1.113	0.487	1.113	0.487	1.113	0.251
90%		1.113	1.113	1.113	0.487	1.113	0.487	1.113	0.251
Mesocosm (ETO); mean measured 0.56 µg/L		ETR (PEC/RAC)							
-	D3 ditch	--	3.5	1.6	1.6	0.8	0.8	0.5	0.5
50%		2.6	1.8	0.8	0.8	0.4	0.4	0.2	0.2
75%		1.3	0.9	0.4	0.4	0.2	0.2	0.1	0.1
90%		0.5	0.4	0.2	0.2	0.08	0.08	0.05	0.05
-	D4 stream	--	4.1	1.8	1.8	0.9	0.9	0.6	0.6
50%		2.6	2.1	0.9	0.9	0.5	0.5	0.3	0.3
75%		1.3	1.03	0.5	0.5	0.2	0.2	0.1	0.1
90%		0.5	0.4	0.2	0.2	0.09	0.09	0.06	0.06
-	D5 stream	--	4.4	2.0	2.0	1.0	1.0*	0.6	0.6
50%		2.8	2.2	0.991	0.991	0.5	0.5	0.3	0.3
75%		1.4	1.1	0.5	0.5	0.3	0.3	0.2	0.2
90%		0.6	0.4	0.2	0.2	0.1	0.1	0.06	0.06
-	R1 stream	--	3.1	1.4	1.4	0.7	0.7	0.6	0.4
50%		2.0	1.5	0.7	0.7	0.6	0.3	0.6	0.2
75%		0.988	0.8	0.6	0.3	0.6	0.2	0.6	0.1
90%		0.6	0.6	0.6	0.2	0.6	0.2	0.6	0.1
-	R2 stream	--	4.2	1.9	1.9	0.952	0.952	0.6	0.6
50%		2.7	2.1	0.9	0.9	0.5	0.5	0.3	0.3
75%		1.4	1.1	0.5	0.5	0.2	0.2	0.1	0.1
90%		0.5	0.4	0.2	0.2	0.09	0.09	0.06	0.06
-	R3 stream	--	4.4	2.0	2.0	1.0	1.0*	0.6	0.6
50%		2.8	2.2	0.991	0.991	0.5	0.5	0.3	0.3
75%		1.4	1.1	0.5	0.5	0.3	0.3	0.2	0.2
90%		0.6	0.4	0.2	0.2	0.1	0.1	0.06	0.06

Intended use		Pome/stone fruit, late applications, umbrella use IIa; Jun-Aug / 71-PHI							
Active substance		Acetamiprid							
Application rate (g/ha)		80							
FOCUS Step 4 PEC_{sw, max} (µg/L)									
Nozzle reduction	Non-sprayed buffer (m)	-	5	10	10	15	15	20	20
	Vegetated filter strip (m)	-	-	-	10	-	10 (VFSmod)	-	20
-	R4 stream	--	3.1	2.0	1.4	2.0	0.9	2.0	0.4
50%		2.0	2.0	2.0	0.9	2.0	0.9	2.0	0.4
75%		2.0	2.0	2.0	0.9	2.0	0.9	2.0	0.4
90%		2.0	2.0	2.0	0.9	2.0	0.9	2.0	0.4

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in **bold**; * safe use can be demonstrated with the implementation of the EoP approach

For the intended application of ADM.00150.I.2.A in “pome/stone fruits” at 80 g a.s./ha (umbrella use IIa; Jun-Aug / 71-PHI), the calculated PEC/RAC ratios indicate an acceptable risk from exposure to acetamiprid if certain mitigation measures are considered. For the mesocosm study (ETO; mean measured; aquatic invertebrates, acute and sed. dwell. organisms, prolonged, RAC = 0.56 µg/L), a non-spray buffer of 20 m in combination with a vegetated filter strip of 20 m or 50% drift reducing nozzles and a non-spray buffer of 10 m in combination with a vegetated filter strip of 10 m should be considered. With the implementation of the EoP approach, additional safe use can be demonstrated when considering a non-sprayed buffer zone of 15 m in combination with a vegetated filter strip of 10 m (Figure 2 and Figure 3).

Figure 2: Worst-case (maximum peak) exposure profile analysis for acetamiprid Step 4, 15 m drift buffer zone + 10 m vegetated filter strip, scenario D5 stream for the proposed application (80 g a.s./ha) of ADM.00150.I.2.A in ‘pome/stone fruit, late applications (umbrella use IIa; Jun-Aug / 71-PHI)

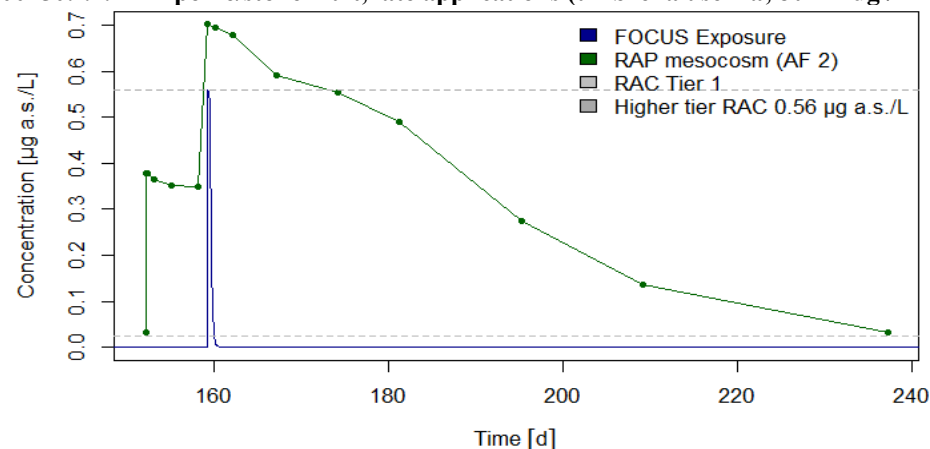


Figure 3: Worst-case (highest AUC) exposure profile analysis for acetamiprid Step 4, 15 m drift buffer zone + 10 m vegetated filter strip, scenario D4 ditch for the proposed application (34 +34 g a.s./ha) of ADM.00150.I.2.A in ‘pome/stone fruit, late applications (umbrella use IIa; Jun-Aug / 71-PHI)

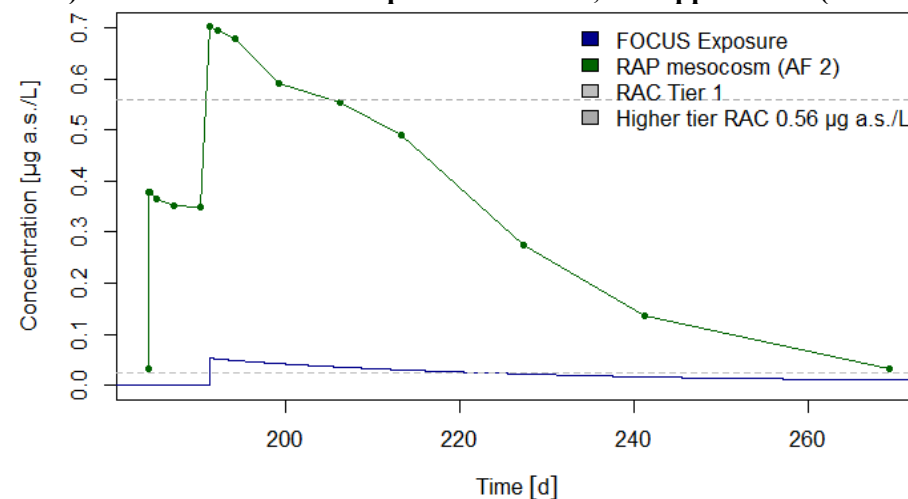


Table 9.5-7: Aquatic organisms - acceptability of risk (PEC/RAC < 1) for acetamiprid for each organism group based on FOCUS Step 1 - 3 calculations for the proposed application (25 +25 g a.s./ha) of ADM.00150.I.2.A in ‘pome/stone fruit, early applications (umbrella use I Ib; June-Aug / BBCH 62-PHI)’

Group		Fish, acute	Fish, prolonged	Invertebrate, acute	Invertebrate, prolonged	Invertebrates incl. sed. dwellers, acute and prolonged	Sed. dwell. organisms, prolonged	Algae
Test Species		<i>C. variegatus</i>	<i>P. promelas</i>	Geomean, Invertebrate acute	<i>D. magna</i>	Mesocosm (ETO); mean measured	<i>C. riparius</i>	<i>A. flos-aquae</i>
Endpoint (µg/L)		LC ₅₀ 100000	EC ₁₀ 9400	EC ₅₀ 8.5	EC ₁₀ 2960	NOEC 1.12	EC ₁₀ 0.235	E _r C ₅₀ >1300
AF		100	10	100	10	2	10	10
RAC (µg/L)		1000	940	0.085	296	0.56	0.024	>130
FOCUS Scenario	PEC _{sw,max} (µg/L)	ETR (PEC/RAC)						
Step 1								
	19.460	0.02	0.02	229	0.07	35	828	<0.1
Step 2								
N-Europe	3.710	0.004	0.004	44	0.01	6.6	158	<0.03
S-Europe	3.710	0.004	0.004	44	0.01	6.6	158	<0.03
Step 3								
D3 ditch	1.678	0.002	0.002	20	0.006	3.0	71	<0.01
D4 pond	0.185	0.0002	0.0002	2.2	0.0006	0.3	7.9	<0.001
D4 stream	1.766	0.002	0.002	21	0.006	3.2	75	<0.01
D5 pond	0.180	0.0002	0.0002	2.1	0.0006	0.3	7.7	<0.001
D5 stream	1.905	0.002	0.002	22	0.006	3.4	81	<0.01
R1 pond	0.174	0.0002	0.0002	2.0	0.0006	0.3	7.4	<0.001
R1 stream	1.351	0.001	0.001	16	0.005	2.4	57	<0.01
R3 stream	1.904	0.002	0.002	22	0.006	3.4	81	<0.01
R4 stream	1.350	0.001	0.001	16	0.005	2.4	57	<0.01

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in **bold**

For the intended application of ADM.00150.I.2.A in “pome/stone fruits” at 25 +25 g a.s./ha (umbrella use I Ib; June-Aug / BBCH 62-PHI), the calculated PEC/RAC ratios indicate an acceptable risk from exposure to acetamiprid for all groups of aquatic organisms based on tier 1 endpoints and FOCUS Step 1-3 PEC values, except the higher tier mesocosm endpoint also covering the lower tier invertebrate endpoints. Additional PEC/RAC ratios were calculated considering FOCUS Step 4 PEC values.

Table 9.5-8: Aquatic organisms - acceptability of risk (PEC/RAC < 1) for acetamiprid for invertebrates incl. sed. dwellers, acute and prolonged based on FOCUS Step 4 calculations for the proposed application (25 +25 g a.s./ha) of ADM.00150.I.2.A in ‘pome/stone fruit, early applications (umbrella use IIb; June-Aug / BBCH 62-PHI)’

Intended use		Pome/stone fruit, early applications, umbrella use IIb; June-Aug / BBCH 62-PHI							
Active substance		Acetamiprid							
Application rate (g/ha)		25 +25							
FOCUS Step 4 PEC _{sw, max} (µg/L)									
Nozzle reduction	Non-sprayed buffer (m)	-	5	10	10	15	15	20	20
	Vegetated filter strip (m)	-	-	-	10	-	10 (VFSmod)	-	20
-	D3 ditch	--	1.294	0.764	0.764	0.419	0.419	0.198	0.198
50%		0.839	0.647	0.382	0.382	0.210	0.210	0.099	0.099
75%		0.419	0.323	0.191	0.191	0.105	0.105	0.049	0.049
90%		0.168	0.129	0.076	0.076	0.042	0.042	0.020	0.020
-	D4 pond	--	0.208	0.118	0.118	0.062	0.062	0.036	0.036
50%		0.093	0.104	0.059	0.059	0.031	0.031	0.018	0.018
75%		0.046	0.052	0.029	0.029	0.015	0.015	0.009	0.009
90%		0.018	0.021	0.012	0.012	0.006	0.006	0.004	0.004
-	D4 stream	--	1.499	0.885	0.885	0.486	0.486	0.229	0.229
50%		0.883	0.749	0.443	0.443	0.243	0.243	0.115	0.115
75%		0.441	0.375	0.221	0.221	0.122	0.122	0.057	0.057
90%		0.177	0.150	0.089	0.089	0.049	0.049	0.023	0.023
-	D5 pond	--	0.201	0.114	0.114	0.060	0.060	0.034	0.034
50%		0.090	0.101	0.057	0.057	0.030	0.030	0.017	0.017
75%		0.045	0.050	0.028	0.028	0.015	0.015	0.009	0.009
90%		0.018	0.020	0.011	0.011	0.006	0.006	0.003	0.003
-	D5 stream	--	1.617	0.955	0.955	0.525	0.525	0.247	0.247
50%		0.953	0.808	0.478	0.478	0.262	0.262	0.124	0.124
75%		0.476	0.404	0.239	0.239	0.131	0.131	0.062	0.062
90%		0.191	0.162	0.096	0.096	0.052	0.052	0.025	0.025
-	R1 pond	--	0.200	0.116	0.113	0.063	0.060	0.039	0.034
50%		0.092	0.103	0.061	0.057	0.034	0.031	0.022	0.018
75%		0.049	0.054	0.033	0.030	0.020	0.017	0.014	0.009
90%		0.023	0.025	0.016	0.013	0.011	0.008	0.009	0.004
-	R1 stream	--	1.147	0.677	0.677	0.372	0.372	0.175	0.175
50%		0.675	0.573	0.339	0.339	0.186	0.186	0.091	0.088
75%		0.338	0.287	0.169	0.169	0.093	0.093	0.091	0.044
90%		0.135	0.115	0.091	0.068	0.091	0.038	0.091	0.020
-	R3 stream	--	1.616	0.955	0.955	0.524	0.524	0.247	0.247
50%		0.952	0.808	0.477	0.477	0.262	0.262	0.124	0.124
75%		0.476	0.404	0.239	0.239	0.131	0.131	0.062	0.062
90%		0.190	0.162	0.095	0.095	0.052	0.052	0.025	0.025
-	R4 stream	--	1.146	0.677	0.677	0.372	0.372	0.175	0.175

Intended use		Pome/stone fruit, early applications, umbrella use IIb; June-Aug / BBCH 62-PHI							
Active substance		Acetamiprid							
Application rate (g/ha)		25 +25							
FOCUS Step 4 PEC _{sw, max} (µg/L)									
Nozzle reduction	Non-sprayed buffer (m)	-	5	10	10	15	15	20	20
	Vegetated filter strip (m)	-	-	-	10	-	10 (VFSmod)	-	20
50%		0.675	0.573	0.339	0.339	0.186	0.186	0.125	0.088
75%		0.338	0.287	0.169	0.169	0.125	0.093	0.125	0.044
90%		0.135	0.125	0.125	0.068	0.125	0.053	0.125	0.027
Mesocosm (ETO); mean measured 0.56 µg/L		ETR (PEC/RAC)							
-	D3 ditch	--	2.3	1.4	1.4	0.7	0.7	0.4	0.4
50%		1.5	1.2	0.7	0.7	0.4	0.4	0.2	0.2
75%		0.7	0.6	0.3	0.3	0.2	0.2	0.09	0.09
90%		0.3	0.2	0.1	0.1	0.08	0.08	0.04	0.04
-	D4 stream	--	2.7	1.6	1.6	0.9	0.9	0.4	0.4
50%		1.6	1.3	0.8	0.8	0.4	0.4	0.2	0.2
75%		0.8	0.7	0.4	0.4	0.2	0.2	0.1	0.1
90%		0.3	0.3	0.2	0.2	0.09	0.09	0.04	0.04
-	D5 stream	--	2.9	1.7	1.7	0.9	0.9	0.4	0.4
50%		1.7	1.4	0.9	0.9	0.5	0.5	0.2	0.2
75%		0.9	0.7	0.4	0.4	0.2	0.2	0.1	0.1
90%		0.3	0.3	0.2	0.2	0.09	0.09	0.04	0.04
-	R1 stream	--	2.0	1.2	1.2	0.7	0.7	0.3	0.3
50%		1.2	1.02	0.6	0.6	0.3	0.3	0.2	0.2
75%		0.6	0.5	0.3	0.3	0.2	0.2	0.2	0.08
90%		0.2	0.2	0.2	0.1	0.2	0.07	0.2	0.04
-	R3 stream	--	2.9	1.7	1.7	0.9	0.9	0.4	0.4
50%		1.7	1.4	0.9	0.9	0.5	0.5	0.2	0.2
75%		0.9	0.7	0.4	0.4	0.2	0.2	0.1	0.1
90%		0.3	0.3	0.2	0.2	0.09	0.09	0.04	0.04
-	R4 stream	--	2.0	1.2	1.2	0.7	0.7	0.3	0.3
50%		1.2	1.02	0.6	0.6	0.3	0.3	0.2	0.2
75%		0.6	0.5	0.3	0.3	0.2	0.2	0.2	0.08
90%		0.2	0.2	0.2	0.1	0.2	0.09	0.2	0.05

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in **bold**

For the intended application of ADM.00150.I.2.A in “pome/stone fruits” at 25 +25 g a.s./ha (umbrella use IIb; June-Aug / BBCH 62-PHI), the calculated PEC/RAC ratios indicate an acceptable risk from exposure to acetamiprid if certain mitigation measures are considered. For the mesocosm study (ETO; mean measured; aquatic

invertebrates, acute and sed. dwell. organisms, prolonged; RAC = 0.56 µg/L), a non-sprayed buffer zone of 15 m or 50% drift reducing nozzles and a non-sprayed buffer zone of 10 m or 75% drift reducing nozzles should be considered.

Table 9.5-9: Aquatic organisms - acceptability of risk (PEC/RAC < 1) for acetamiprid for each organism group based on FOCUS Step 1 - 3 calculations for the proposed application (25 g a.s./ha) of ADM.00150.I.2.A in ‘pome/stone fruit, early applications (umbrella use IIb; June-Aug / BBCH 62-PHI)’

Application (25 g a.s./ha) or FOCUS 150-112-11 in pebble/stone fruit, early applications (ambrosia use 110, June 18g / BBCH 62-111)								
Group		Fish, acute	Fish, prolonged	Invertebrate, acute	Invertebrate, prolonged	Invertebrates incl. sed. dwellers, acute and prolonged	Sed. dwell. organisms, prolonged	Algae
Test Species		<i>C. variegatus</i>	<i>P. promelas</i>	Geomean, Invertebrate acute	<i>D. magna</i>	Mesocosm (ETO); mean measured	<i>C. riparius</i>	<i>A. flos-aquae</i>
Endpoint (µg/L)		LC ₅₀ 100000	EC ₁₀ 9400	EC ₅₀ 8.5	EC ₁₀ 2960	NOEC 1.12	EC ₁₀ 0.235	E _r C ₅₀ >1300
AF		100	10	100	10	2	10	10
RAC (µg/L)		1000	940	0.085	296	0.56	0.024	>130
FOCUS Scenario	PEC _{sw,max} (µg/L)	ETR (PEC/RAC)						
Step 1								
	19.460	0.02	0.02	229	0.07	35	828	<0.1
Step 2								
N-Europe	2.433	0.002	0.003	29	0.008	4.3	104	<0.02
S-Europe	2.433	0.002	0.003	29	0.008	4.3	104	<0.02
Step 3								
D3 ditch	1.948	0.002	0.002	23	0.007	3.5	83	<0.01
D4 pond	0.118	0.0001	0.0001	1.4	0.0004	0.2	5.0	<0.0009
D4 stream	2.065	0.002	0.002	24	0.007	3.7	88	<0.02
D5 pond	0.118	0.0001	0.0001	1.4	0.0004	0.2	5.0	<0.0009
D5 stream	2.230	0.002	0.002	26	0.008	4.0	95	<0.02
R1 pond	0.118	0.0001	0.0001	1.4	0.0004	0.2	5.0	<0.0009
R1 stream	1.582	0.002	0.002	19	0.005	2.8	67	<0.01
R3 stream	2.230	0.002	0.002	26	0.008	4.0	95	<0.02
R4 stream	1.546	0.002	0.002	18	0.005	2.8	66	<0.01

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in **bold**

For the intended application of ADM.00150.I.2.A in “pome/stone fruits” at 25 g a.s./ha (umbrella use IIb; June-Aug / BBCH 62-PHI), the calculated PEC/RAC ratios indicate an acceptable risk from exposure to acetamiprid for all groups of aquatic organisms based on tier 1 endpoints and FOCUS Step 1-3 PEC values, except the higher tier mesocosm endpoint also covering the lower tier invertebrate endpoints. Additional PEC/RAC ratios were calculated considering FOCUS Step 4 PEC values.

Table 9.5-10: Aquatic organisms - acceptability of risk (PEC/RAC < 1) for acetamiprid for invertebrates incl. sed. dwellers, acute and prolonged based on FOCUS Step 4 calculations for the proposed application (25 g a.s./ha) of ADM.00150.I.2.A in ‘pome/stone fruit, early applications (umbrella use IIb; June-Aug / BBCH 62-PHI)’

Intended use		Pome/stone fruit, early applications, umbrella use IIb; June-Aug / BBCH 62-PHI							
Active substance		Acetamiprid							
Application rate (g/ha)		25							
FOCUS Step 4 PEC _{sw, max} (µg/L)									
Nozzle reduction	Non-sprayed buffer (m)	-	5	10	10	15	15	20	20
	Vegetated filter strip (m)	-	-	-	10	-	10 (VFSmod)	-	20
-	D3 ditch	--	1.530	0.940	0.940	0.423	0.423	0.215	0.215
50%		0.974	0.765	0.470	0.470	0.211	0.211	0.107	0.107
75%		0.487	0.383	0.235	0.235	0.106	0.106	0.054	0.054
90%		0.195	0.153	0.094	0.094	0.042	0.042	0.021	0.021
-	D4 pond	--	0.133	0.073	0.073	0.038	0.038	0.024	0.024
50%		0.059	0.066	0.036	0.036	0.019	0.019	0.012	0.012
75%		0.030	0.033	0.018	0.018	0.010	0.010	0.006	0.006
90%		0.012	0.013	0.007	0.007	0.004	0.004	0.002	0.002
-	D4 stream	--	1.774	1.089	1.089	0.490	0.490	0.249	0.249
50%		1.032	0.887	0.545	0.545	0.245	0.245	0.125	0.125
75%		0.516	0.444	0.272	0.272	0.123	0.123	0.062	0.062
90%		0.206	0.177	0.109	0.109	0.049	0.049	0.025	0.025
-	D5 pond	--	0.133	0.073	0.073	0.038	0.038	0.024	0.024
50%		0.059	0.066	0.036	0.036	0.019	0.019	0.012	0.012
75%		0.030	0.033	0.018	0.018	0.010	0.010	0.006	0.006
90%		0.012	0.013	0.007	0.007	0.004	0.004	0.002	0.002
-	D5 stream	--	1.916	1.176	1.176	0.529	0.529	0.269	0.269
50%		1.115	0.958	0.588	0.588	0.265	0.265	0.135	0.135
75%		0.558	0.479	0.294	0.294	0.132	0.132	0.067	0.067
90%		0.223	0.192	0.118	0.118	0.053	0.053	0.027	0.027
-	R1 pond	--	0.133	0.073	0.073	0.038	0.038	0.024	0.024
50%		0.059	0.066	0.036	0.036	0.019	0.019	0.014	0.012
75%		0.030	0.033	0.019	0.018	0.012	0.010	0.010	0.006
90%		0.014	0.015	0.011	0.007	0.008	0.005	0.007	0.003
-	R1 stream	--	1.359	0.835	0.835	0.376	0.376	0.191	0.191
50%		0.791	0.680	0.417	0.417	0.188	0.188	0.095	0.095
75%		0.396	0.340	0.209	0.209	0.094	0.094	0.083	0.048
90%		0.158	0.136	0.083	0.083	0.083	0.038	0.083	0.020
-	R2 stream	--	1.822	1.119	1.119	0.503	0.503	0.256	0.256
50%		1.060	0.911	0.559	0.559	0.252	0.252	0.128	0.128
75%		0.530	0.456	0.280	0.280	0.126	0.126	0.064	0.064
90%		0.212	0.182	0.112	0.112	0.050	0.050	0.026	0.026
-	R3 stream	--	1.916	1.176	1.176	0.529	0.529	0.269	0.269

Intended use		Pome/stone fruit, early applications, umbrella use IIb; June-Aug / BBCH 62-PHI							
Active substance		Acetamiprid							
Application rate (g/ha)		25							
FOCUS Step 4 PEC _{sw, max} (µg/L)									
Nozzle reduction	Non-sprayed buffer (m)	-	5	10	10	15	15	20	20
	Vegetated filter strip (m)	-	-	-	10	-	10 (VFSmod)	-	20
50%	R4 stream	1.115	0.958	0.588	0.588	0.265	0.265	0.135	0.135
75%		0.558	0.479	0.294	0.294	0.132	0.132	0.067	0.067
90%		0.223	0.192	0.118	0.118	0.053	0.053	0.027	0.027
-		--	1.329	0.816	0.816	0.367	0.367	0.187	0.187
50%		0.773	0.664	0.408	0.408	0.184	0.184	0.093	0.093
75%		0.387	0.332	0.204	0.204	0.092	0.092	0.047	0.047
90%		0.155	0.133	0.082	0.082	0.037	0.037	0.019	0.019
Mesocosm (ETO); mean measured 0.56 µg/L		ETR (PEC/RAC)							
-	D3 ditch	--	2.7	1.7	1.7	0.8	0.8	0.4	0.4
50%		1.7	1.4	0.8	0.8	0.4	0.4	0.2	0.2
75%		0.9	0.7	0.4	0.4	0.2	0.2	0.1	0.1
90%		0.3	0.3	0.2	0.2	0.08	0.08	0.04	0.04
-	D4 stream	--	3.2	1.9	1.9	0.9	0.9	0.4	0.4
50%		1.8	1.6	0.973	0.973	0.4	0.4	0.2	0.2
75%		0.9	0.8	0.5	0.5	0.2	0.2	0.1	0.1
90%		0.4	0.3	0.2	0.2	0.09	0.09	0.04	0.04
-	D5 stream	--	3.4	2.1	2.1	0.9	0.9	0.5	0.5
50%		2.0	1.7	1.05	1.05	0.5	0.5	0.2	0.2
75%		0.996	0.9	0.5	0.5	0.2	0.2	0.1	0.1
90%		0.4	0.3	0.2	0.2	0.09	0.09	0.05	0.05
-	R1 stream	--	2.4	1.5	1.5	0.7	0.7	0.3	0.3
50%		1.4	1.2	0.7	0.7	0.3	0.3	0.2	0.2
75%		0.7	0.6	0.4	0.4	0.2	0.2	0.1	0.09
90%		0.3	0.2	0.1	0.1	0.1	0.07	0.1	0.04
-	R3 stream	--	3.4	2.1	2.1	0.9	0.9	0.5	0.5
50%		2.0	1.7	1.05	1.05	0.5	0.5	0.2	0.2
75%		0.996	0.9	0.5	0.5	0.2	0.2	0.1	0.1
90%		0.4	0.3	0.2	0.2	0.09	0.09	0.05	0.05
-	R4 stream	--	2.4	1.5	1.5	0.7	0.7	0.3	0.3
50%		1.4	1.2	0.7	0.7	0.3	0.3	0.2	0.2
75%		0.7	0.6	0.4	0.4	0.2	0.2	0.08	0.08
90%		0.3	0.2	0.1	0.1	0.07	0.07	0.03	0.03

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in **bold**

For the intended application of ADM.00150.I.2.A in “pome/stone fruits” at 25 g a.s./ha (umbrella use IIb; June-Aug / BBCH 62-PHI), the calculated PEC/RAC ratios indicate an acceptable risk from exposure to acetamiprid if certain mitigation measures are considered. For the mesocosm study (ETO; mean measured; aquatic invertebrates, acute and sed. dwell. organisms, prolonged; RAC = 0.56 µg/L), a non-sprayed buffer zone of 15 m or 75% drift reducing nozzles should be considered.

Table 9.5-11: Aquatic organisms - acceptability of risk (PEC/RAC < 1) for acetamiprid for each organism group based on FOCUS Step 1 - 3 calculations for the proposed application (25 +25 g a.s./ha) of ADM.00150.I.2.A in ‘pome/stone fruit, late applications (umbrella use IIb; Jun-Aug/ BBCH 71-PHI)’

Group		Fish, acute	Fish, prolonged	Invertebrate, acute	Invertebrate, prolonged	Invertebrates incl. sed. dwellers, acute and prolonged	Sed. dwell. organisms, prolonged	Algae
Test Species		<i>C. variegatus</i>	<i>P. promelas</i>	Geomean, Invertebrate acute	<i>D. magna</i>	Mesocosm (ETO); mean measured	<i>C. riparius</i>	<i>A. flos-aquae</i>
Endpoint (µg/L)		LC ₅₀ 100000	EC ₁₀ 9400	EC ₅₀ 8.5	EC ₁₀ 2960	NOEC 1.12	EC ₁₀ 0.235	E _r C ₅₀ >1300
AF		100	10	100	10	2	10	10
RAC (µg/L)		1000	940	0.085	296	0.56	0.024	>130
FOCUS Scenario	PEC _{sw,max} (µg/L)	ETR (PEC/RAC)						
Step 1								
	17.215	0.02	0.02	203	0.06	31	733	<0.1
Step 2								
N-Europe	1.763	0.002	0.002	21	0.006	3.1	75	<0.01
S-Europe	1.763	0.002	0.002	21	0.006	3.1	75	<0.01
Step 3								
D3 ditch	0.729	0.0007	0.0008	8.6	0.002	1.3	31	<0.006
D4 pond	0.058	0.00006	0.00006	0.7	0.0002	0.1	2.5	<0.0004
D4 stream	0.722	0.0007	0.0008	8.5	0.002	1.3	31	<0.006
D5 pond	0.062	0.00006	0.00007	0.7	0.0002	0.1	2.6	<0.0005
D5 stream	0.797	0.0008	0.0008	9.4	0.003	1.4	34	<0.006
R1 pond	0.056	0.00006	0.00006	0.7	0.0002	0.1	2.4	<0.0004
R1 stream	0.565	0.0006	0.0006	6.6	0.002	1.01	24	<0.004
R3 stream	0.796	0.0008	0.0008	9.4	0.003	1.4	34	<0.006
R4 stream	0.565	0.0006	0.0006	6.6	0.002	1.01	24	<0.004

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in **bold**

For the intended application of ADM.00150.I.2.A in “pome/stone fruits” at 25 +25 g a.s./ha (umbrella use IIb; Jun-Aug/ BBCH 71-PHI), the calculated PEC/RAC ratios indicate an acceptable risk from exposure to acetamiprid for all groups of aquatic organisms based on tier 1 endpoints and FOCUS Step 1-3 PEC values, except the higher tier mesocosm endpoint also covering the lower tier invertebrate endpoints. Additional PEC/RAC ratios were calculated considering FOCUS Step 4 PEC values.

Table 9.5-12: Aquatic organisms - acceptability of risk (PEC/RAC < 1) for acetamiprid for invertebrates incl. sed. dwellers, acute and prolonged based on FOCUS Step 4 calculations for the proposed application (25 +25 g a.s./ha) of ADM.00150.I.2.A in ‘pome/stone fruit, late applications (umbrella use IIb; Jun-Aug/ BBCH 71-PHI)’

Intended use		Pome/stone fruit, late applications, umbrella use IIb; Jun-Aug/ BBCH 71-PHI							
Active substance		Acetamiprid							
Application rate (g/ha)		25 +25							
FOCUS Step 4 PEC _{sw, max} (µg/L)									
Nozzle reduction	Non-sprayed buffer (m)	-	5	10	10	15	15	20	20
	Vegetated filter strip (m)	-	-	-	10	-	10 (VFSmod)	-	20
-	D3 ditch	--	0.507	0.244	0.244	0.119	0.119	0.069	0.069
50%		0.365	0.254	0.122	0.122	0.060	0.060	0.035	0.035
75%		0.182	0.127	0.061	0.061	0.030	0.030	0.017	0.017
90%		0.073	0.051	0.024	0.024	0.012	0.012	0.007	0.007
-	D4 pond	--	0.066	0.036	0.036	0.022	0.022	0.015	0.015
50%		0.029	0.033	0.018	0.018	0.011	0.011	0.007	0.007
75%		0.014	0.016	0.009	0.009	0.005	0.005	0.004	0.004
90%		0.006	0.007	0.004	0.004	0.002	0.002	0.001	0.001
-	D4 stream	--	0.574	0.276	0.276	0.135	0.135	0.078	0.078
50%		0.361	0.287	0.138	0.138	0.068	0.068	0.039	0.039
75%		0.180	0.144	0.069	0.069	0.034	0.034	0.020	0.020
90%		0.072	0.057	0.028	0.028	0.014	0.014	0.008	0.008
-	D5 pond	--	0.071	0.039	0.039	0.024	0.024	0.016	0.016
50%		0.031	0.035	0.019	0.019	0.012	0.012	0.008	0.008
75%		0.015	0.018	0.010	0.010	0.006	0.006	0.004	0.004
90%		0.006	0.007	0.004	0.004	0.002	0.002	0.002	0.002
-	D5 stream	--	0.634	0.305	0.305	0.149	0.149	0.086	0.086
50%		0.398	0.317	0.152	0.152	0.075	0.075	0.043	0.043
75%		0.199	0.159	0.076	0.076	0.037	0.037	0.022	0.022
90%		0.080	0.063	0.030	0.030	0.015	0.015	0.009	0.009
-	R1 pond	--	0.064	0.035	0.035	0.021	0.021	0.015	0.015
50%		0.028	0.032	0.018	0.018	0.011	0.011	0.007	0.007
75%		0.014	0.016	0.009	0.009	0.005	0.005	0.004	0.004
90%		0.006	0.006	0.004	0.004	0.002	0.002	0.001	0.001
-	R1 stream	--	0.450	0.216	0.216	0.106	0.106	0.061	0.061
50%		0.282	0.225	0.108	0.108	0.053	0.053	0.031	0.031
75%		0.141	0.112	0.054	0.054	0.026	0.026	0.015	0.015
90%		0.056	0.045	0.022	0.022	0.011	0.011	0.006	0.006
-	R2 stream	--	0.603	0.289	0.289	0.142	0.142	0.082	0.082
50%		0.379	0.301	0.145	0.145	0.071	0.071	0.041	0.041
75%		0.189	0.151	0.072	0.072	0.035	0.035	0.021	0.021
90%		0.076	0.060	0.029	0.029	0.014	0.014	0.008	0.008
-	R3 stream	--	0.634	0.304	0.304	0.186	0.149	0.186	0.086

Intended use		Pome/stone fruit, late applications, umbrella use IIb; Jun-Aug/ BBCH 71-PHI							
Active substance		Acetamiprid							
Application rate (g/ha)		25 +25							
FOCUS Step 4 PEC _{sw, max} (µg/L)									
Nozzle reduction	Non-sprayed buffer (m)	-	5	10	10	15	15	20	20
	Vegetated filter strip (m)	-	-	-	10	-	10 (VFSmod)	-	20
50%	R4 stream	0.398	0.317	0.186	0.152	0.186	0.084	0.186	0.044
75%		0.199	0.186	0.186	0.084	0.186	0.084	0.186	0.044
90%		0.186	0.186	0.186	0.084	0.186	0.084	0.186	0.044
-		--	0.450	0.288	0.216	0.288	0.129	0.288	0.067
50%		0.288	0.288	0.288	0.129	0.288	0.129	0.288	0.067
75%		0.288	0.288	0.288	0.129	0.288	0.129	0.288	0.067
90%		0.288	0.288	0.288	0.129	0.288	0.129	0.288	0.067
Mesocosm (ETO); mean measured 0.56 µg/L		ETR (PEC/RAC)							
-	D3 ditch	--	0.9	0.4	0.4	0.2	0.2	0.1	0.1
50%		0.7	0.5	0.2	0.2	0.1	0.1	0.06	0.06
75%		0.3	0.2	0.1	0.1	0.05	0.05	0.03	0.03
90%		0.1	0.09	0.04	0.04	0.02	0.02	0.01	0.01
-	D4 stream	--	1.02	0.5	0.5	0.2	0.2	0.1	0.1
50%		0.6	0.5	0.2	0.2	0.1	0.1	0.07	0.07
75%		0.3	0.3	0.1	0.1	0.06	0.06	0.04	0.04
90%		0.1	0.1	0.05	0.05	0.03	0.03	0.01	0.01
-	D5 stream	--	1.1	0.5	0.5	0.3	0.3	0.2	0.2
50%		0.7	0.6	0.3	0.3	0.1	0.1	0.08	0.08
75%		0.4	0.3	0.1	0.1	0.07	0.07	0.04	0.04
90%		0.1	0.1	0.05	0.05	0.03	0.03	0.02	0.02
-	R1 stream		0.8	0.4	0.4	0.2	0.2	0.1	0.1
50%		0.5	0.4	0.2	0.2	0.09	0.09	0.06	0.06
75%		0.3	0.2	0.1	0.1	0.05	0.05	0.03	0.03
90%		0.1	0.08	0.04	0.04	0.02	0.02	0.01	0.01
-	R3 stream	--	1.1	0.5	0.5	0.3	0.3	0.3	0.2
50%		0.7	0.6	0.3	0.3	0.3	0.2	0.3	0.08
75%		0.4	0.3	0.3	0.2	0.3	0.2	0.3	0.08
90%		0.3	0.3	0.3	0.2	0.3	0.2	0.3	0.08
-	R4 stream	--	0.8	0.5	0.4	0.5	0.2	0.5	0.1
50%		0.5	0.5	0.5	0.2	0.5	0.2	0.5	0.1
75%		0.5	0.5	0.5	0.2	0.5	0.2	0.5	0.1
90%		0.5	0.5	0.5	0.2	0.5	0.2	0.5	0.1

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in **bold**

For the intended application of ADM.00150.I.2.A in “pome/stone fruits” at 25 +25 g a.s./ha (umbrella use IIb; Jun-Aug/ BBCH 71-PHI), the calculated PEC/RAC ratios indicate an acceptable risk from exposure to acetamiprid if certain mitigation measures are considered. For the mesocosm study (ETO; mean measured; aquatic invertebrates, acute and sed. dwell. organisms, prolonged; RAC = 0.56 µg/L), a non-sprayed buffer zone of 10 m or 50% drift reducing nozzles should be considered.

Table 9.5-13: Aquatic organisms - acceptability of risk (PEC/RAC < 1) for acetamiprid for each organism group based on FOCUS Step 1 - 3 calculations for the proposed application (25 g a.s./ha) of ADM.00150.I.2.A in ‘pome/stone fruit, late applications (umbrella use IIb; Jun-Aug/ BBCH 71-PHI)’

Application (20 µg/L) of 12-Misc100M2-1 in polycarbonate flasks, rate applications (ambient use 10, 500 µg/L, 1000 µg/L)								
Group		Fish, acute	Fish, prolonged	Invertebrate, acute	Invertebrate, prolonged	Invertebrates incl. sed. dwellers, acute and prolonged	Sed. dwell. organisms, prolonged	Algae
Test Species		<i>C. variegatus</i>	<i>P. promelas</i>	Geomean, Invertebrate acute	<i>D. magna</i>	Mesocosm (ETO); mean measured	<i>C. riparius</i>	<i>A. flos-aquae</i>
Endpoint (µg/L)		LC ₅₀ 100000	EC ₁₀ 9400	EC ₅₀ 8.5	EC ₁₀ 2960	NOEC 1.12	EC ₁₀ 0.235	E _r C ₅₀ >1300
AF		100	10	100	10	2	10	10
RAC (µg/L)		1000	940	0.085	296	0.56	0.024	>130
FOCUS Scenario	PEC _{sw,max} (µg/L)	ETR (PEC/RAC)						
Step 1								
	17.215	0.02	0.02	203	0.06	31	733	<0.1
Step 2								
N-Europe	1.310	0.001	0.001	15	0.004	2.3	56	<0.01
S-Europe	1.310	0.001	0.001	15	0.004	2.3	56	<0.01
Step 3								
D3 ditch	0.919	0.0009	0.001	11	0.003	1.6	39	<0.007
D4 pond	0.041	0.00004	0.00004	0.5	0.0001	0.07	1.7	<0.0003
D4 stream	0.901	0.0009	0.001	11	0.003	1.6	38	<0.007
D5 pond	0.041	0.00004	0.00004	0.5	0.0001	0.07	1.7	<0.0003
D5 stream	0.995	0.001	0.001	12	0.003	1.8	42	<0.008
R1 pond	0.041	0.00004	0.00004	0.5	0.0001	0.07	1.7	<0.0003
R1 stream	0.706	0.0007	0.0008	8.3	0.002	1.3	30	<0.005
R3 stream	0.994	0.001	0.001	12	0.003	1.8	42	<0.008
R4 stream	0.705	0.0007	0.0007	8.3	0.002	1.3	30	<0.005

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in **bold**

For the intended application of ADM.00150.I.2.A in “pome/stone fruits” at 25 g a.s./ha (umbrella use IIb; Jun-Aug/ BBCH 71-PHI), the calculated PEC/RAC ratios indicate an acceptable risk from exposure to acetamiprid for all groups of aquatic organisms based on tier 1 endpoints and FOCUS Step 1-3 PEC values, except the higher tier mesocosm endpoint also covering the lower tier invertebrate endpoints. Additional PEC/RAC ratios were calculated considering FOCUS Step 4 PEC values.

Table 9.5-14: Aquatic organisms - acceptability of risk (PEC/RAC < 1) for acetamiprid for invertebrates incl. sed. dwellers, acute and prolonged based on FOCUS Step 4 calculations for the proposed application (25 g a.s./ha) of ADM.00150.I.2.A in ‘pome/stone fruit, late applications (umbrella use IIb; Jun-Aug/ BBCH 71-PHI)’

Intended use		Pome/stone fruit, late applications, umbrella use IIb; Jun-Aug/ BBCH 71-PHI							
Active substance		Acetamiprid							
Application rate (g/ha)		25							
FOCUS Step 4 PEC _{sw, max} (µg/L)									
Nozzle reduction	Non-sprayed buffer (m)	-	5	10	10	15	15	20	20
	Vegetated filter strip (m)	-	-	-	10	-	10 (VFSmod)	-	20
-	D3 ditch	--	0.620	0.277	0.277	0.140	0.140	0.086	0.086
50%		0.460	0.310	0.139	0.139	0.070	0.070	0.043	0.043
75%		0.230	0.155	0.069	0.069	0.035	0.035	0.021	0.021
90%		0.092	0.062	0.028	0.028	0.014	0.014	0.009	0.009
-	D4 pond	--	0.047	0.026	0.026	0.017	0.017	0.012	0.012
50%		0.021	0.024	0.013	0.013	0.008	0.008	0.006	0.006
75%		0.010	0.012	0.007	0.007	0.004	0.004	0.003	0.003
90%		0.004	0.005	0.003	0.003	0.002	0.002	0.001	0.001
-	D4 stream	--	0.704	0.314	0.314	0.159	0.159	0.097	0.097
50%		0.451	0.352	0.157	0.157	0.079	0.079	0.049	0.049
75%		0.225	0.176	0.079	0.079	0.040	0.040	0.024	0.024
90%		0.090	0.070	0.031	0.031	0.016	0.016	0.010	0.010
-	D5 pond	--	0.047	0.026	0.026	0.017	0.017	0.012	0.012
50%		0.021	0.024	0.013	0.013	0.008	0.008	0.006	0.006
75%		0.010	0.012	0.007	0.007	0.004	0.004	0.003	0.003
90%		0.004	0.005	0.003	0.003	0.002	0.002	0.001	0.001
-	D5 stream	--	0.777	0.347	0.347	0.175	0.175	0.107	0.107
50%		0.498	0.388	0.174	0.174	0.088	0.088	0.054	0.054
75%		0.249	0.194	0.087	0.087	0.044	0.044	0.027	0.027
90%		0.100	0.078	0.035	0.035	0.018	0.018	0.011	0.011
-	R1 pond	--	0.047	0.026	0.026	0.017	0.017	0.012	0.012
50%		0.021	0.024	0.013	0.013	0.008	0.008	0.006	0.006
75%		0.010	0.012	0.007	0.007	0.004	0.004	0.003	0.003
90%		0.004	0.005	0.003	0.003	0.002	0.002	0.001	0.001
-	R1 stream	--	0.551	0.246	0.246	0.124	0.124	0.076	0.076
50%		0.353	0.275	0.123	0.123	0.062	0.062	0.038	0.038
75%		0.176	0.138	0.062	0.062	0.031	0.031	0.019	0.019
90%		0.071	0.055	0.025	0.025	0.012	0.012	0.008	0.008
-	R2 stream	--	0.738	0.330	0.330	0.167	0.167	0.102	0.102
50%		0.473	0.369	0.165	0.165	0.083	0.083	0.051	0.051
75%		0.236	0.185	0.082	0.082	0.042	0.042	0.025	0.025
90%		0.095	0.074	0.033	0.033	0.017	0.017	0.010	0.010
-	R3 stream	--	0.776	0.347	0.347	0.175	0.175	0.107	0.107

Intended use		Pome/stone fruit, late applications, umbrella use IIb; Jun-Aug/ BBCH 71-PHI							
Active substance		Acetamiprid							
Application rate (g/ha)		25							
FOCUS Step 4 PEC _{sw, max} (µg/L)									
Nozzle reduction	Non-sprayed buffer (m)	-	5	10	10	15	15	20	20
	Vegetated filter strip (m)	-	-	-	10	-	10 (VFSmod)	-	20
50%	R4 stream	0.497	0.388	0.173	0.173	0.088	0.088	0.054	0.054
75%		0.249	0.194	0.087	0.087	0.044	0.044	0.027	0.027
90%		0.099	0.078	0.035	0.035	0.021	0.018	0.021	0.011
-		--	0.551	0.246	0.246	0.124	0.124	0.076	0.076
50%		0.353	0.275	0.123	0.123	0.062	0.062	0.059	0.038
75%		0.176	0.138	0.062	0.062	0.059	0.031	0.059	0.019
90%		0.071	0.059	0.059	0.025	0.059	0.024	0.059	0.012
Mesocosm (ETO); mean measured 0.56 µg/L		ETR (PEC/RAC)							
-	D3 ditch	--	1.1	0.5	0.5	0.3	0.3	0.2	0.2
50%		0.8	0.6	0.2	0.2	0.1	0.1	0.08	0.08
75%		0.4	0.3	0.1	0.1	0.06	0.06	0.04	0.04
90%		0.2	0.1	0.05	0.05	0.03	0.03	0.02	0.02
-	D4 stream	--	1.3	0.6	0.6	0.3	0.3	0.2	0.2
50%		0.8	0.6	0.3	0.3	0.1	0.1	0.09	0.09
75%		0.4	0.3	0.1	0.1	0.07	0.07	0.04	0.04
90%		0.2	0.1	0.06	0.06	0.03	0.03	0.02	0.02
-	D5 stream	--	1.4	0.6	0.6	0.3	0.3	0.2	0.2
50%		0.9	0.7	0.3	0.3	0.2	0.2	0.1	0.1
75%		0.4	0.3	0.2	0.2	0.08	0.08	0.05	0.05
90%		0.2	0.1	0.06	0.06	0.03	0.03	0.02	0.02
-	R1 stream	--	0.984	0.4	0.4	0.2	0.2	0.1	0.1
50%		0.6	0.5	0.2	0.2	0.1	0.1	0.07	0.07
75%		0.3	0.2	0.1	0.1	0.06	0.06	0.03	0.03
90%		0.1	0.1	0.04	0.04	0.02	0.02	0.01	0.01
-	R3 stream	--	1.4	0.6	0.6	0.3	0.3	0.2	0.2
50%		0.9	0.7	0.3	0.3	0.2	0.2	0.1	0.1
75%		0.4	0.3	0.2	0.2	0.08	0.08	0.05	0.05
90%		0.2	0.1	0.06	0.06	0.04	0.03	0.04	0.02
-	R4 stream	--	0.984	0.4	0.4	0.2	0.2	0.1	0.1
50%		0.6	0.5	0.2	0.2	0.1	0.1	0.1	0.07
75%		0.3	0.2	0.1	0.1	0.1	0.06	0.1	0.03
90%		0.1	0.1	0.1	0.04	0.1	0.04	0.1	0.02

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in **bold**

For the intended application of ADM.00150.I.2.A in “pome/stone fruits” at 25 g a.s./ha (umbrella use IIb; Jun-Aug/ BBCH 71-PHI), the calculated PEC/RAC ratios indicate an acceptable risk from exposure to acetamiprid if certain mitigation measures are considered. For the mesocosm study (ETO; mean measured; aquatic invertebrates, acute and sed. dwell. organisms, prolonged; RAC = 0.56 µg/L), a non-sprayed buffer zone of 10 m or 50% drift reducing nozzles should be considered.

Table 9.5-15: Aquatic organisms - acceptability of risk (PEC/RAC < 1) for acetamiprid for each organism group based on FOCUS Step 1 - 3 calculations for the proposed application (36 g a.s./ha) of ADM.00150.I.2.A in ‘potatoes (maize as surrogate for D5 & R4), early (umbrella use III; May-Sep / BBCH 12-79)’

Application (EC 5, 10, 100, 1000 µg/L) of FOCUS scenarios in potatoes (maize as surrogate for EC & R1), early (ammonia use 11, May) & late (BBCH 12-17)								
Group		Fish, acute	Fish, prolonged	Invertebrate, acute	Invertebrate, prolonged	Invertebrates incl. sed. dwellers, acute and prolonged	Sed. dwell. organisms, prolonged	Algae
Test Species		<i>C. variegatus</i>	<i>P. promelas</i>	Geomean, Invertebrate acute	<i>D. magna</i>	Mesocosm (ETO); mean measured	<i>C. riparius</i>	<i>A. flos-aquae</i>
Endpoint (µg/L)		LC ₅₀ 100000	EC ₁₀ 9400	EC ₅₀ 8.5	EC ₁₀ 2960	NOEC 1.12	EC ₁₀ 0.235	E _r C ₅₀ >1300
AF		100	10	100	10	2	10	10
RAC (µg/L)		1000	940	0.085	296	0.56	0.024	>130
FOCUS Scenario	PEC _{sw,max} (µg/L)	ETR (PEC/RAC)						
Step 1								
	10.839	0.01	0.01	128	0.04	19	461	<0.08
Step 2								
N-Europe	0.933	0.0009	0.001	11	0.003	1.7	40	<0.007
S-Europe	0.801	0.0008	0.0009	9.4	0.003	1.4	34	<0.006
Step 3								
D3 ditch	0.189	0.0002	0.0002	2.2	0.0006	0.3	8.0	<0.001
D4 pond	0.008	0.000008	0.000009	0.09	0.00003	0.01	0.3	<0.00006
D4 stream	0.161	0.0002	0.0002	1.9	0.0005	0.3	6.9	<0.001
D5 pond	0.008	0.000008	0.000009	0.09	0.00003	0.01	0.3	<0.00006
D5 stream	0.169	0.0002	0.0002	2.0	0.0006	0.3	7.0	<0.001
R1 pond	0.010	0.00001	0.00001	0.1	0.00003	0.02	0.4	<0.00008
R1 stream	0.165	0.0002	0.0002	1.9	0.0006	0.3	7.0	<0.001
R3 stream	0.209	0.0002	0.0002	2.5	0.0007	0.4	8.9	<0.002
R4 stream	0.331	0.0003	0.0004	3.9	0.0011	0.6	14	<0.003

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in **bold**

For the intended application of ADM.00150.I.2.A in “potatoes (maize as surrogate for D5 & R4)”, early at 36 g a.s./ha (umbrella use III; May-Sep / BBCH 12-79), the calculated PEC/RAC ratios indicate an acceptable risk from exposure to acetamiprid for all groups of aquatic organisms based on the higher tier mesocosm endpoint also covering the lower tier invertebrate endpoints and FOCUS Step 1-3 PEC values. Therefore, no further assessment is necessary.

Table 9.5-16: Aquatic organisms - acceptability of risk (PEC/RAC < 1) for acetamiprid for each organism group based on FOCUS Step 1 - 3 calculations for the proposed application (36 g a.s./ha) of ADM.00150.I.2.A in ‘potatoes (maize as surrogate for D5 & R4), late (umbrella use III; May-Sep / BBCH 12-79)’

Group		Fish, acute	Fish, prolonged	Invertebrate, acute	Invertebrate, prolonged	Invertebrates incl. sed. dwellers, acute and prolonged	Sed. dwell. organisms, prolonged	Algae
Test Species		<i>C. variegatus</i>	<i>P. promelas</i>	Geomean, Invertebrate acute	<i>D. magna</i>	Mesocosm (ETO); mean measured	<i>C. riparius</i>	<i>A. flos-aquae</i>
Endpoint (µg/L)		LC ₅₀ 100000	EC ₁₀ 9400	EC ₅₀ 8.5	EC ₁₀ 2960	NOEC 1.12	EC ₁₀ 0.235	E _r C ₅₀ >1300
AF		100	10	100	10	2	10	10
RAC (µg/L)		1000	940	0.085	296	0.56	0.024	>130
FOCUS Scenario	PEC _{sw,max} (µg/L)	ETR (PEC/RAC)						
Step 1								
	10.839	0.01	0.01	128	0.04	19	461	<0.08
Step 2								
N-Europe	0.933	0.0009	0.001	11	0.003	1.7	40	<0.007
S-Europe	0.801	0.0008	0.0009	9.4	0.003	1.4	34	<0.006
Step 3								
D3 ditch	0.189	0.0002	0.0002	2.2	0.0006	0.4	7.9	<0.002
D4 pond	0.008	0.000008	0.000009	0.09	0.00003	0.01	0.3	<0.00006
D4 stream	0.142	0.0001	0.0002	1.7	0.0005	0.3	5.9	<0.001
D5 pond	0.008	0.000008	0.000009	0.09	0.00003	0.01	0.3	<0.00006
D5 stream	0.185	0.0002	0.0002	2.2	0.0006	0.3	7.7	<0.001
R1 pond	0.024	0.00002	0.00003	0.3	0.00008	0.04	1.0	<0.0002
R1 stream	0.408	0.0004	0.0004	4.8	0.001	0.7	17	<0.003
R3 stream	0.185	0.0002	0.0002	2.2	0.0006	0.3	7.7	<0.001
R4 stream	0.509	0.0005	0.0005	6.0	0.002	0.9	21	<0.004

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in **bold**

For the intended application of ADM.00150.I.2.A in “potatoes (maize as surrogate for D5 & R4)”, late at 36 g a.s./ha (umbrella use III; May-Sep / BBCH 12-79), the calculated PEC/RAC ratios indicate an acceptable risk from exposure to acetamiprid for all groups of aquatic organisms based on the higher tier mesocosm endpoint also covering the lower tier invertebrate endpoints and FOCUS Step 1-3 PEC values. Therefore, no further assessment is necessary.

Table 9.5-17: Aquatic organisms - acceptability of risk (PEC/RAC < 1) for acetamiprid for each organism group based on FOCUS Step 1 - 3 calculations for the proposed application (35 +35 g a.s./ha) of ADM.00150.I.2.A in ‘spring cereals and winter cereals as surrogate for R1, R3 (umbrella use IVa; Mar-Jul / BBCH 40-69 (Spring))

Group		Fish, acute	Fish, prolonged	Invertebrate, acute	Invertebrate, prolonged	Invertebrates incl. sed. dwellers, acute and prolonged	Sed. dwell. organisms, prolonged	Algae
Test Species		<i>C. variegatus</i>	<i>P. promelas</i>	Geomean, Invertebrate acute	<i>D. magna</i>	Mesocosm (ETO); mean measured	<i>C. riparius</i>	<i>A. flos-aquae</i>
Endpoint (µg/L)		LC ₅₀ 100000	EC ₁₀ 9400	EC ₅₀ 8.5	EC ₁₀ 2960	NOEC 1.12	EC ₁₀ 0.235	E _r C ₅₀ >1300
AF		100	10	100	10	2	10	10
RAC (µg/L)		1000	940	0.085	296	0.56	0.024	>130
FOCUS Scenario	PEC _{sw,max} (µg/L)	ETR (PEC/RAC)						
Step 1								
	21.076	0.02	0.02	248	0.07	38	897	<0.2
Step 2								
N-Europe	0.644	0.0006	0.0007	7.6	0.002	1.2	27	<0.005
S-Europe	0.599	0.0006	0.0006	7.0	0.002	1.1	25	<0.005
Step 3								
D1 ditch	0.322	0.0003	0.0003	3.8	0.001	0.6	14	<0.002
D1 stream	0.170	0.0002	0.0002	2.0	0.0006	0.3	7.2	<0.001
D3 ditch	0.194	0.0002	0.0002	2.3	0.0007	0.3	8.3	<0.001
D4 pond	0.009	0.000009	0.00001	0.1	0.00003	0.02	0.4	<0.00007
D4 stream	0.165	0.0002	0.0002	1.9	0.0006	0.3	7.0	<0.001
D5 pond	0.011	0.00001	0.00001	0.1	0.00004	0.02	0.5	<0.00008
D5 stream	0.167	0.0002	0.0002	2.0	0.0006	0.3	7.1	<0.001
R4 stream	0.397	0.0004	0.0004	4.7	0.001	0.7	17	<0.003
R1 pond	0.039	0.00004	0.00004	0.5	0.0001	0.07	1.6	<0.0003
R1 stream	0.282	0.0003	0.0003	3.3	0.001	0.5	12	<0.002
R3 stream	0.604	0.0006	0.0006	7.1	0.002	1.08	25	<0.005

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in **bold**

For the intended application of ADM.00150.I.2.A in “spring cereals and winter cereals as surrogate for R1, R3” at 35 +35 g a.s./ha (umbrella use IVa; Mar-Jul / BBCH 40-69 (Spring)), ~~the calculated PEC/RAC ratios indicate an acceptable risk from exposure to acetamiprid for all groups of aquatic organisms based on the higher tier mesocosm endpoint also covering the lower tier invertebrate endpoints and FOCUS Step 1–3 PEC values. Therefore, no further assessment is necessary~~ - the calculated PEC/RAC ratios indicate an acceptable risk from exposure to acetamiprid for all groups of aquatic organisms based on tier 1 endpoints and FOCUS Step 1-3 PEC values, except the higher tier mesocosm endpoint also covering the lower tier invertebrate endpoints. Additional PEC/RAC ratios were calculated considering FOCUS Step 4 PEC values.

Table 9.5-18: Aquatic organisms - acceptability of risk (PEC/RAC < 1) for acetamiprid for invertebrates incl. sed. dwellers, acute and prolonged based on FOCUS Step 4 calculations for the proposed application (35 +35 g a.s./ha) of ADM.00150.I.2.A in ‘spring cereals and winter cereals as surrogate for R1, R3 (umbrella use IVa; Mar-Jul / BBCH 40-69 (Spring))’

Intended use		Spring cereals, umbrella use IVa; Mar-Jul / BBCH 40-69							
Active substance		Acetamiprid							
Application rate (g/ha)		35 +35							
FOCUS Step 4 PEC _{sw, max} (µg/L)									
Nozzle reduction	Non-sprayed buffer (m)	-	5	10	10	15	15	20	20
	Vegetated filter strip (m)	-	-	-	10	-	10 (VFSmod)	-	20
-	D3 ditch	-	0.050	0.026	0.026	0.018	0.018	0.013	0.013
50%		0.097	0.025	0.013	0.013	0.009	0.009	0.007	0.007
75%		0.049	0.013	0.007	0.007	0.004	0.004	0.003	0.003
90%		0.019	0.005	0.003	0.003	0.002	0.002	0.001	0.001
-	D4 pond	-	0.008	0.006	0.006	0.004	0.004	0.004	0.004
50%		0.005	0.004	0.003	0.003	0.002	0.002	0.002	0.002
75%		0.002	0.002	0.001	0.001	0.001	0.001	0.001	0.001
90%		0.001	0.001	0.001	0.001	< 0.001	< 0.001	< 0.001	< 0.001
-	D4 stream	-	0.058	0.030	0.030	0.020	0.020	0.015	0.015
50%		0.083	0.029	0.015	0.015	0.010	0.010	0.008	0.008
75%		0.041	0.015	0.008	0.008	0.005	0.005	0.004	0.004
90%		0.017	0.006	0.003	0.003	0.002	0.002	0.002	0.002
-	D5 pond	-	0.009	0.006	0.006	0.005	0.005	0.004	0.004
50%		0.005	0.005	0.003	0.003	0.003	0.003	0.002	0.002
75%		0.003	0.002	0.002	0.002	0.001	0.001	0.001	0.001
90%		0.001	0.001	0.001	0.001	0.001	0.001	< 0.001	< 0.001
-	D5 stream	-	0.059	0.031	0.031	0.021	0.021	0.016	0.016
50%		0.084	0.030	0.015	0.015	0.010	0.010	0.008	0.008
75%		0.042	0.015	0.008	0.008	0.005	0.005	0.004	0.004
90%		0.017	0.006	0.003	0.003	0.002	0.002	0.002	0.002
-	D3 ditch	-	0.050	0.026	0.026	0.018	0.018	0.013	0.013
50%		0.097	0.025	0.013	0.013	0.009	0.009	0.007	0.007
75%		0.049	0.013	0.007	0.007	0.004	0.004	0.003	0.003
90%		0.019	0.005	0.003	0.003	0.002	0.002	0.001	0.001
-	R1 pond (winter cereals as surrogate crop)	-	0.038	0.036	0.017	0.036	0.016	0.035	0.009
50%		0.036	0.035	0.034	0.015	0.034	0.015	0.034	0.008
75%		0.034	0.034	0.033	0.014	0.033	0.014	0.033	0.007
90%		0.033	0.033	0.033	0.013	0.032	0.013	0.032	0.007
-	R1 stream (winter cereals as surrogate crop)	-	0.282	0.282	0.128	0.282	0.128	0.282	0.067
50%		0.282	0.282	0.282	0.128	0.282	0.128	0.282	0.067
75%		0.282	0.282	0.282	0.128	0.282	0.128	0.282	0.067
90%		0.282	0.282	0.282	0.128	0.282	0.128	0.282	0.067

For the intended application of ADM.00150.I.2.A in “spring cereals and winter cereals as surrogate for R1, R3 (umbrella use IVa; Mar-Jul / BBCH 40-69 (Spring)), the calculated PEC/RAC ratios indicate an acceptable risk from exposure to acetamiprid if certain mitigation measures are considered. For the mesocosm study (ETO; mean measured; aquatic invertebrates, acute and sed. dwell. organisms, prolonged; RAC = 0.56 µg/L), a non-sprayed buffer zone of 10 m with vegetated filter strip of 10 m should be considered.

Group		Fish, acute	Fish, prolonged	Invertebrate, acute	Invertebrate, prolonged	Invertebrates incl. sed. dwellers, acute and prolonged	Sed. dwell. organisms, prolonged	Algae
Test Species		<i>C. variegatus</i>	<i>P. promelas</i>	Geomean, Invertebrate acute	<i>D. magna</i>	Mesocosm (ETO); mean measured	<i>C. riparius</i>	<i>A. flos-aquae</i>
Endpoint (µg/L)		LC ₅₀ 100000	EC ₁₀ 9400	EC ₅₀ 8.5	EC ₁₀ 2960	NOEC 1.12	EC ₁₀ 0.235	ErC ₅₀ >1300
AF		100	10	100	10	2	10	10
RAC (µg/L)		1000	940	0.085	296	0.56	0.024	>130
FOCUS Scenario	PEC _{sw,max} (µg/L)	ETR (PEC/RAC)						
Step 1								
	21.076	0.02	0.02	248	0.07	38	897	<0.2
Step 2								
N-Europe	0.492	0.0005	0.0005	5.8	0.002	0.9	21	<0.004
S-Europe	0.447	0.0004	0.0005	5.3	0.002	0.8	19	<0.003
Step 3								

Group		Fish, acute	Fish, prolonged	Invertebrate, acute	Invertebrate, prolonged	Invertebrates incl. sed. dwellers, acute and prolonged	Sed. dwell. organisms, prolonged	Algae
Test Species		<i>C. variegatus</i>	<i>P. promelas</i>	Geomean, Invertebrate acute	<i>D. magna</i>	Mesocosm (ETO); mean measured	<i>C. riparius</i>	<i>A. flos-aquae</i>
Endpoint (µg/L)		LC ₅₀ 100000	EC ₁₀ 9400	EC ₅₀ 8.5	EC ₁₀ 2960	NOEC 1.12	EC ₁₀ 0.235	E _r C ₅₀ >1300
AF		100	10	100	10	2	10	10
RAC (µg/L)		1000	940	0.085	296	0.56	0.024	>130
FOCUS Scenario	PEC _{sw,max} (µg/L)	ETR (PEC/RAC)						
D1 ditch	0.225	0.0002	0.0002	2.6	0.0008	0.4	9.6	<0.002
D1 stream	0.196	0.0002	0.0002	2.3	0.0007	0.4	8.3	<0.002
D3 ditch	0.222	0.0002	0.0002	2.6	0.0008	0.4	9.4	<0.002
D4 pond	0.008	0.000008	0.000009	0.09	0.00003	0.01	0.3	<0.00006
D4 stream	0.181	0.0002	0.0002	2.1	0.0006	0.3	7.7	<0.001
D5 pond	0.008	0.000008	0.000009	0.09	0.00003	0.01	0.3	<0.00006
D5 stream	0.193	0.0002	0.0002	2.3	0.0007	0.3	8.2	<0.001
R4 stream	0.397	0.0004	0.0004	4.7	0.001	0.7	17	<0.003
R1 pond	0.015	0.00002	0.00002	0.2	0.00005	0.03	0.6	<0.0001
R1 stream	0.267	0.0003	0.0003	3.1	0.0009	0.5	11	<0.002
R3 stream	0.206	0.0002	0.0002	2.4	0.0007	0.4	8.6	<0.002

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in **bold**

For the intended application of ADM.00150.I.2.A in “spring cereals and winter cereals as surrogate for R1, R3” at 35 g a.s./ha (umbrella use IVa; Mar-Jul / BBCH 40-69 (spring)), the calculated PEC/RAC ratios indicate an acceptable risk from exposure to acetamiprid for all groups of aquatic organisms based on the higher tier mesocosm endpoint also covering the lower tier invertebrate endpoints and FOCUS Step 1-3 PEC values. Therefore, no further assessment is necessary.

Table 9.5-20: Aquatic organisms - acceptability of risk (PEC/RAC < 1) for acetamiprid for each organism group based on FOCUS Step 1 - 3 calculations for the proposed application (35 +35 g a.s./ha) of ADM.00150.I.2.A in ‘spring cereals and winter cereals as surrogate for R1, R3 (umbrella use IVb; Mar-Jul / BBCH 12-69 (spring))’

Application (05-10-2015) of 100 g a.i./ha of 100 kg/ha in spring cereals and winter cereals as surrogate for RE, RE (unintended use FV3, Mar 04) / DDOH 12-07 (Spring)								
Group		Fish, acute	Fish, prolonged	Invertebrate, acute	Invertebrate, prolonged	Invertebrates incl. sed. dwellers, acute and prolonged	Sed. dwell. organisms, prolonged	Algae
Test Species		<i>C. variegatus</i>	<i>P. promelas</i>	Geomean, Invertebrate acute	<i>D. magna</i>	Mesocosm (ETO); mean measured	<i>C. riparius</i>	<i>A. flos-aquae</i>
Endpoint (µg/L)		LC ₅₀ 100000	EC ₁₀ 9400	EC ₅₀ 8.5	EC ₁₀ 2960	NOEC 1.12	EC ₁₀ 0.235	E _r C ₅₀ >1300
AF		100	10	100	10	2	10	10
RAC (µg/L)		1000	940	0.085	296	0.56	0.024	>130
FOCUS Scenario	PEC _{sw,max} (µg/L)	ETR (PEC/RAC)						
Step 1								
	21.076	0.02	0.02	248	0.07	38	897	<0.2
Step 2								
N-Europe	1.098	0.001	0.001	13	0.004	2.0	47	<0.008
S-Europe	0.947	0.0009	0.001	11	0.003	1.7	40	<0.007
Step 3								
D3 ditch	0.194	0.0002	0.0002	2.3	0.0007	0.3	8.3	<0.001
D4 pond	0.010	0.00001	0.00001	0.1	0.00003	0.02	0.4	<0.00008
D4 stream	0.163	0.0002	0.0002	1.9	0.0006	0.3	6.9	<0.001
D5 pond	0.010	0.00001	0.00001	0.1	0.00003	0.02	0.4	<0.00008
D5 stream	0.167	0.0002	0.0002	2.0	0.0006	0.3	7.1	<0.001
R4 stream	0.397	0.0004	0.0004	4.7	0.001	0.7	17	<0.003
R1 pond	0.010	0.00001	0.00001	0.1	0.00003	0.02	0.4	<0.00008
R1 stream	0.167	0.0002	0.0002	2.0	0.0006	0.3	7.0	<0.001
R3 stream	0.337	0.0003	0.0004	4.0	0.001	0.6	14	<0.003

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in **bold**

For the intended application of ADM.00150.I.2.A in “spring cereals and winter cereals as surrogate for R1, R3” at 35 +35 g a.s./ha (umbrella use IVb; Mar-Jul / BBCH 12-69 (spring)), the calculated PEC/RAC ratios indicate an acceptable risk from exposure to acetamiprid for all groups of aquatic organisms based on the higher tier mesocosm endpoint also covering the lower tier invertebrate endpoints and FOCUS Step 1-3 PEC values. Therefore, no further assessment is necessary.

Table 9.5-21: Aquatic organisms - acceptability of risk (PEC/RAC < 1) for acetamiprid for each organism group based on FOCUS Step 1 - 3 calculations for the proposed application (35 g a.s./ha) of ADM.00150.I.2.A in ‘spring cereals and winter cereals as surrogate for R1, R3 (umbrella use IVb; Mar-Jul / BBCH 12-69 (spring))’

Application (SS _g as/ha) of FOCUS 150150121 in spring cereals and winter cereals as surrogate for RE ₁ , RE ₂ (ambrosia use 1.0), and for DDO1 12.07 (spring)								
Group		Fish, acute	Fish, prolonged	Invertebrate, acute	Invertebrate, prolonged	Invertebrates incl. sed. dwellers, acute and prolonged	Sed. dwell. organisms, prolonged	Algae
Test Species		<i>C. variegatus</i>	<i>P. promelas</i>	Geomean, Invertebrate acute	<i>D. magna</i>	Mesocosm (ETO); mean measured	<i>C. riparius</i>	<i>A. flos-aquae</i>
Endpoint (µg/L)		LC ₅₀ 100000	EC ₁₀ 9400	EC ₅₀ 8.5	EC ₁₀ 2960	NOEC 1.12	EC ₁₀ 0.235	E _r C ₅₀ >1300
AF		100	10	100	10	2	10	10
RAC (µg/L)		1000	940	0.085	296	0.56	0.024	>130
FOCUS Scenario	PEC _{sw,max} (µg/L)	ETR (PEC/RAC)						
Step 1								
	21.076	0.02	0.02	248	0.07	38	897	<0.2
Step 2								
N-Europe	1.020	0.001	0.001	12	0.003	1.8	43	<0.008
S-Europe	0.869	0.0009	0.0009	10	0.003	1.6	37	<0.007
Step 3								
D3 ditch	0.222	0.0002	0.0002	2.6	0.0008	0.4	9.4	<0.002
D4 pond	0.008	0.000008	0.000009	0.09	0.00003	0.01	0.3	<0.00006
D4 stream	0.170	0.0002	0.0002	2.0	0.0006	0.3	7.2	<0.001
D5 pond	0.008	0.000008	0.000009	0.09	0.00003	0.01	0.3	<0.00006
D5 stream	0.176	0.0002	0.0002	2.1	0.0006	0.3	7.5	<0.001
R4 stream	0.146	0.0001	0.0002	1.7	0.0005	0.3	6.2	<0.001
R1 pond	0.008	0.000008	0.000009	0.09	0.00003	0.01	0.3	<0.00006
R1 stream	0.146	0.0001	0.0002	1.7	0.0005	0.3	6.1	<0.001
R3 stream	0.205	0.0002	0.0002	2.4	0.0007	0.4	8.5	<0.002

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in **bold**

For the intended application of ADM.00150.I.2.A in “spring cereals and winter cereals as surrogate for R1, R3” at 35 g a.s./ha (umbrella use IVb; Mar-Jul / BBCH 12-69 (spring)), the calculated PEC/RAC ratios indicate an acceptable risk from exposure to acetamiprid for all groups of aquatic organisms based on the higher tier mesocosm endpoint also covering the lower tier invertebrate endpoints and FOCUS Step 1-3 PEC values. Therefore, no further assessment is necessary.

Table 9.5-22: Aquatic organisms - acceptability of risk (PEC/RAC < 1) for acetamiprid for each organism group based on FOCUS Step 1 - 3 calculations for the proposed application (36 +36 g a.s./ha) of ADM.00150.I.2.A in ‘cereals, winter (umbrella use Va; Aug-Nov / BBCH 40-69)’

Application (50-150 g a.i.s./ha) of 100/100/100/120/140 g EC emuls, winter (ambrosia use v.a.; Aug-Nov./DEC/14-15)								
Group		Fish, acute	Fish, prolonged	Invertebrate, acute	Invertebrate, prolonged	Invertebrates incl. sed. dwellers, acute and prolonged	Sed. dwell. organisms, prolonged	Algae
Test Species		<i>C. variegatus</i>	<i>P. promelas</i>	Geomean, Invertebrate acute	<i>D. magna</i>	Mesocosm (ETO); mean measured	<i>C. riparius</i>	<i>A. flos-aquae</i>
Endpoint (µg/L)		LC ₅₀ 100000	EC ₁₀ 9400	EC ₅₀ 8.5	EC ₁₀ 2960	NOEC 1.12	EC ₁₀ 0.235	E _r C ₅₀ >1300
AF		100	10	100	10	2	10	10
RAC (µg/L)		1000	940	0.085	296	0.56	0.024	>130
FOCUS Scenario	PEC _{sw,max} (µg/L)	ETR (PEC/RAC)						
Step 1								
	21.678	0.02	0.02	255	0.07	39	922	<0.2
Step 2								
N-Europe	0.663	0.0007	0.0007	7.8	0.002	1.2	28	<0.005
S-Europe	0.616	0.0006	0.0007	7.2	0.002	1.1	26	<0.005
Step 3								
D3 ditch	0.200	0.0002	0.0002	2.4	0.0007	0.4	8.5	<0.002
D4 pond	0.012	0.00001	0.00001	0.1	0.00004	0.02	0.5	<0.00009
D4 stream	0.157	0.0002	0.0002	1.8	0.0005	0.3	6.7	<0.001
D5 pond	0.011	0.00001	0.00001	0.1	0.00004	0.02	0.5	<0.00008
D5 stream	0.174	0.0002	0.0002	2.0	0.0006	0.3	7.4	<0.001
R1 pond	0.040	0.00004	0.00004	0.5	0.0001	0.07	1.7	<0.0003
R1 stream	0.291	0.0003	0.0003	3.4	0.001	0.5	12	<0.002
R3 stream	0.623	0.0006	0.0007	7.3	0.002	1.1	27	<0.005
R4 stream	1.219	0.001	0.001	14	0.004	2.2	52	<0.009

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in **bold**

For the intended application of ADM.00150.I.2.A in “winter cereals” at 36 +36 g a.s./ha (umbrella use Va; Aug-Nov / BBCH 40-69), the calculated PEC/RAC ratios indicate an acceptable risk from exposure to acetamiprid for all groups of aquatic organisms based on tier 1 endpoints and FOCUS Step 1-3 PEC values, except the higher tier mesocosm endpoint also covering the lower tier invertebrate endpoints. Additional PEC/RAC ratios were calculated considering FOCUS Step 4 PEC values.

Table 9.5-23: Aquatic organisms - acceptability of risk (PEC/RAC < 1) for acetamiprid for invertebrates incl. sed. dwellers, acute and prolonged based on FOCUS Step 4 calculations for the proposed application (36 +36 g a.s./ha) of ADM.00150.I.2.A in ‘cereals, winter (umbrella use Va; Aug-Nov / BBCH 40-69)’

Intended use		Cereals, winter, umbrella use Va; Aug-Nov / BBCH 40-69							
Active substance		Acetamiprid							
Application rate (g/ha)		36 +36							
FOCUS Step 4 PEC _{sw, max} (µg/L)									
Nozzle reduction	Non-sprayed buffer (m)	-	5	10	10	15	15	20	20
	Vegetated filter strip (m)	-	-	-	10	-	10 (VFSmod)	-	20
-	D1 ditch	--	0.054	0.028	0.028	0.019	0.019	0.014	0.014
50%		0.104	0.027	0.014	0.014	0.009	0.009	0.007	0.007
75%		0.052	0.013	0.007	0.007	0.005	0.005	0.004	0.004
90%		0.021	0.005	0.003	0.003	0.002	0.002	0.001	0.001
-	D1 stream	--	0.061	0.032	0.032	0.021	0.021	0.016	0.016
50%		0.086	0.031	0.016	0.016	0.011	0.011	0.008	0.008
75%		0.043	0.015	0.008	0.008	0.005	0.005	0.004	0.004
90%		0.017	0.006	0.003	0.003	0.002	0.002	0.002	0.002
-	D2 ditch	--	0.052	0.027	0.027	0.018	0.018	0.014	0.014
50%		0.101	0.026	0.014	0.014	0.009	0.009	0.007	0.007
75%		0.051	0.013	0.007	0.007	0.005	0.005	0.003	0.003
90%		0.020	0.005	0.003	0.003	0.002	0.002	0.001	0.001
-	D2 stream	--	0.062	0.032	0.032	0.022	0.022	0.016	0.016
50%		0.088	0.031	0.016	0.016	0.011	0.011	0.008	0.008
75%		0.044	0.016	0.008	0.008	0.005	0.005	0.004	0.004
90%		0.018	0.006	0.003	0.003	0.002	0.002	0.002	0.002
-	D3 ditch	--	0.052	0.027	0.027	0.018	0.018	0.014	0.014
50%		0.100	0.026	0.013	0.013	0.009	0.009	0.007	0.007
75%		0.050	0.013	0.007	0.007	0.005	0.005	0.003	0.003
90%		0.020	0.005	0.003	0.003	0.002	0.002	0.001	0.001
-	D4 pond	--	0.010	0.007	0.007	0.006	0.006	0.005	0.005
50%		0.006	0.005	0.004	0.004	0.003	0.003	0.002	0.002
75%		0.003	0.003	0.002	0.002	0.001	0.001	0.001	0.001
90%		0.001	0.001	0.001	0.001	0.001	0.001	0.000	0.000
-	D4 stream	--	0.056	0.029	0.029	0.019	0.019	0.015	0.015
50%		0.079	0.028	0.014	0.014	0.010	0.010	0.007	0.007
75%		0.039	0.014	0.007	0.007	0.005	0.005	0.004	0.004
90%		0.016	0.006	0.003	0.003	0.002	0.002	0.001	0.001
-	D5 pond	--	0.010	0.007	0.007	0.005	0.005	0.005	0.005
50%		0.006	0.005	0.003	0.003	0.003	0.003	0.002	0.002
75%		0.003	0.002	0.002	0.002	0.001	0.001	0.001	0.001
90%		0.001	0.001	0.001	0.001	0.001	0.001	0.000	0.000
-	D5 stream	--	0.061	0.032	0.032	0.022	0.022	0.016	0.016

Intended use		Cereals, winter, umbrella use Va; Aug-Nov / BBCH 40-69							
Active substance		Acetamiprid							
Application rate (g/ha)		36 +36							
FOCUS Step 4 PEC _{sw, max} (µg/L)									
Nozzle reduction	Non-sprayed buffer (m)	-	5	10	10	15	15	20	20
	Vegetated filter strip (m)	-	-	-	10	-	10 (VFSmod)	-	20
50%		0.087	0.031	0.016	0.016	0.011	0.011	0.008	0.008
75%		0.043	0.015	0.008	0.008	0.005	0.005	0.004	0.004
90%		0.017	0.006	0.003	0.003	0.002	0.002	0.002	0.002
-	D6 ditch	--	0.052	0.027	0.027	0.018	0.018	0.014	0.014
50%		0.100	0.026	0.014	0.014	0.009	0.009	0.007	0.007
75%		0.050	0.013	0.007	0.007	0.005	0.005	0.003	0.003
90%		0.020	0.005	0.003	0.003	0.002	0.002	0.001	0.001
-	R1 pond	--	0.039	0.038	0.018	0.037	0.017	0.036	0.010
50%		0.037	0.036	0.035	0.016	0.035	0.015	0.035	0.008
75%		0.035	0.035	0.034	0.015	0.034	0.014	0.034	0.007
90%		0.034	0.034	0.034	0.014	0.034	0.014	0.033	0.007
-	R1 stream	--	0.291	0.291	0.132	0.291	0.132	0.291	0.069
50%		0.291	0.291	0.291	0.132	0.291	0.132	0.291	0.069
75%		0.291	0.291	0.291	0.132	0.291	0.132	0.291	0.069
90%		0.291	0.291	0.291	0.132	0.291	0.132	0.291	0.069
-	R3 stream	--	0.623	0.623	0.284	0.623	0.284	0.623	0.149
50%		0.623	0.623	0.623	0.284	0.623	0.284	0.623	0.149
75%		0.623	0.623	0.623	0.284	0.623	0.284	0.623	0.149
90%		0.623	0.623	0.623	0.284	0.623	0.284	0.623	0.149
-	R4 stream	--	1.219	1.219	0.555	1.219	0.555	1.219	0.291
50%		1.219	1.219	1.219	0.555	1.219	0.555	1.219	0.291
75%		1.219	1.219	1.219	0.555	1.219	0.555	1.219	0.291
90%		1.219	1.219	1.219	0.555	1.219	0.555	1.219	0.291
Mesocosm (ETO); mean measured 0.56 µg/L		ETR (PEC/RAC)							
-	R3 stream	--	1.1	1.1	0.5	1.1	0.5	1.1	0.3
50%		1.1	1.1	1.1	0.5	1.1	0.5	1.1	0.3
75%		1.1	1.1	1.1	0.5	1.1	0.5	1.1	0.3
90%		1.1	1.1	1.1	0.5	1.1	0.5	1.1	0.3
-	R4 stream	--	2.2	2.2	0.991	2.2	0.991	2.2	0.5
50%		2.2	2.2	2.2	0.991	2.2	0.991	2.2	0.5
75%		2.2	2.2	2.2	0.991	2.2	0.991	2.2	0.5
90%		2.2	2.2	2.2	0.991	2.2	0.991	2.2	0.5

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in **bold**

For the intended application of ADM.00150.I.2.A in “winter cereals” at 36 +36 g a.s./ha (umbrella use Va; Aug-Nov / BBCH 40-69), the calculated PEC/RAC ratios indicate an acceptable risk from exposure to acetamiprid if certain mitigation measures are considered. For the mesocosm study (ETO; mean measured; aquatic invertebrates, acute and sed. dwell. organisms, prolonged, RAC = 0.56 µg/L), a non-sprayed buffer of 10 m in combination with a vegetated filter strip of 10 m should be considered.

Table 9.5-24: Aquatic organisms - acceptability of risk (PEC/RAC < 1) for acetamiprid for each organism group based on FOCUS Step 1 - 3 calculations for the proposed application (36 g a.s./ha) of ADM.00150.I.2.A in ‘cereals, winter (umbrella use Va; Aug-Nov / BBCH 40-69)’

Group		Fish, acute	Fish, prolonged	Invertebrate, acute	Invertebrate, prolonged	Invertebrates incl. sed. dwellers, acute and prolonged	Sed. dwell. organisms, prolonged	Algae
Test Species		<i>C. variegatus</i>	<i>P. promelas</i>	Geomean, Invertebrate acute	<i>D. magna</i>	Mesocosm (ETO); mean measured	<i>C. riparius</i>	<i>A. flos-aquae</i>
Endpoint (µg/L)		LC ₅₀ 100000	EC ₁₀ 9400	EC ₅₀ 8.5	EC ₁₀ 2960	NOEC 1.12	EC ₁₀ 0.235	E _r C ₅₀ >1300
AF		100	10	100	10	2	10	10
RAC (µg/L)		1000	940	0.085	296	0.56	0.024	>130
FOCUS Scenario	PEC _{sw,max} (µg/L)	ETR (PEC/RAC)						
Step 1								
	21.678	0.02	0.02	255	0.07	39	922	<0.2
Step 2								
N-Europe	0.506	0.0005	0.0005	6.0	0.002	0.9	22	<0.004
S-Europe	0.459	0.0005	0.0005	5.4	0.002	0.8	20	<0.004
Step 3								
D3 ditch	0.228	0.0002	0.0002	2.7	0.0008	0.4	9.7	<0.002
D4 pond	0.008	0.000008	0.000009	0.09	0.00003	0.01	0.3	<0.00006
D4 stream	0.174	0.0002	0.0002	2.0	0.0006	0.3	7.4	<0.001
D5 pond	0.008	0.000008	0.000009	0.09	0.00003	0.01	0.3	<0.00006
D5 stream	0.182	0.0002	0.0002	2.1	0.0006	0.3	7.7	<0.001
R1 pond	0.016	0.00002	0.00002	0.2	0.00005	0.03	0.7	<0.0001
R1 stream	0.275	0.0003	0.0003	3.2	0.0009	0.5	12	<0.002
R3 stream	0.212	0.0002	0.0002	2.5	0.0007	0.4	9.0	<0.002
R4 stream	0.224	0.0002	0.0002	2.6	0.0008	0.4	9.5	<0.002

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in **bold**

For the intended application of ADM.00150.I.2.A in “winter cereals” at 36 g a.s./ha (umbrella use Va; Aug-Nov / BBCH 40-69), the calculated PEC/RAC ratios indicate an acceptable risk from exposure to acetamiprid for all groups of aquatic organisms based on the higher tier mesocosm endpoint also covering the lower tier invertebrate endpoints and FOCUS Step 1-3 PEC values. Therefore, no further assessment is necessary.

Table 9.5-25: Aquatic organisms - acceptability of risk (PEC/RAC < 1) for acetamiprid for each organism group based on FOCUS Step 1 - 3 calculations for the proposed application (30 g a.s./ha) of ADM.00150.I.2.A in ‘cereals, winter (umbrella use Vb; Aug-Nov; BBCH 12-29)’

Application (Soil, air, water) of FOCUS models for cereals, winter (ambrosia use v3; Aug. 1997; BBOX 12-27)								
Group		Fish, acute	Fish, prolonged	Invertebrate, acute	Invertebrate, prolonged	Invertebrates incl. sed. dwellers, acute and prolonged	Sed. dwell. organisms, prolonged	Algae
Test Species		<i>C. variegatus</i>	<i>P. promelas</i>	Geomean, Invertebrate acute	<i>D. magna</i>	Mesocosm (ETO); mean measured	<i>C. riparius</i>	<i>A. flos-aquae</i>
Endpoint (µg/L)		LC ₅₀ 100000	EC ₁₀ 9400	EC ₅₀ 8.5	EC ₁₀ 2960	NOEC 1.12	EC ₁₀ 0.235	E _r C ₅₀ >1300
AF		100	10	100	10	2	10	10
RAC (µg/L)		1000	940	0.085	296	0.56	0.024	>130
FOCUS Scenario	PEC _{sw,max} (µg/L)	ETR (PEC/RAC)						
Step 1								
	9.033	0.009	0.01	106	0.03	16	384	<0.07
Step 2								
N-Europe	0.874	0.0009	0.0009	10	0.003	1.6	37	<0.007
S-Europe	0.745	0.0007	0.0008	8.8	0.003	1.3	32	<0.006
Step 3								
D3 ditch	0.189	0.0002	0.0002	2.2	0.0006	0.3	8.0	<0.001
D4 pond	0.007	0.000007	0.000007	0.08	0.00002	0.01	0.3	<0.00005
D4 stream	0.164	0.0002	0.0002	1.9	0.0006	0.3	7.0	<0.001
D5 pond	0.007	0.000007	0.000007	0.08	0.00002	0.01	0.3	<0.00005
D5 stream	0.177	0.0002	0.0002	2.1	0.0006	0.3	7.5	<0.001
R1 pond	0.011	0.00001	0.00001	0.1	0.00004	0.02	0.5	<0.00008
R1 stream	0.467	0.0005	0.0005	5.5	0.002	0.8	20	<0.004
R3 stream	0.966	0.001	0.001	11	0.003	1.7	41	<0.007
R4 stream	0.198	0.0002	0.0002	2.3	0.0007	0.4	8.4	<0.002

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in **bold**

For the intended application of ADM.00150.I.2.A in “winter cereals” at 30 g a.s./ha (umbrella use Vb; Aug-Nov; BBCH 12-29), the calculated PEC/RAC ratios indicate an acceptable risk from exposure to acetamiprid for all groups of aquatic organisms based on tier 1 endpoints and FOCUS Step 1-3 PEC values, except the higher tier mesocosm endpoint also covering the lower tier invertebrate endpoints. Additional PEC/RAC ratios were calculated considering FOCUS Step 4 PEC values.

Table 9.5-26: Aquatic organisms - acceptability of risk (PEC/RAC < 1) for acetamiprid for invertebrates incl. sed. dwellers, acute and prolonged based on FOCUS Step 4 calculations for the proposed application (30 g a.s./ha) of ADM.00150.I.2.A in ‘cereals, winter (umbrella use Vb; Aug-Nov; BBCH 12-29)’

Intended use		Cereals, winter, umbrella use Vb; Aug-Nov; BBCH 12-29							
Active substance		Acetamiprid							
Application rate (g/ha)		30							
FOCUS Step 4 PEC _{sw, max} (µg/L)									
Nozzle reduction	Non-sprayed buffer (m)	-	5	10	10	15	15	20	20
	Vegetated filter strip (m)	-	-	-	10	-	10 (VFSmod)	-	20
-	D1 ditch	--	0.052	0.037	0.037	0.037	0.037	0.037	0.037
50%		0.096	0.037	0.037	0.037	0.037	0.037	0.037	0.037
75%		0.048	0.037	0.037	0.037	0.037	0.037	0.037	0.037
90%		0.037	0.037	0.037	0.037	0.037	0.037	0.037	0.037
-	D1 stream	--	0.061	0.033	0.033	0.026	0.026	0.026	0.026
50%		0.084	0.031	0.026	0.026	0.026	0.026	0.026	0.026
75%		0.042	0.026	0.026	0.026	0.026	0.026	0.026	0.026
90%		0.026	0.026	0.026	0.026	0.026	0.026	0.026	0.026
-	D2 ditch	--	0.160	0.160	0.160	0.160	0.160	0.160	0.160
50%		0.160	0.160	0.160	0.160	0.160	0.160	0.160	0.160
75%		0.160	0.160	0.160	0.160	0.160	0.160	0.160	0.160
90%		0.160	0.160	0.160	0.160	0.160	0.160	0.160	0.160
-	D2 stream	--	0.101	0.101	0.101	0.101	0.101	0.101	0.101
50%		0.101	0.101	0.101	0.101	0.101	0.101	0.101	0.101
75%		0.101	0.101	0.101	0.101	0.101	0.101	0.101	0.101
90%		0.101	0.101	0.101	0.101	0.101	0.101	0.101	0.101
-	D3 ditch	--	0.051	0.027	0.027	0.019	0.019	0.014	0.014
50%		0.095	0.026	0.014	0.014	0.009	0.009	0.007	0.007
75%		0.047	0.013	0.007	0.007	0.005	0.005	0.004	0.004
90%		0.019	0.005	0.003	0.003	0.002	0.002	0.001	0.001
-	D4 pond	--	0.006	0.004	0.004	0.003	0.003	0.003	0.003
50%		0.003	0.003	0.002	0.002	0.002	0.002	0.001	0.001
75%		0.002	0.001	0.001	0.001	0.001	0.001	0.001	0.001
90%		0.001	0.001	0.000	0.000	0.000	0.000	0.000	0.000
-	D4 stream	--	0.060	0.032	0.032	0.022	0.022	0.017	0.017
50%		0.082	0.030	0.016	0.016	0.011	0.011	0.008	0.008
75%		0.041	0.015	0.008	0.008	0.005	0.005	0.004	0.004
90%		0.016	0.006	0.003	0.003	0.002	0.002	0.002	0.002
-	D5 pond	--	0.006	0.004	0.004	0.003	0.003	0.003	0.003
50%		0.003	0.003	0.002	0.002	0.002	0.002	0.001	0.001
75%		0.002	0.001	0.001	0.001	0.001	0.001	0.001	0.001
90%		0.001	0.001	0.000	0.000	0.000	0.000	0.000	0.000
-	D5 stream	--	0.065	0.034	0.034	0.023	0.023	0.018	0.018

Intended use		Cereals, winter, umbrella use Vb; Aug-Nov; BBCH 12-29							
Active substance		Acetamiprid							
Application rate (g/ha)		30							
FOCUS Step 4 PEC _{sw, max} (µg/L)									
Nozzle reduction	Non-sprayed buffer (m)	-	5	10	10	15	15	20	20
	Vegetated filter strip (m)	-	-	-	10	-	10 (VFSmod)	-	20
50%		0.089	0.032	0.017	0.017	0.012	0.012	0.009	0.009
75%		0.044	0.016	0.009	0.009	0.006	0.006	0.004	0.004
90%		0.018	0.006	0.003	0.003	0.002	0.002	0.002	0.002
-		--	0.052	0.044	0.044	0.044	0.044	0.044	0.044
50%	D6 ditch	0.096	0.044	0.044	0.044	0.044	0.044	0.044	0.044
75%		0.048	0.044	0.044	0.044	0.044	0.044	0.044	0.044
90%		0.044	0.044	0.044	0.044	0.044	0.044	0.044	0.044
-		--	0.010	0.008	0.005	0.008	0.005	0.007	0.003
50%	R1 pond	0.008	0.007	0.007	0.004	0.006	0.003	0.006	0.002
75%		0.006	0.006	0.006	0.003	0.006	0.003	0.006	0.002
90%		0.006	0.005	0.005	0.002	0.005	0.002	0.005	0.001
-		--	0.467	0.467	0.209	0.467	0.209	0.467	0.109
50%	R1 stream	0.467	0.467	0.467	0.209	0.467	0.209	0.467	0.109
75%		0.467	0.467	0.467	0.209	0.467	0.209	0.467	0.109
90%		0.467	0.467	0.467	0.209	0.467	0.209	0.467	0.109
-		--	0.966	0.966	0.436	0.966	0.436	0.966	0.228
50%	R3 stream	0.966	0.966	0.966	0.436	0.966	0.436	0.966	0.228
75%		0.966	0.966	0.966	0.436	0.966	0.436	0.966	0.228
90%		0.966	0.966	0.966	0.436	0.966	0.436	0.966	0.228
-		--	0.198	0.198	0.090	0.198	0.090	0.198	0.047
50%	R4 stream	0.198	0.198	0.198	0.090	0.198	0.090	0.198	0.047
75%		0.198	0.198	0.198	0.090	0.198	0.090	0.198	0.047
90%		0.198	0.198	0.198	0.09	0.198	0.09	0.198	0.047
Mesocosm (ETO); mean measured 0.56 µg/L		ETR (PEC/RAC)							
-	R3 stream	--	1.7	1.7	0.8	1.7	0.8	1.7	0.4
50%		1.7	1.7	1.7	0.8	1.7	0.8	1.7	0.4
75%		1.7	1.7	1.7	0.8	1.7	0.8	1.7	0.4
90%		1.7	1.7	1.7	0.8	1.7	0.8	1.7	0.4

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in **bold**

For the intended application of ADM.00150.I.2.A in “winter cereals” at 30 g a.s./ha (umbrella use Vb; Aug-Nov; BBCH 12-29), the calculated PEC/RAC ratios indicate an acceptable risk from exposure to acetamiprid if certain mitigation measures are considered. For the mesocosm study (ETO; mean measured; aquatic

invertebrates, acute and sed. dwell. organisms, prolonged, RAC = 0.56 µg/L), a non-sprayed buffer of 10 m in combination with a vegetated filter strip of 10 m should be considered.

Table 9.5-27: Aquatic organisms - acceptability of risk (PEC/RAC < 1) for acetamiprid for each organism group based on FOCUS Step 1 - 3 calculations for the proposed application (60 +60 g a.s./ha) of ADM.00150.I.2.A in ‘oilseed rape (winter cereals as surrogate for R4), winter (umbrella use VIa; Mar-Jun/ BBCH 31-71)’

Group		Fish, acute	Fish, prolonged	Invertebrate, acute	Invertebrate, prolonged	Invertebrates incl. sed. dwellers, acute and prolonged	Sed. dwell. organisms, prolonged	Algae
Test Species		<i>C. variegatus</i>	<i>P. promelas</i>	Geomean, Invertebrate acute	<i>D. magna</i>	Mesocosm (ETO); mean measured	<i>C. riparius</i>	<i>A. flos-aquae</i>
Endpoint (µg/L)		LC ₅₀ 100000	EC ₁₀ 9400	EC ₅₀ 8.5	EC ₁₀ 2960	NOEC 1.12	EC ₁₀ 0.235	E _r C ₅₀ >1300
AF		100	10	100	10	2	10	10
RAC (µg/L)		1000	940	0.085	296	0.56	0.024	>130
FOCUS Scenario	PEC _{sw,max} (µg/L)	ETR (PEC/RAC)						
Step 1								
	36.130	0.04	0.04	425	0.1	65	1537	<0.3
Step 2								
N-Europe	1.140	0.001	0.001	13	0.004	2.0	49	<0.009
S-Europe	1.059	0.001	0.001	12	0.004	1.9	45	<0.008
Step 3								
D2 ditch	0.337	0.0003	0.0004	4.0	0.001	0.6	14	<0.003
D2 stream	0.296	0.0003	0.0003	3.5	0.001	0.5	13	<0.002
D3 ditch	0.332	0.0003	0.0004	3.9	0.001	0.6	14	<0.003
D4 pond	0.019	0.00002	0.00002	0.2	0.00006	0.03	0.8	<0.0001
D4 stream	0.245	0.0002	0.0003	2.9	0.0008	0.4	10	<0.002
D5 pond	0.017	0.00002	0.00002	0.2	0.00006	0.03	0.7	<0.0001
D5 stream	0.266	0.0003	0.0003	3.1	0.0009	0.5	11	<0.002
R1 pond	0.024	0.00002	0.00003	0.3	0.00008	0.04	1.02	<0.0002
R1 stream	0.686	0.0007	0.0007	8.1	0.002	1.2	29	<0.005
R3 stream	0.404	0.0004	0.0004	4.8	0.001	0.7	17	<0.003
R4 stream	0.401	0.0004	0.0004	4.7	0.001	0.7	17	<0.003

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in **bold**

For the intended application of ADM.00150.I.2.A in “winter oilseed rape (winter cereals as surrogate for R4)” at 60 +60 g a.s./ha (umbrella use VIa; Mar-Jun/ BBCH 31-71), the calculated PEC/RAC ratios indicate an acceptable risk from exposure to acetamiprid for all groups of aquatic organisms based on tier 1 endpoints and FOCUS Step 1-3 PEC values, except the higher tier mesocosm endpoint also covering the lower tier invertebrate endpoints. Additional PEC/RAC ratios were calculated considering FOCUS Step 4 PEC values.

Table 9.5-28: Aquatic organisms - acceptability of risk (PEC/RAC < 1) for acetamiprid for invertebrates incl. sed. dwellers, acute and prolonged based on FOCUS Step 4 calculations for the proposed application (60 +60 g a.s./ha) of ADM.00150.I.2.A in ‘oilseed rape (winter cereals as surrogate crop for R4), winter (umbrella use VIa; Mar-Jun/ BBCH 31-71)’

Intended use		Oilseed rape, winter, umbrella use VIa; Mar-Jun/ BBCH 31-71							
Active substance		Acetamiprid							
Application rate (g/ha)		60 +60							
FOCUS Step 4 PEC _{sw, max} (µg/L)									
Nozzle reduction	Non-sprayed buffer (m)	-	5	10	10	15	15	20	20
	Vegetated filter strip (m)	-	-	-	10	-	10 (VFSmod)	-	20
-	D2 ditch	--	0.134	0.134	0.134	0.134	0.134	0.134	0.134
50%		0.168	0.134	0.134	0.134	0.134	0.134	0.134	0.134
75%		0.134	0.134	0.134	0.134	0.134	0.134	0.134	0.134
90%		0.134	0.134	0.134	0.134	0.134	0.134	0.134	0.134
-	D2 stream	--	0.105	0.092	0.092	0.092	0.092	0.092	0.092
50%		0.148	0.092	0.092	0.092	0.092	0.092	0.092	0.092
75%		0.092	0.092	0.092	0.092	0.092	0.092	0.092	0.092
90%		0.092	0.092	0.092	0.092	0.092	0.092	0.092	0.092
-	D3 ditch	--	0.086	0.045	0.045	0.030	0.030	0.023	0.023
50%		0.166	0.043	0.022	0.022	0.015	0.015	0.011	0.011
75%		0.083	0.022	0.011	0.011	0.008	0.008	0.006	0.006
90%		0.033	0.009	0.004	0.004	0.003	0.003	0.002	0.002
-	D4 pond	--	0.016	0.011	0.011	0.009	0.009	0.008	0.008
50%		0.009	0.008	0.006	0.006	0.005	0.005	0.004	0.004
75%		0.005	0.004	0.003	0.003	0.002	0.002	0.002	0.002
90%		0.002	0.002	0.001	0.001	0.001	0.001	0.001	0.001
-	D4 stream	--	0.087	0.045	0.045	0.030	0.030	0.023	0.023
50%		0.123	0.043	0.023	0.023	0.015	0.015	0.011	0.011
75%		0.061	0.022	0.011	0.011	0.008	0.008	0.006	0.006
90%		0.025	0.009	0.005	0.005	0.003	0.003	0.002	0.002
-	D5 pond	--	0.014	0.010	0.010	0.008	0.008	0.007	0.007
50%		0.008	0.007	0.005	0.005	0.004	0.004	0.003	0.003
75%		0.004	0.004	0.003	0.003	0.002	0.002	0.002	0.002
90%		0.002	0.001	0.001	0.001	0.001	0.001	0.001	0.001
-	D5 stream	--	0.094	0.049	0.049	0.033	0.033	0.025	0.025
50%		0.133	0.047	0.024	0.024	0.016	0.016	0.012	0.012
75%		0.067	0.024	0.012	0.012	0.008	0.008	0.006	0.006
90%		0.027	0.009	0.005	0.005	0.003	0.003	0.002	0.002
-	R1 pond	--	0.022	0.018	0.012	0.017	0.011	0.016	0.008
50%		0.017	0.016	0.014	0.008	0.013	0.007	0.013	0.005
75%		0.014	0.013	0.012	0.006	0.012	0.006	0.011	0.003
90%		0.011	0.011	0.011	0.005	0.011	0.005	0.011	0.003

Intended use		Oilseed rape, winter, umbrella use VIa; Mar-Jun/ BBCH 31-71							
Active substance		Acetamiprid							
Application rate (g/ha)		60 +60							
FOCUS Step 4 PEC _{sw, max} (µg/L)									
Nozzle reduction	Non-sprayed buffer (m)	-	5	10	10	15	15	20	20
	Vegetated filter strip (m)	-	-	-	10	-	10 (VFSmod)	-	20
-	R1 stream	--	0.686	0.686	0.289	0.686	0.289	0.686	0.147
50%		0.686	0.686	0.686	0.289	0.686	0.289	0.686	0.147
75%		0.686	0.686	0.686	0.289	0.686	0.289	0.686	0.147
90%		0.686	0.686	0.686	0.289	0.686	0.289	0.686	0.147
-	R3 stream	--	0.404	0.404	0.182	0.404	0.182	0.404	0.095
50%		0.404	0.404	0.404	0.182	0.404	0.182	0.404	0.095
75%		0.404	0.404	0.404	0.182	0.404	0.182	0.404	0.095
90%		0.404	0.404	0.404	0.182	0.404	0.182	0.404	0.095
None	R4 stream	Not calculated – no mitigation required.							
50 %	(winter cereals as surrogate crop)								
75 %									
90 %									
Mesocosm (ETO); mean measured 0.56 µg/L		ETR (PEC/RAC)							
-	R1 stream	--	1.2	1.2	0.5	1.2	0.5	1.2	0.3
50%		1.2	1.2	1.2	0.5	1.2	0.5	1.2	0.3
75%		1.2	1.2	1.2	0.5	1.2	0.5	1.2	0.3
90%		1.2	1.2	1.2	0.5	1.2	0.5	1.2	0.3

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in **bold**

For the intended application of ADM.00150.I.2.A in “winter oilseed rape (winter cereals as surrogate crop for R4)” at 60 +60 g a.s./ha (umbrella use VIa; Mar-Jun/ BBCH 31-71), the calculated PEC/RAC ratios indicate an acceptable risk from exposure to acetamiprid if certain mitigation measures are considered. For the mesocosm study (ETO; mean measured; aquatic invertebrates, acute and sed. dwell. organisms, prolonged, RAC = 0.56 µg/L), a non-spray buffer of 10 m in combination with a vegetated filter strip of 10 m should be considered.

Table 9.5-29: Aquatic organisms - acceptability of risk (PEC/RAC < 1) for acetamiprid for each organism group based on FOCUS Step 1 - 3 calculations for the proposed application (60 g a.s./ha) of ADM.00150.I.2.A in ‘oilseed rape (winter cereals as surrogate crop), winter (umbrella use VIa; Mar-Jun / BBCH 31-71)’

Group		Fish, acute	Fish, prolonged	Invertebrate, acute	Invertebrate, prolonged	Invertebrates incl. sed. dwellers, acute and prolonged	Sed. dwell. organisms, prolonged	Algae
Test Species		<i>C. variegatus</i>	<i>P. promelas</i>	Geomean, Invertebrate acute	<i>D. magna</i>	Mesocosm (ETO); mean measured	<i>C. riparius</i>	<i>A. flos-aquae</i>
Endpoint (µg/L)		LC ₅₀ 100000	EC ₁₀ 9400	EC ₅₀ 8.5	EC ₁₀ 2960	NOEC 1.12	EC ₁₀ 0.235	E _r C ₅₀ >1300
AF		100	10	100	10	2	10	10
RAC (µg/L)		1000	940	0.085	296	0.56	0.024	>130
FOCUS Scenario	PEC _{sw,max} (µg/L)	ETR (PEC/RAC)						
Step 1								
	36.130	0.04	0.04	425	0.1	65	1537	<0.3
Step 2								
N-Europe	0.843	0.0008	0.0009	9.9	0.003	1.5	36	<0.006
S-Europe	0.765	0.0008	0.0008	9.0	0.003	1.4	33	<0.006
Step 3								
D2 ditch	0.385	0.0004	0.0004	4.5	0.001	0.7	16	<0.003
D2 stream	0.343	0.0003	0.0004	4.0	0.001	0.6	15	<0.003
D3 ditch	0.379	0.0004	0.0004	4.5	0.001	0.7	16	<0.003
D4 pond	0.013	0.00001	0.00001	0.2	0.00004	0.02	0.6	<0.0001
D4 stream	0.284	0.0003	0.0003	3.3	0.001	0.5	12	<0.002
D5 pond	0.013	0.00001	0.00001	0.2	0.00004	0.02	0.6	<0.0001
D5 stream	0.303	0.0003	0.0003	3.6	0.001	0.5	13	<0.002
R1 pond	0.013	0.00001	0.00001	0.2	0.00004	0.02	0.6	<0.0001
R1 stream	0.250	0.0003	0.0003	2.9	0.0008	0.4	11	<0.002
R3 stream	0.404	0.0004	0.0004	4.8	0.001	0.7	17	<0.003
R4 stream	0.252	0.0003	0.0003	3.0	0.0009	0.5	11	<0.002

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in **bold**

For the intended application of ADM.00150.I.2.A in “winter oilseed rape (winter cereals as surrogate crop for R4)” at 60 g a.s./ha (umbrella use VIa; Mar-Jun / BBCH 31-71), the calculated PEC/RAC ratios indicate an acceptable risk from exposure to acetamiprid for all groups of aquatic organisms based on the higher tier mesocosm endpoint also covering the lower tier invertebrate endpoints and FOCUS Step 1-3 PEC values. Therefore, no further assessment is necessary.

Table 9.5-30: Aquatic organisms - acceptability of risk (PEC/RAC < 1) for acetamiprid for each organism group based on FOCUS Step 1 - 3 calculations for the proposed application (60 g a.s./ha) of ADM.00150.I.2.A in ‘oilseed rape (winter cereals as surrogate for R4), winter (umbrella use VIb; Aug-Nov / BBCH 11-19)’

Group		Fish, acute	Fish, prolonged	Invertebrate, acute	Invertebrate, prolonged	Invertebrates incl. sed. dwellers, acute and prolonged	Sed. Dwell. Organisms, prolonged	Algae
Test Species		<i>C. variegatus</i>	<i>P. promelas</i>	Geomean, Invertebrate acute	<i>D. magna</i>	Mesocosm (ETO); mean measured	<i>C. riparius</i>	<i>A. flos-aquae</i>
Endpoint (µg/L)		LC ₅₀ 100000	EC ₁₀ 9400	EC ₅₀ 8.5	EC ₁₀ 2960	NOEC 1.12	EC ₁₀ 0.235	E _r C ₅₀ >1300
AF		100	10	100	10	2	10	10
RAC (µg/L)		1000	940	0.085	296	0.56	0.024	>130
FOCUS Scenario	PEC _{sw,max} (µg/L)	ETR (PEC/RAC)						
Step 1								
	18.065	0.02	0.02	213	0.06	32	769	<0.1
Step 2								
N-Europe	1.231	0.001	0.001	14	0.004	2.2	52	<0.009
S-Europe	1.076	0.001	0.001	13	0.004	1.9	46	<0.008
Step 3								
D3 ditch	0.382	0.0004	0.0004	4.5	0.001	0.7	16	<0.003
D4 pond	0.013	0.00001	0.00001	0.2	0.00004	0.02	0.6	<0.0001
D4 stream	0.329	0.0003	0.0004	3.9	0.001	0.6	14	<0.003
D5 pond	0.013	0.00001	0.00001	0.2	0.00004	0.02	0.6	<0.0001
D5 stream	0.355	0.0004	0.0004	4.2	0.001	0.6	15	<0.003
R1 pond	0.013	0.00001	0.00001	0.2	0.00004	0.02	0.6	<0.0001
R1 stream	0.251	0.0003	0.0003	3.0	0.0008	0.4	11	<0.002
R3 stream	0.483	0.0005	0.0005	5.7	0.002	0.9	21	<0.004
R4 stream	0.405	0.0004	0.0004	4.8	0.001	0.7	17	<0.003

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in **bold**

For the intended application of ADM.00150.I.2.A in “winter oilseed rape (winter cereals as surrogate for R4)” at 60 g a.s./ha (umbrella use VIb; Aug-Nov / BBCH 11-19), the calculated PEC/RAC ratios indicate an acceptable risk from exposure to acetamiprid for all groups of aquatic organisms based on the higher tier mesocosm endpoint also covering the lower tier invertebrate endpoints and FOCUS Step 1-3 PEC values. Therefore, no further assessment is necessary.

Table 9.5-31: Aquatic organisms - acceptability of risk (PEC/RAC < 1) for acetamiprid for each organism group based on FOCUS Step 1 - 3 calculations for the proposed application (60 +60 g a.s./ha) of ADM.00150.I.2.A in ‘oilseed rape (legumes as surrogate for R3 & R4), spring (umbrella use VIIa; Apr-Aug / BBCH 31-71)’

Group		Fish, acute	Fish, prolonged	Invertebrate, acute	Invertebrate, prolonged	Invertebrates incl. sed. dwellers, acute and prolonged	Sed. dwell. organisms, prolonged	Algae
Test Species		<i>C. variegatus</i>	<i>P. promelas</i>	Geomean, Invertebrate acute	<i>D. magna</i>	Mesocosm (ETO); mean measured	<i>C. riparius</i>	<i>A. flos-aquae</i>
Endpoint (µg/L)		LC ₅₀ 100000	EC ₁₀ 9400	EC ₅₀ 8.5	EC ₁₀ 2960	NOEC 1.12	EC ₁₀ 0.235	E _r C ₅₀ >1300
AF		100	10	100	10	2	10	10
RAC (µg/L)		1000	940	0.085	296	0.56	0.024	>130
FOCUS Scenario	PEC _{sw,max} (µg/L)	ETR (PEC/RAC)						
Step 1								
	36.130	0.04	0.04	425	0.1	65	1537	<0.3
Step 2								
N-Europe	1.140	0.001	0.001	13	0.004	2.0	49	<0.009
S-Europe	1.059	0.001	0.001	12	0.004	1.9	45	<0.008
Step 3								
D3 ditch	0.333	0.0003	0.0004	3.9	0.001	0.6	14	<0.003
D4 pond	0.020	0.00002	0.00002	0.2	0.00007	0.04	0.9	<0.0002
D4 stream	0.273	0.0003	0.0003	3.2	0.0009	0.5	12	<0.002
D5 pond	0.018	0.00002	0.00002	0.2	0.00006	0.03	0.8	<0.0001
D5 stream	0.287	0.0003	0.0003	3.4	0.001	0.5	12	<0.002
R1 pond	0.047	0.00005	0.00005	0.6	0.0002	0.08	2.0	<0.0004
R1 stream	0.765	0.0008	0.0008	9.0	0.003	1.4	33	<0.006
R3 stream	0.643	0.0006	0.0007	7.6	0.002	1.2	27	<0.005
R4 stream	1.054	0.001	0.001	12	0.004	1.9	44	<0.008

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in **bold**

For the intended application of ADM.00150.I.2.A in “spring oilseed rape (legumes as surrogate for R3 & R4)” at 60 +60 g a.s./ha (umbrella use VIIa; Apr-Aug / BBCH 31-71), the calculated PEC/RAC ratios indicate an acceptable risk from exposure to acetamiprid for all groups of aquatic organisms based on tier 1 endpoints and FOCUS Step 1-3 PEC values, except the higher tier mesocosm endpoint also covering the lower tier invertebrate endpoints. Additional PEC/RAC ratios were calculated considering FOCUS Step 4 PEC values.

Table 9.5-32: Aquatic organisms - acceptability of risk (PEC/RAC < 1) for acetamiprid for invertebrates incl. sed. dwellers, acute and prolonged based on FOCUS Step 4 calculations for the proposed application (60 +60 g a.s./ha) of ADM.00150.I.2.A in ‘oilseed rape, spring (legumes as surrogate for R3 & R4) (umbrella use VIIa; Apr-Aug / BBCH 31-71)’

Intended use		Oilseed rape, spring, umbrella use VIIa; Apr-Aug / BBCH 31-71							
Active substance		Acetamiprid							
Application rate (g/ha)		60 +60							
FOCUS Step 4 PEC _{sw, max} (µg/L)									
Nozzle reduction	Non-sprayed buffer (m)	-	5	10	10	15	15	20	20
	Vegetated filter strip (m)	-	-	-	10	-	10 (VFSmod)	-	20
-	D1 ditch	--	0.156	0.081	0.081	0.054	0.054	0.041	0.041
50%		0.301	0.078	0.040	0.040	0.027	0.027	0.021	0.021
75%		0.150	0.039	0.020	0.020	0.014	0.014	0.010	0.010
90%		0.060	0.016	0.008	0.008	0.006	0.006	0.004	0.004
-	D1 stream	--	0.103	0.053	0.053	0.036	0.036	0.027	0.027
50%		0.146	0.051	0.027	0.027	0.018	0.018	0.014	0.014
75%		0.073	0.026	0.013	0.013	0.009	0.009	0.007	0.007
90%		0.029	0.010	0.005	0.005	0.004	0.004	0.003	0.003
-	D3 ditch	--	0.087	0.045	0.045	0.030	0.030	0.023	0.023
50%		0.167	0.043	0.022	0.022	0.015	0.015	0.011	0.011
75%		0.083	0.022	0.011	0.011	0.008	0.008	0.006	0.006
90%		0.033	0.009	0.004	0.004	0.003	0.003	0.002	0.002
-	D4 pond	--	0.017	0.012	0.012	0.009	0.009	0.008	0.008
50%		0.010	0.008	0.006	0.006	0.005	0.005	0.004	0.004
75%		0.005	0.004	0.003	0.003	0.002	0.002	0.002	0.002
90%		0.002	0.002	0.001	0.001	0.001	0.001	0.001	0.001
-	D4 stream	--	0.097	0.050	0.050	0.034	0.034	0.026	0.026
50%		0.137	0.048	0.025	0.025	0.017	0.017	0.013	0.013
75%		0.068	0.024	0.013	0.013	0.008	0.008	0.006	0.006
90%		0.027	0.010	0.005	0.005	0.003	0.003	0.003	0.003
-	D5 pond	--	0.016	0.011	0.011	0.009	0.009	0.007	0.007
50%		0.009	0.008	0.005	0.005	0.004	0.004	0.004	0.004
75%		0.005	0.004	0.003	0.003	0.002	0.002	0.002	0.002
90%		0.002	0.002	0.001	0.001	0.001	0.001	0.001	0.001
-	D5 stream	--	0.102	0.053	0.053	0.036	0.036	0.027	0.027
50%		0.144	0.051	0.026	0.026	0.018	0.018	0.013	0.013
75%		0.072	0.025	0.013	0.013	0.009	0.009	0.007	0.007
90%		0.029	0.010	0.005	0.005	0.004	0.004	0.003	0.003
-	R1 pond	--	0.044	0.040	0.023	0.037	0.020	0.036	0.013
50%		0.038	0.037	0.035	0.017	0.034	0.016	0.034	0.009
75%		0.034	0.034	0.033	0.014	0.033	0.014	0.033	0.008
90%		0.033	0.033	0.033	0.013	0.033	0.013	0.032	0.007

Intended use		Oilseed rape, spring, umbrella use VIIa; Apr-Aug / BBCH 31-71							
Active substance		Acetamiprid							
Application rate (g/ha)		60 +60							
FOCUS Step 4 PEC _{sw, max} (µg/L)									
Nozzle reduction	Non-sprayed buffer (m)	-	5	10	10	15	15	20	20
	Vegetated filter strip (m)	-	-	-	10	-	10 (VFSmod)	-	20
-	R1 stream	--	0.765	0.765	0.347	0.765	0.347	0.765	0.182
50%		0.765	0.765	0.765	0.347	0.765	0.347	0.765	0.182
75%		0.765	0.765	0.765	0.347	0.765	0.347	0.765	0.182
90%		0.765	0.765	0.765	0.347	0.765	0.347	0.765	0.182
-	R3 stream	-	0.643	0.643	0.643	0.643	0.282	0.282	0.146
50%		0.643	0.643	0.643	0.643	0.643	0.282	0.282	0.146
75%		0.643	0.643	0.643	0.643	0.643	0.282	0.282	0.146
90%		0.643	0.643	0.643	0.643	0.643	0.282	0.282	0.146
-	R4 stream	-	1.054	1.054	1.054	1.054	0.470	0.470	0.244
50%		1.054	1.054	1.054	1.054	1.054	0.470	0.470	0.244
75%		1.054	1.054	1.054	1.054	1.054	0.470	0.470	0.244
90%		1.054	1.054	1.054	1.054	1.054	0.470	0.470	0.244
Mesocosm (ETO); mean measured 0.56 µg/L		ETR (PEC/RAC)							
-	R1 stream	--	1.4	1.4	0.6	1.4	0.6	1.4	0.3
50%		1.4	1.4	1.4	0.6	1.4	0.6	1.4	0.3
75%		1.4	1.4	1.4	0.6	1.4	0.6	1.4	0.3
90%		1.4	1.4	1.4	0.6	1.4	0.6	1.4	0.3
-	R3 stream	-	1.1	1.1	1.1	1.1	0.5	0.5	0.3
50%		1.1	1.1	1.1	1.1	1.1	0.5	0.5	0.3
75%		1.1	1.1	1.1	1.1	1.1	0.5	0.5	0.3
90%		1.1	1.1	1.1	1.1	1.1	0.5	0.5	0.3
-	R4 stream	-	1.9	1.9	1.9	1.9	0.8	0.8	0.4
50%		1.9	1.9	1.9	1.9	1.9	0.8	0.8	0.4
75%		1.9	1.9	1.9	1.9	1.9	0.8	0.8	0.4
90%		1.9	1.9	1.9	1.9	1.9	0.8	0.8	0.4

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in **bold**

For the intended application of ADM.00150.I.2.A in “spring oilseed rape” (legumes as surrogate for R3 & R4) at 60 +60 g a.s./ha (umbrella use VIIa; Apr-Aug / BBCH 31-71), the calculated PEC/RAC ratios indicate an acceptable risk from exposure to acetamiprid if certain mitigation measures are considered. For the mesocosm study (ETO; mean measured; aquatic invertebrates, acute and sed. dwell. organisms, prolonged, RAC = 0.56 µg/L), a non-sprayed buffer of 10 m in combination with a vegetated filter strip of ~~10~~ 15 m should be considered.

Table 9.5-33: Aquatic organisms - acceptability of risk (PEC/RAC < 1) for acetamiprid for each organism group based on FOCUS Step 1 - 3 calculations for the proposed application (60 g a.s./ha) of ADM.00150.I.2.A in ‘oilseed rape, spring (legumes as surrogate for R3 & R4) (umbrella use VIIa; Apr-Aug / BBCH 31-71)’

Group		Fish, acute	Fish, prolonged	Invertebrate, acute	Invertebrate, prolonged	Invertebrates incl. sed. dwellers, acute and prolonged	Sed. dwell. organisms, prolonged	Algae
Test Species		<i>C. variegatus</i>	<i>P. promelas</i>	Geomean, Invertebrate acute	<i>D. magna</i>	Mesocosm (ETO); mean measured	<i>C. riparius</i>	<i>A. flos-aquae</i>
Endpoint (µg/L)		LC ₅₀ 100000	EC ₁₀ 9400	EC ₅₀ 8.5	EC ₁₀ 2960	NOEC 1.12	EC ₁₀ 0.235	E _r C ₅₀ >1300
AF		100	10	100	10	2	10	10
RAC (µg/L)		1000	940	0.085	296	0.56	0.024	>130
FOCUS Scenario	PEC _{sw,max} (µg/L)	ETR (PEC/RAC)						
Step 1								
	36.130	0.04	0.04	425	0.1	65	1537	<0.3
Step 2								
N-Europe	0.843	0.0008	0.0009	9.9	0.003	1.5	36	<0.006
S-Europe	0.765	0.0008	0.0008	9.0	0.003	1.4	33	<0.006
Step 3								
D3 ditch	0.381	0.0004	0.0004	4.5	0.001	0.7	16	<0.003
D4 pond	0.013	0.00001	0.00001	0.2	0.00004	0.02	0.6	<0.0001
D4 stream	0.312	0.0003	0.0003	3.7	0.001	0.6	13	<0.002
D5 pond	0.013	0.00001	0.00001	0.2	0.00004	0.02	0.6	<0.0001
D5 stream	0.331	0.0003	0.0004	3.9	0.001	0.6	14	<0.003
R1 pond	0.043	0.00004	0.00005	0.5	0.0001	0.08	1.8	<0.0003
R1 stream	0.765	0.0008	0.0008	9.0	0.003	1.4	33	<0.006
R3 stream	0.643	0.0006	0.0007	7.6	0.002	1.2	27	<0.005
R4 stream	1.054	0.001	0.001	12	0.004	1.9	44	<0.008

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in **bold**

For the intended application of ADM.00150.I.2.A in “spring oilseed rape” (legumes as surrogate for R3 & R4) at 60 g a.s./ha (umbrella use VIIa; Apr-Aug / BBCH 31-71), the calculated PEC/RAC ratios indicate an acceptable risk from exposure to acetamiprid for all groups of aquatic organisms based on tier 1 endpoints and FOCUS Step 1-3 PEC values, except the higher tier mesocosm endpoint also covering the lower tier invertebrate endpoints. Additional PEC/RAC ratios were calculated considering FOCUS Step 4 PEC values.

Table 9.5-34: Aquatic organisms - acceptability of risk (PEC/RAC < 1) for acetamiprid for invertebrates incl. sed. dwellers, acute and prolonged based on FOCUS Step 4 calculations for the proposed application (60 g a.s./ha) of ADM.00150.I.2.A in ‘oilseed rape, spring (legumes as surrogate for R3 & R4) (umbrella use VIIa; Apr-Aug / BBCH 31-71)’

Intended use		Oilseed rape, spring, umbrella use VIIa; Apr-Aug / BBCH 31-71							
Active substance		Acetamiprid							
Application rate (g/ha)		60							
FOCUS Step 4 PEC _{sw, max} (µg/L)									
Nozzle reduction	Non-sprayed buffer (m)	-	5	10	10	15	15	20	20
	Vegetated filter strip (m)	-	-	-	10	-	10 (VFSmod)	-	20
-	D1 ditch	--	0.104	0.055	0.055	0.038	0.038	0.029	0.029
50%		0.192	0.052	0.028	0.028	0.019	0.019	0.014	0.014
75%		0.096	0.026	0.014	0.014	0.009	0.009	0.007	0.007
90%		0.038	0.010	0.006	0.006	0.004	0.004	0.003	0.003
-	D1 stream	--	0.123	0.065	0.065	0.045	0.045	0.034	0.034
50%		0.168	0.061	0.033	0.033	0.022	0.022	0.017	0.017
75%		0.084	0.031	0.016	0.016	0.011	0.011	0.008	0.008
90%		0.034	0.012	0.007	0.007	0.004	0.004	0.003	0.003
-	D3 ditch	--	0.103	0.055	0.055	0.037	0.037	0.028	0.028
50%		0.190	0.052	0.027	0.027	0.019	0.019	0.014	0.014
75%		0.095	0.026	0.014	0.014	0.009	0.009	0.007	0.007
90%		0.038	0.010	0.005	0.005	0.004	0.004	0.003	0.003
-	D4 pond	--	0.011	0.008	0.008	0.007	0.007	0.005	0.005
50%		0.007	0.006	0.004	0.004	0.003	0.003	0.003	0.003
75%		0.003	0.003	0.002	0.002	0.002	0.002	0.001	0.001
90%		0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001
-	D4 stream	--	0.114	0.060	0.060	0.041	0.041	0.031	0.031
50%		0.156	0.057	0.030	0.030	0.021	0.021	0.016	0.016
75%		0.078	0.028	0.015	0.015	0.010	0.010	0.008	0.008
90%		0.031	0.011	0.006	0.006	0.004	0.004	0.003	0.003
-	D5 pond	--	0.011	0.008	0.008	0.007	0.007	0.005	0.005
50%		0.007	0.006	0.004	0.004	0.003	0.003	0.003	0.003
75%		0.003	0.003	0.002	0.002	0.002	0.002	0.001	0.001
90%		0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001
-	D5 stream	--	0.121	0.064	0.064	0.044	0.044	0.033	0.033
50%		0.166	0.060	0.032	0.032	0.022	0.022	0.017	0.017
75%		0.083	0.030	0.016	0.016	0.011	0.011	0.008	0.008
90%		0.033	0.012	0.006	0.006	0.004	0.004	0.003	0.003
-	R1 pond	--	0.042	0.039	0.020	0.038	0.019	0.037	0.011
50%		0.038	0.037	0.036	0.016	0.035	0.016	0.034	0.009
75%		0.035	0.035	0.034	0.015	0.033	0.014	0.033	0.008
90%		0.033	0.033	0.033	0.014	0.033	0.013	0.033	0.007

Intended use		Oilseed rape, spring, umbrella use VIIa; Apr-Aug / BBCH 31-71							
Active substance		Acetamiprid							
Application rate (g/ha)		60							
FOCUS Step 4 PEC _{sw, max} (µg/L)									
Nozzle reduction	Non-sprayed buffer (m)	-	5	10	10	15	15	20	20
	Vegetated filter strip (m)	-	-	-	10	-	10 (VFSmod)	-	20
-	R1 stream	--	0.765	0.765	0.347	0.765	0.347	0.765	0.182
50%		0.765	0.765	0.765	0.347	0.765	0.347	0.765	0.182
75%		0.765	0.765	0.765	0.347	0.765	0.347	0.765	0.182
90%		0.765	0.765	0.765	0.347	0.765	0.347	0.765	0.182
-	R3 stream	-	0.643	0.643	0.643	0.643	0.282	0.282	0.146
50%		0.643	0.643	0.643	0.643	0.643	0.282	0.282	0.146
75%		0.643	0.643	0.643	0.643	0.643	0.282	0.282	0.146
90%		0.643	0.643	0.643	0.643	0.643	0.282	0.282	0.146
-	R4 stream	-	1.054	1.054	1.054	1.054	0.470	0.470	0.244
50%		1.054	1.054	1.054	1.054	1.054	0.470	0.470	0.244
75%		1.054	1.054	1.054	1.054	1.054	0.470	0.470	0.244
90%		1.054	1.054	1.054	1.054	1.054	0.470	0.470	0.244
Mesocosm (ETO); mean measured 0.56 µg/L		ETR (PEC/RAC)							
-	R1 stream	--	1.4	1.4	0.6	1.4	0.6	1.4	0.3
50%		1.4	1.4	1.4	0.6	1.4	0.6	1.4	0.3
75%		1.4	1.4	1.4	0.6	1.4	0.6	1.4	0.3
90%		1.4	1.4	1.4	0.6	1.4	0.6	1.4	0.3
-	R3 stream	-	1.1	1.1	1.1	1.1	0.5	0.5	0.3
50%		1.1	1.1	1.1	1.1	1.1	0.5	0.5	0.3
75%		1.1	1.1	1.1	1.1	1.1	0.5	0.5	0.3
90%		1.1	1.1	1.1	1.1	1.1	0.5	0.5	0.3
-	R4 stream	-	1.9	1.9	1.9	1.9	0.8	0.8	0.4
50%		1.9	1.9	1.9	1.9	1.9	0.8	0.8	0.4
75%		1.9	1.9	1.9	1.9	1.9	0.8	0.8	0.4
90%		1.9	1.9	1.9	1.9	1.9	0.8	0.8	0.4

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in **bold**

For the intended application of ADM.00150.I.2.A in “spring oilseed rape” (legumes as surrogate for R3 & R4) at 60 g a.s./ha (umbrella use VIIa; Apr-Aug / BBCH 31-71), the calculated PEC/RAC ratios indicate an acceptable risk from exposure to acetamiprid if certain mitigation measures are considered. For the mesocosm study (ETO; mean measured; aquatic invertebrates, acute and sed. dwell. organisms, prolonged, RAC = 0.56 µg/L), a non-sprayed buffer of 10 m in combination with a vegetated filter strip of 10-15 m should be considered.

Table 9.5-35: Aquatic organisms - acceptability of risk (PEC/RAC < 1) for acetamiprid for each organism group based on FOCUS Step 1 - 3 calculations for the proposed application (50 +50 g a.s./ha) of ADM.00150.I.2.A in ‘sugar beet (maize as surrogate for D5 & R4) (umbrella use VIIIa; Apr-Aug / BBCH 12-39)’

Application (50 + 50 g a.i.s/ha) of FIBROBIOSTIM 24 F in sugar beet (maize as surrogate for DS & R7) (ambrosia use v.i.i.i.i., April-Aug, 1998) (BIOF 12-97)								
Group		Fish, acute	Fish, prolonged	Invertebrate, acute	Invertebrate, prolonged	Invertebrates incl. sed. dwellers, acute and prolonged	Sed. dwell. organisms, prolonged	Algae
Test Species		<i>C. variegatus</i>	<i>P. promelas</i>	Geomean, Invertebrate acute	<i>D. magna</i>	Mesocosm (ETO); mean measured	<i>C. riparius</i>	<i>A. flos-aquae</i>
Endpoint (µg/L)		LC ₅₀ 100000	EC ₁₀ 9400	EC ₅₀ 8.5	EC ₁₀ 2960	NOEC 1.12	EC ₁₀ 0.235	E _r C ₅₀ >1300
AF		100	10	100	10	2	10	10
RAC (µg/L)		1000	940	0.085	296	0.56	0.024	>130
FOCUS Scenario	PEC _{sw,max} (µg/L)	ETR (PEC/RAC)						
Step 1								
	30.108	0.03	0.03	354	0.1	54	1281	<0.2
Step 2								
N-Europe	1.508	0.002	0.002	18	0.005	2.7	64	<0.01
S-Europe	1.329	0.001	0.001	16	0.004	2.4	57	<0.01
Step 3								
D3 ditch	0.228	0.0002	0.0002	2.7	0.0008	0.4	9.7	<0.002
D4 pond	0.016	0.00002	0.00002	0.2	0.00005	0.03	0.7	<0.0001
D4 stream	0.189	0.0002	0.0002	2.2	0.0006	0.3	8.0	<0.001
D5 pond	0.015	0.00002	0.00002	0.2	0.00005	0.03	0.6	<0.0001
D5 stream	0.213	0.0002	0.0002	2.5	0.0007	0.4	8.9	<0.002
R1 pond	0.016	0.00002	0.00002	0.2	0.00005	0.03	0.7	<0.0001
R1 stream	0.156	0.0002	0.0002	1.8	0.0005	0.3	6.6	<0.001
R3 stream	0.328	0.0003	0.0003	3.9	0.001	0.6	14	<0.003
R4 stream	0.884	0.0009	0.0009	10	0.003	1.6	37	<0.007

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in **bold**

For the intended application of ADM.00150.I.2.A in “sugar beet” (maize as surrogate for D5 & R4) at 50 +50 g a.s./ha (umbrella use VIIIa; Apr-Aug / BBCH 12-39), the calculated PEC/RAC ratios indicate an acceptable risk from exposure to acetamiprid for all groups of aquatic organisms based on the higher tier mesocosm endpoint also covering the lower tier invertebrate endpoints and FOCUS Step 1-3 PEC values. Therefore, no further assessment is necessary. the calculated PEC/RAC ratios indicate an acceptable risk from exposure to acetamiprid for all groups of aquatic organisms based on tier 1 endpoints and FOCUS Step 1-3 PEC values, except the higher tier mesocosm endpoint also covering the lower tier invertebrate endpoints. Additional PEC/RAC ratios were calculated considering FOCUS Step 4 PEC values.

Table 9.5-36: Aquatic organisms - acceptability of risk (PEC/RAC < 1) for acetamiprid for invertebrates incl. sed. dwellers, acute and prolonged based on FOCUS Step 4 calculations for the proposed application (50+50 g a.s./ha) of ADM.00150.I.2.A in ‘sugar beet (maize as surrogate for D5 & R4) (umbrella use VIIIa; Apr-Aug / BBCH 12-39)’

Intended use		Sugar beet, umbrella use VIIIa; Apr-Aug / BBCH 12-39							
Active substance		Acetamiprid							
Application rate (g/ha)		50+50							
FOCUS Step 4 PEC _{sw, max} (µg/L)									
Nozzle reduction	Non-sprayed buffer (m)	-	5	10	10	15	15	20	20
	Vegetated filter strip (m)	-	-	-	10	-	10 (VFSmod)	-	20
-	D3 ditch	-	0.072	0.037	0.025	0.019	0.037	0.025	0.019
50%		0.114	0.036	0.019	0.013	0.010	0.019	0.013	0.010
75%		0.057	0.018	0.009	0.006	0.005	0.009	0.006	0.005
90%		0.023	0.007	0.004	0.003	0.002	0.004	0.003	0.002
-	D4 pond	-	0.014	0.010	0.008	0.007	0.010	0.008	0.007
50%		0.008	0.007	0.005	0.004	0.003	0.005	0.004	0.003
75%		0.004	0.004	0.003	0.002	0.002	0.003	0.002	0.002
90%		0.002	0.001	0.001	0.001	0.001	0.001	0.001	0.001
-	D4 stream	-	0.077	0.040	0.027	0.020	0.040	0.027	0.020
50%		0.094	0.039	0.020	0.014	0.010	0.020	0.014	0.010
75%		0.047	0.019	0.010	0.007	0.005	0.010	0.007	0.005
90%		0.019	0.008	0.004	0.003	0.002	0.004	0.003	0.002
-	D5 pond	-	0.013	0.009	0.007	0.006	0.009	0.007	0.006
50%		0.007	0.007	0.005	0.004	0.003	0.005	0.004	0.003
75%		0.004	0.003	0.002	0.002	0.002	0.002	0.002	0.002
90%		0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001
-	D5 stream	-	0.087	0.045	0.031	0.023	0.045	0.031	0.023
50%		0.107	0.044	0.023	0.015	0.012	0.023	0.015	0.012
75%		0.053	0.022	0.011	0.008	0.006	0.011	0.008	0.006
90%		0.021	0.009	0.005	0.003	0.002	0.005	0.003	0.002
-	R1 pond	-	0.014	0.010	0.008	0.007	0.010	0.008	0.007
50%		0.008	0.007	0.005	0.005	0.004	0.005	0.004	0.003
75%		0.005	0.004	0.004	0.003	0.003	0.003	0.002	0.002
90%		0.003	0.003	0.003	0.003	0.002	0.002	0.001	0.001
-	R1 stream	-	0.091	0.091	0.091	0.091	0.037	0.037	0.018
50%		0.091	0.091	0.091	0.091	0.091	0.037	0.037	0.018
75%		0.091	0.091	0.091	0.091	0.091	0.037	0.037	0.018
90%		0.091	0.091	0.091	0.091	0.091	0.037	0.037	0.018
-	R3 stream	-	0.328	0.328	0.328	0.328	0.150	0.150	0.078
50%		0.328	0.328	0.328	0.328	0.328	0.150	0.150	0.078
75%		0.328	0.328	0.328	0.328	0.328	0.150	0.150	0.078
90%		0.328	0.328	0.328	0.328	0.328	0.150	0.150	0.078

Intended use		Sugar beet, umbrella use VIIIa; Apr-Aug / BBCH 12-39							
Active substance		Acetamiprid							
Application rate (g/ha)		50+50							
FOCUS Step 4 PEC _{sw, max} (µg/L)									
Nozzle reduction	Non-sprayed buffer (m)	-	5	10	10	15	15	20	20
	Vegetated filter strip (m)	-	-	-	10	-	10 (VFSmod)	-	20
-	R4 stream	-	0.884	0.884	0.884	0.884	0.400	0.400	0.209
50%		0.884	0.884	0.884	0.884	0.884	0.400	0.400	0.209
75%		0.884	0.884	0.884	0.884	0.884	0.400	0.400	0.209
90%		0.884	0.884	0.884	0.884	0.884	0.400	0.400	0.209
Mesocosm (ETO); mean measured 0.56 µg/L		ETR (PEC/RAC)							
-	R4 stream	-	1.6	1.6	1.6	1.6	0.7	0.7	0.4
50%		1.6	1.6	1.6	1.6	1.6	0.7	0.7	0.4
75%		1.6	1.6	1.6	1.6	1.6	0.7	0.7	0.4
90%		1.6	1.6	1.6	1.6	1.6	0.7	0.7	0.4

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in **bold**

For the intended application of ADM.00150.I.2.A in “sugar beet” (maize as surrogate for D5 & R4) at 50+50 g a.s./ha (umbrella use VIIIa; Apr-Aug / BBCH 12-39), the calculated PEC/RAC ratios indicate an acceptable risk from exposure to acetamiprid if certain mitigation measures are considered. For the mesocosm study (ETO; mean measured; aquatic invertebrates, acute and sed. dwell. organisms, prolonged, RAC = 0.56 µg/L), a non-sprayed buffer of 15 m in combination with a vegetated filter strip of 10 m should be considered.

Table 9.5-37: Aquatic organisms - acceptability of risk (PEC/RAC < 1) for acetamiprid for each organism group based on FOCUS Step 1 - 3 calculations for the proposed application (50 g a.s./ha) of ADM.00150.I.2.A in ‘sugar beet (maize as surrogate for D5 & R4) (umbrella use VIIIa; Apr-Aug / BBCH 12-39)’

Group		Fish, acute	Fish, prolonged	Invertebrate, acute	Invertebrate, prolonged	Invertebrates incl. sed. dwellers, acute and prolonged	Sed. Dwell. Organisms, prolonged	Algae
Test Species		<i>C. variegatus</i>	<i>P. promelas</i>	Geomean, Invertebrate acute	<i>D. magna</i>	Mesocosm (ETO); mean measured	<i>C. riparius</i>	<i>A. flos-aquae</i>
Endpoint (µg/L)		LC ₅₀ 100000	EC ₁₀ 9400	EC ₅₀ 8.5	EC ₁₀ 2960	NOEC 1.12	EC ₁₀ 0.235	E _r C ₅₀ >1300
AF		100	10	100	10	2	10	10
RAC (µg/L)		1000	940	0.085	296	0.56	0.024	>130
FOCUS Scenario	PEC _{sw,max} (µg/L)	ETR (PEC/RAC)						
Step 1								
	30.108	0.03	0.03	354	0.1	54	1281	<0.2
Step 2								
N-Europe	1.242	0.001	0.001	15	0.004	2.2	53	<0.01
S-Europe	1.069	0.001	0.001	13	0.004	1.9	45	<0.008

Group		Fish, acute	Fish, prolonged	Invertebrate, acute	Invertebrate, prolonged	Invertebrates incl. sed. dwellers, acute and prolonged	Sed. Dwell. Organisms, prolonged	Algae
Test Species		<i>C. variegatus</i>	<i>P. promelas</i>	Geomean, Invertebrate acute	<i>D. magna</i>	Mesocosm (ETO); mean measured	<i>C. riparius</i>	<i>A. flos-aquae</i>
Endpoint (µg/L)		LC ₅₀ 100000	EC ₁₀ 9400	EC ₅₀ 8.5	EC ₁₀ 2960	NOEC 1.12	EC ₁₀ 0.235	E _r C ₅₀ >1300
AF		100	10	100	10	2	10	10
RAC (µg/L)		1000	940	0.085	296	0.56	0.024	>130
FOCUS Scenario	PEC _{sw,max} (µg/L)	ETR (PEC/RAC)						
Step 3								
D3 ditch	0.262	0.0003	0.0003	3.1	0.0009	0.5	11	<0.002
D4 pond	0.011	0.00001	0.00001	0.1	0.00004	0.02	0.5	<0.00008
D4 stream	0.214	0.0002	0.0002	2.5	0.0007	0.4	9.1	<0.002
D5 pond	0.011	0.00001	0.00001	0.1	0.00004	0.02	0.5	<0.00008
D5 stream	0.234	0.0002	0.0002	2.8	0.0008	0.4	9.7	<0.002
R1 pond	0.011	0.00001	0.00001	0.1	0.00004	0.02	0.5	<0.00008
R1 stream	0.181	0.0002	0.0002	2.1	0.0006	0.3	7.7	<0.001
R3 stream	0.256	0.0003	0.0003	3.0	0.0009	0.5	11	<0.002
R4 stream	0.469	0.0005	0.0005	5.5	0.002	0.8	20	<0.004

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in **bold**

For the intended application of ADM.00150.I.2.A in “sugar beet” (maize as surrogate for D5 & R4) at 50 g a.s./ha (umbrella use VIIa; Apr-Aug / BBCH 12-39), the calculated PEC/RAC ratios indicate an acceptable risk from exposure to acetamiprid for all groups of aquatic organisms based on the higher tier mesocosm endpoint also covering the lower tier invertebrate endpoints and FOCUS Step 1-3 PEC values. Therefore, no further assessment is necessary.

Table 9.5-38: Aquatic organisms - acceptability of risk (PEC/RAC < 1) for acetamiprid for each organism group based on FOCUS Step 1 - 3 calculations for the proposed application (46 g a.s./ha) of ADM.00150.I.2.A in ‘flower bulbs (legumes as surrogate for D5) (umbrella use IXa; Mar-Jul/ BBCH 12 - 91)’

Group		Fish, acute	Fish, prolonged	Invertebrate, acute	Invertebrate, prolonged	Invertebrates incl. sed. dwellers, acute and prolonged	Sed. dwell. organisms, prolonged	Algae
Test Species		<i>C. variegatus</i>	<i>P. promelas</i>	Geomean, Invertebrate acute	<i>D. magna</i>	Mesocosm (ETO); mean measured	<i>C. riparius</i>	<i>A. flos-aquae</i>
Endpoint (µg/L)		LC ₅₀ 100000	EC ₁₀ 9400	EC ₅₀ 8.5	EC ₁₀ 2960	NOEC 1.12	EC ₁₀ 0.235	E _r C ₅₀ >1300
AF		100	10	100	10	2	10	10
RAC (µg/L)		1000	940	0.085	296	0.56	0.024	>130
FOCUS Scenario	PEC _{sw,max} (µg/L)	ETR (PEC/RAC)						
Step 1								
	13.850	0.01	0.01	163	0.05	25	589	<0.1
Step 2								
N-Europe	1.242	0.001	0.001	15	0.004	2.2	53	<0.01
S-Europe	1.063	0.001	0.001	13	0.004	1.9	45	<0.008
Step 3								
D3 ditch	0.292	0.0003	0.0003	3.4	0.001	0.5	12	<0.002
D4 pond	0.010	0.00001	0.00001	0.1	0.00003	0.02	0.4	<0.00008
D4 stream	0.224	0.0002	0.0002	2.6	0.0008	0.4	9.5	<0.002
D5 pond	0.010	0.00001	0.00001	0.1	0.00003	0.02	0.4	<0.00008
D5 stream	0.201	0.0002	0.0002	2.4	0.0007	0.4	8.4	<0.002
D6 ditch	0.293	0.0003	0.0003	3.4	0.001	0.5	12	<0.002
D6 ditch, late	0.294	0.0003	0.0003	3.5	0.001	0.5	13	<0.002
R1 pond	0.014	0.00001	0.00001	0.2	0.00005	0.03	0.6	<0.0001
R1 stream	0.251	0.0003	0.0003	3.0	0.0008	0.4	11	<0.002
R2 stream	0.254	0.0003	0.0003	3.0	0.0009	0.5	11	<0.002
R3 stream	0.270	0.0003	0.0003	3.2	0.0009	0.5	11	<0.002
R4 stream	0.659	0.0007	0.0007	7.8	0.002	1.2*	28	<0.005

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in **bold**; * safe use can be demonstrated with the implementation of the EoP approach

For the intended application of ADM.00150.I.2.A in “flower bulbs” (legumes as surrogate for D5) at 46 g a.s./ha (umbrella use IXa; Mar-Jul/ BBCH 12 - 91), the calculated PEC/RAC ratios indicate an acceptable risk from exposure to acetamiprid for all groups of aquatic organisms based on tier 1 endpoints and FOCUS Step 1-3 PEC values, except the higher tier mesocosm endpoint also covering the lower tier invertebrate endpoints. With the implementation of the EoP approach, safe use can be demonstrated in Step 3, based on the higher tier mesocosm endpoint, covering the lower tier invertebrate endpoints (Figure 1) and FOCUS Step 1-3 PEC values (Figure 4 and Figure 5). Therefore, no further assessment is necessary.

Figure 4: Worst-case (maximum peak) exposure profile analysis for acetamiprid Step 3, scenario R4 stream for the proposed application (46 g a.s./ha) of ADM.00150.I.2.A in ‘flower bulbs (umbrella use IXa; Mar-Jul/ BBCH 12 - 91)

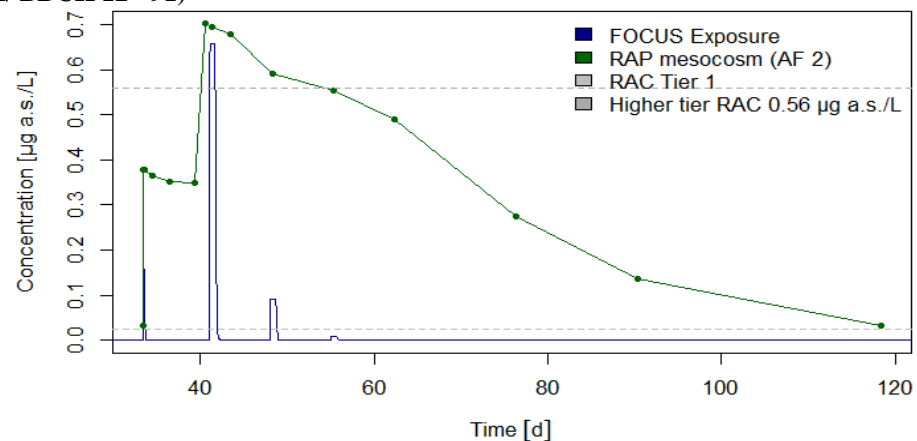


Figure 5: Worst-case (highest AUC) exposure profile analysis for acetamiprid Step 3, scenario D6 ditch for the proposed application (46 g a.s./ha) of ADM.00150.I.2.A in ‘flower bulbs (umbrella use IXa; Mar-Jul/ BBCH 12 - 91)

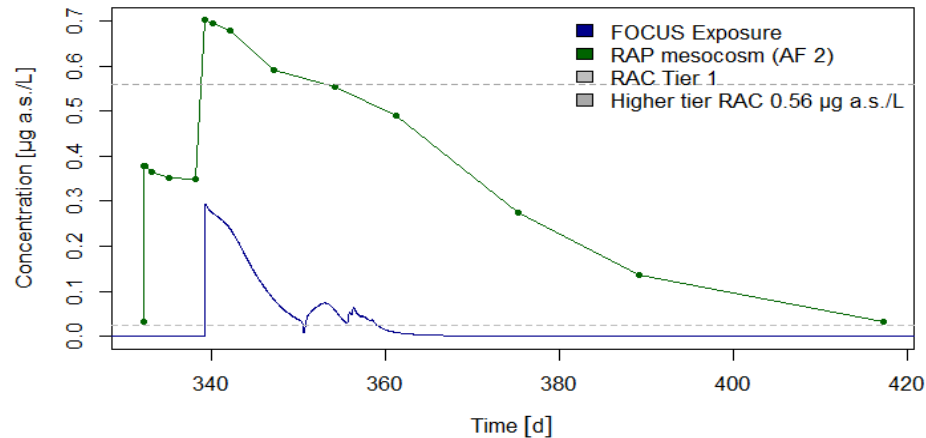


Table 9.5-39: Aquatic organisms - acceptability of risk (PEC/RAC < 1) for acetamiprid for each organism group based on FOCUS Step 1 - 3 calculations for the proposed application (34 +34 g a.s./ha) of ADM.00150.I.2.A in ‘flower bulbs (legumes as surrogate for D5) (umbrella use IXb; Mar-Jul/ BBCH 20 - 91)’

Group		Fish, acute	Fish, prolonged	Invertebrate, acute	Invertebrate, prolonged	Invertebrates incl. sed. dwellers, acute and prolonged	Sed. dwell. organisms, prolonged	Algae
Test Species		<i>C. variegatus</i>	<i>P. promelas</i>	Geomean, Invertebrate acute	<i>D. magna</i>	Mesocosm (ETO); mean measured	<i>C. riparius</i>	<i>A. flos-aquae</i>
Endpoint (µg/L)		LC ₅₀ 100000	EC ₁₀ 9400	EC ₅₀ 8.5	EC ₁₀ 2960	NOEC 1.12	EC ₁₀ 0.235	E _r C ₅₀ >1300
AF		100	10	100	10	2	10	10
RAC (µg/L)		1000	940	0.085	296	0.56	0.024	>130
FOCUS Scenario	PEC _{sw,max} (µg/L)	ETR (PEC/RAC)						
Step 1								
	20.474	0.02	0.02	241	0.07	37	871	<0.2
Step 2								
N-Europe	0.987	0.001	0.001	12	0.003	1.8	42	<0.008
S-Europe	0.874	0.0009	0.0009	10	0.003	1.6	37	<0.007
Step 3								
D3 ditch	0.189	0.0002	0.0002	2.2	0.0006	0.3	8.0	<0.001
D4 pond	0.011	0.00001	0.00001	0.1	0.00004	0.02	0.5	<0.00008
D4 stream	0.144	0.0001	0.0002	1.7	0.0005	0.3	6.1	<0.001
D5 pond	0.010	0.00001	0.00001	0.1	0.00003	0.02	0.4	<0.00008
D5 stream	0.140	0.0001	0.0001	1.6	0.0005	0.3	5.8	<0.001
R1 pond	0.035	0.00004	0.00004	0.4	0.0001	0.06	1.5	<0.0003
R1 stream	0.383	0.0004	0.0004	4.5	0.001	0.7	16	<0.003
R3 stream	0.366	0.0004	0.0004	4.3	0.001	0.7	16	<0.003
R4 stream	0.740	0.0007	0.0008	8.7	0.003	1.3	31	<0.006

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in **bold**

For the intended application of ADM.00150.I.2.A in “flower bulbs” (legumes as surrogate for D5) at 34 +34 g a.s./ha (umbrella use IXb; Mar-Jul/ BBCH 20 - 91), the calculated PEC/RAC ratios indicate an acceptable risk from exposure to acetamiprid for all groups of aquatic organisms based on tier 1 endpoints and FOCUS Step 1-3 PEC values, except the higher tier mesocosm endpoint also covering the lower tier invertebrate endpoints. Additional PEC/RAC ratios were calculated considering FOCUS Step 4 PEC values.

Table 9.5-40: Aquatic organisms - acceptability of risk (PEC/RAC < 1) for acetamiprid for invertebrates incl. sed. dwellers, acute and prolonged based on FOCUS Step 4 calculations for the proposed application (34 +34 g a.s./ha) of ADM.00150.I.2.A in 'flower bulbs (legumes as surrogate for D5) (umbrella use IXb; Mar-Jul/ BBCH 20 - 91)'

Intended use		Flower bulbs, umbrella use IXb; Mar-Jul/ BBCH 20 – 91							
Active substance		Acetamiprid							
Application rate (g/ha)		34 +34							
FOCUS Step 4 PEC _{sw, max} (µg/L)									
Nozzle reduction	Non-sprayed buffer (m)	-	5	10	10	15	15	20	20
	Vegetated filter strip (m)	-	-	-	10	-	10 VFSmod	-	20
-	D3 ditch	--	0.049	0.025	0.025	0.017	0.017	0.013	0.013
50%		0.094	0.024	0.013	0.013	0.009	0.009	0.006	0.006
75%		0.047	0.012	0.006	0.006	0.004	0.004	0.003	0.003
90%		0.019	0.005	0.003	0.003	0.002	0.002	0.001	0.001
-	D4 pond	--	0.009	0.007	0.007	0.005	0.005	0.004	0.004
50%		0.005	0.005	0.003	0.003	0.003	0.003	0.002	0.002
75%		0.003	0.002	0.002	0.002	0.001	0.001	0.001	0.001
90%		0.001	0.001	0.001	0.001	0.001	0.001	0.000	0.000
-	D4 stream	--	0.051	0.026	0.026	0.018	0.018	0.013	0.013
50%		0.072	0.025	0.013	0.013	0.009	0.009	0.007	0.007
75%		0.036	0.013	0.007	0.007	0.004	0.004	0.003	0.003
90%		0.014	0.005	0.003	0.003	0.002	0.002	0.001	0.001
-	D5 pond (legumes as surrogate crop)	Not calculated – no mitigation needed.							
50%									
75%									
90%	D5 stream (legumes as surrogate crop)	Not calculated – no mitigation needed.							
-									
50%									
75%	D6 ditch	--	0.070	0.036	0.036	0.024	0.024	0.018	0.018
50%		0.135	0.035	0.018	0.018	0.012	0.012	0.009	0.009
75%		0.067	0.017	0.009	0.009	0.006	0.006	0.005	0.005
90%		0.027	0.007	0.004	0.004	0.002	0.002	0.002	0.002
-	D6 ditch, late	--	0.049	0.026	0.026	0.017	0.017	0.013	0.013
50%		0.095	0.025	0.013	0.013	0.009	0.009	0.007	0.007
75%		0.047	0.012	0.007	0.007	0.004	0.004	0.003	0.003
90%		0.019	0.005	0.003	0.003	0.002	0.002	0.001	0.001
-	R1 pond	--	0.034	0.032	0.016	0.031	0.015	0.031	0.009
50%		0.031	0.031	0.030	0.014	0.029	0.013	0.029	0.007
75%		0.029	0.029	0.029	0.012	0.028	0.012	0.028	0.006
90%		0.028	0.028	0.028	0.012	0.028	0.011	0.028	0.006
-	R1 stream	--	0.383	0.383	0.171	0.383	0.171	0.383	0.089

Intended use		Flower bulbs, umbrella use IXb; Mar-Jul/ BBCH 20 – 91							
Active substance		Acetamiprid							
Application rate (g/ha)		34 +34							
FOCUS Step 4 PEC _{sw, max} (µg/L)									
Nozzle reduction	Non-sprayed buffer (m)	-	5	10	10	15	15	20	20
	Vegetated filter strip (m)	-	-	-	10	-	10 VFSmod	-	20
50%		0.383	0.383	0.383	0.171	0.383	0.171	0.383	0.089
75%		0.383	0.383	0.383	0.171	0.383	0.171	0.383	0.089
90%		0.383	0.383	0.383	0.171	0.383	0.171	0.383	0.089
-		--	0.134	0.134	0.060	0.134	0.060	0.134	0.031
50%	R2 stream	0.134	0.134	0.134	0.060	0.134	0.060	0.134	0.031
75%		0.134	0.134	0.134	0.060	0.134	0.060	0.134	0.031
90%		0.134	0.134	0.134	0.060	0.134	0.060	0.134	0.031
-	R3 stream	--	0.366	0.366	0.166	0.366	0.166	0.366	0.087
50%		0.366	0.366	0.366	0.166	0.366	0.166	0.366	0.087
75%		0.366	0.366	0.366	0.166	0.366	0.166	0.366	0.087
90%		0.366	0.366	0.366	0.166	0.366	0.166	0.366	0.087
-	R4 stream	--	0.740	0.740	0.334	0.740	0.334	0.740	0.175
50%		0.740	0.740	0.740	0.334	0.740	0.334	0.740	0.175
75%		0.740	0.740	0.740	0.334	0.740	0.334	0.740	0.175
90%		0.74	0.74	0.74	0.334	0.74	0.334	0.74	0.175
Mesocosm (ETO); mean measured 0.56 µg/L		ETR (PEC/RAC)							
-	R4 stream	--	1.3	1.3	0.6	1.3	0.6	1.3	0.3
50%		1.3	1.3	1.3	0.6	1.3	0.6	1.3	0.3
75%		1.3	1.3	1.3	0.6	1.3	0.6	1.3	0.3
90%		1.3	1.3	1.3	0.6	1.3	0.6	1.3	0.3

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in **bold**

For the intended application of ADM.00150.I.2.A in “flower bulbs” (legumes as surrogate for D5) at 34 +34 g a.s./ha (umbrella use IXb; Mar-Jul/ BBCH 20 - 91), the calculated PEC/RAC ratios indicate an acceptable risk from exposure to acetamiprid if certain mitigation measures are considered. For the mesocosm study (ETO; mean measured; aquatic invertebrates, acute and sed. dwell. organisms, prolonged, RAC = 0.56 µg/L), a non-sprayed buffer of 10 m in combination with a vegetated filter strip of 10 m should be considered.

Table 9.5-41: Aquatic organisms - acceptability of risk (PEC/RAC < 1) for acetamiprid for each organism group based on FOCUS Step 1 - 3 calculations for the proposed application (34 g a.s./ha) of ADM.00150.I.2.A in ‘flower bulbs (legumes as surrogate for D5) (umbrella use IXb Mar-Jul / BBCH 20-91)’

Group		Fish, acute	Fish, prolonged	Invertebrate, acute	Invertebrate, prolonged	Invertebrates incl. sed. dwellers, acute and prolonged	Sed. dwell. organisms, prolonged	Algae
Test Species		<i>C. variegatus</i>	<i>P. promelas</i>	Geomean, Invertebrate acute	<i>D. magna</i>	Mesocosm (ETO); mean measured	<i>C. riparius</i>	<i>A. flos-aquae</i>
Endpoint (µg/L)		LC ₅₀ 100000	EC ₁₀ 9400	EC ₅₀ 8.5	EC ₁₀ 2960	NOEC 1.12	EC ₁₀ 0.235	E _r C ₅₀ >1300
AF		100	10	100	10	2	10	10
RAC (µg/L)		1000	940	0.085	296	0.56	0.024	>130
FOCUS Scenario	PEC _{sw,max} (µg/L)	ETR (PEC/RAC)						
Step 1								
	20.474	0.02	0.02	241	0.07	37	871	<0.2
Step 2								
N-Europe	0.808	0.0008	0.0009	9.5	0.003	1.4	34	<0.006
S-Europe	0.698	0.0007	0.0007	8.2	0.002	1.2	30	<0.005
Step 3								
D3 ditch	0.215	0.0002	0.0002	2.5	0.0007	0.4	9.1	<0.002
D4 pond	0.007	0.000007	0.000007	0.08	0.00002	0.01	0.3	<0.00005
D4 stream	0.164	0.0002	0.0002	1.9	0.0006	0.3	7.0	<0.001
D5 pond	0.007	0.000007	0.000007	0.08	0.00002	0.01	0.3	<0.00005
D5 stream	0.148	0.0001	0.0002	1.7	0.0005	0.3	6.2	<0.001
R1 pond	0.030	0.00003	0.00003	0.4	0.0001	0.05	1.3	<0.0002
R1 stream	0.383	0.0004	0.0004	4.5	0.001	0.7	16	<0.003
R3 stream	0.366	0.0004	0.0004	4.3	0.001	0.7	16	<0.003
R4 stream	0.740	0.0007	0.0008	8.7	0.003	1.3	31	<0.006

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in **bold**

For the intended application of ADM.00150.I.2.A in “flower bulbs” (legumes as surrogate for D5) at 34 g a.s./ha (umbrella use IXb Mar-Jul / BBCH 20-91), the calculated PEC/RAC ratios indicate an acceptable risk from exposure to acetamiprid for all groups of aquatic organisms based on tier 1 endpoints and FOCUS Step 1-3 PEC values, except the higher tier mesocosm endpoint also covering the lower tier invertebrate endpoints. Additional PEC/RAC ratios were calculated considering FOCUS Step 4 PEC values.

Table 9.5-42: Aquatic organisms - acceptability of risk (PEC/RAC < 1) for acetamiprid for invertebrates incl. sed. dwellers, acute and prolonged based on FOCUS Step 4 calculations for the proposed application (34 g a.s./ha) of ADM.00150.I.2.A in ‘flower bulbs (legumes as surrogate for D5) (umbrella use IXb Mar-Jul / BBCH 20-91)’

Intended use		Flower bulbs, umbrella use IXb Mar-Jul / BBCH 20-91							
Active substance		Acetamiprid							
Application rate (g/ha)		34							
FOCUS Step 4 PEC _{sw, max} (µg/L)									
Nozzle reduction	Non-sprayed buffer (m)	-	5	10	10	15	15	20	20
	Vegetated filter strip (m)	-	-	-	10	-	10 (VFSmod)	-	20
-	D3 ditch	--	0.058	0.031	0.031	0.021	0.021	0.016	0.016
50%		0.108	0.029	0.015	0.015	0.011	0.011	0.008	0.008
75%		0.054	0.015	0.008	0.008	0.005	0.005	0.004	0.004
90%		0.022	0.006	0.003	0.003	0.002	0.002	0.002	0.002
-	D4 pond	--	0.006	0.005	0.005	0.004	0.004	0.003	0.003
50%		0.004	0.003	0.002	0.002	0.002	0.002	0.002	0.002
75%		0.002	0.002	0.001	0.001	0.001	0.001	0.001	0.001
90%		0.001	0.001	0.000	0.000	0.000	0.000	0.000	0.000
-	D4 stream	--	0.060	0.032	0.032	0.022	0.022	0.016	0.016
50%		0.082	0.030	0.016	0.016	0.011	0.011	0.008	0.008
75%		0.041	0.015	0.008	0.008	0.005	0.005	0.004	0.004
90%		0.016	0.006	0.003	0.003	0.002	0.002	0.002	0.002
-	D5 pond (legumes as surrogate crop)	Not calculated – no mitigation needed.							
50%									
75%									
90%									
-	D5 stream (legumes as surrogate crop)	Not calculated – no mitigation needed.							
50%									
75%									
90%									
-	D6 ditch	--	0.059	0.031	0.031	0.021	0.021	0.016	0.016
50%		0.109	0.029	0.016	0.016	0.011	0.011	0.008	0.008
75%		0.054	0.015	0.008	0.008	0.005	0.005	0.004	0.004
90%		0.022	0.006	0.003	0.003	0.002	0.002	0.002	0.002
-	D6 ditch, late	--	0.058	0.031	0.031	0.021	0.021	0.016	0.016
50%		0.107	0.029	0.015	0.015	0.011	0.011	0.008	0.008
75%		0.053	0.015	0.008	0.008	0.005	0.005	0.004	0.004
90%		0.021	0.006	0.003	0.003	0.002	0.002	0.002	0.002
-	R1 pond	--	0.029	0.028	0.013	0.028	0.013	0.027	0.007
50%		0.028	0.027	0.027	0.012	0.026	0.011	0.026	0.006
75%		0.026	0.026	0.026	0.011	0.026	0.011	0.026	0.006
90%		0.026	0.025	0.025	0.010	0.025	0.010	0.025	0.005

Intended use		Flower bulbs, umbrella use IXb Mar-Jul / BBCH 20-91							
Active substance		Acetamiprid							
Application rate (g/ha)		34							
FOCUS Step 4 PEC _{sw, max} (µg/L)									
Nozzle reduction	Non-sprayed buffer (m)	-	5	10	10	15	15	20	20
	Vegetated filter strip (m)	-	-	-	10	-	10 (VFSmod)	-	20
-	R1 stream	--	0.383	0.383	0.171	0.383	0.171	0.383	0.089
50%		0.383	0.383	0.383	0.171	0.383	0.171	0.383	0.089
75%		0.383	0.383	0.383	0.171	0.383	0.171	0.383	0.089
90%		0.383	0.383	0.383	0.171	0.383	0.171	0.383	0.089
-	R2 stream	--	0.134	0.134	0.060	0.134	0.060	0.134	0.031
50%		0.134	0.134	0.134	0.060	0.134	0.060	0.134	0.031
75%		0.134	0.134	0.134	0.060	0.134	0.060	0.134	0.031
90%		0.134	0.134	0.134	0.060	0.134	0.060	0.134	0.031
-	R3 stream	--	0.366	0.366	0.166	0.366	0.166	0.366	0.087
50%		0.366	0.366	0.366	0.166	0.366	0.166	0.366	0.087
75%		0.366	0.366	0.366	0.166	0.366	0.166	0.366	0.087
90%		0.366	0.366	0.366	0.166	0.366	0.166	0.366	0.087
-	R4 stream	--	0.740	0.740	0.334	0.740	0.334	0.740	0.175
50%		0.740	0.740	0.740	0.334	0.740	0.334	0.740	0.175
75%		0.740	0.740	0.740	0.334	0.740	0.334	0.740	0.175
90%		0.74	0.74	0.74	0.334	0.74	0.334	0.74	0.175
Mesocosm (ETO); mean measured 0.56 µg/L		ETR (PEC/RAC)							
-	R4 stream	--	1.3	1.3	0.6	1.3	0.6	1.3	0.3
50%		1.3	1.3	1.3	0.6	1.3	0.6	1.3	0.3
75%		1.3	1.3	1.3	0.6	1.3	0.6	1.3	0.3
90%		1.3	1.3	1.3	0.6	1.3	0.6	1.3	0.3

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in **bold**

For the intended application of ADM.00150.I.2.A in “flower bulbs” (legumes as surrogate for D5) at 34 g a.s./ha (umbrella use IXb Mar-Jul / BBCH 20-91), the calculated PEC/RAC ratios indicate an acceptable risk from exposure to acetamiprid if certain mitigation measures are considered. For the mesocosm study (ETO; mean measured; aquatic invertebrates, acute and sed. dwell. organisms, prolonged, RAC = 0.56 µg/L), a non-spray buffer of 10 m in combination with a vegetated filter strip of 10 m should be considered.

Table 9.5-43: Aquatic organisms - acceptability of risk (PEC/RAC < 1) for acetamiprid for each organism group based on FOCUS Step 1 - 3 calculations for the proposed application (46 g a.s./ha) of ADM.00150.I.2.A in ‘vegetables, leafy (legumes as surrogate for D5) (umbrella use Xa; Mar-Aug / BBCH 12)’

Group		Fish, acute	Fish, prolonged	Invertebrate, acute	Invertebrate, prolonged	Invertebrates incl. sed. dwellers, acute and prolonged	Sed. dwell. organisms, prolonged	Algae
Test Species		<i>C. variegatus</i>	<i>P. promelas</i>	Geomean, Invertebrate acute	<i>D. magna</i>	Mesocosm (ETO); mean measured	<i>C. riparius</i>	<i>A. flos-aquae</i>
Endpoint (µg/L)		LC ₅₀ 100000	EC ₁₀ 9400	EC ₅₀ 8.5	EC ₁₀ 2960	NOEC 1.12	EC ₁₀ 0.235	E _r C ₅₀ >1300
AF		100	10	100	10	2	10	10
RAC (µg/L)		1000	940	0.085	296	0.56	0.024	>130
FOCUS Scenario	PEC _{sw,max} (µg/L)	ETR (PEC/RAC)						
Step 1								
	13.850	0.01	0.01	163	0.05	25	589	<0.1
Step 2								
N-Europe	1.093	0.001	0.001	13	0.004	2.0	47	<0.008
S-Europe	0.944	0.0009	0.001	11	0.003	1.7	40	<0.007
Step 3								
D3 ditch	0.292	0.0003	0.0003	3.4	0.001	0.5	12	<0.002
D4 pond	0.010	0.00001	0.00001	0.1	0.00003	0.02	0.4	<0.00008
D4 stream	0.236	0.0002	0.0003	2.8	0.0008	0.4	10	<0.002
D5 pond	0.010	0.00001	0.00001	0.1	0.00003	0.02	0.4	<0.00008
D5 stream	0.201	0.0002	0.0002	2.4	0.0007	0.4	8.4	<0.002
R1 pond	0.010	0.00001	0.00001	0.1	0.00003	0.02	0.4	<0.00008
R1 stream	0.192	0.0002	0.0002	2.3	0.0006	0.3	8.2	<0.001
R3 stream	0.270	0.0003	0.0003	3.2	0.0009	0.5	11	<0.002
R4 stream	0.655	0.0007	0.0007	7.7	0.002	1.2*	28	<0.005

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in **bold**; * safe use can be demonstrated with the implementation of the EoP approach

For the intended application of ADM.00150.I.2.A in “leafy vegetables” (legumes as surrogate for D5) at 46 g a.s./ha (umbrella use Xa; Mar-Aug / BBCH 12), the calculated PEC/RAC ratios indicate an acceptable risk from exposure to acetamiprid for all groups of aquatic organisms based on tier 1 endpoints and FOCUS Step 1-3 PEC values, except the higher tier mesocosm endpoint also covering the lower tier invertebrate endpoints. With the implementation of the EoP approach, safe use can be demonstrated in Step 3, based on the higher tier mesocosm endpoint, covering the lower tier invertebrate endpoints (**Figure 6**). and FOCUS Step 1-3 PEC values. Therefore, no further assessment is necessary.

Figure 6: Worst-case (maximum peak and highest AUC) exposure profile analysis for acetamiprid Step 3, scenario R4 stream for the proposed application (46 g a.s./ha) of ADM.00150.I.2.A in ‘vegetables, leafy (umbrella use Xa; Mar-Aug / BBCH 12)

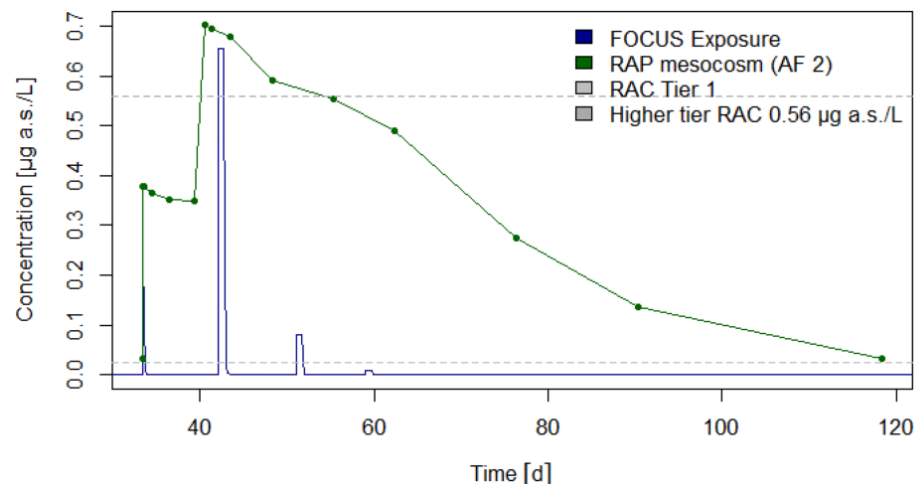


Table 9.5-44: Aquatic organisms - acceptability of risk (PEC/RAC < 1) for acetamiprid for each organism group based on FOCUS Step 1 - 3 calculations for the proposed application (46 g a.s./ha) of ADM.00150.I.2.A in ‘pome/stone fruit, early applications (umbrella use Xa; Mar-Aug / BBCH 12)’

Group		Fish, acute	Fish, prolonged	Invertebrate, acute	Invertebrate, prolonged	Invertebrates incl. sed. dwellers, acute and prolonged	Sed. dwell. organisms, prolonged	Algae
Test Species		<i>C. variegatus</i>	<i>P. promelas</i>	Geomean, Invertebrate acute	<i>D. magna</i>	Mesocosm (ETO); mean measured	<i>C. riparius</i>	<i>A. flos-aquae</i>
Endpoint (µg/L)		LC ₅₀ 100000	EC ₁₀ 9400	EC ₅₀ 8.5	EC ₁₀ 2960	NOEC 1.12	EC ₁₀ 0.235	E _r C ₅₀ >1300
AF		100	10	100	10	2	10	10
RAC (µg/L)		1000	940	0.085	296	0.56	0.024	>130
FOCUS Scenario	PEC _{sw,max} (µg/L)	ETR (PEC/RAC)						
Step 1								
	17.904	0.02	0.02	211	0.06	32	762	<0.1
Step 2								
N-Europe	4.484	0.004	0.005	53	0.02	8.0	191	<0.03
S-Europe	4.477	0.004	0.005	53	0.02	8.0	191	<0.03
Step 3								
D3 ditch	3.572	0.004	0.004	42	0.01	6.4	152	<0.03
D4 pond	0.217	0.0002	0.0002	2.6	0.0007	0.4	9.2	<0.002
D4 stream	3.442	0.003	0.004	40	0.01	6.1	146	<0.03

Group		Fish, acute	Fish, prolonged	Invertebrate, acute	Invertebrate, prolonged	Invertebrates incl. sed. dwellers, acute and prolonged	Sed. dwell. organisms, prolonged	Algae
Test Species		<i>C. variegatus</i>	<i>P. promelas</i>	Geomean, Invertebrate acute	<i>D. magna</i>	Mesocosm (ETO); mean measured	<i>C. riparius</i>	<i>A. flos-aquae</i>
Endpoint (µg/L)		LC ₅₀ 100000	EC ₁₀ 9400	EC ₅₀ 8.5	EC ₁₀ 2960	NOEC 1.12	EC ₁₀ 0.235	E _r C ₅₀ >1300
AF		100	10	100	10	2	10	10
RAC (µg/L)		1000	940	0.085	296	0.56	0.024	>130
FOCUS Scenario	PEC _{sw,max} (µg/L)	ETR (PEC/RAC)						
D5 pond	0.217	0.0002	0.0002	2.6	0.0007	0.4	9.2	<0.002
D5 stream	3.543	0.004	0.004	42	0.01	6.3	151	<0.03
R1 pond	0.217	0.0002	0.0002	2.6	0.0007	0.4	9.2	<0.002
R1 stream	2.889	0.003	0.003	34	0.01	5.2	123	<0.02
R2 stream	3.826	0.004	0.004	45	0.01	6.8	163	<0.03
R3 stream	4.087	0.004	0.004	48	0.01	7.3	174	<0.03
R4 stream	2.906	0.003	0.003	34	0.01	5.2	124	<0.02

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in **bold**

For the intended application of ADM.00150.I.2.A in “pome/stone fruits” at 46 g a.s./ha (umbrella use Xa; Mar-Aug / BBCH 12-91), the calculated PEC/RAC ratios indicate an acceptable risk from exposure to acetamiprid for all groups of aquatic organisms based on tier 1 endpoints and FOCUS Step 1-3 PEC values, except the higher tier mesocosm endpoint also covering the lower tier invertebrate endpoints. Additional PEC/RAC ratios were calculated considering FOCUS Step 4 PEC values.

Table 9.5-45: Aquatic organisms - acceptability of risk (PEC/RAC < 1) for acetamiprid for invertebrates incl. sed. dwellers, acute and prolonged based on FOCUS Step 4 calculations for the proposed application (46 g a.s./ha) of ADM.00150.I.2.A in ‘pome/stone fruit, early applications (umbrella use Xa; Mar-Aug / BBCH 12)’

Intended use		Pome/stone fruit, early applications, umbrella use Xa; Mar-Aug / BBCH 12							
Active substance		Acetamiprid							
Application rate (g/ha)		46							
FOCUS Step 4 PEC _{sw, max} (µg/L)									
Nozzle reduction	Non-sprayed buffer (m)	-	5	10	10	15	15	20	20
	Vegetated filter strip (m)	-	-	-	10	-	10 (VFSmod)	-	20
-	D3 ditch	--	2.807	1.724	1.724	0.776	0.776	0.394	0.394
50%		1.786	1.403	0.862	0.862	0.388	0.388	0.197	0.197
75%		0.893	0.702	0.431	0.431	0.194	0.194	0.099	0.099
90%		0.357	0.281	0.172	0.172	0.078	0.078	0.039	0.039
-	D4 pond	--	0.244	0.134	0.134	0.071	0.071	0.043	0.043
50%		0.109	0.122	0.067	0.067	0.035	0.035	0.022	0.022
75%		0.054	0.061	0.034	0.034	0.018	0.018	0.011	0.011
90%		0.022	0.024	0.013	0.013	0.007	0.007	0.004	0.004
-	D4 stream	--	2.958	1.816	1.816	0.817	0.817	0.415	0.415

Intended use		Pome/stone fruit, early applications, umbrella use Xa; Mar-Aug / BBCH 12							
Active substance		Acetamiprid							
Application rate (g/ha)		46							
FOCUS Step 4 PEC _{sw, max} (µg/L)									
Nozzle reduction	Non-sprayed buffer (m)	-	5	10	10	15	15	20	20
	Vegetated filter strip (m)	-	-	-	10	-	10 (VFSmod)	-	20
50%		1.721	1.479	0.908	0.908	0.409	0.409	0.208	0.208
75%		0.861	0.739	0.454	0.454	0.204	0.204	0.104	0.104
90%		0.344	0.296	0.182	0.182	0.082	0.082	0.042	0.042
-		--	0.244	0.134	0.134	0.071	0.071	0.043	0.043
50%	D5 pond	0.109	0.122	0.067	0.067	0.035	0.035	0.022	0.022
75%		0.054	0.061	0.034	0.034	0.018	0.018	0.011	0.011
90%		0.022	0.024	0.013	0.013	0.007	0.007	0.004	0.004
-		--	3.045	1.869	1.869	0.841	0.841	0.427	0.427
50%	D5 stream	1.772	1.522	0.935	0.935	0.421	0.421	0.214	0.214
75%		0.886	0.761	0.467	0.467	0.210	0.210	0.107	0.107
90%		0.354	0.305	0.187	0.187	0.084	0.084	0.043	0.043
-		--	0.244	0.134	0.134	0.071	0.071	0.043	0.043
50%	R1 pond	0.109	0.122	0.067	0.067	0.035	0.035	0.022	0.022
75%		0.054	0.061	0.034	0.034	0.018	0.018	0.011	0.011
90%		0.022	0.024	0.013	0.013	0.007	0.007	0.004	0.004
-		--	2.483	1.524	1.524	0.686	0.686	0.348	0.348
50%	R1 stream	1.444	1.241	0.762	0.762	0.343	0.343	0.174	0.174
75%		0.722	0.620	0.381	0.381	0.171	0.171	0.087	0.087
90%		0.289	0.248	0.152	0.152	0.069	0.069	0.035	0.035
-		--	3.289	2.019	2.019	0.908	0.908	0.462	0.462
50%	R2 stream	1.913	1.644	1.009	1.009	0.454	0.454	0.231	0.231
75%		0.957	0.822	0.505	0.505	0.227	0.227	0.115	0.115
90%		0.383	0.329	0.202	0.202	0.091	0.091	0.046	0.046
-		--	3.512	2.156	2.156	0.970	0.970	0.493	0.493
50%	R3 stream	2.043	1.755	1.078	1.078	0.485	0.485	0.247	0.247
75%		1.022	0.878	0.539	0.539	0.243	0.243	0.123	0.123
90%		0.409	0.351	0.216	0.216	0.097	0.097	0.049	0.049
-		--	2.497	1.533	1.533	0.690	0.690	0.350	0.350
50%	R4 stream	1.453	1.248	0.767	0.767	0.345	0.345	0.175	0.175
75%		0.727	0.624	0.383	0.383	0.172	0.172	0.088	0.088
90%		0.29	0.25	0.153	0.153	0.069	0.069	0.035	0.035
Mesocosm (ETO); mean measured 0.56 µg/L		ETR (PEC/RAC)							
-	D3 ditch	--	5.0	3.1	3.1	1.4	1.4	0.7	0.7

Intended use		Pome/stone fruit, early applications, umbrella use Xa; Mar-Aug / BBCH 12							
Active substance		Acetamiprid							
Application rate (g/ha)		46							
FOCUS Step 4 PEC _{sw, max} (µg/L)									
Nozzle reduction	Non-sprayed buffer (m)	-	5	10	10	15	15	20	20
	Vegetated filter strip (m)	-	-	-	10	-	10 (VFSmod)	-	20
50%		3.2	2.5	1.5	1.5	0.7	0.7	0.4	0.4
75%		1.6	1.3	0.8	0.8	0.3	0.3	0.2	0.2
90%		0.6	0.5	0.3	0.3	0.1	0.1	0.07	0.07
-	D4 stream	--	5.3	3.2	3.2	1.5	1.5	0.7	0.7
50%		3.1	2.6	1.6	1.6	0.7	0.7	0.4	0.4
75%		1.5	1.3	0.8	0.8	0.4	0.4	0.2	0.2
90%		0.6	0.5	0.3	0.3	0.1	0.1	0.08	0.08
-	D5 stream	--	5.4	3.3	3.3	1.5	1.5	0.8	0.8
50%		3.2	2.7	1.7	1.7	0.8	0.8	0.4	0.4
75%		1.6	1.4	0.8	0.8	0.4	0.4	0.2	0.2
90%		0.6	0.5	0.3	0.3	0.2	0.2	0.08	0.08
-	R1 stream	--	4.4	2.7	2.7	1.2	1.2	0.6	0.6
50%		2.6	2.2	1.4	1.4	0.6	0.6	0.3	0.3
75%		1.3	1.1	0.7	0.7	0.3	0.3	0.2	0.2
90%		0.5	0.4	0.3	0.3	0.1	0.1	0.06	0.06
-	R2 stream	--	5.9	3.6	3.6	1.6	1.6	0.8	0.8
50%		3.4	2.9	1.8	1.8	0.8	0.8	0.4	0.4
75%		1.7	1.5	0.9	0.9	0.4	0.4	0.2	0.2
90%		0.7	0.6	0.4	0.4	0.2	0.2	0.08	0.08
-	R3 stream	--	6.3	3.9	3.9	1.7	1.7	0.9	0.9
50%		3.6	3.1	1.9	1.9	0.9	0.9	0.4	0.4
75%		1.8	1.6	0.962	0.962	0.4	0.4	0.2	0.2
90%		0.7	0.6	0.4	0.4	0.2	0.2	0.09	0.09
-	R4 stream	--	4.5	2.7	2.7	1.2	1.2	0.6	0.6
50%		2.6	2.2	1.4	1.4	0.6	0.6	0.3	0.3
75%		1.3	1.1	0.7	0.7	0.3	0.3	0.2	0.2
90%		0.5	0.4	0.3	0.3	0.1	0.1	0.06	0.06

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in **bold**

For the intended application of ADM.00150.I.2.A in “pome/stone fruits” at 46 g a.s./ha (umbrella use Xa; Mar-Aug / BBCH 12-91), the calculated PEC/RAC ratios indicate an acceptable risk from exposure to acetamiprid if certain mitigation measures are considered. For the mesocosm study (ETO; mean measured; aquatic invertebrates, acute and sed. dwell. organisms, prolonged, RAC = 0.56 µg/L), a non-sprayed buffer zone of 20 m or 50% drift reducing nozzles and a non-sprayed buffer zone of 15 m or 75% drift reducing nozzles and a non-sprayed buffer zone of 10 m or 90% drift reducing nozzles should be considered.

Table 9.5-46: Aquatic organisms - acceptability of risk (PEC/RAC < 1) for acetamiprid for each organism group based on FOCUS Step 1 - 3 calculations for the proposed application (46 g a.s./ha) of ADM.00150.I.2.A in ‘pome/stone fruit, late applications (umbrella use Xa; Mar-Aug / BBCH 91)’

Group		Fish, acute	Fish, prolonged	Invertebrate, acute	Invertebrate, prolonged	Invertebrates incl. sed. dwellers, acute and prolonged	Sed. dwell. organisms, prolonged	Algae
Test Species		<i>C. variegatus</i>	<i>P. promelas</i>	Geomean, Invertebrate acute	<i>D. magna</i>	Mesocosm (ETO); mean measured	<i>C. riparius</i>	<i>A. flos-aquae</i>
Endpoint (µg/L)		LC ₅₀ 100000	EC ₁₀ 9400	EC ₅₀ 8.5	EC ₁₀ 2960	NOEC 1.12	EC ₁₀ 0.235	E _r C ₅₀ >1300
AF		100	10	100	10	2	10	10
RAC (µg/L)		1000	940	0.085	296	0.56	0.024	>130
FOCUS Scenario	PEC _{sw,max} (µg/L)	ETR (PEC/RAC)						
Step 1								
	15.838	0.02	0.02	186	0.05	28	674	<0.1
Step 2								
N-Europe	2.781	0.003	0.003	33	0.009	5.0	118	<0.02
S-Europe	2.623	0.003	0.003	31	0.009	4.7	112	<0.02
Step 3								
D3 ditch	1.697	0.002	0.002	20	0.006	3.0	72	<0.01
D4 pond	0.076	0.00008	0.00008	0.9	0.0003	0.1	3.2	<0.0006
D4 stream	1.657	0.002	0.002	19	0.006	3.0	71	<0.01
D5 pond	0.076	0.00008	0.00008	0.9	0.0003	0.1	3.2	<0.0006
D5 stream	1.831	0.002	0.002	22	0.006	3.3	78	<0.01
R1 pond	0.076	0.00008	0.00008	0.9	0.0003	0.1	3.2	<0.0006
R1 stream	1.298	0.001	0.001	15	0.004	2.3	55	<0.01
R3 stream	1.830	0.002	0.002	22	0.006	3.3	78	<0.01
R4 stream	1.298	0.001	0.001	15	0.004	2.3	55	<0.01

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in **bold**

For the intended application of ADM.00150.I.2.A in “pome/stone fruits” at 46 g a.s./ha (umbrella use Xa; Mar-Aug / BBCH 91), the calculated PEC/RAC ratios indicate an acceptable risk from exposure to acetamiprid for all groups of aquatic organisms based on tier 1 endpoints and FOCUS Step 1-3 PEC values, except the higher tier mesocosm endpoint also covering the lower tier invertebrate endpoints. Additional PEC/RAC ratios were calculated considering FOCUS Step 4 PEC values.

Table 9.5-47: Aquatic organisms - acceptability of risk (PEC/RAC < 1) for acetamiprid for invertebrates incl. sed. dwellers, acute and prolonged based on FOCUS Step 4 calculations for the proposed application (46 g a.s./ha) of ADM.00150.I.2.A in ‘pome/stone fruit, late applications (umbrella use Xa; Mar-Aug / BBCH 91)’

Intended use		Pome/stone fruit, late applications, umbrella use Xa; Mar-Aug / BBCH 91							
Active substance		Acetamiprid							
Application rate (g/ha)		46							
FOCUS Step 4 PEC _{sw, max} (µg/L)									
Nozzle reduction	Non-sprayed buffer (m)	-	5	10	10	15	15	20	20
	Vegetated filter strip (m)	-	-	-	10	-	10 (VFSmod)	-	20
-	D3 ditch	--	1.146	0.512	0.512	0.259	0.259	0.158	0.158
50%		0.849	0.573	0.256	0.256	0.129	0.129	0.079	0.079
75%		0.424	0.286	0.128	0.128	0.065	0.065	0.039	0.039
90%		0.170	0.115	0.051	0.051	0.026	0.026	0.016	0.016
-	D4 pond	--	0.087	0.048	0.048	0.031	0.031	0.022	0.022
50%		0.038	0.043	0.024	0.024	0.015	0.015	0.011	0.011
75%		0.019	0.022	0.012	0.012	0.008	0.008	0.005	0.005
90%		0.008	0.009	0.005	0.005	0.003	0.003	0.002	0.002
-	D4 stream	--	1.293	0.578	0.578	0.292	0.292	0.178	0.178
50%		0.829	0.647	0.289	0.289	0.146	0.146	0.089	0.089
75%		0.414	0.323	0.144	0.144	0.073	0.073	0.045	0.045
90%		0.166	0.129	0.058	0.058	0.029	0.029	0.018	0.018
-	D5 pond	--	0.087	0.048	0.048	0.031	0.031	0.022	0.022
50%		0.038	0.043	0.024	0.024	0.015	0.015	0.011	0.011
75%		0.019	0.022	0.012	0.012	0.008	0.008	0.005	0.005
90%		0.008	0.009	0.005	0.005	0.003	0.003	0.002	0.002
-	D5 stream	--	1.429	0.639	0.639	0.322	0.322	0.197	0.197
50%		0.916	0.714	0.319	0.319	0.161	0.161	0.099	0.099
75%		0.458	0.357	0.160	0.160	0.081	0.081	0.049	0.049
90%		0.183	0.143	0.064	0.064	0.032	0.032	0.020	0.020
-	R1 pond	--	0.087	0.048	0.048	0.031	0.031	0.022	0.022
50%		0.038	0.043	0.024	0.024	0.015	0.015	0.011	0.011
75%		0.019	0.022	0.012	0.012	0.008	0.008	0.005	0.005
90%		0.008	0.009	0.005	0.005	0.003	0.003	0.002	0.002
-	R1 stream	--	1.013	0.453	0.453	0.229	0.229	0.140	0.140
50%		0.649	0.507	0.226	0.226	0.114	0.114	0.070	0.070
75%		0.325	0.253	0.113	0.113	0.057	0.057	0.035	0.035
90%		0.130	0.101	0.045	0.045	0.023	0.023	0.014	0.014
-	R2 stream	--	1.358	0.607	0.607	0.306	0.306	0.187	0.187
50%		0.870	0.679	0.303	0.303	0.153	0.153	0.094	0.094
75%		0.435	0.340	0.152	0.152	0.077	0.077	0.047	0.047
90%		0.174	0.136	0.061	0.061	0.031	0.031	0.019	0.019
-	R3 stream	--	1.428	0.638	0.638	0.367	0.322	0.367	0.197

Intended use		Pome/stone fruit, late applications, umbrella use Xa; Mar-Aug / BBCH 91							
Active substance		Acetamiprid							
Application rate (g/ha)		46							
FOCUS Step 4 PEC _{sw, max} (µg/L)									
Nozzle reduction	Non-sprayed buffer (m)	-	5	10	10	15	15	20	20
	Vegetated filter strip (m)	-	-	-	10	-	10 (VFSmod)	-	20
50%	R4 stream	0.915	0.714	0.367	0.319	0.367	0.166	0.367	0.098
75%		0.457	0.367	0.367	0.166	0.367	0.166	0.367	0.087
90%		0.367	0.367	0.367	0.166	0.367	0.166	0.367	0.087
-		--	1.013	0.453	0.453	0.229	0.229	0.188	0.140
50%		0.649	0.506	0.226	0.226	0.188	0.114	0.188	0.070
75%		0.324	0.253	0.188	0.113	0.188	0.085	0.188	0.045
90%		0.188	0.188	0.188	0.085	0.188	0.085	0.188	0.045
Mesocosm (ETO); mean measured 0.56 µg/L		ETR (PEC/RAC)							
-	D3 ditch	--	2.0	0.9	0.9	0.5	0.5	0.3	0.3
50%		1.5	1.02	0.5	0.5	0.2	0.2	0.1	0.1
75%		0.8	0.5	0.2	0.2	0.1	0.1	0.07	0.07
90%		0.3	0.2	0.09	0.09	0.05	0.05	0.03	0.03
-	D4 stream	--	2.3	1.03*	1.03	0.5	0.5	0.3	0.3
50%		1.5	1.2	0.5	0.5	0.3	0.3	0.2	0.2
75%		0.7	0.6	0.3	0.3	0.1	0.1	0.08	0.08
90%		0.3	0.2	0.1	0.1	0.05	0.05	0.03	0.03
-	D5 stream	--	2.6	1.1*	1.1	0.6	0.6	0.4	0.4
50%		1.6	1.3	0.6	0.6	0.3	0.3	0.2	0.2
75%		0.8	0.6	0.3	0.3	0.1	0.1	0.09	0.09
90%		0.3	0.3	0.1	0.1	0.06	0.06	0.04	0.04
-	R1 stream	--	1.8	0.8	0.8	0.4	0.4	0.3	0.3
50%		1.2	0.9	0.4	0.4	0.2	0.2	0.1	0.1
75%		0.6	0.5	0.2	0.2	0.1	0.1	0.06	0.06
90%		0.2	0.2	0.08	0.08	0.04	0.04	0.03	0.03
-	R3 stream	--	2.6	1.1*	1.1	0.7	0.6	0.7	0.4
50%		1.6	1.3	0.7	0.6	0.7	0.3	0.7	0.2
75%		0.8	0.7	0.7	0.3	0.7	0.3	0.7	0.2
90%		0.7	0.7	0.7	0.3	0.7	0.3	0.7	0.2
-	R4 stream	--	1.8	0.8	0.8	0.4	0.4	0.3	0.3
50%		1.2	0.9	0.4	0.4	0.3	0.2	0.3	0.1
75%		0.6	0.5	0.3	0.2	0.3	0.2	0.3	0.08
90%		0.3	0.3	0.3	0.2	0.3	0.2	0.3	0.08

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in **bold**; * safe use can be demonstrated with the implementation of the EoP approach

For the intended application of ADM.00150.I.2.A in “pome/stone fruits” at 46 g a.s./ha (umbrella use Xa; Mar-Aug / BBCH 91), the calculated PEC/RAC ratios indicate an acceptable risk from exposure to acetamiprid if certain mitigation measures are considered. For the mesocosm study (ETO; mean measured; aquatic invertebrates, acute and sed. dwell. organisms, prolonged, RAC = 0.56 µg/L), a non-sprayed buffer zone of 15 m or 50% drift reducing nozzles and a non-sprayed buffer zone of 10 m or 75% drift reducing nozzles should be considered. With the implementation of the EoP approach, additional safe use can be demonstrated when considering a non-sprayed buffer zone of 10 m only (**Figure 7** and **Figure 8**).

Figure 7: Worst-case (maximum peak) exposure profile analysis for acetamiprid Step 4, 10 m non-sprayed buffer, scenario D5 stream for the proposed application (46 g a.s./ha) of ADM.00150.I.2.A in pome/stone fruit, late applications (umbrella use Xa; Mar-Aug / BBCH 91)

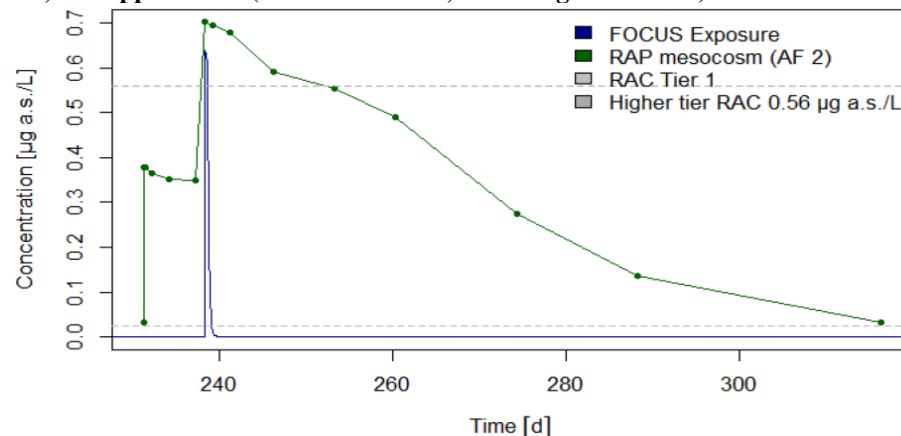


Figure 8: Worst-case (highest AUC) exposure profile analysis for acetamiprid Step 4, 10 m non-sprayed buffer, scenario D3 ditch for the proposed application (46 g a.s./ha) of ADM.00150.I.2.A in pome/stone fruit, late applications (umbrella use Xa; Mar-Aug / BBCH 91)

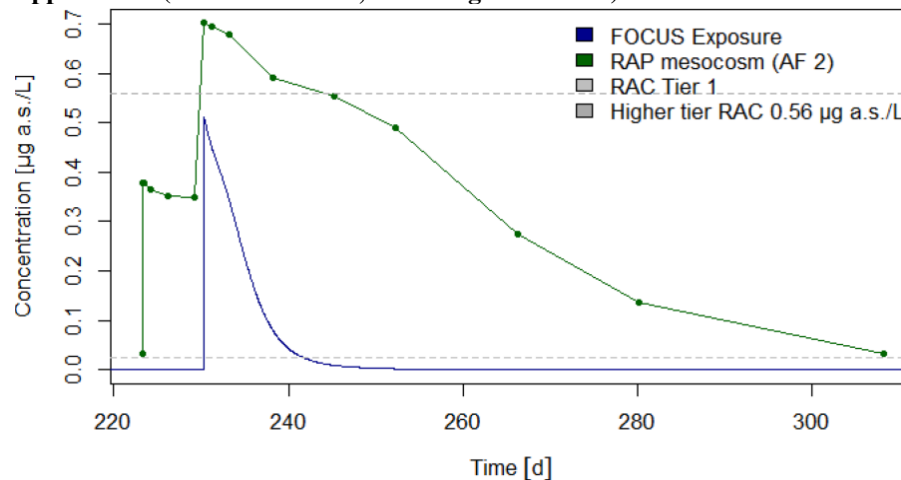


Table 9.5-48: Aquatic organisms - acceptability of risk (PEC/RAC < 1) for acetamiprid for each organism group based on FOCUS Step 1 - 3 calculations for the proposed application (34 +34 g a.s./ha) of ADM.00150.I.2.A in ‘vegetables, leafy (legumes as surrogate for D5) (umbrella use Xb; Mar-Aug / BBCH 12)’

Application (0.4-0.4 g a.s./ha) of 100-1000 L/m ² in vegetables, ready (regimes as surrogate for EC) (umbrella use AD, Max. RAC = 1000 L)								
Group		Fish, acute	Fish, prolonged	Invertebrate, acute	Invertebrate, prolonged	Invertebrates incl. sed. dwellers, acute and prolonged	Sed. dwell. organisms, prolonged	Algae
Test Species		<i>C. variegatus</i>	<i>P. promelas</i>	Geomean, Invertebrate acute	<i>D. magna</i>	Mesocosm (ETO); mean measured	<i>C. riparius</i>	<i>A. flos-aquae</i>
Endpoint (µg/L)		LC ₅₀ 100000	EC ₁₀ 9400	EC ₅₀ 8.5	EC ₁₀ 2960	NOEC 1.12	EC ₁₀ 0.235	E _r C ₅₀ >1300
AF		100	10	100	10	2	10	10
RAC (µg/L)		1000	940	0.085	296	0.56	0.024	>130
FOCUS Scenario	PEC _{sw,max} (µg/L)	ETR (PEC/RAC)						
Step 1								
	20.474	0.02	0.02	241	0.07	37	871	<0.2
Step 2								
N-Europe	0.987	0.001	0.001	12	0.003	1.8	42	<0.008
S-Europe	0.874	0.0009	0.0009	10	0.003	1.6	37	<0.007
Step 3								
D3 ditch	0.189	0.0002	0.0002	2.2	0.0006	0.3	8.0	<0.001
D4 pond	0.010	0.00001	0.00001	0.1	0.00003	0.02	0.4	<0.00008
D4 stream	0.151	0.0002	0.0002	1.8	0.0005	0.3	6.4	<0.001
D5 pond	0.010	0.00001	0.00001	0.1	0.00003	0.02	0.42	<0.00008
D5 stream	0.140	0.0001	0.0001	1.7	0.0005	0.3	5.8	<0.001
R1 pond	0.015	0.00001	0.00002	0.2	0.00005	0.03	0.6	<0.0001
R1 stream	0.151	0.0002	0.0002	1.8	0.0005	0.3	6.4	<0.001
R3 stream	0.174	0.0002	0.0002	2.0	0.0006	0.3	7.4	<0.001
R4 stream	0.630	0.0006	0.0007	7.4	0.002	1.1	27	<0.005

For the intended application of ADM.00150.I.2.A in “leafy vegetables” (legumes as surrogate for D5) at 34 +34 g a.s./ha (umbrella use Xb; Mar-Aug / BBCH 12), the calculated PEC/RAC ratios indicate an acceptable risk from exposure to acetamiprid for all groups of aquatic organisms based on tier 1 endpoints and FOCUS Step 1-3 PEC values, except the higher tier mesocosm endpoint also covering the lower tier invertebrate endpoints. Additional PEC/RAC ratios were calculated considering FOCUS Step 4 PEC values.

Table 9.5-49: Aquatic organisms - acceptability of risk (PEC/RAC < 1) for acetamiprid for invertebrates incl. sed. dwellers, acute and prolonged based on FOCUS Step 4 calculations for the proposed application (34 +34 g a.s./ha) of ADM.00150.I.2.A in ‘vegetables, leafy (legumes as surrogate for D5) (umbrella use Xb; Mar-Aug / BBCH 12)’

Intended use		Vegetables, leafy, umbrella use Xb; Mar-Aug / BBCH 12							
Active substance		Acetamiprid							
Application rate (g/ha)		34 +34							
FOCUS Step 4 PEC _{sw, max} (µg/L)									
Nozzle reduction	Non-sprayed buffer (m)	-	5	10	10	15	15	20	20
	Vegetated filter strip (m)	-	-	-	10	-	10 (VFSmod)	-	20
-	D3 ditch	--	0.049	0.025	0.025	0.017	0.017	0.013	0.013
50%		0.094	0.024	0.013	0.013	0.009	0.009	0.006	0.006
75%		0.047	0.012	0.006	0.006	0.004	0.004	0.003	0.003
90%		0.019	0.005	0.003	0.003	0.002	0.002	0.001	0.001
-	D4 pond	--	0.008	0.006	0.006	0.005	0.005	0.004	0.004
50%		0.005	0.004	0.003	0.003	0.002	0.002	0.002	0.002
75%		0.002	0.002	0.001	0.001	0.001	0.001	0.001	0.001
90%		0.001	0.001	0.001	0.001	0.000	0.000	0.000	0.000
-	D4 stream	--	0.053	0.028	0.028	0.019	0.019	0.014	0.014
50%		0.075	0.027	0.014	0.014	0.009	0.009	0.007	0.007
75%		0.038	0.013	0.007	0.007	0.005	0.005	0.004	0.004
90%		0.015	0.005	0.003	0.003	0.002	0.002	0.001	0.001
-	D5 pond (legumes as surrogate crop)	Not calculated – no mitigation needed.							
50%									
75%									
-	D5 stream (legumes as surrogate crop)	Not calculated – no mitigation needed.							
50%									
75%									
-	D6 ditch	--	0.048	0.025	0.025	0.017	0.017	0.013	0.013
50%		0.093	0.024	0.013	0.013	0.008	0.008	0.006	0.006
75%		0.046	0.012	0.006	0.006	0.004	0.004	0.003	0.003
90%		0.019	0.005	0.003	0.003	0.002	0.002	0.001	0.001
-	R1 pond	--	0.014	0.012	0.008	0.011	0.007	0.010	0.005
50%		0.011	0.010	0.009	0.005	0.009	0.005	0.008	0.003
75%		0.009	0.008	0.008	0.004	0.008	0.004	0.007	0.002
90%		0.007	0.007	0.007	0.003	0.007	0.003	0.007	0.002
-	R1 stream	--	0.151	0.151	0.068	0.151	0.068	0.151	0.036
50%		0.151	0.151	0.151	0.068	0.151	0.068	0.151	0.036
75%		0.151	0.151	0.151	0.068	0.151	0.068	0.151	0.036
90%		0.151	0.151	0.151	0.068	0.151	0.068	0.151	0.036
-	R2 stream	--	0.072	0.072	0.032	0.072	0.032	0.072	0.017

Intended use		Vegetables, leafy, umbrella use Xb; Mar-Aug / BBCH 12							
Active substance		Acetamiprid							
Application rate (g/ha)		34 +34							
FOCUS Step 4 PEC _{sw, max} (µg/L)									
Nozzle reduction	Non-sprayed buffer (m)	-	5	10	10	15	15	20	20
	Vegetated filter strip (m)	-	-	-	10	-	10 (VFSmod)	-	20
50%	R3 stream	0.081	0.072	0.072	0.032	0.072	0.032	0.072	0.017
75%		0.072	0.072	0.072	0.032	0.072	0.032	0.072	0.017
90%		0.072	0.072	0.072	0.032	0.072	0.032	0.072	0.017
-		--	0.116	0.116	0.053	0.116	0.053	0.116	0.028
50%		0.116	0.116	0.116	0.053	0.116	0.053	0.116	0.028
75%		0.116	0.116	0.116	0.053	0.116	0.053	0.116	0.028
90%		0.116	0.116	0.116	0.053	0.116	0.053	0.116	0.028
-		R4 stream	--	0.630	0.630	0.286	0.630	0.286	0.630
50%	0.630		0.630	0.630	0.286	0.630	0.286	0.630	0.150
75%	0.630		0.630	0.630	0.286	0.630	0.286	0.630	0.150
90%	0.63		0.63	0.63	0.286	0.63	0.286	0.63	0.15
Mesocosm (ETO); mean measured 0.56 µg/L		ETR (PEC/RAC)							
-	R4 stream	--	1.1	1.1	0.5	1.1	0.5	1.1	0.3
50%		1.1	1.1	1.1	0.5	1.1	0.5	1.1	0.3
75%		1.1	1.1	1.1	0.5	1.1	0.5	1.1	0.3
90%		1.1	1.1	1.1	0.5	1.1	0.5	1.1	0.3

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in **bold**

For the intended application of ADM.00150.I.2.A in “leafy vegetables” (legumes as surrogate for D5) at 34 +34 g a.s./ha (umbrella use Xb; Mar-Aug / BBCH 12), the calculated PEC/RAC ratios indicate an acceptable risk from exposure to acetamiprid if certain mitigation measures are considered. For the mesocosm study (ETO; mean measured; aquatic invertebrates, acute and sed. dwell. organisms, prolonged, RAC = 0.56 µg/L), a non-sprayed buffer zone of 10 m and a vegetated filter strip of 10 m should be considered.

Table 9.5-50: Aquatic organisms - acceptability of risk (PEC/RAC < 1) for acetamiprid for each organism group based on FOCUS Step 1 - 3 calculations for the proposed application (34 g a.s./ha) of ADM.00150.I.2.A in ‘vegetables, leafy (legumes as surrogate for D5) (umbrella use Xb; Mar-Aug / BBCH 12)’

Application (0.4 g a.s./ha) of AD-M0615012/1 in vegetables, ready (regimes as surrogate for D3) (ambrosia use AD; Max. RAC: 7.500E+12)								
Group		Fish, acute	Fish, prolonged	Invertebrate, acute	Invertebrate, prolonged	Invertebrates incl. sed. dwellers, acute and prolonged	Sed. dwell. organisms, prolonged	Algae
Test Species		<i>C. variegatus</i>	<i>P. promelas</i>	Geomean, Invertebrate acute	<i>D. magna</i>	Mesocosm (ETO); mean measured	<i>C. riparius</i>	<i>A. flos-aquae</i>
Endpoint (µg/L)		LC ₅₀ 100000	EC ₁₀ 9400	EC ₅₀ 8.5	EC ₁₀ 2960	NOEC 1.12	EC ₁₀ 0.235	E _r C ₅₀ >1300
AF		100	10	100	10	2	10	10
RAC (µg/L)		1000	940	0.085	296	0.56	0.024	>130
FOCUS Scenario	PEC _{sw,max} (µg/L)	ETR (PEC/RAC)						
Step 1								
	20.474	0.02	0.02	241	0.07	37	871	<0.2
Step 2								
N-Europe	0.808	0.0008	0.0009	9.5	0.003	1.4	34	<0.006
S-Europe	0.698	0.0007	0.0007	8.2	0.002	1.2	30	<0.005
Step 3								
D3 ditch	0.216	0.0002	0.0002	2.5	0.0007	0.4	9.2	<0.002
D4 pond	0.007	0.000007	0.000007	0.08	0.00002	0.01	0.3	<0.00005
D4 stream	0.174	0.0002	0.0002	2.0	0.0006	0.3	7.4	<0.001
D5 pond	0.007	0.000007	0.00001	0.08	0.00002	0.01	0.3	<0.00005
D5 stream	0.148	0.0001	0.0002	1.7	0.0005	0.3	6.2	<0.001
R1 pond	0.007	0.000007	0.000007	0.08	0.00002	0.01	0.3	<0.00005
R1 stream	0.142	0.0001	0.0002	1.7	0.0005	0.3	6.0	<0.001
R3 stream	0.200	0.0002	0.0002	2.4	0.0007	0.4	8.5	<0.002
R4 stream	0.475	0.0005	0.0005	5.6	0.002	0.8	20	<0.004

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in **bold**

For the intended application of ADM.00150.I.2.A in “leafy vegetables” (legumes as surrogate for D5) at 34 g a.s./ha (umbrella use Xb; Mar-Aug / BBCH 12-91), the calculated PEC/RAC ratios indicate an acceptable risk from exposure to acetamiprid for all groups of aquatic organisms based on the higher tier mesocosm endpoint also covering the lower tier invertebrate endpoints and FOCUS Step 1-3 PEC values. Therefore, no further assessment is necessary.

Table 9.5-51: Aquatic organisms - acceptability of risk (PEC/RAC < 1) for acetamiprid for each organism group based on FOCUS Step 1 - 3 calculations for the proposed application (34 +34 g a.s./ha) of ADM.00150.I.2.A in ‘pome/stone fruit, early applications (umbrella use Xb; Mar-Aug / BBCH 12)’

Application (S4 + S								
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AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in **bold**

For the intended application of ADM.00150.I.2.A in “pome/stone fruits” at 34 +34 g a.s./ha (umbrella use Xb; Mar-Aug / BBCH 12), the calculated PEC/RAC ratios indicate an acceptable risk from exposure to acetamiprid for all groups of aquatic organisms based on tier 1 endpoints and FOCUS Step 1-3 PEC values, except the higher tier mesocosm endpoint also covering the lower tier invertebrate endpoints. Additional PEC/RAC ratios were calculated considering FOCUS Step 4 PEC values.

Table 9.5-52: Aquatic organisms - acceptability of risk (PEC/RAC < 1) for acetamiprid for invertebrates incl. sed. dwellers, acute and prolonged based on FOCUS Step 4 calculations for the proposed application (34 +34 g a.s./ha) of ADM.00150.I.2.A in ‘pome/stone fruit, early applications (umbrella use Xb; Mar-Aug / BBCH 12)’

Intended use		Pome/stone fruit, early applications, umbrella use Xb; Mar-Aug / BBCH 12							
Active substance		Acetamiprid							
Application rate (g/ha)		34 +34							
FOCUS Step 4 PEC _{sw, max} (µg/L)									
Nozzle reduction	Non-sprayed buffer (m)	-	5	10	10	15	15	20	20
	Vegetated filter strip (m)	-	-	-	10	-	10 (VFSmod)	-	20
-	D3 ditch	--	1.752	1.035	1.035	0.568	0.568	0.268	0.268
50%		1.136	0.876	0.518	0.518	0.284	0.284	0.134	0.134
75%		0.568	0.438	0.259	0.259	0.142	0.142	0.067	0.067
90%		0.227	0.175	0.104	0.104	0.057	0.057	0.027	0.027
-	D4 pond	--	0.242	0.137	0.137	0.072	0.072	0.041	0.041
50%		0.108	0.121	0.069	0.069	0.036	0.036	0.021	0.021
75%		0.054	0.060	0.034	0.034	0.018	0.018	0.010	0.010
90%		0.021	0.024	0.014	0.014	0.007	0.007	0.004	0.004
-	D4 stream	--	1.951	1.152	1.152	0.633	0.633	0.298	0.298
50%		1.149	0.976	0.576	0.576	0.316	0.316	0.149	0.149
75%		0.574	0.488	0.288	0.288	0.158	0.158	0.075	0.075
90%		0.230	0.195	0.115	0.115	0.063	0.063	0.030	0.030
-	D5 pond	--	0.280	0.159	0.159	0.083	0.083	0.048	0.048
50%		0.125	0.140	0.079	0.079	0.042	0.042	0.024	0.024
75%		0.062	0.070	0.040	0.040	0.021	0.021	0.012	0.012
90%		0.025	0.028	0.016	0.016	0.008	0.008	0.005	0.005
-	D5 stream	--	2.067	1.221	1.221	0.671	0.671	0.316	0.316
50%		1.218	1.034	0.611	0.611	0.335	0.335	0.158	0.158
75%		0.609	0.517	0.305	0.305	0.168	0.168	0.079	0.079
90%		0.244	0.207	0.122	0.122	0.067	0.067	0.032	0.032
-	R1 pond	--	0.291	0.165	0.165	0.087	0.087	0.050	0.050
50%		0.130	0.146	0.083	0.083	0.043	0.043	0.025	0.025
75%		0.065	0.073	0.041	0.041	0.022	0.022	0.012	0.012
90%		0.026	0.029	0.016	0.016	0.009	0.009	0.005	0.005
-	R1 stream	--	1.547	0.914	0.914	0.502	0.502	0.237	0.237
50%		0.911	0.774	0.457	0.457	0.251	0.251	0.118	0.118
75%		0.456	0.387	0.229	0.229	0.125	0.125	0.059	0.059
90%		0.182	0.155	0.091	0.091	0.050	0.050	0.024	0.024
-	R2 stream	--	2.053	1.213	1.213	0.666	0.666	0.314	0.314
50%		1.209	1.026	0.606	0.606	0.333	0.333	0.157	0.157
75%		0.604	0.513	0.303	0.303	0.166	0.166	0.078	0.078
90%		0.242	0.205	0.121	0.121	0.067	0.067	0.031	0.031
-	R3 stream	--	2.189	1.293	1.293	0.710	0.710	0.335	0.335

Intended use		Pome/stone fruit, early applications, umbrella use Xb; Mar-Aug / BBCH 12							
Active substance		Acetamiprid							
Application rate (g/ha)		34 +34							
FOCUS Step 4 PEC _{sw, max} (µg/L)									
Nozzle reduction	Non-sprayed buffer (m)	-	5	10	10	15	15	20	20
	Vegetated filter strip (m)	-	-	-	10	-	10 (VFSmod)	-	20
50%	R4 stream	1.289	1.095	0.647	0.647	0.355	0.355	0.179	0.167
75%		0.645	0.547	0.323	0.323	0.179	0.178	0.179	0.084
90%		0.258	0.219	0.179	0.129	0.179	0.081	0.179	0.042
-		--	1.556	0.919	0.919	0.505	0.505	0.279	0.238
50%		0.917	0.778	0.460	0.460	0.279	0.252	0.279	0.119
75%		0.458	0.389	0.279	0.230	0.279	0.126	0.279	0.060
90%		0.279	0.279	0.279	0.116	0.279	0.116	0.279	0.059
Mesocosm (ETO); mean measured 0.56 µg/L		ETR (PEC/RAC)							
-	D3 ditch	--	3.1	1.8	1.8	1.01	1.01	0.5	0.5
50%		2.0	1.6	0.9	0.9	0.5	0.5	0.2	0.2
75%		1.01	0.8	0.5	0.5	0.3	0.3	0.1	0.1
90%		0.4	0.3	0.2	0.2	0.1	0.1	0.05	0.05
-	D4 stream	--	3.5	2.1	2.1	1.1	1.1	0.5	0.5
50%		2.1	1.7	1.03	1.03	0.6	0.6	0.3	0.3
75%		1.02	0.9	0.5	0.5	0.3	0.3	0.1	0.1
90%		0.4	0.3	0.2	0.2	0.1	0.1	0.05	0.05
-	D5 stream	--	3.7	2.2	2.2	1.2	1.2	0.6	0.6
50%		2.2	1.8	1.1	1.1	0.6	0.6	0.3	0.3
75%		1.1	0.9	0.5	0.5	0.3	0.3	0.1	0.1
90%		0.4	0.4	0.2	0.2	0.1	0.1	0.06	0.06
-	R1 stream	--	2.8	1.6	1.6	0.9	0.9	0.4	0.4
50%		1.6	1.4	0.8	0.8	0.4	0.4	0.2	0.2
75%		0.8	0.7	0.4	0.4	0.2	0.2	0.1	0.1
90%		0.3	0.3	0.2	0.2	0.09	0.09	0.04	0.04
-	R3 stream	--	3.9	2.3	2.3	1.3	1.3	0.6	0.6
50%		2.3	2.0	1.2	1.2	0.6	0.6	0.3	0.3
75%		1.2	0.977	0.6	0.6	0.3	0.3	0.3	0.2
90%		0.5	0.4	0.3	0.2	0.3	0.1	0.3	0.08
-	R4 stream	--	2.8	1.6	1.6	0.9	0.9	0.5	0.4
50%		1.6	1.4	0.8	0.8	0.5	0.5	0.5	0.2
75%		0.8	0.7	0.5	0.4	0.5	0.2	0.5	0.1
90%		0.5	0.5	0.5	0.2	0.5	0.2	0.5	0.1

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in **bold**

For the intended application of ADM.00150.I.2.A in “pome/stone fruits” at 34 +34 g a.s./ha (umbrella use Xb; Mar-Aug / BBCH 12), the calculated PEC/RAC ratios indicate an acceptable risk from exposure to acetamiprid if certain mitigation measures are considered. For the mesocosm study (ETO; mean measured; aquatic invertebrates, acute and sed. dwell. organisms, prolonged, RAC = 0.56 µg/L), a non-sprayed buffer zone of 20 m or 50% drift reducing nozzles and a non-sprayed buffer zone of 15 m or 75% drift reducing nozzles and a non-sprayed buffer zone of 5 m or 90% drift reducing nozzles should be considered.

Table 9.5-53: Aquatic organisms - acceptability of risk (PEC/RAC < 1) for acetamiprid for each organism group based on FOCUS Step 1 - 3 calculations for the proposed application (34 g a.s./ha) of ADM.00150.I.2.A in ‘pome/stone fruit, early applications (umbrella use Xb; Mar-Aug / BBCH 12)’

Application (54 g a.s./ha) of AD-M:00150.12/1 in pebble/stone fruit, early applications (ambrosia use AB, Mar-Aug / BDEC12)								
Group		Fish, acute	Fish, prolonged	Invertebrate, acute	Invertebrate, prolonged	Invertebrates incl. sed. dwellers, acute and prolonged	Sed. dwell. organisms, prolonged	Algae
Test Species		<i>C. variegatus</i>	<i>P. promelas</i>	Geomean, Invertebrate acute	<i>D. magna</i>	Mesocosm (ETO); mean measured	<i>C. riparius</i>	<i>A. flos-aquae</i>
Endpoint (µg/L)		LC ₅₀ 100000	EC ₁₀ 9400	EC ₅₀ 8.5	EC ₁₀ 2960	NOEC 1.12	EC ₁₀ 0.235	E _r C ₅₀ >1300
AF		100	10	100	10	2	10	10
RAC (µg/L)		1000	940	0.085	296	0.56	0.024	>130
FOCUS Scenario	PEC _{sw,max} (µg/L)	ETR (PEC/RAC)						
Step 1								
	26.466	0.03	0.03	311	0.09	47	1126	<0.2
Step 2								
N-Europe	3.314	0.003	0.004	39	0.01	5.9	141	<0.03
S-Europe	3.309	0.003	0.004	39	0.01	5.9	141	<0.03
Step 3								
D3 ditch	2.640	0.003	0.003	31	0.009	4.7	112	<0.02
D4 pond	0.161	0.0002	0.0002	1.9	0.0005	0.3	6.9	<0.001
D4 stream	2.544	0.003	0.003	30	0.009	4.5	108	<0.02
D5 pond	0.161	0.0002	0.0002	1.9	0.0005	0.3	6.9	<0.001
D5 stream	2.619	0.003	0.003	31	0.009	4.7	111	<0.02
R1 pond	0.161	0.0002	0.0002	1.9	0.0005	0.3	6.9	<0.001
R1 stream	2.135	0.002	0.002	25	0.007	3.8	91	<0.02
R3 stream	3.020	0.003	0.003	36	0.01	5.4	129	<0.02
R4 stream	2.148	0.002	0.002	25	0.007	3.8	91	<0.02

For the intended application of ADM.00150.I.2.A in “pome/stone fruits” at 34 g a.s./ha (umbrella use Xb; Mar-Aug / BBCH 12), the calculated PEC/RAC ratios indicate an acceptable risk from exposure to acetamiprid for all groups of aquatic organisms based on tier 1 endpoints and FOCUS Step 1-3 PEC values, except the higher tier mesocosm endpoint also covering the lower tier invertebrate endpoints. Additional PEC/RAC ratios were calculated considering FOCUS Step 4 PEC values.

Table 9.5-54: Aquatic organisms - acceptability of risk (PEC/RAC < 1) for acetamiprid for invertebrates incl. sed. dwellers, acute and prolonged based on FOCUS Step 4 calculations for the proposed application (34 g a.s./ha) of ADM.00150.I.2.A in ‘pome/stone fruit, early applications (umbrella use Xb; Mar-Aug / BBCH 12)’

Intended use		Pome/stone fruit, early applications, umbrella use Xb; Mar-Aug / BBCH 12							
Active substance		Acetamiprid							
Application rate (g/ha)		34							
FOCUS Step 4 PEC _{sw, max} (µg/L)									
Nozzle reduction	Non-sprayed buffer (m)	-	5	10	10	15	15	20	20
	Vegetated filter strip (m)	-	-	-	10	-	10 (VFSmod)	-	20
-	D3 ditch	--	2.075	1.274	1.274	0.573	0.573	0.291	0.291
50%		1.320	1.037	0.637	0.637	0.287	0.287	0.146	0.146
75%		0.660	0.519	0.319	0.319	0.143	0.143	0.073	0.073
90%		0.264	0.207	0.127	0.127	0.057	0.057	0.029	0.029
-	D4 pond	--	0.181	0.099	0.099	0.052	0.052	0.032	0.032
50%		0.080	0.090	0.050	0.050	0.026	0.026	0.016	0.016
75%		0.040	0.045	0.025	0.025	0.013	0.013	0.008	0.008
90%		0.016	0.018	0.010	0.010	0.005	0.005	0.003	0.003
-	D4 stream	--	2.186	1.342	1.342	0.604	0.604	0.307	0.307
50%		1.272	1.093	0.671	0.671	0.302	0.302	0.154	0.154
75%		0.636	0.546	0.336	0.336	0.151	0.151	0.077	0.077
90%		0.254	0.219	0.134	0.134	0.060	0.060	0.031	0.031
-	D5 pond	--	0.181	0.099	0.099	0.052	0.052	0.032	0.032
50%		0.080	0.090	0.050	0.050	0.026	0.026	0.016	0.016
75%		0.040	0.045	0.025	0.025	0.013	0.013	0.008	0.008
90%		0.016	0.018	0.010	0.010	0.005	0.005	0.003	0.003
-	D5 stream	--	2.250	1.382	1.382	0.622	0.622	0.316	0.316
50%		1.309	1.125	0.691	0.691	0.311	0.311	0.158	0.158
75%		0.655	0.562	0.346	0.346	0.155	0.155	0.079	0.079
90%		0.262	0.225	0.138	0.138	0.062	0.062	0.032	0.032
-	R1 pond	--	0.181	0.099	0.099	0.052	0.052	0.032	0.032
50%		0.080	0.090	0.050	0.050	0.026	0.026	0.016	0.016
75%		0.040	0.045	0.025	0.025	0.013	0.013	0.008	0.008
90%		0.016	0.018	0.010	0.010	0.005	0.005	0.003	0.003
-	R1 stream	--	1.834	1.126	1.126	0.507	0.507	0.258	0.258
50%		1.067	0.917	0.563	0.563	0.253	0.253	0.129	0.129
75%		0.534	0.458	0.282	0.282	0.127	0.127	0.064	0.064
90%		0.214	0.183	0.113	0.113	0.051	0.051	0.026	0.026
-	R2 stream	--	2.430	1.492	1.492	0.671	0.671	0.341	0.341
50%		1.414	1.215	0.746	0.746	0.336	0.336	0.171	0.171
75%		0.707	0.607	0.373	0.373	0.168	0.168	0.085	0.085
90%		0.283	0.243	0.149	0.149	0.067	0.067	0.034	0.034
-	R3 stream	--	2.595	1.594	1.594	0.717	0.717	0.364	0.364

Intended use		Pome/stone fruit, early applications, umbrella use Xb; Mar-Aug / BBCH 12							
Active substance		Acetamiprid							
Application rate (g/ha)		34							
FOCUS Step 4 PEC _{sw, max} (µg/L)									
Nozzle reduction	Non-sprayed buffer (m)	-	5	10	10	15	15	20	20
	Vegetated filter strip (m)	-	-	-	10	-	10 (VFSmod)	-	20
50%	R4 stream	1.510	1.298	0.797	0.797	0.358	0.358	0.182	0.182
75%		0.755	0.649	0.399	0.399	0.179	0.179	0.091	0.091
90%		0.302	0.260	0.159	0.159	0.072	0.072	0.036	0.036
-		--	1.845	1.133	1.133	0.510	0.510	0.259	0.259
50%		1.074	0.923	0.567	0.567	0.255	0.255	0.130	0.130
75%		0.537	0.461	0.283	0.283	0.127	0.127	0.073	0.065
90%		0.215	0.185	0.113	0.113	0.073	0.051	0.073	0.026
Mesocosm (ETO); mean measured 0.56 µg/L		ETR (PEC/RAC)							
-	D3 ditch	--	3.7	2.3	2.3	1.02	1.02	0.5	0.5
50%		2.4	1.9	1.1	1.1	0.5	0.5	0.3	0.3
75%		1.2	0.9	0.6	0.6	0.3	0.3	0.1	0.1
90%		0.5	0.4	0.2	0.2	0.1	0.1	0.05	0.05
-	D4 stream	--	3.9	2.4	2.4	1.1	1.1	0.5	0.5
50%		2.3	2.0	1.2	1.2	0.5	0.5	0.3	0.3
75%		1.1	0.975	0.6	0.6	0.3	0.3	0.1	0.1
90%		0.5	0.4	0.2	0.2	0.1	0.1	0.06	0.06
-	D5 stream	--	4.0	2.5	2.5	1.1	1.1	0.6	0.6
50%		2.3	2.0	1.2	1.2	0.6	0.6	0.3	0.3
75%		1.2	1.0	0.6	0.6	0.3	0.3	0.1	0.1
90%		0.5	0.4	0.2	0.2	0.1	0.1	0.06	0.06
-	R1 stream	--	3.3	2.0	2.0	0.9	0.9	0.5	0.5
50%		1.9	1.6	1.01	1.01	0.5	0.5	0.2	0.2
75%		0.954	0.8	0.5	0.5	0.2	0.2	0.1	0.1
90%		0.4	0.3	0.2	0.2	0.09	0.09	0.05	0.05
-	R3 stream	--	4.6	2.8	2.8	1.3	1.3	0.6	0.6
50%		2.7	2.3	1.4	1.4	0.6	0.6	0.3	0.3
75%		1.3	1.2	0.7	0.7	0.3	0.3	0.2	0.2
90%		0.5	0.5	0.3	0.3	0.1	0.1	0.06	0.06
-	R4 stream	--	3.3	2.0	2.0	0.9	0.9	0.5	0.5
50%		1.9	1.6	1.01	1.01	0.5	0.5	0.2	0.2
75%		0.959	0.8	0.5	0.5	0.2	0.2	0.1	0.1
90%		0.4	0.3	0.2	0.2	0.1	0.09	0.1	0.05

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in **bold**

For the intended application of ADM.00150.I.2.A in “pome/stone fruits” at 34 g a.s./ha (umbrella use Xb; Mar-Aug / BBCH 12), the calculated PEC/RAC ratios indicate an acceptable risk from exposure to acetamiprid if certain mitigation measures are considered. For the mesocosm study (ETO; mean measured; aquatic invertebrates, acute and sed. dwell. organisms, prolonged, RAC = 0.56 µg/L), a non-sprayed buffer zone of 20 m or 50% drift reducing nozzles and a non-sprayed buffer zone of 15 m or a 75% drift reducing nozzles and a non-sprayed buffer zone of 10 m or 90% drift reducing nozzles should be considered.

Table 9.5-55: Aquatic organisms - acceptability of risk (PEC/RAC < 1) for acetamiprid for each organism group based on FOCUS Step 1 - 3 calculations for the proposed application (34 +34 g a.s./ha) of ADM.00150.I.2.A in ‘pome/stone fruit, late applications (umbrella use Xb; Mar-Aug / BBCH 91)’

Group		Fish, acute	Fish, prolonged	Invertebrate, acute	Invertebrate, prolonged	Invertebrates incl. sed. dwellers, acute and prolonged	Sed. dwell. organisms, prolonged	Algae
Test Species		<i>C. variegatus</i>	<i>P. promelas</i>	Geomean, Invertebrate acute	<i>D. magna</i>	Mesocosm (ETO); mean measured	<i>C. riparius</i>	<i>A. flos-aquae</i>
Endpoint (µg/L)		LC ₅₀ 100000	EC ₁₀ 9400	EC ₅₀ 8.5	EC ₁₀ 2960	NOEC 1.12	EC ₁₀ 0.235	E _r C ₅₀ >1300
AF		100	10	100	10	2	10	10
RAC (µg/L)		1000	940	0.085	296	0.56	0.024	>130
FOCUS Scenario	PEC _{sw,max} (µg/L)	ETR (PEC/RAC)						
Step 1								
	23.413	0.02	0.02	275	0.08	42	996	<0.2
Step 2								
N-Europe	2.687	0.003	0.003	32	0.009	4.8	114	<0.02
S-Europe	2.566	0.003	0.003	30	0.009	4.6	109	<0.02
Step 3								
D3 ditch	1.141	0.001	0.001	13	0.004	2.0	49	<0.009
D4 pond	0.080	0.00008	0.00009	0.9	0.0003	0.1	3.4	<0.0006
D4 stream	0.981	0.001	0.001	12	0.003	1.8	42	<0.008
D5 pond	0.085	0.00009	0.00009	1.0	0.0003	0.2	3.6	<0.0007
D5 stream	1.084	0.001	0.001	13	0.004	1.9	46	<0.008
R1 pond	0.070	0.00007	0.00007	0.8	0.0002	0.1	3.0	<0.0005
R1 stream	0.768	0.0008	0.0008	9.0	0.003	1.4	33	<0.006
R3 stream	1.083	0.001	0.001	13	0.004	1.9	46	<0.008
R4 stream	0.768	0.0008	0.0008	9.0	0.003	1.4	33	<0.006

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in **bold**

For the intended application of ADM.00150.I.2.A in “pome/stone fruits” at 34 +34 g a.s./ha (umbrella use Xa; Mar-Aug / BBCH 12-91), the calculated PEC/RAC ratios indicate an acceptable risk from exposure to acetamiprid for all groups of aquatic organisms based on tier 1 endpoints and FOCUS Step 1-3 PEC values, except the higher tier mesocosm endpoint also covering the lower tier invertebrate endpoints. Additional PEC/RAC ratios were calculated considering FOCUS Step 4 PEC values.

Table 9.5-56: Aquatic organisms - acceptability of risk (PEC/RAC < 1) for acetamiprid for invertebrates incl. sed. dwellers, acute and prolonged based on FOCUS Step 4 calculations for the proposed application (34 +34 g a.s./ha) of ADM.00150.I.2.A in ‘pome/stone fruit, late applications (umbrella use Xb; Mar-Aug / BBCH 91)’

Intended use		Pome/stone fruit, late applications, umbrella use Xb; Mar-Aug / BBCH 12-91							
Active substance		Acetamiprid							
Application rate (g/ha)		34 +34							
FOCUS Step 4 PEC _{sw, max} (µg/L)									
Nozzle reduction	Non-sprayed buffer (m)	-	5	10	10	15	15	20	20
	Vegetated filter strip (m)	-	-	-	10	-	10 (VFSmod)	-	20
-	D3 ditch	--	0.793	0.381	0.381	0.187	0.187	0.108	0.108
50%		0.570	0.397	0.191	0.191	0.093	0.093	0.054	0.054
75%		0.285	0.198	0.095	0.095	0.047	0.047	0.027	0.027
90%		0.114	0.079	0.038	0.038	0.019	0.019	0.011	0.011
-	D4 pond	--	0.092	0.050	0.050	0.031	0.031	0.021	0.021
50%		0.040	0.046	0.025	0.025	0.015	0.015	0.010	0.010
75%		0.020	0.023	0.013	0.013	0.008	0.008	0.005	0.005
90%		0.008	0.009	0.005	0.005	0.003	0.003	0.002	0.002
-	D4 stream	--	0.780	0.375	0.375	0.183	0.183	0.106	0.106
50%		0.490	0.390	0.187	0.187	0.092	0.092	0.053	0.053
75%		0.245	0.195	0.094	0.094	0.046	0.046	0.027	0.027
90%		0.098	0.078	0.037	0.037	0.018	0.018	0.011	0.011
-	D5 pond	--	0.097	0.053	0.053	0.032	0.032	0.022	0.022
50%		0.043	0.049	0.027	0.027	0.016	0.016	0.011	0.011
75%		0.021	0.024	0.013	0.013	0.008	0.008	0.006	0.006
90%		0.008	0.010	0.005	0.005	0.003	0.003	0.002	0.002
-	D5 stream	--	0.862	0.414	0.414	0.203	0.203	0.118	0.118
50%		0.542	0.431	0.207	0.207	0.101	0.101	0.059	0.059
75%		0.271	0.216	0.104	0.104	0.051	0.051	0.029	0.029
90%		0.108	0.086	0.041	0.041	0.020	0.020	0.012	0.012
-	R1 pond	--	0.080	0.044	0.044	0.026	0.026	0.018	0.018
50%		0.035	0.040	0.022	0.022	0.013	0.013	0.009	0.009
75%		0.017	0.020	0.011	0.011	0.007	0.007	0.005	0.005
90%		0.007	0.008	0.004	0.004	0.003	0.003	0.002	0.002
-	R1 stream	--	0.611	0.294	0.294	0.144	0.144	0.083	0.083
50%		0.384	0.306	0.147	0.147	0.072	0.072	0.042	0.042
75%		0.192	0.153	0.073	0.073	0.036	0.036	0.021	0.021
90%		0.077	0.061	0.029	0.029	0.014	0.014	0.008	0.008
-	R2 stream	--	0.819	0.394	0.394	0.193	0.193	0.112	0.112
50%		0.515	0.410	0.197	0.197	0.096	0.096	0.056	0.056
75%		0.258	0.205	0.098	0.098	0.048	0.048	0.028	0.028
90%		0.103	0.082	0.039	0.039	0.019	0.019	0.011	0.011
-	R3 stream	--	0.862	0.415	0.414	0.209	0.203	0.209	0.118

Intended use		Pome/stone fruit, late applications, umbrella use Xb; Mar-Aug / BBCH 12-91							
Active substance		Acetamiprid							
Application rate (g/ha)		34 +34							
FOCUS Step 4 PEC _{sw, max} (µg/L)									
Nozzle reduction	Non-sprayed buffer (m)	-	5	10	10	15	15	20	20
	Vegetated filter strip (m)	-	-	-	10	-	10 (VFSmod)	-	20
50%	R4 stream	0.542	0.431	0.209	0.207	0.209	0.102	0.209	0.059
75%		0.271	0.216	0.209	0.104	0.209	0.094	0.209	0.049
90%		0.209	0.209	0.209	0.094	0.209	0.094	0.209	0.049
-		--	0.611	0.294	0.294	0.144	0.144	0.137	0.083
50%		0.384	0.306	0.147	0.147	0.137	0.072	0.137	0.042
75%		0.192	0.153	0.137	0.073	0.137	0.062	0.137	0.033
90%		0.137	0.137	0.137	0.062	0.137	0.062	0.137	0.033
Mesocosm (ETO); mean measured 0.56 µg/L		ETR (PEC/RAC)							
-	D3 ditch	--	1.4	0.7	0.7	0.3	0.3	0.2	0.2
50%		1.02*	0.7	0.3	0.3	0.2	0.2	0.1	0.1
75%		0.5	0.4	0.2	0.2	0.08	0.08	0.05	0.05
90%		0.2	0.1	0.07	0.07	0.03	0.03	0.02	0.02
-	D4 stream	--	1.4	0.7	0.7	0.3	0.3	0.2	0.2
50%		0.9	0.7	0.3	0.3	0.2	0.2	0.09	0.09
75%		0.4	0.3	0.2	0.2	0.08	0.08	0.05	0.05
90%		0.2	0.1	0.07	0.07	0.03	0.03	0.02	0.02
-	D5 stream	--	1.5	0.7	0.7	0.4	0.4	0.2	0.2
50%		0.968	0.8	0.4	0.4	0.2	0.2	0.1	0.1
75%		0.5	0.4	0.2	0.2	0.09	0.09	0.05	0.05
90%		0.2	0.2	0.07	0.07	0.04	0.04	0.02	0.02
-	R1 stream	--	1.1	0.5	0.5	0.3	0.3	0.1	0.1
50%		0.7	0.5	0.3	0.3	0.1	0.1	0.08	0.08
75%		0.3	0.3	0.1	0.1	0.06	0.06	0.04	0.04
90%		0.1	0.1	0.05	0.05	0.03	0.03	0.01	0.01
-	R3 stream	--	1.5	0.7	0.7	0.4	0.4	0.4	0.2
50%		0.968	0.8	0.4	0.4	0.4	0.2	0.4	0.1
75%		0.5	0.4	0.4	0.2	0.4	0.2	0.4	0.09
90%		0.4	0.4	0.4	0.2	0.4	0.2	0.4	0.09
-	R4 stream	--	1.1	0.5	0.5	0.3	0.3	0.2	0.1
50%		0.7	0.5	0.3	0.3	0.2	0.1	0.2	0.08
75%		0.3	0.3	0.2	0.1	0.2	0.1	0.2	0.06
90%		0.2	0.2	0.2	0.1	0.2	0.1	0.2	0.06

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in **bold**; * safe use can be demonstrated with the implementation of the EoP approach

For the intended application of ADM.00150.I.2.A in “pome/stone fruits” at 34 +34 g a.s./ha (umbrella use Xb; Mar-Aug / BBCH 12-91), the calculated PEC/RAC ratios indicate an acceptable risk from exposure to acetamiprid if certain mitigation measures are considered. For the mesocosm study (ETO; mean measured; aquatic invertebrates, acute and sed. dwell. organisms, prolonged, RAC = 0.56 µg/L), a non-sprayed buffer zone of 10 m or 50% drift reducing nozzles and a non-sprayed buffer zone of 5 m or 75% drift reducing nozzles should be considered. With the implementation of the EoP approach, additional safe use can be demonstrated when considering only 50% drift reducing nozzles (**Figure 9**).

Figure 9: Worst-case (maximum peak and highest AUC) exposure profile analysis for acetamiprid Step 4, 50% drift reducing nozzles, scenario D3 ditch for the proposed application (34 +34 g a.s./ha) of ADM.00150.I.2.A in ‘pome/stone fruit, late applications (umbrella use Xb; Mar-Aug / BBCH 91)’

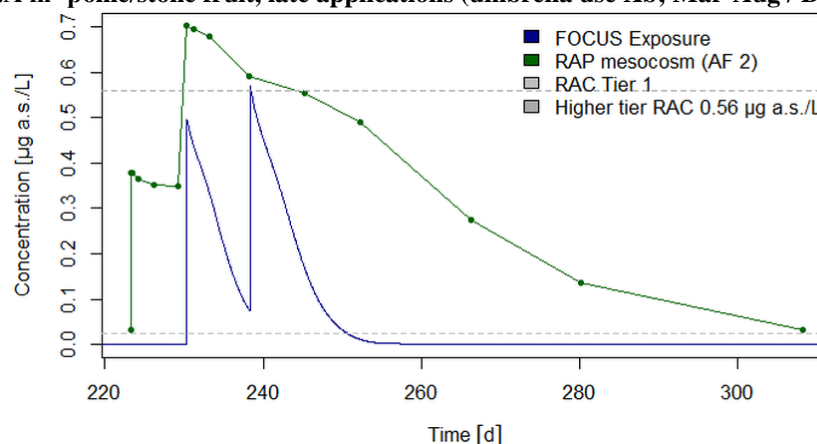


Table 9.5-57: Aquatic organisms - acceptability of risk (PEC/RAC < 1) for acetamiprid for each organism group based on FOCUS Step 1 - 3 calculations for the proposed application (34 g a.s./ha) of ADM.00150.I.2.A in ‘pome/stone fruit, late applications (umbrella use Xb; Mar-Aug / BBCH 12-91)’

Group		Fish, acute	Fish, prolonged	Invertebrate, acute	Invertebrate, prolonged	Invertebrates incl. sed. dwellers, acute and prolonged	Sed. dwell. organisms, prolonged	Algae
Test Species		<i>C. variegatus</i>	<i>P. promelas</i>	Geomean, Invertebrate acute	<i>D. magna</i>	Mesocosm (ETO); mean measured	<i>C. riparius</i>	<i>A. flos-aquae</i>
Endpoint (µg/L)		LC ₅₀	EC ₁₀	EC ₅₀	EC ₁₀	NOEC	EC ₁₀	E _r C ₅₀
AF		100000	9400	8.5	2960	1.12	0.235	>1300
RAC (µg/L)		100	10	100	10	2	10	10
RAC (µg/L)		1000	940	0.085	296	0.56	0.024	>130
FOCUS Scenario	PEC _{sw,max} (µg/L)	ETR (PEC/RAC)						
Step 1								
	23.413	0.02	0.02	275	0.08	42	996	<0.2
Step 2								

Group		Fish, acute	Fish, prolonged	Invertebrate, acute	Invertebrate, prolonged	Invertebrates incl. sed. dwellers, acute and prolonged	Sed. dwell. organisms, prolonged	Algae
Test Species		<i>C. variegatus</i>	<i>P. promelas</i>	Geomean, Invertebrate acute	<i>D. magna</i>	Mesocosm (ETO); mean measured	<i>C. riparius</i>	<i>A. flos-aquae</i>
Endpoint (µg/L)		LC ₅₀ 100000	EC ₁₀ 9400	EC ₅₀ 8.5	EC ₁₀ 2960	NOEC 1.12	EC ₁₀ 0.235	E _r C ₅₀ >1300
AF		100	10	100	10	2	10	10
RAC (µg/L)		1000	940	0.085	296	0.56	0.024	>130
FOCUS Scenario	PEC _{sw,max} (µg/L)	ETR (PEC/RAC)						
N-Europe	2.056	0.002	0.002	24	0.007	3.7	87	<0.02
S-Europe	1.938	0.002	0.002	23	0.007	3.5	82	<0.01
Step 3								
D3 ditch	1.254	0.001	0.001	15	0.004	2.2	53	<0.01
D4 pond	0.056	0.00006	0.00006	0.7	0.0002	0.1	2.4	<0.0004
D4 stream	1.225	0.001	0.001	14	0.004	2.2	52	<0.009
D5 pond	0.056	0.00006	0.00006	0.7	0.0002	0.1	2.4	<0.0004
D5 stream	1.353	0.001	0.001	16	0.005	2.4	58	<0.01
R1 pond	0.056	0.00006	0.00006	0.7	0.0002	0.1	2.4	<0.0004
R1 stream	0.960	0.001	0.001	11	0.003	1.7	41	<0.007
R3 stream	1.352	0.001	0.001	16	0.005	2.4	58	<0.01
R4 stream	0.959	0.001	0.001	11	0.003	1.7	41	<0.007

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in **bold**

For the intended application of ADM.00150.I.2.A in “pome/stone fruits” at 34 g a.s./ha (umbrella use Xa; Mar-Aug / BBCH 12-91), the calculated PEC/RAC ratios indicate an acceptable risk from exposure to acetamiprid for all groups of aquatic organisms based on tier 1 endpoints and FOCUS Step 1-3 PEC values, except the higher tier mesocosm endpoint also covering the lower tier invertebrate endpoints. Additional PEC/RAC ratios were calculated considering FOCUS Step 4 PEC values.

Table 9.5-58: Aquatic organisms - acceptability of risk (PEC/RAC < 1) for acetamiprid for invertebrates incl. sed. dwellers, acute and prolonged based on FOCUS Step 4 calculations for the proposed application (34 g a.s./ha) of ADM.00150.I.2.A in ‘pome/stone fruit, late applications (umbrella use Xb; Mar-Aug / BBCH 12-91)’

Intended use		Pome/stone fruit, late applications, umbrella use Xb; Mar-Aug / BBCH 12-91							
Active substance		Acetamiprid							
Application rate (g/ha)		34							
FOCUS Step 4 PEC _{sw, max} (µg/L)									
Nozzle reduction	Non-sprayed buffer (m)	-	5	10	10	15	15	20	20
	Vegetated filter strip (m)	-	-	-	10	-	10 VFS _{mod}	-	20
-	D3 ditch	--	0.847	0.378	0.378	0.191	0.191	0.117	0.117
50%		0.627	0.423	0.189	0.189	0.096	0.096	0.058	0.058
75%		0.314	0.212	0.095	0.095	0.048	0.048	0.029	0.029
90%		0.125	0.085	0.038	0.038	0.019	0.019	0.012	0.012
-	D4 pond	--	0.064	0.036	0.036	0.023	0.023	0.016	0.016
50%		0.028	0.032	0.018	0.018	0.011	0.011	0.008	0.008

Intended use		Pome/stone fruit, late applications, umbrella use Xb; Mar-Aug / BBCH 12-91							
Active substance		Acetamiprid							
Application rate (g/ha)		34							
FOCUS Step 4 PEC _{sw, max} (µg/L)									
Nozzle reduction	Non-sprayed buffer (m)	-	5	10	10	15	15	20	20
	Vegetated filter strip (m)	-	-	-	10	-	10 VFS _{mod}	-	20
75%	D4 stream	0.014	0.016	0.009	0.009	0.006	0.006	0.004	0.004
90%		0.006	0.006	0.004	0.004	0.002	0.002	0.002	0.002
-		--	0.956	0.427	0.427	0.216	0.216	0.132	0.132
50%		0.613	0.478	0.214	0.214	0.108	0.108	0.066	0.066
75%		0.306	0.239	0.107	0.107	0.054	0.054	0.033	0.033
90%		0.123	0.096	0.043	0.043	0.022	0.022	0.013	0.013
-	D5 pond	--	0.064	0.036	0.036	0.023	0.023	0.016	0.016
50%		0.028	0.032	0.018	0.018	0.011	0.011	0.008	0.008
75%		0.014	0.016	0.009	0.009	0.006	0.006	0.004	0.004
90%		0.006	0.006	0.004	0.004	0.002	0.002	0.002	0.002
-	D5 stream	--	1.056	0.472	0.472	0.238	0.238	0.146	0.146
50%		0.677	0.528	0.236	0.236	0.119	0.119	0.073	0.073
75%		0.338	0.264	0.118	0.118	0.060	0.060	0.036	0.036
90%		0.135	0.106	0.047	0.047	0.024	0.024	0.015	0.015
-	R1 pond	--	0.064	0.035	0.035	0.023	0.023	0.016	0.016
50%		0.028	0.032	0.018	0.018	0.011	0.011	0.008	0.008
75%		0.014	0.016	0.009	0.009	0.006	0.006	0.004	0.004
90%		0.006	0.006	0.004	0.004	0.002	0.002	0.002	0.002
-	R1 stream	--	0.749	0.335	0.335	0.169	0.169	0.103	0.103
50%		0.480	0.375	0.167	0.167	0.084	0.084	0.052	0.052
75%		0.240	0.187	0.084	0.084	0.042	0.042	0.026	0.026
90%		0.096	0.075	0.033	0.033	0.017	0.017	0.010	0.010
-	R2 stream	--	1.004	0.449	0.449	0.227	0.227	0.138	0.138
50%		0.643	0.502	0.224	0.224	0.113	0.113	0.069	0.069
75%		0.322	0.251	0.112	0.112	0.057	0.057	0.035	0.035
90%		0.129	0.100	0.045	0.045	0.023	0.023	0.014	0.014
-	R3 stream	--	1.056	0.472	0.472	0.262	0.238	0.262	0.146
50%		0.676	0.528	0.262	0.236	0.262	0.119	0.262	0.073
75%		0.338	0.264	0.262	0.119	0.262	0.119	0.262	0.062
90%		0.262	0.262	0.262	0.119	0.262	0.119	0.262	0.062
-	R4 stream	--	0.749	0.335	0.335	0.169	0.169	0.137	0.103
50%		0.480	0.375	0.167	0.167	0.137	0.084	0.137	0.052
75%		0.240	0.187	0.137	0.084	0.137	0.062	0.137	0.033
90%		0.137	0.137	0.137	0.062	0.137	0.062	0.137	0.033
Mesocosm (ETO); mean measured		ETR (PEC/RAC)							

Intended use		Pome/stone fruit, late applications, umbrella use Xb; Mar-Aug / BBCH 12-91							
Active substance		Acetamiprid							
Application rate (g/ha)		34							
FOCUS Step 4 PEC _{sw, max} (µg/L)									
Nozzle reduction	Non-sprayed buffer (m)	-	5	10	10	15	15	20	20
	Vegetated filter strip (m)	-	-	-	10	-	10 VFS _{mod}	-	20
0.56 µg/L									
-	D3 ditch	--	1.5	0.7	0.7	0.3	0.3	0.2	0.2
50%		1.1	0.8	0.3	0.3	0.2	0.2	0.1	0.1
75%		0.6	0.4	0.2	0.2	0.09	0.09	0.05	0.05
90%		0.2	0.2	0.07	0.07	0.03	0.03	0.02	0.02
-	D4 stream	--	1.7	0.8	0.8	0.4	0.4	0.2	0.2
50%		1.1	0.9	0.4	0.4	0.2	0.2	0.1	0.1
75%		0.5	0.4	0.2	0.2	0.1	0.1	0.06	0.06
90%		0.2	0.2	0.08	0.08	0.04	0.04	0.02	0.02
-	D5 stream	--	1.9	0.8	0.8	0.4	0.4	0.3	0.3
50%		1.2	0.9	0.4	0.4	0.2	0.2	0.1	0.1
75%		0.6	0.5	0.2	0.2	0.1	0.1	0.06	0.06
90%		0.2	0.2	0.08	0.08	0.04	0.04	0.03	0.03
-	R1 stream	--	1.3	0.6	0.6	0.3	0.3	0.2	0.2
50%		0.9	0.7	0.3	0.3	0.2	0.2	0.09	0.09
75%		0.4	0.3	0.2	0.2	0.08	0.08	0.05	0.05
90%		0.2	0.1	0.06	0.06	0.03	0.03	0.02	0.02
-	R3 stream	--	1.9	0.8	0.8	0.5	0.4	0.5	0.3
50%		1.2	0.9	0.5	0.4	0.5	0.2	0.5	0.1
75%		0.6	0.5	0.5	0.2	0.5	0.2	0.5	0.1
90%		0.5	0.5	0.5	0.2	0.5	0.2	0.5	0.1
-	R4 stream	--	1.3	0.6	0.6	0.3	0.3	0.2	0.2
50%		0.9	0.7	0.3	0.3	0.2	0.2	0.2	0.09
75%		0.4	0.3	0.2	0.2	0.2	0.1	0.2	0.06
90%		0.2	0.2	0.2	0.1	0.2	0.1	0.2	0.06

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in **bold**

For the intended application of ADM.00150.I.2.A in “pome/stone fruits” at 34 g a.s./ha (umbrella use Xb; Mar-Aug / BBCH 12-91), the calculated PEC/RAC ratios indicate an acceptable risk from exposure to acetamiprid if certain mitigation measures are considered. For the mesocosm study (ETO; mean measured; aquatic invertebrates, acute and sed. dwell. organisms, prolonged, RAC = 0.56 µg/L), a non-sprayed buffer zone of 10 m or 50% drift reducing nozzles and a non-sprayed buffer zone of 5 m or 75% drift reducing nozzles should be considered.

Metabolites of acetamiprid

In the following, the exposure-toxicity ratios (ETR) between predicted environmental concentrations in surface water bodies (PEC_{sw/sed}) and regulatory acceptable concentrations (RAC) for aquatic organisms are given for the relevant metabolites of acetamiprid. Only the worst-case FOCUS PEC values out of all application scenarios are considered, covering all proposed uses. For details on the PEC calculations please refer to Part B, Section 8.9.

Table 9.5-59: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for IM-1-2 a metabolite of Acetamiprid for each organism group based on worst-case FOCUS Step 1-3 calculations for the proposed application of ADM.00150.I.2.A in ‘various crops (global worst case)’

Group		Invertebrate, acute	Invertebrate, acute
Test Species		<i>C. riparius</i>	<i>D. magna</i>
Endpoint (µg/L)		EC ₅₀	EC ₅₀
AF		15000	>99800
RAC (µg/L)		100	100
FOCUS Scenario	PEC _{sw,max} (µg/L)	150	>998
ETR (PEC/RAC)			
Step 1			
	27.707 ¹	0.2	<0.03
Step 2			
N-Europe	1.403 ²	0.009	<0.001
S-Europe	1.246 ³	0.008	<0.001

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in **bold**

¹ Step 1 PEC_{sw}, max in Oilseed rape (winter), 2x60 g a.s./ha, BBCH 31

² Step 2 NEU PEC_{sw}, max in Pome fruit (early) (tree nursery), 1x46 g a.s./ha, BBCH 12

³ Step 2 SEU PEC_{sw}, max in Pome fruit (early) (tree nursery), 1x46 g a.s./ha, BBCH 12

For the intended application of ADM.00150.I.2.A in “various crops” at up to 120 g a.s./ha (global worst case), the calculated PEC/RAC ratios indicate an acceptable risk from exposure to IM-1-2 for all groups of aquatic organisms based on tier 1 endpoints and FOCUS Step 1-2 PEC values. Therefore, no further assessment is necessary.

Table 9.5-60: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for IM-1-4 a metabolite of Acetamiprid for each organism group based on worst-case FOCUS Step 1-3 calculations for the proposed application of ADM.00150.I.2.A in ‘various crops (global worst case)’

Group		Fish, acute	Invertebrate, acute	Invertebrate, acute	Invertebrate, acute
Test Species		<i>O. mykiss</i>	<i>A. bahia</i>	<i>C. riparius</i>	<i>D. magna</i>
Endpoint (µg/L)		LC ₅₀	EC ₅₀	EC ₅₀	EC ₅₀
AF		98100	19000	76000	43900
RAC (µg/L)		100	100	100	100
FOCUS Scenario	PEC _{sw,max} (µg/L)	981	190	760	439
ETR (PEC/RAC)					
Step 1					
	35.746 ¹	0.04	0.2	0.05	0.08
Step 2					
N-Europe	4.776 ²	0.005	0.03	0.006	0.01

Group		Fish, acute	Invertebrate, acute	Invertebrate, acute	Invertebrate, acute
Test Species		<i>O. mykiss</i>	<i>A. bahia</i>	<i>C. riparius</i>	<i>D. magna</i>
Endpoint (µg/L)		LC ₅₀ 98100	EC ₅₀ 19000	EC ₅₀ 76000	EC ₅₀ 43900
AF		100	100	100	100
RAC (µg/L)		981	190	760	439
FOCUS Scenario	PEC _{sw,max} (µg/L)	ETR (PEC/RAC)			
S-Europe	4.227 ³	0.004	0.02	0.006	0.01

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in **bold**

¹ Step 1 PEC_{sw, max} in Oilseed rape (winter), 2x60 g a.s./ha, BBCH 31

² Step 2 NEU PEC_{sw, max} in Sugar beet, 2x50 g a.s./ha, BBCH 12

³ Step 2 SEU PEC_{sw, max} in Pome fruit (early) (tree nursery), 1x46 g a.s./ha, BBCH 12

For the intended application of ADM.00150.I.2.A in “various crops” at up to 120 g a.s./ha (global worst case), the calculated PEC/RAC ratios indicate an acceptable risk from exposure to IM-1-4 for all groups of aquatic organisms based on tier 1 endpoints and FOCUS Step 1-2 PEC values. Therefore, no further assessment is necessary.

Table 9.5-61: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for IM-1-5 a metabolite of Acetamiprid for each organism group based on worst-case FOCUS Step 1-3 calculations for the proposed application of ADM.00150.I.2.A in ‘various crops (global worst case)’

1.5 calculations for the proposed application of AD-M00135612/1 in various crops (Global worst case)				
Group		Invertebrate, acute	Invertebrate, acute	Invertebrate, prolonged
Test Species		<i>C. riparius</i>	<i>D. magna</i>	<i>D. magna</i>
Endpoint		EC ₅₀	EC ₅₀	NOEC
(µg/L)		68000	25000	26000
AF		100	100	10
RAC (µg/L)		680	250	2600
FOCUS Scenario	PEC _{sw,max} (µg/L)	ETR (PEC/RAC)		
Step 1				
	4.947 ¹	0.007	0.02	0.002
Step 2				
N-Europe	1.632 ²	0.002	0.007	0.0006
S-Europe	1.306 ³	0.002	0.005	0.0005

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in **bold**

¹ Step 1 PEC_{sw}, max in Oilseed rape (winter), 2x60 g a.s./ha, BBCH 31

² Step 2 NEU PEC_{sw}, max in Sugar beet, 2x50 g a.s./ha, BBCH 12

³ Step 2 SEU PEC_{sw}, max in Sugar beet, 2x50 g a.s./ha, BBCH 12

For the intended application of ADM.00150.I.2.A in “various crops” at up to 120 g a.s./ha (global worst case), the calculated PEC/RAC ratios indicate an acceptable risk from exposure to IM-1-5 for all groups of aquatic organisms based on tier 1 endpoints and FOCUS Step 1-2 PEC values. Therefore, no further assessment is necessary.

Table 9.5-62: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for IB-1-1 a metabolite of Acetamiprid for each organism group based on worst-case FOCUS Step 1-3 calculations for the proposed application of ADM.00150.I.2.A in ‘oilseed rape (winter), 2x60 g a.s./ha, BBCH 31’

Group		Invertebrate, acute	Invertebrate, acute
Test Species		<i>C. riparius</i>	<i>D. magna</i>
Endpoint		EC ₅₀	EC ₅₀
(µg/L)		>100000	>100800
AF		100	100
RAC (µg/L)		>1000	>1008
FOCUS Scenario	PEC _{sw,max} (µg/L)	ETR (PEC/RAC)	
Step 1			
	13.175	<0.01	<0.01

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in **bold**

For the intended application of ADM.00150.I.2.A in oilseed rape (winter), 2x60 g a.s./ha, BBCH 31 (global worst case), the calculated PEC/RAC ratios indicate an acceptable risk from exposure to IB-1-1 for all groups of aquatic organisms based on tier 1 endpoints and FOCUS Step 1 PEC values. Therefore, no further assessment is necessary.

Table 9.5-63: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for IC-0 a metabolite of Acetamiprid for each organism group based on worst-case FOCUS Step 1-3 calculations for the proposed application of ADM.00150.I.2.A in ‘various crops (global worst case)’

Group		Invertebrate, acute	Invertebrate, acute
Test Species		<i>C. riparius</i>	<i>D. magna</i>
Endpoint		EC ₅₀	EC ₅₀
(µg/L)		>100000	>95100
AF		100	100
RAC (µg/L)		>1000	>951
FOCUS Scenario	PEC _{sw,max} (µg/L)	ETR (PEC/RAC)	
Step 1			
	10.147	<0.01	<0.01
Step 2			
N-Europe	1.153	<0.001	<0.001
S-Europe	1.090	<0.001	<0.001

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in **bold**

¹ Step 1 PEC_{sw}, max in Oilseed rape (winter), 2x60 g a.s./ha, BBCH 31

² Step 2 NEU PEC_{sw}, max in Pome fruit (early) (tree nursery), 1x46 g a.s./ha, BBCH 12

³ Step 2 SEU PEC_{sw}, max in Pome fruit (early) (tree nursery), 1x46 g a.s./ha, BBCH 12

For the intended application of ADM.00150.I.2.A in “various crops” at up to 120 g a.s./ha (global worst case), the calculated PEC/RAC ratios indicate an acceptable risk from exposure to IC-0 for all groups of aquatic organisms based on tier 1 endpoints and FOCUS Step 1-2 PEC values. Therefore, no further assessment is necessary.

zRMS comments:

Aquatic risk assessment based on FOCUS Step 1-4 surface water exposure has been checked by the zRMS and agreed. The lowest endpoints derived from either active substance or formulation studies were taken into account for particular species. Surface water exposure as agreed in area of Section 8 was considered.

The risk to aquatic invertebrates from acetamiprid was refined using endpoint derived from mesocosm study performed with ADM.00150.I.2.A and assessment factor of 2, which was agreed by the zRMS.

Based on performed evaluation acceptable acute and chronic risk from acetamiprid in ADM.00150.I.2.A could be concluded for application for spring and winter cereals (group IVb), OSR (VIb), potatoes and maize with no need for risk mitigation measures.

For remaining uses the risk mitigation measures based on FOCUS STEP 4 calculations are required.

The EoP approach and VFS_{mod} PEC_{sw} calculation have been used in refined risk and considered acceptable.

Based on the calculations provided in the Tables above the risk mitigation measures are required to conclude acceptable risk to aquatic organism.

As different scenarios are considered representative in various cMS and required risk mitigation measures varied among scenarios, the summary table presenting mitigation measures for each scenario separately has been prepared by the zRMS for convenience of the cMS.

Uses	Application pattern							
		D3	D4	D5	R1	R2	R3	R4
Apple, BBCH 71-PHI, 1 x 80 g a.s./ha, late application covering 60 g a.s./ha	IIa 4, 6, 9, 11, 13, 15	90% DRN or 5 m BZ + 75 % DRN or 10 m BZ +50% DRN or 15 m BZ	90% DRN or 10 m BZ +50 %DRN or 15 m BZ	90% DRN or 10 m BZ +50% DRN or 20 m BZ	75 % DRN or 10 m BZ +50% DRN or 15 m BZ	90 % DRN or 10 m BZ +50% DRN or 15 m BZ	90% DRN or 10 m BZ + 50% DRN or 15 m BZ with 10 m VFS _{mod} or 20 m BZ	10 m VFS+50% DRN or 15 m with 10 m VFS _{mod} or 20 m VFS
Apple, BBCH 62-PHI, 2 x 25 g a.s./ha (8 days interval), early application	IIb 5, 7, 8, 10, 12, 14, 16	75 % DRN or 10 m BZ+ 50 % DRN or 15 m BZ	75% DRN or 10 +50% DRN or 15 m BZ	75% DRN or 10 +50% DRN or 15 m BZ	75 % DRN or 10 m BZ +50% DRN or 15 m BZ	-	75% DRN or 10 m BZ + 50% DRN or 15 m BZ	75% DRN or 10 m +50% DRN or 15 m BZ
Apple, BBCH 62-PHI, 1 x 25 g a.s./ha (early application)		75 % DRN or 10 m BZ+ 50 % DRN or 15 m BZ	75 % DRN or 10 +50% DRN or 15 m BZ	75% DRN or 15 m BZ	75 % DRN or 10 m BZ +50% DRN or 15 m BZ	-	-	75% DRN or 10 m +50% DRN or 15 m BZ
Apple, BBCH (71 BBCH), 2 x 25 g a.s./ha (8 days interval), late application	IIb 5, 7, 8, 10, 12, 14, 16	50% DRN or 5 m BZ	50% DRN or 10 m BZ	50% DRN or 10 m BZ	50% DRN or 5 m BZ	-	50% or 10 m BZ	50% DRN
Apple, BBCH (71 BBCH), 1 x 25 g a.s./ha (late application)		50% DRN or 10 m BZ	50% DRN or 10 m BZ	50% DRN or 10 m BZ	50% DRN or 5 m BZ	-	50% DRN or 5 m BZ	50% DRN or 5 m BZ

Spring cereals and winter cereals as surrogate for R1, R3, BBCH 40-69, 2 x 35 g a.s./ha (10 days interval)	IVa 23, 25, 27, 28, 29,	None	None		None	-	10 m VFS	None
Spring cereals and winter cereals as surrogate for R1, R3, BBCH 40-69, 1 x 35 g a.s./ha		None	None	None	None	-	None	None
Winter cereals, BBCH 40 – 69, 1-2 x 36 g a.s./ha (10 days interval)	Va 32, 34, 36, 39	None	None	None	None	-	10 m VFS	10 m VFS
Winter cereals, BBCH 40 – 69, 1 x 36 g a.s./ha	Va	None	None	None	None	-	None	None
Winter cereals, 12 – 29, 1 x 30 g a.s./ha	Vb 33, 35, 37, 38, 40	None	None	None	None	-	10 m VFS	None
Winter oilseed rape, BBCH 31 – 71, 1-2 x 60 g a.s./ha (7 days interval)	VIa 41, 42, 43, 46, 47, 48, 49, 50, 51, 53, 54, 55, 56, 57, 58, 62, 63, 64	None	None	None	10 m VFS	-	None	None
Winter oilseed rape, BBCH 31 – 71, 1 x 60 g a.s./ha (7 days interval)		None	None	None	None	-	None	None
Spring oilseed rape, BBCH 31 – 69, 1-2 x 60 g a.s./ha (7 days interval)	VIIa 66 - 79	None	None	None	10 m VFS	-	20 m BZ or 15 m BZ with 10m VFSmod	20 m BZ or 15 m BZ with 10 m VFSmod
Spring oilseed rape, BBCH 31 – 69, 1 x 60 g a.s./ha		None	None	None	10 m VFS	-	20 m BZ or 15 m BZ with 10 m VFSmod	20 m BZ or 15 m BZ with 10 m VFSmod
Sugar beet, BBCH 12-39, 2 x 50 g a.s./ha (7 days interval)	VIIIa 80 – 84	None	None	None	None	-	None	20 m BZ or 15 m BZ with 10 m VFSmod
Sugar beet, BBCH 12-39, 1 x 50 g a.s./ha		None	None	None	None	-	None	None
Flower bulbs and flower tubers, BBCH 20 – 91, 2 x 34 g a.s./ha (7 days interval)	IXb 86, 87	None	None	None	None	-	None	10 m VFS

Flower bulbs and flower tubers, BBCH 20–91, 1 × 34 g a.s./ha (None	None	None	None	-	-	10 m VFS
Floriculture, Tree nursery and Perennial nursery crops, BBCH 12-91, 1 x 46 g a.s./ha (Pome and stone fruit early)	Xa 88	90% DRN or 10 m +75% DRN or 15 m +50% DRN or 20 m BZ	90% DRN or 10 m +75% DRN or 15 m + 50% DRN or 20 m BZ	90%DRN or 10 m +75% DRN or 15 m +50% DRN or 20 m BZ	90% DRN or 10 m BZ +75% DRN or 15 m +50% DRN or 20 m BZ	90% DRN or 10m BZ+75% DRN or 15 m +50% DRN or 20 m BZ	90% DRN or 10 m +75% DRN or 15 m +50% DRN or 20 m BZ	90% DRN or 10 m +75% DRN or 15 m +50% DRN or 20 m BZ
Floriculture, Tree nursery and Perennial nursery crops, BBCH 12-91, 1 x 46 g a.s./ha (Pome and stone fruit late)	Xa 88	75 % DRN or 10 m BZ	75%DRN or 10 m BZ	75%DRN or 10 m BZ	75% DRN or 5 m BZ +50% DRN or 10 m BZ	none	75% DRN or 10 m BZ	75 % DRN or 5 m +50% DRN or 10 m BZ
Floriculture, Tree nursery and Perennial nursery crops, BBCH 20–91, 2 x 34 g a.s./ha (Leafy vegetables)	Xb 88	None	None	None	None	None	None	10 m VFS
Floriculture, Tree nursery and Perennial nursery crops, BBCH 20–91, 1 x 34 g a.s./ha (Leafy vegetables)		None	None	None	None	None	None	None
Floriculture, Tree nursery and Perennial nursery crops, BBCH 20–91, 2 x 34 g a.s./ha (Pome and stone fruit early)	Xb 88, 89	90 % DRN or 5 m BZ+ 75 % DRN or 10 m BZ +50% DRN or 20 m BZ	90 % DRN or 5 m BZ+ 75 % DRN or 15 m BZ +50% or 20 m BZ	90% DRN or 10 m +75% or 15 m BZ +50% or 20 m BZ	75 % DRN or 15 m BZ	None	90% DRN or 5 m BZ + 75% DRN or 15 m +50% DRN or 20 m BZ	75% DRN or 10 m +50% DRN or 15 m BZ
Floriculture, Tree nursery and Perennial nursery crops, BBCH 20–91, 1 x 34 g a.s./ha (Pome and stone fruit early)		90 % DRN or 5 m BZ+ 75 % DRN or 15 m BZ	90 % DRN or 5 m BZ+ 75 % DRN or 15 m BZ +50% or 20 m BZ	90% DRN or 10 m +75% or 15 m BZ +50% or 20 m BZ	75 % DRN or 15 m BZ	None	90% DRN or 10 m BZ + 75% DRN or 15 m +50% DRN or 20 m BZ	75% DRN or 15 m BZ

Floriculture, Tree nursery and Perennial nursery crops, BBCH 20– 91, 2 x 34 g a.s./ha (Pome and stonefruit late) 91 BBCH Late application	Xb 88, 89	75 % DRN or 5 m +50% DRN or 10 m BZ	50 % DRN or 10 m BZ	50% DRN or 10 m BZ	50 % DRN or 10 m BZ	None	75% DRN or 10 m BZ	50% DRN or 10 m BZ
Floriculture, Tree nursery and Perennial nursery crops, BBCH 20– 91, 1 x 34 g a.s./ha (Pome and stonefruit late) 91 BBCH		75 % DRN or 5 m +50%DRN or 10 m BZ	75 % DRN or 5 m +50% or 10 m BZ	75% DRN or 5 m +50% or 10 m BZ	50 % DRN or 10 m BZ	-	75% DRN or 10 m BZ	50% DRN or 10 m BZ

BZ: unsprayed buffer zone; **VFS:** vegetated filter strip; **DRN:** drift reducing nozzles VFSmod: vegetated filter strip modified
* Mitigation reductions based on the EoP approach are also included

9.5.3 Overall conclusions

The standard and refined risk assessment provided for the insecticidal product ADM.00150.I.2.A, the active substance acetamiprid and its major metabolites demonstrate that the application of ADM.00150.I.2.A as intended in the GAP according to good agricultural practice is of low risk to aquatic ecosystems if certain mitigation measures are considered:

Umbrella GAP number	Intended uses	Single GAP uses covered	Mitigating measures*
Ila	Apple, BBCH 71-PHI, 1 x 80 g a.s./ha, late	4, 6, 9, 11, 13, 15	- 20 m DBZ plus 20 m VFS or - 10 m DBZ plus 10 m VFS plus 50% DRN or - 10 m DBZ plus 10 m VFS
Iib	Apple, BBCH 62-PHI, 2 x 25 g a.s./ha (8 days interval), early	5, 7, 8, 10, 12, 14, 16	- 15 m DBR or - 10 m DBZ plus 50% DRN or - 75% DRN
Iib	Apple, BBCH 62-PHI, 2 x 25 g a.s./ha (8 days interval), late	5, 7, 8, 10, 12, 14, 16	- 10 m DBZ or - 50% DRN
IVa	Spring cereals and winter cereals as surrogate for R1, R3, BBCH 40-69, 2 x 35 g a.s./ha (10 days interval)	23, 25, 27, 28, 29	- 10 m DBZ plus 10 m VFS
Va	Winter cereals, BBCH 40 – 69, 1-2 x 36 g a.s./ha (10 days interval)	32, 34, 36, 39	- 10 m DBZ plus 10 m VFS
Vb	Winter cereals, 12 – 29, 1 x 30 g a.s./ha	33, 35, 37, 38, 40	- 10 m DBZ plus 10 m VFS
VIa	Winter oilseed rape, BBCH 31 – 71, 1-2 x 60 g a.s./ha (7 days interval)	41, 42, 43, 46, 47, 48, 49, 50, 51, 53, 54, 55, 56, 57, 58, 62, 63, 64	- 10 m DBZ plus 10 m VFS
VIIa	Spring oilseed rape, BBCH 31 – 69, 1-2 x 60 g a.s./ha (7 days interval)	66 - 79	- 10 15 m DBZ plus 10 m VFS
VIIIa	Sugar beet, BBCH 12-39, 2 x 50 g a.s./ha (7 days interval)	80 – 84	- 15 m DBZ plus 10 m VFS
IXb	Flower bulbs and flower tubers, BBCH 20 – 91, 2 x 34 g a.s./ha (7 days interval)	86, 87	- 10 m DBZ plus 10 m VFS
Xa	Floriculture, Tree nursery and Perennial nursery crops, BBCH 12-91, 1 x 46 g a.s./ha (Pome and stonefruit early)	88	- 20 m DBZ or - 15 m DBZ plus 50% DRN or - 10 m DBZ plus 75% DRN or - 90% DRN
Xa	Floriculture, Tree nursery and Perennial nursery crops, BBCH 12-91, 1 x 46 g a.s./ha (Pome and stonefruit late)	88	- 10 m DBZ or - 75% DRN
Xb	Floriculture, Tree nursery and	89, 90	- 10 m DBZ plus 10 m VFS

Umbrella GAP number	Intended uses	Single GAP uses covered	Mitigating measures*
	Perennial nursery crops, BBCH 20– 91, 2 x 34 g a.s./ha (Leafy vegetables)		
Xb	Floriculture, Tree nursery and Perennial nursery crops, BBCH 20– 91, 2 x 34 g a.s./ha (Pome and stonefruit early)	89, 90	<ul style="list-style-type: none"> - 20 m DBZ or - 15 m DBZ plus 50% DRN or - 10 m DBZ plus 75% DRN or - 90% DRN
Xb	Floriculture, Tree nursery and Perennial nursery crops, BBCH 20– 91, 2 x 34 g a.s./ha (Pome and stonefruit late)	89, 90	<ul style="list-style-type: none"> - 10 m DBZ or - 50% DRN

DBZ: drift buffer zone; DRN: drift reducing nozzles; VFS: vegetated filter strip; VFS_{mod}: vegetated filter strip modified

* Mitigation reductions based on the EoP approach are also included

zRMS comments:

The risk assessment provided for the product ADM.00150.I.2.A, the active substance acetamiprid and its major metabolites indicated an acceptable risk to aquatic organism if the relevant risk mitigation measures are considered.

Concerned Member State must decide on acceptability and applicability of the proposed risk mitigation measures in their countries.

Additional calculations may be required by cMS that do not accept surface water exposure derived using FOCUS models.

The following text is added due to agreements during the Central Zone harmonisation meetings. It should be noted that this text has no impact on the outcome of zonal evaluation of formulation ADM.00150.I.2.A which was performed in line with the EU agreed methodology.

“The endpoint E_rC_{50} is selected in this Core Assessment but there are some uncertainties regarding the level of protection reached for primary producers. This is indicated for macrophytes in the aquatic Guidance Document (EFSA Journal 2013;11(7):3290) that recommends: “... a proper calibration between different tiers (higher and lower tier data) for macrophytes should be performed in the future”. Such calibration should be extended to algae. Until available relevant information on the level of protection reached is considered at EU level, it is recommended to address this uncertainty at each Member State level in the National Addendum if considered necessary, although it would be highly appreciated to have a harmonised approach in the Central Zone.”

9.6 Effects on bees (KCP 10.3.1)

9.6.1 Toxicity data

Studies on the toxicity to bees have been carried out with acetamiprid. Full details of these studies are provided in the respective EFSA conclusion (2016) and related documents as well as in Appendix 2 of this document (new studies).

Effects on bees of ADM.00150.I.2.A (containing 200 g/L acetamiprid) were not evaluated as part of the EU assessment of acetamiprid. New data submitted with this application are listed in Appendix 1 and summarised in Appendix 2.

In total, 10 higher tier studies (*i.e.* 7 semi-field and 3 field studies) on effects of acetamiprid on bees are available for MCW-2222.

Table 9.6-1: Endpoints and effect values relevant for the risk assessment for bees derived from laboratory studies - acetamiprid

Species	Substance	Exposure System	Results	Reference
<i>Apis mellifera</i>	a.s.*	Acute, oral	LD₅₀ = 8.85 µg a.s./bee	EFSA, 2016
		Acute, contact	LD₅₀ = 9.26 µg a.s./bee	
<i>Bombus terrestris</i>	a.s.*	Acute, contact	LD ₅₀ > 100 µg a.s./bee	EFSA, 2016
<i>Apis mellifera</i>	a.s.	Chronic, oral, 10 days	LDD ₅₀ = 11.7 µg a.s./bee/day	EFSA, 2016
<i>Apis mellifera</i>	a.s.	Chronic larvae, oral feeding for 6 days	EC ₁₀ = 1.3 µg/larvae/developmental period (LD ₅₀ = 21.7 µg a.s./larva, total dose over 6 days of feeding = 3.6 µg a.s./day)	EFSA, 2016 (As published in RAR, Vol. 3 – annex B, CA-B 9, August 2016)

* tested as EXP 60707A - the representative formulation of acetamiprid in the EFSA conclusion (2016); values shown in **bold** are used for the risk assessment acc. to EPPO

Table 9.6-2: Endpoints and effect values relevant for the risk assessment for bees derived from laboratory studies - MCW-2222 (ADM.00150.I.2.A)

Species	Substance	Exposure System	Results	Reference
<i>Apis mellifera</i>	MCW-2222	Acute, Oral	LD₅₀ = 9.1 µg a.s./bee	Franke, M., 2014 R-33834 KCP 10.3.1.1.1/01 KCP 10.3.1.1.2/01
		Acute, Contact	LD₅₀ = 3.8 µg a.s./bee	
<i>Bombus terrestris</i>	MCW-2222	Acute, Oral	LD ₅₀ = 24.3 µg a.s./bumble bee	Röhlig, U, 2014 R-33837 KCP 10.3.1.2.1/01 KCP 10.3.1.2.2/01
		Acute, Contact	LD ₅₀ > 200 µg a.s./bumble bee	
<i>Apis mellifera</i>	MCW-2222	Chronic, Oral	LDD ₅₀ = 3.994 µg a.s./bee/day NOEDD = 0.546 µg a.s./bee/day	Kleebaum, K., 2015a R-33835 KCP 10.3.1.2/01
<i>Apis mellifera</i>	CA3573 Acetamiprid 200 SL (Carnadine) = MCW-2222	Chronic, Oral	LDD ₅₀ = 3.71 µg a.s./bee/day NOEDD = 1.54 µg a.s./bee/day	Dressler, K., 2019 19 48 BAC 0028 KCP 10.3.1.2/02
<i>Apis mellifera</i>	MCW-2222	Chronic, Oral, larvae	8d LD ₅₀ = 10.2 µg a.s./larvae (total dose over 4 days of feeding = 2.55 µg a.s./day) 8d NOEDD = 3.8 µg a.s./larvae	Kleebaum, K. 2015b R-33836 KCP 10.3.1.3/01
<i>Apis mellifera</i>	CA3573 Acetamiprid 200 SL (Carnadine) = MCW-2222	Chronic, Oral, larvae	22d ED ₅₀ > 0.486 µg a.s./larvae (total dose over 4 days of feeding is > 0.1215 µg a.s./day) 22d NOEDD ≥ 0.486 µg a.s./larvae	Scheller, K. 2020 19 48 BLC 0033 KCP 10.3.1.3/02

Values shown in **bold** are used for the risk assessment acc. to EPPO

Table 9.6-3: Summary and results of semi-field and field studies supporting the evaluation risk with MCW-2222 (ADM.00150.I.2.A)

Higher-tier studies – semi-field studies (tunnels)			
Species	Substance	Endpoint used for risk assessment	Reference
<i>Apis mellifera</i>	MCW 2222, applied once at 0.5 L/ha (100 g/ha acetamiprid) during (T1) and after (T2) bee flight to winter wheat, which was daily sprayed with sugar syrup as artificial honeydew to make the crop attractive for foraging bees	<ul style="list-style-type: none"> • Temporary, significant effects on daily adult mortality until D+2 in T1 and T2, • No significant differences on cumulative adult mortality in T1 and T2, • Temporary effects on foraging activity until D+1 and behaviour (few bees with signs of intoxication) until D+2 in T1 and T2, • No impact on colony strength and colony development in T1 and T2. 	Mamet, O. & Molitor, C., 2015a, R-34874 KCP 10.3.1.5/01
<i>Apis mellifera</i>	MCW 2222, applied once at 0.5 L/ha (100 g/ha acetamiprid) during (T1) and after (T2) bee flight to winter wheat, which was daily sprayed with sugar syrup as artificial honeydew to make the crop attractive for foraging bees	<p>T1:</p> <ul style="list-style-type: none"> • Statistically significant effect on daily mortality from D+1 to D+3, • No significant difference on cumulative adult mortality, • Effects on foraging activity were observed until D+3, • Bees hesitated to forage and few bees displayed signs of intoxication until D+1, • No impact on colony strength and colony development. <p>T2:</p> <ul style="list-style-type: none"> • Statistically significant effect on daily mortality at D+2 and D+3, • No significant difference on cumulative adult mortality, • Effects on foraging activity were observed until D+3, • Bees hesitated to forage until D+2 and few bees displayed signs of intoxication until D+1, • No impact on colony strength and colony development. 	Mamet, O., 2015a, R-35845 KCP 10.3.1.5/02
<i>Apis mellifera</i>	MCW 2222, applied once at 0.5 L/ha (100 g/ha acetamiprid) during (T1) and after (T2) bee flight to winter wheat, which was daily sprayed with sugar syrup as artificial honeydew to make the crop attractive for foraging bees	<p>T1:</p> <ul style="list-style-type: none"> • Statistically significant effect on daily mortality on D+1, • No significant difference on cumulative adult mortality, • Effects on foraging activity were observed until D+1 and on behaviour on the day of application, • No signs of intoxication, • No impact on colony strength and colony development. <p>T2:</p> <ul style="list-style-type: none"> • No significant effects on daily mortality, • No significant difference on cumulative adult mortality, • Effects on foraging activity were observed until D+3 and effects on behaviour until D+2, • No signs of intoxication, • No impact on colony strength and colony development. 	Mamet, O., 2015b, R-35846 KCP 10.3.1.5/03
<i>Apis mellifera</i>	MCW 2222, applied once at 0.5 L/ha (100 g/ha acetamiprid) during (T1) and after (T2) bee flight to flowering <i>Phacelia</i>	<ul style="list-style-type: none"> • Slight but significant effect on daily adult mortality on D+1 in T1, no effect on adult mortality in T2, • No significant differences on cumulative adult mortality in T1 and T2, • No effects on foraging activity and behaviour in T1 and T2, • No impact on colony strength and colony development in T1 and T2. <p>Observations up to 8 DAA.</p> <p>No residue analysis, but treated crop was the only food source during the study.</p>	Mamet, O. & Molitor, C., 2015b, R-34875 KCP 10.3.1.5/04
<i>Apis mellifera</i>	MCW 2222, applied once at 0.5 L/ha (100 g/ha acetamiprid) during	<p>T1 and T2</p> <ul style="list-style-type: none"> • No significant effects on daily adult mortality, • No significant differences on cumulative adult mortality, 	Mamet, O. & Molitor, C., 2015c, R-34876 KCP 10.3.1.5/05

	(T1) and after (T2) bee flight to flowering <i>Phacelia</i>	<ul style="list-style-type: none"> No effects on foraging activity and behaviour, No impact on colony strength and colony development. Observations up to 8 DAA. No residue analysis, but treated crop was the only food source during the study. 	
<i>Apis mellifera</i>	MCW 2222, applied once at 0.5 L/ha (100 g/ha acetamiprid) during (T1) and after (T2) bee flight to flowering <i>Phacelia</i>	<p>T1 and T2</p> <ul style="list-style-type: none"> No significant effects on daily adult mortality, No significant differences on cumulative adult mortality, No effects on foraging activity and behaviour, No impact on colony strength and colony development. Observations up to 8 DAA. No residue analysis, but treated crop was the only food source during the study 	Molitor, C., 2015a, R-35847 KCP 10.3.1.5/06
<i>Apis mellifera</i>	MCW 2222, applied twice at 0.4443 kg/ha (80 g/ha acetamiprid) to <i>Phacelia</i> ; 1 st application just before the flowering period and 2 nd application during the flowering period in the evening after the flight	<ul style="list-style-type: none"> No impact on adult and pupal bee mortality, No impact on foraging activity and behaviour, No impact on colony strength and colony development, No impact on the detailed brood development based on the assessed indices, i.e. brood termination rate, brood index, brood compensation index, Residue analysis prove clearly that bees and colonies were exposed to MCW-2222. Slight rainfall observed on DALA 1, DALA 2 and DALA 3 at 1, 1 and 0.5 mm; however residue analysis confirmed that despite precipitation bees were exposed to the test item. Observations up to 28 DALA (8 days of exposure in the tunnels followed by 20 days at the monitoring site, in line with OECD 75). <p>Under these experimental conditions, the use of the test item MCW-2222 can be considered of low effect on the honey bee brood development when two times applied in the field to oil-seed rape <i>Phacelia tanacetifolia</i> at rate of 80 g a.s./ha before and during the flowering period outside the foraging activity of honey bees.</p>	Hecht-Rost, S. & Mayer, O., 2018, R-37336 KCP 10.3.1.5/07

Higher-tier studies - field studies

Species	Substance	Endpoint used for risk assessment	Reference
<i>Apis mellifera</i>	MCW 2222, applied once at 0.5 L/ha (100 g/ha acetamiprid) to flowering <i>Phacelia</i> in the field in the evening after bee flight	<ul style="list-style-type: none"> No impact on adult and pupal bee mortality (although adult mortality elevated in test item fields on 18 and 19 DAA, but seems to be not treatment related; pupae mortality elevated comparing to controls on 4, 5, 6 DAA, but still at low level comparable with mortality in treatment groups before application, difference between test item and control groups visible due to very low pupae mortality in controls). No impact on foraging activity and behaviour, No impact on colony strength and colony development, No impact on the detailed brood development based on the assessed indices, i.e. brood termination rate, brood index, brood compensation index Residue analysis prove clearly that colonies were exposed to MCW-2222. Rainfall observed on DAA 2, DAA 3, DAA 4 and DAA 5 at 3, 6, 1 and 13 mm. Although residues of acetamiprid were detected in chemical analyses in nectar and bee bread up to 8 DAA (in pollen low levels were detected) and in honey at 20 DAA, exposure could be reduced to some extent. Brood measurements made up to 28 BFD, covering one full brood cycle and beginning of a new one (but statistical analyses performed up to BFD 22); no brood measurements at test termination (41 DAA). Colonies used for the test not particularly strong; 3 test item and one control hives lost their queens; low reproductive performance of queens in some control hives, this effect less 	Molitor, C., 2015b, R-34877 KCP 10.3.1.6/01

		<p>pronounced in test item groups; in some hives the number of nursery bees too low to assure correct development of brood cells; colonies at test termination not stronger than at test initiation with likely loss of some of the colonies at the end of the season. The pattern in development/underdevelopment of bee colonies was equally observed in both, test item and control groups, so considered not to be treatment related.</p> <ul style="list-style-type: none"> Overwintering success not investigated. <p>Under these experimental conditions, the use of the test item MCW-2222 can be considered of low effect on the honey bee brood development when applied in the field to <i>Phacelia</i> at a rate of 100 g a.s./ha during the flowering period outside the foraging activity of honey bees.</p>	
<i>Apis mellifera</i>	<p>MCW 2222, applied twice at 0.5 L/ha (100 g/ha acetamiprid) to <u>oil seed rape</u> in the field; 1st application just before the flowering period and 2nd application during the flowering period in the evening after the flight</p>	<ul style="list-style-type: none"> No impact on adult and pupal bee mortality, No impact on foraging activity and behaviour, No impact on colony strength and colony development, No impact on the detailed brood development based on the assessed indices, i.e. brood termination rate, brood index, brood compensation index, Residue analysis prove clearly that colonies were exposed to MCW-2222. Brood measurements made up to 28 BFD, covering one full brood cycle and beginning of a new one (but statistical analyses performed up to BFD 22); no brood measurements at test termination (41 DALA). Other fields of flowering oilseed rape were present at least 1 km from the treated field (accurate distance not specified), so bees could potentially forage on uncontaminated pollen, leading to reduction of the exposure to acetamiprid in hives. This issue was further consulted with the zRMS apiary expert who indicated that in general, bees will forage first on the nearest bee attractive crop and will not risk the energy losses to fly to forage on the same crop even only 1 km away. As no repellent effect of MCW-2222 was observed and foraging activity in control and test item fields was comparable, there was no reason for bees to fly to another OSR field. Taking this into account, flying of bees to neighbouring OSR fields could not be fully excluded, but was not likely. Overwintering success not investigated. <p>Under these experimental conditions, the use of the test item MCW-2222 can be considered of low effect on the honey bee brood development when two times applied in the field to oil seed rape at a rate of 100 g a.s./ha before and during the flowering period outside the foraging activity of honey bees.</p>	<p>Molitor, C., 2015c, R-35844 KCP 10.3.1.6/02</p>
<i>Apis mellifera</i>	<p>MCW 2222, applied twice at 0.5 L/ha (100 g/ha acetamiprid) to <u>apple</u> trees in an orchard; 1st application just before the flowering period and 2nd application during the flowering period in the evening after the flight</p>	<ul style="list-style-type: none"> No impact on adult, larval and pupal bee mortality, No impact on foraging and flight activity, Foraging activity was statistically significantly lower in both test item fields comparing to controls. However, this has been also observed before application, so most probably effects is not treatment related, especially foraging activity in test item plots was at level comparable with activity before treatment on -3, -2, -1 and 0 DALA. No statistically significant impact on colony strength and brood amount, <ul style="list-style-type: none"> No impact on the detailed brood development based on the assessed indices, i.e. brood termination rate and brood index. However, brood termination rates in treatment fields were higher than in control, while brood indices in treatment plots after application were lower (statistically not significant). Compensation indices were not calculated. Rain at 0.8, 7.0 and 10.6 mm was observed on 1 DALA, 	<p>Aucejo, C., 2015, R-35961 KCP 10.3.1.6/03</p>

		<p>2 DALA and 3 DALA respectively. Precipitation on 2 and 3 DALA was high enough to significantly reduce the exposure</p> <ul style="list-style-type: none"> Residue analysis of nectar and pollen prove clearly that colonies were exposed to MCW-2222 but but could be reduced due to rain on 1 DALA, 2 DALA nad 3 DALA. Flowers were not analysed for acetamiprid residues. <p>Under these experimental conditions, the use of the test item MCW-2222 can be considered of low effect on the honey bee brood development when two times applied in an orchard to apple trees at a rate of 100 g a.s./ha before and during the flowering period in the evening after the flight.</p>	
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zRMS comments:

Endpoints for acetamiprid and representative formulation presented in Table 9.6-1 are in line with values reported in EFSA Journal 2016;14(11):4610.

Studies on acute toxicity ADM.00150.I.2.A (formerly MCW-2222) to bees listed in Table 9.7-2 were already evaluated in the course of the first zonal authorisation in April 2018 and considered acceptable. The guidelines against which the studies were validated have not changed since that time, so re-evaluation of the studies was not necessary. Provided endpoints are confirmed to be correct. Summaries of studies together with zRMS conclusions on acceptability are provided in Appendix 2.

In support of evaluation of d ADM.00150.I.2.A also chronic and larvae toxicity studies performed with the product were provided. Studies were evaluated and accepted by the zRMS-PL previously, during authorisation of the product CA3573 in 2021, so re-evaluation of these studies is not necessary. For study summaries and zRMS-PL conclusions, please refer to Appendix 2.

Studies on chronic and larvae toxicity performed with MCW-2222 (Kleebaum 2014a and 2014b) were not performed in line with respective OECD guidelines and are superseded by studies performed with CA3573 (Dressler, 2019 and Scheller, 2020).

For the quantitative risk assessment endpoints from studies performed with MCW-2222 were selected as being lower than EU agreed values and representing thus worst case.

Most of semi-field and field studies (with exception of Hecht-Rost, 2018) were already agreed in the course of the first zonal evaluation. Although the test guidelines have not changed since that time, the studies were re-evaluated by the zRMS-PL during authorization of the product CA3573 in 2021.

Summaries of studies together with zRMS-PL previously evaluation and conclusions are presented in Appendix 2.

Additional information resulting from zRMS evaluation as well as changed conclusions were added in Table 9.6-3, if necessary.

Semi-field studies performed on cereals in Table 9.6-3 were not used by zRMS in the current assessment as the risk are concluded based on the laboratory studies.

9.6.1.1 Justification for new endpoints

Not required.

9.6.2 Risk assessment

The evaluation of the risk for bees was performed in accordance with the recommendations of the “Guidance Document on Terrestrial Ecotoxicology”, as provided by the Commission Services (SANCO/10329/2002 rev.2 (final), October 17, 2002).

The acute risk assessment was conducted based on the revised EPPO scheme (EPPO/OEPP, 2010¹), following the HQ-approach. As the ‘Guidance Document on Terrestrial Ecotoxicology’ lacks guidance how to evaluate the chronic risk for adult honey bees as well as for honey bee larvae, the respective endpoints are presented and compared to the endpoints of the acute oral studies.

The applicant regards that the risk assessment according to the EFSA GD “Guidance on the risk assessment of plant protection products on bees (*Apis mellifera*, *Bombus* spp. and solitary bees)” (EFSA Journal 2013; 11(7):3295 – updated 2014) (hereafter called EFSA Bee GD) is not appropriate for regulatory decision making at EU level as it has not yet been adopted by the Standing Committee on Plants, Animals, Food and Feed (SCoPAFF). In fact, it is currently under revision by EFSA (EFSA, 2019²) and modified triggers may be expected due to modified background mortality (EFSA, 2020³) and acceptable colony level variation (EFSA 2021⁴). Originally, it was envisaged to complete the revision of the assessment scheme by March 2021 (EFSA, 2019), but current information suggests a finalisation not by mid of 2023. Due to these developments a bee risk assessment according to the EFSA risk assessment scheme is not considered relevant for the renewal of registration.

The risk assessment is presented in chapter 9.6.2.1 and was conducted with the respective single application rates of the respective umbrella GAP as worst-case scenarios (see 9.1.2), covering the risk for bees from all uses within these umbrella GAPs.

The risk for honey bees and their developmental stages due to exposure to metabolites is considered to be covered in the higher-tier studies. Hence, no separate risk assessment for metabolites needs to be performed.

9.6.2.1 Hazard quotients for bees

The acute risk to honey bees from use of ADM.00150.I.2.A was assessed using the maximum single application rate in the respective umbrella GAP (see 9.1.2) and the acute oral and contact LD₅₀ values to calculate hazard quotients (EPPO 2010) as follows:

$$\text{Hazard Quotient (HQ)} = \frac{\text{Maximum single application rate [g/ha]}}{\text{Acute LD}_{50} [\mu\text{g/bee}]}$$

A HQ value of ≤ 50 indicates an acceptable acute risk to pollinators after the application of ADM.00150.I.2.A to the intended crops. The calculated HQ values for acetamiprid and ADM.00150.I.2.A from the proposed worst-case uses of ADM.00150.I.2.A according to the respective umbrella GAPs are presented in the tables below.

¹ EPPO/OEPP (2010): Environmental risk assessment scheme for plant protection products, Chapter 10: Honey-bees (PP 3/10(3). Bulletin OEPP/EPPO Bulletin 40: 323-331.

² EFSA (2019): Outline of the revision of the Guidance on the risk assessment of plant protection products on bees (*Apis mellifera*, *Bombus* spp. and solitary bees) (EFSA, 2013).

³ EFSA (2020): Review of the evidence on bee background mortality. EFSA Supporting publication 2020: EN-1880.

⁴ EFSA (2021): Analysis of background variability of honey bee colony size. EFSA Supporting publication 2021: EN-6518.

Table 9.6-4: First-tier assessment of the acute risk for honey bees due to the use of acetamiprid in the respective umbrella GAP

Group number (crop)	Maximum single application rate [g a.s./ha]	Endpoint	Hazard quotient (HQ)	Exposure route
IIa (apple)	60	LD ₅₀ oral: 8.85 µg a.s./bee	6.8	Oral
I (corn), VIa & VIb (winter OSR), VIIa (spring OSR)	60		6.8	
VIIIa (sugar beet)	50		5.6	
IXa (flower bulbs and flower tubers), Xa (floriculture, tree nursery & perennial nursery crops)	46		5.2	
III (potato), Va (winter cereals)	36		4.1	
IVa & IVb (spring cereals)	35		4.0	
IXb (flower bulbs and flower tubers), Xb (floriculture, tree nursery & perennial nursery crops)	34		3.8	
Vb (winter cereals)	30		3.4	
IIb (apple)	25		2.8	
IIa (apple)	60		6.5	
I (corn), VIa & VIb (winter OSR), VIIa (spring OSR)	60	LD ₅₀ contact: 9.26 µg a.s./bee	6.5	Contact
VIII (sugar beet)	50		5.4	
IXa (flower bulbs and flower tubers), Xa (floriculture, tree nursery & perennial nursery crops)	46		5.0	
III (potato), Va (winter cereals)	36		3.9	
IVa & IVb (spring cereals)	35		3.8	
IXb (flower bulbs and flower tubers), Xb (floriculture, tree nursery & perennial nursery crops)	34		3.7	
Vb (winter cereals)	30		3.2	
IIb (apple)	25		2.7	

Hazard quotients for oral and contact exposure. Q_H values shown in bold breach the relevant trigger.

Table 9.6-5: First-tier assessment of the acute risk for honey bees due to the use of ADM.00150.I.2.A in the respective umbrella GAP

Group number (crop)	Maximum single application rate [g a.s./ha]	Endpoint	Hazard quotient (HQ)	Exposure route
IIa (apple)	60	LD ₅₀ oral: 9.1 µg a.s./bee	6.6	Oral
I (corn), VIa & VIb (winter OSR), VIIa (spring OSR)	60		6.6	
VIII (sugar beet)	50		5.5	
IXa (flower bulbs and flower tubers), Xa (floriculture, tree nursery & perennial nursery crops)	46		5.1	
III (potato), Va (winter cereals)	36		4.0	
IVa & IVb (spring cereals)	35		3.8	
IXb (flower bulbs and flower tubers), Xb (floriculture, tree nursery & perennial nursery crops)	34		3.7	
Vb (winter cereals)	30		3.3	
IIb (apple)	25		2.7	
IIa (apple)	80		21.1	
I (corn), VIa & VIb (winter OSR), VIIa (spring OSR)	60	LD ₅₀ contact: 3.8 µg a.s./bee	15.8	Contact
VIII (sugar beet)	50		13.2	
IXa (flower bulbs and flower tubers), Xa (floriculture, tree nursery & perennial nursery crops)	46		12.1	

III (potato), Va (winter cereals)	36		9.5	
IVa & IVb (spring cereals)	35		9.2	
IXb (flower bulbs and flower tubers), Xb (floriculture, tree nursery & perennial nursery crops)	34		8.9	
Vb (winter cereals)	30		7.9	
IIb (apple)	25		6.6	

Hazard quotients for oral and contact exposure. Q_H values shown in bold breach the relevant trigger.

Based on the proposed maximum application rates of the respective umbrella GAPs which overall cover application rates of up to ~~60~~ ~~80~~ g a.s./ha, the resulting honey bee HQ values for oral and contact exposure were always below the trigger of 50. This indicates a low acute risk to honey bees from the proposed uses even when using the worst-case endpoints and the worst-case application rates.

Based on the daily doses, a comparison of the endpoints for the active ingredient acetamiprid as well for the formulation ADM.00150.I.2.A indicate that the chronic exposure of adult bees and honey bee larva display similar toxicity levels compared to the acute oral exposure of adult honey bees (factor smaller than 3.6) (see table below). This is below a factor of 5, indicating that there is no increased toxicity and similar toxicity can be assumed (EFSA, 2013).

Table 9.6-6: Comparison of toxicity endpoints for adult honey bees and honey bee larvae to the acetamiprid and ADM.00150.I.2.A

Test item	Acute oral exposure of adult bees, LD ₅₀ [µg a.s./bee]	Chronic oral exposure of adult bees, LDD ₅₀ [µg a.s./bee/day]	Chronic oral exposure of honey bee larvae, LD ₅₀ [µg a.s./bee larvae/day]
Acetamiprid	8.85	11.7 (0.75x of acute LD ₅₀)	3.6 (2.5x of acute LD ₅₀)
ADM.00150.I.2.A (MCW-2222)	9.1	3.994 (2.3x of acute LD ₅₀)	2.55 (3.6x of acute LD ₅₀)
CA3573 Acetamiprid 200 SL (Carnadine)		3.71 (2.5x of acute LD ₅₀)	Not applicable as the endpoint display to be a ">" value

* deriving from LD₅₀ values for larvae in Table 9.6-1 and Table 9.6-2, corrected to the daily dose

For bumble bees, the oral LD₅₀ was > 24.3 µg a.s./bumble bee, while the contact LD₅₀ was > 200.0 µg a.s./bumble bee. Thus, both endpoints exceed the acute endpoints for honey bees, indicating that bumblebees are not more susceptible to acetamiprid than honey bees.

As a mitigation measure to protect bees and other pollinators, the application of ADM.00150.I.2.A to bee attractive, flowering crops (e.g. apples [umbrella use IIb], potatoes [III], oil seed rape [VIa & VIIa], flower bulbs/tubers and floriculture [umbrella uses IX and X]) will be limited to take place after daily bee flight. Moreover, the application of the maximum rate of ~~60~~ ~~80~~ g a.s./ha in apple orchards will be carried out after the flowering period, i.e. BBCH ≥ 71 (umbrella use IIa).

The outcome of the theoretical risk assessment and the effectiveness to apply ADM.00150.I.2.A to flowering, bee attractive crops after daily bee flight is confirmed by the results of one semi-field bee brood study and three full field studies with detailed bee brood assessments (6 semi-field studies according to CEB 230 were not considered as they do not comply with current accepted guidelines). The results of the respective endpoints of the respective higher tier studies are shortly presented and discussed below.

zRMS comments:

The acute risk assessment performed in compliance with SANCO/10329/2002 rev 2 final is agreed by the zRMS. Calculations provided in Tables 9.6-5 and 9.6-6 above were performed with consideration of the maximum intended application rate of acetamiprid in and on their basis acceptable acute risk to bees may be concluded from all intended uses of

In general, the evaluation could be finalised with this conclusion, as SANCO/10329/2002 rev 2 final does not require any further evaluation when HQ values based on acute toxicity endpoints are below the trigger of 50. However, as already indicated in the introductory part of point 9.6.2 above, the chronic and larvae risk should be also addressed due to acetamiprid specific mode of action. In opinion of the zRMS indications of EFSA (2013) are more relevant than EPPO scheme to address this issue.

Therefore, zRMS provided the risk assessment according to EFSA GD 2013

All steps for the risk assessment, i.e. the screening step, 1st and 2nd oral tier calculations were performed using the corresponding EFSA Bee calculator Tool (Bee-Tool v.3) provided by EFSA.

Screening step risk assessment

The acute and chronic risks to adult honey bees and honey bee larvae as well as the acute risk for bumble bees and solitary bees from the use of were assessed using the maximum single application rates and the respective 'hazard quotients' (HQs) and 'exposure toxicity ratios' (ETRs).

Table 9.6-7: Screening step risk assessment of ADM.00150.I.2.A for crops with a maximum single application rate of 0.060 kg a.s./ha (worst case)

Test	Endpoint	Calculation factor ^{a)}	HQ or ETR ^{a)}	Trigger ^{a)}	Risk acceptable?
<i>Contact route of exposure</i>					
Honey bee	3.8 µg a.s./bee	1	21.1	42 / 85	Yes
Bumble bee	> 200 µg a.s./bee		< 0.4	7 / 14	Yes
<i>Oral route of exposure</i>					
Honey bee, acute	9.1 µg a.s./bee	7.6 / 10.6	0.05/ 0.07	0.2	Yes
Honey bee, chronic	3.71 µg a.s./bee/day	7.6 / 10.6	0.123 / 0.171	0.03	No
Honey bee, larvae	≥ 0.486 µg a.s./larva	4.4 / 6.1	≤ 0.54 / ≤ 0.75	0.2	No
Bumble bee, acute	24.3 µg a.s./bee	11.2 / 13.3	0.03 / 0.03	0.036	Yes

HQ/ETR values in **bold** are above the trigger value

^{a)} Application scenario used for calculations: downward spraying / up- and sideward spraying

Considering the proposed uses of ADM.00150.I.2.A at a maximum application rate of 0.06 kg a.s./ha, no unacceptable effects are expected for honey bees following acute oral and contact exposure, respectively.

However, a potential risk of acetamiprid for is still indicated following the chronic exposure of adults and for honey bee larvae at this stage of testing. Therefore, 1st tier oral risk assessments were carried out (see 9.6-9).

In case of bumble bee, the ETR value for oral end contact exposure is below triggers.

Therefore, 1st tier for acute oral risk assessments is not required.

Table 9.6-9: 1st tier oral risk assessment for honey bees (chronic and larvae) of ADM.00150.I.2.A.

Crop (Crop group according to EFSA tool)	Endpoint	ETR (oral exposure scenario) ^{a)}					Trigger
		Treated crop	Weeds	Field margin	Adjacent crop	Next crop	
Maximum single application rate: 0.06 kg a.s./ha, BBCH 51-75							
Corn	adult, chronic	0.011	0.008	0.000	0.000	0.006	0.03
	larvae	0.02	0.06	0.00	0.00	0.04	0.2
Maximum single application rate: 0.036 kg a.s./ha, BBCH 12-69							
Cereals	adult, chronic	0.006	0.02	0.000	0.00	0.00	0.03
	Larvae	0.01	0.00	0.00	0.00	0.03	0.2
Maximum single application rate: 0.060 kg a.s./ha, BBCH 71							
Apple	adult, chronic	0.000	0.010	0.002	0.002	0.006	0.03
BBCH 71	larvae	0.00	0.07	0.01	0.01	0.04	0.2
Maximum single application rate: 0.025 kg a.s./ha, BBCH 62							
Apple 1	adult, chronic	0.040	0.004	0.001	0.001	0.003	0.03
BBCH 62	larvae	0.27	0.01	0.01	0.01	0.02	0.2
Maximum single application rate: 0.025 kg a.s./ha, BBCH 71							
Apple	adult, chronic	0.000	0.004	0.001	0.001	0.003	0.03
BBCH 71	larvae	0.00	0.03	0.01	0.01	0.02	0.2
Maximum single application rate: 0.036 kg a.s./ha, BBCH 12-79							
Potato (potatoes)	adult, chronic	0.006	0.020	0.000	0.000	0.004	0.03
	larvae	0.01	0.14	0.00	0.00	0.03	0.2

Maximum single application rate: 0.06 kg a.s./ha BBCH 31-71							
Spring and winter oil seed rape (31-69 BBCH)	adult, chronic	0.068	0.010	0.000	0.000	0.006	0.03
	larvae	0.46	0.07	0.00	0.00	0.04	0.2
Spring and winter oil seed rape > BBCH 70-71	adult, chronic	0.000	0.008	0.000	0.000	0.006	0.03
	larvae	0.00	0.06	0.00	0.00	0.04	0.2
Maximum single application rate: 0.048 kg a.s./ha BBCH 11-19							
Winter oil seed rape (BBCH 10-29)*	adult, chronic	0.054	0.027	0.00	0.000	0.005	0.03
	larvae	0.37	0.18	0.00	0.000	0.003	0.2
Maximum single application rate: 0.050 kg a.s./ha, BBCH 12-39							
Sugar beet 12-39 BBCH	adult, chronic	0.052	0.026	0.000	0.000	0.005	0.03
	larvae	0.35	0.18	0.00	0.00	0.03	0.2
Maximum single application rate: 0.046 kg a.s./ha, BBCH 12-91							
Flower bulbs and flower tubers, BBCH 12-69	adult, chronic	0.052	0.026	0.000	0.000	0.005	0.03
	larvae	0.35	0.18	0.00	0.000	0.03	0.2
Flower bulbs and flower tubers, BBCH>70	adult, chronic	0.00	0.016	0.000	0.005	0.11	0.03
	larvae	0.00	0.11	0.00	0.00	0.03	0.2
Maximum single application rate: 0.034 kg a.s./ha, BBCH 20-91							
Flower bulbs and flower tubers, BBCH 10-69	adult, chronic	0.038	0.019	0.000	0.000	0.000	0.03
	larvae	0.26	0.13	0.00	0.00	0.002	0.2
Flower bulbs and flower tubers, BBCH >70	adult, chronic	0.00	0.011	0.000	0.004	0.004	0.03
	larvae	0.00	0.08	0.00	0.00	0.02	0.2
Maximum single application rate: 0.046 kg a.s./ha, Floriculture, Tree nursery & Perennial nursery crops, BBCH 12-91							
Floriculture, Tree nursery & Perennial nursery crops, BBCH 10-69 (Orchards 1)	adult, chronic	0.073	0.021	0.003	0.003	0.005	0.03
	larvae	0.49	0.14	0.02	0.02	0.03	0.2
Floriculture, Tree nursery & Perennial nursery crops, BBCH >70 (Orchards 1) BBCH>70	adult, chronic	0.00	0.008	0.003	0.003	0.003	0.03
	larvae	0.00	0.05	0.02	0.02	0.03	0.2
Maximum single application rate: 0.034 kg a.s./ha, Floriculture, Tree nursery & Perennial nursery crops, BBCH 12-91							
Floriculture, Tree nursery & Perennial nursery crops, BBCH 10-69 (Orchards 1)	adult, chronic	0.054	0.015	0.002	0.003	0.004	0.03
	larvae	0.36	0.10	0.01	0.02	0.02	0.03
Floriculture, Tree nursery & Perennial nursery crops, BBCH >70 (Orchards 1)	adult, chronic	0.00	0.006	0.002	0.002	0.003	0.03
	larvae	0.00	0.04	0.01	0.02	0.02	0.2

ETR values in **bold** are above the trigger value

- a) All BBCH scenarios were used according to the proposed application timing. In the table only the worst-case (highest) values are presented
b) not relevant as the application is in autumn.

For the use in potatoes and maize no unacceptable effects are expected considering all oral routes of exposure. As already mentioned in the introductory part of point 9.6.2 above, due to specific mode of action of acetamiprid and in absence of any other validated guidance enabling evaluation of the chronic and larvae risk, in opinion of the zRMS consideration of indications of EFSA (2013) is justified even if the guidance itself is not noted yet.

Calculations provided above using EFSA Bee-Tool v.3 for all intended uses acceptable acute contact and oral risk could be concluded at the screening step for the worst-case application rate (60 g a.s./ha), covering all intended uses.

However, the oral chronic and larvae risk was not acceptable and for this reason 1st tier oral risk assessment has been performed.

Based on provided above calculations acceptable risk with no need for further refinement could be concluded for intended uses in potatoes, cereals and maize (all considered scenarios).

It is noted that 1st tier risk assessment scheme in EFSA (2013) allows for distinguishing between particular BBCH stages of the crop in question. Therefore, it was decided by the zRMS to perform separate risk assessment for particular stages at which will be applied to proposed uses of ADM.00150.I.2.A.

Based on the zRMS calculations chronic risk for adult honey bees and honey bee larvae cannot be ruled out based on the oral exposure in the 'treated field' scenario for the following uses:

- apple at maximum application rate of rate 1 x 25 g a.s./ha and BBCH 62,
- Spring and winter oil seed rape at BBCH 31-71,
- Sugar beet at BBCH 12-39,
- Floriculture, Tree nursery & Perennial nursery crops at rates 46 g a.s./ha and 34 g a.s./ha at BBCH 10-69
- flower bulbs at BBCH 10-69 at rates 34 and 46 g a.s./ha

Although intended application pattern to oilseed rape also includes wide range of BBCH stages (31-71) it is noted that application after flowering is intended only during short period at BBCH 70-71, so potential restriction of the application to period after flowering would be pointless from the agronomical perspective.

In case of oilseed rape at BBCH 10-19 with application in autumn for „treated crop” scenario is not relevant.

Generally, zRMS's calculations demonstrated acceptable risk for the “treated crop” scenario when the product is applied from BBCH 70 onward (i.e. after the flowering period) for application rate 1 x 60 g a.s./ ha (apples), 1 x 25 g a.s./ha (apples), Floriculture, Tree nursery & Perennial nursery crops and Flower bulbs and flower tubers, at rates 1 x 34 g a.s./ha and 1 x 46 g a.s./ha.

With regard to exposure of bees to acetamiprid metabolite the following is concluded in EFSA Journal 2016;14(11):4610:

Insufficient information was available to perform a first-tier risk assessment to honeybees for relevant metabolites in pollen and nectar. However, most of the plant metabolites were reported in the RAR as not having an insecticidal activity and the exposure from these metabolites is expected to be very low. Therefore, the experts concluded that the risk from metabolites could be expected as low.

Based on that no unacceptable risk to bees exposed to acetamiprid metabolites via nectar and pollen is expected.

Therefore a 2nd tier oral risk assessment is necessary to address potential risk of adult honey bees for chronic oral exposure as well as for honey bee larvae exposed to ADM.00150.I.2.A.

9.6.2.2 Higher-tier risk assessment for bees (tunnel test, field studies)

Next to the laboratory studies, seven semi-field studies in tunnels and three field studies were conducted with honey bees.

~~Six out of the seven tunnel tests were carried out according to the CEB 230 methodology and followed the EPPO 170/4 (2010) protocol for semi-field tests. Three out of these six studies were conducted on *Phacelia* during the flowering period (Mamet & Molitor, 2015b &c; Molitor, 2015a), whereas the three others were performed on winter wheat (Mamet & Molitor, 2015a; Mamet, 2015a & b). This range of growth stage on winter wheat fits with period of aphid infestations.~~

~~According to EPPO 170/4 and CEB 230, *Phacelia* is considered a standard attractive crop for application during the flowering period, whereas winter wheat is the standard crop for application during the honeydew production period. Winter wheat crop is sprayed with syrup before application in order to mimic the honeydew produced by sucking pests.~~

~~According to CEB 230 methodology, all the plots inside the tunnels are treated.~~

Four treatments are generally studied:

- Water is applied during the foraging activity (C)
- Test item when it is applied during the foraging activity (T1)
- Test item when it is applied outside the foraging activity (T2)
- Tunnel allocated to toxic reference applied during the foraging activity (R)

In all these studies MCW 2222 was applied once at a rate of 0.5 L/ha (= 100 g acetamiprid/ha). However, these studies are not considered for the risk assessment as they do not comply with current accepted guidelines. Nevertheless, summaries of these studies are presented in Appendix 2 for completeness.

Furthermore, another semi-field bee brood study (Hecht Rost & Mayer, 2018) is available. It was conducted according to OECD GD 75 (2007) in flowering *Phacelia*, and thus under worst-case semi-field exposure conditions. The study mainly focused on potential effects on adult and pupal mortality, foraging activity, behaviour as well as on the colony strength and colony development in the course of one brood cycle. Especially, it aimed to investigate the development success of a certain number of marked brood cells which were filled with eggs at the initial assessment. The current study covers a GAP with two applications at a rate of 0.4 L/ha (80 g acetamiprid/ha), the 1st application before the flowering period and the 2nd application during the flowering period but after the daily bee flight activity which is a risk mitigation measure applied during this period. It therefore covers the maximum intended application of ADM.00150.I.2.A, which is foreseen in apple crops (umbrella use IIb) under the consideration that the product is only applied when bees are not actively foraging during the crop flowering period.

Finally, three honey bee full-field studies were carried out with MCW 2222 according to EPPO 170/4 (2010) and OECD GD 75 (2007), one in *Phacelia* (Molitor, 2015b), one in oilseed rape (Molitor, 2015c) and one in an apple orchard (Aujejo, 2015) to investigate potential impacts on the adult and pupal mortality, foraging activity and behaviour under realistic field exposure conditions, which covered the acute and chronic exposure of adult bees and larvae. Special attention was paid on the assessment of the colony strength, colony development and detailed bee brood assessment (marking of cells with eggs, young and old larvae with subsequent assessment of the development, only cells with eggs in the apple study).

In the *Phacelia* study (Molitor, 2015b), MCW 2222 was applied once at 0.5 L/ha (= 100 g acetamiprid/ha) during the flowering period of the crop, whereas in the oilseed rape (Molitor, 2015c) and apple (Aujejo, 2015) studies it was applied twice at a rate of 100 g acetamiprid/ha, just before and during the flowering period. This rate is higher than the intended maximum application rate of 80 g a.s./ha in apple. In all the studies, the application during the flowering period was carried out when bees were not actively foraging, i.e. at dusk after bee flight activity. As already outlined, the application during the flowering period when bees are not actively foraging is regarded as a risk mitigation measure.

The results of the semi-field bee brood study (Hecht Rost & Mayer, 2018) did not reveal any effects caused by the use of acetamiprid when applied at a time when bees are not foraging. The daily and overall adult and pupal mortality (covering acute and chronic exposure of adult bees and larvae) were not increased. Neither the foraging activity nor the behaviour were affected at observations in the days following the application. Furthermore, the regular assessments of the colony strength and the colony development as well as the detailed assessment of marked brood cells indicated no impact of the test item on the bee brood and the colonies. In fact, the brood termination rate of the test item group was even lower than in the control, and brood index and brood compensation index was thus higher compared to the control.

The results of the semi-field bee brood study were confirmed by the field studies (Molitor, 2015b & c; Aujejo, 2015). In all three field studies no effects on the daily and overall adult and pupal mortality (covering acute and chronic exposure of adult bees and larvae), foraging activity and behaviour were recorded. Moreover, the assessments of the colony strength and the colony development as well as the detailed assessment of marked brood cells indicated no adverse effects of the test item on the bee brood and the colonies. In fact, the brood termination rates, brood indices and brood compensation indices for cells filled with eggs, young and old larvae were on the control level. Especially when MCW 2222 was applied to flowering apple trees at a rate of 100 g a.s./ha and bees thus were directly exposed for a period of 11 days in the orchard (i.e. from the second application to the end of flowering on 11 DAB) and for additional 23

days at the monitoring site, no effects on the investigated parameters were observed, especially on the adult and pupal mortality as well as on the brood relevant endpoints, i.e. colony strength, colony development and brood termination rates with its respective indices

In the 'Phacelia' field study (Molitor 2015b) low precipitation of about 3 mm and 1 mm was recorded on 2DAA and 4DAA, respectively. Moreover, it can be assumed that the rain has no influence on the quality of the results because foraging activity was very high at 10.4 bees/m² (i.e. 208,000 bees foraging on the 2 ha field) on 1DAA, the day before the rain. And even the following day a good number of foraging of bees was observed with 3.5 bees/m² (i.e. 70,000 bees foraging) in 2DAA and 0.9 bees/m² (i.e. 18,000 bees foraging) on 3DAA, indicating a significant exposure.

Overall, it can be concluded, that the application of ADM.00150.I.2.A to flowering, bee attractive crops, after daily bee flight does not adversely affect the survival and fitness of adult and pupal honey bees, honey bee brood and their colonies after acute and chronic exposure when applied to flowering, bee attractive crops up to a rate of 100 g acetamiprid/ha, which is above the maximum intended use rate of 80 g a.s./ha in apple. The application in the evening after bee flight is regarded as a suitable risk mitigation measure providing a good margin of safety for applications in the flowering period on bee attractive crops.

zRMS comments:

The semi-field and field studies were evaluated by zRMS-PL during authorisation of the product CA3573 in 2021 and the zRMS-PL revealed some deficiencies, which were not taken into account in the Applicants' evaluation above. As no new higher tier studies are provided to bees for the current evaluation of the product ADM.00150.I.2.A (formerly MCW-2222) the conclusions from these studies are still valid.

The summaries of higher-tier studies together with detailed zRMS-PL evaluation may be found in in Appendix 2. Obtained results and observed deficiencies are also shortly summarised in Table 9.6-3 above.

The conclusion of zRMS-PL from higher tier studies provided with MCW-222 is relevant also for the current evaluation of the product ADM.00150.I.2.A and is presented below:

Apples

No semi-field studies on effects of application of CA3573 (formerly MCW-2222) to apples were performed and only one field study was performed on this crop (Aucejo, 2015). The full study summary together with zRMS comments are presented in Appendix 2 under KCP 10.3.1.6/03, while short information on the study results is presented in Table 9.6-3 in point 9.6.1 above.

Overall, the zRMS-PL was of the opinion that results of the field study on apples are not fully reliable due to significant deficiencies of the study noted in the course of the evaluation and including too small bee colonies used for the trial, no information on flowering weeds and trees in the field surroundings (they could be in flower), no information on flowering orchard crops in field surroundings (they could be in flower) and rainfall during first 3 days after the second application.

In addition to that, despite potentially reduced exposure due to presence of flowering weeds and trees as well as the rainfall, the brood termination rates in the test item groups were clearly higher comparing to controls. This effect was statistically not significant, but the statistical power of the study may be also questioned as BTR in treated fields were several times higher than in controls with brood indices reduced at the same time. Therefore, in opinion of the zRMS-PL, observed effects were of biological relevance. Lack of calculation of compensation indices makes interpretation of the study results even more difficult, as potential recovery of affected brood could not be confirmed.

Taking all this into account in opinion of the zRMS-PL the study by Aucejo (2015) indicates that application of CA3573 to flowering apples may have some adverse effects on the bee brood, but due to deficiencies noted no firm conclusion may be derived and further study would be necessary to confirm or exclude these effects.

Overall, unacceptable risk to bees following application of CA3573. to flowering apples cannot be excluded based on available data and for this reason the authorisation for application in this crop may be granted only for post-flowering period from BBCH 70 to PHI.

The conclusion is still valid for current evaluation of the product ADM.00150.I.2.A in uses in Apples at rate 1-2 x 25 g a.s./ha at BBCH from 62.

In order to remove this restriction, the applicant should provide reliable field study performed in line with current recommendations regarding the bee field studies. Preferably, the study should include investigation of effects on the overwintering success. In case this parameter is not included in the study, the bee brood observations should cover at least two brood cycles with last brood assessment performed at 42 BFD. Nevertheless, study including overwintering success is the preferred option.

Oilseed rape

Only one field study has been performed on flowering oilseed rape (Molitor, 2015). However, several higher tier studies (tunnel, semi-field and field trials) were performed on flowering *Phacelia tanacetifolia*, which due to comparable crop structure and attractiveness may be used as a surrogate crop to conclude on effects expected following application to flowering oilseed rape.

Summaries of all higher tier studies performed on *Phacelia* together with zRMS-PL evaluation are presented in Appendix 2, while the summary of obtained results is presented in Table 9.6-3.

In general, application of CA3573 at 80-100 g a.s./ha to flowering *Phacelia* after the bee flight had no significant effects on mortality of various bee stages (adult, larvae, pupae). Slight and transient effects were observed on bee foraging activity on the day of application in the tunnel tests, but they were not confirmed in semi-field and field studies. Application of the product during the bee activity in the tunnels increased the bee mortality during first days after the application. Effects of the direct overspray under the field conditions could not be confirmed, as in all semi-field and field studies the product was applied in the evening, after the bee flight.

The bee brood parameters as well as adult, pupae or larvae mortality were not affected in the semi-field bee brood study (Hecht-Rost & Mayer, 2018) performed in line with OECD 75 with CA3573 applied to flowering *Phacelia* after the bee flight.

No treatment related effects on the investigated bee and bee brood parameters were observed in the field studies performed in flowering *Phacelia* and winter OSR with CA3573 applied after the bee flight at 100 g a.s./ha once (*Phacelia* study) or twice (OSR study, with first application carried out just before the flowering period at BBCH 59 and second carried out in full flowering at BBCH 64). Although both field studies had some deficiencies, the zRMS is of the opinion that they complement each other and indicate that application of CA3573 had no adverse effects on the adult bees, bee brood and the general status of the tested bee colonies. Especially in the study performed on OSR the increase in strength of the colonies was observed in all treatment groups at the test termination, indicating correct development. All deficiencies of the studies are described in detail and discussed in the zRMS evaluation presented in Appendix 2 under KCP 10.3.1.6/01 (*Phacelia* study) and KCP 10.3.1.6/02 (OSR study).

None of the studies performed on *Phacelia* or oilseed rape included investigation of effects on overwintering success. Nevertheless, none of the brood parameters was not affected by the treatment and the colonies were stronger at test termination comparing to test initiation. The exception was the field study performed on *Phacelia*, however in this study the weak status of the colonies at test termination was not a result of the treatment, since similar effects were seen in both, test item and control groups with reproductive performance lower in some of control hives. Therefore, in this study application of CA3573 also had no effect on the colony strength, which is especially important as the colonies were in general weak already at the test initiation.

Overall, in opinion of the zRMS-PL results of the field studies performed on *Phacelia* and oilseed rape together with results of the tunnel and semi-field bee brood tests performed on *Phacelia* are sufficient to conclude that CA3573 applied to OSR at 60 g a.s./ha in the evening after the bee flight will not pose unacceptable risk to adult bees and the bee colonies.

It should be noted that the results from the field study provided in OSR , (Molitor, 2015) does not cover double application during flowering OSR at rate 2 x 60 g.a./ha.

For this reason, restriction to only one application during flowering in OSR crops , applied in the evening after the bee flight is granted.

In order to remove this restriction, the applicant should provide reliable field study performed in line with current recommendations regarding the bee field studies. Preferably, the study should include investigation of effects on the overwintering success. In case this parameter is not included in the study, the bee brood observations should cover at least two brood cycles with last brood assessment performed at 42 BFD. Nevertheless, study including overwintering success is the preferred option.

Remaining crops:

Floriculture, Tree nursery & Perennial nursery crops and Flower bulbs and flower tubers, at rates 1 x 34 g a.s./ha and 1 x 46 g a.s./ha at BBCH 12-91.

The risk mitigation for bees for these crops are based on higher tier study provided in apple, phacelia and oilseed rape.

As unacceptable risk to bees following application of ADM.00150.I.2.A. to flowering apples cannot be excluded based on available data and for this reason the authorisation for application in this crop may be granted only for post-flowering period from BBCH 70 to PHI.

This same risk mitigation measures to bees are proposed by zRMS for flowering Tree nursery crops and flowering Floriculture crops. For nurseries of non-flowering trees and bushes no risk mitigation are required as no exposure to bees is expected.

In case of remaining ornamental plants and perennial nursery crops at BBCH 12-91, the cumulative application rate (2 x 34 g a.s./ha) is lower than in used in field study in OSR or Phacelia, therefore the application in the evening after bee flight is sufficient to protect bees.

The same conclusion is valid flower bulb and flower tubers at rates 34 g a.s./ha and 46 g a.s./ha at BBCH 12-91, therefore application in the evening after bee flight is proposed.

Sugar beet

As the sugar beet crop is not attractive to bees and it is not expected that last application will be closed to flowering stage in this crop no additional risk mitigation measures to bees are proposed.

Based on the evaluation of higher tier studies for bees, the following restriction is applied to protect bees and other pollinators:

Apple, 25 g a.s./ha:

- application from BBCH 70 (Don't apply during flowering)

Floriculture crops:

Flowering ornamental bushes:

- application from BBCH 70 (Don't apply during flowering)

Remaining ornamental plants and perennial nursery crops at BBCH 12-91:

- application during flowering in the evening after bee flight

Tree nursery crops:

Flowering tree nursery:

- application from BBCH 70 (Don't apply during flowering)

Nurseries of non-flowering trees and bushes:

No risk mitigation is required.

Flower bulb and flower tubers at rate 34 g a.s./ha and 46 g a.s./ha at BBCH 12-91:

Application during flowering period in the evening after bee flight

Oil seed rape: Only one application during flowering allowed, applied in the evening after bee flight.

Sugar beet: no risk mitigation is required

9.6.3 Effects on bumble bees

See chapter 9.6.1, Table 9.6-2.

9.6.4 Effects on solitary bees

There is no experimental data available for solitary bees as it is not a data requirement of Regulation (EU) 283/2013 or Regulation (EU) 284/2013. Moreover, no valid testing guidelines are available. Therefore, risk assessments are not performed.

9.6.5 Overall conclusions

~~All HQ values for the oral and contact exposure of adult honey bees were below the trigger of 50, based on the proposed maximum application rates of the respective umbrella GAPs. This demonstrated that no negative effects on honey bees are expected when ADM.00150.I.2.A (containing 200 g/L acetamiprid) is applied according to the intended application rates up to 80 g a.s./ha.~~

Furthermore, a comparison of the LD₅₀ values deriving from chronic adult and larvae laboratory studies indicate that toxicity is very similar compared to the acute oral endpoint, and no difference in sensitivity was observed. For bumble bees, the acute endpoints indicated that bumblebees are not more susceptible to acetamiprid than honey bees.

The outcome of the theoretical risk assessment is confirmed by the results of one semi-field bee brood study and three full field studies with detailed bee brood assessments. The data showed that the application of ADM.00150.I.2.A to flowering, bee attractive crops after daily bee flight does not adversely affect the survival and fitness of adult and pupal honey bees, honey bee brood and their colonies after acute and chronic exposure when applied to flowering, bee attractive crops up to a rate of 100 g acetamiprid/ha. The application in the evening after bee flight is regarded as a suitable risk mitigation measure providing a good margin of safety for applications in the flowering and bee attractive crops.

Overall, it can be concluded that ADM.00150.I.2.A is of low risk for bees, their brood and their colonies when used according to the proposed GAP.

zRMS comments:

The risk assessment performed in line with SANCO/1039/2002 demonstrated acceptable risk to bees following application of ADM.00150.I.2.A to all intended crops.

However, as acetamiprid is an insecticide with the specific mode of action, evaluation of the chronic risk to adult bees and bee larvae was also deemed necessary. In absence of the chronic and larvae risk assessment scheme, the zRMS concluded that the risk assessment as provided in EFSA (2013) will be most relevant to cover the risk to all bee stages and all exposure patterns, even though the guidance is not noted yet at the EU level.

Evaluation based on indications of EFSA (2013) demonstrated acceptable acute and chronic risk to adult bees and larvae exposed following intended uses of ADM.00150.I.2.A in maize, cereals and potatoes.

Based on the evaluation of higher tier studies for bees, the following restriction is applied to protect bees and other pollinators:

Apple, 25 g a.s./ha:

- application from BBCH 70 (Don't apply during flowering)

Floriculture crops:

Flowering ornamental bushes:

- application from BBCH 70 (Don't apply during flowering)

Remaining ornamental plants and perennial nursery crops at BBCH 12-91:

- application during flowering in the evening after bee flight

Tree nursery crops:

Flowering tree nursery:

- application from BBCH 70 (Don't apply during flowering)

Nurseries of non-flowering trees and bushes:

No risk mitigation is required.

Flower bulb and flower tubers at rate 34 g a.s./ha and 46 g a.s./ha at BBCH 12-91:

Application during flowering period in the evening after bee flight

Oil seed rape: Only one application during flowering allowed, applied in the evening after bee flight

Sugar beet: no risk mitigation is required

9.7 Effects on arthropods other than bees (KCP 10.3.2)

9.7.1 Toxicity data

Studies on the toxicity to non-target arthropods have been carried out with ADM.00150.I.2.A. Full details of these studies are provided in the respective EU DAR and related documents as well as in Appendix 2 of this document (new studies).

Effects on non-target arthropods of ADM.00150.I.2.A were not evaluated as part of the EU assessment of acetamiprid. Data submitted with this application are listed in Appendix 1 and summarised in Appendix 2.

Table 9.7-1: Endpoints and effect values relevant for the risk assessment for non-target arthropods of ADM.00150.I.2.A

Species	Substance	Exposure System	Results	Reference
Laboratory studies				
<i>Typhlodromus pyri</i> (protonymphs)	ADM.00150.I.2.A	Laboratory test glass plates (2D)	LR₅₀ = 9.13 g a.s./ha ER ₅₀ = > 6.17 g a.s./ha	Röhlig, U., 2014 R-33838 KCP 10.3.2.1/01
<i>Aphidius rhopalosiphi</i> (adults)	ADM.00150.I.2.A	Laboratory test glass plates (2D)	LR₅₀ = 0.0243 g a.s./ha ER -	Röhlig, U., 2014 R-33839 KCP 10.3.2.1/02
Extended laboratory studies				
<i>Typhlodromus pyri</i> (protonymphs)	ADM.00150.I.2.A	Extended laboratory test, bean leafs (2D)	LR₅₀ = 31.9 g a.s./ha ER₅₀ = > 12.5 g a.s./ha	Röhlig, U., 2014 R-34780 KCP 10.3.2.2/01
<i>Aphidius rhopalosiphi</i> (adults)	ADM.00150.I.2.A	Extended laboratory bean leafs (2D)	LR₅₀ = 0.111 g a.s./ha ER ₅₀ = 0.1 g a.s./ha	Stevens, J., 2015 R-35026 KCP 10.3.2.2/02
<i>Aphidius rhopalosiphi</i> (adults)	ADM.00150.I.2.A	Extended laboratory test, barley plants (3D)	LR₅₀ = 3.56 g/ha ER ₅₀ = - ^a g/ha	Röhlig, U., 2014 R-33839A KCP 10.3.2.2/03
<i>Chrysoperla carnea</i>	ADM.00150.I.2.A	Extended laboratory test, bean leafs (2D)	LR₅₀ = 106 g a.s./ha ER ₅₀ > 116 g a.s./ha	Röhlig, U., 2014 R-34781 KCP 10.3.2.2/04
<i>Coccinella septempunctata</i>	ADM.00150.I.2.A	Extended laboratory test, bean leaf (2D)	LR₅₀ = 22.1 g a.s./ha ER ₅₀ = 20.7 g a.s./ha	Röhling, U. 2014 R-34782 KCP 10.3.2.2/05

Species	Substance	Exposure System	Results	Reference
Agede residue studies				
<i>Typhlodromus pyri</i>	ADM.00150.I.2.A	Aged Residue Test (leaves of potted apples plants, 2D 3D)	Mortality at 102 g a.s./ha: 1.06% at 0 DAT -4.30% at 35 DAT 2.13% at 42 DAT Red. of reproduction at 102 g a.s./ha: 7.41% at 0 DAT -16.61% at 35 DAT -5.66% at 42 DAT Mortality at 170 g a.s./ha: 42.55% at 0 DAT 0% at 35 DAT 3.19% at 42 DAT Red of reproduction at 170 g a.s./ha: 27.65% at 0 DAT -3.37% at 35 DAT 9.24% at 42 DAT	Luna, F., 2017b R-37335 KCP 10.3.2.3/01
<i>Aphidius rhopalosiphi</i>	ADM.00150.I.2.A	Aged Residue Test (leaves of potted bean plants, 2D 3D)	Mortality at 45 g.a./ha: 100% at 0 DAT 10% at 28 DAT 5% at 36 DAT Red. of reproduction at 45 g a.s./ha: N/A at 0 DAT -34.97% at 28 DAT 11.97% at 36 DAT	Luna, F., 2016a R-36938A / TRC15-242BA KCP 10.3.2.3/02
<i>Aphidius rhopalosiphi</i>	ADM.00150.I.2.A	Aged Residue Test (leaves of potted bean plants 2D, 3D)	Mortality at 70 g.a./ha: 100% at 0 DAT 27.5% at 28 DAT 20% at 36 DAT Red. of reproduction at 70 g a.s./ha: N/A at 0 DAT -6.56% at 28 DAT 15.88 % at 36 DAT	Luna, F., 2016b R-36938A TRC15-243BA KCP 10.3.2.3/03
<i>Aphidius rhopalosiphi</i>	ADM.00150.I.2.A	Aged Residue Test (leaves of potted bean plants, 2D 3D)	Mortality at 102 g.a./ha: 100% at 0 DAT 75% at 28 DAT 42.5% at 36 DAT 25% at 42 DAT Red. of reproduction at 102 g a.s./ha: N/A at 0 DAT, 28 DAT and 36 DAT 11.61% at 42 DAT	Luna, F., 2016c R-36938A TRC15-244BA KCP 10.3.2.3/04

Species	Substance	Exposure System	Results	Reference
<i>Aphidius rhopalosiphi</i>	ADM.00150.I.2.A	Aged Residue Test (branches leaves of potted apple plants, 2D , 3D)	Mortality at 170 g a.s./ha: 100% at 0 DAT 28.57% at 42 DAT 14.29% at 49 DAT Red. of reproduction at 170 g a.s./ha: N/A at 0 DAT 41.97% at 42 DAT -8.41% at 49 DAT	Luna, F., 2017a R-37333 / TRC16-073BA KCP 10.3.2.3/05
<i>Coccinella septempunctata</i>	ADM.00150.I.2.A	Aged Residue Test (branches leaves of potted apple plants, 3D)	Mortality at 102 g a.s./ha: 48.72% at 0 DAT 5.26% at 35 DAT 7.69% at 42 DAT Mortality at 170 g a.s./ha: 61.54% at 0 DAT 5.13% at 35 DAT 3.05% at 42 DAT Red. of reproduction at 102 g a.s./ha: 7.41% at 0 DAT -16.61% at 35 DAT -5.66% at 42 DAT Red. of reproduction at 170 g a.s./ha: 27.65% at 0 DAT -3.37% at 35 DAT 9.24% at 42 DAT	Luna, F., 2017c R-37334 / TRC16-075BA KCP 10.3.2.3/06
Higher-tier studies				
Species	Substance	Endpoint used for risk assessment		Reference

Non-target arthropod fauna	ADM.00150.I.2.A	Lowest Observed Ecological Adverse Effect Rate (LOEAER) for population = 7.2 g a.s./ha. No Observed Ecological Adverse Effect Rate (NOEAER) = 3.4 g a.s./ha. No Observed Effect Rate (NOER) = 2.38^b g a.s./ha No Observed Ecological Effect rate (NOER) = 1.4 g a.s./ha	Appeltauer, A 2016, R-35848, S15-01184 KCP 10.3.2.4/01
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Values shown in **bold** used for risk assessment

^a No ER₅₀ could be determined in this study

^b NOER of 2.38 g a.s./ha (single application rate of 1.4 g a.s./ha multiplied by the foliar multiple application factor 1.7)

zRMS comments:

All the laboratory and extended laboratory studies on effects of MCW-2222 to non-target arthropods listed in Table 9.7-1 were already evaluated in the course of the first zonal authorisation in April 2018 and considered acceptable. The guidelines against which the studies were validated have not changed since that time, so re-evaluation of the studies was not necessary. Provided endpoints are confirmed to be correct. Study summaries together with zRMS conclusions on acceptability are provided in Appendix 2.

The field study by Appeltauer (2016) also has been accepted in the course of the first zonal evaluation and is still considered to be valid. However, NOEAER of 3.4 g a.s./ha was agreed as an endpoint relevant for purposes of the risk refinement, while the NOER was set to 1.4 g a.s./ha. Respective corrections were thus made in Table 9.7-1. The study summary together with zRMS-PL previously evaluation and conclusions on acceptability are provided in Appendix 2.

Aged residue studies were submitted. Summaries of the studies together with their evaluation by the zRMS may be found in Appendix 2.

It was noted that in the aged residue study with *Coccinella septempunctata* the mean number of eggs per female per day and mean number of viable eggs per female per day were reduced by more than 50% comparing to control in test groups exposed to residues aged for 42 days. However, based on results of available research high variability of reproductive performance of ladybird beetles is observed in laboratory tests it is proposed that for regulatory purposes the effect is considered as treatment related when the number of viable eggs/female/day falls below the lower limit of the observed ranges of 2-10. The same is proposed in guideline of Schmuck et al. (2000), which states that due to the high variability, the reproductive performance of this species may be evaluated only qualitatively. Furthermore, it should be also noted that in the submitted study by Luna (2017c) >50% reduction in reproductive capacity was observed only in groups exposed to residues aged for 42 days and no such a reduction was observed in groups exposed to residues aged for shorter period of time or exposed to fresh residues at 102 g a.s./ha. Taking this into account it seems to be highly unlikely that residues aged for longer period of time would have more pronounced adverse effects than fresh residues and the observed reduction seems to be rather due to unexpectedly high production of eggs in controls.

Overall, all aged residues studies were considered valid with endpoints relevant for the risk assessment purposes.

9.7.2 Justification for new endpoints

ADM.00150.I.2.A was not the representative formulation for the renewal of the active substance acetamiprid. Studies on effects of the formulation ADM.00150.I.2.A on non-target arthropods were carried out as required by Regulation (EU) 284/2013. The studies with ADM.00150.I.2.A were conducted in accordance with the most recent guidelines.

9.7.3 Risk assessment

The evaluation of the risk for non-target arthropods was performed in accordance with the recommendations of the “Guidance Document on Terrestrial Ecotoxicology”, as provided by the Commission Services (SANCO/10329/2002 rev.2 (final), October 17, 2002), and in consideration of the recommendations of the guidance document ESCORT 2.

9.7.3.1 Risk assessment for in-field exposure

The results of the first- and higher tier risk assessments are summarised in the following tables.

Table 9.7-2: First- and higher-tier assessment of the in-field risk for non-target arthropods due to the use of ADM.00150.I.2.A in corn (Use No. I)

Intended use	Corn		
Product	ADM.00150.I.2.A		
Application rate (g a.s./ha)	1 × 60		
MAF	1		
Test species Tier I	LR₅₀ (lab.) (g a.s./ha)	PER_{in-field} (g a.s./ha)	HQ_{in-field} criterion: HQ ≤ 2
<i>Typhlodromus pyri</i>	LR ₅₀ = 9.13	60	6.57
<i>Aphidius rhopalosiphi</i>	LR ₅₀ = 0.0243		2469
Extended laboratory studies	Rate with ≤ 50 % effect* (g a.s./ha)	PER_{in-field} (g a.s./ha)	PER_{in-field} below rate with ≤ 50 % effect?
<i>Typhlodromus pyri</i>	LR ₅₀ = 31.9 ER ₅₀ = > 12.5	60	no
<i>Aphidius rhopalosiphi</i>	LR ₅₀ = 0.111 ER ₅₀ = 0.100 g a.s./ha		no
<i>Aphidius rhopalosiphi</i>	LR ₅₀ = 3.56 ER ₅₀ < 0.64 g a.s./ha		no
<i>Chrysoperla carnea</i>	LR ₅₀ = 106 ER ₅₀ = 116 g a.s./ha		yes
<i>Coccinella septempunctata</i>	LR ₅₀ = 22.1 g a.s./ha ER ₅₀ = 20.7 g a.s./ha		no
Aged residue studies	Rate with ≤ 50 % effect (g a.s./ha) at xxx DAT	PER_{in-field} (g a.s./ha)	PER_{in-field} below rate with ≤ 50 % effect?
<i>Typhlodromus pyri</i>	102 at 0 DAT	60	yes
<i>Aphidius rhopalosiphi</i>	45 at 28 DAT		no
<i>Aphidius rhopalosiphi</i>	70 at 28 DAT		yes
<i>Aphidius rhopalosiphi</i>	102 at 42 36 DAT		yes
<i>Coccinella septempunctata</i>	102 at 0 DAT		yes

MAF: Multiple application factor; PER: Predicted environmental rate; HQ: Hazard quotient. DAT: Days after treatment.
Criteria values shown in **bold** breach the relevant trigger.

* If an LR₅₀ or ER₅₀ from a relevant extended laboratory test is available, it should be considered in place of the rate with ≤ 50 % effect.

Table 9.7-3: First- and higher-tier assessment of the in-field risk for non-target arthropods due to the use of ADM.00150.I.2.A in apple (Use No. IIa)

Intended use	Apple		
Product	ADM.00150.I.2.A		
Application rate (g a.s./ha)	1 x 60 80		
MAF	1		
3D crop correction factor	1.2		
Test species Tier I	LR₅₀ (lab.) (g a.s./ha)	PER_{in-field} (g a.s./ha)	HQ_{in-field} criterion: HQ ≤ 2
<i>Typhlodromus pyri</i>	LR ₅₀ = 9.13	60 40	6.57 3.38 4.38
<i>Aphidius rhopalosiphi</i>	LR ₅₀ = 0.0243		2469.14 1234.6 1646
Extended laboratory studies	Rate with ≤ 50 % effect* (g a.s./ha)	PER_{in-field} (g a.s./ha)	PER_{in-field} below rate with ≤ 50 % effect?
<i>Typhlodromus pyri</i>	LR ₅₀ = 31.9 ER ₅₀ = > 12.5	60 40	no
<i>Aphidius rhopalosiphi</i>	LR ₅₀ = 0.111 ER ₅₀ = 0.100 g a.s./ha		no
<i>Aphidius rhopalosiphi</i>	LR ₅₀ = 3.56 ER ₅₀ < 0.64 g a.s./ha		no
<i>Chrysoperla carnea</i>	LR ₅₀ = 106 ER ₅₀ = 116 g a.s./ha		yes
<i>Coccinella septempunctata</i>	LR ₅₀ = 22.1 g a.s./ha ER ₅₀ = 20.7 g a.s./ha		no
Aged residue studies	Rate with ≤ 50 % effect (g a.s./ha) at xxx DAT	PER_{in-field} (g a.s./ha)	PER_{in-field} below rate with ≤ 50 % effect?
<i>Typhlodromus pyri</i>	102 at 0 DAT	60 40	yes
<i>Aphidius rhopalosiphi</i>	45 at 28 DAT		yes
<i>Aphidius rhopalosiphi</i>	70 at 28 DAT		yes
<i>Aphidius rhopalosiphi</i>	102 at 42 36 DAT		yes
<i>Coccinella septempunctata</i>	102 at 0 DAT		yes

MAF: Multiple application factor; PER: Predicted environmental rate; HQ: Hazard quotient. DAT: Days after treatment.
Criteria values shown in **bold** breach the relevant trigger.

* If an LR₅₀ or ER₅₀ from a relevant extended laboratory test is available, it should be considered in place of the rate with ≤ 50 % effect.

Table 9.7-4: First- and higher-tier assessment of the in-field risk for non-target arthropods due to the use of ADM.00150.I.2.A in apple (Use No. IIb)

Intended use	Apple		
Product	ADM.00150.I.2.A		
Application rate (g a.s./ha)	2 x 25 g a.s./ha		
MAF	1.7 (foliar) 1.9 (soil)		
3D crop correction factor	0.5		
Test species Tier I	LR₅₀ (lab.) (g a.s./ha)	PER_{in-field} (g a.s./ha)	HQ_{in-field} criterion: HQ ≤ 2
<i>Typhlodromus pyri</i>	LR ₅₀ = 9.13	21.25 (foliar) 23.75 (soil)	2.32 (foliar) 2.60 (soil)
<i>Aphidius rhopalosiphi</i>	LR ₅₀ = 0.0243		874 (foliar) 977 (soil)
Extended laboratory studies	Rate with ≤ 50 % effect* (g a.s./ha)	PER_{in-field} (g a.s./ha)	PER_{in-field} below rate with ≤ 50 % effect?
<i>Typhlodromus pyri</i>	LR ₅₀ = 31.9 ER ₅₀ = > 12.5	21.25 (foliar) 23.75 (soil)	no (foliar) no (soil)
<i>Aphidius rhopalosiphi</i>	LR ₅₀ = 0.111 ER ₅₀ = 0.100		no (foliar) no (soil)
<i>Aphidius rhopalosiphi</i>	LR ₅₀ = 3.56 ER ₅₀ < 0.64		no (foliar) no (soil)
<i>Chrysoperla carnea</i>	LR ₅₀ = 106 g a.s./ha ER ₅₀ = 116 g a.s./ha		yes (foliar) yes (soil)
<i>Coccinella septempunctata</i>	LR ₅₀ = 22.1 g a.s./ha ER ₅₀ = 20.7		no (foliar) no (soil)
Aged residue studies	Rate with ≤ 50 % effect (g a.s./ha) at xxx DAT	PER_{in-field} (g a.s./ha)	PER_{in-field} below rate with ≤ 50 % effect?
<i>Typhlodromus pyri</i>	102 at 0 DAT	21.25 (foliar) 23.75 (soil)	yes (foliar) yes (soil)
<i>Aphidius rhopalosiphi</i>	45 at 28 DAT		yes (foliar) yes (soil)
<i>Aphidius rhopalosiphi</i>	70 at 28 DAT		yes (foliar) yes (soil)
<i>Aphidius rhopalosiphi</i>	102 at 42 36 DAT		yes (foliar) yes (soil)
<i>Coccinella septempunctata</i>	102 at 0 DAT		yes (foliar) yes (soil)

MAF: Multiple application factor; PER: Predicted environmental rate; HQ: Hazard quotient. DAT: Days after treatment.

Criteria values shown in **bold** breach the relevant trigger.

* If an LR₅₀ or ER₅₀ from a relevant extended laboratory test is available, it should be considered in place of the rate with ≤ 50 % effect.

Table 9.7-5: First- and higher-tier assessment of the in-field risk for non-target arthropods due to the use of ADM.00150.I.2.A in potato (Use No. III)

Intended use	Potato		
Product	ADM.00150.I.2.A		
Application rate (g a.s./ha)	1 × 36		
MAF	1		
Test species Tier I	LR₅₀ (lab.) (g a.s./ha)	PER_{in-field} (g a.s./ha)	HQ_{in-field} criterion: HQ ≤ 2
<i>Typhlodromus pyri</i>	LR ₅₀ = 9.13	36	3.94
<i>Aphidius rhopalosiphi</i>	LR₅₀ = 0.0243		1481
Extended laboratory studies	Rate with ≤ 50 % effect* (g a.s./ha)	PER_{in-field} (g a.s./ha)	PER_{in-field} below rate with ≤ 50 % effect?
<i>Typhlodromus pyri</i>	LR ₅₀ = 31.9 ER ₅₀ = > 12.5	36	no
<i>Aphidius rhopalosiphi</i>	LR ₅₀ = 0.111 ER ₅₀ = 0.100		no
<i>Aphidius rhopalosiphi</i>	LR ₅₀ = 3.56 ER ₅₀ < 0.64		no
<i>Chrysoperla carnea</i>	LR ₅₀ = 106 ER ₅₀ = 116		yes
<i>Coccinella septempunctata</i>	LR ₅₀ = 22.1 ER ₅₀ = 20.7		no
Aged residue studies	Rate with ≤ 50 % effect (g a.s./ha) at xxx DAT	PER_{in-field} (g a.s./ha)	PER_{in-field} below rate with ≤ 50 % effect?
<i>Typhlodromus pyri</i>	102 at 0 DAT	36	yes
<i>Aphidius rhopalosiphi</i>	45 at 28 DAT		yes
<i>Aphidius rhopalosiphi</i>	70 at 28 DAT		yes
<i>Aphidius rhopalosiphi</i>	102 at 42 36 DAT		yes
<i>Coccinella septempunctata</i>	102 at 0 DAT		yes

MAF: Multiple application factor; PER: Predicted environmental rate; HQ: Hazard quotient. DAT: Days after treatment.
Criteria values shown in **bold** breach the relevant trigger.

* If an LR₅₀ or ER₅₀ from a relevant extended laboratory test is available, it should be considered in place of the rate with ≤ 50 % effect.

Table 9.7-6: First- and higher-tier assessment of the in-field risk for non-target arthropods due to the use of ADM.00150.I.2.A in spring/winter cereals (Use No. IVa, IVb, Va)

Intended use	Spring/winter cereals		
Product	ADM.00150.I.2.A		
Application rate (g a.s./ha)	2 × 36 (covering use no. IVa and IVb of 2 x 35)		
MAF	1.7 (foliar) 1.9 (soil)		
Test species	LR₅₀ (lab.)	PER_{in-field}	HQ_{in-field}
Tier I	(g a.s./ha)	(g a.s./ha)	criterion: HQ ≤ 2
<i>Typhlodromus pyri</i>	LR ₅₀ = 9.13	61.2 (foliar) 68.4 (soil)	6.70 (foliar) 7.49 (soil)
<i>Aphidius rhopalosiphi</i>	LR ₅₀ = 0.0243		2519 (foliar) 2815 (soil)
Extended laboratory studies	Rate with ≤ 50 % effect*	PER_{in-field}	PER_{in-field} below rate with
	(g a.s./ha)	(g/ha)	≤ 50 % effect?
<i>Typhlodromus pyri</i>	LR ₅₀ = 31.9 ER ₅₀ = > 12.5	61.2 (foliar) 68.4 (soil)	no (foliar) no (soil)
<i>Aphidius rhopalosiphi</i>	LR ₅₀ = 0.111 ER ₅₀ = 0.100		no (foliar) no (soil)
<i>Aphidius rhopalosiphi</i>	LR ₅₀ = 3.56 ER ₅₀ < 0.64		no (foliar) no (soil)
<i>Chrysoperla carnea</i>	LR ₅₀ = 106 ER ₅₀ = 116		yes (foliar) yes (soil)
<i>Coccinella septempunctata</i>	LR ₅₀ = 22.1 ER ₅₀ = 20.7		no (foliar) no (soil)
Aged residue studies	Rate with ≤ 50 % effect	PER_{in-field}	PER_{in-field} below rate with
	(g a.s./ha) at xxx DAT	(g a.s./ha)	≤ 50 % effect?
<i>Typhlodromus pyri</i>	102 at 0 DAT	61.2 (foliar) 68.4 (soil)	yes (foliar) yes (soil)
<i>Aphidius rhopalosiphi</i>	45 at 28 DAT		no (foliar) no (soil)
<i>Aphidius rhopalosiphi</i>	70 at 28 DAT		yes (foliar) yes (soil)
<i>Aphidius rhopalosiphi</i>	102 at 42 36 DAT		yes (foliar) yes (soil)
<i>Coccinella septempunctata</i>	102 at 0 DAT		yes (foliar) yes (soil)

MAF: Multiple application factor; PER: Predicted environmental rate; HQ: Hazard quotient. DAT: Days after treatment.

Criteria values shown in **bold** breach the relevant trigger.

* If an LR₅₀ or ER₅₀ from a relevant extended laboratory test is available, it should be considered in place of the rate with ≤ 50 % effect.

Table 9.7-7: First- and higher-tier assessment of the in-field risk for non-target arthropods due to the use of ADM.00150.I.2.A in winter cereals (Use No. Vb)

Intended use	Winter cereals		
Product	ADM.00150.I.2.A		
Application rate (g a.s./ha)	1 × 30		
MAF	1		
Test species Tier I	LR₅₀ (lab.) (g a.s./ha)	PER_{in-field} (g a.s./ha)	HQ_{in-field} criterion: HQ ≤ 2
<i>Typhlodromus pyri</i>	LR ₅₀ = 9.13	30	3.26
<i>Aphidius rhopalosiphi</i>	LR ₅₀ = 0.0243		1235
Extended laboratory studies	Rate with ≤ 50 % effect* (g a.s./ha)	PER_{in-field} (g/ha)	PER_{in-field} below rate with ≤ 50 % effect?
<i>Typhlodromus pyri</i>	LR ₅₀ = 31.9 ER ₅₀ = > 12.5	30	no
<i>Aphidius rhopalosiphi</i>	LR ₅₀ = 0.111 ER ₅₀ = 0.100		no
<i>Aphidius rhopalosiphi</i>	LR ₅₀ = 3.56 ER ₅₀ < 0.64		no
<i>Chrysoperla carnea</i>	LR ₅₀ = 106 ER ₅₀ = 116		yes
<i>Coccinella septempunctata</i>	LR ₅₀ = 22.1 ER ₅₀ = 20.7		no
Aged residue studies	Rate with ≤ 50 % effect (g a.s./ha) at xxx DAT	PER_{in-field} (g a.s./ha)	PER_{in-field} below rate with ≤ 50 % effect?
<i>Typhlodromus pyri</i>	102 at 0 DAT	30	yes
<i>Aphidius rhopalosiphi</i>	45 at 28 DAT		yes
<i>Aphidius rhopalosiphi</i>	70 at 28 DAT		yes
<i>Aphidius rhopalosiphi</i>	102 at 42-36 DAT		yes
<i>Coccinella septempunctata</i>	102 at 0 DAT		yes

MAF: Multiple application factor; PER: Predicted environmental rate; HQ: Hazard quotient. DAT: Days after treatment.
Criteria values shown in **bold** breach the relevant trigger.

* If an LR₅₀ or ER₅₀ from a relevant extended laboratory test is available, it should be considered in place of the rate with ≤ 50 % effect.

Table 9.7-8: First- and higher-tier assessment of the in-field risk for non-target arthropods due to the use of ADM.00150.I.2.A in winter/spring OSR (Use No. VIa, VII)

Intended use	Winter/spring OSR		
Product	ADM.00150.I.2.A		
Application rate (g a.s./ha)	2 × 60		
MAF	1.7 (foliar) 1.9 (soil)		
Test species	LR₅₀ (lab.)	PER_{in-field}	HQ_{in-field}
Tier I	(g a.s./ha)	(g a.s./ha)	criterion: HQ ≤ 2
<i>Typhlodromus pyri</i>	LR ₅₀ = 9.13	102 (foliar) 114 (soil)	11.17 (foliar) 12.49 (soil)
<i>Aphidius rhopalosiphi</i>	LR ₅₀ = 0.0243		4198 (foliar) 4691 (soil)
Extended laboratory studies	Rate with ≤ 50 % effect*	PER_{in-field}	PER_{in-field} below rate with
	(g a.s./ha)	(g/ha)	≤ 50 % effect?
<i>Typhlodromus pyri</i>	LR ₅₀ = 31.9 ER ₅₀ = > 12.5	102 (foliar) 114 (soil)	no (foliar) no (soil)
<i>Aphidius rhopalosiphi</i>	LR ₅₀ = 0.111 ER ₅₀ = 0.100		no (foliar) no (soil)
<i>Aphidius rhopalosiphi</i>	LR ₅₀ = 3.56 ER ₅₀ < 0.64		no (foliar) no (soil)
<i>Chrysoperla carnea</i>	LR ₅₀ = 106 ER ₅₀ = 116		yes (foliar) yes (soil)
<i>Coccinella septempunctata</i>	LR ₅₀ = 22.1 ER ₅₀ = 20.7		no (foliar) no (soil)
Aged residue studies	Rate with ≤ 50 % effect	PER_{in-field}	PER_{in-field} below rate with
	(g a.s./ha) at xxx DAT	(g a.s./ha)	≤ 50 % effect?
<i>Typhlodromus pyri</i>	102 at 0 DAT	102 (foliar) 114 (soil)	yes (foliar) no (soil)
<i>Typhlodromus pyri</i>	170 at 0 DAT		yes (foliar) yes (soil)
<i>Aphidius rhopalosiphi</i>	45 at 28 DAT		no (foliar) no (soil)
<i>Aphidius rhopalosiphi</i>	70 at 28 DAT		no (foliar) no (soil)
<i>Aphidius rhopalosiphi</i>	102 at 42 36 DAT		yes (foliar) no (soil)
<i>Aphidius rhopalosiphi</i>	170 at 42 DAT		yes (foliar) yes (soil)
<i>Coccinella septempunctata</i>	102 at 0 DAT		yes (foliar) no (soil)
<i>Coccinella septempunctata</i>	170 at 35 DAT		yes (foliar) yes (soil)

MAF: Multiple application factor; PER: Predicted environmental rate; HQ: Hazard quotient. DAT: Days after treatment.

Criteria values shown in **bold** breach the relevant trigger.

* If an LR₅₀ or ER₅₀ from a relevant extended laboratory test is available, it should be considered in place of the rate with ≤ 50 % effect.

Table 9.7-9: First- and higher-tier assessment of the in-field risk for non-target arthropods due to the use of ADM.00150.I.2.A in winter OSR (Use No. VIb, 129, 144, 150)

Intended use	Winter OSR		
Product	ADM.00150.I.2.A		
Application rate (g a.s./ha)	1 × 60 (covering use no. 129, 144 and 150 of 1 x 40)		
MAF	1		
Test species Tier I	LR₅₀ (lab.) (g a.s./ha)	PER_{in-field} (g a.s./ha)	HQ_{in-field} criterion: HQ ≤ 2
<i>Typhlodromus pyri</i>	LR ₅₀ = 9.13	60	6.57
<i>Aphidius rhopalosiphi</i>	LR ₅₀ = 0.0243		2469
Extended laboratory studies	Rate with ≤ 50 % effect* (g a.s./ha)	PER_{in-field} (g/ha)	PER_{in-field} below rate with ≤ 50 % effect?
<i>Typhlodromus pyri</i>	LR ₅₀ = 31.9 ER ₅₀ = > 12.5	60	no
<i>Aphidius rhopalosiphi</i>	LR ₅₀ = 0.111 LR ₅₀ =0.1000		no
<i>Aphidius rhopalosiphi</i>	LR ₅₀ = 3.56 ER ₅₀ <0.64		no
<i>Chrysoperla carnea</i>	LR ₅₀ = 106 ER ₅₀ =116		yes
<i>Coccinella septempunctata</i>	LR ₅₀ =22.1 ER ₅₀ = 20.7		no
Aged residue studies	Rate with ≤ 50 % effect (g a.s./ha) at xxx DAT	PER_{in-field} (g a.s./ha)	PER_{in-field} below rate with ≤ 50 % effect?
<i>Typhlodromus pyri</i>	102 at 0 DAT	60	yes
<i>Aphidius rhopalosiphi</i>	45 at 28 DAT		no
<i>Aphidius rhopalosiphi</i>	70 at 28 DAT		yes
<i>Aphidius rhopalosiphi</i>	102 at 42 ³⁶ DAT		yes
<i>Coccinella septempunctata</i>	102 at 0 DAT		yes

MAF: Multiple application factor; PER: Predicted environmental rate; HQ: Hazard quotient. DAT: Days after treatment.
Criteria values shown in **bold** breach the relevant trigger.

* If an LR₅₀ or ER₅₀ from a relevant extended laboratory test is available, it should be considered in place of the rate with ≤ 50 % effect.

Table 9.7-10: First- and higher-tier assessment of the in-field risk for non-target arthropods due to the use of ADM.00150.I.2.A in sugar beet (Use No.VIII)

Intended use	Sugar beet		
Product	ADM.00150.I.2.A		
Application rate (g a.s./ha)	2 × 50		
MAF	1.7 (foliar) 1.9 (soil)		
Test species	LR₅₀ (lab.)	PER_{in-field}	HQ_{in-field}
Tier I	(g a.s./ha)	(g a.s./ha)	criterion: HQ ≤ 2
<i>Typhlodromus pyri</i>	LR ₅₀ = 9.13	80 (foliar) 95 (soil)	9.31 (foliar) 10.41 (soil)
<i>Aphidius rhopalosiphi</i>	LR ₅₀ = 0.0243		3498 (foliar) 3909 (soil)
Extended laboratory studies	Rate with ≤ 50 % effect*	PER_{in-field}	PER_{in-field} below rate with
	(g a.s./ha)	(g a.s./ha)	≤ 50 % effect?
<i>Typhlodromus pyri</i>	LR ₅₀ = 31.9 ER ₅₀ = > 12.5	80 (foliar) 95 (soil)	no (foliar) no (soil)
<i>Aphidius rhopalosiphi</i>	LR ₅₀ = 0.111 ER=0.100		no (foliar) no (soil)
<i>Aphidius rhopalosiphi</i>	LR ₅₀ = 3.56 ER ₅₀ <0.64		no (foliar) no (soil)
<i>Chrysoperla carnea</i>	LR ₅₀ = 106 ER ₅₀ =116		yes (foliar) yes (soil)
<i>Coccinella septempunctata</i>	LR ₅₀ =22.1 ER ₅₀ = 20.7		no (foliar) no (soil)
Aged residue studies	Rate with ≤ 50 % effect	PER_{in-field}	PER_{in-field} below rate with
	(g a.s./ha) at xxx DAT	(g a.s./ha)	≤ 50 % effect?
<i>Typhlodromus pyri</i>	102 at 0 DAT	80 (foliar) 95 (soil)	yes (foliar) yes (soil)
<i>Aphidius rhopalosiphi</i>	45 at 28 DAT		no (foliar) no (soil)
<i>Aphidius rhopalosiphi</i>	70 at 28 DAT		no (foliar) no (soil)
<i>Aphidius rhopalosiphi</i>	102 at 42 36 DAT		yes (foliar) yes (soil)
<i>Coccinella septempunctata</i>	102 at 0 DAT		yes (foliar) yes (soil)

MAF: Multiple application factor; PER: Predicted environmental rate; HQ: Hazard quotient. DAT: Days after treatment.

Criteria values shown in **bold** breach the relevant trigger.

* If an LR₅₀ or ER₅₀ from a relevant extended laboratory test is available, it should be considered in place of the rate with ≤ 50 % effect.

Table 9.7-11: First- and higher-tier assessment of the in-field risk for non-target arthropods due to the use of ADM.00150.I.2.A in flower bulbs and flower tubers (Use no. IXa)

Intended use	Flower bulbs and flower tubers		
Product	ADM.00150.I.2.A		
Application rate (g a.s./ha)	1 × 46		
MAF	1		
Test species Tier I	LR₅₀ (lab.) (g a.s./ha)	PER_{in-field} (g a.s./ha)	HQ_{in-field} criterion: HQ ≤ 2
<i>Typhlodromus pyri</i>	LR ₅₀ = 9.13	46	5.04
<i>Aphidius rhopalosiphi</i>	LR ₅₀ = 0.0243		1893
Extended laboratory studies	Rate with ≤ 50 % effect* (g a.s./ha)	PER_{in-field} (g/ha)	PER_{in-field} below rate with ≤ 50 % effect?
<i>Typhlodromus pyri</i>	LR ₅₀ = 31.9 ER ₅₀ = > 12.5	46	no
<i>Aphidius rhopalosiphi</i>	LR ₅₀ = 0.111 ER ₅₀ = 0.100		no
<i>Aphidius rhopalosiphi</i>	LR ₅₀ = 3.56 ER ₅₀ < 0.64		no
<i>Chrysoperla carnea</i>	LR ₅₀ = 106 ER ₅₀ = 116		yes
<i>Coccinella septempunctata</i>	LR ₅₀ = 22.1 ER ₅₀ = 20.7		no
Aged residue studies	Rate with ≤ 50 % effect (g a.s./ha) at xxx DAT	PER_{in-field} (g a.s./ha)	PER_{in-field} below rate with ≤ 50 % effect?
<i>Typhlodromus pyri</i>	102 at 0 DAT	46	yes
<i>Aphidius rhopalosiphi</i>	45 at 28 DAT		no
<i>Aphidius rhopalosiphi</i>	70 at 28 DAT		yes
<i>Aphidius rhopalosiphi</i>	102 at 42 36 DAT		yes
<i>Coccinella septempunctata</i>	102 at 0 DAT		yes

MAF: Multiple application factor; PER: Predicted environmental rate; HQ: Hazard quotient. DAT: Days after treatment.
Criteria values shown in **bold** breach the relevant trigger.

* If an LR₅₀ or ER₅₀ from a relevant extended laboratory test is available, it should be considered in place of the rate with ≤ 50 % effect.

Table 9.7-12: First- and higher-tier assessment of the in-field risk for non-target arthropods due to the use of ADM.00150.I.2.A in flower bulbs and flower tubers (Use no. IXb, IXc)

Intended use		Flower bulbs and flower tubers	
Product		ADM.00150.I.2.A	
Application rate (g a.s./ha)		2 × 34	
MAF		1.7 (foliar) 1.9 (soil)	
Test species	LR₅₀ (lab.)	PER_{in-field}	HQ_{in-field}
Tier I	(g a.s./ha)	(g a.s./ha)	criterion: HQ ≤ 2
<i>Typhlodromus pyri</i>	LR ₅₀ = 9.13	57.8 (foliar) 64.6 (soil)	6.33 (foliar) 7.08 (soil)
<i>Aphidius rhopalosiphi</i>	LR ₅₀ = 0.0243		2379 (foliar) 2658 (soil)
Extended laboratory studies	Rate with ≤ 50 % effect*	PER_{in-field}	PER_{in-field} below rate with
	(g a.s./ha)	(g/ha)	≤ 50 % effect?
<i>Typhlodromus pyri</i>	LR ₅₀ = 31.9 ER ₅₀ = > 12.5	57.8 (foliar) 64.6 (soil)	no (foliar) no (soil)
<i>Aphidius rhopalosiphi</i>	LR ₅₀ = 0.111 LR ₅₀ = 0.100		no (foliar) no (soil)
<i>Aphidius rhopalosiphi</i>	LR ₅₀ = 3.56 ER ₅₀ < 0.64		no (foliar) no (soil)
<i>Chrysoperla carnea</i>	LR ₅₀ = 106 ER ₅₀ = 116		yes (foliar) yes (soil)
<i>Coccinella septempunctata</i>	LR ₅₀ = 22.1 ER ₅₀ = 20.7		no (foliar) no (soil)
Aged residue studies	Rate with ≤ 50 % effect	PER_{in-field}	PER_{in-field} below rate with
	(g a.s./ha) at xxx DAT	(g a.s./ha)	≤ 50 % effect?
<i>Typhlodromus pyri</i>	102 at 0 DAT	57.8 (foliar) 64.6 (soil)	yes (foliar) yes (soil)
<i>Aphidius rhopalosiphi</i>	45 at 28 DAT		no (foliar) no (soil)
<i>Aphidius rhopalosiphi</i>	70 at 28 DAT		yes (foliar) yes (soil)
<i>Aphidius rhopalosiphi</i>	102 at 42 ³⁶ DAT		yes (foliar) yes (soil)
<i>Coccinella septempunctata</i>	102 at 0 DAT		yes (foliar) yes (soil)

MAF: Multiple application factor; PER: Predicted environmental rate; HQ: Hazard quotient. DAT: Days after treatment.
Criteria values shown in **bold** breach the relevant trigger.

* If an LR₅₀ or ER₅₀ from a relevant extended laboratory test is available, it should be considered in place of the rate with ≤ 50 % effect.

Table 9.7-13: First- and higher-tier assessment of the in-field risk for non-target arthropods due to the use of ADM.00150.I.2.A in floriculture, tree nursery & perennial nursery crops (Use no. Xb, Xc)

Intended use	Floriculture, tree nursery & perennial nursery crops		
Product	ADM.00150.I.2.A		
Application rate (g a.s./ha)	2 × 34		
MAF	1.7 (foliar) 1.9 (soil)		
3D crop correction factor	0.5		
Test species Tier I	LR₅₀ (lab.) (g a.s./ha)	PER_{in-field} (g a.s./ha)	HQ_{in-field} criterion: HQ ≤ 2
<i>Typhlodromus pyri</i>	LR ₅₀ = 9.13	28.9 (foliar) 32.3 (soil)	3.17 (foliar) 3.54 (soil)
<i>Aphidius rhopalosiphi</i>	LR ₅₀ = 0.0243		1189 (foliar) 1329 (soil)
Extended laboratory studies	Rate with ≤ 50 % effect* (g a.s./ha)	PER_{in-field} (g a.s./ha)	PER_{in-field} below rate with ≤ 50 % effect?
<i>Typhlodromus pyri</i>	LR ₅₀ = 31.9 ER ₅₀ = > 12.5	28.9 (foliar) 32.3 (soil)	no (foliar) no (soil)
<i>Aphidius rhopalosiphi</i>	LR ₅₀ = 0.111 ER ₅₀ = 0.100		no (foliar) no (soil)
<i>Aphidius rhopalosiphi</i>	LR ₅₀ = 3.56 ER ₅₀ < 0.64		no (foliar) no (soil)
<i>Chrysoperla carnea</i>	LR ₅₀ = 106 ER ₅₀ = 116		yes (foliar) yes (soil)
<i>Coccinella septempunctata</i>	LR ₅₀ = 22.1 ER ₅₀ = 20.7		no (foliar) no (soil)
Aged residue studies	Rate with ≤ 50 % effect (g a.s./ha) at xxx DAT	PER_{in-field} (g a.s./ha)	PER_{in-field} below rate with ≤ 50 % effect?
<i>Typhlodromus pyri</i>	102 at 0 DAT	28.9 (foliar) 32.3 (soil)	yes (foliar) yes (soil)
<i>Aphidius rhopalosiphi</i>	45 at 28 DAT		yes (foliar) yes (soil)
<i>Aphidius rhopalosiphi</i>	70 at 28 DAT		yes (foliar) yes (soil)
<i>Aphidius rhopalosiphi</i>	102 at 42 36 DAT		yes (foliar) yes (soil)
<i>Coccinella septempunctata</i>	102 at 0 DAT		yes (foliar) yes (soil)

MAF: Multiple application factor; PER: Predicted environmental rate; HQ: Hazard quotient. DAT: Days after treatment.

Criteria values shown in **bold** breach the relevant trigger.

* If an LR₅₀ or ER₅₀ from a relevant extended laboratory test is available, it should be considered in place of the rate with ≤ 50 % effect.

Table 9.7-14: First- and higher-tier assessment of the in-field risk for non-target arthropods due to the use of ADM.00150.I.2.A in floriculture, tree nursery & perennial nursery crops (Use no. Xa)

Intended use		Floriculture, tree nursery & perennial nursery crops	
Product		ADM.00150.I.2.A	
Application rate (g a.s./ha)		1 x 46	
MAF		1	
3D crop correction factor		0.5	
Test species	LR₅₀ (lab.) (g a.s./ha)	PER_{in-field} (g a.s./ha)	HQ_{in-field} criterion: HQ ≤ 2
Tier I			
<i>Typhlodromus pyri</i>	LR ₅₀ = 9.13	23	2.52
<i>Aphidius rhopalosiphi</i>	LR ₅₀ = 0.0243		947
Extended laboratory studies	Rate with ≤ 50 % effect* (g a.s./ha)	PER_{in-field} (g a.s./ha)	PER_{in-field} below rate with ≤ 50 % effect?
<i>Typhlodromus pyri</i>	LR ₅₀ = 31.9 ER ₅₀ = > 12.5	23	no
<i>Aphidius rhopalosiphi</i>	LR ₅₀ = 0.111 ER ₅₀ = 0.100		no
<i>Aphidius rhopalosiphi</i>	LR ₅₀ = 3.56 ER ₅₀ < 0.64		no
<i>Chrysoperla carnea</i>	LR ₅₀ = 106 ER ₅₀ = 116		yes
<i>Coccinella septempunctata</i>	LR ₅₀ = 22.1 ER ₅₀ = 20.7		no
Aged residue studies	Rate with ≤ 50 % effect (g a.s./ha) at xxx DAT	PER_{in-field} (g a.s./ha)	PER_{in-field} below rate with ≤ 50 % effect?
<i>Typhlodromus pyri</i>	102 at 0 DAT	23	yes
<i>Aphidius rhopalosiphi</i>	45 at 28 DAT		yes
<i>Aphidius rhopalosiphi</i>	70 at 28 DAT		yes
<i>Aphidius rhopalosiphi</i>	102 at 42 36 -DAT		yes
<i>Coccinella septempunctata</i>	102 at 0 DAT		yes

MAF: Multiple application factor; PER: Predicted environmental rate; HQ: Hazard quotient. DAT: Days after treatment.

Criteria values shown in **bold** breach the relevant trigger.

* If an LR₅₀ or ER₅₀ from a relevant extended laboratory test is available, it should be considered in place of the rate with ≤ 50 % effect.

zRMS comments:

In-field risk assessment for non-target arthropods presented by the Applicant in tables above is agreed by the zRMS. Endpoints for reproduction from extended lab studies has been added for completeness.

It was also noted that in an aged residue study with *A. rhopalosiphi* no unacceptable effects of application rate of 102 g a.s./ha were observed after 42 days of aging (and not 36 days as initially reported). This has been corrected.

No acceptable risk could be concluded with Tier I toxicity data, while at Tier II acceptable risk for most of uses could be concluded for *Chrysoperla carnea* only.

The in-field risk for species of concern was further refined with consideration of results of aged-residues studies, which demonstrated that for applications at rate up to 102 g a.s./ha no unacceptable effects on all tested species (including most sensitive *A. rhopalosiphi*) are observed after maximum 42 days of aging.

Based on that it may be concluded that after application of ADM.00150.I.2.A at the maximum application rate indicated in GAP (i.e. 60 g a.s./ha) there is a potential for re-colonisation of the treated field within less than one year and acceptable risk to in-field population of non-target arthropods may be thus concluded for the intended uses of ADM.00150.I.2.A.

9.7.3.2 Risk assessment for off-field exposure

The results of the first- and higher tier risk assessments are summarised in the following tables.

Table 9.7-15: First- and higher-tier assessment of the off-field risk for non-target arthropods due to the use of ADM.00150.I.2.A in corn (Use no. I)

Intended use		Corn				
Active substance/product		ADM.00150.I.2.A				
Application rate (g a.s./ha)		1 x 60				
MAF		1				
vdf		5				
Test species Tier I	LR₅₀ (lab.) (g a.s./ha)	Drift rate	PER_{off-field} (g a.s./ha)	CF	PER_{off-field, corr.} (g a.s./ha)	HQ_{off-field} criterion: HQ ≤ 2
<i>Typhlodromus pyri</i>	LR ₅₀ = 9.13	2.77	0.332	10	3.32	0.36
<i>Aphidius rhopalosiphi</i>	LR ₅₀ = 0.0243					137
Extended laboratory studies	Rate with ≤ 50 % effect* (g a.s./ha)	Drift rate	PER_{off-field} (g a.s./ha)	CF	PER_{off-field, corr.} (g a.s./ha)	corr. PER_{off-field} below rate with ≤ 50 % effect?
<i>Typhlodromus pyri</i>	ER ₅₀ = > 12.5	2.77	0.332	5	1.66	yes
<i>Aphidius rhopalosiphi</i>	LR ₅₀ =0.100 LR ₅₀ = 0.111		0.332		1.66	no
<i>Aphidius rhopalosiphi</i>	LR ₅₀ = 3.56 ER ₅₀ <0.64		1.662		8.31	no
<i>Coccinella septempunctata</i>	ER ₅₀ = 20.7		0.332		1.66	yes
<i>Chrysoperla carnea</i>	LR ₅₀ = 106		0.332		1.66	yes

MAF: Multiple application factor; vdf: Vegetation distribution factor; (corr.) PER: (corrected) Predicted environmental rate; CF: Correction factor; HQ: Hazard quotient. Criteria values shown in **bold** breach the relevant trigger.

* If an LR₅₀ or ER₅₀ from a relevant extended laboratory test is available, it should be considered in place of the rate with ≤ 50 % effect.

Table 9.7-16: First- and higher-tier assessment of the off-field risk for non-target arthropods due to the use of ADM.00150.I.2.A in apple (Use no. IIa)

Intended use		Apple				
Active substance/product		ADM.00150.I.2.A				
Application rate (g a.s./ha)		1 x 60 ³⁹				
MAF		1				
vdf		5				
Test species Tier I	LR₅₀ (lab.) (g a.s./ha)	Drift rate	PER_{off-field} (g a.s./ha)	CF	PER_{off-field, corr.} (g a.s./ha)	HQ_{off-field} criterion: HQ ≤ 2
<i>Typhlodromus pyri</i>	LR ₅₀ = 9.13	15.73	1.89 2.517	10	18.9 25.17	2.07 2.76
<i>Aphidius rhopalosiphi</i>	LR ₅₀ = 0.0243					777.7 1036
Extended laboratory studies	Rate with ≤ 50 % effect* (g a.s./ha)	Drift rate	PER_{off-field} (g a.s./ha)	CF	PER_{off-field, corr.} (g a.s./ha)	corr. PER_{off-field} below rate with ≤ 50 % effect?
<i>Typhlodromus pyri</i>	ER ₅₀ = > 12.5	15.73	1.89 2.517	5	9.45 12.58	yes
<i>Aphidius rhopalosiphi</i>	LR₅₀=0.100 LR₅₀=0.111		1.89 2.517		9.45 12.58	no
<i>Aphidius rhopalosiphi</i>	LR ₅₀ = 3.56 ER ₅₀ <0.64		9.45 12.58		47.25 62.92	no
<i>Coccinella septempunctata</i>	ER ₅₀ = 20.7		1.89 2.517		9.45 12.58	yes
<i>Chrysoperla carnea</i>	LR ₅₀ = 106		1.89 2.517		9.45 12.58	yes

MAF: Multiple application factor; vdf: Vegetation distribution factor; (corr.) PER: (corrected) Predicted environmental rate; CF: Correction factor; HQ: Hazard quotient. Criteria values shown in **bold** breach the relevant trigger.

* If an LR₅₀ or ER₅₀ from a relevant extended laboratory test is available, it should be considered in place of the rate with ≤ 50 % effect.

Table 9.7-17: First- and higher-tier assessment of the off-field risk for non-target arthropods due to the use of ADM.00150.I.2.A in apple (Use no. IIb)

Intended use		Apple				
Active substance/product		ADM.00150.I.2.A				
Application rate (g a.s./ha)		2 x 25				
MAF		1.7				
vdf		5				
Test species Tier I	LR₅₀ (lab.) (g a.s./ha)	Drift rate	PER_{off-field} (g a.s./ha)	CF	PER_{off-field, corr.} (g a.s./ha)	HQ_{off-field} criterion: HQ ≤ 2
<i>Typhlodromus pyri</i>	LR ₅₀ = 9.13	12.13	1.031	10	10.31	1.13
<i>Aphidius rhopalosiphi</i>	LR ₅₀ = 0.0243					424
Extended laboratory studies	Rate with ≤ 50 % effect* (g a.s./ha)	Drift rate	PER_{off-field} (g a.s./ha)	CF	PER_{off-field, corr.} (g a.s./ha)	corr. PER_{off-field} below rate with ≤ 50 % effect?
<i>Typhlodromus pyri</i>	ER ₅₀ = > 12.5	12.13	1.031	5	5.16	yes
<i>Aphidius rhopalosiphi</i>	ER₅₀=0.100 LR₅₀=0.111		1.031		5.16	no
<i>Aphidius rhopalosiphi</i>	LR ₅₀ = 3.56 ER ₅₀ <0.64		5.155		25.78	no
<i>Coccinella septempunctata</i>	ER ₅₀ = 20.7		1.031		5.16	yes
<i>Chrysoperla carnea</i>	LR ₅₀ = 106		1.031		5.16	yes

MAF: Multiple application factor; vdf: Vegetation distribution factor; (corr.) PER: (corrected) Predicted environmental rate; CF: Correction factor; HQ: Hazard quotient. Criteria values shown in **bold** breach the relevant trigger.

* If an LR₅₀ or ER₅₀ from a relevant extended laboratory test is available, it should be considered in place of the rate with ≤ 50 % effect.

Table 9.7-18: First- and higher-tier assessment of the off-field risk for non-target arthropods due to the use of ADM.00150.I.2.A in potato (Use no. III)

Intended use		Potato				
Active substance/product		ADM.00150.I.2.A				
Application rate (g a.s./ha)		1 x 36				
MAF		1				
vdf		5				
Test species Tier I	LR₅₀ (lab.) (g a.s./ha)	Drift rate	PER_{off-field} (g a.s./ha)	CF	PER_{off-field, corr.} (g a.s./ha)	HQ_{off-field} criterion: HQ ≤ 2
<i>Typhlodromus pyri</i>	LR ₅₀ = 9.13	2.77	0.19944	10	1.99	0.22
<i>Aphidius rhopalosiphi</i>	LR ₅₀ = 0.0243					82.1
Extended laboratory studies	Rate with ≤ 50 % effect* (g a.s./ha)	Drift rate	PER_{off-field} (g a.s./ha)	CF	PER_{off-field, corr.} (g a.s./ha)	corr. PER_{off-field} below rate with ≤ 50 % effect?
<i>Typhlodromus pyri</i>	ER ₅₀ = > 12.5	2.77	0.199	5	1.00	yes
<i>Aphidius rhopalosiphi</i>	LR ₅₀ = 0.100 LR₅₀ = 0.111		0.199		1.00	no
<i>Aphidius rhopalosiphi</i>	LR ₅₀ = 3.56 ER ₅₀ < 0.64		0.997		4.99	no
<i>Coccinella septempunctata</i>	ER ₅₀ = 20.7		0.199		1.00	yes
<i>Chrysoperla carnea</i>	LR ₅₀ = 106		0.199		1.00	yes

MAF: Multiple application factor; vdf: Vegetation distribution factor; (corr.) PER: (corrected) Predicted environmental rate; CF: Correction factor; HQ: Hazard quotient. Criteria values shown in **bold** breach the relevant trigger.

* If an LR₅₀ or ER₅₀ from a relevant extended laboratory test is available, it should be considered in place of the rate with ≤ 50 % effect.

Table 9.7-19: First- and higher-tier assessment of the off-field risk for non-target arthropods due to the use of ADM.00150.I.2.A in spring/winter cereals (Use no. IVa, IVb, Va)

Intended use		Spring/winter cereals				
Active substance/product		ADM.00150.I.2.A				
Application rate (g a.s./ha)		2 x 36 (covering use no. IVa and IVb of 2 x 35)				
MAF		1.7 (foliar)				
vdf		5				
Test species Tier I	LR₅₀ (lab.) (g a.s./ha)	Drift rate	PER_{off-field} (g a.s./ha)	CF	PER_{off-field, corr.} (g a.s./ha)	HQ_{off-field} criterion: HQ ≤ 2
<i>Typhlodromus pyri</i>	LR ₅₀ = 9.13	2.38	0.291312	10	2.91	0.32
<i>Aphidius rhopalosiphi</i>	LR ₅₀ = 0.0243					120
Extended laboratory studies	Rate with ≤ 50 % effect* (g a.s./ha)	Drift rate	PER_{off-field} (g a.s./ha)	CF	PER_{off-field, corr.} (g a.s./ha)	corr. PER_{off-field} below rate with ≤ 50 % effect?
<i>Typhlodromus pyri</i>	ER ₅₀ = > 12.5	2.38	0.291	5	1.46	yes
<i>Aphidius rhopalosiphi</i>	LR ₅₀ = 0.100 LR₅₀ = 0.111		0.291		1.46	no
<i>Aphidius rhopalosiphi</i>	LR ₅₀ = 3.56 ER ₅₀ < 0.64		1.457		7.28	no
<i>Coccinella septempunctata</i>	ER ₅₀ = 20.7		0.291		1.46	yes
<i>Chrysoperla carnea</i>	LR ₅₀ = 106		0.291		1.46	yes

MAF: Multiple application factor; vdf: Vegetation distribution factor; (corr.) PER: (corrected) Predicted environmental rate; CF: Correction factor; HQ: Hazard quotient. Criteria values shown in **bold** breach the relevant trigger.

* If an LR₅₀ or ER₅₀ from a relevant extended laboratory test is available, it should be considered in place of the rate with ≤ 50 % effect.

Table 9.7-20: First- and higher-tier assessment of the off-field risk for non-target arthropods due to the use of ADM.00150.I.2.A in winter cereals (Use no. Vb)

Intended use		Winter cereals				
Active substance/product		ADM.00150.I.2.A				
Application rate (g a.s./ha)		1 x 30				
MAF		1				
vdf		5				
Test species Tier I	LR₅₀ (lab.) (g a.s./ha)	Drift rate	PER_{off-field} (g a.s./ha)	CF	PER_{off-field, corr.} (g a.s./ha)	HQ_{off-field} criterion: HQ ≤ 2
<i>Typhlodromus pyri</i>	LR ₅₀ = 9.13	2.77	0.166	10	1.66	0.18
<i>Aphidius rhopalosiphi</i>	LR ₅₀ = 0.0243					168
Extended laboratory studies	Rate with ≤ 50 % effect* (g a.s./ha)	Drift rate	PER_{off-field} (g a.s./ha)	CF	PER_{off-field, corr.} (g a.s./ha)	corr. PER_{off-field} below rate with ≤ 50 % effect?
<i>Typhlodromus pyri</i>	ER ₅₀ = > 12.5	2.77	0.166	5	0.83	yes
<i>Aphidius rhopalosiphi</i>	LR ₅₀ = 0.100 LR₅₀ = 0.111		0.166		0.83	no
<i>Aphidius rhopalosiphi</i>	LR ₅₀ = 3.56 ER ₅₀ < 0.64		0.83		4.12	no
<i>Coccinella septempunctata</i>	ER ₅₀ = 20.7		0.166		0.83	yes
<i>Chrysoperla carnea</i>	LR ₅₀ = 106		0.166		0.83	yes

MAF: Multiple application factor; vdf: Vegetation distribution factor; (corr.) PER: (corrected) Predicted environmental rate; CF: Correction factor; HQ: Hazard quotient. Criteria values shown in **bold** breach the relevant trigger.

* If an LR₅₀ or ER₅₀ from a relevant extended laboratory test is available, it should be considered in place of the rate with ≤ 50 % effect.

Table 9.7-21: First- and higher-tier assessment of the off-field risk for non-target arthropods due to the use of ADM.00150.I.2.A in winter/spring OSR (Use no. VIa & VII)

Intended use		Winter/spring OSR				
Active substance/product		ADM.00150.I.2.A				
Application rate (g a.s./ha)		2 x 60				
MAF		1.7				
vdf		5				
Test species Tier I	LR₅₀ (lab.) (g a.s./ha)	Drift rate	PER_{off-field} (g a.s./ha)	CF	PER_{off-field, corr.} (g a.s./ha)	HQ_{off-field} criterion: HQ ≤ 2
<i>Typhlodromus pyri</i>	LR ₅₀ = 9.13	2.38	0.486	10	4.86	0.53
<i>Aphidius rhopalosiphi</i>	LR ₅₀ = 0.0243					200
Extended laboratory studies	Rate with ≤ 50 % effect* (g a.s./ha)	Drift rate	PER_{off-field} (g a.s./ha)	CF	PER_{off-field, corr.} (g a.s./ha)	corr. PER_{off-field} below rate with ≤ 50 % effect?
<i>Typhlodromus pyri</i>	ER ₅₀ = > 12.5	2.38	0.486	5	2.43	yes
<i>Aphidius rhopalosiphi</i>	LR ₅₀ = 0.100 LR₅₀ = 0.111		0.486		2.43	no
<i>Aphidius rhopalosiphi</i>	LR ₅₀ = 3.56 ER ₅₀ < 0.64		2.428		12.14	no
<i>Coccinella septempunctata</i>	ER ₅₀ = 20.7		0.486		2.43	yes
<i>Chrysoperla carnea</i>	LR ₅₀ = 106		0.486		2.43	yes

MAF: Multiple application factor; vdf: Vegetation distribution factor; (corr.) PER: (corrected) Predicted environmental rate; CF: Correction factor; HQ: Hazard quotient. Criteria values shown in **bold** breach the relevant trigger.

* If an LR₅₀ or ER₅₀ from a relevant extended laboratory test is available, it should be considered in place of the rate with ≤ 50 % effect.

Table 9.7-22: First- and higher-tier assessment of the off-field risk for non-target arthropods due to the use of ADM.00150.I.2.A in winter OSR (Use no.Vib), 129, 144, 150)

Intended use		Winter OSR				
Active substance/product		ADM.00150.I.2.A				
Application rate (g a.s./ha)		1 x 60 (covering use no. 129, 144 and 150 of 1 x 40)				
MAF		1				
vdf		5				
Test species Tier I	LR₅₀ (lab.) (g a.s./ha)	Drift rate	PER_{off-field} (g a.s./ha)	CF	PER_{off-field, corr.} (g a.s./ha)	HQ_{off-field} criterion: HQ ≤ 2
<i>Typhlodromus pyri</i>	LR ₅₀ = 9.13	2.77	0.332	10	3.32	0.36
<i>Aphidius rhopalosiphi</i>	LR ₅₀ = 0.0243					137
Extended laboratory studies	Rate with ≤ 50 % effect* (g a.s./ha)	Drift rate	PER_{off-field} (g a.s./ha)	CF	PER_{off-field, corr.} (g a.s./ha)	corr. PER_{off-field} below rate with ≤ 50 % effect?
<i>Typhlodromus pyri</i>	ER ₅₀ = > 12.5	2.77	0.332	5	1.66	yes
<i>Aphidius rhopalosiphi</i>	LR ₅₀ = 0.100 LR₅₀ = 0.111		0.332		1.66	no
<i>Aphidius rhopalosiphi</i>	LR ₅₀ = 3.56 ER ₅₀ < 0.64		1.66		8.31	no
<i>Coccinella septempunctata</i>	ER ₅₀ = 20.7		0.332		1.66	yes
<i>Chrysoperla carnea</i>	LR ₅₀ = 106		0.332		1.66	yes

MAF: Multiple application factor; vdf: Vegetation distribution factor; (corr.) PER: (corrected) Predicted environmental rate; CF: Correction factor; HQ: Hazard quotient. Criteria values shown in **bold** breach the relevant trigger.

* If an LR₅₀ or ER₅₀ from a relevant extended laboratory test is available, it should be considered in place of the rate with ≤ 50 % effect.

Table 9.7-23: First- and higher-tier assessment of the off-field risk for non-target arthropods due to the use of ADM.00150.I.2.A in sugar beet (Use no. VIII)

Intended use		Sugar beet				
Active substance/product		ADM.00150.I.2.A				
Application rate (g a.s./ha)		2 x 50				
MAF		1.7				
vdf		5				
Test species Tier I	LR₅₀ (lab.) (g a.s./ha)	Drift rate	PER_{off-field} (g a.s./ha)	CF	PER_{off-field, corr.} (g a.s./ha)	HQ_{off-field} criterion: HQ ≤ 2
<i>Typhlodromus pyri</i>	LR ₅₀ = 9.13	2.38	0.405	10	4.05	0.32368
<i>Aphidius rhopalosiphi</i>	LR ₅₀ = 0.0243					167
Extended laboratory studies	Rate with ≤ 50 % effect* (g a.s./ha)	Drift rate	PER_{off-field} (g a.s./ha)	CF	PER_{off-field, corr.} (g a.s./ha)	corr. PER_{off-field} below rate with ≤ 50 % effect?
<i>Typhlodromus pyri</i>	ER ₅₀ = > 12.5	2.38	0.405	5	2.02	yes
<i>Aphidius rhopalosiphi</i>	LR ₅₀ = 0.100 LR₅₀ = 0.111		0.405		2.02	no
<i>Aphidius rhopalosiphi</i>	LR ₅₀ = 3.56 ER ₅₀ < 0.64		2.023		10.12	no
<i>Coccinella septempunctata</i>	ER ₅₀ = 20.7		0.405		2.02	yes
<i>Chrysoperla carnea</i>	LR ₅₀ = 106		0.405		2.02	yes

MAF: Multiple application factor; vdf: Vegetation distribution factor; (corr.) PER: (corrected) Predicted environmental rate; CF: Correction factor; HQ: Hazard quotient. Criteria values shown in **bold** breach the relevant trigger.

* If an LR₅₀ or ER₅₀ from a relevant extended laboratory test is available, it should be considered in place of the rate with ≤ 50 % effect.

Table 9.7-24: First- and higher-tier assessment of the off-field risk for non-target arthropods due to the use of ADM.00150.I.2.A in flower bulbs and flower tubers (Use no. IXb)

Intended use		Flower bulbs and flower tubers				
Active substance/product		ADM.00150.I.2.A				
Application rate (g a.s./ha)		2 x 34				
MAF		1.7				
vdf		5				
Test species Tier I	LR₅₀ (lab.) (g a.s./ha)	Drift rate	PER_{off-field} (g a.s./ha)	CF	PER_{off-field, corr.} (g a.s./ha)	HQ_{off-field} criterion: HQ ≤ 2
<i>Typhlodromus pyri</i>	LR ₅₀ = 9.13	2.38	0.275	10	2.75	0.30
<i>Aphidius rhopalosiphi</i>	LR ₅₀ = 0.0243					1113
Extended laboratory studies	Rate with ≤ 50 % effect* (g a.s./ha)	Drift rate	PER_{off-field} (g a.s./ha)	CF	PER_{off-field, corr.} (g a.s./ha)	corr. PER_{off-field} below rate with ≤ 50 % effect?
<i>Typhlodromus pyri</i>	ER ₅₀ = > 12.5	2.38	0.275	5	1.38	yes
<i>Aphidius rhopalosiphi</i>	LR ₅₀ = 0.100 LR₅₀ = 0.111		0.275		1.38	no
<i>Aphidius rhopalosiphi</i>	LR ₅₀ = 3.56 ER ₅₀ < 0.64		1.376		6.88	no
<i>Coccinella septempunctata</i>	ER ₅₀ = 20.7		0.275		1.38	yes
<i>Chrysoperla carnea</i>	LR ₅₀ = 106		0.275		1.38	yes

MAF: Multiple application factor; vdf: Vegetation distribution factor; (corr.) PER: (corrected) Predicted environmental rate; CF: Correction factor; HQ: Hazard quotient. Criteria values shown in **bold** breach the relevant trigger.

* If an LR₅₀ or ER₅₀ from a relevant extended laboratory test is available, it should be considered in place of the rate with ≤ 50 % effect.

Table 9.7-25: First- and higher-tier assessment of the off-field risk for non-target arthropods due to the use of ADM.00150.I.2.A in flower bulbs and flower tubers (Use no. IXa)

Intended use		Flower bulbs and flower tubers				
Active substance/product		ADM.00150.I.2.A				
Application rate (g a.s./ha)		1 x 46				
MAF		1				
vdf		5				
Test species Tier I	LR₅₀ (lab.) (g a.s./ha)	Drift rate	PER_{off-field} (g a.s./ha)	CF	PER_{off-field, corr.} (g a.s./ha)	HQ_{off-field} criterion: HQ ≤ 2
<i>Typhlodromus pyri</i>	LR ₅₀ = 9.13	2.77	0.255	10	2.5484	0.28
<i>Aphidius rhopalosiphi</i>	LR ₅₀ = 0.0243					105
Extended laboratory studies	Rate with ≤ 50 % effect* (g a.s./ha)	Drift rate	PER_{off-field} (g a.s./ha)	CF	PER_{off-field, corr.} (g a.s./ha)	corr. PER_{off-field} below rate with ≤ 50 % effect?
<i>Typhlodromus pyri</i>	ER ₅₀ = > 12.5	2.77	0.255	5	1.27	yes
<i>Aphidius rhopalosiphi</i>	LR ₅₀ = 0.100 LR₅₀ = 0.111		0.255		1.27	no
<i>Aphidius rhopalosiphi</i>	LR ₅₀ = 3.56 ER ₅₀ < 0.64		0.274		6.37	no
<i>Coccinella septempunctata</i>	ER ₅₀ = 20.7		0.255		1.27	yes
<i>Chrysoperla carnea</i>	LR ₅₀ = 106		0.255		1.27	yes

MAF: Multiple application factor; vdf: Vegetation distribution factor; (corr.) PER: (corrected) Predicted environmental rate; CF: Correction factor; HQ: Hazard quotient. Criteria values shown in **bold** breach the relevant trigger.

* If an LR₅₀ or ER₅₀ from a relevant extended laboratory test is available, it should be considered in place of the rate with ≤ 50 % effect.

Table 9.7-26: First- and higher-tier assessment of the off-field risk for non-target arthropods due to the use of ADM.00150.I.2.A in floriculture, tree nursery & perennial nursery crops (Use no. Xb)

Intended use		Floriculture, tree nursery & perennial nursery crops				
Active substance/product		ADM.00150.I.2.A				
Application rate (g a.s./ha)		2 x 34				
MAF		1.7				
vdf		5				
Test species	LR₅₀ (lab.) (g a.s./ha)	Drift rate	PER_{off-field} (g a.s./ha)	CF	PER_{off-field, corr.} (g a.s./ha)	HQ_{off-field} criterion: HQ ≤ 2
Tier I						
<i>Typhlodromus pyri</i>	LR ₅₀ = 9.13	12.13	1.402	10	14.02	1.53
<i>Aphidius rhopalosiphi</i>	LR ₅₀ = 0.0243					577
Extended laboratory studies	Rate with ≤ 50 % effect* (g a.s./ha)	Drift rate	PER_{off-field} (g a.s./ha)	CF	PER_{off-field, corr.} (g a.s./ha)	corr. PER_{off-field} below rate with ≤ 50 % effect?
<i>Typhlodromus pyri</i>	ER ₅₀ = > 12.5	12.13	1.402	5	7.01	yes
<i>Aphidius rhopalosiphi</i>	LR ₅₀ = 0.100 LR₅₀ = 0.111		1.402		7.01	no
<i>Aphidius rhopalosiphi</i>	LR ₅₀ = 3.56 ER ₅₀ < 0.64		7.011		35.06	no
<i>Coccinella septempunctata</i>	ER ₅₀ = 20.7		1.402		7.01	yes
<i>Chrysoperla carnea</i>	LR ₅₀ = 106		1.402		7.01	yes

MAF: Multiple application factor; vdf: Vegetation distribution factor; (corr.) PER: (corrected) Predicted environmental rate; CF: Correction factor; HQ: Hazard quotient. Criteria values shown in **bold** breach the relevant trigger.

* If an LR₅₀ or ER₅₀ from a relevant extended laboratory test is available, it should be considered in place of the rate with ≤ 50 % effect.

Table 9.7-27: First- and higher-tier assessment of the off-field risk for non-target arthropods due to the use of ADM.00150.I.2.A in floriculture, tree nursery & perennial nursery crops (Use no. Xa)

Intended use		Floriculture, tree nursery & perennial nursery crops				
Active substance/product		ADM.00150.I.2.A				
Application rate (g a.s./ha)		1 x 46				
MAF		1				
vdf		5				
Test species	LR₅₀ (lab.) (g a.s./ha)	Drift rate	PER_{off-field} (g a.s./ha)	CF	PER_{off-field, corr.} (g a.s./ha)	HQ_{off-field} criterion: HQ ≤ 2
Tier I						
<i>Typhlodromus pyri</i>	LR ₅₀ = 9.13	15.73	1.447	10	14.4716	1.59
<i>Aphidius rhopalosiphi</i>	LR ₅₀ = 0.0243					596
Extended laboratory studies	Rate with ≤ 50 % effect* (g a.s./ha)	Drift rate	PER_{off-field} (g a.s./ha)	CF	PER_{off-field, corr.} (g a.s./ha)	corr. PER_{off-field} below rate with ≤ 50 % effect?
<i>Typhlodromus pyri</i>	ER ₅₀ = > 12.5	15.73	1.447	5	7.24	yes
<i>Aphidius rhopalosiphi</i>	LR ₅₀ = 0.100 LR₅₀ = 0.111		1.447		7.24	no
<i>Aphidius rhopalosiphi</i>	LR ₅₀ = 3.56 ER ₅₀ < 0.64		7.236		36.18	no
<i>Coccinella septempunctata</i>	ER ₅₀ = 20.7		1.447		7.24	yes
<i>Chrysoperla carnea</i>	LR ₅₀ = 106		1.447		7.24	yes

MAF: Multiple application factor; vdf: Vegetation distribution factor; (corr.) PER: (corrected) Predicted environmental rate; CF: Correction factor; HQ: Hazard quotient. Criteria values shown in **bold** breach the relevant trigger.

* If an LR₅₀ or ER₅₀ from a relevant extended laboratory test is available, it should be considered in place of the rate with ≤ 50 % effect.

Regarding non-target arthropods in in-field habitats, no acceptable risk could be concluded with Tier I toxicity data, while at Tier II acceptable risk for all of uses could be concluded for *Chrysoperla carnea* only. The in-field risk for species of concern was further refined with consideration of results of aged-residues studies. Based on available data from these studies, acceptable risk can be concluded for all non-target arthropods for all of the intended uses.

Regarding non-target arthropods in off-field habitats, the data from the available extended laboratory studies show an acceptable risk to most of the non-target arthropods from all intended uses of ADM.00150.I.2.A with an exception to *Aphidius rhopalosiphi*, where no acceptable risk can be concluded. Here, the risk should be further refined on the basis of a higher tier field study.

zRMS comments:

zRMS agrees with f Tier I and Tier II calculations presented above.

As a worst case the VDF of 5 has been considered, since available investigations indicate that VDF of 10 recommended by ESCORT 2 guidance document is not appropriate and may lead to underestimation of the exposure. It should be, however, noted that according to EFSA Supporting publication 2019:EN-1673, VDF of 5 should be considered as the interim solution that will be reflected in the SANCO/10329/2002 rev 2 final with its implementation considered further. Since use of VDF of 5 was not reflected in the current SANCO terrestrial guidance, its use is not yet mandatory. Nevertheless, the risk assessment performed with VDF of 5 is more protective and is thus agreed by the zRMS.

In evaluation for orchards only the late applications were considered, resulting with lower drift values comparing to “early applications” scenario. In general, the threshold for late and early applications to orchards (and vineyards) is not defined in ESCORT 2, so indications of FOCUS surface water guidance were consulted, as in calculation of surface water exposure separate scenarios early and late are defined for uses in orchards and vineyards. No clear information enabling determination of the BBCH stage from which the late application scenario should be considered is given in the guidance mentioned, however the following is indicated:

As spray drift deposition varies considerably for fruit trees and vines, a distinction has been made between their early and late crop growth stage, representing respectively a growth stage with no or few leaves and a growth stage in which the leaves are well developed [...]

Based on indications of the FOCUS surface water guidance, full canopy development is relevant for BBCH 40 onwards. In orchards is intended to be applied from BBCH 62, so late application scenario is considered to be more relevant for the risk assessment for NTAs as leaves will be fully developed at that time.

Based on presented above calculations, acceptable risk may be concluded for most of the non-target arthropods from all intended uses of ADM.00150.I.2.A .With regard to *Aphidius rhopalosiphi*, no acceptable risk could be concluded based on Tier I toxicity data and the $PER_{\text{off-field}}$ was higher than Tier II LR_{50} . However, no reproduction endpoint could be determined from this Tier II study, as >50% effects were seen at 0.64 g a.s./ha, the lowest rate tested

The risk was further refined on the basis of the field study by Appeltauer (2016). For details, see point 9.7.2.3 below.

9.7.3.3 Additional higher-tier risk assessment

As potential risks to non-target arthropods in off-field habitats cannot be excluded based on extended laboratory studies, a higher tier risk assessment based on data from an off-field field study is presented in the following. The available field study assessed potential effects of acetamiprid, applied at four drift rates (nominally 2 x 7.2 g a.s./ha, 2 x 3.4 g a.s./ha, 2 x 1.4 g a.s./ha and 2 x 0.7 g a.s./ha), on the non-target arthropod fauna in a meadow in Germany. Four different sampling methods were used. Detailed information are provided in A 2.3.2.4.

In the present study, no effects on the community level have been observed at any of the tested rates, hence, the community NOER is given as 7.2 g a.s./ha in the study report.

Taking single taxa into account, one taxon (juvenile specimens of the order Thysanoptera) did not recover within the assessed sampling period of 27 days after the 2nd application at the highest test rate. Therefore, the rate 7.2 g a.s./ha is given as the population LOEAER (Lowest Observed Ecologically Adverse Effect Rate) in the original report.

For all other taxa recorded in the study, effect class 3b (pronounced short term effects; recovery within 2 months after first application) was the largest effect observed at any of the tested rates.

Such class 3b effects were recorded for Polyphaga at the highest test rate, while only a slight and transient effect (class 2) was observed at 2 x 3.4 g a.s./ha. At the lower test rates no effects were observed on this taxon.

Furthermore, class 3b effects were also recorded for Aphidoidea at the highest test rate. Aphidoidea are a target taxon of acetamiprid and consequently can be considered as one of the most sensitive taxa with regard to potential effects of acetamiprid. Nevertheless, no effect was observed at the lowest rate (2 x 0.7 g a.s./ha) during the entire course of the study for any kind of sampling type and a slight and transient effect (effect class 2; according to de Jong et al. (2010)) was observed at 2 x 1.4 g a.s./ha, with no further effects on any later sampling date (in vortis suction samples only) and hence recovery within few days. At the next higher rate of 2 x 3.4 g a.s./ha a pronounced effect on this taxon has been observed (effect class 3a in vortis suction samples only; 3rd and 4th sampling), however recovery was achieved within one month (20 DAA2; 6 days after the 4th sampling) after application.

A further taxon that can be considered as very sensitive, are braconid wasps, as a member of this family, *Aphidius rhopalosiphii* (Braconidae) was by far the most sensitive species tested in laboratory studies. In extended laboratory studies with a 2D exposure system the LR₅₀ and ER₅₀ values for *A. rhopalosiphii* are 287 and 125 times lower than for *Typhlodromus pyri*. Consequently, special attention needs to be paid on potential effects on braconid wasps in the field study.

Braconid wasps (parasitic wasps) were recorded in the field study and no adverse effects on this family of hymenopterans occurred at any of the tested rates. In the toxic reference, a pronounced effect was recorded, demonstrating that the test system is suitable to detect potential effects on this taxon.

Regarding Alticinae, a slight and transient effect has been observed at test rates of 0.7, 1.4 and 3.4 g a.s./ha at the second sampling event. After that the population recovered and no effects have been observed at any further sampling event. The 3rd sampling event was performed 10 DAA2 and thus recovery was observed within few days after the 2nd application.

All other evaluated taxa only showed slight transient effects (effect class 2) at 2 x 3.4 g a.s./ha.

According to de Jong et al. (2010), the highest rate causing effects with subsequent recovery can be considered the NOEAER of the study. In the study report the NOEAER was set to 3.4 g a.s./ha based on class 3a effects on Aphidoidea only. Recovery for this taxon was achieved within 1 month after application.

In a conservative approach, the endpoint used for the higher tier off-field risk assessment is based on class 2 effects and thus on slight transient effects where recovery was observed in very short time. As laid down above, the highest tested rate at which no effects or only class 2 effects occurred was 2 x 1.4 g a.s./ha. In the original study report the endpoints are based on single application rates, while the product was actually applied twice at an interval of 6 days. In order to account for the two-fold application in the field study, the endpoint is multiplied by the foliar multiple application factor (foliar MAF) of 1.7. Consequently, the NOER of 2.38 g a.s./ha (1.7 x 1.4 g a.s./ha) is considered to be the relevant endpoint to assess potential risks to non-target arthropods in off-field habitats.

Table 9.7-28: Assessment of the off-field risk for non-target arthropods for all intended uses of ADM.00150.I.2.A considering higher tier NOER

Use No. of the umbrella GAP	Crop	Application rate [g a.s./ha]	MAF	Drift rate	PER _{off-field} [g a.s./ha]	NOER* [g a.s./ha]	PER _{off-field} < NOER?
I	Corn	1 x 60	1	2.77%	1.66	2.38	yes
IIa	Apple	1 x 80	1	15.73%	12.58		no
IIb	Apple	2 x 25	1.7	12.13%	5.16		no
III	Potato	1 x 36	1	2.77%	1.00		yes
IVa, IVb	Spring cereals	2 x 35	1.7	2.38%	1.42		yes
Va	Winter cereals	2 x 36	1.7	2.38%	1.46		yes
Vb	Winter cereals	1 x 30	1	2.77%	0.83		yes
VIa, VII	Winter/spring OSR	2 x 60	1.7	2.38%	2.43		no
VIIb	Winter OSR	1 x 60	1	2.77%	1.66		yes
129, 144, 150	Winter OSR	1 x 40	1	2.77%	1.11		yes
VIII	Sugar beet	2 x 50	1.7	2.77%	2.35		yes
IXb, IXc	Flower bulbs and flower tubers	2 x 34	1.7	2.38%	1.38		yes
IXa	Flower bulbs and flower tubers	1 x 46	1	2.38%	1.09		yes
Xb, Xc	Floriculture, tree nursery & perennial nursery crops	2 x 34	1.7	12.13%	7.01		no
Xa	Floriculture, tree nursery & perennial nursery crops	1 x 46	1	15.73%	7.24		no

MAF: Multiple application factor; PER: Predicted environmental rate; PER values shown in **bold** exceed NOER of 2.38 g a.s./ha.
* NOER of 2.38 g a.s./ha (single application rate of 1.4 g a.s./ha multiplied by the foliar multiple application factor 1.7)

The intended application to apples (1 x 80 g a.s./ha and 2 x 25 g a.s./ha), spring and winter OSR (2 x 60 g a.s./ha) and floriculture, tree nursery & perennial nursery crops (2 x 34 g a.s./ha and 1 x 46 g a.s./ha) pose a potential risk to off-field non-target arthropods, which needs further refinement via risk mitigation measures.

zRMS comments:

The field study by Appeltauer (2016) on effects of on off-field population of non-target arthropods has been evaluated and agreed by the zRMS-PL previously for the product (for details, please, refer to Appendix 2).

The study was performed on a meadow in Germany and covered two applications formerly MCW-2222 with 6 days interval, The application rates were based on drift rates of the product after application to most of crops indicated in the GAP, with exception of application to apples at 60 g a.s./ha.

Although effects on all caught species were evaluated by the zRMS-PL, special attention was paid to the *Braconidae* family (parasitic wasps), as in the laboratory studies *Aphidius rhopalosphi* turned out to be particularly sensitive to acetamiprid in. During the field study the *Braconidae* family was present on the study plots but no effects of the treatment with MCW-2222 were observed. The only statistically significant and treatment-related effects were seen in the toxic standard group, confirming that the design of the study was sufficient to detect effects on these insects.

Overall, application of MCW-2222 on non-target arthropod populations up to and including application rate 1.4 g a.s./ha resulted with no or only minor effects class 1 and 2 over the whole study period, so this rate was determined

to be the NOER. Clear treatment related effects followed by recovery were seen at rate of 3.4 g a.s./ha, while treatment related effects class 8 were observed at application rate 7.2 g a.s./ha.

Taking this into account, the NOEAER from the study was set to 3.4 g a.s./ha by zRMS-PL previously and in line with indications of ESCORT 2, this endpoint is relevant for purposes of refinement of the risk.

In order to address the risk to off-field population of NTAs, both values NOER and NOEAER from the study was compared directly with the drift rates expected after application of MCW-2222 to particular crops. As evaluation is based on results of the field study which covered enormous number of species/families and subfamilies of non-target arthropods, the drift rates do not need to be corrected by a factor of 5, relevant in situation when toxicity data for limited number of species is available.

In using the data from the field study, it has been taken into account that 2 applications of the off-field treatments were applied. Therefore, it is not necessary to apply a MAF in the PER calculation, as this has been accounted for in the study design. In addition, as a single application is now considered for the PER, the drift values for a single application have been used (i.e. 90th percentile drift values). Therefore, the Raw PER values in the table below differ somewhat from those in the table provided by the Applicant above.

Use No. of the umbrella GAP	Crop	Application rate [g a.s./ha]	MAF	Drift rate	PER _{off-field} [g a.s./ha]	NOER* [g a.s./ha]	PER _{off-field} < NOER?	NOEAR	PER _{off-field} < NOEAR?
I	Corn	1 x 60	1	2.77%	1.66	1.4	no	3.4	yes
IIa	Apple	1 x 60	1	15.73%	9.43		no		no
IIb	Apple	2 x 25	1	15.73%	3.93		no		no
III	Potato	1 x 36	1	2.77%	1.00		yes		yes
IVa, IVb	Spring cereals	2 x 35	1	2.77%	1.00		yes		yes
Va	Winter cereals	2 x 36	1	2.77%	1.00		yes		yes
Vb	Winter cereals	1 x 30	1	2.77%	0.83		yes		yes
VIa, VII	Winter/spring OSR	2 x 60	1	2.77%	1.66		no		yes
VIb	Winter OSR	1 x 48	1	2.77%	1.32		yes		yes
129, 144, 150	Winter OSR	1 x 40	1	2.77%	1.11		yes		yes
VIII	Sugar beet	2 x 50	1	2.77%	1.38		yes		yes
IXb, IXc	Flower bulbs and flower tubers	2 x 34	1	2.77%	1.38		yes		yes
IXa	Flower bulbs and flower tubers	1 x 46	1	2.77%	1.09		yes		yes
Xb, Xc	Floriculture, tree nursery & perennial nursery crops	2 x 34	1	15.73%	5.34		no		no
Xa	Floriculture, tree nursery & perennial nursery crops	1 x 46	1	15.73%	7.23		no		no

MAF: Multiple application factor; PER: Predicted environmental rate; PER values shown in **bold** exceed NOER or NOEAER values.

Taking this into account the risk mitigation measures must be identified in order to reduce the exposure to acceptable level. Respective calculations are presented in point 9.7.3.4 below.

In a conservative approach, the endpoint used for the higher tier off-field risk assessment is based NOER from field study.

Taking this into account, the NOEAER from the study was set to 3.4 g a.s./ha by zRMS-PL previously and in line with indications of ESCORT 2, this endpoint is considered relevant for purposes of refinement of the risk.

9.7.3.4 Risk mitigation measures

In order to reduce the off-field exposure, risk mitigation measures can be implemented. These correspond to unsprayed in-field buffer strips of a given width and/or the usage of drift reducing nozzles. The results of the risk assessment using typical mitigation measures (no-spray buffer zones of 5 or 10 m; drift-reducing nozzles with reduction by 50 %, 75 %, or 90 %) are summarised in the following table.

Table 9.7-29: Assessment of the off-field risk for non-target arthropods due to the use of ADM.00150.I.2.A in apple (Use No. IIa) considering risk mitigation (in-field no-spray buffer zones, and drift-reducing nozzles)

Intended use		Apple			
Active substance/product		ADM.00150.I.2.A			
Application rate (g a.s./ha)		1 x 80			
MAF		1			
vdf		Not relevant for higher tier assessment based on field data			
Buffer strip (m)	Drift rate (%)	corr. PER _{off-field} (g a.s./ha)	corr. PER _{off-field} 50 % drift red. (g a.s./ha)	corr. PER _{off-field} 75 % drift red. (g a.s./ha)	corr. PER _{off-field} 90 % drift red. (g a.s./ha)
-	15.73	12.58	6.29	3.15	1.26
5	8.41	6.73	3.36	1.68	-
10	3.60	2.88	1.44	-	-
15	1.81	1.45	-	-	-

MAF: Multiple application factor; PER: Predicted environmental rates; PER values shown in **bold** exceed NOER of 2.38 g a.s./ha.

Table 9.7-30: Assessment of the off-field risk for non-target arthropods due to the use of ADM.00150.I.2.A in apple (Use No. IIb) considering risk mitigation (in-field no-spray buffer zones, and drift-reducing nozzles)

Intended use		Apple			
Active substance/product		ADM.00150.I.2.A			
Application rate (g a.s./ha)		2 x 25			
MAF		1.7			
vdf		Not relevant for higher tier assessment based on field data			
Buffer strip (m)	Drift rate (%)	corr. PER _{off-field} (g a.s./ha)	corr. PER _{off-field} 50 % drift red. (g a.s./ha)	corr. PER _{off-field} 75 % drift red. (g a.s./ha)	corr. PER _{off-field} 90 % drift red. (g a.s./ha)
-	12.13	5.16	2.58	1.29	-
5	6.81	2.89	1.45	-	-
10	3.11	1.32	-	-	-

MAF: Multiple application factor; PER: Predicted environmental rates; PER values shown in **bold** exceed NOER of 2.38 g a.s./ha.

Table 9.7-31: Assessment of the off-field risk for non-target arthropods due to the use of ADM.00150.I.2.A in apple (Use No. VIa, VII) considering risk mitigation (in-field no-spray buffer zones, and drift-reducing nozzles)

Intended use		Winter/Spring OSR			
Active substance/product		ADM.00150.I.2.A			
Application rate (g a.s./ha)		2 x 60			
MAF		1.7			
vdf		Not relevant for higher tier assessment based on field data			

Buffer-strip (m)	Drift rate (%)	corr. PER _{off-field} (g a.s./ha)	corr. PER _{off-field} 50 %-drift red. (g a.s./ha)	corr. PER _{off-field} 75 %-drift red. (g a.s./ha)	corr. PER _{off-field} 90 %-drift red. (g a.s./ha)
–	2.38	2.42	1.21	–	–
5	0.47	0.48	–	–	–
10	0.24	–	–	–	–

MAF: Multiple application factor; PER: Predicted environmental rates; PER values shown in **bold** exceed NOER of 2.38 g a.s./ha.

Table 9.7-32: Assessment of the off-field risk for non-target arthropods due to the use of ADM.00150.I.2.A in floriculture, tree nursery & perennial nursery crops (Use No. Xa) considering risk mitigation (in-field no-spray buffer zones, and drift-reducing nozzles)

Intended use		Floriculture, tree nursery & perennial nursery crops			
Active substance/product		ADM.00150.I.2.A			
Application rate (g a.s./ha)		1 x 46			
MAF		1.7			
vdf		Not relevant for higher tier assessment based on field data			
Buffer-strip (m)	Drift rate (%)	corr. PER _{off-field} (g a.s./ha)	corr. PER _{off-field} 50 %-drift red. (g a.s./ha)	corr. PER _{off-field} 75 %-drift red. (g a.s./ha)	corr. PER _{off-field} 90 %-drift red. (g a.s./ha)
–	15.73	7.24	3.62	1.81	–
5	8.41	3.87	1.93	–	–
10	3.60	1.66	–	–	–

MAF: Multiple application factor; PER: Predicted environmental rates; Criteria values shown in **bold** breach the relevant trigger. PER values shown in **bold** exceed NOER of 2.38 g a.s./ha.

Table 9.7-33: Assessment of the off-field risk for non-target arthropods due to the use of ADM.00150.I.2.A in floriculture, tree nursery & perennial nursery crops (Use No. Xb, Xc) considering risk mitigation (in-field no-spray buffer zones, and drift-reducing nozzles)

Intended use		Floriculture, tree nursery & perennial nursery crops			
Active substance/product		ADM.00150.I.2.A			
Application rate (g/ha)		2 x 34			
MAF		1.7			
vdf		Not relevant for higher tier assessment based on field data			
Buffer-strip (m)	Drift rate (%)	corr. PER _{off-field} (g a.s./ha)	corr. PER _{off-field} 50 %-drift red. (g a.s./ha)	corr. PER _{off-field} 75 %-drift red. (g a.s./ha)	corr. PER _{off-field} 90 %-drift red. (g a.s./ha)
–	12.13	7.01	3.51	1.75	–
5	6.81	3.94	1.97	–	–
10	3.11	1.80	–	–	–

MAF: Multiple application factor; PER: Predicted environmental rates; PER values shown in **bold** exceed NOER of 2.38 g a.s./ha.

zRMS comments:

The risk assessment performed with consideration of risk mitigation measures presented by the Applicant above has been based on not agreed endpoint by zRMS. The study was previously evaluated and the NOER of 1.4 g a.s./ha and NOEAER of 3.4 g a.s./ha values were agreed.

Taking this into account, table above has been struck through and respective calculations were performed by the zRMS in tables below.

Off-field exposure greater than has been highlighted in bold indicating unacceptable risk.

Assessment of the off-field risk for non-target arthropods due to the use of ADM.00150.I.2.A in corn (Use No. I) considering risk mitigation (in-field no-spray buffer zones, and drift-reducing nozzles)

Intended use		Corn			
Active substance/product		ADM.00150.I.2.A			
Application rate (g a.s./ha)		1 x 60			
MAF		1			
vdf		n.a			
Buffer strip (m)	Drift rate (%)	PER_{off-field} (g a.s./ha)	PER_{off-field} 50 % drift red. (g a.s./ha)	PER_{off-field} 75 % drift red. (g a.s./ha)	PER_{off-field} 90 % drift red. (g a.s./ha)
-	2.77	1.66	0.83	0.42	0.17
5	0.057	0.34	0.17	0.09	0.03
10	0.029	0.17	0.09	0.04	0.02
15	0.02	0.12	0.06	0.03	0.01

MAF: Multiple application factor; PER: Predicted environmental rates; PER values shown in **bold** exceed NOER of 1.4 g a.s./ha.

Assessment of the off-field risk for non-target arthropods due to the use of ADM.00150.I.2.A in apple (Use No. IIa) considering risk mitigation (in-field no-spray buffer zones, and drift-reducing nozzles)

Intended use		Apple			
Active substance/product		ADM.00150.I.2.A			
Application rate (g a.s./ha)		1 x 60			
MAF		1			
vdf		n.a			
Buffer strip (m)	Drift rate (%)	PER_{off-field} (g a.s./ha)	PER_{off-field} 50 % drift red. (g a.s./ha)	PER_{off-field} 75 % drift red. (g a.s./ha)	PER_{off-field} 90 % drift red. (g a.s./ha)
-	15.73	9.44	4.72	2.36	0.94
5	8.41	5.05	2.52	1.26	0.50
10	3.60	2.16	1.08	0.54	0.22
15	1.81	1.09	0.54	0.27	0.11

MAF: Multiple application factor; PER: Predicted environmental rates; PER values shown in **bold** exceed NOER of 1.4 g a.s./ha.

Assessment of the off-field risk for non-target arthropods due to the use of ADM.00150.I.2.A in apple (Use No. IIb) considering risk mitigation (in-field no-spray buffer zones, and drift-reducing nozzles)

Intended use		Apple			
Active substance/product		ADM.00150.I.2.A			
Application rate (g a.s./ha)		2 x 25			
MAF		1			

vdf		n.a			
Buffer strip (m)	Drift rate (%)	PER _{off-field} (g a.s./ha)	PER _{off-field} 50 % drift red. (g a.s./ha)	PER _{off-field} 75 % drift red. (g a.s./ha)	PER _{off-field} 90 % drift red. (g a.s./ha)
	15.73	3.93	1.97	0.98	0.39
5	8.41	2.10	1.05	0.53	0.21
10	3.60	0.90	0.45	0.23	0.09

MAF: Multiple application factor; PER: Predicted environmental rates; PER values shown in **bold** exceed NOER of 1.4 g a.s./ha.

Assessment of the off-field risk for non-target arthropods due to the use of ADM.00150.I.2.A in Winter/spring OSR (Use No. VIa, VII) considering risk mitigation (in-field no-spray buffer zones, and drift-reducing nozzles)

Intended use		Winter/Spring OSR			
Active substance/product		ADM.00150.I.2.A			
Application rate (g a.s./ha)		2 x 60			
MAF		1			
vdf		n.a			
Buffer strip (m)	Drift rate (%)	PER _{off-field} (g a.s./ha)	PER _{off-field} 50 % drift red. (g a.s./ha)	PER _{off-field} 75 % drift red. (g a.s./ha)	PER _{off-field} 90 % drift red. (g a.s./ha)
-	2.77	1.66	0.83	0.42	0.17
5	0.057	0.34	0.17	0.09	0.03
10	0.029	0.17	0.09	0.04	0.02
15	0.02	0.12	0.06	0.03	0.01

MAF: Multiple application factor; PER: Predicted environmental rates; PER values shown in **bold** exceed NOER of 1.4 g a.s./ha.

Assessment of the off-field risk for non-target arthropods due to the use of ADM.00150.I.2.A in floriculture, tree nursery & perennial nursery crops (Use No. Xa) considering risk mitigation (in-field no-spray buffer zones, and drift-reducing nozzles)

Intended use		Floriculture, tree nursery & perennial nursery crops			
Active substance/product		ADM.00150.I.2.A			
Application rate (g a.s./ha)		1 x 46			
MAF		1			
vdf		n.a			
Buffer strip (m)	Drift rate (%)	PER _{off-field} (g a.s./ha)	PER _{off-field} 50 % drift red. (g a.s./ha)	PER _{off-field} 75 % drift red. (g a.s./ha)	PER _{off-field} 90 % drift red. (g a.s./ha)
-	15.73	7.24	3.62	1.81	0.72
5	8.41	3.87	1.93	0.97	0.39
10	3.60	1.66	0.83	0.41	0.17
15	1.80	0.83	0.42	0.21	0.08

MAF: Multiple application factor; PER: Predicted environmental rates; Criteria values shown in bold breach the relevant trigger.
PER values shown in **bold** exceed NOER of 1.4 g a.s./ha.

Assessment of the off-field risk for non-target arthropods due to the use of ADM.00150.I.2.A in floriculture, tree nursery & perennial nursery crops (Use No. Xb, Xc) considering risk mitigation (in-field no-spray buffer zones, and drift-reducing nozzles)

Intended use		Floriculture, tree nursery & perennial nursery crops			
Active substance/product		ADM.00150.I.2.A			
Application rate (g/ha)		2 x 34			
MAF		1			
vdf		n.a			
Buffer strip (m)	Drift rate (%)	corr. PER_{off-field} (g a.s./ha)	corr. PER_{off-field} 50 % drift red. (g a.s./ha)	corr. PER_{off-field} 75 % drift red. (g a.s./ha)	corr. PER_{off-field} 90 % drift red. (g a.s./ha)
-	15.73	5.35	2.67	1.34	0.53
5	8.41	2.86	1.43	0.71	0.29
10	3.60	1.22	0.61	0.31	0.12

MAF: Multiple application factor; PER: Predicted environmental rates; PER values shown in **bold** exceed NOER of 1.4 g a.s./ha.

The risk to off-field non-target arthropods is acceptable following use of ADM.00150.I.2.A in pome fruit (1 x 60 g a.s./ha), provided when the following risk mitigation measures are applied:

- 15 m or
- 10 m + 50% DRN or
- 5 m + 75% DRN or
- 90% DRN

The risk to off-field non-target arthropods is acceptable following use of ADM.00150.I.2.A in pome fruit (2 x 25 g a.s./ha), provided the following risk mitigation measures are applied:

- 10 m buffer or
- 5 m + 50% DRN or
- 75% DRN or

The risk to off-field non-target arthropods is acceptable following use of ADM.00150.I.2.A in floriculture, tree nursery & perennial nursery crops (1 x 46 g a.s./ha), provided the following risk mitigation measures are applied:

- 15 m or
- 10 m + 50% DRN or
- 5 m + 75% DRN or
- 90% DRN

The risk to off-field non-target arthropods is acceptable following use of ADM.00150.I.2.A in floriculture, tree nursery & perennial nursery crops (2 x 34 g a.s./ha), provided the following risk mitigation measures are applied

- 10 m or
- 5 m + 50% DRN or
- 90% DRN

The risk to off-field non-target arthropods is acceptable following use of ADM.00150.I.2.A in corn (1 x 50, use I) and OSR winter (uses: VIa, VII) provided the following risk mitigation measures are applied

- 5 m + or
- 50% DRN

The risk mitigation measures based on the NOEAER of 3.4 g a.s./ha value has been used in the refined risk assessment and it presented below:

Assessment of the off-field risk for non-target arthropods due to the use of ADM.00150.I.2.A in apple (Use No. IIa) considering risk mitigation (in-field no-spray buffer zones, and drift-reducing nozzles)

Intended use		Apple			
Active substance/product		ADM.00150.I.2.A			
Application rate (g a.s./ha)		1 x 60			
MAF		1			
vdf		n.a			
Buffer strip (m)	Drift rate (%)	PER_{off-field} (g a.s./ha)	PER_{off-field} 50 % drift red. (g a.s./ha)	PER_{off-field} 75 % drift red. (g a.s./ha)	PER_{off-field} 90 % drift red. (g a.s./ha)
-	15.73	9.44	4.72	2.36	0.94
5	8.41	5.05	2.52	1.26	0.50
10	3.60	2.16	1.08	0.54	0.22
15	1.81	1.09	0.54	0.27	0.11

MAF: Multiple application factor; PER: Predicted environmental rates; PER values shown in **bold** exceed NOEAER of 3.4 g a.s./ha.

Assessment of the off-field risk for non-target arthropods due to the use of ADM.00150.I.2.A in apple (Use No. IIb) considering risk mitigation (in-field no-spray buffer zones, and drift-reducing nozzles)

Intended use		Apple			
Active substance/product		ADM.00150.I.2.A			
Application rate (g a.s./ha)		2 x 25			
MAF		1			
vdf		n.a			
Buffer strip (m)	Drift rate (%)	PER_{off-field} (g a.s./ha)	PER_{off-field} 50 % drift red. (g a.s./ha)	PER_{off-field} 75 % drift red. (g a.s./ha)	PER_{off-field} 90 % drift red. (g a.s./ha)
	15.73	3.93	1.97	0.98	0.39
5	8.41	2.10	1.05	0.53	0.21
10	3.60	0.90	0.45	0.23	0.09

MAF: Multiple application factor; PER: Predicted environmental rates; PER values shown in **bold** exceed NOEAER of 3.4 g a.s./ha.

Assessment of the off-field risk for non-target arthropods due to the use of ADM.00150.I.2.A in floriculture, tree nursery & perennial nursery crops (Use No. Xa) considering risk mitigation (in-field no-spray buffer zones, and drift-reducing nozzles)

Intended use		Floriculture, tree nursery & perennial nursery crops			
Active substance/product		ADM.00150.I.2.A			
Application rate (g a.s./ha)		1 x 46			
MAF		1			
vdf		n.a			
Buffer strip (m)	Drift rate (%)	PER_{off-field} (g a.s./ha)	PER_{off-field} 50 % drift red. (g a.s./ha)	PER_{off-field} 75 % drift red. (g a.s./ha)	PER_{off-field} 90 % drift red. (g a.s./ha)
-	15.73	7.24	3.62	1.81	0.72

5	8.41	3.87	1.93	0.97	0.39
10	3.60	1.66	0.83	0.41	0.17
15	1.80	0.83	0.42	0.21	0.08

MAF: Multiple application factor; PER: Predicted environmental rates; Criteria values shown in bold breach the relevant trigger.

PER values shown in **bold** exceed NOEAER of 3.4 g a.s./ha.

Assessment of the off-field risk for non-target arthropods due to the use of ADM.00150.I.2.A in floriculture, tree nursery & perennial nursery crops (Use No. Xb, Xc) considering risk mitigation (in-field no-spray buffer zones, and drift-reducing nozzles)

Intended use		Floriculture, tree nursery & perennial nursery crops			
Active substance/product		ADM.00150.I.2.A			
Application rate (g/ha)		2 x 34			
MAF		1			
vdf		n.a			
Buffer strip (m)	Drift rate (%)	corr. PER_{off-field} (g a.s./ha)	corr. PER_{off-field} 50 % drift red. (g a.s./ha)	corr. PER_{off-field} 75 % drift red. (g a.s./ha)	corr. PER_{off-field} 90 % drift red. (g a.s./ha)
-	15.73	5.35	2.67	1.34	0.53
5	8.41	2.86	1.43	0.71	0.29
10	3.60	1.22	0.61	0.31	0.12

MAF: Multiple application factor; PER: Predicted environmental rates; PER values shown in **bold** exceed NOEAER of 3.4 g a.s./ha.

The risk to off-field non-target arthropods based on NOEAER of 3.4 g a.s./ha is acceptable following use of ADM.00150.I.2.A in pome fruit (1 x 60 g a.s./ha), provided when the following risk mitigation measures are applied:

- 10 m or
- 5 m+ 50% DRN or
- 75% DRN

The risk to off-field non-target arthropods based on NOEAER of 3.4 g a.s./ha is acceptable following use of ADM.00150.I.2.A in pome fruit (2 x 25 g a.s./ha), provided the following risk mitigation measures are applied:

- 5 m buffer or
- 50% DRN

The risk to off-field non-target arthropods based on NOEAER of 3.4 g a.s./ha is acceptable following use of ADM.00150.I.2.A in floriculture, tree nursery & perennial nursery crops (1 x 46 g a.s./ha), provided the following risk mitigation measures are applied:

- 10 m or
- 5 m + 50% DRN or
- 75% DRN

The risk to off-field non-target arthropods based on NOEAER of 3.4 g a.s./ha is acceptable following use of ADM.00150.I.2.A in floriculture, tree nursery & perennial nursery crops (2 x 34 g a.s./ha), provided the following risk mitigation measures are applied

- 5 m or
- 50% DRN

9.7.4 Overall conclusions

Regarding non-target arthropods in in-field habitats, the available data from aged residue studies clearly demonstrate that recovery within an ecologically relevant timeframe can be expected, especially as the available field study demonstrates that recolonization from the off-field is not impaired.

Regarding non-target arthropods in off-field habitats, the data from the available field study show that no unacceptable risks are to be expected when ADM.00150.I.2.A is applied according to good agricultural practice, except for the intended application in pomefruit (1 x 60 g a.s./ha and 2 x 25 g a.s./ha) and floriculture, tree nursery & perennial nursery crops (2 x 34 g a.s./ha and 1 x 46 g a.s./ha), corn and winter/spring OSR (Use No. VIa, VII), based NOER of 1.4 g a.s./ha from field study. When NOEAER of 3.4 g a.s./ha value from the field study were used an unacceptable risk is indicated only for pome fruit (1 x 60 g a.s./ha and 2 x 25 g a.s./ha) and floriculture, tree nursery & perennial nursery crops (2 x 34 g a.s./ha and 1 x 46 g a.s./ha). Therefore, the risk mitigation measures are applied to confirmed safe use of the product to NTA.

zRMS comments:

The risk to off-field non-target arthropods based on NOEAER of 3.4 g a.s./ha is acceptable following use of ADM.00150.I.2.A in pome fruit (1 x 60 g a.s./ha), provided when the following risk mitigation measures are applied:

- 10 m or
- 5 m+ 50% DRN or
- 75% DRN

The risk to off-field non-target arthropods based on NOEAER of 3.4 g a.s./ha is acceptable following use of ADM.00150.I.2.A in pome fruit (2 x 25 g a.s./ha), provided the following risk mitigation measures are applied:

- 5 m buffer or
- 50% DRN

The risk to off-field non-target arthropods based on NOEAER of 3.4 g a.s./ha is acceptable following use of ADM.00150.I.2.A in floriculture, tree nursery & perennial nursery crops (1 x 46 g a.s./ha), provided the following risk mitigation measures are applied:

- 10 m or
- 5 m + 50% DRN or
- 75% DRN

The risk to off-field non-target arthropods based on NOEAER of 3.4 g a.s./ha is acceptable following use of ADM.00150.I.2.A in floriculture, tree nursery & perennial nursery crops (2 x 34 g a.s./ha), provided the following risk mitigation measures are applied

- 5 m or
- 50% DRN

The final risk mitigation are left at MSs level.

Commenting period process:

During commenting period process some of MSs did not accept the study results as a refinement option to off-field risk assessment. Due that the study was previously evaluated by zRMS in 2018 for MCW-222 (equivalent to Leaxo 200 SL) and after again in 2021/22 according to art. 43 for the other product Kestrel 200 SL (by Nufarm) and no comments were received on this area, the zRMS is still in the opinion that the study is useful for the risk assessment but decision of use of this study for the current evaluation of Leaxo 200 SL is left at MSs level.

The risk to off field non target arthropods is acceptable following use of ADM.00150.I.2.A in pomefruit (1 x 80 g a.s./ha), provided when the following risk mitigation measures are applied:

- 90% drift reduction ~~or~~
- 5 m buffer and 75% drift reduction ~~or~~
- 10 m buffer and 50% drift reduction ~~or~~
- 15 m buffer

The risk to off field non target arthropods is acceptable following use of ADM.00150.I.2.A in pomefruit (2 x 25 g a.s./ha), provided the following risk mitigation measures are applied:

- 5 m buffer and 50% drift reduction ~~or~~
- 10 m buffer

The risk to off field non target arthropods is acceptable following use of ADM.00150.I.2.A in winter/spring OSR (2 x 60 g a.s./ha), provided the following risk mitigation measures are applied:

- 50% drift reduction ~~or~~
- 5 m buffer

The risk to off field non target arthropods is acceptable following use of ADM.00150.I.2.A in floriculture, tree nursery & perennial nursery crops (1 x 46 g a.s./ha and 2 x 34 g a.s./ha), provided the following risk mitigation measures are applied:

- 75% drift reduction ~~or~~
- 5 m buffer and 50% drift reduction
- 10 m buffer

In conclusion, no unacceptable risks for non target arthropods are expected when ADM.00150.I.2.A is applied according to good agricultural practice and considering risk mitigation measures as specified above for the use in pomefruit (1 x 80 g a.s./ha and 2 x 25 g a.s./ha), spring and winter OSR (2 x 60 g a.s./ha) and in floriculture, tree nursery & perennial nursery crops (2 x 34 g a.s./ha and 1 x 46 g a.s./ha).

9.8 Effects on non-target soil meso- and macrofauna (KCP 10.4)

9.8.1 Toxicity data

Studies on the toxicity to earthworms and other non-target soil organisms (meso- and macrofauna) have been carried out with acetamiprid and its relevant metabolites. Full details of these studies are provided in the respective EU DAR and related documents as well as in Appendix 2 of this document (new studies).

Effects on earthworms and other non-target soil organisms (meso- and macrofauna) of ADM.00150.I.2.A were not evaluated as part of the EU assessment of acetamiprid. Data submitted with this application are listed in Appendix 1 and summarised in Appendix 2.

Table 9.8-1: Endpoints and effect values relevant for the risk assessment for earthworms and other non-target soil organisms (meso- and macrofauna)

Species	Substance	Exposure System	Results	Reference
<i>Eisenia fetida</i>	IM-1-5	Homogenous mixing, chronic	Growth, reproduction, behaviour NOEC = 62.5 mg/kg d.w. soil	EFSA, 2016
<i>Folsomia candida</i>	IM-1-5	Homogenous mixing, chronic	NOEC _{mortality} = 62.7 mg/kg soil d.w. No EC values could be calculated as there were no effects below the highest tested value. NOEC _{reproduction} = 12.5 mg/kg soil d.w.	EFSA, 2016

Species	Substance	Exposure System	Results	Reference
			NOEC values were calculated as the data were not appropriate for modelling.	
<i>Eisenia fetida</i>	ADM.00150.I.2.A	Mixed into substrate, 56 d, chronic, 10 % peat content	NOEC = 0.85 mg a.s./kg dw EC ₁₀ = 0.90 mg a.s./kg dw	Friedrich, S. 2014a, R-33840 KCP 10.4.1.1/01
<i>Folsomia candida</i>	ADM.00150.I.2.A	Mixed into substrate, 28 d, chronic, 5 % peat content	NOEC = 0.18 mg a.s./kg soil dry weight EC ₁₀ = 0.41 mg a.s./kg dw	Friedrich, S. 2014b, R-33841 KCP 10.4.2.1/01
<i>Hypoaspis aculeifer</i>	ADM.00150.I.2.A	Mixed into substrate, 14 d, chronic, 5 % peat content	NOEC = 100 mg a.s./kg dw NOEC = 200 mg a.s./kg dw EC₁₀ > 200 mg a.s./kg dw	Schulz, L., 2014, R-33842 KCP 10.4.2.1/02
Field studies				
Collembola community	ADM.00150.I.2.A	NOER = 2 x 80 g a.s./ha		Schulz, L., 2022 21 48 FCM 0002 KCP 10.4.2.2/01

Values shown in **bold** used for risk assessment

zRMS comments:

During the EU renewal the toxicity to soil macro- and meso-fauna was investigated only with the representative formulation and metabolite IM-1-5, as according to data requirements as set by the Commission Regulation (EU) No 283/2013, in case of testing of soil organisms it is more appropriate to use the formulated product than the active substance. Taking this into account, the risk to soil macro- and meso-fauna may be sufficiently addressed based on toxicity data for ADM.00150.I.2.A and the metabolite tested during the EU review.

Endpoints for metabolite IM-1-5 provided in Table 9.8-1 above are in line with EU agreed values reported in EFSA Journal 2016;14(11):4610.

Studies on effects of ADM.00150.I.2.A to earthworms, *Folsomia candida* and *Hypoaspis aculeifer* listed in Table 9.8-2 were already evaluated in the course of the first zonal authorisation in April 2018 and considered acceptable. The guidelines against which the studies were validated have not changed since that time, so re-evaluation of the studies was not necessary with exception of assessment of reliability of EC₁₀ values, required by EFSA Supporting publication 2019:EN-1673. Provided endpoints are in general confirmed to be correct, however the NOEC for *Hypoaspis aculeifer* has been changed by the zRMS based on the review of the effects observed in the study.

Study summaries together with zRMS conclusions on acceptability are provided in Appendix 2.

The risk assessment for soil macro- and meso-fauna is performed with consideration of the lower of EC₁₀ and NOEC values. In case of study by Friedrich (2014b), NOEC is lower than EC₁₀ and is thus relevant for the risk assessment purposes.

9.8.2 Justification for new endpoints

ADM.00150.I.2.A was not the representative formulation for the renewal of the active substance acetamiprid. Studies on effects of the formulation ADM.00150.I.2.A on soil macro-organisms were carried out as required by Regulation (EU) 284/2013. The studies with ADM.00150.I.2.A were conducted in accordance with the most recent guidelines.

9.8.3 Risk assessment

The evaluation of the risk for earthworms and other non-target soil organisms (meso- and macrofauna) was performed in accordance with the recommendations of the “Guidance Document on Terrestrial Ecotoxicology”, as provided by the Commission Services (SANCO/10329/2002 rev 2 (final), October 17, 2002). Since according to the Commission Regulation 283/2013 tests on acute effects on earthworms are no longer required, only an assessment of chronic effects on soil macro-organisms is conducted.

9.8.3.1 First-tier risk assessment

The relevant PEC_{soil} for risk assessments covering the proposed use pattern are taken from Section 8 (Environmental Fate), Chapter 8.7.2. According to the assessment of environmental-fate data, multi-annual accumulation in soil does not need to be considered for acetamiprid. For the metabolite IM-1-5, multi-annual accumulation in soil needs to be considered.

In accordance with the recent EFSA conclusion on acetamiprid (2016), the metabolites IM-1-2, IM-1-4 and IC-0 are not expected to be more toxic to earthworms and collembolans than the most persistent metabolite IM-1-5. No study on toxicity of IM-1-5 to soil mites is available. Thus, the toxicity of the metabolites is based on the toxicity of acetamiprid. As IM-1-5 is considerably less toxic than acetamiprid in studies on earthworms and collembolans, it is highly likely that soil mites are also more sensitive towards the parent. In a worst case approach, the toxicity of the metabolites is considered to be increased by a factor of 10 compared to acetamiprid in the present risk assessment.

The results of the risk assessments are summarised in the following tables.

Table 9.8-2: First-tier assessment of the chronic risk for earthworms and other non-target soil organisms (meso- and macrofauna) due to the use of ADM.00150.I.2.A in corn BBCH 51-75, annual (Use No. I)

SP-75, annual (USE No. 1)			
Intended use	Corn		
Application rate (g a.s./ha)	1 x 60		
Chronic effects on earthworms			
Product/active substance	NOEC (mg/kg dw)	PEC _{soil} (mg/kg dw)	TER _a (criterion TER ≥ 5)
Acetamiprid (tested as ADM.00150.I.2.A)	0.85	0.020	42.5
IM-1-5	62.5	0.007 ^a	8929
Chronic effects on other soil macro- and mesofauna - <i>Folsomia</i>			
Product/active substance	NOEC (mg/kg dw)	PEC _{soil} (mg/kg dw)	TER _{It} (criterion TER ≥ 5)
Acetamiprid (tested as ADM.00150.I.2.A)	0.18	0.020	9.0
IM-1-5	12.5	0.007 ^a	1786
Chronic effects on other soil macro- and mesofauna - <i>Hypoaspis</i>			
Product/active substance	NOEC (mg/kg dw)	PEC _{soil} (mg/kg dw)	TER _{It} (criterion TER ≥ 5)
Acetamiprid (tested as ADM.00150.I.2.A)	100 200	0.020	5000 10000
IM-1-5	10 20^d	0.007 ^a	1428 2857

TER values shown in **bold** fall below the relevant trigger.

^a Maximum PEC_{soil} , accumulation

^d Chronic toxicity of the metabolite is assumed to be 10-fold higher than the parent

Table 9.8-3: First-tier assessment of the chronic risk for earthworms and other non-target soil organisms (meso- and macrofauna) due to the use of ADM.00150.I.2.A in apple BBCH 71-PHI, annual (Use No. IIa)

Intended use	Apple		
Application rate (g a.s./ha)	1 x 80		
Chronic effects on earthworms			
Product/active substance	NOEC (mg/kg dw)	PEC _{soil} (mg/kg dw)	TER _a (criterion TER ≥ 5)
Acetamiprid (tested as ADM.00150.I.2.A)	0.85	0.037	23.0
IM-1-5	62.5	0.030 ^a	2083
Chronic effects on other soil macro- and mesofauna - <i>Folsomia</i>			
Product/active substance	NOEC (mg/kg dw)	PEC _{soil} (mg/kg dw)	TER _{It} (criterion TER ≥ 5)
Acetamiprid (tested as ADM.00150.I.2.A)	0.18	0.028 0.037	6.42 4.86
IM-1-5	12.5	0.030 ^a	417
Chronic effects on other soil macro- and mesofauna - <i>Hypoaspis</i>			
Product/active substance	NOEC (mg/kg dw)	PEC _{soil} (mg/kg dw)	TER _{It} (criterion TER ≥ 5)
Acetamiprid (tested as ADM.00150.I.2.A)	100 200	0.037	2702.70 5405
IM-1-5	10 20^d	0.030 ^a	333.3 667

TER values shown in **bold** fall below the relevant trigger.

^a Maximum PEC_{soil}, accumulation

^d Chronic toxicity of the metabolite is assumed to be 10-fold higher than the parent

Table 9.8-4: First-tier assessment of the chronic risk for earthworms and other non-target soil organisms (meso- and macrofauna) due to the use of ADM.00150.I.2.A in apple BBCH 62-PHI, annual (Use No. IIb)

Intended use	Apple		
Application rate (g a.s./ha)	2 x 25		
Chronic effects on earthworms			
Product/active substance	NOEC (mg/kg dw)	PEC _{soil} (mg/kg dw)	TER _a (criterion TER ≥ 5)
Acetamiprid (tested as ADM.00150.I.2.A)	0.85	0.022	38.6
IM-1-5	62.5	0.021 ^a	2976
Chronic effects on other soil macro- and mesofauna - <i>Folsomia</i>			
Product/active substance	NOEC (mg/kg dw)	PEC _{soil} (mg/kg dw)	TER _{It} (criterion TER ≥ 5)
Acetamiprid (tested as ADM.00150.I.2.A)	0.18	0.022	8.18
IM-1-5	12.5	0.021 ^a	595
Chronic effects on other soil macro- and mesofauna - <i>Hypoaspis</i>			
Product/active substance	NOEC (mg/kg dw)	PEC _{soil} (mg/kg dw)	TER _{It} (criterion TER ≥ 5)
Acetamiprid (tested as ADM.00150.I.2.A)	100 200	0.022	4545.5 9091
IM-1-5	10 20 ^d	0.021 ^a	476.2 952

TER values shown in **bold** fall below the relevant trigger.

^a Maximum PEC_{soil}, accumulation

^d Chronic toxicity of the metabolite is assumed to be 10-fold higher than the parent

Table 9.8-5: First-tier assessment of the chronic risk for earthworms and other non-target soil organisms (meso- and macrofauna) due to the use of ADM.00150.I.2.A in potato BBCH 12-79, annual (Use No. III)

Intended use	Potato		
Application rate (g a.s./ha)	1 x 36		
Chronic effects on earthworms			
Product/active substance	NOEC (mg/kg dw)	PEC _{soil} (mg/kg dw)	TER _a (criterion TER ≥ 5)
Acetamiprid (tested as ADM.00150.I.2.A)	0.85	0.041	20.7
IM-1-5	62.5	0.014 ^a	4464
Chronic effects on other soil macro- and mesofauna - <i>Folsomia</i>			
Product/active substance	NOEC (mg/kg dw)	PEC _{soil} (mg/kg dw)	TER _{It} (criterion TER ≥ 5)
Acetamiprid (tested as ADM.00150.I.2.A)	0.18	0.041	4.39
IM-1-5	12.5	0.014 ^a	893
Chronic effects on other soil macro- and mesofauna - <i>Hypoaspis</i>			
Product/active substance	NOEC (mg/kg dw)	PEC _{soil} (mg/kg dw)	TER _{It} (criterion TER ≥ 5)
Acetamiprid (tested as ADM.00150.I.2.A)	100 200	0.041	24390 4878
IM-1-5	10 20 ^d	0.014 ^a	714.3 1429

TER values shown in **bold** fall below the relevant trigger.

^a Maximum PEC_{soil, accumulation}

^d Chronic toxicity of the metabolite is assumed to be 10-fold higher than the parent

Table 9.8-6: First-tier assessment of the chronic risk for earthworms and other non-target soil organisms (meso- and macrofauna) due to the use of ADM.00150.I.2.A in spring cereals BBCH 40-69, annual (Use No. IVa)

Intended use	Spring cereals		
Application rate (g a.s./ha)	2 x 35		
Chronic effects on earthworms			
Product/active substance	NOEC (mg/kg dw)	PEC _{soil} (mg/kg dw)	TER _a (criterion TER ≥ 5)
Acetamiprid (tested as ADM.00150.I.2.A)	0.85	0.007	121
IM-1-5	62.5	0.003 ^a	20833
Chronic effects on other soil macro- and mesofauna - <i>Folsomia</i>			
Product/active substance	NOEC (mg/kg dw)	PEC _{soil} (mg/kg dw)	TER _{It} (criterion TER ≥ 5)
Acetamiprid (tested as ADM.00150.I.2.A)	0.18	0.007	25.7
IM-1-5	12.5	0.003 ^a	4167
Chronic effects on other soil macro- and mesofauna - <i>Hypoaspis</i>			
Product/active substance	NOEC (mg/kg dw)	PEC _{soil} (mg/kg dw)	TER _{It} (criterion TER ≥ 5)
Acetamiprid (tested as ADM.00150.I.2.A)	100 200	0.007	14285.7 28571
IM-1-5	10 20^d	0.003 ^a	33333.33 6667

TER values shown in **bold** fall below the relevant trigger.

^a Maximum PEC_{soil, accumulation}

^d Chronic toxicity of the metabolite is assumed to be 10-fold higher than the parent

Table 9.8-7: First-tier assessment of the chronic risk for earthworms and other non-target soil organisms (meso- and macrofauna) due to the use of ADM.00150.I.2.A in spring cereals 1st appl.: BBCH 12-69, 2nd appl.: BBCH 40, annual and biennial (Use No. IVb)

Intended use	Spring cereals		
Application rate (g a.s./ha)	2 x 35		
Chronic effects on earthworms			
Product/active substance	NOEC (mg/kg dw)	PEC _{soil} (mg/kg dw)	TER _a (criterion TER ≥ 5)
Acetamiprid (tested as ADM.00150.I.2.A)	0.85	0.047	18.1
IM-1-5	62.5	0.017 ^a	3676
Chronic effects on other soil macro- and mesofauna - <i>Folsomia</i>			
Product/active substance	NOEC (mg/kg dw)	PEC _{soil} (mg/kg dw)	TER _{lt} (criterion TER ≥ 5)
Acetamiprid (tested as ADM.00150.I.2.A)	0.18	0.047	3.83
IM-1-5	12.5	0.017 ^a	735
Chronic effects on other soil macro- and mesofauna - <i>Hypoaspis</i>			
Product/active substance	NOEC (mg/kg dw)	PEC _{soil} (mg/kg dw)	TER _{lt} (criterion TER ≥ 5)
Acetamiprid (tested as ADM.00150.I.2.A)	100 200	0.047	2127.65 4255
IM-1-5	10 20^d	0.017 ^a	588.23 1176

TER values shown in **bold** fall below the relevant trigger.

^a Maximum PEC_{soil, accumulation}

^d Chronic toxicity of the metabolite is assumed to be 10-fold higher than the parent

Table 9.8-8: First-tier assessment of the chronic risk for earthworms and other non-target soil organisms (meso- and macrofauna) due to the use of ADM.00150.I.2.A in winter cereals BBCH 40-69, annual (Use No. Va)

Intended use	Winter cereals		
Application rate (g a.s./ha)	2 x 36		
Chronic effects on earthworms			
Product/active substance	NOEC (mg/kg dw)	PEC _{soil} (mg/kg dw)	TER _a (criterion TER ≥ 5)
Acetamiprid (tested as ADM.00150.I.2.A)	0.85	0.008	106
IM-1-5	62.5	0.003 ^a	20833
Chronic effects on other soil macro- and mesofauna - <i>Folsomia</i>			
Product/active substance	NOEC (mg/kg dw)	PEC _{soil} (mg/kg dw)	TER _{lt} (criterion TER ≥ 5)
Acetamiprid (tested as ADM.00150.I.2.A)	0.18	0.008	22.5
IM-1-5	12.5	0.003 ^a	4167
Chronic effects on other soil macro- and mesofauna - <i>Hypoaspis</i>			
Product/active substance	NOEC (mg/kg dw)	PEC _{soil} (mg/kg dw)	TER _{lt} (criterion TER ≥ 5)
Acetamiprid (tested as ADM.00150.I.2.A)	100 200	0.008	12500 25000
IM-1-5	10 20^d	0.003 ^a	3333.33 6667

TER values shown in **bold** fall below the relevant trigger.

^a Maximum PEC_{soil, accumulation}

^d Chronic toxicity of the metabolite is assumed to be 10-fold higher than the parent

Table 9.8-9: First-tier assessment of the chronic risk for earthworms and other non-target soil organisms (meso- and macrofauna) due to the use of ADM.00150.I.2.A in winter cereals BBCH 12-29, annual (Use No. Vb)

Intended use	Winter cereals		
Application rate (g a.s./ha)	1 x 30		
Chronic effects on earthworms			
Product/active substance	NOEC (mg/kg dw)	PEC _{soil} (mg/kg dw)	TER _a (criterion TER ≥ 5)
Acetamiprid (tested as ADM.00150.I.2.A)	0.85	0.040	21.3
IM-1-5	62.5	0.013 ^a	4808
Chronic effects on other soil macro- and mesofauna - <i>Folsomia</i>			
Product/active substance	NOEC (mg/kg dw)	PEC _{soil} (mg/kg dw)	TER _{It} (criterion TER ≥ 5)
Acetamiprid (tested as ADM.00150.I.2.A)	0.18	0.040	4.5
IM-1-5	12.5	0.013 ^a	962
Chronic effects on other soil macro- and mesofauna – <i>Hypoaspis</i>			
Product/active substance	NOEC (mg/kg dw)	PEC _{soil} (mg/kg dw)	TER _{It} (criterion TER ≥ 5)
Acetamiprid (tested as ADM.00150.I.2.A)	100 200	0.040	2500 5000
IM-1-5	10 20 ^d	0.013 ^a	769.23 1538

TER values shown in **bold** fall below the relevant trigger.

^a Maximum PEC_{soil}, accumulation

^d Chronic toxicity of the metabolite is assumed to be 10-fold higher than the parent

Table 9.8-10: First-tier assessment of the chronic risk for earthworms and other non-target soil organisms (meso- and macrofauna) due to the use of ADM.00150.I.2.A in winter/spring OSR BBCH 31-71, annual (Use No. VIa and VII)

winter/spring OSR DEC 01-11, annual (Use No. VII and VII)			
Intended use	Winter/spring OSR		
Application rate (g a.s./ha)	2 x 60		
Chronic effects on earthworms			
Product/active substance	NOEC (mg/kg dw)	PEC _{soil} (mg/kg dw)	TER _a (criterion TER ≥ 5)
Acetamiprid (tested as ADM.00150.I.2.A)	0.85	0.027	31.5
IM-1-5	62.5	0.011 ^a	5682
Chronic effects on other soil macro- and mesofauna - <i>Folsomia</i>			
Product/active substance	NOEC (mg/kg dw)	PEC _{soil} (mg/kg dw)	TER _{It} (criterion TER ≥ 5)
Acetamiprid (tested as ADM.00150.I.2.A)	0.18	0.027	6.67
IM-1-5	12.5	0.011 ^a	1136
Chronic effects on other soil macro- and mesofauna - <i>Hypoaspis</i>			
Product/active substance	NOEC (mg/kg dw)	PEC _{soil} (mg/kg dw)	TER _{It} (criterion TER ≥ 5)
Acetamiprid (tested as ADM.00150.I.2.A)	100 200	0.027	3703.7 7407
IM-1-5	10 20 ^d	0.011 ^a	909.1 1818

TER values shown in **bold** fall below the relevant trigger.

^a Maximum PEC_{soil}, accumulation

^d Chronic toxicity of the metabolite is assumed to be 10-fold higher than the parent

Table 9.8-11: First-tier assessment of the chronic risk for earthworms and other non-target soil organisms (meso- and macrofauna) due to the use of ADM.00150.I.2.A in winter OSR BBCH 11-19 annual, biennial (Use No. VIb)

Intended use	Winter OSR		
Application rate (g a.s./ha)	1 x 60		
Chronic effects on earthworms			
Product/active substance	NOEC (mg/kg dw)	PEC _{soil} (mg/kg dw)	TER _a (criterion TER ≥ 5)
Acetamiprid (tested as ADM.00150.I.2.A)	0.85	0.038 0.048	22.4 17.7
IM-1-5	62.5	0.012 ^a	5208
Chronic effects on other soil macro- and mesofauna - <i>Folsomia</i>			
Product/active substance	NOEC (mg/kg dw)	PEC _{soil} (mg/kg dw)	TER _{lt} (criterion TER ≥ 5)
Acetamiprid (tested as ADM.00150.I.2.A)	0.18	0.038 0.048	4.73 3.75
IM-1-5	12.5	0.012 ^a	1042
Chronic effects on other soil macro- and mesofauna - <i>Hypoaspis</i>			
Product/active substance	NOEC (mg/kg dw)	PEC _{soil} (mg/kg dw)	TER _{lt} (criterion TER ≥ 5)
Acetamiprid (tested as ADM.00150.I.2.A)	100 200	0.038 0.048	2631.6 4167
IM-1-5	10 20^d	0.012 ^a	833.33 1667

TER values shown in **bold** fall below the relevant trigger.

^a Maximum PEC_{soil}, accumulation

^d Chronic toxicity of the metabolite is assumed to be 10-fold higher than the parent

Table 9.8-12: First-tier assessment of the chronic risk for earthworms and other non-target soil organisms (meso- and macrofauna) due to the use of ADM.00150.I.2.A in sugar beet BBCH 12-39, triennial (Use No. VIII)

Intended use	Sugar beet		
Application rate (g a.s./ha)	2 x 50		
Chronic effects on earthworms			
Product/active substance	NOEC (mg/kg dw)	PEC _{soil} (mg/kg dw)	TER _a (criterion TER ≥ 5)
Acetamiprid (tested as ADM.00150.I.2.A)	0.85	0.090	9.44
IM-1-5	62.5	0.023 ^a	2717
Chronic effects on other soil macro- and mesofauna - <i>Folsomia</i>			
Product/active substance	NOEC (mg/kg dw)	PEC _{soil} (mg/kg dw)	TER _{lt} (criterion TER ≥ 5)
Acetamiprid (tested as ADM.00150.I.2.A)	0.18	0.090	2.0
IM-1-5	12.5	0.023 ^a	543
Chronic effects on other soil macro- and mesofauna - <i>Hypoaspis</i>			
Product/active substance	NOEC (mg/kg dw)	PEC _{soil} (mg/kg dw)	TER _{lt} (criterion TER ≥ 5)
Acetamiprid (tested as ADM.00150.I.2.A)	100 200	0.090	1111.11 2222
IM-1-5	10 20 ^d	0.023 ^a	434.80 870

TER values shown in **bold** fall below the relevant trigger.

^a Maximum PEC_{soil}, accumulation

^d Chronic toxicity of the metabolite is assumed to be 10-fold higher than the parent

Table 9.8-13: First-tier assessment of the chronic risk for earthworms and other non-target soil organisms (meso- and macrofauna) due to the use of ADM.00150.I.2.A in flower bulbs and flower tubers BBCH 12-91, annual (Use No. IXa)

Intended use	Flower bulbs and flower tubers		
Application rate (g a.s./ha)	1 x 46		
Chronic effects on earthworms			
Product/active substance	NOEC (mg/kg dw)	PEC _{soil} (mg/kg dw)	TER _a (criterion TER ≥ 5)
Acetamiprid (tested as ADM.00150.I.2.A)	0.85	0.055	15.5
IM-1-5	62.5	0.018 ^a	3472
Chronic effects on other soil macro- and mesofauna - <i>Folsomia</i>			
Product/active substance	NOEC (mg/kg dw)	PEC _{soil} (mg/kg dw)	TER _{It} (criterion TER ≥ 5)
Acetamiprid (tested as ADM.00150.I.2.A)	0.18	0.055	3.27
IM-1-5	12.5	0.018 ^a	694
Chronic effects on other soil macro- and mesofauna - <i>Hypoaspis</i>			
Product/active substance	NOEC (mg/kg dw)	PEC _{soil} (mg/kg dw)	TER _{It} (criterion TER ≥ 5)
Acetamiprid (tested as ADM.00150.I.2.A)	100 200	0.055	1818 3636
IM-1-5	10 20 ^d	0.018 ^a	555.55 1111

TER values shown in **bold** fall below the relevant trigger.

^a Maximum PEC_{soil}, accumulation

^d Chronic toxicity of the metabolite is assumed to be 10-fold higher than the parent

Table 9.8-14: First-tier assessment of the chronic risk for earthworms and other non-target soil organisms (meso- and macrofauna) due to the use of ADM.00150.I.2.A in flower bulbs and flower tubers BBCH 12, annual **biennial** (Use No. IXb)

Intended use	Flower bulb and flower tubers		
Application rate (g a.s./ha)	2 x 34		
Chronic effects on earthworms			
Product/active substance	NOEC (mg/kg dw)	PEC _{soil} (mg/kg dw)	TER _a (criterion TER ≥ 5)
Acetamiprid (tested as ADM.00150.I.2.A)	0.85	0.069	12.3
IM-1-5	62.5	0.020 ^a	3125
Chronic effects on other soil macro- and mesofauna - <i>Folsomia</i>			
Product/active substance	NOEC (mg/kg dw)	PEC _{soil} (mg/kg dw)	TER _{It} (criterion TER ≥ 5)
Acetamiprid (tested as ADM.00150.I.2.A)	0.18	0.069	2.61
IM-1-5	12.5	0.020 ^a	625
Chronic effects on other soil macro- and mesofauna - <i>Hypoaspis</i>			
Product/active substance	NOEC (mg/kg dw)	PEC _{soil} (mg/kg dw)	TER _{It} (criterion TER ≥ 5)
Acetamiprid (tested as ADM.00150.I.2.A)	100 200	0.069	1449.3 2899
IM-1-5	10 20 ^d	0.020 ^a	500 1000

TER values shown in **bold** fall below the relevant trigger.

^a Maximum PEC_{soil}, accumulation

^d Chronic toxicity of the metabolite is assumed to be 10-fold higher than the parent

Table 9.8-15: First-tier assessment of the chronic risk for earthworms and other non-target soil organisms (meso- and macrofauna) due to the use of ADM.00150.I.2.A in flower bulbs and flower tubers BBCH 20, annual (Use No. IXb)

Intended use	Flower bulbs and flower tubers		
Application rate (g a.s./ha)	2 x 34		
Chronic effects on earthworms			
Product/active substance	NOEC (mg/kg dw)	PEC _{soil} (mg/kg dw)	TER _a (criterion TER ≥ 5)
Acetamiprid (tested as ADM.00150.I.2.A)	0.85	0.057	14.9
IM-1-5	62.5	0.023 ^a	2717
Chronic effects on other soil macro- and mesofauna – <i>Folsomia</i>			
Product/active substance	NOEC (mg/kg dw)	PEC _{soil} (mg/kg dw)	TER _{It} (criterion TER ≥ 5)
Acetamiprid (tested as ADM.00150.I.2.A)	0.18	0.057	3.16
IM-1-5	12.5	0.023 ^a	543
Chronic effects on other soil macro- and mesofauna - <i>Hypoaspis</i>			
Product/active substance	NOEC (mg/kg dw)	PEC _{soil} (mg/kg dw)	TER _{It} (criterion TER ≥ 5)
Acetamiprid (tested as ADM.00150.I.2.A)	100 200	0.057	1754.4 3509
IM-1-5	10 20 ^d	0.023 ^a	434.8 870

TER values shown in **bold** fall below the relevant trigger.

^a Maximum PEC_{soil}, accumulation

^d Chronic toxicity of the metabolite is assumed to be 10-fold higher than the parent

Table 9.8-16: First-tier assessment of the chronic risk for earthworms and other non-target soil organisms (meso- and macrofauna) due to the use of ADM.00150.I.2.A in floriculture & perennial nursery crops BBCH 12-91, annual (Use No. Xa)

Intended use	Floriculture, tree nursery & perennial nursery crops		
Application rate (g a.s./ha)	1 x 46		
Chronic effects on earthworms			
Product/active substance	NOEC (mg/kg dw)	PEC _{soil} (mg/kg dw)	TER _a (criterion TER ≥ 5)
Acetamiprid (tested as ADM.00150.I.2.A)	0.85	0.046	18.5
IM-1-5	62.5	0.015 ^a	4167
Chronic effects on other soil macro- and mesofauna - <i>Folsomia</i>			
Product/active substance	NOEC (mg/kg dw)	PEC _{soil} (mg/kg dw)	TER _{It} (criterion TER ≥ 5)
Acetamiprid (tested as ADM.00150.I.2.A)	0.18	0.046	3.91
IM-1-5	12.5	0.015 ^a	833
Chronic effects on other soil macro- and mesofauna - <i>Hypoaspis</i>			
Product/active substance	NOEC (mg/kg dw)	PEC _{soil} (mg/kg dw)	TER _{It} (criterion TER ≥ 5)
Acetamiprid (tested as ADM.00150.I.2.A)	100 200	0.046	2173.9 4348
IM-1-5	10 20 ^d	0.015 ^a	666.66 13333

TER values shown in **bold** fall below the relevant trigger.

^a Maximum PEC_{soil}, accumulation

^d Chronic toxicity of the metabolite is assumed to be 10-fold higher than the parent

Table 9.8-17: First-tier assessment of the chronic risk for earthworms and other non-target soil organisms (meso- and macrofauna) due to the use of ADM.00150.I.2.A in floriculture & perennial nursery crops BBCH 12-91, annual ~~and biennial~~ (Use No. Xb)

Intended use	Floriculture, tree nursery & perennial nursery crops		
Application rate (g a.s./ha)	2 x 34		
Chronic effects on earthworms			
Product/active substance	NOEC (mg/kg dw)	PEC _{soil} (mg/kg dw)	TER _a (criterion TER ≥ 5)
Acetamiprid (tested as ADM.00150.I.2.A)	0.85	0.057	14.9
IM-1-5	62.5	0.023 ^a	2717
Chronic effects on other soil macro- and mesofauna - <i>Folsomia</i>			
Product/active substance	NOEC (mg/kg dw)	PEC _{soil} (mg/kg dw)	TER _{lt} (criterion TER ≥ 5)
Acetamiprid (tested as ADM.00150.I.2.A)	0.18	0.057	3.16
IM-1-5	12.5	0.023 ^a	543
Chronic effects on other soil macro- and mesofauna - <i>Hypoaspis</i>			
Product/active substance	NOEC (mg/kg dw)	PEC _{soil} (mg/kg dw)	TER _{lt} (criterion TER ≥ 5)
Acetamiprid (tested as ADM.00150.I.2.A)	100 200	0.057	1754.4 3509
IM-1-5	10 20 ^d	0.023 ^a	434.8 870

TER values shown in **bold** fall below the relevant trigger.

^a Maximum PEC_{soil}, accumulation

^d Chronic toxicity of the metabolite is assumed to be 10-fold higher than the parent

Table 9.8-18: First-tier assessment of the chronic risk for earthworms and other non-target soil organisms (meso- and macrofauna) due to the use of ADM.00150.I.2.A in tree nursery BBCH 12-91, annual (Use No. Xa)

Intended use	Tree nursery		
Application rate (g a.s./ha)	1 x 46		
Chronic effects on earthworms			
Product/active substance	NOEC (mg/kg dw)	PEC _{soil} (mg/kg dw)	TER _a (criterion TER ≥ 5)
Acetamiprid (tested as ADM.00150.I.2.A)	0.85	0.025	34
IM-1-5	62.5	0.019 ^a	3289
Chronic effects on other soil macro- and mesofauna - <i>Folsomia</i>			
Product/active substance	NOEC (mg/kg dw)	PEC _{soil} (mg/kg dw)	TER _{lt} (criterion TER ≥ 5)
Acetamiprid (tested as ADM.00150.I.2.A)	0.18	0.025	7.2
IM-1-5	12.5	0.019 ^a	658
Chronic effects on other soil macro- and mesofauna - <i>Hypoaspis</i>			
Product/active substance	NOEC (mg/kg dw)	PEC _{soil} (mg/kg dw)	TER _{lt} (criterion TER ≥ 5)
Acetamiprid (tested as ADM.00150.I.2.A)	100 200	0.025	4000 8000
IM-1-5	10 20 ^d	0.019 ^a	526.31 1053

TER values shown in **bold** fall below the relevant trigger.

^a Maximum PEC_{soil}, accumulation

^d Chronic toxicity of the metabolite is assumed to be 10-fold higher than the parent

Table 9.8-19: First-tier assessment of the chronic risk for earthworms and other non-target soil organisms (meso- and macrofauna) due to the use of ADM.00150.I.2.A in tree nursery BBCH 12-91, annual (Use No. Xb)

Intended use	Tree nursery		
Application rate (g a.s./ha)	2 x 34		
Chronic effects on earthworms			
Product/active substance	NOEC (mg/kg dw)	PEC _{soil} (mg/kg dw)	TER _a (criterion TER ≥ 5)
Acetamiprid (tested as ADM.00150.I.2.A)	0.85	0.031	27.4
IM-1-5	62.5	0.029 ^a	2155
Chronic effects on other soil macro- and mesofauna - <i>Folsomia</i>			
Product/active substance	NOEC (mg/kg dw)	PEC _{soil} (mg/kg dw)	TER _{lt} (criterion TER ≥ 5)
Acetamiprid (tested as ADM.00150.I.2.A)	0.18	0.031	5.81
IM-1-5	12.5	0.029 ^a	431
Chronic effects on other soil macro- and mesofauna - <i>Hypoaspis</i>			
Product/active substance	NOEC (mg/kg dw)	PEC _{soil} (mg/kg dw)	TER _{lt} (criterion TER ≥ 5)
Acetamiprid (tested as ADM.00150.I.2.A)	100 200	0.031	3225.80 6452
IM-1-5	10 20 ^d	0.029 ^a	344.82 690

TER values shown in **bold** fall below the relevant trigger.

^a Maximum PEC_{soil, accumulation}

^d Chronic toxicity of the metabolite is assumed to be 10-fold higher than the parent

The risk of ADM.00150.I.2.A to earthworms and other non-target soil macro-organisms, was assessed from long-term toxicity exposure ratios (TERs) between the selected no-effect concentrations, derived from laboratory tests on ADM.00150.I.2.A and relevant acetamiprid soil metabolites, and the maximum PEC_{soil}.

Acceptable risk could be concluded for earthworms and *Hypoaspis aculeifer* from all relevant compounds and *Folsomia candida* exposed to metabolite IM-1-5. However, unacceptable risk was concluded for *Folsomia candida* exposed to acetamiprid in ADM.00150.I.2.A following intended uses in apple (1 x 80 g a.s./ha), potato (1 x 36 g a.s./ha), spring cereals (2 x 35 g a.s./ha), winter cereals (1 x 30 g a.s./ha), winter OSR (1 x 60 g a.s./ha), sugar beet (2 x 50 g a.s./ha), flower bulbs and flower tubers (1 x 46 g a.s./ha and 2 x 34 g a.s./ha), floriculture & perennial nursery crops (1 x 46 g a.s./ha and 2 x 34 g a.s./ha), resulting with the highest exposure.

Therefore, the risk should be further refined on the basis of a higher Tier field study.

zRMS comments:

The risk assessment for earthworm and other soil organism from acetamiprid (formulated as ADM.00150.I.2.A) is agreed by the zRMS.

Based on the calculations provided in the Tables from 9.8-2 to 9.8-19 an acceptable risk could be concluded for earthworms and *Hypoaspis aculeifer* from all relevant compounds and *Folsomia candida* exposed to metabolite IM-1-5.

An unacceptable risk was concluded for *Folsomia candida* exposed to acetamiprid in ADM.00150.I.2

A following intended uses in potato (1 x 36 g a.s./ha), spring cereals (2 x 35 g a.s./ha), winter cereals (1 x 30 g a.s./ha), winter OSR (1 x 60 g a.s./ha), sugar beet (2 x 50 g a.s./ha), flower bulbs and flower tubers (1 x 46 g a.s./ha and 2 x 34 g a.s./ha), floriculture & perennial nursery crops (1 x 46 g a.s./ha and 2 x 34 g a.s./ha), resulting with the highest exposure.

Therefore, higher Tier study for Collembola Taxa has been considered by zRMS.

9.8.3.2 Higher-tier risk assessment

Tests at rates up to 2 x 80 g a.s./ha were conducted with ADM.00150.I.2.A and had no adverse effects on single Collembola taxa and total Collembola as well as on the community structure of Collembola of the upper 5 cm of the soil and the soil surface one year after application. The limit test rates exceed the highest field application rate (2 x 60 g a.s./ha in OSR and 1 x 680 g a.s./ha in apple) and consequently it is concluded that the risk for *Folsomia candida* ~~non-target terrestrial plants~~ is acceptable following the use of ADM.00150.I.2.A according to the proposed use pattern.

zRMS comments:

The study at rates up to 2 x 80 g a.s./ha were conducted with ADM.00150.I.2.A and had no adverse effects on single Collembola taxa and total Collembola as well as on the community structure of Collembola of the upper 5 cm of the soil and the soil surface one year after application.

Based on the results it can be concluded that the risk for *Folsomia candida* at application rates in potato (1 x 36 g a.s./ha), spring cereals (2 x 35 g a.s./ha), winter cereals (1 x 30 g a.s./ha), winter OSR (1 x 60 g a.s./ha), sugar beet (2 x 50 g a.s./ha), flower bulbs and flower tubers (1 x 46 g a.s./ha and 2 x 34 g a.s./ha), floriculture & perennial nursery crops (1 x 46 g a.s./ha and 2 x 34 g a.s./ha) is considered acceptable.

Commenting period process:

During commenting period process some of MSs did not accept the study results as a refinement option for *Folsomia candida* and the decision of using this study is left at MSs level.

9.8.4 Overall conclusions

In a one year field study, no effects occurred at tested rates up to 2 x 80 g a.s./ha, indicating that the risk to soil meso- and macrofauna is acceptable following the use of ADM.00150.I.2.A according to the proposed use patterns.

9.9 Effects on soil microbial activity (KCP 10.5)

9.9.1 Toxicity data

Studies on effects of soil microorganisms have been carried out with acetamiprid and its relevant metabolites. Full details of these studies are provided in the respective EU DAR and related documents as well as in Appendix 2 of this document (new studies).

Table 9.9-1: Endpoints and effect values relevant for the risk assessment for soil microorganisms

Endpoint	Substance	Exposure System	Results	Reference
N-mineralisation	NI-25 ^{a)}	28 d, aerobic	No significant effects > 25% at 0.2 kg a.s./ha	EFSA, 2016
N-mineralisation	ADM.00150.I.2.A	28 d, aerobic loamy sand	NOEC = 22.74 mg test item/kg dw corresponding to 4.01 mg a.s./kg dw ^{b)}	Schulz, L., 2014 R-33843 KCP 10.5/01

Values shown in **bold** used for risk assessment

^{a)} Representative formulation, Acetamiprid 20 SL

^{b)} Calculated based on the test item amount of 22.47 mg/kg and an a.s. content of 17.83 % w/w provided in the CoA

zRMS comments:

During the EU renewal the effects on soil micro-organisms were investigated only with the representative formulation, as according to data requirements as set by the Commission Regulation (EU) No 283/2013, in case of testing of soil organisms it is more appropriate to use the formulated product than the active substance. Taking this

into account, the risk to soil macro- and meso-fauna may be sufficiently addressed based on toxicity data for ADM.00150.I.2.A.

Study on effects of ADM.00150.I.2.A on soil nitrogen transformation listed in Table 9.9-1 was already evaluated in the course of the first zonal authorisation of MCW-222 in April 2018 and considered acceptable. The guideline against which the study was validated has not changed since that time, so re-evaluation of the study was not necessary. Provided endpoint is confirmed to be correct.

Summary of the study together with zRMS conclusions on acceptability is provided in Appendix 2.

9.9.2 Justification for new endpoints

Effects on soil microorganisms of ADM.00150.I.2.A were not evaluated as part of the EU assessment of acetamiprid. Data submitted with this application are listed in Appendix 1 and summarised in Appendix 2.

9.9.3 Risk assessment

The evaluation of the risk for soil microorganisms was performed in accordance with the recommendations of the “Guidance Document on Terrestrial Ecotoxicology”, as provided by the Commission Services (SANCO/10329/2002 rev 2 (final), October 17, 2002).

The relevant PEC_{soil} for risk assessments covering the proposed use pattern are taken from Section 8 (Environmental Fate), Chapter 8.7.2 and were already used in the risk assessment for earthworms and other non-target soil organisms (meso- and macrofauna) (see 9.16).

To achieve a concise risk assessment, the risk envelope approach is applied. Here, the assessment for the use in sugar beet also covers the risk for the soil microorganisms from all other intended uses (see Chapter 9.1-1).

Table 9.9-2: Assessment of the risk for effects on soil micro-organisms due to the use of ADM.00150.I.2.A in sugar beet BBCH 12-39, triennial (Use No. VIII)

Intended use	Sugar beet		
Application rate (g a.s./ha)	2 x 50		
N-mineralisation			
Product/active substance	Max. conc. with effects ≤ 25 % (mg/kg dw)	PEC _{soil} (mg/kg dw)	Risk acceptable?
Acetamiprid	> 4.01 (at 28 d)	0.090	yes
IM-1-5	> 0.401 (at 28 d) ^a	0.023 ^b	yes

^a 10 times toxicity of the parent assumed as a worst case

^b Maximum PEC_{soil} , accumulation

zRMS comments:

The risk assessment for soil micro-organisms from acetamiprid in ADM.00150.I.2.A presented in Table 9.9-2 above is agreed by the zRMS. As the maximum expected concentration of acetamiprid in soil is lower than concentration at which effects <25% were seen in the respective study, acceptable risk from all intended uses of ADM.00150.I.2.A may be concluded.

No toxicity data for metabolites were available from the EU review and hence the risk assessment could not be performed. However, during the EU review no risk assessment was performed for metabolites and no data gap for respective toxicity studies with metabolites was identified in EFSA Journal 2016;14(11):4610.

Furthermore, based on data from soil metabolism studies it may be expected that metabolites IM-1-2, IM-1-4 and IC-0 were formed in soil during studies performed with the formulated product, as in the route of degradation studies their maximum occurrence in soil was observed on day 1, 14 and 2, respectively, while the study duration is 28 days. Thus, based on the available information it may be concluded that the risk to soil micro-organisms from metabolites IM-1-2, IM-1-4 and IC-0 is sufficiently covered by evaluation performed for acetamiprid in formulation ADM.00150.I.2.A.

Metabolite IM-1-5 was most probably not formed in the study, as according to information available from the EU

review, this compound is formed in calcareous soils. For this reason, risk assessment for this metabolite has been performed using the maximum accumulated PEC_{soil} agreed in area of Section 8 and assuming 10 times toxicity of the parent. Based on these worst case assumptions, acceptable risk may be concluded from IM-1-5 for all intended uses of ADM.00150.I.2.A.

9.9.4 Overall conclusions

The risk of ADM.00150.I.2.A to soil microorganisms was evaluated by comparison of the maximum concentrations with effects <25% derived from laboratory tests, with maximum PEC_{soil}. For metabolite IM-1-5 the evaluation was performed with consideration of the maximum agreed accumulated PEC_{soil} and assumption that metabolite is 10 times more toxic for the parent.

No effects > 25% occurred at tested rates exceeding the relevant PEC_{soil} values, indicating that the risk to soil microorganisms is acceptable following the use of ADM.00150.I.2.A according to the proposed use patterns. Risk from metabolites IM-1-2, IM-1-4 and IC-0 is considered to be covered by evaluation performed for the parent.

9.10 Effects on non-target terrestrial plants (KCP 10.6)

9.10.1 Toxicity data

Studies on the toxicity to non-target terrestrial plants have been carried out with ADM.00150.I.2.A. Full details of these studies are provided in the respective EU DAR and related documents as well as in Appendix 2 of this document (new studies).

Effects on non-target terrestrial plants of ADM.00150.I.2.A were not evaluated as part of the EU assessment of acetamiprid. Data submitted with this application are listed in Appendix 1 summarised in Appendix 2.

Table 9.10-1: Endpoints and effect values relevant for the risk assessment for non-target terrestrial plants

Species	Substance	Exposure System	Results	Reference
<i>Avena sativa</i> _m <i>Lolium perenne</i> _m <i>Brassica rapa</i> _d <i>Lycopersicon esculentum</i> _d <i>Cucumis sativus</i> _d <i>Glycine max</i> _d	ADM.00150.I.2.A	21 d Vegetative vigour	ER₅₀ plant weight > 510 g a.s./ha	Friedrich, S., 2014 14 10 48 002 P KCP 10.6.2/01

m: monocotyledonous, d: dicotyledonous

Values shown in **bold** used for risk assessment

zRMS comments:

Study on effects of ADM.00150.I.2.A on non-target terrestrial plants listed in Table 9.10-1 was already evaluated in the course of the first zonal authorisation in April 2018 and considered acceptable. The guideline against which the study was validated has not changed since that time, so re-evaluation of the study was not necessary. Provided endpoint is confirmed to be correct.

Summary of the study together with zRMS conclusions on acceptability is provided in Appendix 2,

9.10.2 Justification for new endpoints

ADM.00150.I.2.A was not the representative formulation for the renewal of the active substance acetamiprid. Studies on effects of the formulation ADM.00150.I.2.A on non-target terrestrial plants were

carried out as required by Regulation (EU) 284/2013.

9.10.3 Risk assessment

9.10.3.1 Tier-1 risk assessment (based screening data)

To achieve a concise risk assessment, the risk envelope approach is applied. Here, the assessment for the use in oil seed rape also covers the risk for non-target terrestrial plants from all other intended uses (see 9.1-1).

Limit tests at rates up to 510 g/ha were conducted with ADM.00150.I.2.A and effects were below the critical threshold as defined by the “Guidance Document on Terrestrial Ecotoxicology”, (SANCO/10329/2002 rev.2 final, 2002). The limit test rates exceed the highest field application rate (2 x 60 g a.s./ha in OSR and 1 x 80 g a.s./ha in apple) and consequently it is concluded that the risk for non-target terrestrial plants is acceptable following the use of ADM.00150.I.2.A according to the proposed use pattern.

zRMS comments:

Although standard vegetative vigour study is not considered to be the screening study, the evaluation provided by the Applicant above is agreed by the zRMS.

The available study was performed as a limit test with single application rate of 510 g a.s./ha, at which no effects on investigated parameters were seen (i.e. phytotoxicity and fresh shoot weight).

The maximum intended application rate of (60 g a.s./ha) is more than 6 times lower than rate at which no effects were seen in the study.

Based on that the risk to non-target plants from all intended uses of is concluded to be acceptable and calculation of TER values is deemed not necessary. Concluded to be acceptable and calculation of TER values is deemed not necessary.

9.10.3.2 Tier-2 risk assessment (based on dose-response data)

Not relevant

9.10.3.3 Higher-tier risk assessment

Not relevant.

9.10.3.4 Risk mitigation measures

No risk mitigation needed.

9.10.4 Overall conclusions

The application of ADM.00150.I.2.A according to the proposed use pattern will pose an acceptable risk to non-target terrestrial plants.

9.11 Effects on other terrestrial organisms (flora and fauna) (KCP 10.7)

No additional studies on terrestrial organisms (flora and fauna) have been conducted.

9.12 Monitoring data (KCP 10.8)

No monitoring data are available.

9.13 Classification and Labelling

According to (EC) No 1272/2008 (CLP) classification has to be made for plant protection products for their environmental hazard (acute and chronic). Classification is based on acute and chronic product data if adequate data is available. When product data for all three trophic levels is not available, the summation method is carried out instead.

For the product ADM.00150.I.2.A /MCW-2222 the following data is available:

Acute data: fish, daphnia and algae

Chronic data: algae

An overview is presented in Table 9.13-1:

Table 9.13-1: Ecotoxicology/Environment data relevant for classification of ADM.00150.I.2.A

Substance tested	Study Type (duration)	Findings	Triggered classification and labelling	Reference
Acute (short-term) aquatic hazard				
MCW-2222 ¹⁾	<i>Oncorhynchus mykiss</i> (96 h)	96 h LC ₅₀ = 15.3 mg a.s./L	No aquatic acute hazard cat.	R-33831 KCP 10.2.1/01
	<i>Daphnia magna</i> (48 h)	48 h EC ₅₀ = 22.8 mg a.s./L	No aquatic acute hazard cat.	Juckeland, D., 2014b R-33832 KCP 10.2.1/02
	<i>Chironomus riparius</i> (48 h)	48 h EC₅₀ = 0.0929 mg a.s./L nom	Aquatic acute hazard cat. 1 (H400)	Juckeland, D., 2015a R-34873 KCP 10.2.1/03
	<i>Desmodesmus subspicatus</i> (72 h)	72 h ErC ₅₀ = 553.5 mg a.s./L 72 h ErC ₁₀ = 146.6 mg a.s./L	No aquatic acute hazard cat. No aquatic chronic hazard cat	Juckeland, D., 2014b R-33833 KCP 10.2.1/04
Long-term aquatic hazard				
Acetamiprid ²⁾	<i>P. promelas</i> (35 d)	NOEC = 9.4 mg a.s./L	No aquatic chronic hazard cat	EFSA, 2016
	<i>D. magna</i> (21 d)	EC ₁₀ = 2.96 mg a.s./L	No aquatic chronic hazard cat	EFSA, 2016
	<i>C. riparius</i> (28 d)	EC₁₀ = 0.000235 mg a.s./L	Aquatic chronic hazard cat 1 (H410), M = 100	EFSA, 2016
	--	--	Aquatic chronic hazard cat. 3 (H412)	legal classification of acetamiprid in Annex VI of (EC) No 1272/2008 (CLP)
	Biodegradation	not readily biodegradable	--	EFSA, 2016

¹⁾ Tested as MCW-2222 equivalent to ADM.00150.I.2.A

²⁾ Nominal contents within the formulated product ADM.00150.I.2.A: 200 g acetamiprid/L.

Acute aquatic hazard category 1 (H400) is given according to (EC) No 1272/2008 (CLP) according to the lowest acute aquatic toxicity endpoint of ADM.00150.I.2.A.

For the chronic classification of the product ADM.00150.I.2.A the summation method is applied considering all components that are classified aquatic chronic 1, i.e. acetamiprid (M = 100, 20% (w/v)) in the first equation according to CLP (Chronic 1 x M ≥ 25 %). The resulting value exceeds the trigger of 25% (Table 9.13-2). Hence, ADM.00150.I.2.A is classified as Chronic 1 (H410).

Table 9.13-2: Chronic classification of acetamiprid ADM.00150.I.2.A using the summation method according to (EC) No 1272/2008

Chronic classification of ADM.00150.I.2.A						
Formulation component						
Name	Chronic Category	M-Factor	Content in ADM.00150.I.2.A Acetamiprid 200 SL/ LEAXO [%]	Result (% Content x M-Factor)		
Acetamiprid	1	100	20	200		
1 st equation	SUM (<i>M x Chronic 1</i>)			2000	≥ 25 %	ADM.00150.I.2.A: Aquatic Chronic Hazard Category 1

Conclusion

In conclusion the following classification and labelling is proposed for ADM.00150.I.2.A: aquatic acute hazard category 1 (H400) and aquatic chronic hazard category 1 (H410) according to GHS following Regulation (EC) No 1272/2008.

zRMS comments:

CLP classification of ADM.00150.I.2.A. presented by the Applicant above is agreed by the zRMS.

It is noted that according to Regulation (EC) No 1272/2008, acetamiprid is classified for chronic aquatic hazard in category 3. However, this classification is obviously based on old studies and does not take into account the more recent studies performed with aquatic insects which demonstrated that this group of species is extremely sensitive to acetamiprid, which should not be ignored in classification of the product. No studies on chronic toxicity of ADM.00150.I.2.A. to *Chironomus riparius* were performed, but it may be expected that they would result with similarly low endpoints. Therefore, the zRMS agrees with the Applicant that ADM.00150.I.2.A is classified for acute and chronic aquatic hazard in category 1.

Following phrases must be included in the label:

- Hazard statement:** H410
- Signal word:** Warning
- Pictogram:** GHS09
- Safety phrases:** P391, P501

Reference list

Bastiansen, F. (2018): EPAT v. 1.2 – Exposure Pattern Analysis Tool – Program Manual; Rifcon GmbH Report No. R1860108 (European Crop Protection Association) (https://rifcon.de/wp-content/uploads/2018/08/rifcon_epat_2010.pdf)

Candolfi MP, Barrett KL, Campbell P, Forster R, Grandy N, Huet M-C, Lewis G, Oomen PA, Schmuck R and Vogt H, 2001. Guidance document on regulatory testing and risk assessment procedures for plant protection products with non-target arthropods. Report of the SETAC/ESCORT 2 Workshop, Wageningen, the Netherlands, and SETAC-Europe, Brussels, Belgium.

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EFSA (European Food Safety Authority), 2009. EFSA Journal 2009; 7(12):1438, Guidance of EFSA, Risk Assessment for Birds and Mammals

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EFSA (European Food Safety Authority), 2013. EFSA Guidance Document on the risk assessment of plant production products on bees (*Apis mellifera*, *Bombus* spp. and solitary bees) (published on July 04, 2013, updated on 04 July 2014). EFSA Journal 11(7): 3295.

European Commission Guidance Document - Guidelines on comparability, extrapolation, group tolerances and data requirements for setting MRLs. SANCO 7525/VI/95, Rev. 10.3, 13 June 2017 available at https://ec.europa.eu/food/sites/food/files/plant/docs/pesticides_mrl_guidelines_app-d.pdf (accessed 2020 January 10).

EFSA (European Food Safety Authority), 2016a. Conclusion on the peer review of the pesticide risk assessment of the active substance acetamiprid. EFSA Journal 2016;14(11):4610, 26 pp. doi:10.2903/j.efsa.2016.4610

EFSA (European Food Safety Authority), 2016b. Conclusion on the peer review of the pesticide risk assessment of the active substance acetamiprid. Appendix to EFSA Journal 2016;14(11):4610, Appendix A – List of end points for the active substance and the representative formulation, 91 pp. doi:10.2903/j.efsa.2016.4610

EFSA (European Food Safety Authority), 2019. Technical report on the outcome of the Pesticides Peer Review Meeting on general recurring issues in ecotoxicology. EFSA supporting publication 2019:EN-1673. 117 pp. doi:10.2903/sp.efsa.2019.EN-1673

EFSA (European Food Safety Authority), 2019. Outline of the revision of the Guidance on the risk assessment of plant protection products on bees (*Apis mellifera*, *Bombus* spp. and solitary bees)

EFSA (European Food Safety Authority), 2020. Review of the evidence on bee background mortality. EFSA Supporting publication 2020: EN-1880

EFSA (European Food Safety Authority), 2021. Analysis of background variability of honey bee colony size. EFSA Supporting publication 2021: EN-6518

EFSA (European Food Safety Authority), 2021. Draft Guidance Risk assessment for Birds and Mammals. Available online at: <https://lynxee.consulting/en/europe-efsa-public-consultation-on-updated-bird-and-mammals-risk-assessment> (last access: May 2022)

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Van Vlaardingen PLA, Traas TP, Wintersen AM and Aldenberg T (2004) ETX 2.0 – A program to calculate hazardous concentrations and fractions affected, based on normally distributed toxicity data. RIVM report 601501028/2004

Appendix 1 Lists of data considered in support of the evaluation

List of data submitted by the applicant and relied on

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
KCP 10.1.1/01	Finch, E., Payne, M., Crocker, J.	2006	Bird and mammal risk assessment: Refining the proportion of diet obtained in the treated crop area (PT) through the use of radio-tracking data. Pages 1–48 in Advisory Committee on Pesticides SC 11419. GLP: no Published: no	N	Public
KCP 10.1.1/02	Lahr J, Krämer W, Mazerolles V, Poulsen V, Jölli D, Müller M, McVey E, Wassenberg J, Derks R, Brouwer A, Deneer D, Beltman W, Lammertsma D, Jansman H, Buij R	2018	Data collection for the estimation of ecological data (specific focal species, time spent in treated areas collecting food, composition of diet), residue level and residue decline on food items to be used in the risk assessment for birds and mammals. EFSA supporting publication 2018:EN 1513. 155 pp.	N	Public
KCP 10.1.2/01	Weick, S., Henkes, K.	2017	Residues of acetamiprid in foliage-dwelling arthropods and ground vegetation after spray application of Acetamiprid 200 SL in a pome fruit orchard in Italy – magnitude of residues and time course of residue decline, unpubl. RIFCON GmbH report, 08 June 2017 Report No.: R1640039 GLP No. 299 Published: no	N	ADAMA Makhteshim Ltd
KCP 10.1.2/02	Staffel, J., Brehm, C.	2021	Residues of acetamiprid in monocotyledonous and dicotyledonous plants after spray application of Acetamiprid 200 SL in early vegetation stages in Spain – magnitude of residues and time course of residue decline. unpubl. RIFCON GmbH report, 29 July 2021. Report No.: R2040056 GLP No.513 Published: no	N	ADAMA Makhteshim Ltd
KCP 10.1.2/03	Staffel, J., Brehm, C.	2021	Residues of acetamiprid in monocotyledonous and dicotyledonous plants after spray application of Acetamiprid 200 SL in early vegetation stages in Germany – magnitude of residues and time course of residue decline. unpubl. RIFCON GmbH report, 05 August 2021. Report No.: R2040057 GLP No. 517 Published: no	N	ADAMA Makhteshim Ltd
KCP 10.1.2/04	Staffel, J., Brehm, C.	2022	Residues of acetamiprid in monocotyledonous and dicotyledonous plants after spray application of Acetamiprid 200 SL in early vegetation stages in spring in Germany – magnitude of residues and time course of residue decline. unpubl. RIFCON GmbH report, 28 February 2022. Report No.: R2040059 GLP No.519 Published: no	N	ADAMA Makhteshim Ltd

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
KCP 10.1.2/05	Gräf, K.	2022	Residues of acetamiprid in monocotyledonous and dicotyledonous plants after spray application of Acetamiprid 200 SL in early vegetation stages in Northern Europe – magnitude of residues and time course of residue decline. unpubl. RIFCON GmbH report, 01 March 2022. Report No.: R2040060 GLP No. 520 Published: no	N	ADAMA Makhteshim Ltd
KCP 10.1.2/06	Ebeling, M., Wang, M.	2018	Dissipation of Plant Protection Products From Foliage. Environmental Toxicology and Chemistry 37:1926–1932 GLP: no Published: yes	N	Public
KCP 10.1.2/07	Kaetzke, P., Niedermeier, J., Masseti, M.	2003	Oryctolagus cuniculus (Linné, 1758) Europäisches Wildkaninchen. In: Handbuch der Säugetiere Europas, Lagomorpha (Ed. by Niethammer, J. & Krapp, F.), pp. 189–289. AULA Verlag. GLP: no Published: yes	N	Public
KCP 10.1.2/08	Gea-Izquierdo, G., Muñoz-Igualada, J., San Miguel-Ayanz, A.	2005	Gea-Izquierdo, G., Muñoz-Igualada, J. & San Miguel-Ayanz, A. 2005. Rabbit warren distribution in relation to pasture communities in Mediterranean habitats: consequences for management of rabbit populations. Wildlife Research, 32, 723–731. GLP No. Published: no	N	Public
KCP 10.1.2/09	Calvete, C., Estrada, R., Angulo, E., Cabezas-Ruiz, S.	2004	Habitat factors related to wild rabbit conservation in an agricultural landscape. Landscape Ecology 19:531–542.	N	Public
KCP 10.1.2/10	Katzschner, I., Ludwigs, J-D., Grimm, T. Blanckenhagen, F. von	2015	Generic monitoring of hares and rabbits to determine proportion of time spent foraging in oilseed rape, sunflower and sugar beet in Central Europe. ADAMA Irvita NV; unpublished RIFCON GmbH report, 04 December 2015 Report No.: R12244-1 GLP No. Published: no		ADAMA Makhteshim Ltd
KCP 10.1.2/11	Schabacker, J. Hahne, J., Ludwigs, J.-D., Vallon, M., Foudoulakis, M., Murfitt, R., Ristau, K.	2020	Residue levels of pesticides on fruits for use in wildlife risk assessments. Integrated Environmental Assessment and Management. https://doi.org/10.1002/ieam.4345 ; https://setac.onlinelibrary.wiley.com/doi/epdf/10.1002/ieam.4345 GLP: no Published: yes	N	Public
KCP 10.1.2/12	Jacob, J., Manson, P., Barfknecht, R., Fredricks, T.	2013	Common Vole (<i>Microtus Arvalis</i>) Ecology and Management: Implications for Risk Assessment of Plant Protection Products. Pest Management Science 70: 869–878. GLP: no Published: yes	N	Public
KCP 10.1.2/13	Rinke, T.	1991	Percentage of volume versus number of species: availability and intake of grasses and forbs in <i>Microtus arvalis</i> . Folia Zoologica, 40(2), 143–151. GLP: no Published: yes	N	Public

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
KCP 10.1.2/14	Leutert, A.	1983	Einfluss der Feldmaus, <i>Microtus arvalis</i> (Pall.), auf die floristische Zusammensetzung von Wiesen-Ökosystemen. Veröffentlichung des Geobotanischen Institutes der Eidgen. Techn. Hochschule, Stiftung Rübel, Zürich. GLP: no Published: yes	N	Publie
KCP 10.1.2/15	Lüthi, M., W. Nentwig, and J. P. Airoldi	2010	Nutritional ecology of <i>Microtus arvalis</i> (Pallas, 1779) in sown wild flower fields and quasi-natural habitats. <i>Revue Suisse de Zoologie</i> 4:811-828. GLP: no Published: yes		
KCP 10.2.1/01	■■■■	2014 a	Acute toxicity of MCW-2222 to the rainbow trout <i>Oncorhynchus mykiss</i> in a 96-hour static test Report No.: R-33831 ■■■■ GLP: yes Published: no	Y	Adama
KCP 10.2.1/02	Juckeland, D.	2014 b	Acute toxicity of MCW-2222 to <i>Daphnia magna</i> in a 48-hour static test Report No.: R-33832 BioChem agrar Labor für biologische und chemische Analytik GmbH. Kupferstraße 6. 04827 Gerichshain, Germany. GLP: yes Published: no	N	Adama
KCP 10.2.1/03	Juckeland, D.,	2015	Acute toxicity of MCW-2222 to <i>Chironomus riparius</i> in a 48-hour static test Report No.: R-34873 BioChem agrar Labor für biologische und chemische Analytik GmbH. Kupferstraße 6. 04827 Gerichshain, Germany. GLP: yes Published: no	N	Adama
KCP 10.2.1/04	Juckeland, D.	2014 c	Effects of MCW-2222 on <i>Desmodesmus subspicatus</i> in an algal growth inhibition test Report No.: R-33833 BioChem agrar Labor für biologische und chemische Analytik GmbH. Kupferstraße 6. 04827 Gerichshain, Germany. GLP: yes Published: no	N	Adama
KCP 10.2.3/01	Hommen, U.	2022	Acetamiprid – Outdoor mesocosm study Report No.: ADM-025/7-52 Fraunhofer Institute for Molecular Biology and Applied Ecology (IME) Auf dem Aberg 1 57392 Schmallenberg, Germany GLP: yes Published: no	N	Adama
KCP 10.2.3/02	Taylor, S. & Joyce, F., D	2015	Acetamiprid 200 SL – Acute Toxicity to Aquatic Organisms Report Nr.: R-35057 Cambridge Environmental Assessments Battlegate Road, Boxworth Cambridgeshire, CB23 4NN / UK GLP: yes Published: no	N	Adama
KCP 10.2.3/03	Koerner, O.	2015	MCW-2222: Evaluation of Aquatic Invertebrate Toxicity Tests to Derive a Regulatory Acceptable Concentration Report Nr.: R-36040 ADAMA Europe	N	Adama

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
			Edmund-Rumpler-Straße 5 51149 Köln Germany GLP: no Published: no		
KCP 10.3.1.1.1/01 & KCP 10.3.1.1.2/01	Franke, M.	2014	Acute toxicity of MCW-2222 to the honeybee <i>Apis mellifera</i> L. under laboratory conditions Sponsor ID: R-33834; Study No.: 14 10 48 076 B BioChem agrar Labor für biologische und chemische Analytik GmbH. Kupferstraße 6. 04827 Gerichshain, Germany. GLP: yes Published: no	N	Adama
KCP 10.3.1.2.1/01 & KCP 10.3.1.2.2/01	Röhlig, U.	2014	Acute toxicity of MCW-2222 to the bumblebee <i>Bombus terrestris</i> L. under laboratory conditions Sponsor ID: R-33837; Study No.: 14 10 48 024 A BioChem agrar Labor für biologische und chemische Analytik GmbH. Kupferstraße 6. 04827 Gerichshain, Germany. GLP: yes Published: no	N	Adama
KCP 10.3.1.2/01	Kleebaum, K.	2015 a	Chronic toxicity of MCW-2222 to the honeybee <i>Apis mellifera</i> L. under laboratory conditions Sponsor ID: R-33835; Study No.: 14 10 48 077 B BioChem agrar Labor für biologische und chemische Analytik GmbH. Kupferstraße 6. 04827 Gerichshain, Germany. GLP: yes Published: no	N	Adama
KCP 10.3.1.2/02	Dreßler, K.	2019	Chronic oral toxicity of CA3573 Acetamiprid 200 SL (Carnadine) to the honey bee <i>Apis mellifera</i> L. under laboratory conditions Project No. 19 48 BAC 0028 BioChem agrar Labor für biologische und chemische Analytik GmbH. Kupferstraße 6. 04827 Gerichshain, Germany. GLP: yes Published: no	N	Nufarm
KCP 10.3.1.3/01	Kleebaum, K.	2015 b	Chronic toxicity of MCW-2222 to honeybee larvae (<i>Apis mellifera</i> L.) under laboratory conditions (in vitro) Sponsor ID: R-33836; Study No.: 14 10 48 078 B BioChem agrar Labor für biologische und chemische Analytik GmbH. Kupferstraße 6. 04827 Gerichshain, Germany. GLP: yes Published: no	N	Adama
KCP 10.3.1.3/02	Scheller, K.	2020	CA3573 Acetamiprid 200 SL (Carnadine) - Repeated exposure of honey bee larvae (<i>Apis mellifera</i> L.) under laboratory conditions Project No. 19 48 BLC 0033 BioChem agrar Labor für biologische und chemische Analytik GmbH. Kupferstraße 6. 04827 Gerichshain, Germany. GLP: yes Published: no	N	Nufarm
KCP 10.3.1.5/01	Mamet, O. & Molitor, C.	2015 a	Assessment of toxicity on honey bees (<i>Apis mellifera</i>) of the product MCW-2222 (acetamiprid 200 g/L) applied under insect proof tunnels on a cereal crop Sponsor ID: R-34874; Study No.: 216-2014 TESTAPI, Sarré, 49350 Gennes, France. GLP: yes Published: no	N	Adama

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
KCP 10.3.1.5/02	Mamet, O.	2015 a	Assessment of toxicity on honeybees (<i>Apis mellifera</i>) of MCW-2222 on wheat crop in a tunnel trial in France. Sponsor ID: R-35845; Study No.: 223-2015 TESTAPI, Sarré, 49350 Gennes, France. GLP: yes Published: no	N	Adama
KCP 10.3.1.5/03	Mamet, O.	2015 b	Assessment of toxicity on honey bees (<i>Apis mellifera</i>) of the product MCW-2222 on cereals in a tunnel trial in France. Sponsor ID: R-35846; Study No.: 224-2015 TESTAPI, Sarré, 49350 Gennes, France. GLP: yes Published: no	N	Adama
KCP 10.3.1.5/04	Mamet, O. & Molitor, C.	2015 b	Assessment of toxicity on honeybees (<i>Apis mellifera</i>) of the product MCW-2222 (acetamiprid 200 g/L) applied under insect proof tunnels on a phacelia crop in Northern France. Sponsor ID: R-34875; Study No.: 217-2014 TESTAPI, Sarré, 49350 Gennes, France. GLP: yes Published: no	N	Adama
KCP 10.3.1.5/05	Mamet, O. & Molitor, C.	2015 c	Assessment of toxicity on honey bees (<i>Apis mellifera</i>) of the product MCW-2222 (acetamiprid 200 g/L) applied under insect proof tunnels on a phacelia crop during summer in France Sponsor ID: R-34876; Study No.: 218-2014 TESTAPI, Sarré, 49350 Gennes, France. GLP: yes Published: no	N	Adama
KCP 10.3.1.5/06	Molitor, C.	2015 a	Assessment of toxicity on honey bees (<i>Apis mellifera</i>) of the product MCW-2222 (acetamiprid 200 g/L) applied under insect proof tunnels on a phacelia crop during summer in France. Sponsor ID: R-35847; Study No.: 225-2015 TESTAPI, Sarré, 49350 Gennes, France. GLP: yes Published: no	N	Adama
KCP 10.3.1.5/07	Hecht-Rost, S. & Mayer, O.	2018	Semi-field brood study to evaluate potential effects of MCW-2222 on brood development of honeybees (<i>Apis mellifera</i> L.) Sponsor ID: R-37336; Report No.: R1640035 RIFCON GmbH Goldbeckstr. 13 D-69493 Hirschberg, Germany. GLP: no Published: no	N	Adama
KCP 10.3.1.6/01	Molitor, C.	2015 b	Field Study to Evaluate Potential Side Effects of the product MCW-2222 (acetamiprid 200 g/L) on Brood Development, Foraging Activity, Mortality and Behaviour of Adult Honeybees <i>Apis mellifera</i> L. (Hymenoptera: Apidae) Following Application after Bee-Flight on <i>Phacelia tanacetifolia</i> Sponsor ID: R-34877; Study No.: 215-2014 TESTAPI, Sarré, 49350 Gennes, France. GLP: yes Published: no	N	Adama
KCP 10.3.1.6/02	Molitor, C.	2015 c	Field Study to Evaluate Potential Side Effects of MCW-2222 on Brood Development, Foraging Activity, Mortality and Behaviour of Adult Honeybees (<i>Apis mellifera</i>) on Oilseed rape & Final Report Amendment N°1 Sponsor ID: R-35844; Study No.: 230-2015 TESTAPI, Sarré, 49350 Gennes, France.	N	Adama

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
			GLP: yes Published: no		
KCP 10.3.1.6/03	Aucejo, S.	2015 a	Effects and Determination of Residues of Acetamiprid 200 SL on the Honeybee (<i>Apis mellifera</i> L.) Brood in Apple, under Field Conditions, in Italy 2015. Sponsor ID: R-35961; Study No: 307SRES15C01 TESTAPI, Sarré, 49350 Gennes, France. GLP: yes Published: no	N	Adama
KCP 10.3.2.1/01	Röhlig, U.	2014	Effects of MCW-2222 on the predatory mite <i>Typhlodromus pyri</i> SCHEUTEN in a laboratory test -Rate-Response-Test (LR50) - Report No.: R-33838 BioChem agrar Labor für biologische und chemische Analytik GmbH. Kupferstraße 6. 04827 Gerichshain, Germany. GLP: yes Published: no	N	ADAMA
KCP 10.3.2.1/02	Röhlig, U.	2014	Effects of MCW-2222 on the parasitic wasp <i>Aphidius rhopalosiphi</i> (DESTEFANI-PEREZ) in a laboratory test - Rate-Response-Test (LR50) - Report No.: R-33839 BioChem agrar Labor für biologische und chemische Analytik GmbH. Kupferstraße 6. 04827 Gerichshain, Germany. GLP: yes Published: no	N	ADAMA
KCP 10.3.2.2/01	Röhlig, U.	2014	Effects of MCW-2222 on the predatory mite <i>Typhlodromus pyri</i> SCHEUTEN in an extended laboratory test - Rate-Response-Test (LR50) - Report No.: R-34780 BioChem agrar Labor für biologische und chemische Analytik GmbH. Kupferstraße 6. 04827 Gerichshain, Germany. GLP: yes Published: no	N	ADAMA
KCP 10.3.2.2/02	Stevens, J.	2015	MCW-2222 – A rate-response extended laboratory bioassay of the effects of fresh residues on the parasitic wasp <i>Aphidius rhopalosiphi</i> (Hymenoptera, Braconidae) Report No.: R-35026 Mambo-Tox Ltd., 2 Venture Road, University Science Park, Southampton SO16 7NP, UK GLP: yes Published: no	N	ADAMA
KCP 10.3.2.2/03	Röhlig, U.	2014	Effects of MCW-2222 on the parasitic wasp <i>Aphidius rhopalosiphi</i> (DESTEFANI-PEREZ) STEPH. in an extended laboratory test - Rate-Response-Test (LR50) - Report No.:R-33839A BioChem agrar Labor für biologische und chemische Analytik GmbH. Kupferstraße 6. 04827 Gerichshain, Germany. GLP: yes Published: no	N	ADAMA
KCP 10.3.2.2/04	Röhlig, U.	2014	Effects of MCW-2222 on the green lacewing <i>Chrysoperla carnea</i> STEPH. in an extended laboratory test - Rate-Response-Test (LR50) - Report No.: R-34781 BioChem agrar Labor für biologische und chemische Analytik GmbH. Kupferstraße 6. 04827 Gerichshain, Germany. GLP: yes Published: no	N	ADAMA

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
KCP 10.3.2.2/05	Röhlig, U.	2014	Effects of MCW-2222 on the ladybird <i>Coccinella septempunctata</i> L. in an extended laboratory test -Rate-Response-Test (LR50) - Report No.: R-34782 BioChem agrar Labor für biologische und chemische Analytik GmbH, Kupferstraße 6, 04827 Gerichshain, Germany. GLP: yes Published: no	N	ADAMA
KCP 10.3.2.3/01	Luna, F.	2016	Aged residue test with the formulation “MCW-2222” (Acetamiprid20% w/v SL) at 45 g a.s./ha on the parasitic wasp <i>Aphidius rhopalosiphii</i> (Hymenoptera: Braconidae) Report No.: TRC15-242BA TRIALCAMP, Poligono Industrial de L’Alter Av. Antic Regne de València, 25, 46290 Alcàsser (Valencia) Spain GLP: Yes Published: no	N	ADAMA
KCP 10.3.2.3/02	Luna, F.	2016	Aged residue test with the formulation “MCW-2222” (Acetamiprid20% w/v SL) at 70 g a.s. /ha on the parasitic wasp <i>Aphidius rhopalosiphii</i> (Hymenoptera: Braconidae) Report No.: TRC15-243BA TRIALCAMP, Poligono Industrial de L’Alter Av. Antic Regne de València, 25, 46290 Alcàsser (Valencia) Spain GLP: Yes Published: no	N	ADAMA
KCP 10.3.2.3/03	Luna, F.	2016	Aged residue test with the formulation “MCW-2222” (Acetamiprid20% w/v SL) at 102 g a.s. /ha on the parasitic wasp <i>Aphidius rhopalosiphii</i> (Hymenoptera: Braconidae) Report No.: TRC15-244BA TRIALCAMP, Poligono Industrial de L’Alter Av. Antic Regne de València, 25, 46290 Alcàsser (Valencia) Spain GLP: Yes Published: no	N	ADAMA
KCP 10.3.2.3/04	Luna, F.	2017 a	Aged residue test with the formulation “MCW-2222” at 170 g a.s. /ha on the parasitic wasp <i>Aphidius rhopalosiphii</i> (Hymenoptera: Braconidae) Report No.: TRC16-073BA TRIALCAMP, Poligono Industrial de L’Alter Av. Antic Regne de València, 25, 46290 Alcàsser (Valencia) Spain GLP: Yes Published: no	N	ADAMA
KCP 10.3.2.3/05	Luna, F.	2017 b	Aged residue test with the formulation “MCW-2222” on the predatory mite <i>Typhlodromus pyri</i> (Acari: phytoseiidae) Report No.: R-37335 TRIALCAMP, Poligono Industrial de L’Alter Av. Antic Regne de València, 25, 46290 Alcàsser (Valencia) Spain GLP: Yes Published: no		Adama
KCP 10.3.2.3/06	Luna, F.	2017 c	Aged residue test with the formulation “MCW-2222” on <i>Coccinella septempunctata</i> (Coleoptera: coccinellidae) Report No.: TRC16-075BA / R-37334 TRIALCAMP, Poligono Industrial de L’Alter Av. Antic Regne de València, 25, 46290 Alcàsser (Valencia) Spain GLP: Yes Published: no		Adama
KCP 10.3.2.4/01	Appeltaue A.	2016	A field study assessing the impact of drift rates of acetamiprid on the non-target arthropod fauna on a meadow in Germany	N	Adama

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
			Report No.: R-35848 Eurofins agrosience services EcoChem GmbH/Eurofins. Eutinger Straße 24, D-75223 Niefern-Öschelbronn, Germany. GLP: yes Published: no		
KCP 10.4.1.1/01	Friedrich, S.	2014	MCW-2222 - Sublethal toxicity to the earthworm <i>Eisenia fetida</i> in artificial soil Report No.: R-33840 BioChem agrar Labor für biologische und chemische Analytik GmbH. Kupferstraße 6. 04827 Gerichshain, Germany. GLP: yes Published: no	N	Adama
KCP 10.4.2.1/01	Friedrich, S.	2014	MCW-2222 - Effects on the reproduction of the collembolan <i>Folsomia candida</i> Report No.: R-33841 BioChem agrar Labor für biologische und chemische Analytik GmbH. Kupferstraße 6. 04827 Gerichshain, Germany. GLP: yes Published: no	N	Adama
KCP 10.4.2.1/02	Schulz, L.	2014	Effects of MCW-2222 on the reproduction of the predatory mite <i>Hypoaspis aculeifer</i> Report No.: R-33842 BioChem agrar Labor für biologische und chemische Analytik GmbH. Kupferstraße 6. 04827 Gerichshain, Germany. GLP: yes Published: no	N	Adama
KCP 10.4.2.2/01	Schulz, L.	2022	Effects of Acetamiprid 200 SL on Collembola under field conditions BioChem agrar Labor für biologische und chemische Analytik GmbH, Kupferstraße 6, 04827 Gerichshain, Germany. Project No: 21 48 FCM 0002 GLP: yes Published: no	N	Adama
KCP 10.5/01	Schulz, L.	2014	MCW-2222 - Effects on the activity of soil microflora (Nitrogen transformation test) Report No.: R-33843 BioChem agrar Labor für biologische und chemische Analytik GmbH. Kupferstraße 6. 04827 Gerichshain, Germany. GLP: yes Published: no	N	Adama
KCP 10.6.2/01	Friedrich, S.	2014	Terrestrial plant test with MCW-2222: Vegetative vigour test Report No.: 14 10 48 002 P BioChem agrar Labor für biologische und chemische Analytik GmbH. Kupferstraße 6. 04827 Gerichshain, Germany. GLP: yes Published: no	N	Adama

List of data submitted or referred to by the applicant and relied on, but already evaluated at EU peer review

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner

List of data submitted by the applicant and not relied on

Data point	Author(s)	Year	Title Company Report No. _____ Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
KCP 10.1.1/01	Finch, E., Payne, M., Crocker, J.	2006	Bird and mammal risk assessment: Refining the proportion of diet obtained in the treated crop area (PT) through the use of radio tracking data. Pages 1-48 in: Advisory Committee on Pesticides SC 11419. GLP: no Published: no	N	Public
KCP 10.1.1/02	Lahr J, Krämer W, Mazerolles V, Poulsen V, Jölli D, Müller M, McVey E, Wassenberg J, Derckx R, Brouwer A, Deneer D, Beltman W, Lammertsma D, Jansman H, Buij R	2018	Data collection for the estimation of ecological data (specific focal species, time spent in treated areas collecting food, composition of diet), residue level and residue decline on food items to be used in the risk assessment for birds and mammals. EFSA supporting publication 2018:EN-1513. 155 pp.	N	Public
KCP 10.1.2/14	Leutert, A.	1983	Einfluss der Feldmaus, <i>Microtus arvalis</i> (Pall.), auf die floristische Zusammensetzung von Wiesen-Ökosystemen. Veröffentlichung des Geobotanischen Institutes der Eidg. Techn. Hochschule, Stiftung Rübel, Zürich. GLP: no Published: yes	N	Public
KCP 10.1.2/15	Lüthi, M., W. Nentwig, and J. P. Airolidi	2010	Nutritional ecology of <i>Microtus arvalis</i> (Pallas, 1779) in sown wild flower fields and quasi-natural habitats. <i>Revue Suisse de Zoologie</i> 4:811-828. GLP: no Published: yes		
KCP 10.1.2/12	Jacob, J., Manson, P., Barfknecht, R., Fredricks, T	2013	Common Vole (<i>Microtus Arvalis</i>) Ecology and Management: Implications for Risk Assessment of Plant Protection Products. <i>Pest Management Science</i> 70: 869-878. GLP: no Published: yes	N	Public
KCP 10.1.2/13	Rinke, T.	1991	Percentage of volume versus number of species: availability and intake of grasses and forbs in <i>Microtus arvalis</i> . <i>Folia Zoologica</i> , 40(2), 143-151. GLP: no Published: yes	N	Public
KCP 10.1.2/06	Ebeling, M., Wang, M.	2018	Dissipation of Plant Protection Products From Foliage. <i>Environmental Toxicology and Chemistry</i> 37:1926-1932 GLP: no Published: yes	N	Public
KCP 10.1.2/07	Kaetzke, P., Niedermeier, J., Masseti, M.	2003	<i>Oryctolagus cuniculus</i> (Linné, 1758) Europäisches Wildkaninchen. In: <i>Handbuch der Säugetiere Europas, Lagomorpha</i> (Ed. by Niethammer, J. & Krapp, F.), pp. 189-289: AULA-Verlag. GLP: no Published: yes	N	Public
KCP 10.1.2/08	Gea-Izquierdo, G., Muñoz-Igualada, J., San Miguel-	2005	Gea-Izquierdo, G., Muñoz-Igualada, J. & San Miguel-Ayanz, A. 2005. Rabbit-warren distribution in relation to pasture communities in Mediterranean habitats: consequences for management of rabbit populations. <i>Wildlife Research</i> , 32, 723-731. GLP No.	N	Public

Data point	Author(s)	Year	Title Company Report No. _____ Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
	Ayanz, A.		Published: no		
KCP 10.1.2/09	Calvete, C., Estrada, R., Angulo, E., Cabezas- Ruiz, S.	2004	Habitat factors related to wild rabbit conservation in an agricultural landscape. Landscape Ecology 19:531-542.	N	Public

List of data relied on not submitted by the applicant but necessary for evaluation

Data point	Author(s)	Year	Title Company Report No. _____ Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner

Appendix 2 Detailed evaluation of the new studies

A 2.1	KCP 10.1	Effects on birds and other terrestrial vertebrates
A 2.1.1	KCP 10.1.1	Effects on birds
A 2.1.1.1	KCP 10.1.1.1	Acute oral toxicity
A 2.1.1.2	KCP 10.1.1.2	Higher tier data on birds
A 2.1.2	KCP 10.1.2	Effects on terrestrial vertebrates other than birds

Literature data

Comments of zRMS:	<p>The literature data were evaluated by zRMS-PL for authorisation of the CA3573 in 2021 and was considered as reliable to use in the risk assessment.</p> <p>The zRMS-PL evaluation of residue data has been provided below:</p> <p>First of all, the zRMS would like to point out that very limited options to refine the risk for frugivorous birds and mammals are available and are mainly restricted to refinement of the initial residue levels since residue decline studies in fruits or monitoring studies to determine focal species and refine PT values for frugivores are performed very rarely.</p> <p>In general, at the time of the development of EFSA (2009) extent database of the residue trials was provided by the industry for grass, cereals, non-grass weeds, seeds and tomato. For fruits no respective information has been available from the industry and RUD values for various fruits (including large fruits from orchards) were taken from Baril et al. (2005)⁵. The study authors analysed literature data published between 1970 and 1999 reporting concentrations of pesticides on various crop plants. In addition to that also 25 regulatory residue trials were taken into account, which most probably originated from the registration procedure in the United States (no exact information given). From the whole dataset of 1488 residue values, 33 were relevant for the large fruits from orchards resulting with the mean RUD of 19.5 mg/kg.</p> <p>It is, however, noted that actually no details regarding the residue dataset are available in the publication and only very general information is presented with no description of the methods used in the considered residue trials, so it is not known if they were performed in line with the guidelines relevant for the residue section or methods relevant for derivation of the residue data to be used in the risk assessment for non-target organisms.</p> <p>Furthermore, the study authors indicated that there was a high variability among the residue levels in particular fruit trees, which may be seen on a graph presented in the publication showing that the RUD values ranged from ~0.3 mg/kg to ~30 mg/kg with ~60% of RUD values up to 10 mg/kg and ~40% in range >10-30 mg/kg (it should be noted that these values are read by the zRMS from not very clearly outlined graph, so they are not fully accurate). However, in absence of information on residues in particular large fruits it is not possible to conclude to which group apples belong. Furthermore, in opinion of the zRMS, in case such a variation in results is observed calculation of the mean value to be used as a generic RUD may be questionable as it may lead to under- or overestimation of exposure, depending on the group to which the fruits belong to.</p> <p>The study authors indicated that variation in residue level in fruit trees could not be explained by the tree morphology but the performed analyses showed that the fruit size may play a role, which was the basis to divide orchard fruits into two categories of small and large fruits. It is, however, noted that according to Figure 3 in the publication, the residue level in some large fruits was lower than in small fruits, so there could be also some other factors that had impact on the residue level. As the residue level in particular fruits is not given, it is not known if apples belonged to group containing higher or lower residue levels.</p>
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⁵ Baril A., Whiteside M., Boutin C., 2005: Analysis of a database of pesticide residues on plants for wildlife risk assessment. Environmental Toxicology and Chemistry, Vol. 24, No 2, pp. 360-371, 2005

Since the time when study of Baril et al. (2005) was published and EFSA (2009) issued, multiple regulatory residue trials performed by the industry in orchard crops became available and were gathered by several authors and first published by Hahne et al. in 2019 as a SETAC poster in 2019⁶, referenced in the Applicants' text above. However, the very limited information presented in the poster is not sufficient to be the basis for refinement of the RUD value in fruits. Nevertheless, during the literature review performed by the zRMS the literature study by Schabacker et al. (2020)⁷ has been found, which presents the same results but in the form of the full publication with more information regarding the data collection. The mean RUD of 0.9 mg/kg proposed for food category "large fruits from orchards" has been derived based on results of 127 regulatory residue trials performed according to GLP and accepted either at the EU or MS level. As in case of Baril et al. (2005), large fruits were not divided into sub-categories such as pome fruits (apple, pear), soft fruits (peach, nectarine, apricot) and citrus fruits, but merging of the data for all large fruits was preceded by statistical analysis which demonstrated that no significant differences in residue levels are observed between particular groups of large fruits. The RUD values in large fruits ranged from 0.2 to 4.8 mg/kg and it is noted by the zRMS that the variation in the residue levels was much lower comparing to Baril et al. (2005).

Overall, in opinion of the zRMS the study by Schabacker et al. (2020) seems to be fully reliable and could be potentially used for refinement of the RUD value in apples to address the risk to frugivorous mammals from acetamiprid following application of CA3573. However, the information presented in Schabacker et al. (2020) has been not implemented into the regulatory risk assessment default values and the zRMS has some reservation to refine the RUD based on generic data before they are officially accepted at the EU level and implemented in the revised B&M guidance documents, especially there is large difference between current (19.5 mg/kg) and proposed (0.9 mg/kg) RUD values.

For this reason, it was decided by the zRMS to check first what where the acetamiprid residue levels in regulatory studies submitted in area of Section 7 for CA3573. Table below presents respective data together with RUD values calculated specifically for acetamiprid. Please note that in order to cover worst case, the RUD values were calculated based on maximum residue level, regardless of the DALA. All considered studies were accepted by the zRMS residue expert and their summaries together with zRMS evaluation may be found in the Core Assessment, Part B, Section 7 of May 2021.

Trial	Variety	BBCH at last treatment	No of applications ¹⁾	Rate [kg/ha]	Sampling day	Matrix	Residue level [mg/kg]	RUD [mg/kg] ¹⁾
ChR 14 17311 FR01 Nord Pas de Calais 59400 Fontaine Notre Dame, Northern France N-EU 2014	Idared	85	1	104	0 DALA 3 DALA 7 DALA 14 DALA 21 DALA	Whole fruit	0.08 <u>0.09</u> 0.07 0.03 0.03	1.06
		85	2	104 105	0 DALA 3 DALA 7 DALA 14 DALA 21 DALA	Whole fruit	<u>0.11</u> 0.11 0.11 0.06 0.07	1.06
ChR 14 17311 DE02 Rheinland-Pfalz 67551 Worms Pfeddersheim Germany N-EU 2014	Braeburn	87	2	102 103	0 DALA 3 DALA 7 DALA 14 DALA 21 DALA	Whole fruit	0.20 0.18 0.16 <u>0.21</u> 0.20	2.06

⁶ Hahne J., Schabacker J., Foudoulakis M., Ludwigs J-D., Murfitt R., Ristau K.: New proposed residues on fruits (RUD's) for frugivore scenarios in EFSA bird and mammal risk assessment. Poster at SETAC 2019

⁷ Schabacker J., Hahne J., Ludwigs J-D., Vallon M., Foudoulakis M., Murfitt R., Ristau K., 2020: Residue levels of pesticides on fruits for use in wildlife risk assessments. Integrated Environmental Assessment and Management, Volume 17, Number 3, pp. 552-561

ChR 14 17311 PL03 Lodzkie 99307 Strzelce Poland N-EU 2014	Topaz	85	2	101 101	0 DALA 3 DALA 7 DALA 14 DALA 21 DALA	Whole fruit	0.09 <u>0.10</u> 0.08 0.08 0.06	0.99
DMC-13- 16134 FR01 Centre 37110 Dame Marie les Bois Northern France N-EU 2014	Antares	85	1	98	0 DALA 3 DALA 7 DALA 14 DALA 21 DALA	Whole fruit	<u>0.11</u> 0.09 0.07 0.06 0.06	1.12
		85	2	97 102	0 DALA 3 DALA 7 DALA 14 DALA 21 DALA	Whole fruit	0.17 0.15 <u>0.18</u> 0.11 0.12	1.86

¹⁾ Interval between applications not given as not relevant for the intended GAP with only single application intended in orchards
²⁾ RUD based on maximum residues and lowest application rate in the trial (maximum residue underlined)

Although the range of the RUD values calculated on the basis of results of the residue trials performed with CA3573 (0.99 to 2.06 mg/kg) is well within the range obtained by Schabacker et al. (2020), values obtained for acetamiprid are in general higher than the proposed refined generic RUD of 0.9. For this reason, the zRMS would prefer to use the RUD calculated specifically for acetamiprid, but the number of trials (only 6 with measurements at 0 DALA) is not sufficient for RUD refinement. Nevertheless, all residue data available from the regulatory studies indicate that mean RUD value of 19.5 mg/kg based on Baril et al. (2005) and indicated in EFSA (2009) is highly overestimated.

Taking all available information into account, the zRMS is of the opinion that the risk refinement based on the maximum RUD of 4.8 mg/kg obtained by Schabacker et al. (2020) will be sufficiently protective, as this is the maximum value obtained in 127 trials performed in orchards and is two times higher than maximum RUD calculated specifically for acetamiprid. In opinion of the zRMS this will also cover situation of higher acetamiprid RUD in case more studies with CA3573 were available.

Reference:	KCP 10.1.2.2/29
Report	Residue levels of pesticides on fruits for use in wildlife risk assessments. Schabacker, J., Hahne, J., Ludwigs, J.-D., Vallon, M., Foudoulakis, M., Murfitt, R. and Ristau, K. 2021 Integr Environ Assess Manag, 17: 552-561. https://doi.org/10.1002/ieam.4345
Guideline(s):	Not applicable (publication)
Deviations:	Not applicable (publication)
GLP:	No
Acceptability:	Yes
Duplication (if vertebrate study)	Not applicable

Executive Summary

Data on pesticide residues in fruit crops have been compiled from field studies and are analysed in this publication. The field studies were carried out in the EU over the last 26 years. In the final dataset, 291 studies provided 1002 residue levels in different fruit crops, including grapes, berries (currants, raspberries, gooseberries), orchard fruits (apple, peach, pear, lemon, mandarin, orange, apricot, cherry, plum), pumpkins (gourds, cucumbers, squash, melons) and strawberries. This dataset provides a basis for revising the registration-relevant RUD values for fruit as a potential food for birds and mammals in the context of environmental wildlife risk assessments.

The aim of this study was to estimate the resulting residue levels in different fruits determined under field conditions following the application of pesticides in their growing areas within the EU in different climatic zones, which can be directly used in wildlife risk assessments. The large dataset of generally more than 100 residue values per "fruit group", all evaluated at EU Member State level, resulted in significantly lower RUDs compared to the current EFSA/2009/1438 default RUDs. These new RUD values for fruit should be considered as default values for future risk assessments of birds and mammals and the corresponding guidance documents.

Materials and methods

291 field studies were analysed, conducted between 1991 and 2017. Residue levels on fruit were measured in a varying number of separate field trials (n = 1-8) per study after the application of pesticides (insecticides and fungicides). All study protocols followed regulatory relevant study guidelines (e.g. OECD TG 509, OCSPP 860.1500) and were evaluated by EU member state authorities as being acceptable within the European regulatory processes. Samples were collected on the day(s) of application and on subsequent days.

The final dataset comprised 1002 initial or maximum residue values (each from a field trial conducted to determine the level of pesticide residues in fruit) from the following fruit species: Grapes, currants, raspberries, gooseberries, apples, peaches, pears, lemons, mandarins, oranges, apricots, cherries, plums, pumpkins, cucumbers, gourds, melons and strawberries.

The RUD values for each residue value were calculated by dividing the highest measured value by the amount of pesticide applied (or the amount in the last treatment in case of more than one application) to be conservative.

The data set was analysed in terms of identifiable groups (subgroups) within the relevant EFSA/2009/1438 Guidance fruit groups to identify possible different residue loads due to the fruit type, geographical area from which the data originated etc.

Based on the data distributions, medians and quantiles were calculated as representative parameters for each subset. Means and standard deviations were also calculated and are presented in tabular form, as is the case for the current standard residue data in the EFSA/2009/1438.

Results

The study examined the relationship between pesticide application rates and residue levels in fruits treated together in risk assessments, as specified in the crop groups. The results in the table below are presented in relation to the crop groups specified in EFSA/2009/1438.

Table A3: Proposed new default RUD values calculated for fruit groups calculated according to EFSA/2009/1438

EFSA (2009) crop group	Vineyard	Bush and cane fruit	Orchard	Orchard	Fruiting vegetables	Strawberries
Fruit group analysed	Grapes	Berries ¹	Large fruits ²	Small fruits ³	Gourds ⁴	Strawberries
BBCH stages covered by evaluated studies	79 - 95	75-89	74 - 87	77 - 88	71 - 89	73 - 89
Number of trials = residue values (n)	98	180	127	44	209	138
Mean RUD (sd)	1.6 (1.1)	5.0 (3.6)	0.9 (0.6)	2.8 (1.3)	0.7 (0.7)	1.2 (0.7)
Lower 95% conf. limit	1.4	4.4	0.7	2.4	0.6	1.0
Upper 95% conf. limit	1.8	5.5	1.0	3.2	0.8	1.2
Maximum	5.5	25.2	4.8	6.4	6.3	3.8
90th percentile	2.9	9.2	1.5	4.3	1.3	2.2
Median	1.3	4.6	0.7	2.6	0.6	1.0

Minimum	0.2	0.4	0.2	0.8	0	0.1
¹ Currants, raspberries and gooseberries						
² Apple, peach, pear, lemon, mandarin and orange						
³ RUD value from Cherries (C-EU), covering apricot and plum (C-EU), and cherry apricot, and plum (S-EU) (192 trials)						
⁴ Pumpkins, cucumbers, squash and melons from studies conducted in S-EU (covering 58 additional RUD values from C-EU)						

Discussion

The current default RUD values for fruits in the EFSA/2009/1438 come from the open literature as reviewed by Baril et al. (2005) and are based on a relatively small number of trials (n = 9-33, depending on the fruit group).

In contrast, the RUD values presented here (see table) are based on 291 studies with more than 1000 residue trials. The database available here covers the last 26 years and is therefore more up-to-date, both in terms of pesticides and study design of the residue studies. All studies used in this analysis were conducted according to regulatory study designs and were assessed by EU Member State authorities as acceptable within the European regulatory processes. The fruit residues sampled on the day of application (or the residue peaks reached shortly afterwards) are reported for all required fruit types (including strawberries).

These RUD values are mostly significantly lower compared to the standard RUDs (EFSA 2009). However, compared to the current standard RUD values, the RUD values presented here are considered more relevant for European regulatory processes, as the underlying residue trials were all conducted in European member states and according to the current EU agricultural standards and the data set is much larger overall.

Conclusion

Based on a large data set of residue measurements from a total of more than 1000 independent residue trials, relevant data on fruits as food for birds and mammals could be obtained from usually about ≥100 trials per plant group defined in EFSA/2009/1438. For the calculation of RUDs, the highest residue levels after the last application were used. In addition, specific data on strawberries, currently missing in the EFSA/2009/143 guidance, were provided. The data further confirms that the subdivision of fruit from orchards into small and large orchards (in EFSA/2009/1438) is justified from the RUD concept. These new RUD values are considered relevant and appropriate for use in wildlife risk assessments of pesticides in Europe.

A 2.1.2.1 KCP 10.1.2.1 Acute oral toxicity to mammals

A 2.1.2.2 KCP 10.1.2.2 Higher tier data on mammals

A 2.1.2.2.1 Residue decline study, Weick, S. and Henkes, K., 2017, GLP 299

<p>Comments of zRMS:</p>	<p>The residues of the active substance acetamiprid were determined in foliage-dwelling arthropods and ground vegetation following foliar after spray application of Acetamiprid 200 SL to an apple orchard in Northern Italy. The application of Acetamiprid 200 SL took place on 30 May and 07 June 2016 with a nominal application rate of 0.5 L product/ha (corresponding to 100 g a.s./ha per application) in a nominal spray volume of 1240 L water/ha at pome fruit BBCH stage 73 and 74.</p> <p><u>Ground vegetation</u></p> <p>Ground vegetation was sampled within 3 study plots (= replicates) on DAT -2 and 0, 1, 2, 3, 7, 8, 9, 10, 11, 15, 22 and 29. Samples of ground vegetation were collected by cutting the ground vegetation along transects with scissors just above the soil. Acetamiprid was analysed in the final sample extracts by using LC MS/MS detection. The maximum residue concentration of acetamiprid (arithmetic mean, n=3) in ground vegetation after the first application was 4.77 ± 0.57 mg a.s./kg f.w. (DAT 0) and 7.57 ± 1.16 mg a.s./kg f.w. after the second application (DAT 8).</p> <p>The maximum 90th percentile of acetamiprid residue concentrations (n=3) after the first application was 5.24 mg a.s./kg f.w. (DAT 0) and 8.52 mg a.s./kg f.w. after the second application (DAT 8).</p> <p>Based on calculation the DT₅₀ for residue dissipation of acetamiprid in ground vegetation after the first application was 1.07 days and 1.50 days after the second application using a single first order kinetic (SFO).</p> <div data-bbox="427 1146 975 1697"> <table border="1"> <caption>Data points estimated from Figure 1</caption> <thead> <tr> <th>Time [DAT]</th> <th>Residue levels of acetamiprid [mg a.s./kg f.w.]</th> </tr> </thead> <tbody> <tr> <td>0</td> <td>4.77</td> </tr> <tr> <td>1</td> <td>2.5</td> </tr> <tr> <td>2</td> <td>1.0</td> </tr> <tr> <td>3</td> <td>1.5</td> </tr> <tr> <td>7</td> <td>0.5</td> </tr> </tbody> </table> </div> <p>Figure 1. Parameter estimation of DT₅₀ of acetamiprid (SFO kinetic) in ground vegetation for the time between the first and the second application (DAT 0-7)</p> <p>DT₅₀= 1.07 d, Chi² error 15.93%; t-test: p<0.01; time period: DAT 0-7; carried out with kinetics software tool KinGUII Version 2.1 following single-first order kinetics.</p>	Time [DAT]	Residue levels of acetamiprid [mg a.s./kg f.w.]	0	4.77	1	2.5	2	1.0	3	1.5	7	0.5
Time [DAT]	Residue levels of acetamiprid [mg a.s./kg f.w.]												
0	4.77												
1	2.5												
2	1.0												
3	1.5												
7	0.5												

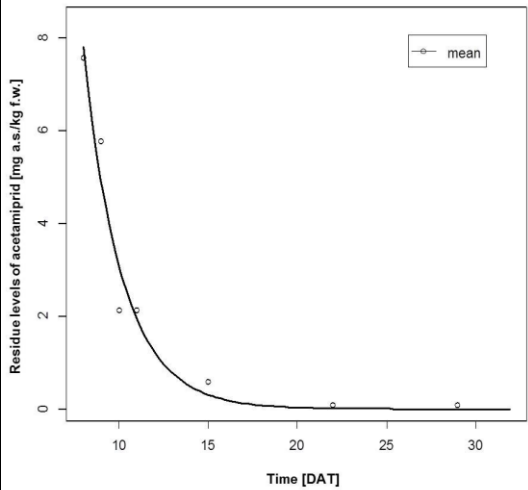


Figure 2. Parameter estimation of DT₅₀ of acetamiprid (SFO kinetic) in ground vegetation for the time after the second application (DAT 8-29)
DT₅₀= 1.50 d, Chi² error 15.54 %; t-test: p<0.001; time period: DAT 8-29; carried out with kinetics software tool KinGUII Version 2.1 following single-first order kinetics.

In general, the kinetic evaluation is considered acceptable by the fate expert. The performed SFO analysis are accepted with good visual fit, however it is noted some overestimation of the model in the degradation rate of the active substance acetamiprid. Since the statistic (Chi² around 15 %) and p-value lower <0.01 are acceptable and the SFO fit give fair visual fit, thus it is considered reliable.

Foliage dwelling arthropods

Foliage-dwelling arthropods were sampled within 3 study plots (= replicates) on DAT -2 and 0, 1, 2, 3, 7, 8, 9, 10, 11, 15, 22 and 29. The number of replicates for arthropods is in line with recommendation given in EFSA for B&M 2009. Foliage-dwellers were collected by inventory spraying. The maximum residue concentration of acetamiprid (arithmetic mean, n=3) in foliage-dwelling arthropods after the first application was 1.01 ± 0.09 mg a.s./kg f.w. (DAT 0) and 1.41 ± 0.44 mg a.s./kg f.w. after the second application (DAT 8). The maximum 90th percentile of acetamiprid residue concentrations (n=3) after the first application was 1.08 mg a.s./kg f.w. (DAT 0) and 1.74 mg a.s./kg f.w. after the second application (DAT 8). The DT₅₀ for residue dissipation of acetamiprid in foliage-dwelling arthropods after the first application was 1.40 days and 1.88 days after the second application using a Single First Order kinetic (SFO). The data for foliage dwelling organism was not used in the risk assessment as the risk is resolved based on Tier 1.

Data point	KCP 10.1.2/01
Report	Residues of acetamiprid in foliage-dwelling arthropods and ground vegetation after spray application of Acetamiprid 200 SL in a pome fruit orchard in Italy – magnitude of residues and time course of residue decline, Weick, S. and Henkes, K., 2017
Report No.:	R1640039
Document No.:	R-37376
Guideline(s):	No guidelines available / The study design follows the general

recommendations of the EFSA Guidance Document on Risk assessments for Birds and Mammals (EFSA 2009)

Deviations:	None to guidance; two minor deviations to the study plan without any impact on the study outcome.
GLP:	Yes, certified laboratory
Acceptability:	Yes, study considered acceptable
Duplication (if vertebrate study)	Not applicable

Executive Summary

Arthropods and ground vegetation are important food sources for insectivorous, herbivorous and omnivorous birds and mammals in agricultural habitats. Application of MCW-2222 (synonym Acetamiprid 200 SL) may cause residues on arthropods. In order to provide a basis for assessing the risk of Acetamiprid 200 SL to insectivorous, herbivorous and omnivorous birds and mammals, residues of the active substance acetamiprid were determined following foliar spray application of Acetamiprid 200 SL to an apple orchard in Northern Italy. The application of Acetamiprid 200 SL took place in compliance with GLP on 30 May and 07 June 2016 with a nominal application rate of 0.5 L product/ha (corresponding to 100 g a.s./ha per application) in a nominal spray volume of 1240 L water/ha at pome fruit BBCH stage 73 and 74.

Foliage-dwelling arthropods were sampled within 3 study plots (= replicates) on DAT -2 and 0, 1, 2, 3, 7, 8, 9, 10, 11, 15, 22 and 29. Foliage-dwellers were collected by inventory spraying. Acetamiprid was analysed in the final sample extracts by using LC MS/MS detection. The maximum residue concentration of acetamiprid (arithmetic mean, n=3) in foliage-dwelling arthropods after the first application was 1.01 ± 0.09 mg a.s./kg f.w. (DAT 0) and 1.41 ± 0.44 mg a.s./kg f.w. after the second application (DAT 8). The maximum 90th percentile of acetamiprid residue concentrations (n=3) after the first application was 1.08 mg a.s./kg f.w. (DAT 0) and 1.74 mg a.s./kg f.w. after the second application (DAT 8). The DT50 for residue dissipation of acetamiprid in foliage-dwelling arthropods after the first application was 1.40 days and 1.88 days after the second application using a Single First Order kinetic (SFO).

Ground vegetation was sampled within 3 study plots (= replicates) on DAT -2 and 0, 1, 2, 3, 7, 8, 9, 10, 11, 15, 22 and 29. Samples of ground vegetation were collected by cutting the ground vegetation along transects with scissors just above the soil. Acetamiprid was analysed in the final sample extracts by using LC MS/MS detection. The maximum residue concentration of acetamiprid (arithmetic mean, n=3) in ground vegetation after the first application was 4.77 ± 0.57 mg a.s./kg f.w. (DAT 0) and 7.57 ± 1.16 mg a.s./kg f.w. after the second application (DAT 8). The maximum 90th percentile of acetamiprid residue concentrations (n=3) after the first application was 5.24 mg a.s./kg f.w. (DAT 0) and 8.52 mg a.s./kg f.w. after the second application (DAT 8). The DT50 for residue dissipation of acetamiprid in ground vegetation after the first application was 1.07 days and 1.50 days after the second application using a single first order kinetic (SFO).

Materials and Methods

Materials

Test item	Acetamiprid 200 SL
Batch #	469-129-01
Content of active substance	200 g acetamiprid/L (nominal), 205.1 g acetamiprid/L (analysed)
Description	Clear yellow to brown

Test organism

Species	Foliage-dwelling arthropods
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Study design and methods

Study location	Saluzzo (Province: Cuneo), Northern Italy
Experimental dates	25 May 2016 to 31 Aug 2016 (including Analytical Phase)
Application date	30 May 2016 and 07 June 2016

Test concentrations	Acetamiprid 200 SL was applied twice at a nominal application rate of 0.5 L product/ha (corresponding to 100 g a.s./ha per application). Actual application rates ^{a)} were 102.74 g a.s./ha in a spray volume of 1242.26 L water/ha (first application) and 102.78 g a.s./ha in a spray volume of 1242.73 L water/ha (second application) for study plot 1,
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	102.57 g a.s./ha in a spray volume of 1240.25L water/ha (first application) and 102.55 g a.s./ha in a spray volume of 1240.05 L water/ha (second application) for study plot 2, and 102.19 g a.s./ha in a spray volume of 1235.62 L water/ha (first application) and 102.30 g a.s./ha in a spray volume of 1236.94 L water/ha (second application) for study plot 3.
Study plots	Within the study orchard, three study plots (replicates) were established each with an area of approximately 1 ha.
Group size/replicates	13 sampling events took place with samples taken on each of the 3 study plots (n=3) in a period of 32 days on DAT (Days After Treatment) -2 and 0, 1, 2, 3, 7, 8, 9, 10, 11, 15, 22 and 29. At each sampling event, between 31-51 trees were sampled in the inventory spraying to collect foliage-dwelling arthropods. For the ground vegetation sampling, the size of the sampling area was 350 x 10 cm at each sampling.
Test duration	32 days
BBCH growth stage at time of application	73-74
Environmental conditions	
Temperature	Min-max: 9.8° – 36.6 °C (Non-GLP)
Precipitation	77 mm in total, 15 rain events on DAT -1, 0, 1, 2, 4, 5, 6, 10, 11, 13, 15, 17, 18, 19 and 26 (Non-GLP)
a) Calculated based on actual Acetamiprid content stated in the certificate of analysis.	

Study design and methods

The study was conducted at an apple orchard in Saluzzo (Province: Cuneo), Northern Italy. Within the study orchard, three study plots (replicates) were established each with an area of approximately 1 ha. All arthropods and ground vegetation were collected within these three study plots.

Foliage-dwelling arthropods were collected by inventory spraying (using a knock-down insecticide AquaPy (natural pyrethrins: 30 g/L and Piperonylbutoxid: 150 g/L); Non GLP spraying). Each study plot was divided into 24 spray sampling areas (=lots) for inventory spraying. Within each lot >70 m tree rows were treated to obtain the foliage-dwelling arthropods and collected from cotton sheets. No lot was treated twice with AquaPy. A total of 39 samples were collected between 28 May 2016 and 28 June 2016. Foliage-dwellers were sampled two days before application of the test item (pre-sampling, DAT -2) to obtain reference matrix for residue analysis. Further samples were taken on DAT 0 (approximately 4 hours after first application), 1, 2, 3, 7, 8 (approximately 4 hours after second application), 9, 10, 11, 15, 22 and 29. After determination of the taxonomic composition of each arthropod sample to order or family level, the arthropod samples were weighed and stored at a temperature of ≤ -18 °C until transport to the Test Site of the Analytical Phase.

For choosing the area of vegetation sampling, the lot set-up of inventory spraying was used. In each study plot/lot a transect from row to row, crossing the grass stripe between the tree rows was defined. The ground vegetation was cut with scissors just above the soil along each transect. The size of each transect (sampling area) was 350 cm x 10 cm. The minimum matrix mass per sample of 25 g was obtained on every sampling day. Sampling was conducted at comparable sites within the sampling areas of each study plot (comparable ground vegetation composition). No area was sampled twice in the course of the Sampling Phase. The vegetation cover and height was recorded at each sampling.

A total of 39 samples were collected between 28 May 2016 and 28 June 2016. Ground vegetation was sampled two days before the first application of the test item (DAT -2) to obtain reference matrix for residue analysis. Further samples were taken on DAT 0 (approx. 4 hours after first application), 1, 2, 3, 7, 8 (approx. 4 hours after second application), 9, 10, 11, 15, 22, and 29.

The time between the end of sampling and storage in the freezer never exceeded four hours. Following each sampling event, the ground vegetation sampled was directly transported in an insulated box to the

accommodation for further processing. The cut plant material was weighted and separately bagged in plastic bag samples and stored at a temperature of $\leq -18^{\circ}\text{C}$ until transport to the Test Site of the Analytical Phase.

Analytical measurements

Acetamiprid were analysed in the final sample extracts by using LC MS/MS detection.

Data evaluation and statistics

The initial and maximal concentrations of acetamiprid on foliage-dwelling arthropods and ground vegetation were calculated based on the arithmetic mean of three study plots (=replicates, $n=3$). AUC (Area Under the Curve), a time-weighted average residue, mean residue concentrations of acetamiprid were calculated for 21-day periods (moving window) and the maximum AUC out of them is reported. Residue concentrations of acetamiprid were used to calculate the RUDs (Residue per Unit Dose), based on an application rate of 1.0 kg a.s./ha. The DT_{50} of acetamiprid was calculated using single first order kinetics (SFO). Goodness of fit was evaluated with three criteria: visual fit, Chi^2 error level ($<15\%$) and t-test ($p < 0.05$). The DT_{50} was calculated with KinGUII.

Results and Discussion

Analytical measurements

The Limit of Quantification (LOQ) and the Limit of Detection (LOD) for acetamiprid in arthropods and ground vegetation were 0.01 mg a.s./kg f.w. and 0.003 mg a.s./kg fresh weight (f.w.), respectively.

Procedural recovery/quality control data

The analytical method used in the current study was previously validated in study R1640039, (Henkes, 2017). See Section 5.1.2. In order to confirm the analytical method was functioning correctly when used in the current study, procedural recovery samples were processed and analysed concurrently with the test sample and are summarised in the table below.

Table A 1: Procedural recovery data for acetamiprid in arthropods and ground vegetation reported in study R1640039

Matrix	Fortification level [mg/kg]	Recovery (%)		RSD (%)	n
		Range	Mean		
Arthropods	0.01 (LOQ)	70 – 102	81	18	4
	0.1	84 – 85	84	0.7	3
	1.0	101	-	-	1
	10	100	-	-	1
Ground vegetation	0.01 (LOQ)	94 – 104	99	4.3	4
	0.1	97 – 102	100	2.5	3
	1.0	104	-	-	1
	10	89	-	-	1

Analytical results & statistics

The acetamiprid residue content in foliage-dwelling arthropods collected from all study plots two days before application (DAT -2) of the formulation Acetamiprid 200 SL was below the LOD of the analytical method, i.e. below 0.003 mg a.s./kg f.w.

Measured residue concentrations of acetamiprid on pea and wheat plants, RUD values and the calculated DT_{50} are given in the following table.

Table A 2: Residues of acetamiprid on foliage-dwelling arthropods and ground vegetation following the application of Acetamiprid 200 SL (nominally containing 200 g acetamiprid/L) at a rate of 0.5 L product/ha (nominally 100 g a.s./ha)

	Foliage-dwellers (mg a.s./kg f.w.)		Ground vegetation (mg a.s./kg f.w.)	
	After first application	After second application	After first application	After second application
Initial concentration of mean residues \pm SD (n=3) ^{a)}	1.01 \pm 0.09 (DAT 0)	1.41 \pm 0.44 (DAT 8)	4.77 \pm 0.57 (DAT 0)	7.57 \pm 1.16 (DAT 8)
Initial concentration of mean residues \pm SD (n=3), RUD ^{b)}	9.82 \pm 0.87 (DAT 0)	13.75 \pm 4.32 (DAT 8)	46.51 \pm 5.68 (DAT 0)	73.80 \pm 11.36 (DAT 8)
Maximum concentration of mean residues \pm SD (n=3) ^{a)}	1.01 \pm 0.09 (DAT 0)	1.41 \pm 0.44 (DAT 8)	4.77 \pm 0.57 (DAT 0)	7.57 \pm 1.16 (DAT 8)
Maximum concentration of mean residues \pm SD (n=3), RUD ^{b)}	9.82 \pm 0.87 (DAT 0)	13.75 \pm 4.32 (DAT 8)	46.51 \pm 5.68 (DAT 0)	73.80 \pm 11.36 (DAT 8)
Maximum 90 th percentile ^{a)}	1.08 (DAT 0)	1.74 (DAT 8)	5.24 (DAT 0)	8.52 (DAT 8)
Maximum 90 th percentile, RUD ^{b)}	10.52 (DAT 0)	16.97 (DAT 8)	51.24 (DAT 0)	83.12 (DAT 8)
Maximum AUC over 21 days ^{a)}	0.36 (DAT 0-20)		1.71 (DAT 0-20)	
DT ₅₀ (calculated with SFO) Chi2 error (%), t-test (p value)	1.40 d 8.36, p<0.01	1.88 d 8.67, p<0.001	1.07 d 15.93, p<0.01	1.50 d 15.54, p<0.001

^{a)} residues based on a nominal application rate corresponding to 100 g a.s./ha

^{b)} calculated with the actual application rate and converted to an application rate of 1.0 kg a.s./ha

RUD=Residue per Unit Dose

AUC=Area Under the Curve

d=days

DAT=Days After first Treatment

f.w.=fresh weight

SD=standard deviation

SFO=Single First Order kinetic

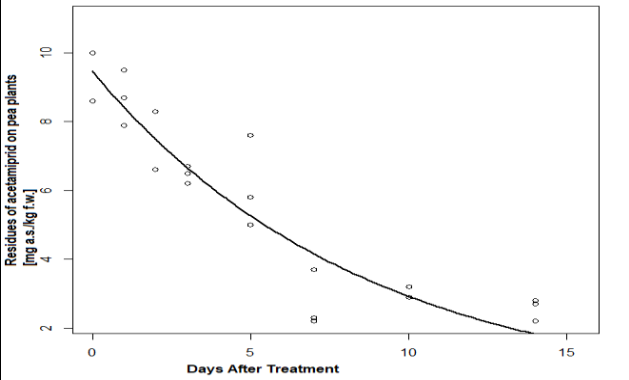
Conclusions

The study provides field data on the magnitude of initial residue levels and the subsequent time course of residue decline of acetamiprid in foliage-dwelling arthropods and ground vegetation. The maximum residue concentration of acetamiprid (arithmetic mean, n=3) in foliage-dwelling arthropods after the first application was 1.01 \pm 0.09 mg a.s./kg f.w. (DAT 0) and 1.41 \pm 0.44 mg a.s./kg f.w. after the second application (DAT 8). The maximum 90th percentile of acetamiprid residue concentrations (n=3) after the first application was 1.08 mg a.s./kg f.w. (DAT 0) and 1.74 mg a.s./kg f.w. after the second application (DAT 8). The DT₅₀ for residue dissipation of acetamiprid in foliage-dwelling arthropods after the first application was 1.40 days and 1.88 days after the second application using a Single First Order kinetic (SFO).

The maximum residue concentration of acetamiprid (arithmetic mean, n=3) in ground vegetation after the first application was 4.77 \pm 0.57 mg a.s./kg f.w. (DAT 0) and 7.57 \pm 1.16 mg a.s./kg f.w. after the second application (DAT 8). The maximum 90th percentile of acetamiprid residue concentrations (n=3) after the first application was 5.24 mg a.s./kg f.w. (DAT 0) and 8.52 mg a.s./kg f.w. after the second application (DAT 8). The DT₅₀ for residue dissipation of acetamiprid in ground vegetation after the first application was 1.07 days and 1.50 days after the second application using a single first order kinetic (SFO).

These data provide reliable values of initial and maximum residues, AUC and DT₅₀ values for use in higher tier risk assessments for insectivorous, herbivorous and omnivorous birds and mammals.

A 2.1.2.2.2 **Residue decline study, Brehm, C. and Staffel, J., 2021, GLP 513**

Comments of zRMS:	<p>The study was provided to estimate the residues of acetamiprid in monocotyledonous and dicotyledonous plants after spray application of Acetamiprid 200 SL The study was conducted on an arable field in Alcarrás (Region: Catalonia, Province: Lleida) in Northern Spain.</p> <p>The application of Acetamiprid 200 SL took place on 29 September 2020 with a nominal application rate of 0.875 L product/ha (175 g acetamiprid/ha) in a spray volume of 200 L/ha. The application took place at BBCH growth stage 15-18 on the wheat and 17-19 on the pea study plots.</p> <p>Within the study field 3 pea study plots (=replicates) and 3 wheat study plots (=replicates) were set up with areas of 0.052 ha. Each study plots was equally divided into 9 sampling areas (=lots). All plants were collected within the 6 study plots. Vegetation was cut with scissors just above the soil (without roots or seed remains). The sampling was conducted at 5 different locations within the each lot. Samples were collected at DAT -1, 0 (~ 1 - 3 h after application), 1, 2, 3, 5, 7, 10 and 14.</p> <p>The matrix mass per sample was 43.23 g – 127.96 g (at least 20 plants).</p> <p>Residue concentrations of acetamiprid were used to calculate the RUDs (Residue per Unit Dose), based on an application rate of 1.0 kg a.s./ha.</p> <p>The DT₅₀ of acetamiprid was calculated using single first order kinetics (SFO). Goodness of fit was evaluated with three criteria: visual fit, Chi² error level and t test.</p> <p><u>Pea plants:</u></p> <p>The initial and maximum residue concentration (arithmetic mean, n=3) of acetamiprid on pea plants was 9.5 ± 0.08 mg a.s./kg f.w. at DAT 0 (~ 1 - 3 h after application). Towards the last sampling event on DAT 14 the detected acetamiprid residues (arithmetic mean, n=3) declined to 2.57 ± 0.32 mg a.s./kg f.w.</p>  <p>Figure 1. The DT₅₀ of 5.9 days (SFO kinetic, Chi² 9.21%, t test: p < 0.001) was calculated considering residue concentrations from DAT 0 (~ 1-3 h after application) to DAT 14.</p> <p><u>Wheat plants</u></p> <p>The initial and maximum residue concentration (arithmetic mean, n=3) of acetamiprid on wheat plants was 8.3 ± 0.7 mg a.s./kg f.w. at DAT 0 (approximately 1-3 h after application).</p>
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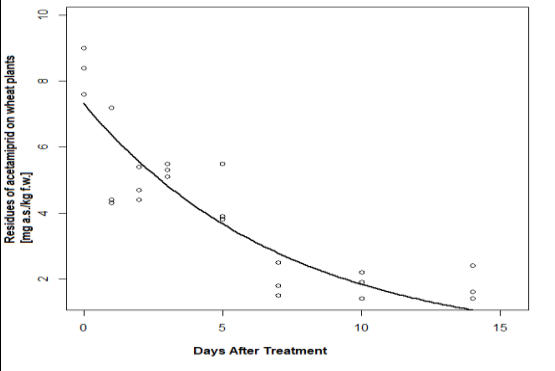


Figure 2. The DT₅₀ of 5.0 days (SFO kinetic, Chi² 14.52%, t test: p < 0.001) was calculated considering residue concentrations from DAT 0 (~ 1 - 3 h after application) to DAT 14.

In general, the kinetic evaluation is considered acceptable by e fate expert. The performed SFO analysis are accepted with good visual fit, however some overestimation of the model in the degradation rate of the active substance acetamiprid was noted. The residue of acetamiprid on pea plants of 3.1 mg/kg observed on DAT 10 could be an outlier as it was considerably higher than the residue of 2.6 mg/kg observed on DAT 14, however it could be considered within an acceptable margin of error. For the wheat plants it was noted that the residue of acetamiprid of 5.3 mg/kg observed on DAT3 could be also an outlier as it was higher than the residue of 4.4 mg/kg observed on DAT 5. Taking this into account, additional SFO analysis has been performed by the zRMS efate expert using CAKE Version 3.7 with residue at DAT10 excluded for pea plants and DAT3 for wheat plants. Since not improvement was observed (Chi2 at the same level 9.22% for pea plants and even increased from 14.52 % to 15.7% for the wheat plants, p-value below 0.01) and the SFO fit give fair visual fit, presented study is consider reliable.

Data point	KCP 10.1.2/02
Report	Residues of acetamiprid in monocotyledonous and dicotyledonous plants after spray application of Acetamiprid 200 SL in early vegetation stages in Spain – magnitude of residues and time course of residue decline, Brehm, C. and Staffel, J., 2021
Report No.:	R2040056
Document No.:	000106551
Guideline(s):	No guidelines available / The study design follows the general recommendations of the EFSA Guidance Document on Risk assessments for Birds and Mammals (EFSA 2009)
Deviations:	None to guidance; a minor deviation to the study plan without any impact on the study outcome.
GLP:	Yes, certified laboratory
Acceptability:	Yes, study considered acceptable
Duplication (if vertebrate study)	Not applicable

Executive Summary

Monocotyledonous and dicotyledonous plants are important food sources for herbivorous and omnivorous birds and mammals in agricultural habitats. Application of MCW-2222, ADM.00150.I.2.A (synonym Acetamiprid 200 SL) may cause residues on the plants. In order to provide a basis for the assessment of the risk for herbivorous and omnivorous birds and mammals, residue levels of the active substance acetamiprid were determined after spray application of Acetamiprid 200 SL in a wheat (representing monocotyledonous

plants) and pea (representing dicotyledonous plants) field in Spain ~~Western Germany~~. The application of Acetamiprid 200 SL took place in compliance with GLP on 29 September 2020 with a nominal application rate of 0.875 L product/ha (175 g acetamiprid/ha) in a spray volume of 200 L/ha.

Pea and wheat plants were collected at 3 study plots (=replicates) on DAT -1, 0 (~1 - 3 h after application), 1, 2, 3, 5, 7, 10 and 14. Acetamiprid was analysed in the final sample extracts by using LC MS/MS detection. The initial and maximum residue concentration (arithmetic mean, n=3) of acetamiprid on pea plants was 9.5 ± 0.08 mg a.s./kg f.w. at DAT 0 (~1 - 3 h after application). The DT₅₀ of 5.9 days (SFO kinetic, Chi² 9.21%, t-test: p < 0.001) was calculated considering residue concentrations from DAT 0 (~1 - 3 h after application) to DAT 14. The initial and maximum residue concentration (arithmetic mean, n=3) of acetamiprid on wheat plants was 8.3 ± 0.7 mg a.s./kg f.w. at DAT 0 (approximately 1-3 h after application). The DT₅₀ of 5.0 days (SFO kinetic, Chi² 14.52%, t-test: p < 0.001) was calculated considering residue concentrations from DAT 0 (~1 - 3 h after application) to DAT 14.

Materials and Methods

Materials

Test item	Acetamiprid 200 SL
Lot #	99191024
Content of active substance	200 g acetamiprid/L (nominal), 200.1 g acetamiprid/L (analysed)
Description	Clear yellow to brown
Test organism	
Species	Monocotyledonous plants represented by wheat (variety: Balbona) and dicotyledonous represented by pea (variety: Mitic)

Study design and methods

Study location	Alcarrás (Region: Catalonia, Province: Lleida), Northern Spain
Experimental dates	06 Aug 2020 to 13 Jan 2021 (including Analytical Phase)
Sowing	Sowing (Non-GLP) took place on 13 July 2020 with a sowing rate of 240 kg wheats/ha and 300 kg peas/ha.
Application date	10 May 2019
Test concentrations	Nominal application rate of 0.875 L product/ha (175 g acetamiprid/ha). Actual application rates ^{a)} were 176.7 g a.s./ha in a spray volume of 202.8 L water/ha for study plot 1, 180.3 g a.s./ha in a spray volume of 206.9 L water/ha for study plot 2 and 174.1 g a.s./ha in a spray volume of 199.8 L water/ha for study plot 3, 176.7 g a.s./ha in a spray volume of 202.7 L water/ha for study plot 4, 172.2 g a.s./ha in a spray volume of 196.6 L water/ha for study plot 5 and 174.0 g a.s./ha in a spray volume of 199.7 L water/ha for study plot 6.
Study plots	Within the study field three wheat study plots (replicates 1 – 3) and three pea study plots (replicates 4 – 6) were established, each with sizes of 0.052 ha.
Group size/replicates	9 sampling events took place with samples taken on each of the 3 study plots per crop separately (n=3) in a period of 16 days on DAT (Days After Treatment) -1, 0 (~1 - 3 h after application), 2, 3, 5, 7, 10 and 14. At each sampling event wheat and pea plants were collected at 5 different locations within a predefined separate sampling area (=lot) in each study plot, respectively.
Test duration	16 days
BBCH growth stage at time of application	17-19 (pea), 17-19 (wheat)
Environmental conditions	
Temperature	22.9° – 28.6 °C (Non-GLP)
Precipitation	3.2 mm in total, 5 rain events on DAT -1, 3, 5, 11 and 14 (Non-GLP)

^{a)} Calculated with the analysed content of 20.01% acetamiprid/L

Study design and methods

The study was conducted on an arable field (0.052 ha) in Alcarrás (Region: Catalonia, Province: Lleida) in

Northern Spain. Within the study field 3 pea study plots (=replicates) and 3 wheat study plots (=replicates) were set up with areas of 0.052 ha. Each study plots was equally divided into 9 sampling areas (=lots). All plants were collected within the 6 study plots. Vegetation was cut with scissors just above the soil (without roots or seed remains). The sampling was conducted at 5 different locations within the each lot. Samples were collected at DAT -1, 0 (~ 1 - 3 h after application), 1, 2, 3, 5, 7, 10 and 14. The matrix mass per sample was 43.23 g – 127.96 g (at least 20 plants).

All samples were stored at a temperature of $\leq -18^{\circ}\text{C}$ until transport to the Test Site of the Analytical Phase (Eurofins GmbH). The storage temperature at the Analytical Test Site was $\leq -18^{\circ}\text{C}$ with no exceedance.

Analytical measurements

Acetamiprid were analysed in the final sample extracts by using LC MS/MS detection.

Data evaluation and statistics

The initial and maximal concentrations of acetamiprid on pea and wheat plants were calculated based on the arithmetic mean of three study plots (=replicates, $n=3$). Residue concentrations of acetamiprid were used to calculate the RUDs (Residue per Unit Dose), based on an application rate of 1.0 kg a.s./ha. The DT_{50} of acetamiprid was calculated using single first order kinetics (SFO). Goodness of fit was evaluated with three criteria: visual fit, χ^2 error level and t-test.

Results and Discussion

Analytical measurements

The Limit of Quantification (LOQ) and the Limit of Detection (LOD) for acetamiprid on pea and wheat plants were 0.01 mg a.s./kg fresh weight (f.w.) and 0.003 mg a.s./kg f.w., respectively.

Procedural recovery/quality control data

The analytical method used in the current study was previously validated in study R1640039, (Henkes, 2017). See Section 5.1.2. In order to confirm the analytical method was functioning correctly when used in the current study, procedural recovery samples were processed and analysed concurrently with the test sample and are summarised in the table below.

Table A 3: Procedural recovery data for acetamiprid in arthropods reported in study R2040056

Matrix	Fortification level [mg/kg]	Recovery (%)		RSD (%)	n
		Range	Mean		
Wheat plant	0.01	97 – 100	98	1.8	3
	0.10	100 – 105	102	2.2	3
	20	104 – 118	110	6.4	3
Pea plant	0.01	103 – 107	104	1.4	3
	0.10	104 – 112	108	3.3	3
	20	98 – 108	104	5.3	3

Analytical results & statistics

The acetamiprid residue content on pea and wheat plants collected from all study plots before application (DAT -1) of Acetamiprid 200 SL was below the LOD of the analytical method, i.e. < 0.003 mg a.s./kg f.w.. Measured residue concentrations of acetamiprid on pea and wheat plants, RUD values and the calculated DT_{50} are given in the following table.

Table A 4: Residues of acetamiprid on pea and wheat plants following the application of Acetamiprid 200 SL (nominally containing 200 g acetamiprid/L) at a rate of 0.875 L product/ha (nominally 175 g a.s./ha)

Day after treatment	Mean residue (\pm Standard Deviation) [mg acetamiprid/kg f.w.] ^{a)}	
	Pea plants	Wheat plants
-1	< LOD	< LOD
0 (~1-3 h)	9.5 (\pm 0.8)	8.3 (\pm 0.7)
1	8.7 (\pm 0.8)	5.3 (\pm 1.7)
2	7.2 (\pm 1.0)	4.8 (\pm 0.5)
3	6.5 (\pm 0.3)	5.3 (\pm 0.2)
5	6.1 (\pm 1.3)	4.4 (\pm 1.0)
7	2.7 (\pm 0.8)	1.9 (\pm 0.5)
10	3.1 (\pm 0.2)	1.8 (\pm 0.4)
14	2.6 (\pm 0.3)	1.8 (\pm 0.5)
Initial concentration	9.5	8.30
Maximal concentration	9.5	8.30
DT ₅₀ (days)	5.9	5.0
Kinetic model	SFO	SFO
Chi ² error	9.21%	14.52%
t-test (p value)	p < 0.001	p < 0.001

LOD = < 0.003 mg a.s./kg f.w.

^{a)} Values shown are the mean of 3 replicate samples

Table A 5: Residues of acetamiprid on pea and wheat plants based on an application rate of 1.0 kg a.s./ha (RUDs)

Day after treatment	Mean residue (\pm Standard Deviation) [mg acetamiprid/kg f.w.] ^{a)}	
	Pea plants ^{b)}	Wheat plants ^{b)}
-1	< LOD	< LOD
0 (~1-3 h)	57.4 (\pm 0.7)	47.5 (\pm 0.8)
1	49.9 (\pm 4.9)	30.0 (\pm 9.4)
2	41.1 (\pm 5.1)	27.3 (\pm 3.3)
3	37.1 (\pm 1.7)	30.0 (\pm 1.5)
5	35.1 (\pm 7.3)	24.9 (\pm 5.5)
7	15.6 (\pm 4.6)	10.9 (\pm 2.9)
10	17.8 (\pm 0.8)	10.4 (\pm 2.4)
14	14.7 (\pm 1.9)	10.2 (\pm 3.0)
Initial concentration	57.4	47.5
Maximal concentration	57.4	47.5

LOD = < 0.003 mg a.s./kg f.w.

^{a)} Values shown are the mean of 3 replicate samples;

^{b)} Calculated with the actual application rates

Conclusions

The study provides field data on the magnitude of initial residue levels and the subsequent time course of residue decline of the active substance acetamiprid on wheat (monocotyledonous) and pea (dicotyledonous) plants.

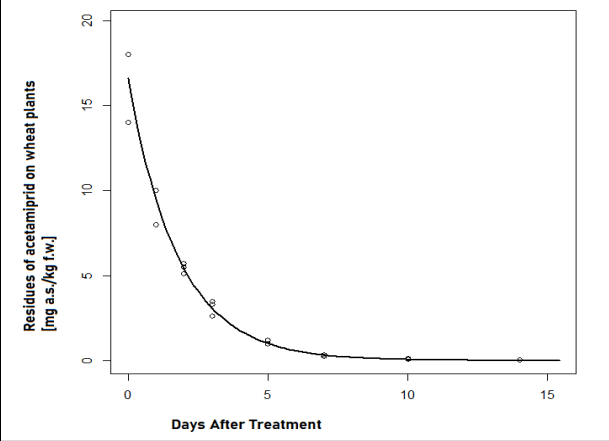
The initial and maximum residue concentration (arithmetic mean, n=3) of acetamiprid on pea plants was 9.5 ± 0.08 mg a.s./kg f.w. at DAT 0 (~ 1 - 3 h after application; arithmetic mean, n=3). Towards the last sampling event at DAT 14 the detected acetamiprid declined to 2.6 ± 0.3 mg a.s./kg f.w. The initial and maximum RUD concentration of acetamiprid (arithmetic mean, n=3) on pea plants was 57.4 ± 0.7 mg a.s./kg f.w. (DAT 0, ~ 1 - 3 h after application; arithmetic mean, n=3).

The initial and maximum residue concentration of acetamiprid (arithmetic mean, n=3) on wheat plants was 47.5 ± 0.8 mg a.s./kg f.w. at DAT 0 (~ 1 - 3 h h after application). Thereafter residue concentrations declined to 8.3 ± 0.7 mg a.s./kg f.w. (DAT 14; arithmetic mean, n=3). The initial and maximum RUD of acetamiprid on pea plants was 10.2 ± 3.0 at DAT 0 (~ 1 - 3 h after application; arithmetic mean, n=3).

The DT₅₀ of 5.9 days (SFO kinetic, Chi² 9.21%, t-test: p < 0.001) on pea plants was calculated considering residue concentrations from DAT 0 (~ 1 - 3 h after application) to DAT 14. The DT₅₀ of 5.0 days (SFO kinetic, Chi² 14.52%, t-test: p < 0.001) on wheat plants was calculated considering residue concentrations

from DAT 0 (~ 1 - 3 h after application) to DAT 14.
These data provide a reliable basis of initial and maximum residues, as well as DT₅₀ values for use in higher tier risk assessment for herbivorous and omnivorous birds and mammals.

A 2.1.2.2.3 Residue decline study, Brehm, C. and Staffel, J., 2021, GLP 517

Comments of zRMS:	<p>The active substance acetamiprid were determined after spray application of Acetamiprid 200 SL in a wheat (representing monocotyledonous plants) and pea (representing dicotyledonous plants) field in central Germany. The application of Acetamiprid 200 SL took place in compliance with GLP on 29 September 2020 with a nominal application rate of 0.875 L product/ha (175 g acetamiprid/ha) in a spray volume of 300 L/ha. The application took place at BBCH growth stage 13 on the wheat and 14 on the pea study plots.</p> <p>Pea and wheat plants were collected at 3 study plots (=replicates) on DAT 0 (before application), 0 (~1 h after application), 1, 2, 3, 5, 7, 10 and 14. Acetamiprid was analysed in the final sample extracts by using LC MS/MS detection.</p> <p><u>Wheat plants</u></p> <p>The initial and maximum residue concentration of acetamiprid (arithmetic mean, n=3) on wheat plants was 16.67 ± 2.31 mg a.s./kg f.w. on DAT 0. Towards the last sampling event on DAT 14 the detected acetamiprid residue concentration (arithmetic mean, n=3) declined to 0.03 ± 0.001 mg a.s./kg f.w.</p>  <table border="1"><caption>Approximate data points from the residue decline graph on wheat plants</caption><thead><tr><th>Days After Treatment</th><th>Residues of acetamiprid [mg a.s./kg f.w.]</th></tr></thead><tbody><tr><td>0</td><td>16.67 ± 2.31</td></tr><tr><td>1</td><td>~10</td></tr><tr><td>2</td><td>~6</td></tr><tr><td>3</td><td>~4</td></tr><tr><td>5</td><td>~2</td></tr><tr><td>7</td><td>~1</td></tr><tr><td>10</td><td>~0.5</td></tr><tr><td>14</td><td>0.03 ± 0.001</td></tr></tbody></table> <p>The DT₅₀ of 1.24 days (SFO kinetic, Chi² 1.39%, t-test: p<0.001) was calculated considering residue concentrations from DAT 0 to DAT 14.</p> <p><u>Pea plants</u></p> <p>The initial and maximum residue concentration of acetamiprid (arithmetic mean, n=3) on pea plants was 4.57 ± 0.31 mg a.s./kg f.w. on DAT 0. Towards the last sampling event on DAT 14 the detected acetamiprid residues (arithmetic mean, n=3) declined to 0.62 ± 0.07 mg a.s./kg f.w.</p>	Days After Treatment	Residues of acetamiprid [mg a.s./kg f.w.]	0	16.67 ± 2.31	1	~10	2	~6	3	~4	5	~2	7	~1	10	~0.5	14	0.03 ± 0.001
Days After Treatment	Residues of acetamiprid [mg a.s./kg f.w.]																		
0	16.67 ± 2.31																		
1	~10																		
2	~6																		
3	~4																		
5	~2																		
7	~1																		
10	~0.5																		
14	0.03 ± 0.001																		

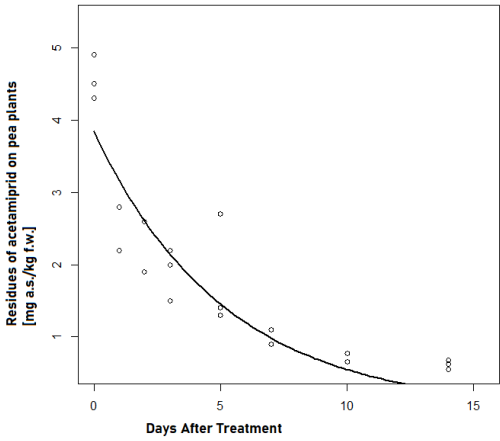


Figure 2. The DT₅₀ for residue dissipation of acetamiprid on pea plants was 3.56 days using a single first order kinetic (SFO, Chi² error: 17.99 %, t-test: p<0.001).

In general, the kinetic evaluation of study performed in Germany is considered acceptable. The performed SFO analysis are accepted with low Chi2 error level (9.21%) and t test below 0.01 and good visual fit of the model in the degradation rate of the active substance acetamiprid on pea plants.

Since the SFO fit give very good visual fit and statistic is acceptable, thus study performed on wheat and pea plants is consider reliable.

Data point	KCP 10.1.2/03
Report	Residues of acetamiprid in monocotyledonous and dicotyledonous plants after spray application of Acetamiprid 200 SL in early vegetation stages in Germany – magnitude of residues and time course of residue decline, Brehm, C. and Staffel, J., 2021
Report No.:	R2040057
Document No.:	000106552
Guideline(s):	No guidelines available / The study design follows the general recommendations of the EFSA Guidance Document on Risk assessments for Birds and Mammals (EFSA 2009)
Deviations:	None to guidance; a minor deviation to the study plan without any impact on the study outcome.
GLP:	Yes, certified laboratory
Acceptability:	Yes, study considered acceptable
Duplication (if vertebrate study)	Not applicable

Executive Summary

Monocotyledonous and dicotyledonous plants are important food sources for herbivorous and omnivorous birds and mammals in agricultural habitats. Application of MCW-2222, ADM.00150.I.2.A (synonym Acetamiprid 200 SL) may cause residues on the plants. In order to provide a basis for the assessment of the risk for herbivorous and omnivorous birds and mammals, residue levels of the active substance acetamiprid were determined after spray application of Acetamiprid 200 SL in a wheat (representing monocotyledonous plants) and pea (representing dicotyledonous plants) field in central Germany. The application of

Acetamiprid 200 SL took place in compliance with GLP on 29 September 2020 with a nominal application rate of 0.875 L product/ha (175 g acetamiprid/ha) in a spray volume of 300 L/ha.

Pea and wheat plants were collected at 3 study plots (=replicates) on DAT 0 (before application), 0 (~1 h after application), 1, 2, 3, 5, 7, 10 and 14. Acetamiprid was analysed in the final sample extracts by using LC MS/MS detection. The initial and maximum residue concentration (arithmetic mean, n=3) of acetamiprid on pea plants was 4.57 ± 0.31 mg a.s./kg f.w. at DAT 0 (approximately 1 h after application). The DT₅₀ of 3.56 days (SFO kinetic, Chi² 17.99%, t-test: p < 0.001) was calculated considering residue concentrations from DAT 0 (~ 1 h after application) to DAT 14. The initial and maximum residue concentration (arithmetic mean, n=3) of acetamiprid on wheat plants was 16.67 ± 2.31 mg a.s./kg f.w. at DAT 0 (~ 1 h after application). The DT₅₀ of 1.24 days (SFO kinetic, Chi² 1.39%, t-test: p < 0.001) was calculated considering residue concentrations from DAT 0 (~ 1 h after application) to DAT 14.

Materials and Methods

Materials

Test item	Acetamiprid 200 SL
Lot #	99191024
Content of active substance	200 g acetamiprid/L (nominal), 200.1 g acetamiprid/L (analysed)
Description	Clear yellow to brown
Test organism	
Species	Monocotyledonous plants represented by wheat (variety: Solehio) and dicotyledonous represented by pea (variety: Navarro)

Study design and methods

Study location	Brensbach (Hesse, Germany)
Experimental dates	29 Sep 2020 to 06 Apr 2021 (including Analytical Phase)
Sowing	Sowing (Non-GLP) took place on 01 Oct 2020 with a sowing rate of 250 kg wheat/ha and 280 kg pea/ha.
Application date	29 Sep 2020
Test concentrations	Nominal application rate of 0.875 L product/ha (175 g acetamiprid/ha). Actual application rates ^{a)} were 171.8 g a.s./ha in a spray volume of 295 L water/ha for study plot 1, 188.4 g a.s./ha in a spray volume of 324 L water/ha for study plot 2 and 182.9 g a.s./ha in a spray volume of 314 L water/ha for study plot 3, 177.4 g a.s./ha in a spray volume of 305 L water/ha for study plot 4, 177.4 g a.s./ha in a spray volume of 305 L water/ha for study plot 5 and 177.4 g a.s./ha in a spray volume of 305 L water/ha for study plot 6.
Study plots	Within the study field three wheat study plots (replicates 1 – 3) and three pea study plots (replicates 4 – 6) were established, each with sizes of 0.105 ha.
Group size/replicates	9 sampling events took place with samples taken on each of the 3 study plots per crop separately (n=3) in a period of 15 days on DAT (Days After Treatment) 0 (before application, 0 (~ 1 h after application), 1, 2, 3, 5, 7, 10 and 14. At each sampling event wheat and pea plants were collected at 5 different locations within a predefined separate sampling area (=lot) in each study plot, respectively.
Test duration	15 days
BBCH growth stage at time of application	13 (pea), 13 (wheat)
Environmental conditions	
Temperature	6.4° – 15.1 °C (Non-GLP)
Precipitation	17.8 mm in total, 12 rain events on DAT 0, 1, 2, 4, 5, 6, 7, 8, 9, 10, 13 and 14 (Non-GLP)

^{a)} Calculated with the analysed content of 20.01% acetamiprid/L

Study design and methods

The study was conducted on an arable field (0.63 ha) in Brensbach (state: Hesse) in Germany. Within the

study field 3 pea study plots (=replicates) and 3 wheat study plots (=replicates) were set up with areas of 0.105 ha. Each study plots was equally divided into 9 sampling areas (=lots). All plants were collected within the 6 study plots. Vegetation was cut with scissors just above the soil (without roots or seed remains). The sampling was conducted at 5 different locations within the each lot. Samples were collected at DAT 0 (before application), 0 (~ 1 h after application), 1, 2, 3, 5, 7, 10 and 14. The matrix mass per sample was 17.89 g – 150.61 g (at least 20 plants).

All samples were stored at a temperature of ≤ -18 °C until transport to the Test Site of the Analytical Phase (Eurofins GmbH). The storage temperature at the Analytical Test Site was ≤ -18 °C with no exceedance.

Analytical measurements

Acetamiprid were analysed in the final sample extracts by using LC MS/MS detection.

Data evaluation and statistics

The initial and maximal concentrations of acetamiprid on pea and wheat plants were calculated based on the arithmetic mean of three study plots (=replicates, n=3). Residue concentrations of acetamiprid were used to calculate the RUDs (Residue per Unit Dose), based on an application rate of 0.175 kg a.s./ha. The DT₅₀ of acetamiprid was calculated using single first order kinetics (SFO). Goodness of fit was evaluated with three criteria: visual fit, Chi² error level and t-test.

Results and Discussion

Analytical measurements

The Limit of Quantification (LOQ) and the Limit of Detection (LOD) for acetamiprid on pea and wheat plants were 0.01 mg a.s./kg fresh weight (f.w.) and 0.003 mg a.s./kg f.w., respectively.

Procedural recovery/quality control data

The analytical method used in the current study was previously validated in study R1640039, (Henkes, 2017). See Section 5.1.2. In order to confirm the analytical method was functioning correctly when used in the current study, procedural recovery samples were processed and analysed concurrently with the test sample and are summarised in the table below.

Table A 6: Procedural recovery data for acetamiprid in arthropods reported in study R2040057

Matrix	Fortification level [mg/kg]	Recovery (%)		RSD (%)	n
		Range	Mean		
Wheat plant	0.01	76 – 79	78	2.3	3
	0.10	81 – 84	83	2.0	3
	50	84 – 87	85	1.8	3
Pea plant	0.01	92 – 101	97	4.7	3
	0.10	99 – 106	103	3.3	3
	10	97 – 100	98	1.8	3

Analytical results & statistics

The acetamiprid residue content on pea and wheat plants collected from all study plots before application (DAT 0) of Acetamiprid 200 SL was below the LOD of the analytical method, i.e. < 0.003 mg a.s./kg f.w. Measured residue concentrations of acetamiprid on pea and wheat plants, RUD values and the calculated DT₅₀ are given in the following table.

Table A 7: Residues of acetamiprid on pea and wheat plants following the application of Acetamiprid 200 SL (nominally containing 200 g acetamiprid/L) at a rate of 0.875 L product/ha (nominally 175 g a.s./ha)

Day after treatment	Mean residue (\pm Standard Deviation) [mg acetamiprid/kg f.w.] ^{a)}	
	Pea plants	Wheat plants
0 (before application)	< LOD	< LOD
0 (~1 h)	4.57 (\pm 0.31)	16.67 (\pm 2.31)
1	2.40 (\pm 0.35)	9.33 (\pm 1.15)
2	2.37 (\pm 0.40)	5.43 (\pm 0.31)
3	1.90 (\pm 0.36)	3.13 (\pm 0.47)
5	1.80 (\pm 0.78)	1.13 (\pm 0.12)
7	1.03 (\pm 0.12)	0.27 (\pm 0.06)
10	0.73 (\pm 0.06)	0.11 (\pm 0.02)
14	0.62 (\pm 0.07)	0.03 (\pm 0.01)
Initial concentration	4.57	16.67
Maximal concentration	4.57	16.67
DT₅₀ (days)	3.56	1.24
Kinetic model	SFO	SFO
Chi² error	17.99%	1.39%
t-test (p value)	p < 0.001	p < 0.001

LOD = < 0.003 mg a.s./kg f.w.

^{a)} Values shown are the mean of 3 replicate samples

Table A 8: Residues of acetamiprid on pea and wheat plants based on an application rate of 1.0 kg a.s./ha (RUDs)

Day after treatment	Mean residue (\pm Standard Deviation) [mg acetamiprid/kg f.w.] ^{a)}	
	Pea plants ^{b)}	Wheat plants ^{b)}
0 (before application)	< LOD	< LOD
0 (~1 h)	28 (\pm 0.0)	77 (\pm 3.7)
1	14 (\pm 2.0)	52 (\pm 8.3)
2	13 (\pm 2.3)	30 (\pm 2.6)
3	11 (\pm 2.0)	17 (\pm 2.0)
5	10 (\pm 4.4)	6.3 (\pm 0.76)
7	5.8 (\pm 0.65)	1.5 (\pm 0.31)
10	4.1 (\pm 0.36)	0.61 (\pm 0.07)
14	3.5 (\pm 0.37)	0.19 (\pm 0.04)
Initial concentration	28	77
Maximal concentration	28	77

LOD = < 0.003 mg a.s./kg f.w.

^{a)} Values shown are the mean of 3 replicate samples;

^{b)} Calculated with the actual application rates

Conclusions

The study provides field data on the magnitude of initial residue levels and the subsequent time course of residue decline of the active substance acetamiprid on wheat (monocotyledonous) and pea (dicotyledonous) plants.

The initial and maximum residue concentration (arithmetic mean, n=3) of acetamiprid on pea plants was 4.57 ± 0.31 mg a.s./kg f.w. at DAT 0 (~ 1 h after application; arithmetic mean, n=3). Towards the last sampling event at DAT 14 the detected acetamiprid declined to 0.62 ± 0.07 mg a.s./kg f.w. The initial and maximum RUD concentration of acetamiprid (arithmetic mean, n=3) on pea plants was 28 ± 0.0 mg a.s./kg f.w. (DAT 0, ~ 1 h after application; arithmetic mean, n=3).

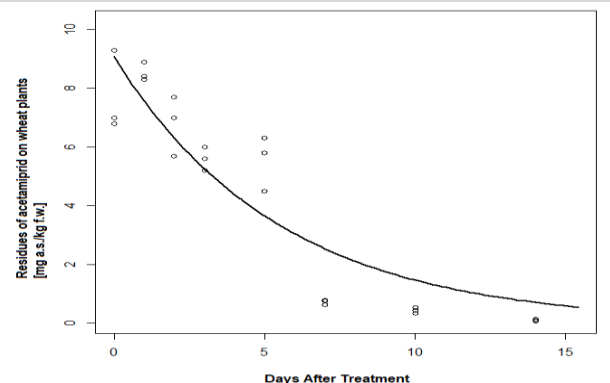
The initial and maximum residue concentration of acetamiprid (arithmetic mean, n=3) on wheat plants was 16.67 ± 2.31 mg a.s./kg f.w. at DAT 0 (~ 1 h after application). Thereafter residue concentrations declined to 0.03 ± 0.01 mg a.s./kg f.w. (DAT 14; arithmetic mean, n=3). The initial and maximum RUD of acetamiprid on wheat plants was 77 ± 3.7 at DAT 0 (~ 1 h after application; arithmetic mean, n=3).

The DT₅₀ of 3.56 days (SFO kinetic, Chi² 17.99%, t-test: p < 0.001) on pea plants was calculated considering residue concentrations from DAT 0 (~ 1 h after application) to DAT 14. The DT₅₀ of 1.24 days (SFO kinetic, Chi² 1.39%, t-test: p < 0.001) on wheat plants was calculated considering residue concentrations from DAT

0 (~1 h after application) to DAT 14.

These data provide a reliable basis of initial and maximum residues, as well as DT₅₀ values for use in higher tier risk assessment for herbivorous and omnivorous birds and mammals.

A 2.1.2.2.4 Residue decline study, Staffel, J. and Brehm, C., 2022, GLP 519

<p>Comments of zRMS:</p>	<p>The study was conducted on an arable field (1.56 ha) in Brensbach (state: Hesse) in Germany. The application of Acetamiprid 200 SL took place on 22 April 2021 with a nominal application rate of 0.875 L product/ha (175 g acetamiprid/ha) in a spray volume of 300 L/ha. The application took place at BBCH growth stage 12 on the wheat and at 10- 11 on the pea study plots.</p> <p>Within the study field 3 pea study plots (=replicates) and 3 wheat study plots (=replicates) were set up with areas of 0.120 ha. Each study plot was equally divided into 9 sampling areas (=lots). All plants were collected within the 6 study plots. Vegetation was cut with scissors just above the soil (without roots or seed remains). The sampling was conducted at 5 different locations within each lot. Samples were collected at DAT -1, 0 (~ 3 h after application), 1, 2, 3, 5, 7, 10 and 14. The matrix mass per sample was 15.25 g – 15.68 g (at least 20 plants).</p> <p><u>Wheat plants</u></p> <p>The initial residue concentration (arithmetic mean, n=3) of acetamiprid in wheat plants was 7.70 mg a.s./kg f.w. on DAT 0 and the maximum residue concentration (arithmetic mean, n=3) of acetamiprid in wheat plants was 8.53 mg a.s./kg f.w. on DAT 1.</p>  <p>Figure 1. The DT₅₀ of 3.8 days (SFO kinetic, Chi² error 21.54%, t-test: p < 0.001) was calculated considering residue concentrations from DAT 0 to DAT 14.</p> <p><u>Pea plants</u></p> <p>The initial and maximum residue concentration (arithmetic mean, n=3) of acetamiprid in pea plants was 7.27 mg a.s./kg f.w. on DAT 0. The DT₅₀ of 2.4 days (SFO kinetic, Chi² error 5.78 %, t-test: p<0.001) was calculated considering residue concentrations from DAT 0 to DAT 14. The initial and maximum residue concentration of acetamiprid (arithmetic mean, n=3) on pea plants was 7.27 ± 1.68 mg a.s./kg f.w. on DAT 0.</p>
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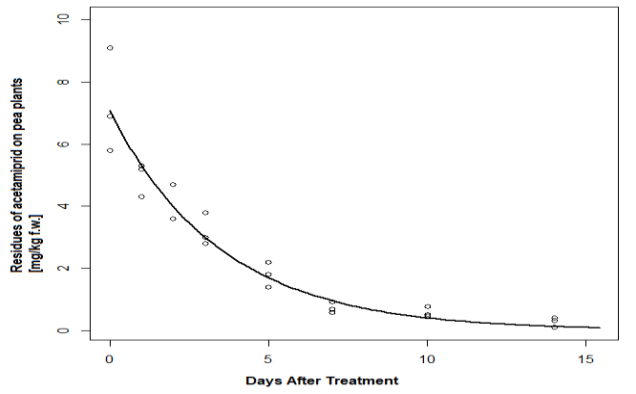


Figure 2. The DT₅₀ of 2.4 days (SFO kinetic, Chi² error 5.78 %, t-test: p<0.001) was calculated considering residue concentrations from DAT 0 to DAT 14.

In general, the kinetic evaluation of study performed in Germany is considered acceptable by e fate expert.

The performed SFO analysis are accepted with low Chi2 error level and t test below 0.01 and good visual fit of the model in the degradation rate of the active substance acetamiprid on pea plants.

However, it is noted that the evaluation of the degradation kinetics performed by the Applicant on wheat plants showed that the residue of acetamiprid of 8.53 mg/kg observed on DAT1 could be an outlier as it was considerably higher than the residue of 7.70 mg/kg observed directly after the application. Taking this into account, additional SFO analysis has been performed by the zRMS efate expert using CAKE Version 3.7 with residue at DAT0 excluded. Since not significant improvement was observed (Chi2 was reduced from 21.54% to 20.01%, p-value below 0.01) and anyway the SFO fit give acceptable visual fit, presented study of degradation kinetics on wheat plants is consider reliable

Data point	KCP 10.1.2/04
Report	Residues of acetamiprid in monocotyledonous and dicotyledonous plants after spray application of Acetamiprid 200 SL in early vegetation stages in spring in Germany – magnitude of residues and time course of residue decline Staffel, J. and Brehm, C., 2022
Report No.:	R2040059
Document No.:	000106554
Guideline(s):	No guidelines available / The study design follows the general recommendations of the EFSA Guidance Document on Risk assessments for Birds and Mammals (EFSA 2009)
Deviations:	None to guidance; three minor deviations to the study plan without any impact on the study outcome
GLP:	Yes, certified laboratory
Acceptability:	Yes, study considered acceptable
Duplication (if vertebrate study)	Not applicable

Executive Summary

Monocotyledonous and dicotyledonous plants are important food sources for herbivorous and omnivorous birds and mammals in agricultural habitats. Application of MCW-2222, ADM.00150.I.2.A (synonym Acetamiprid 200 SL) may cause residues on the plants. In order to provide a basis for the assessment of the risk for herbivorous and omnivorous birds and mammals, residue levels of the active substance acetamiprid were determined after spray application of Acetamiprid 200 SL in a wheat (representing monocotyledonous plants) and pea (representing dicotyledonous plants) field in central Germany. The application of

Acetamiprid 200 SL took place in compliance with GLP on 22 April 2021 with a nominal application rate of 0.875 L product/ha (175 g acetamiprid/ha) in a spray volume of 300 L/ha.

Pea and wheat plants were collected at 3 study plots (=replicates) on DAT -1 (before application), 0 (~3 h after application), 1, 2, 3, 5, 7, 10 and 14. Acetamiprid was analysed in the final sample extracts by using LC MS/MS detection. The initial residue concentration (arithmetic mean, n=3) of acetamiprid in wheat plants was 7.70 mg a.s./kg f.w. on DAT 0 and the maximum residue concentration (arithmetic mean, n=3) of acetamiprid in wheat plants was 8.53 mg a.s./kg f.w. on DAT 1. The DT₅₀ of 3.8 days (SFO kinetic, Chi² error 21.54%, t-test: p < 0.001) was calculated considering residue concentrations from DAT 0 to DAT 14. The initial and maximum residue concentration (arithmetic mean, n=3) of acetamiprid in pea plants was 7.27 mg a.s./kg f.w. on DAT 0. The DT₅₀ of 2.4 days (SFO kinetic, Chi² error 5.78 %, t-test: p<0.001) was calculated considering residue concentrations from DAT 0 to DAT 14.

Materials and Methods

Materials

Test item	Acetamiprid 200 SL
Batch #	99191024
Content of active substance	200 g acetamiprid/L (nominal), 200.1 g acetamiprid/L (analysed)
Description	Clear yellow to brown
Test organism	
Species	Monocotyledonous plants represented by wheat (feed wheat) and dicotyledonous represented by pea (variety: Trendy)

Study design and methods

Study location	Brensbach (Hesse, Germany)
Experimental dates	12 Apr 2021 to 22 Sep 2021 (including Analytical Phase)
Sowing	Sowing (Non-GLP) took place on 29 Mar 2021 with a sowing rate of 180 kg wheat/ha and 250 kg pea/ha.
Application date	22 Apr 2021
Test concentrations	The nominal application rate was 0.875 L product/ha (175 g acetamiprid/ha). The actual application rates ^{a)} were 174.5 g a.s./ha in a spray volume of 300 L water/ha for study plot 1, 169.7 g a.s./ha in a spray volume of 292 L water/ha for study plot 2 and 179.4 g a.s./ha in a spray volume of 308 L water/ha for study plot 3, 174.5 g a.s./ha in a spray volume of 300 L water/ha for study plot 4, 174.5 g a.s./ha in a spray volume of 300 L water/ha for study plot 5 and 160.0 g a.s./ha in a spray volume of 275 L water/ha for study plot 6.
Study plots	Within the study field three wheat study plots (replicates 1 – 3) and three pea study plots (replicates 4 – 6) were established, each with a size of 0.120 ha.
Group size/replicates	9 sampling events took place with samples taken on each of the 3 study plots per crop separately (n=3) in a period of 16 days, on the day before application (DAT (Day After Treatment) -1), and after application (approximately 3 hours) on DAT 0, 1, 2, 3, 5, 7, 10 and 14. At each sampling event wheat and pea plants were collected at 5 different locations within a predefined separate sampling area (=lot) in each study plot, respectively.
Test duration	16 days
BBCH growth stage at time of application	12 (wheat), 10-11 (pea)
Environmental conditions	
Temperature	-1.5° – 25.4 °C (Non-GLP)
Precipitation	12.0 mm in total, 2 rain events on DAT 7 and 14 (Non-GLP)

^{a)} Calculated with the analysed content of 20.01% acetamiprid/L

Study design and methods

The study was conducted on an arable field (1.56 ha; size non-GLP) in Brensbach (state: Hesse) in

Germany. Within the study field 3 pea study plots (=replicates) and 3 wheat study plots (=replicates) were set up with areas of 0.120 ha. Each study plot was equally divided into 9 sampling areas (=lots). All plants were collected within the 6 study plots. Vegetation was cut with scissors just above the soil (without roots or seed remains). The sampling was conducted at 5 different locations within each lot. Samples were collected at DAT -1, 0 (~ 3 h after application), 1, 2, 3, 5, 7, 10 and 14. The matrix mass per sample was 15.25 g – 15.68 g (at least 20 plants).

All samples were stored at a temperature of $\leq -18^{\circ}\text{C}$ until transport to the Test Site of the Analytical Phase (Eurofins Chem GmbH, see Deviation 1). The storage temperature at the Analytical Test Site was $\leq -18^{\circ}\text{C}$ with an exception from 21 April 2021, 17:25 to 22 April 2021, 10:25, where the temperature up to -8.27°C , due to a malfunction of the freezer. However, the samples were always in a frozen condition

Deviation from the Study Plan

Results and Discussion Deviation 1: Sample storage

All samples should be stored at $\leq -18^{\circ}\text{C}$ in a freezer and temperatures from $>-18^{\circ}\text{C}$ to 15°C for a maximal time period of 12 hours or from -15°C to -8°C for a maximal time period of 6 hours are tolerable and are no deviations. Due to a malfunction of the freezer, the temperature of the samples exceeded -15°C from 17:25 (21 April 2021) until 10:25 (22 April 2021), the highest temperature was -8.27°C . However, the samples were always in a frozen condition.

Results and Discussion Deviation 2: Sample transport

Due to a malfunction of the data logger for temperature recording, there was no temperature recording during the shipment. However, when arriving at the Analytical Test Site, there was dry ice remaining and the samples were deep frozen.

Results and Discussion Deviation 3: Study field

By mistake, the buffer zone around the lots was 0.5 m instead of 1 m.

Analytical measurements

Acetamiprid were analysed in the final sample extracts by using LC MS/MS detection.

Data evaluation and statistics

The initial and maximal concentrations of acetamiprid on pea and wheat plants were calculated based on the arithmetic mean of three study plots (=replicates, $n=3$). Residue concentrations of acetamiprid were used to calculate the RUDs (Residue per Unit Dose), based on an application rate of 0.175 kg a.s./ha. The DT_{50} of acetamiprid was calculated using single first order kinetics (SFO). Goodness of fit was evaluated with three criteria: visual fit, Chi^2 error level and t-test.

Results and Discussion

Analytical measurements

The Limit of Quantification (LOQ) and the Limit of Detection (LOD) for acetamiprid on pea and wheat plants were 0.01 mg a.s./kg and 0.0025 mg a.s./kg, respectively.

Procedural recovery/quality control data

The analytical method used in the current study was previously validated in study R1640039, (Henkes, 2017). See Section 5.1.2. In order to confirm the analytical method was functioning correctly when used in the current study, procedural recovery samples were processed and analysed concurrently with the test sample and are summarised in the table below.

Table A 9: Procedural recovery data for acetamiprid in arthropods reported in study R2040059

Matrix	Fortification level [mg/kg]	Recovery (%)		RSD (%)	n
		Range	Mean		
Wheat plant	0.01	107 – 113	110	3.0	3
	0.10	93 – 97	95	2.4	3
	10	104	104	0.3	3
Pea plant	0.01	101 – 102	102	0.5	3
	0.10	109 – 111	110	1.0	3
	10	107 – 110	108	1.3	3

Analytical results & statistics

The acetamiprid residue content in wheat plants sampled one day before the application (DAT -1) was

below the LOD of the analytical method, i.e. below 0.0025 mg a.s./kg in all three study plots.
Measured residue concentrations of acetamiprid on pea and wheat plants, RUD values and the calculated DT₅₀ are given in the following table.

Table A 10: Residues of acetamiprid on pea and wheat plants following the application of Acetamiprid 200 SL (nominally containing 200 g acetamiprid/L) at a rate of 0.875 L product/ha (nominally 175 g a.s./ha)

Day after treatment	Mean residue (± Standard Deviation) [mg acetamiprid/kg f.w.] ^{a)}	
	Pea plants	Wheat plants
-1 (before application)	< LOD	< LOD
0	7.27 (±1.68)	7.70 (±1.39)
1	4.93 (±0.55)	8.53 (±0.32)
2	3.97 (±0.64)	6.80 (±1.01)
3	3.20 (±0.53)	5.60 (±0.40)
4	2.50 (±0.46)	5.57 (±0.62)
5	1.80 (±0.40)	5.53 (±0.93)
6	1.27 (±0.19)	3.13 (±0.45)
7	0.73 (±0.17)	0.73 (±0.08)
8	0.68 (±0.09)	0.63 (±0.03)
9	0.63 (±0.08)	0.53 (±0.04)
10	0.59 (±0.16)	0.43 (±0.10)
11	0.51 (±0.15)	0.35 (±0.08)
12	0.43 (±0.15)	0.27 (±0.06)
13	0.35 (±0.15)	0.19 (±0.04)
14	0.28 (±0.16)	0.10 (±0.04)
Initial concentration	7.27	7.70
Maximal concentration	7.27	8.53
DT₅₀ (days)	2.4	3.8
Kinetic model	SFO	SFO
Chi² error	5.78%	21.54%
t-test (p value)	p < 0.001	p < 0.001

LOD = < 0.0025 mg a.s./kg

^{a)} Values shown are the mean of 3 replicate samples

Table A 11: Residues of acetamiprid on pea and wheat plants based on an application rate of 1.0 kg a.s./ha (RUDs)

Day after treatment	Mean residue (± Standard Deviation) [mg acetamiprid/kg f.w.] ^{a)}	
	Pea plants ^{b)}	Wheat plants ^{b)}
0 (before application)	< LOD	< LOD
0	42.65 (±8.39)	44.01 (±6.78)
1	29.17 (±4.06)	48.89 (±1.15)
2	23.35 (±3.24)	39.09 (±6.86)
3	18.86 (±2.87)	32.06 (±1.40)
4	14.74 (±2.59)	31.85 (±2.90)
5	10.63 (±2.35)	31.65 (±4.83)
6	7.48 (±1.32)	17.91 (±2.35)
7	4.34 (±1.26)	4.17 (±0.58)
8	4.05 (±0.74)	3.60 (±0.24)
9	3.75 (±0.52)	3.03 (±0.14)
10	3.45 (±0.86)	2.45 (±0.48)
11	3.00 (±0.84)	1.99 (±0.37)
12	2.55 (±0.85)	1.52 (±0.28)
13	2.09 (0.88)	1.06 (±0.21)
14	1.64 (±0.93)	0.59 (±0.20)
Initial concentration	42.65	44.01
Maximal concentration	42.65	48.89

LOD = < 0.0025 mg a.s./kg

^{a)} Values shown are the mean of 3 replicate samples;

^{b)} Calculated with the actual application rates

Conclusions

The study provides field data on the magnitude of initial residue levels and the subsequent time course of residue decline of acetamiprid on monocotyledonous (wheat) plants and dicotyledonous (pea) plants after spray application of Acetamiprid 200 SL.

The initial residue concentration (arithmetic mean, n=3) of acetamiprid on wheat plants was 7.70 ± 1.39 mg a.s./kg f.w. on DAT 0, the maximum residue concentration of acetamiprid on wheat plants was 8.53 ± 0.32 mg a.s./kg f.w. on DAT 1. The DT₅₀ of 3.8 days (SFO kinetic, Chi² error 21.54 %, t-test: p<0.001) was calculated considering residue concentrations from DAT 0 to DAT 14.

The initial and maximum residue concentration of acetamiprid (arithmetic mean, n=3) on pea plants was 7.27 ± 1.68 mg a.s./kg f.w. on DAT 0. The DT₅₀ of 2.4 days (SFO kinetic, Chi² error 5.78 %, t-test: p<0.001) was calculated considering residue concentrations from DAT 0 to DAT 14.

Weather conditions were adequate for the conduct of the study.

These data provide a reliable basis of initial and maximum residues, as well as DT₅₀ values and residue decline of acetamiprid in monocotyledonous and dicotyledonous plants for the use in higher tier risk assessments for herbivorous and omnivorous birds and mammals.

A 2.1.2.2.5 Residue decline study, Gräf, K., 2022, GLP 520

Comments of zRMS:	<p>The field study to estimate residues of acetamiprid in monocotyledonous and dicotyledonous plants after spray application of Acetamiprid 200 SL in Northern Europe-Denmark has been conducted. The application took place at BBCH growth stage 14-15 on the wheat and 15 on the pea study plots.</p> <p>The residue levels of the active substance acetamiprid were determined after spray application of Acetamiprid 200 SL in a wheat (representing monocotyledonous plants) and pea (representing dicotyledonous plants) field in Demark, Northern Europe. The application of Acetamiprid 200 SL took place in compliance with GLP on 29 May 2021 with a nominal application rate of 0.875 L product/ha (175 g acetamiprid/ha) in a spray volume of 300 L/ha. Pea and wheat plants were collected at 3 study plots (=replicates) on DAT -1 (before application), on DAT 0 (1.5-2 h after application), 1, 2, 3, 5, 7, 10 and 14. Acetamiprid was analysed in the final sample extracts by using LC MS/MS detection.</p> <p><u>Wheat plants</u></p> <p>The initial and maximum residue concentration (arithmetic mean, n=3) of acetamiprid in wheat plants was 14.3 ± 1.5 mg a.s./kg f.w. on DAT 0.</p> <p>Towards the last sampling event on DAT 14 the detected acetamiprid residue concentration (arithmetic mean, n=3) declined to < LOQ.</p> <div data-bbox="427 1422 1110 1845"> <table border="1"> <caption>Approximate data points from the residue decline graph for wheat plants</caption> <thead> <tr> <th>Days After Treatment</th> <th>Residues of acetamiprid [mg a.s./kg f.w.]</th> </tr> </thead> <tbody> <tr><td>0</td><td>14.3</td></tr> <tr><td>1</td><td>12.5</td></tr> <tr><td>2</td><td>10.5</td></tr> <tr><td>3</td><td>8.5</td></tr> <tr><td>5</td><td>5.5</td></tr> <tr><td>7</td><td>3.5</td></tr> <tr><td>10</td><td>1.5</td></tr> <tr><td>14</td><td>< LOQ</td></tr> </tbody> </table> </div> <p>The DT₅₀ of 1.3 days (SFO kinetic, Chi² error 8.25%, t-test: p < 0.001) was calculated considering residue concentrations from DAT 0 to DAT 14.</p> <p><u>Pea plants</u></p> <p>The initial and maximum residue concentration of acetamiprid (arithmetic mean, n=3) in</p>	Days After Treatment	Residues of acetamiprid [mg a.s./kg f.w.]	0	14.3	1	12.5	2	10.5	3	8.5	5	5.5	7	3.5	10	1.5	14	< LOQ
Days After Treatment	Residues of acetamiprid [mg a.s./kg f.w.]																		
0	14.3																		
1	12.5																		
2	10.5																		
3	8.5																		
5	5.5																		
7	3.5																		
10	1.5																		
14	< LOQ																		

pea plants was 10.1 mg a.s./kg f.w. on DAT 0. Towards the last sampling event on DAT 14 the detected acetamiprid residues (arithmetic mean, n=3) declined to 0.19 ± 0.07 mg a.s./kg f.w.

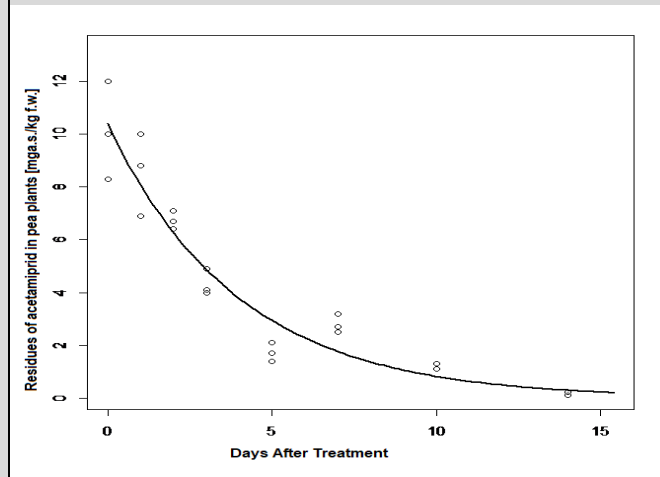


Figure 2. The DT₅₀ of **2.8 days** (SFO kinetic, Chi² error 11.84 %, t-test: p<0.001) was calculated considering residue concentrations from DAT 0 to DAT 14.

Data point	KCP 10.1.2/05
Report	Residues of acetamiprid in monocotyledonous and dicotyledonous plants after spray application of Acetamiprid 200 SL in early vegetation stages in Northern Europe – magnitude of residues and time course of residue decline Gräf, K., 2022
Report No.:	R2040060
Document No.:	000106555
Guideline(s):	No guidelines available / The study design follows the general recommendations of the EFSA Guidance Document on Risk assessments for Birds and Mammals (EFSA 2009)
Deviations:	None to guidance; none to the study plan
GLP:	Yes, certified laboratory
Acceptability:	Yes, study considered acceptable
Duplication (if vertebrate study)	Not applicable

Executive Summary

Monocotyledonous and dicotyledonous plants are important food sources for herbivorous and omnivorous birds and mammals in agricultural habitats. Application of MCW-2222, ADM.00150.I.2.A (synonym Acetamiprid 200 SL) may cause residues on the plants. In order to provide a basis for the assessment of the risk for herbivorous and omnivorous birds and mammals, residue levels of the active substance acetamiprid were determined after spray application of Acetamiprid 200 SL in a wheat (representing monocotyledonous plants) and pea (representing dicotyledonous plants) field in Denmark, Northern Europe. The application of Acetamiprid 200 SL took place in compliance with GLP on 29 May 2021 with a nominal application rate of 0.875 L product/ha (175 g acetamiprid/ha) in a spray volume of 300 L/ha.

Pea and wheat plants were collected at 3 study plots (=replicates) on DAT -1 (before application), on DAT 0 (1.5-2 h after application), 1, 2, 3, 5, 7, 10 and 14. Acetamiprid was analysed in the final sample extracts by using LC MS/MS detection. The initial and maximum residue concentration (arithmetic mean, n=3) of acetamiprid in wheat plants was 14.3 mg a.s./kg f.w. on DAT 0. The DT₅₀ of 1.3 days (SFO kinetic, Chi² error 8.25%, t-test: p < 0.001) was calculated considering residue concentrations from DAT 0 to DAT 14.

The initial and maximum residue concentration of acetamiprid (arithmetic mean, n=3) in pea plants was 10.1 mg a.s./kg f.w. on DAT 0. The DT₅₀ of 2.8 days (SFO kinetic, Chi² error 11.84 %, t-test: p<0.001) was calculated considering residue concentrations from DAT 0 to DAT 14.

Materials and Methods

Materials

Test item	Acetamiprid 200 SL
Batch #	99191024
Content of active substance	200 g acetamiprid/L (nominal), 200.1 g acetamiprid/L (analysed)
Description	Clear yellow to brown
Test organism	
Species	Monocotyledonous plants represented by wheat (variety: Thorus) and dicotyledonous represented by pea (variety: Maxigolt)

Study design and methods

Study location	Middelfart (region: Syddanmark, Denmark)
Experimental dates	13 May 2021 to 23 Sep 2021 (including Analytical Phase)
Sowing	Sowing of wheat (non-GLP) took place on 19 Apr 2021 with a sowing rate of 200 kg wheat seeds/ha; sowing of pea (non-GLP) took place on 21 Apr 2021 with a sowing rate of 130 kg pea seeds/ha
Application date	29 May 2021
Test concentrations	The nominal application rate was 0.875 L product/ha (175 g acetamiprid/ha). The actual application rate ^{a)} of all study plots was 180.4 a.s./ha in a spray volume of 310 L water/ha.
Study plots	Within the study field three wheat study plots (replicates 1 – 3) and three pea study plots (replicates 4 – 6) were established, each with a size of 0.049 ha.
Group size/replicates	9 sampling events took place with samples taken on each of the 3 study plots per crop separately (n=3) in a period of 16 days, on the day before application (DAT (Day After Treatment) -1), and after application (approximately 3 hours) on DAT 0, 1, 2, 3, 5, 7, 10 and 14. At each sampling event wheat and pea plants were collected at 5 different locations within a predefined separate sampling area (=lot) in each study plot, respectively.
Test duration	16 days
BBCH growth stage at time of application	14-15 (wheat), 15 (pea)
Environmental conditions	
Temperature	11.1° – 18.4 °C (Non-GLP)
Precipitation	28.0 mm in total, 3 rain events on DAT 5, 8 and 9 (Non-GLP)

^{a)} Calculated with the analysed content of 20.01% acetamiprid/L

Study design and methods

The study was conducted on an arable field (14 ha ha; size non-GLP) Middelfart (region: Syddanmark) in Denmark. Within the study field 3 pea study plots (=replicates) and 3 wheat study plots (=replicates) were set up with areas of 0.049 ha. Each study plot was equally divided into 9 sampling areas (=lots). All plants were collected within the 6 study plots. Vegetation was cut with scissors just above the soil (without roots or seed remains). Samples were taken at least at five different locations within the respective lot. Samples were collected at DAT -1, 0 (~ 1.5-2 h after application), 1, 2, 3, 5, 7, 10 and 14. The matrix mass per sample was 18.9 g – 24.7 g (at least 20 plants).

All samples were stored at a temperature of ≤ -18 °C until transport to the Test Site of the Analytical Phase (Eurofins GmbH). The storage temperature at the Analytical Test Site was ≤ -18 °C.

Analytical measurements

Acetamiprid were analysed in the final sample extracts by using LC MS/MS detection.

Data evaluation and statistics

The initial and maximal concentrations of acetamiprid on pea and wheat plants were calculated based on the arithmetic mean of three study plots (=replicates, n=3). Residue concentrations of acetamiprid were used to calculate the RUDs (Residue per Unit Dose), based on an application rate of 0.175 kg a.s./ha. The DT₅₀ of acetamiprid was calculated using single first order kinetics (SFO). Goodness of fit was evaluated with three criteria: visual fit, Chi² error level and t-test.

Results and Discussion

Analytical measurements

The Limit of Quantification (LOQ) and the Limit of Detection (LOD) for acetamiprid on pea and wheat plants were 0.01 mg a.s./kg and 0.0025 mg a.s./kg, respectively.

Procedural recovery/quality control data

The analytical method used in the current study was previously validated in study R1640039, (Henkes, 2017). See Section 5.1.2. In order to confirm the analytical method was functioning correctly when used in the current study, procedural recovery samples were processed and analysed concurrently with the test sample and are summarised in the table below.

Table A 12: Procedural recovery data for acetamiprid in arthropods reported in study R2040060

Matrix	Fortification level [mg/kg]	Recovery (%)		RSD (%)	n
		Range	Mean		
Wheat plant	0.01	104 – 116	109	5.8	3
	0.10	87 – 96	91	5.1	3
	20	88 – 102	95	7.5	3
Pea plant	0.01	71 – 74	73	2.1	3
	0.10	91 – 94	92	1.7	3
	20	89 – 105	98	8.5	3

Analytical results & statistics

The acetamiprid residue content in wheat plants sampled one day before the application (DAT -1) was below the LOD of the analytical method, i.e. below 0.0025 mg a.s./kg in all three study plots.

Measured residue concentrations of acetamiprid on pea and wheat plants, RUD values and the calculated DT₅₀ are given in the following table.

Table A 13: Residues of acetamiprid on pea and wheat plants following the application of Acetamiprid 200 SL (nominally containing 200 g acetamiprid/L) at a rate of 0.875 L product/ha (nominally 175 g a.s./ha)

Day after treatment	Mean residue (\pm Standard Deviation) [mg acetamiprid/kg f.w.] ^{a)}	
	Pea plants	Wheat plants
-1 (before application)	< LOD	< LOD
0	10.1 (\pm 1.9)	14.3 (\pm 1.5)
1	8.6 (\pm 1.9)	8.9 (\pm 1.5)
2	6.7 (\pm 1.6)	5.6 (\pm 0.6)
3	4.3 (\pm 0.4)	2.7 (\pm 1.3)
4	3.0 (\pm 0.2)	1.46 (\pm 0.07)
5	1.7 (\pm 0.4)	0.18 (\pm 0.04)
6	2.3 (\pm 0.2)	0.11 (\pm 0.02)
7	2.8 (\pm 0.4)	0.03 (\pm 0.01)
8	2.3 (\pm 0.3)	0.03 (\pm 0.00)
9	1.8 (\pm 0.2)	0.02 (\pm 0.00)
10	1.2 (\pm 0.1)	0.01 (\pm 0.00)
11	0.97 (\pm 0.10)	0.01 (\pm 0.00)
12	0.71 (\pm 0.09)	0.01 (\pm 0.00)
13	0.45 (\pm 0.08)	0.01 (\pm 0.00)
14	0.19 (\pm 0.07)	0.01 (\pm 0.00)
Initial concentration	10.1	14.3
Maximal concentration	10.1	14.3
DT ₅₀ (days)	2.8	1.3
Kinetic model	SFO	SFO
Chi ² error	11.84%	8.25%
t-test (p value)	p < 0.001	p < 0.001

LOD = < 0.0025 mg a.s./kg

^{a)} Values shown are the mean of 3 replicate samples

Table A 14: Residues of acetamiprid on pea and wheat plants based on an application rate of 1.0 kg a.s./ha (RUDs)

Day after treatment	Mean residue (\pm Standard Deviation) [mg acetamiprid/kg f.w.] ^{a)}	
	Pea plants ^{b)}	Wheat plants ^{b)}
0	56.0 (\pm 10.3)	79.5 (\pm 8.5)
1	47.5 (\pm 8.7)	49.3 (\pm 3.4)
2	37.3 (\pm 1.9)	31.0 (\pm 7.2)
3	24.0 (\pm 2.7)	15.2 (\pm 0.8)
4	16.8 (\pm 0.9)	8.1 (\pm 0.4)
5	9.6 (\pm 1.9)	0.98 (\pm 0.21)
6	12.6 (\pm 1.1)	0.58 (\pm 0.12)
7	15.5 (\pm 2.0)	0.18 (\pm 0.03)
8	12.6 (\pm 1.5)	0.14 (\pm 0.02)
9	9.7 (\pm 1.0)	0.10 (\pm 0.01)
10	6.8 (\pm 0.6)	0.06 (\pm 0.00)
11	5.39 (\pm 0.58)	0.05 (\pm 0.01)
12	3.95 (\pm 0.51)	0.04 (\pm 0.01)
13	2.50 (\pm 0.45)	0.04 (\pm 0.02)
14	1.05 (\pm 0.39)	0.03 (\pm 0.02)
Initial concentration	56.0	79.5
Maximal concentration	56.0	79.5

^{a)} Values shown are the mean of 3 replicate samples;

^{b)} Calculated with the actual application rates

Conclusions

The study provides field data on the magnitude of initial residue levels and the subsequent time course of residue decline of acetamiprid in monocotyledonous (wheat) plants and dicotyledonous (pea) plants after spray application of Acetamiprid 200 SL.

The initial and maximum residue concentration of acetamiprid (arithmetic mean, n=3) in wheat plants was 14.3 \pm 1.5 mg a.s./kg f.w. on DAT 0. The DT₅₀ of 1.3 days (SFO kinetic, Chi² 8.25%, t-test: p<0.001) was

calculated considering residue concentrations from DAT 0 to DAT 14.

The initial and maximum residue concentration of acetamiprid (arithmetic mean, n=3) in pea plants was 10.1 ± 1.9 mg a.s./kg f.w. on DAT 0. The DT₅₀ of 2.8 days (SFO kinetic, Chi² 11.84%, t-test: p<0.001) was calculated considering residue concentrations from DAT 0 to DAT 14.

The f_{TWA} of acetamiprid in wheat plants was 0.16 mg a.s./kg f.w. (DAT 0-14), in pea plants it was 0.31 (DAT 0-14).

Weather conditions were adequate for the conduct of the study.

These data provide a reliable basis of initial and maximum residues, as well as DT₅₀ values, residue declines and Time-Weighted Average factors (f_{TWA}) of acetamiprid in monocotyledonous and dicotyledonous plants for the use in higher tier risk assessments for herbivorous and omnivorous birds and mammals.

A 2.1.2.2.6 Field study, Katzschner et al. (2015), GLP 186

Comments of zRMS:	<p>The study was focused on two species: European hares (<i>Lepus europaeus</i>) and rabbits (<i>Oryctolagus cuniculus</i>) that use oilseed rape, sunflower and sugar beet fields as foraging habitat, and determinate respective PT values via 24-hour radio-tracking of multiple individuals monthly over the entire growing season for each crop.</p> <p>For hares the study was conducted at three different study sites: near Erding (Bavaria, Germany) for oilseed rape, near Ochsenfurt (Bavaria, Germany) for sugar beet, and near Szany (Győr-Moson-Sopron, Hungary) for oilseed rape and sunflower. For rabbits the study was conducted at two different sites. One site was located near Würzburg (Bavaria, Germany), a typical region for oilseed rape and sugar beet cultivation. Another site was located near Paks (Tolna, Hungary), a typical region for sunflower growing.</p> <p>In total 25 individual rabbits at two study sites and 26 individual hares at three different study sites were radio-tracked during the entire crop development of oilseed rape, sugar beet and sunflower. Data recording lasted for 19 months, from April 2013 to October 2014, and in total 267 sessions of 24h telemetry were analysed (based on 228 sessions conducted). The study is considered acceptable by zRMS and reliable for use in the risk assessment.</p> <p>The following conclusion of PT values for both species are provided below:</p> <p>PT rabbits:</p> <p><u>Oilseed rape</u></p> <p>In total, twelve different individuals were radio-tracked during the entire growing period of oilseed rape (September to July) at the study site Würzburg (Germany). For PT calculations 69 tracking sessions were analysed. The monthly mean PT values ranged from 0 to 0.57 and the 90th percentile ranged from 0 to 0.97. The highest PT values were calculated for April to July during late crop development stages and before harvest (BBCH growth stages 57 to 85). The average time potentially foraging in the crop of concern during these months ranged between 0.41 and 0.57 (90th percentile between 0.81 and 0.97). Lowest values were calculated for early crop development stages (BBCH growth stages 09 to 24) from September to January and in March. The mean PT in oilseed rape from September to January ranged between 0 and 0.18 (90th percentile between 0 and 0.51). In March (BBCH growth stages 40 to 59) low PT values of an average of 0.01 (90th percentile = 0.02) were calculated.</p> <p><u>Sugar beet</u></p> <p>At the study site Würzburg (Germany) in total 13 different individuals were radio-tracked during the growing period of sugar beet (April to October). Forty-two tracking sessions were analysed (see Table 2). The mean PT value per month ranged from 0 to 0.27 and the 90th percentile from 0 to 0.67. During early crop development in April (BBCH growth stages 00 to 10) no tagged animal spent potentially foraging time in the crop. During the remaining time of crop development from May to October (BBCH growth stages 14 to 49) mean PT values remained low except for July (see below). The PT in sugar beet during this growing period generally ranged from 0.02 to 0.06 (90th percentile 0.04 to 0.18). Only during the month of July (BBCH growth stages 37 to > 39) rabbits spent more time potentially foraging in sugar beet (mean PT = 0.27, 90th percentile = 0.67).</p>
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Sunflower

During the entire growing period of sunflower (May to August) in total nine different individuals were radio-tracked at the study site Paks (Hungary). For PT calculations 21 tracking sessions were analysed. The monthly mean PT value ranged from 0 to 0.32 (90th percentile 0 to 0.66). None of the tagged animals spent potentially foraging time in the crop during the early development stages (BBCH growth stages 03 to 18) in May. During further crop development until August (up to BBCH growth stage 85) the mean PT value for each month increased continuously. The mean PT in sunflower during these months ranged from 0.17 to 0.32 (90th percentile 0.35 to 0.66).

Agreed endpoints for rabbit:

Oilseed rape

PT = 0.82 (90th percentile)
PT= 0.31 (mean)
(based on 69 tracking session)

Sugar beet

PT = 0.18 (90th percentile)
PT= 0.06 (mean)
(based on 42 tracking session)

Sunflower

PT = 0.47 (90th percentile)
PT= 0.17 (mean)
(based on 21 tracking session)

PT hares

Oilseed rape

In total eleven different individuals were radio-tracked during the entire growing period of oilseed rape (September to July) at the study site Erding (Germany) and additional seven individuals at the study site Szany (Hungary) from March to June. For PT calculations presented here, the telemetry sessions were grouped by BBCH growth stages irrespective of the months in which the sessions were conducted (see Table 3), as it is regarded most relevant to consider the PT by crop development stage. However, data for each site grouped by calendar month are given in Table A 4. It should be noted that the BBCH growth stages were not covering the same months at both study sites. Whereas in Erding (Germany) oilseed rape fields in March/April showed BBCH growth stages of 19-31, the first PT session in Hungary in March on oilseed rape fields was conducted during BBCH growth stage 53. In Hungary BBCH growth stages during April were 63-67 and 71-80 in May. In Erding a BBCH growth stage of 80 for oilseed rape was not reached until July.

All together 73 sessions for oilseed rape were analysed (see Table 3). The monthly mean PT value (based on combined BBCH growth stages from both study sites) ranged from 0.11 to 0.56 and the 90th percentile from 0.29 to 0.81.

The mean PT in the crop of concern was increasing from values between 0.15 and 0.33 (90th percentile 0.29 – 0.50) in the early crop development stages from September to January (BBCH growth stages 00 to 21) to 0.55 and 0.56 (90th percentile = 0.81 and 0.78) in February and March (BBCH growth stages 19 - 31), and was decreasing again with further crop development until June (Hungary)/August (Germany) (up to BBCH growth stage 99) from 0.37 to 0.11 (90th percentile 0.62 to 0.30).

Sugar beet

At the study site Ochsenfurt (Germany) in total eight different individuals were radio-tracked during the growing period of sugar beet (April to October). Thirty-five tracking sessions were analysed. The mean PT value per month ranged from 0.05 to 0.58 and the 90th percentile from 0.11 to 0.91.

In the early crop development (April and May; BBCH growth stages 03 to 18) hares showed mean PT values of less than 0.08 (90th percentile = 0.12) for sugar beet. During the remaining crop development from June to October (BBCH growth stages 19 to 49) the mean PT values ranged from 0.30 to 0.58 (90th percentile 0.56 to 0.91).

Sunflower

During the entire growing period of sunflower (April to August) in total seven different individuals were radio-tracked at the study site Szany (Hungary). Twenty-seven tracking sessions were analysed (see Table 3). The average PT value for each month ranged from 0.09 to 0.45 and the 90th percentile from 0.24 to 0.79.

The mean PT values for sunflower were less than 0.15 (90th percentile < 0.34) during the early development stages in April (BBCH growth stages 00 to 12) and the late development stages (BBCH growth stages 65 to 89) in July and August. During May (BBCH growth stages 30 to 32) the average PT value for this crop was 0.26 (90th percentile = 0.51), and 0.45 (90th percentile = 0.79) in June (BBCH growth stages 51 to 53).

Agreed endpoints for rabbit:

Oilseed rape

PT = 0.82 (90th percentile)

PT= 0.31 (mean)

Sugar beet

PT = 0.18 (90th percentile)

PT= 0.06 (mean)

Sunflower

PT = 0.47 (90th percentile)

PT= 0.17 (mean)

Agreed endpoints for hare:

Oilseed rape

PT = 0.60 (90th percentile)

PT= 0.27 (mean)

Sugar beet

PT = 0.82 (90th percentile)

PT=0.36 (mean)

Sunflower

PT = 0.50 (90th percentile)

PT= 0.21 (mean)

Data point	KCP 10.1.2/06
Report	Generic monitoring of hares and rabbits to determine proportion of time spent foraging in oilseed rape, sunflower and sugar beet in Central Europe. I Katzschner, J-D Ludwigs, T Grimm and F von Blanckenhagen (2015)
Report No.:	R12244-1
Document No.:	R-30856
Guideline(s):	No guidelines available / The study design follows the general recommendations of the EFSA Guidance Document on Risk assessments for Birds and Mammals (EFSA 2009)
Deviations:	None to guidance; none to the study plan
GLP:	Yes, certified laboratory
Acceptability:	Yes
Duplication (if vertebrate study)	Not applicable

Executive summary

The aim of this generic study was to investigate European hares (*Lepus europaeus*) and rabbits (*Oryctolagus cuniculus*) that use oilseed rape, sunflower and sugar beet fields as foraging habitat, and to determine respective PT values (i.e. proportion of diet obtained in treated area, calculated as proportion of foraging time spent potentially foraging in the crop of concern) via 24-hour radio-tracking of multiple individuals monthly over the entire growing season for each crop. In total 25 individual rabbits at two study sites and 26 individual hares at three different study sites were radio-tracked during the entire crop development of oilseed rape, sugar beet and sunflower. Data recording lasted for 19 months, from April 2013 to October 2014, and in total 267 sessions of 24h telemetry were analysed (based on 228 sessions conducted).

For PT calculations 69 tracking sessions of rabbits in oilseed rape were analysed and the mean and 90th percentile PT were 0.31 and 0.82, respectively.

For PT calculations in sugar beet, 42 tracking sessions of rabbits were analysed and the mean and 90th percentile PT were 0.06 and 0.18, respectively.

For PT calculations in sunflower, 21 tracking sessions of rabbits were analysed and the mean and 90th percentile PT were 0.17 and 0.47, respectively.

Material and Methods

Study sites

To assure high availability of the crops of concern within the activity ranges of tracked lagomorphs, study sites were selected carefully regarding the cultivation of oilseed rape, sugar beet and sunflower, respectively, and the presence of the two lagomorph species in suitable numbers. This resulted in different sites being used for each species.

The study was conducted at two different study sites for rabbits (Würzburg, Germany; and Paks, Hungary) and at three different study sites for hares (Erding and Ochsenfurt, Germany; and Szany, Hungary).

Trapping

The majority of animals were trapped and equipped with radio tags at the beginning of the Field Phase. Lagomorphs were captured either on fields covered with the crop of concern or nearby (e.g. in surrounding off-crop structures). Only adult animals were tagged.

Hares were trapped using lines of nets. Hares were chased into the nets by drivers walking towards the nets. Rabbits were trapped using three different trapping methods:

1. By placing wired box live traps outside the burrows
2. By using ferrets to chase rabbits out of their burrow into nets placed at burrow exits
3. By using net lines (comparable to hares; see above)

Each trapped animal was sexed, weighed and equipped with a radio tag (Biotrack Ltd., UK; www.biotrack.co.uk) and released at the trapping site.

Radio-tracking

For radio-tracking, animals were located with Yagi antennae according to two different approaches: 24h telemetry and single check telemetry. During 24h telemetry the animal was radio-tracked continuously (for 24 hours) by two observers, locating the animal from two different positions, which allows to triangulate the animal's position.

To allow calculation of the proportion of time rabbits and hares spent in the specific crop at least five different animals per species per crop were radio-tracked per month. Each change of habitat and/or each change of behaviour (i.e. active/inactive) was recorded with time (exact to the minute). During single check telemetry each animal was located at least once per month in order to survey its presence in the study area. If possible, animals were observed with binoculars, scopes and night observation devices. Individuals were followed for as long as possible during the Field Phase, and dead or missing individuals or tags were replaced with newly tagged animals.

Habitat mapping

Each study area comprised the area around the trapping locations of the tagged animals and the positions in which they were recorded during single check telemetry and 24h telemetry. At least this area was mapped for habitat types approximately once per month. Moreover the BBCH growth stage of the crop of concern in the study area was recorded approximately every two weeks, following Meier (2001) crop growth stages.

Calculation of PT in crops of concern

For each telemetry session the proportion of diet obtained in treated area (PT) was calculated as the proportion of the total 'potentially foraging' time that an individual hare or rabbit spent 'potentially foraging' in the crop of concern. The total time 'potentially foraging' was the sum of the time periods covered by all behavioural categories when foraging could not be excluded. All instances when the animal was definitely known to be performing non-foraging activities were excluded from PT calculations. For each 24h telemetry the time 'potentially foraging' within the crop of concern was compared with the total time 'potentially foraging' in all habitats to give the PT for that crop (i.e. $PT(crop) = \frac{\text{time in crop}}{\text{total time}}$).

A mean PT value (plus standard deviation and 90th percentile values) was calculated based on all single PT values for each studied species in each studied crop per month. Furthermore PT values were calculated for different BBCH growth stages, and as overall mean including all PT sessions conducted in the course of the Field Phase per species, crop and study site.

Results

In total, 25 individual rabbits at two study sites and 26 individual hares at three different study sites were radio-tracked during the entire crop development of oilseed rape, sugar beet and sunflower. Data recording lasted for 19 months, from March 2013 to October 2014 for all three crops investigated. The number of analysed 24h telemetry sessions was 142 for oilseed rape (69 for rabbits and 73 for hares), 77 for sugar beet (42 for rabbits and 35 for hares) and 48 for sunflower (21 for rabbits and 27 for hares). These numbers are related to the time such crops are available to lagomorphs in the course of the year. Results of calculated PT values are summarised in the table below.

For rabbits, the results for all crops showed that rabbits generally exhibited higher crop-related PT values if crop plants were in advanced BBCH growth stages (i.e. grown high) and give good shelter. Oilseed rape and sunflower were used as a minor foraging habitat during early growth stages and were utilized more frequently after the crop elongation phase, whereas sugar beet fields were used as minor foraging habitat during the entire vegetation period of this crop.

For hares, similarly to rabbits, the results for all crops showed that in general hares also exhibited lower crop-related PT values as long as the plants were not grown high; but also the late stages of sunflower and oilseed rape were not frequently used. However, sugar beet fields were consistently used as foraging habitat from BBCH growth stage 19 onwards, though not more than on average 58% of the total potentially foraging time.

Table A 15: Summary of calculated PT values per crop per species at different BBCH growth stages in different months

Mean PT values and 90th percentile are presented. The growing period of oilseed rape has been covered during data recordings of two different years for both species and at two different study sites for hares. The given BBCH growth stages are summarised for species and sites.

Crop	BBCH growth stage (month)	Rabbit PT		Hare PT	
		mean	90 th percentile	mean	90 th percentile
Oilseed rape	00-14 (Sep)	0.18	0.45	0.15	0.29
	12-17 (Oct)	0.00	0.00	0.20	0.36
	16-20 (Nov)	0.17	0.51	0.26	0.60
	16-21 (Dec)	0.16	0.46	0.33	0.48
	19-24 (Jan)	0.11	0.32	0.31	0.50
	19-29 (Feb)	0.38	0.87	0.55	0.81
	19-59 (March)	0.01	0.02	0.56	0.78
	24-65 (April)	0.42	0.82	0.37	0.62
	53-70 (May ^G /March ^H)	0.57	0.83	0.31	0.58
	63-80 (June ^G /April ^H)	0.55	0.87	0.19	0.53
	69-85 (July ^G /May ^H)	0.55	0.98	0.21	0.51
	86-99 (Aug ^G /June ^H)	no crop	no crop	0.11	0.30
	00-99 (Sep-Aug)	0.31	0.82	0.27	0.60
Sugar beet	00-10 (April)	0.00	0.00	0.07	0.11
	14-18 (May)	0.02	0.04	0.05	0.12
	18-36 (June)	0.04	0.11	0.40	0.65
	37- >39 (July)	0.27	0.66	0.57	0.87
	>39 (Aug)	0.06	0.18	0.47	0.91
	<49 (Sep)	0.03	0.10	0.30	0.56
	49 (Oct)	0.03	0.08	0.58	0.77
	00-49 (April-Oct)	0.06	0.18	0.36	0.82
Sunflower	00-12 (April)	no crop	no crop	0.09	0.24
	03-32 (May)	0.00	0.00	0.26	0.51
	10-53 (June)	0.17	0.35	0.45	0.79
	57-73 (July)	0.20	0.44	0.13	0.33
	80-89 (Aug)	0.32	0.66	0.12	0.32
	00-89 (April-Aug)	0.17	0.47	0.21	0.50

^G= Germany, ^H = Hungary

Conclusion

According to recommendations of EFSA (2009) and meeting highest standards of most exact and continuous radio-tracking of hares and rabbits in the course of the entire vegetation period of each crop, this study gives reliable and robust PT values for both species utilizing oilseed rape, sugar beet and sunflower fields in Central Europe for use in pesticide risk assessments for lagomorphs. The basis for the reported PT values are 228 full sessions of 24h continuous telemetry (some of which could be used for two crops of concern, resulting in 267 sessions total) conducted simultaneously by two field workers positioned at different angles to the tracked individual resulting in almost 11000 working hours of animal tracking.

A 2.1.3 KCP 10.1.3 Effects on other terrestrial vertebrate wildlife (reptiles and amphibians)

A 2.2 KCP 10.2 Effects on aquatic organisms

A 2.2.1 KCP 10.2.1 Acute toxicity to fish, aquatic invertebrates, or effects on aquatic algae and macrophytes

A 2.2.1.1 KCP 10.2.1/01 Acute toxicity to fish

Comments of zRMS:	<p>The study has been already evaluated and accepted by the zRMS-PL in the course of the first zonal evaluation of CA3573 (formerly MCW-2222) finalised in April 2018. ADM.001501. is the same product as MCW-2222 and the evaluation are still valid. As the test guideline has not changed since that time, re-evaluation of the study was not necessary and presented below conclusions were taken from the Core Assessment, Part B, Section 6 of April 2018.</p> <p>The study was performed in line with OECD 203 and met all validity criteria. Following endpoints based on nominal concentrations were agreed:</p> <p>LC₅₀ = 85.8 mg product/L (corresponding to 15.3 mg a.s./L)</p>
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Reference:	KCP 10.2.1/01
Report	Acute toxicity of MCW-2222 to the rainbow trout <i>Oncorhynchus mykiss</i> in a 96-hour static test, [REDACTED] R-33831
Guideline(s):	OECD 203 (1992)
Deviations:	<p>Minor deviation to OECD 203 (2019):</p> <p>Due to a recent change in respective guidance, the test temperature was slightly outside the recommended range of 10-14°C (actually 13.4 – 14.5 °C)</p> <p>This is not considered to have any impact on the study integrity or outcome</p>
GLP:	Yes, certified laboratory
Acceptability:	Yes
Duplication (if vertebrate study)	No

Executive Summary

In a 96 hour acute toxicity study rainbow trout *Oncorhynchus mykiss* was exposed to MCW-2222 at nominal concentrations of 9.70, 21.3, 47.0, 103.3, 227.3 and 500.0 mg test item/L corresponding to 1.73, 3.80, 8.37, 18.4, 40.5 and 89.1 mg a.s./L under static conditions and in accordance with the OECD guideline 203.

Analytical measurements were conducted for the control and all test item concentration at t = 0 and 96 hours, using HPLC methods. The mean measured concentrations ranged between 90.6 – 96.2% of nominal values at test start and ranged from 90.8 – 97.7% at test end after 96 hours. Therefore, the biological results are reported based on nominal concentrations.

At test end the LC₁₀, LC₂₀, LC₅₀ were determined at 34.5, 47.1 and 85.8 mg test item/L corresponding to 6.14, 8.40 and 15.3 mg a.s./L, (nominal). The NOEC was determined at 47.0 mg test item/L (nominal) corresponding to 8.37 mg a.s./L (nominal).

Materials and methods

Materials

Test item	MCW-2222
Batch #	611-280413-01
Purity	Acetamiprid200 g/L (nominal); 202.7 g/L (analysed)

Description	Yellowish liquid
Control	Test medium
Test organism	
Species	Rainbow trout <i>Oncorhynchus mykiss</i> mean length: 5.0 ± 0.2 cm, mean weight: 1.18 ± 0.2 g.
Source	Forellenzucht Trostadt GbR” Dorfstraße 7, 98646 Trostadt OT Reurieth, Germany
Study design and methods	
Test duration and exposure	96 hours, static
Experimental dates	03 to 14 February 2014
Test concentrations	9.70, 21.3, 47.0, 103.3, 227.3, 500.0 mg test item/L corresponding to 1.73, 3.80, 8.37, 18.4, 40.5, 89.1 mg a.s./L
Test units	One 13 L stainless steel container per concentration, each filled with 10 L of test solution
Group size/replicates	8 organisms per concentration; 1 replicate per concentration
Test medium	Reconstituted water according to ISO 6341 Conductivity of deionised water: ≤ 10 μ S/cm (measured 1.9 μ S/cm)
Adaptation	The test fish were in good health and were acclimatised in test medium of the same quality as was used in the test for 73 days.
Aeration	Yes
Environmental conditions	
Temperature	13.4 – 14.5 °C
Photoperiod	16 hours light / 8 hours darkness
pH	7.56 - 8.22
Dissolved oxygen	≥ 7.46

Analytical measurements

Analytical verification of test item concentrations was conducted using an HPLC-method with UV-detection. Analytical samples were analysed from all test concentrations and control(s) at test start and at test end after 96 hours.

Biological observations

Determination of the number of dead fish (including loss of equilibrium, swimming, behaviour, respiratory function, pigmentation etc.) was done at 3, 6, 24, 48, 72 and 96 hours after start of exposure.

Statistics

The 96 hour EC_x values were calculated by Probit analysis. The NOEC was determined using Fisher’s Exact Binominal Test, $p \leq 0.05$

Results and discussion

Analytical measurements

Analytical results are given in the table below.

Table A 16: Nominal and measured concentrations of test item

	Measured concentration [mg a.s./L]						
Nominal concentration	0.0	1.73	3.80	8.37	18.4	40.5	89.1
Test start (0 h)							
Measured concentration	-	1.57	3.67	8.00	17.5	38.6	85.7
% of nominal t= 0 h	-	90.6	96.5	95.1	94.8	95.4	96.2
Range	90.6 – 96.5						
Test end (96 h)							
Measured concentration	-	1.57	3.66	7.79	17.5	38.2	87.1
% of nominal	-	90.8	96.1	93.0	95.0	94.3	97.7
Range	90.8 – 97.7						

Limit of quantification: 0.185 mg/L

Biological results

Mortality data are given in the table below.

Table A 17: Cumulative mortality of rainbow trout exposed to MCW-2222

MCW-2222 (mg test item/L, nominal)	Control	9.7	21.3	47.0	103.3	227.3	500
Acetamiprid (mg a.s./L, nominal)	Control	1.73	3.80	8.37	18.4	40.5	89.1
Cumulative mortality [%]							
24 h	0	0	0	0	0	37.5	100*
48 h	0	0	0	0	25.0	62.5*	100*
72 h	0	0	0	0	37.5	87.5*	100*
96 h	0	0	0	25.0	62.5*	87.5*	100*

*Significantly different from the control (Fisher's Exact Binominal Test, $p \geq 0.05$)

Sub-lethal effects:

At the test concentration of 47.0 mg test item/L, some fish were positioned on their sides or backs at 72 and 96 hours. At the test concentrations of 103.3 and 227.3 mg test item/L, some fish were positioned on their sides or backs and some fish showed a bloated abdomen at 24, 48, 72 and 96 hours. At the test concentration of 500.0 mg test item/L, fish were positioned on their sides or backs, some fish showed a bloated abdomen and some fish gasped for air at 3 and 6 hours.

Table A 18: Endpoints after 96 hours

	Concentration [mg test item/L]	Concentration [mg a.s./L]
EC10 (95%-CI)	34.5 (12.2 – 54.0)	6.14 (2.17 – 9.62)
EC20 (95%-CI)	47.1 (21.5 – 70.2)	8.40 (3.83 – 12.5)
EC50 (95%-CI)	85.8 (55.0 – 134.0)	15.3 (9.81 – 23.9)
NOEC	47.0	8.37
LOEC	103.3	18.4

Validity criteria

As shown in the following table, all validity criteria were met.

Table A 19: Validity criteria

Validity criteria according to OECD 203 (2019)	Observed in study
Mortality in the control $\leq 10\%$	0%
O ₂ concentration $\geq 60\%$ of saturation value throughout the test	$\geq 95\%$

Conclusion

In a 96 hour acute toxicity study rainbow trout *O. mykiss* was exposed to MCW-2222 at nominal concentrations of 9.70, 21.3, 47.0, 103.3, 227.3, 500.0 mg test item/L corresponding to 1.73, 3.80, 8.37, 18.4, 40.5, 89.1 mg a.s./L under static conditions and in accordance with the OECD guideline 203.

At the test end LC₁₀, LC₂₀, LC₅₀ were determined at 9.52, 34.5, 47.1, 85.8 mg test item/L corresponding to 1.70, 6.14, 8.40, 15.3 mg a.s./L, (nominal). The NOEC was determined at 47.0 mg test item/L (nominal) corresponding to 8.37 mg a.s./L (nominal).

A 2.2.1.2 KCP 10.2.1/02 Acute toxicity to Invertebrates – *Daphnia*

Comments of zRMS:	<p>The study has been already evaluated and accepted by the zRMS in the course of the first zonal evaluation of CA3573 (formerly MCW-2222) finalised in April 2018.</p> <p>As the test guideline has not changed since that time, re-evaluation of the study was not necessary and presented below conclusions were taken from the Core Assessment, Part B, Section 6 of April 2018.</p> <p>The study was performed in line with OECD 202 and met all validity criteria. Following endpoints based on nominal concentrations were agreed:</p> <p>EC₅₀ = 100.2 mg product/L (corresponding to 17.9 mg a.s./L)</p>
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Reference:	KCP 10.2.1/02
Report	Acute toxicity of MCW-2222 to <i>Daphnia magna</i> in a 48-hour static test Juckeland, D., 2014b, R-33832
Guideline(s):	OECD 202 (2004)
Deviations:	No
GLP:	Yes, certified laboratory
Acceptability:	Yes
Duplication (if vertebrate study)	Not applicable

Executive Summary

In a 48 hour acute toxicity study, neonate daphnids were exposed to MCW-2222 at nominal concentrations of 0, 19.2, 42.2, 93.1, 204.5, 450.2 mg test item/L corresponding to corresponding to 3.42, 7.53, 16.6, 36.5, 80.3 mg a.s./L under static conditions and in accordance with the OECD guideline 202. Immobility was observed at the end of the test after 48 hours.

Analytical measurements were conducted for the control and all test item concentration at t = 0 and 48 hours, using HPLC methods. The mean measured concentrations ranged between 87.7 – 92.2% of nominal values at test start and between 99.7 – 109.5% at test end. Therefore, all toxicity results are based on the nominal concentrations of the test item.

The EC₁₀, EC₂₀ and EC₅₀ values for immobilisation based on nominal concentrations were calculated to be 36.0, 51.4 and 100.2 mg test item/L at 48 hours (nominal) corresponding to 6.42, 9.16 and 17.9 mg a.s./L (nominal). The NOEC at 48 hours was determined to be 42.2 mg test item/L (nominal) corresponding to 16.6 mg a.s./L (nominal).

Materials and methods

Materials

Test item	MCW-2222
Batch #	611-280413-01
Content of active substance	Acetamiprid 200 g/L (nominal); 202.7 g/L (analysed)
Description	Yellowish liquid
Control	Test medium
Toxic reference	Potassium chloride was tested in a separate study
Test organism	
Species	<i>Daphnia magna</i> ; neonate (less than 24 hours old)
Source	In-house culture, originally obtained from Landesanstalt für Umweltschutz Baden-Württemberg, Griesbachstr. 1, 76185 Karlsruhe, Germany

Study design and methods

Test duration and exposure	48 hours, static exposure
Experimental dates	04 to 06 Feb 2014
Test concentrations	19.2, 42.2, 93.1, 204.5, 450.2 mg test item/L corresponding to 3.42,

Test units	7.53, 16.6, 36.5, 80.3 mg a.s./L
Group size/replicates	150 mL glass beakers, each filled with 10 mL of test solution.
Test medium	20 organisms per concentration; 5 in each of 4 replicates
Environmental conditions	M-4 Medium (OECD 202, 2004)
Temperature	19.7 – 20.7 °C
Photoperiod	16 hours light / 8 hours darkness
pH	7.77 - 8.28
Dissolved oxygen	≥ 8.48 mg/L

Analytical measurements

Analytical verification of test item concentrations was conducted using an HPLC method using UV detection. Analytical samples were analysed from all test concentrations and the control at test start and test end after 48 hours.

Biological observations

Immobilisation of daphnids was recorded 24 and 48 hours after the test start. Those animals not able to swim within 15 seconds after gentle agitation of the test beaker were considered to be immobile.

Statistics

The 48 hour EC_x values were calculated by Probit analysis. The NOEC was determined using Fisher's Exact Test with a Bonferroni correction.

Results and discussion

Analytical measurements

Analytical results are given in the table below.

Table A 20: Nominal and measured concentrations of MCW-2222

Measured concentration [mg a.s./L]						
Nominal concentration	0.0	3.42	7.53	16.6	36.5	80.3
Test start (0 h)						
Measured concentration	-	3.0	6.87	15.3	33.3	74.0
% of nominal	-	87.7	91.2	92.2	91.3	92.2
Range	87.7 – 92.2%					
Test end (48 h)						
Measured concentration	-	3.75	7.64	16.8	36.3	81.2
% of nominal	-	109.5	101.5	101.4	99.7	101.2
Range	99.7 – 109.5					

Limit of quantification: 0.367 mg a.s./L

Biological results

Biological results are given in the table below.

Table A 21: Percent of immobilised daphnids in a 48 hour acute immobilisation test exposed to MCW-2222

Nominal concentration [mg test item/L]	Control	19.2	42.2	93.1	204.5	450.2
Nominal concentration [mg a.s./L]	Control	3.42	7.53	16.6	36.5	80.3
Immobilisation [%]						
24 h	0	0	0	0	5	65*
48 h	0	0	0	45*	75*	100*

* Significantly different from the control (Fisher's Exact Binominal Test with Bonferroni correction, $p \geq 0.05$)

Table A 22: Endpoints after 48 hours

	Concentration [mg test item/L]	Concentration [mg a.s./L]
EC ₁₀ (95%-CI)	36.0 (24.0 – 54.7)	6.42 (4.28 – 9.75)
EC ₂₀ (95%-CI)	51.4 (36.8 – 71.7)	9.16 (6.56 – 12.8)
EC ₅₀ (95%-CI)	100.2 (77.2 – 130.0)	17.9 (13.8 – 23.2)
NOEC	42.2	80.3
LOEC	93.1	16.6

Validity criteria

As shown in the following table, all validity criteria were met.

Table A 23: Validity criteria

Validity criteria according to OECD 202	Observed in study
Number of immobilised daphnids must be ≤ 10%	0%
Dissolved oxygen concentration at the end of the test must be ≥ 3 mg/L in control(s) and test solutions.	≥ 8.4 mg/L
Daphnids in the control group must not have been trapped at the surface of the water.	none

Conclusion

In a 48 hour acute toxicity study, neonate daphnids were exposed to MCW-2222 at nominal concentrations of 0, 19.2, 42.2, 93.1, 204.5, 450.2 mg test item/L corresponding to corresponding to 3.42, 7.53, 16.6, 36.5, 80.3 mg a.s./L under static conditions and in accordance with the OECD guideline 202.

The EC₁₀, EC₂₀ and EC₅₀ values for immobilisation based on nominal concentrations were calculated to be 36.0, 51.4 and 100.2 mg test item/L at 48 hours (nominal) corresponding to 6.42, 9.16 and 17.9 mg a.s./L (nominal). The NOEC at 48 hours was determined to be 42.2 mg test item/L (nominal) corresponding to 16.6 mg a.s./L (nominal).

A 2.2.1.3 KCP 10.2.1/03 Acute toxicity to Invertebrates – Chironomus

Comments of zRMS:	<p>The study has been already evaluated and accepted by the zRMS in the course of the first zonal evaluation of CA3573 (formerly MCW-2222) finalised in April 2018.</p> <p>1 is the same product as MCW-222 and the evaluation is still valid. As the test guideline has not changed since that time, re-evaluation of the study was not necessary and presented below conclusions were taken from the Core Assessment, Part B, Section 6 of April 2018.</p> <p>The study was performed in line with OECD 235 and met all validity criteria. Following endpoints based on nominal concentrations were agreed:</p> <p>EC₅₀ = 0.0521 mg product/L (corresponding to 0.00929 mg a.s./L)</p>
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Reference:	KCP 10.2.1/03
Report	Acute toxicity of MCW-2222 to <i>Chironomus riparius</i> in a 48-hour static test, Juckeland, D., 2015, R-34873
Guideline(s):	OECD 235 (2011)
Deviations:	No
GLP:	Yes, certified laboratory
Acceptability:	Yes
Duplication (if vertebrate study)	Not applicable

Executive Summary

In a 48 hour acute toxicity study, first instar larvae of *Chironomus riparius* were exposed to MCW-2222 at nominal concentrations of 0, 26.1, 36.4, 51.0, 71.4, 100 µg test item/L under static conditions in accordance with the OECD guideline 235. Immobility was observed at the end of the test after 48 hours.

Analytical measurements were conducted for the control and all test item concentration at t = 0 and 48 hours, using HPLC-MS/MS. The mean measured concentrations ranged between 97.6 – 103% of nominal values at test start and between 103.0 – 106% at test end. Therefore, all toxicity results are based on the nominal concentrations for the test item.

The EC₁₀, EC₂₀ and EC₅₀ values for immobilisation based on nominal concentrations were calculated to be 37.9, 42.3 and 52.1 µg test item/L at 48 hours (nominal) corresponding to 6.76, 7.54 and 9.29 µg a.s/L (nominal). The NOEC at 48 hours was determined to be 36.4 µg test item/L (nominal) corresponding to 6.49 µg a.s/L (nominal).

Materials and methods

Materials

Test item	MCW-2222
Batch #	611-280413-01
Content of active substance	Acetamiprid 200 g/L (nominal); 202.7 g/L (analysed)
Description	Yellowish liquid
Control	Test medium
Toxic reference	Potassium chloride was tested in a separate study.
Test organism	
Species	<i>Chironomus riparius</i> ; first instar (~48 hours old)
Source	In-house culture, originally obtained from RWTH Aachen, Institut für Umweltforschung (Biologie V) Lehrstuhl für Umweltbiologie und Chemodynamik, Worringerweg 1, 52074 Aachen, Germany

Study design and methods

Test duration and exposure	48 hours, static exposure
Experimental dates	29 to 31 Jul 2014
Test concentrations	26.1, 36.4, 51.0, 71.4, 100.0 µg test item/L corresponding to 4.65, 6.49, 9.10, 12.7, 17.8 µg a.s./L
Test units	Glass beakers, each filled with 10 mL of test solution
Group size/replicates	20 organisms per concentration; 5 in each of 4 replicates
Test medium	M-4 Medium (OECD 235, 2011)
Environmental conditions	
Temperature	19.8 – 20.4 °C
Photoperiod	16 hours light / 8 hours darkness
pH	7.87 - 8.17
Dissolved oxygen	≥ 7.91 mg/L

Analytical measurements

Analytical verification of test item concentrations was conducted using an HPLC MS/MS. Analytical samples were analysed from all test concentrations and the control at test start and test end after 48 hours.

Biological observations

Immobilisation of chironomids was recorded 12, 24, 36 and 48 hours after the test start and compared with control values. Those animals not able to swim within 15 seconds after gentle agitation of the test beaker were considered to be immobile. In addition, any abnormal behaviour or appearance was recorded (e.g. trapping at surface).

Statistics

The 48 hour EC_x values were calculated by Probit analysis. The NOEC was determined using Fisher's Exact Test with a Bonferroni correction.

Results and discussion

Analytical measurements

Analytical results are given in the table below.

Table A 24: Nominal and measured concentrations of acetamiprid

Measured concentration [µg a.s./L]						
Nominal concentration	0.0	4.65	6.50	9.01	12.8	17.8

Measured concentration [µg a.s./L]						
	Test start (0 h)					
Measured concentration	-	4.80	6.7	9.00	12.4	17.8
% of nominal	-	103	104	98.9	97.6	100
Range	97.6 – 103%					
	Test end (48 h)					
Measured concentration	-	4.91	6.90	9.54	13.2	18.3
% of nominal	-	106	106	105	104	103
Range	103 – 106%					

Limit of quantification: 0.470 µg a.s./L

Biological results

Biological results are given in the table below.

Table A 25: Percent of immobilised chironomids in a 48-hour acute immobilisation test exposed to MCW-2222

Nominal concentration [µg test item/L]	Control	26.1	36.4	51.0	71.4	100.0
Nominal concentration [µg a.s./L]	Control	4.65	6.49	9.10	12.7	17.8
	Immobilisation [%]					
24 h	0.0	0.0	0.0	0.0	25.0	75.0*
48 h	0.0	0.0	5.0	55.0*	85.0*	100.0*

* Significantly different from the control (Fisher's Exact Binominal Test with Bonferroni correction, $p < 0.05$, one-sided greater)

Table A 26: Endpoints after 48 hours

	Concentration [µg test item/L]	Concentration [µg a.s./L]
EC ₁₀ (95%-CI)	37.9 (32.8 – 43.8)	6.76 (5.85 – 7.81)
EC ₂₀ (95%-CI)	42.3 (37.6 – 47.6)	7.54 (6.70 – 8.49)
EC ₅₀ (95%-CI)	52.1 (47.4 – 57.3)	9.29 (8.45 – 10.2)
NOEC	36.4	6.49
LOEC	51.0	9.10

Validity criteria

As shown in the following table, all validity criteria were met.

Table A 27: Validity criteria

Validity criteria according to OECD 235	Observed in study
Number of immobilised larvae must be ≤ 15%	0%
Dissolved oxygen concentration at the end of the test must be ≥ 3 mg/L in control(s) and test solutions.	≥ 7.91 mg/L
<i>Chironomus</i> larvae in the control group must not have been trapped at the surface of the water.	none

Conclusion

In a 48 hour acute toxicity study, first instar larvae of *Chironomus riparius* were exposed to MCW-2222 at nominal concentrations of 0, 26.1, 36.4, 51.0, 71.4, 100 µg test item/L under static conditions in accordance with the OECD guideline 235.

The EC₁₀, EC₂₀ and EC₅₀ values for immobilisation based on nominal concentrations were calculated to be 37.9, 42.3 and 52.1 µg test item/L at 48 hours (nominal) corresponding to 6.76, 7.54 and 9.29 µg a.s./L (nominal). The NOEC at 48 hours was determined to be 36.4 µg test item/L (nominal) corresponding to 6.49 µg a.s./L (nominal).

A 2.2.1.4 KCP 10.2.1/04 Toxicity to green algae

Comments of zRMS:	<p>The study has been already evaluated and accepted by the zRMS in the course of the first zonal evaluation of CA3573 (formerly MCW-2222) finalised in April 2018. Leaxo is the same product as MCW-222 and the evaluation is still valid. As the test guideline has not changed since that time, re-evaluation of the study was not necessary and presented below conclusions were taken from the Core Assessment, Part B, Section 6 of April 2018.</p> <p>The study was performed in line with OECD 235 and met all validity criteria. Following endpoints based on nominal concentrations were agreed:</p> <p>EC₅₀ = 0.0521 mg product/L (corresponding to 0.00929 mg a.s./L)</p>
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Reference:	KCP 10.2.1/04
Report	Effects of MCW-2222 on <i>Desmodesmus subspicatus</i> in an algal growth inhibition test, Juckeland, D., 2014; R-33833
Guideline(s):	OECD 201 (2006/2011)
Deviations:	No
GLP:	Yes, certified laboratory
Acceptability:	Yes
Duplication (if vertebrate study)	Not applicable

Executive Summary

In a 72 hour toxicity study, cultures of *Desmodesmus subspicatus* were exposed to MCW-2222 at nominal concentrations of 218.8, 437.5, 875.1, 1750.2, 3500.3 mg test item/L under static conditions in accordance with the OECD guideline 201. Growth rate and yield were observed by means of microscopic cell counting during the test.

Analytical measurements were conducted for the control and all test item concentration at t = 0 and 72 hours, using HPLC analysis. The mean measured concentrations ranged between 90.6 and 97.3% of nominal values.

At the test end an ErC₅₀ of 554.5 and an EyC₅₀ of 204.9 mg a.s./L were determined. The NOEC was determined to be 39 mg a.s./L.

Materials and methods

Materials

Test item	MCW-2222
Batch#	611-280413-01
Content of active substance	Acetamiprid 200 g/L (nominal), 202.7 g/L (analysed)
Description	Yellowish liquid
Control	Test medium
Toxic reference	The reference item potassium dichromate was tested in a separate study to verify the sensitivity of the test system.

Test organism

Species	<i>Desmodesmus subspicatus</i>
Source	ScienceBridge GmbH, Hans-Adolf-Krebs-Weg 1, 37077 Göttingen, Germany; strain: 86.81 SAG

Study design and methods

Test duration and exposure	72 hours, static
Experimental dates	04 Feb to 07 Feb 2014
Test concentrations	218.8, 437.5, 875.1, 1750.2, 3500.3 mg test item/L corresponding to 39.0, 78.0, 156.0, 312.0, 624.0 mg a.s./L (based on

Test units	analysed content of a.s.)
Group size/replicates	250 mL glass vessels filled with 100 mL test solution.
Test medium	3 replicates for each test concentration and 6 replicates for the control
Preculture	OECD medium
	Preculture was established in 1000 mL glass flasks with OECD-medium; algae were kept at the similar temperature and light conditions as in the test.
Aeration	None
Renewal of test solutions	None
Initial cell density	Approximately 5×10^3 cells/mL
Environmental conditions	
Temperature	22.7 – 22.8 °C
Lighting	Continuously at $95 \mu\text{E m}^{-2}\text{s}^{-1}$
pH	7.96 – 8.82

Analytical measurements

Analytical verification of test item concentrations was conducted using an HPLC-method with UV-detection. Analytical samples were analysed from all test concentrations and control at test start and test end after 72 hours.

Biological observations

At 24, 48 and 72 hours after the start of the test, the biomass (number of cells per millilitre) in all test vessels including control was determined by direct counting using a Neubauer counting chamber.

Statistics

The 72 h EC_{50} values were calculated by probit analysis. NOEC/LOEC values were calculated by Willams test or Welch test with Boferroni adjustment ($p \leq 0.05$, one-sided).

Results and discussion

Analytical measurements

Measured concentrations of the test item ranged from 90.6 and 93.2% of nominal concentrations at test initiation and from 94.9 and 97.3% of nominal at test termination. Hence, biological results are based on nominal concentrations.

Table A 28: Nominal and measured concentrations of test item

Nominal and measured concentrations of test item						
	Measured concentration [mg a.s./L]					
Nominal concentration	0.0	39.0	78.0	156	312	624
Test start (0 h)						
Measured concentration	-	35.3	71.1	145	284	581
% of nominal	-	90.6	91.2	93.2	90.9	93.2
Range	90.6 - 93.2%					
Test end (72 h)						
Measured concentration	-	37.4	74.0	150	297	607
% of nominal	-	95.8	94.9	96.1	95.3	97.3
Range	94.9 - 97.3%					

Limit of quantification: 0.344 mg/L

Biological results

Biological result are given in the following table:

Table A 29: Percentage of inhibition of growth rate and yield of *Desmodesmus subspicatus* after 72 h exposure to MCW-2222

Nominal concentration [mg test item/L]	Biomass [x 10 ⁴ cells/mL]	% Inhibition (0-72 h)	
		Growth rate	Yield
0 (Control)	23.21	0	0
218.8	23.50	0 (-0.3) ^a	0 (-1.3) ^a
437.5	18.83	5.4*	19.3*
875.1	14.58	12.1*	38.0*
1750.2	8.42	26.5*	65.1*
3500.3	2.75	55.8*	90.1*

* Significantly different from control (Williams t-test, $p \leq 0.05$, one-sided)

a) Negative values in % inhibition indicate an increase in growth relative to that of

Table A 30: EC₅₀-values and 95% confidence intervals (0 – 72 h) of MCW-2222 based on nominal test item concentrations [mg test item/L]

Endpoints (0 – 72 h)	Nominal concentration [mg test item/L]
E _r C ₅₀	3110.8 (2701.4 – 3754.0)
E _y C ₅₀	1149.5 (956.3 – 1386.1)
LOEC	437.5
NOEC	218.8

Validity criteria

As shown in the following table, all validity criteria were met.

Table A 31: Validity criteria

Validity criteria according to OECD 201	Observed in study
Exponential biomass increase in the control cultures by a factor of at least 16 within the 72-hour test period, corresponding to a specific growth rate of 0.92 day ⁻¹ .	46.4
Mean coefficient of variation for section-by-section specific growth rates (days 0-1, 1-2 and 2-3, for 72-hour tests) in the control cultures $\leq 35\%$.	34.8%
Coefficient of variation of average specific growth rates during the whole test period in replicate control cultures $\leq 7\%$ in tests with <i>Pseudokirchneriella subcapitata</i> and <i>Desmodesmus subspicatus</i> (for other less frequently tested species, the value should not exceed 10%).	1.9%

Conclusion

In a 72 hour growth inhibition test algae cells of *Desmodesmus subspicatus* were exposed to a range of test item concentrations. Based on nominal concentrations the E_rC₁₀, E_rC₂₀ and E_rC₅₀ values (0-72 h) for the average specific growth rate were calculated to be 822.6, 1298.7 and 3110.8 mg test item/L (corresponding to 146.6, 231.5 and 554.5 mg a.s./L, nominal). Based on nominal concentrations the E_yC₁₀, E_yC₂₀ and E_yC₅₀ values (0-72 h) for yield were calculated to be 339.4, 515.9 and 1149.5 mg test item/L (equivalent to 60.5, 92.0 and 204.9 mg a.s./L, nominal). the NOEC (no observed effect concentration) for the average specific growth rate and yield was determined to be 218.8 mg test item/L (equivalent to 39.0 mg a.s./L, nominal).

A 2.2.2 KCP 10.2.2 Additional long-term and chronic toxicity studies on fish, aquatic invertebrates and sediment dwelling organisms

A 2.2.3 KCP 10.2.3 Further testing on aquatic organisms

A 2.2.3 KCP 10.2.3/01 Mesocosm study

Comments of zRMS:	<p>The newly submitted mesocosm study with (Hommen et al., 2022) has been validated by the zRMS against criteria given in:</p> <ul style="list-style-type: none">• EFSA aquatic guidance (2013),• OECD 53 (2006): Guidance document on simulated freshwater lentic field tests (outdoor microcosms and mesocosms),• Giddings et al. (2002): Community Level Aquatic System Studies – Interpretation Criteria (CLASSIC). <p>In the study formulation ADM.00150.1.2.A was applied to established systems twice with 7 days interval. The test item has been introduced directly to the water column using the separating funnels followed with mixing (“toxicological approach”) which was relevant for situations when run-off or drainage are the main route of migration of acetamiprid into the surface water bodies and represented worst case for situations when spray drift is the main route of migration of the active substance to surface water bodies, as the test item was mixed with the pond water resulting with instantaneous exposure of the tested species (in case of application to the pond surface it takes some time before the test item is distributed in the water column).</p> <p>Three replicates per test item group and 5 replicates for controls were used, which is more than minimum 2 replicates recommended by OECD 53.</p> <p>The test duration was 86 days (12 weeks after dosing in order to allow detection of delayed effects on e.g. emergence of insects) and 79 after second application.</p> <p>The concentration of acetamiprid in water was verified approximately 3-3.5 hours after the both applications day 0 and 7 and on days: 1, 3, 6, 8, 10, 15, 22, 29 ,43, 57, 85, and which provided detailed information on fate and behaviour of the active compound in the water column. Sediment was analysed for residues of acetamiprid on days 6, 14, 22, 29, 43, 57, 85 which was sufficient to confirm dissipation of the active substance to this compartment and exposure of sediment dwelling organisms.</p> <p>The biological sampling closely followed the sampling schedule during the mesocosm study evaluated and agreed at the EU level (Hommen, 2015) and was in line with recommendations of OECD 53.</p> <p>In order to evaluate the scientific reliability of the mesocosm, the questions listed in point 9.3.3 of EFSA (2013) has been addressed below.</p> <p>The reliability of the study was further evaluated using checklist provided in Table 32 in point 9.3.3 of EFSA (2013).</p> <table><tr><th>Items</th><th>Notes</th><th>Reliability index 1-3</th></tr><tr><td colspan="3">Methodology and test description</td></tr><tr><td>1. Substance</td><td>Properly characterised and reported?</td><td>1</td></tr><tr><td>1.1 Concentration</td><td>Identity and amount of a.s. per litre test water?</td><td>Yes</td></tr><tr><td>1.2 Formulation and purity</td><td>Substances in the formulation influencing the working action of the a.s. should be reported</td><td>Tested formulation as will be used in the field, but no substances influencing working action of the a.s.</td></tr></table>	Items	Notes	Reliability index 1-3	Methodology and test description			1. Substance	Properly characterised and reported?	1	1.1 Concentration	Identity and amount of a.s. per litre test water?	Yes	1.2 Formulation and purity	Substances in the formulation influencing the working action of the a.s. should be reported	Tested formulation as will be used in the field, but no substances influencing working action of the a.s.
Items	Notes	Reliability index 1-3														
Methodology and test description																
1. Substance	Properly characterised and reported?	1														
1.1 Concentration	Identity and amount of a.s. per litre test water?	Yes														
1.2 Formulation and purity	Substances in the formulation influencing the working action of the a.s. should be reported	Tested formulation as will be used in the field, but no substances influencing working action of the a.s.														

			identified.
	1.3 Vehicle	In case a vehicle - other than in the formulation - is used, identity and concentration?	No vehicle used (test item diluted in water)
	1.4 Chemical analyses	Method, LOQ, LOD, recovery	Yes, in the analytical phase report
	1.5 Properties	Relevant for potential fate and effects in test system	Yes, water and sediment samples taken up to the test termination at sufficient frequency.
	2. Test site, duration	Properly characterised and reported?	1
	2.1 Location	Necessary to make a link between the effects and local environmental conditions, representativeness	Yes, all details given
	2.2 Test date/duration	Application dates and experimental period?	Yes, all details given
	2.4 General climatic conditions	Necessary to make a link between the effects and local climatic conditions	Yes, all details given
	3. Application	Properly characterised and reported?	1
	3.1 Mode of application	Exposure route; spraying or homogenising the a.s. into the test medium?	All details given, test solutions introduced using separating funnels in a way assuring uniform distribution in the water column.
	3.2 Dosage and exposure	Actual concentrations during the test? Chemical analysis of dosing solution?	Actual concentrations were verified until the test termination (day 84). Sampling frequency sufficient to determine the exposure profile. Stock solutions analysed to calculate theoretical loading and verify intended concentrations.
	3.3 Application scheme	Necessary to make a link between the test and the intended use of the PPP	All details given in the study report, linking of the application in the test and in the field possible.
	3.4 Conditions during applications	Weather conditions during application, wind speed and temperature?	Yes, all details given
	4. Test design	Properly designed and reported?	1
	4.1 Type and size	e.g. outdoor microcosm, outdoor pond or mesocosm; dimensions	All details regarding the type of mesocosm, its size and set up provided in the study report.
	4.2 Pre-treatment	Proper equilibration?	Yes (9 months, September 2019)

	4.3 Treatment period	Number and spacing of treatments?	Yes (2 applications, 7 days interval).
	4.3 Post-treatment	Period long enough to allow expression of effects and recovery?	Yes (12 weeks after first application)
	4.4 Untreated control	Sufficient number; solvent applied?	Sufficient number of controls (5), no solvent used since test item dissolved in water.
	4.5 Replications	Sufficient replications for proper statistical analysis?	Yes, 3 replicates per test item group and 5 per control (minimum 2 indicated in OECD 53).
	4.6 Statistics	Univariate and multivariate techniques applied	All relevant methods used for statistical analysis of results (Williams-test to determine the NOEC, diversity analysis, ordination analysis, principal component analysis (PCA), redundancy analysis (RDA) and principal response curve analysis (PCR)). MDD's calculated, in line with requirements of EFSA (2013).
	4.8 Dose-response	Number of test concentrations for finding a dose–response relation (controls excl.)	Yes, sufficient number of test concentrations (5).
	4.9 Quality assurance	Study conducted under GLP?	Yes
	5. Biological system	Representative and properly reported?	1
	5.1 Populations	Enough sensitive/vulnerable species of the relevant taxonomic group?	Yes, sufficient taxa of macroinvertebrates, emerging insects and zooplankton. Efforts made to assure sufficient abundance of species identified at the EU level as most sensitive.
	5.2 Community	The community/ecosystem representative and complete?	Yes
	6. Sampling	Is sampling adequate for risk assessment	Yes, sampling in line with indications of OECD 53 and sampling regime in the EU agreed mesocosm study.
	6.1 General features	Relevance selected measurement endpoints	Yes
	6.2 Actual concentration	Actual concentrations measured in medium and other compartments or biota?	Yes, actual concentrations measured in water column and sediment.
	6.3 Biological sampling	Appropriate methods and frequency?	Yes, sampling in line with indications of OECD 53 and sampling regime in the EU agreed mesocosm study.

Results		
7. Endpoints	Properly reported?	1
7.1 Type	Reported endpoints relevant for objective of study?	Yes
7.2 Value	Are measured data consistently presented?	Yes, detailed data for particular species, populations and communities presented in the study report in tabular and graphical form.
7.3 Verification of endpoint	Test results are verifiable and source data reported	Yes, all raw data available in the biological phase report.
8. Elaboration of results	Are conclusions based on measured data? Methodology correct?	1
8.1 Statistical comparison	Data meet requirements for method used?	Yes
8.2 Dose-effect relationship	Minimal detectable difference; consistence of response	Yes, MDD calculated for species and populations
8.3 Population-level responses	Sufficiently reported?	Yes
8.3 Community-level responses	Sufficiently reported?	Yes
9. Control		1
9.1 Untreated control	Unexpected effects or disappearance of species?	No, but as in case of such experiments, abundance of some taxa declined during or by the end of the study (consistent in all test groups).
9.2 Solvent control	Possible effects caused by solvent?	No solvent control required (test item dissolved in water).
10 Classification of effects	Properly derivable?	Yes
11 Biological meaning of statistically significant differences	Sufficiently explained?	Yes
<p>Explanation to reliability index:</p> <p>1 Reliable 2 Less reliable 3 Not reliable</p> <p>Mean recoveries in the enclosure water were 94 % of the nominal concentration after the first application and 92 % of the nominal concentration after the second application. The first water samples were taken 3 to 3.5 hours after application with a mean recovery of 94 % after the first and 172 % after the second application (due to accumulated residues from the first application) if related to the refined nominal concentrations. The mean maximum measured concentrations per treatment level were 0.55, 0.87, 1.4, 2.5 and 4.4 µg a.s./L.</p>		

If for each enclosure the measured concentration on day 6 is subtracted from the concentrations measured three hours after the second application, on average 92 % of the refined nominal concentration was found after the second application. Variability between replicates is probably caused by not complete homogenous distribution shortly after application.

In test item groups concentration of acetamiprid after first application exceeded 80% of nominal up to day 6 of the study, with exception two lowest test concentration groups : 0.30 and 0.51 µg a.s./L, in which the measured concentration on day 6 was at 78.2% and 79.1% of nominal (information based on raw data from the Analytical phase report). It is also noted that after the second application (day 7) the measured concentrations increased in all test group concentration as follows: 185% (at 30 µg a.s./L), 170% (at 0.51 µg a.s./L), 161 % (at 0.87 µg a.s./L) % , 168% (at 1.5 µg a.s./L) and 176 % (at 2.5 µg a.s./L) of nominal,. It gives a a mean value of 172%). The were were in range 50-60% of nominal up to day 46. Clear decrease in measured concentrations was observed on day 84 in all test item groups, (5.44 to 14.8 % of nominal, with mean approximately 10%).

The table below giving clearer overview of exposure to acetamiprid during the study has been copied from the study report and presented below:

Table 25: Analytical results of acetamiprid in aqueous main test samples – control treatment.
Nominal water concentration = 0 µg a.s./L

Sampling Time [d]	Analyte concentration measured [µg a.s./L] Mesocosm enclosure no.				
	A1	A2	A3	A4	A5
0.125	0.0242	0.0250	0.0247	0.0239	0.0236
1					<LOQ
3		<LOQ			
6			<LOQ		
7.125	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
8				<LOQ	
10					<LOQ
15			<LOQ		
22				<LOQ	
29					<LOQ
43	<LOQ				
57		<LOQ			
85			<LOQ		

LOQ = 10 ng a.s./L

Table 26: Analytical results of acetamiprid in aqueous main test samples – treatment level 1.
Nominal water concentration = 0.30 µg a.s./L

Sampling Time [d]	Analyte concentration measured [µg a.s./L] Mesocosm enclosure no.				Percent of nominal [%]
	A6	A7	A8	Mean	
0.125	0.308	0.296	0.292	0.299	99.6
1	0.265	0.256	0.246	0.256	85.3
3	0.253	0.242	0.230	0.242	80.6
6	0.248	0.232	0.224	0.235	78.2
7.125	0.548	0.491	0.623	0.554	185
8	0.525	0.457	0.446	0.476	159
10	0.488	0.456	0.435	0.460	153
15	0.450	0.403	0.410	0.421	140
22	0.412	0.342	0.369	0.374	125
29	0.384	0.282	0.327	0.331	110
43	0.226	0.129	0.198	0.184	61.5
57	0.133	0.0792	0.151	0.121	40.4
85	0.0356	0.0224	0.0753	0.0445	14.8

LOQ = 10 ng a.s./L

Table 27: Analytical results of acetamiprid in aqueous main test samples – treatment level 2.
Nominal water concentration = 0.51 µg a.s./L

Sampling Time [d]	Analyte concentration measured [µg a.s./L] Mesocosm enclosure no.			Mean	Percent of nominal [%]
	A9	A10	A11		
0.125	0.523	0.527	0.535	0.529	104
1	0.436	0.445	0.457	0.446	87.4
3	0.427	0.416	0.426	0.423	82.9
6	0.403	0.388	0.419	0.403	79.1
7.125	0.881	0.832	0.896	0.869	170
8	0.906	0.771	0.862	0.846	166
10	0.800	0.792	0.822	0.804	158
15	0.745	0.705	0.708	0.719	141
22	0.663	0.640	0.647	0.650	127
29	0.572	0.550	0.592	0.571	112
43	0.377	0.325	0.362	0.355	69.6
57	0.218	0.194	0.239	0.217	42.6
85	0.0651	0.0793	0.0642	0.0695	13.6

LOQ = 10 ng a.s./L

Table 28: Analytical results of acetamiprid in aqueous main test samples – treatment level 3.
Nominal water concentration = 0.87 µg a.s./L

Sampling Time [d]	Analyte concentration measured [µg a.s./L] Mesocosm enclosure no.			Mean	Percent of nominal [%]
	A12	A13	A14		
0.125	0.760	0.745	0.773	0.760	87.3
1	0.730	0.730	0.736	0.732	84.2
3	0.725	0.671	0.718	0.705	81.0
6	0.701	0.720	0.676	0.699	80.4
7.125	1.46	1.31	1.45	1.40	161
8	1.32	1.39	1.46	1.39	160
10	1.39	1.39	1.31	1.36	156
15	1.18	1.24	1.13	1.18	136
22	1.18	1.10	1.04	1.11	127
29	1.079	0.945	0.921	0.982	113
43	0.650	0.523	0.467	0.547	62.8
57	0.347	0.239	0.231	0.272	31.3
85	0.0947	0.0484	0.0589	0.0673	7.74

LOQ = 10 ng a.s./L

Table 29: Analytical results of acetamiprid in aqueous main test samples – treatment level 4.
Nominal water concentration = 1.5 µg a.s./L

Sampling Time [d]	Analyte concentration measured [µg a.s./L] Mesocosm enclosure no.			Mean	Percent of nominal [%]
	A15	A16	A17		
0.125	1.34	1.35	1.30	1.33	88.6
1	1.27	1.33	1.29	1.30	86.6
3	1.18	1.30	1.23	1.24	82.6
6	1.15	1.24	1.24	1.21	80.7
7.125	2.44	2.59	2.55	2.53	168
8	2.43	2.49	2.37	2.43	162
10	2.34	2.49	2.43	2.42	161
15	2.03	2.13	2.06	2.07	138
22	1.75	1.94	1.87	1.85	123
29	1.53	1.67	1.56	1.59	106
43	0.596	0.907	0.784	0.762	50.8
57	0.185	0.491	0.390	0.355	23.7
85	0.0287	0.145	0.113	0.095	6.36

LOQ = 10 ng a.s./L

Table 30: Analytical results of acetamiprid in aqueous main test samples – treatment level 5.
Nominal water concentration = 2.5 µg a.s./L

Sampling Time [d]	Analyte concentration measured [µg a.s./L] Mesocosm enclosure no.			Mean	Percent of nominal [%]
	A18	A19	A20		
0.125	2.34	2.28	2.29	2.30	92.2
1	2.22	2.24	2.16	2.21	88.3
3	2.04	2.15	2.09	2.09	83.7
6	2.03	2.06	2.02	2.04	81.5
7.125	4.29	4.57	4.32	4.39	176
8	4.08	4.53	4.09	4.23	169
10	4.75	4.20	4.23	4.39	176
15	3.42	3.63	3.54	3.53	141
22	3.20	3.49	3.29	3.33	133
29	2.86	2.97	2.86	2.90	116
43	1.53	1.71	1.36	1.53	61.3
57	0.789	0.852	0.520	0.720	28.8
85	0.187	0.177	0.0439	0.136	5.44

LOQ = 10 ng a.s./L

We agree that since the dissipation of acetamiprid in the enclosure water was relatively slow these maximum measured concentrations of 0.55, 0.88, **1.4**, 2.5 and 4.5 µg a.s./L are considered to be most relevant for a comparison with maximum PEC values.

The study is robust and valid and due to the exposure regime applied, the geometric mean concentration between nominal and mean measured concentrations at t = 7 days is most suitable to derive the actual test concentrations. This results in a mean measured concentration of 1.12 µg a.s./L (6.4 µg test item/L) used to derive an ETO-RAC. This is considered a conservative approach as it could be justified that the overall peak measured concentration (1.40 µg a.s./L) at the NOEC should be used to derive the ETO-RAC. Considering the conservatism of using the geometric mean measured concentration in addition to the high comparability of recent results with findings of the mesocosm study conducted with acetamiprid during the active substance renewal process, an assessment factor of 2 is justified according to EFSA (2013).

An ETO-RAC of 0.56 µg a.s./L (3.18 µg test item/L) is used in the risk assessment.

It should be noted that if the mean measured value is 1.4 µg a.s./L and nominal value is 0.87 µg a.s./L is considered the geomean mean concentration is 1.104 µg a.s./L not 1.12 µg a.s./L and RAC=0.55 µg a.s./L. Due to that difference is small the **RAC of 0.56 is acceptable by zRMS**.

Concentration of acetamiprid in control samples was <LOQ at all sampling occasions.

	<p>The study is acceptable and sufficiently reliable to be used for purposes of refinement of the risk assessment for aquatic invertebrates.</p> <p>The following effects were observed at the different test concentrations:</p> <ul style="list-style-type: none"> • 0.55 µg a.s./L maximum measured (0.30 µg a.s./L nominal), effect class 1: No treatment related effects were found. • 0.88 µg a.s./L maximum measured (0.51 µg a.s./L nominal), effect class 1: No treatment related effects were found. • Maximum measured 1.4 µg a.s./L (0.87 µg a.s./L nominal), effect class 2: No treatment effects were found except a slight effect on the mayfly <i>Cloeon dipterum</i>. Single statistical findings with NOECs of 1.4 µg a.s./L were found not to be ecotoxicologically relevant due to very low numbers of animals in the samples, missing concentration-response, and / or implausible timing of the statistical findings. • 2.5 µg a.s./L maximum measured (1.5 µg a.s./L nominal) effect class 5B: This concentration had only slight effects on mayfly larvae abundance but pronounced effects on the emergence of <i>Cloeon dipterum</i> with recovery of emergence demonstrated at the end of the study (class 5A). <i>Gammarus</i> survival was slightly affected but Naididae were affected without a clear demonstration of recovery until the end of the study (class 5B). At the community level, the macroinvertebrates and the emergence of insects were affected without recovery since <i>Cloeon</i> and Naididae dominated the community response. In the Zooplankton, <i>Chydorus sphaericus</i> abundance might have been slightly promoted. Temporarily higher oxygen levels indicate an indirect promotion of primary production but no effects on algae and macrophytes were detected. • 4.5 µg a.s./L maximum measured (2.5 µg a.s./L nominal), effect class 5B: Compared to 2.5 µg a.s./L (max. measured), some effects became more pronounced and for additional species slight direct or indirect effects were found. The number of mayfly larvae were clearly reduced but recovered towards the end of the study. The abundance of <i>Asellus</i> was affected but did not recover within the course of the study. Emergence of some chironomids was temporarily reduced (effect class 3A) while damselflies emerged at lower numbers until the end of the study (class 5B). The water beetle <i>Helophorus</i> sp. was found in higher numbers in the emergence traps shortly after the second application. The potential promotion of <i>Chydorus sphaericus</i> became more pronounced and slight promotion might also be given for Ostracoda and Copepoda (abundance of nauplius larvae) in the zooplankton. The promotion of the zooplankton taxa which also graze on periphyton might be an indirect effect of reduced competition by <i>Cloeon</i> larvae affected directly by the test item. <p>Based on the effect classes and statistical evaluation it may be concluded that no effects were observed up to the concentration of 1.12 µg a.s./L (peak measured) and this concentration is considered to be the NOEC from the study to derive the ETO-RAC.</p>
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KCP 10.2.3/01

Data point	
Report	Acetamiprid - Outdoor mesocosm study Test item: Acetamiprid 200 SL (Code: ADM.00150.1.2.A)
Report No.:	Study number: ADM-025/7-52
Document No.:	000106190 (ADAMA reference number)
Guideline(s):	OECD Guidance Document “Freshwater Lentic Field Tests - Outdoor Microcosms and Mesocosms (OECD, 2006) EFSA guidance document on tiered risk assessment for plant protection products for aquatic organisms in edge-of-field surface waters (EFSA PPR panel, 2013)
Deviations:	By mistake (due to the wrong information on water volumes), the concentrations in the application solutions were 21% lower than the nominal concentrations given in the study plan. Therefore, the refined nominal concentrations are 79% of the originally planned nominal

concentrations. This refinement of the nominal concentrations has no effects on the measured water concentrations.

This deviation is considered minor with no influence on the integrity or outcome of the study.

GLP: Yes, certified laboratory
Acceptability: Yes, study considered acceptable
Duplication (if vertebrate study) Not applicable

Executive Summary

An 86-day outdoor mesocosm study was performed to investigate effects of two applications of the insecticide acetamiprid on freshwater ecosystems in outdoor mesocosms. The test system was located in Germany and consisted of steel enclosures with a volume of approximately 1600 L within an artificial pond. Nominal concentrations of 0.30, 0.51, 0.87, 1.5, 2.5 µg a.s./L plus an untreated control were tested. The main focus of the study was to investigate the direct effects of acetamiprid on aquatic insects, benthic macroinvertebrates and zooplankton. However, algae and plants were also monitored to detect indirect effects. In addition to the biological aspects, the correct dosing, fate and distribution of the active substance was monitored in the water body and the sediment.

The tested community was representative for lentic and slow flowing water bodies (excluding vertebrates). The test ponds included macrophytes and a high number of algae and invertebrate species from a large variety of taxonomic groups. Since the study was conducted in enclosures located within an artificial pond, typical stream taxa like stoneflies, caddisflies (Plecoptera and Trichoptera) and Amphipoda like *Gammarus* sp., were not present or only in low numbers. However, *Gammarus* was successfully introduced and tested in an in-situ bioassay.

Mean recoveries in the enclosure water were 94 % of the nominal concentration after the first application and 92 % of the nominal concentration after the second application.

Dissipation of acetamiprid from the water was relatively slow. The average DT₅₀ was calculated to be 19 days. The DT₅₀ of the single enclosures showed a slight trend of faster dissipation with increasing test concentration (about 22 d at the two lower concentrations and about 16 d at the two highest concentrations). Thus, six weeks after the first application still more than 50 % of the nominal concentrations were present in the water. No acetamiprid was detected in water or sediment samples of the controls.

In total 15 potentially sensitive taxa fulfil the MDD criterion proposed by Brock *et al.* (2015), including mayflies (*Cloeon dipterum*), midges (*Chaoborus crystallinus*), Chironominae (*Tanypodinae*, *Orthocladinae*), damselflies (Zygoptera, *Coenagrionidae*), *Helophorus* sp., Isopoda (*Asellus aquaticus*), *Gammarus* sp. (bioassay on survival), Cladocera (three species), Copepoda (Cyclopidae), Ostracoda and Naididae.

Due to the long-term exposure of the organisms in the mesocosm, the MDD criterion by Brock *et al.* (2015), based on all MDDs after the first application seems appropriate. If only the first six weeks after application were considered for macroinvertebrates and zooplankton, when exposure was on average higher than 50 % of the nominal, still more than eight taxa (including *Cloeon* and Naididae) revealed at least once MDDs up to 70 % which is sufficient to detect medium effects following the EFSA PPR panel (2013).

The maximum measured concentration of 1.4 µg a.s./L (0.87 µg a.s./L nominal) is the overall Class 2 concentration which can be used to derive an ETO-RAC. Uncertainty related to this concentration is considered small since clearly no pronounced effects on potentially sensitive taxa were found. These results are in line with the findings of a previous mesocosm study with acetamiprid (EFSA 2016).

An ERO-RAC cannot be derived from this study according to the current guidance (EFSA PPR panel, 2013) since at the next higher test concentration effects on mayflies lasted longer than eight weeks.

Materials and methods

Materials

Test item

Acetamiprid 200 SL (Code: ADM.00150.1.2.A)

Code No.

ADM.00150.1.2.A
(old Code: MCW-2222)

Batch #	99191024
Content a.s. nominal	200 g /L
Content a.s. measured	200.1 ± 3 g
Description	Liquid, clear, yellow to brown
Test organisms	<p>The representative aquatic community included:</p> <ul style="list-style-type: none"> - Algae species from several classes - Zooplankton (several species of cladocerans, copepods, rotifers) as well as midge larvae of <i>Chaoborus</i> sp. - Macroinvertebrates (e.g. <i>Asellus aquaticus</i>, Chironomidae, Ephemeroptera, Odonata, Hirudinea, Oligochaeta, Gastropoda, Bivalvia. - Rooting macrophytes (e.g. <i>Chara globularis</i>, <i>Myriophyllum spicatum</i>) covering not more than 30% of the sediment surface at application. - <i>In-situ</i> bioassays with 20 individuals of <i>Gammarus</i> sp. (introduced into each replicate one day before the 1st application.) - Introduction of vertebrates e.g. amphibians or fish was avoided. <p>Organisms were introduced with the sediment and the water from an uncontaminated source site as well as via aerial colonisation.</p>
Source	Natural water bodies on the test site of Mesocosm GmbH, Homberg/Ohm, Germany
Study design and methods	
Test duration and exposure	86 days after the first and 79 days after the second test item application. To allow detection of delayed effects, e.g. the emergence of insects the duration of the study after dosing was > 12 weeks.
Application no. and dates	<p>1st application on June 3rd 2020 = day 0</p> <p>2nd application on June 10th 2020 = day 7</p> <p>End of in-life phase on 28 August 2020 = day 86</p>
Application method	Application was conducted following the so-called toxicological approach, i.e. the application solution (including the rinse water) was introduced directly into the water column by means of separating funnels.
Test concentrations	<p>Nominal concentrations:</p> <p>0.0 (control), 0.30, 0.51, 0.87, 1.5, 2.5 µg a.s./L corresponding to: 0.0 (control), 0.55, 0.88, 1.4, 2.5, 4.5 µg a.s./L maximum measured concentrations and to 0.0 (control), 1.7, 2.9, 4.9, 8.5, 14 µg test item/L.</p>
Test units	<p>Stainless-steel enclosures each with a diameter of approximately 1.43 m (surface approximately 1.6 m²) and a depth of approximately 1.5 m. With a depth of the water body of about 100 cm ± 15%, the total volume of each enclosure was approximately 1600 L.</p> <p>Stainless steel enclosures were pressed into the sediment of “Big Pond A”.</p>
Group size/replicates	20 enclosures were used in the study. One enclosure represented one replicate. Three replicates were used per test concentration and five replicates were used for the control.
Test medium	The water of “Big Pond A” was originally taken from an uncontaminated lake on site in September 2019. The water was mixed with rain water to give the ultimate water body.
Sediment	Big Pond A was filled with a clay layer of about 10 cm. another layer of about 10 cm consisted of natural sediment (upper 40 cm horizon collected from a lake site at a depth of 0.7 m). It was mixed with washed sand to achieve in sediment with a TOC content of about 1 %.
Adaptation	The period for equilibration of the enclosures was about nine months starting in September 2019 and ending at start of the study in June

2020.

Environmental conditions

Temperature

The mean water temperature was 18.4 °C and ranged between a minimum of 15.0 °C at the end of May (27.06.2020, day -7) and a maximum of 22.2°C mid of August (12.08.2020, day 70).

Photoperiod

Natural

pH

Mean of 9.37 with a range of 7.9 – 10.7

Dissolved oxygen

Mean of 11.7 with a range of 7.2 – 16.7 mg/L

Conductivity

Mean of 224 with a range of 166 – 310 µS/cm

Samplings and measurements

The following parameters were measured over the course of the study:

- Water quality: Temperature pH, conductivity, oxygen concentration, concentrations of ammonium, nitrate and phosphate, water hardness, total and dissolve organic carbon (TOC, DOC).
- Zooplankton: Abundances of taxa per liter
- Macroinvertebrates via different sampling techniques, including different samplers, e.g. for sediment dwellers and netting
- In-situ *Gammarus* assay: 20 individuals of *Gammarus* sp. were introduced one day before the first application into each enclosure in stainless-steel cages to allow monitoring of survival.
- Emerging insects: Abundance of emerged insect by means of two emergence traps per enclosure.
- Phytoplankton chlorophyll-a: Delayed fluorescence analysis of chlorophyll-a, which allows to differentiate four major algae groups (green, blue-green, chromophyte and cryptophyte algae)
- Periphyton: Delayed fluorescence analysis of chlorophyll-a as for phytoplankton
- Macrophytes: Mapping of area coverage.
- Acetamiprid concentrations in water (depth integrated sampling) and sediment samples

Analytical measurements

Acetamiprid concentrations in water and sediment samples were analysed by Ultra-High-Performance Liquid Chromatography (UHPLC) coupled to a triple quadrupole mass spectrometer (MS). The MS was operated in the tandem mass spectrometry mode (MS/MS). The validation of the analytical method was performed based on the guidance document SANCO/3029/99 rev.4 (11/07/00) in a separate GLP study (study ADM-026/6-22). The analytical method was validated at a concentration of 10.0 ng a.s./L as LOQ for the water phase and at a concentration of 50.0 ng a.s./kg dw as the LOQ for the sediment (see ADM-036-22; Hennecke, 2020).

Statistics

The biological data sets were statistically analysed by uni- and multivariate statistics. For each taxon (phylum down to species level, if appropriate), univariate statistics were used to test differences between the treated enclosures and the controls, and to calculate respective NOECs (No Observed Effect Concentration).

Effects on the community levels were analyzed by Principal Response Curves (PRC) for macroinvertebrates, emerged insects and zooplankton. For the PRC, all taxa including the rare ones which don't give reliable results in the univariate analysis, were considered.

Williams-test, NOEC calculation, MDDs

For each taxon (species or higher taxonomic level if appropriate) and sampling date, Williams t-test was used to test the differences between means in controls and treatments and to calculate the NOEC (No Observed Effect Concentration) on the population level. Minimum Detectable Differences (MDD) at the NOEC are reported in accordance with the EFSA aquatic guidance document (EFSA PPR panel, 2013) and Brock et al. (2015). The abundance data of the organisms were log-transformed ($y' = \ln(y + 1)$) before the analysis to approximate normality and homoscedasticity (homogeneity of variances) requirements (van den Brink et al., 2000). All Williams' tests were performed one-sided with $\alpha = 0.05$ (5% level of significance).

MDDs are reported together with the NOECs. The MDD describes the % effect on the non-transformed abundance at the NOEC which would be needed to result in a significant difference compared to the controls.

Table A 32: Classification of MDDs as suggested in EFSA PPR panel (2013)

Class	MDD	Comment
0	> 100%	No effects can be determined
I	90 -100%	Only large effects can be determined
II	70 -90%	Large to medium effects can be determined
III	50 -70%	Medium effects can be determined
IV	< 50%	Small effects can be determined

According to Brock et al. (2015) the NOEC calculation for a given endpoint was considered reliable for effect classification (for a decline in abundance) in this report if the % MDD related to abundance was:

- < 100% (so, at least MDD class I) for at least 5 or
- < 90% (so, at least MDD class II) for at least 4 or
- < 70% (so, at least MDD class III) for at least 3 or
- < 50% (so, MDD class IV) for at least 2 sampling dates after application.

If one of these conditions is fulfilled, the taxon is considered MDD category 1, allowing the assessment of direct effects. Based on the MDDs after the first application, the taxa of a data set were classified into three categories following the proposal by Brock *et al.* (2015):

1. Taxa with sufficiently low MDDs to allow a reliable statistical analysis of direct effects, thus fulfilling the MDD criterion above.
2. Taxa with higher MDDs not fulfilling the MDD criteria but which show a significant difference to the control (decline or increase) on at least one sampling date after first application.
3. Taxa with high MDDs and no significance difference to the control (after first application).

Taxa of MDD category 1 were counted to check whether a statistical analysis of direct effect was possible for at least eight potentially sensitive populations (EFSA PPR panel, 2013). From taxonomically overlapping MDD category 1 taxa (e.g. *Chaoborus* and Diptera) only the lowest taxonomic level was counted. Due to the insecticide mode of action of acetamiprid, insects and crustaceans were considered potentially sensitive. In addition, Naididae were considered relevant since they showed signs of being sensitive in a previous mesocosm study.

Taxa of MDD category 2, i.e. not fulfilling the MDD criterion but with at least one significant difference to the control found after the application, were also assessed further to check if the data indicate a treatment effect. Taxa falling into MDD category 3 (high MDDs and no significant difference from control) were not considered further.

Ordination analysis (PRC, RDA, RDA)

Principal Response Curves (PRCs, van den Brink & Ter Braak, 1998, 1999) including the calculation of Community-NOECs were used for the analysis on the phytoplankton and periphyton data sets. PRCs are a type of ordination analysis especially developed to analyse community level effects e.g. in mesocosm studies. PRCs are calculated via the ordination technique redundancy analysis (RDA), which can be seen as a canonical form of a principal component analysis (PCA) because RDA uses only the variance, which can be attributed to the explanatory variables. Usually the original abundances are log transformed before the analysis, e.g. $y' = \ln(a y + 1)$. In the following, the term abundance is used for the transformed data.

Software

The program Community Analysis V4.3 (CA) was used for NOEC, MDD and diversity calculations. A former version of the CA program is described in Hommen et al. (1994). Calculations of the CA program have been validated by means of example data and of calculations using MS-Excel™ (Microsoft® Corp.) and ToxRat® (Vers. 2.09). The PRC analysis was performed with CANOCO™ 4.5 (DLO, Wageningen, The Netherlands).

Effect evaluation

The biological effects on a taxon were classified for each treatment level according to the recommendations of the EFSA guidance document (2013) and Brock *et al.* (2015), considering also the MDDs (⁸). In order to differentiate cases where recovery is clearly not shown (effect class 5A or 5B) from cases where recovery cannot be demonstrated (e.g. the taxon is declining or absent in the controls during the recovery period, the effect is found at the end of the study, or the MDD is too large to demonstrate recovery), effect class 4 was further differentiated. Originally class 4 has been used for cases when the study was too short to test recovery within 8 weeks. This is considered class 4A now. If potential treatment effects were found at the end of the study, these were indicated as 2 - 4A or 3A - 4A because the duration of the effect could not be assessed. Class 4B is used if recovery cannot be assessed due to high MDD or decline of abundance also in the controls.

Effect class 0 (treatment related effects cannot be statistically evaluated) does not fit well with the other effect classes because this is a property of the full data set for a taxon over all treatment levels including the controls, while the other effect classes are related to the effect at the different treatment levels. Thus, if treatment related effects cannot be statistically evaluated for a taxon, class 0 would apply for each treatment level. However, these cases are covered already by the MDD categorization of taxa according to Brock *et al.* (2015): all taxa of MDD category 3 are the ones with effect class 0. Therefore, no effect classification was conducted for MDD category 3 taxa.

The aim of the study was to provide endpoints for deriving an ETO-RAC (Ecological Threshold Option – Regulatory Acceptable Concentration) and an ERO-RAC (Ecological Recovery Option RAC) according to EFSA (2013), i.e. to identify the treatment levels with effect classes up to 3A only based on the identification of the most sensitive taxa. Therefore, the focus of the effect evaluation was on the MDD category 1 taxa, i.e. those with sufficiently low MDDs to allow an effect assessment. Taxa of category 2, i.e. those with relatively high MDDs, but nevertheless at least once with a significant difference to controls, are only discussed if, based on the statistical finding they might have been more sensitive than category 1 taxa. Category 3 taxa are not considered further because of high MDD values and missing statistical significance, and, in most cases, their low abundances. However, these taxa were included in the community level analysis.

It should be noted that the MDD evaluation is related to direct effects, i.e. reduction of abundances. If a test item has an indirect effect shown as a treatment related increase of abundance, the MDD classification is not applicable because the effects can be larger than 100 %. Thus, MDD category 2 taxa can be used for the assessment of indirect effect, even if MDDs are high. A promotion effect is indicated by a '+' sign added to the effect class, e.g. 3A+ indicates a pronounced but temporary promotion.

With hundreds of taxa and many sampling dates, a large number of statistical tests were conducted. Using an error level of 5 % means that many positive findings are to be expected just by chance. In addition, by the default use of the Williams' test as a most conservative multiple test, low NOECs can be obtained also in cases without a monotonous (or almost monotonous) concentration response relation – just by the moving average procedure used in the Williams' test. Therefore, the statistical findings were evaluated for their ecotoxicological relevance based on different criteria:

- Does the time of a potential direct effect fit to the exposure dynamics?
- Were effects found over more than one sampling date?
- Was there a reasonable concentration response relation?
- If the effect was potentially indirect, was there a direct effect which could have caused the indirect effect?

⁸ These effect classes should not be confused with MDD classes or the MDD categorization of taxa. Each taxon monitored falls into one of three categories based on its MDD at the different sampling dates but independently from the magnitude of effects. Effect classes are used to summarize the magnitude and duration of effects for a specific taxon at each treatment level considering also the uncertainty indicated by the MDDs.

Table A 33: Definition of effect classes based on Brock et al. (2015)

Effect class	Description	Criteria
1	No treatment-related effects demonstrated	No (statistically and/or ecologically significant) effects observed as a result of the treatment. Observed differences between treatment and controls show no clear causal relationship.
2	Slight effects	Effects concern short-term and/or quantitatively restricted responses usually observed at individual samplings only.*
3A	Pronounced short-term effects (effect period < 8 weeks), followed by recovery	Clear response of sensitive endpoints, but full recovery within 8 weeks after the first application, or in the case of delayed responses and repeated applications, the duration of the effect period is less than 8 weeks and followed by full recovery.* Treatment-related effects demonstrated on consecutive samplings.
3B	Pronounced effects longer than 8 weeks but recovery within 8 weeks after last application	Clear response of the endpoint in micro-/mesocosm experiment repeatedly treated with the test substance and that lasts longer than eight weeks (responses already start in treatment period), but full recovery of affected endpoint within eight weeks post last application.*
4A	Significant effects in short-term study	Clear effects (e.g. large reductions in densities of the population) observed, but the study is too short to demonstrate complete recovery within eight weeks after the (last) application. If delayed response is observed on the last sampling(s) only, this may be indicated as effect class 2-4A or 3A-4A.
4B	Significant short-term effects but MDD too high in recovery period	Significant short-term effects demonstrated but recovery cannot be properly evaluated due to high %MDD values in recovery period or the population in the controls is declining or even absent. If significant treatment related response is demonstrated on one sampling but recovery cannot be interpreted due to high MDD this may be indicated as class 2-4B, in other case it can be 3A-4B.
5A	Pronounced long-term effect followed by recovery	Clear response of sensitive endpoint, effect period longer than 8 weeks and recovery did not yet occur within 8 weeks after the last application but full recovery is demonstrated to occur in the year of application.*
5B	Pronounced long-term effects without recovery	Clear response of sensitive endpoints (> 8 weeks post last application) and full recovery cannot be demonstrated before termination of the experiment or before the start of the winter period.

* Note that following Brock *et al.* (2015) recovery can only be considered if the MDDs during the recovery period are < 70% on at least one sampling or < 90% on at least two samplings or if the deviation to controls is less than 20%. If this is not the case, an appropriate higher class has to be selected.

Results

Analytical measurements

Water

The nominal concentrations in the application solutions were calculated from the volume of the application solutions, the measured water volume of the enclosures and the nominal concentrations intended for the enclosure. The measured recovery in the application solutions was on average 86 % for the first application and 90 % for the second application and thus, 88 % overall.

The first water samples were taken 3 to 3.5 hours after application with a mean recovery of 94 % after the first and 172 % after the second application (due to accumulated residues from the first application) compared to the refined nominal concentrations. The mean maximum measured concentrations per treatment level were 0.55, 0.88, 1.4, 2.5 and 4.5 µg a.s./L.

The course of acetamiprid concentrations in the water is shown in the figures below for the measured concentrations per enclosure and for the percent of nominal concentrations per treatment level. At least until day 28, the variability between the replicates per test concentration was very low and the general pattern over time was similar across the different test concentrations. In the first six weeks after the first application, the concentrations stayed above 50 % of the nominal concentrations. At the end of the study,

eleven weeks after the second application, on average 10 % of the nominal concentrations were still found in the water.

No acetamiprid was found in the control samples (all measured concentrations were below the LOQ).

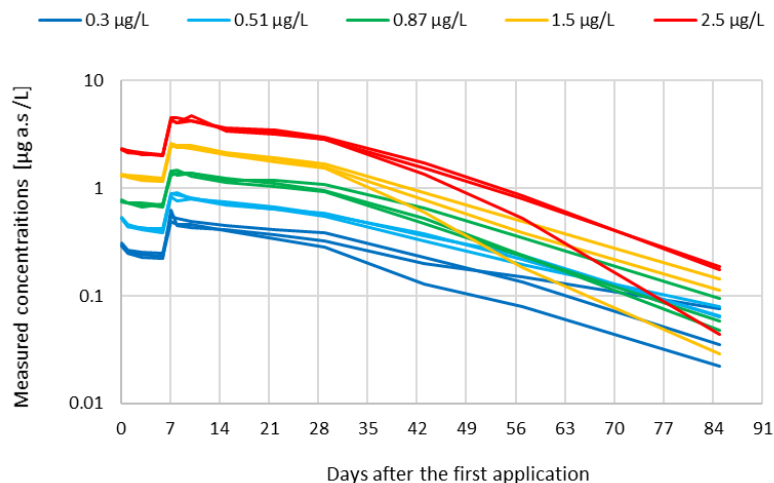


Figure A 1: Measured acetamiprid concentrations in the enclosure water over time

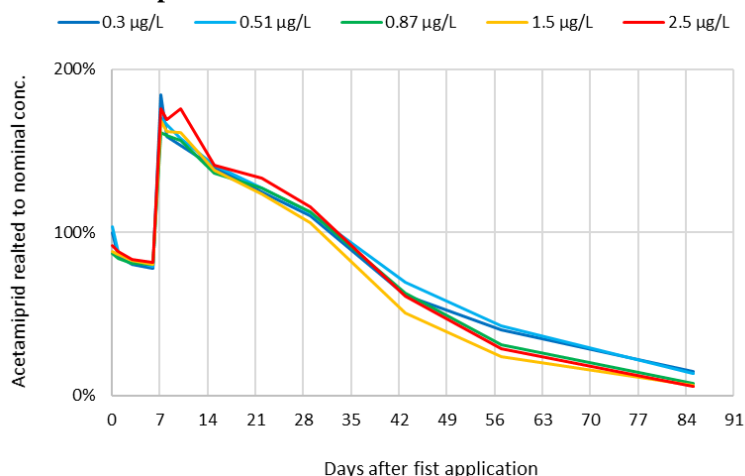


Figure A 2: Acetamiprid concentrations in the water expressed as mean % of nominal concentration per treatment level

Sediment

Concentrations of acetamiprid in the sediment increased slowly during the first few weeks and reached a mean maximum in the highest treatment level of 2.3 µg/kg dw four weeks after the first application (see figure below). Until the end of the study, the mean concentration decreased to an average of 1.2 µg/kg dw in the highest treatment level. The general pattern was similar but the mean measured concentrations partly overlapped for the different treatment levels which can be attributed to the relatively small spacing factor and the spatial heterogeneity of the sediment.

No concentrations above the LOQ were found in the control samples.

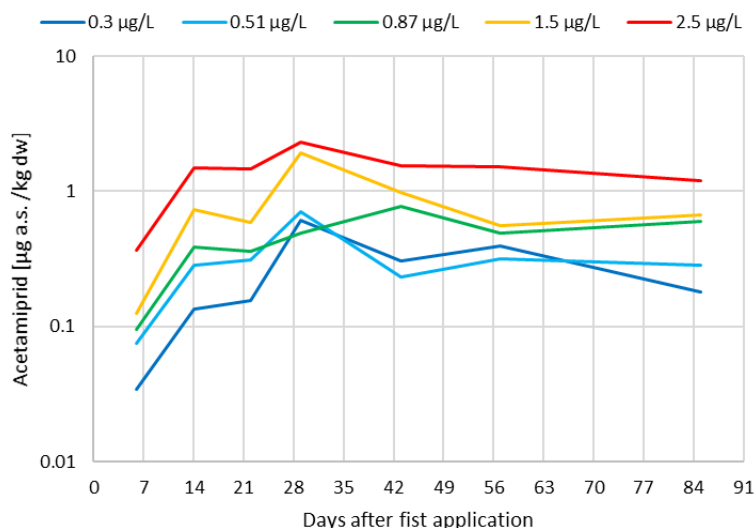


Figure A 3: Concentrations of acetamiprid in the sediment samples as means per treatment level.

A summary of the analytical results in the water and sediment phases is given in the table below.

Table A 34: Nominal and measured concentrations of acetamiprid

	Treatment level				
	1	2	3	4	5
Nominal concentration [$\mu\text{g a.s./L}$]	0.30	0.51	0.87	1.5	2.5
Maximum measured means in water [$\mu\text{g a.s./L}$]	0.55	0.88	1.4	2.5	4.5
Maximum measured means in sediment [$\mu\text{g a.s./kg dw}$]	0.61	0.71	0.78	1.9	2.3

Since the dissipation of acetamiprid in the enclosure water was relatively slow **these maximum measured concentrations of 0.55, 0.88, 1.4, 2.5 and 4.5 $\mu\text{g a.s./L}$ are considered to be most relevant for a comparison with maximum PEC values.** Nevertheless, in the report and accordingly the summary including relevant graphs nominal concentrations were used to indicate the exposure per treatment level in diagrams and tables as well as for defining NOECs and effect concentrations.

Biological results

Macroinvertebrates

Thirty-seven taxa or stages, including 31 taxa, were differentiated in the samples of the macroinvertebrate data set. The most abundant was the snail *Planorbis planorbis* followed by the mayfly *Cloeon dipterum* and the phantom midge *Chaoborus* sp. Other commonly observed taxa were worms of the family of Naididae, the leech *Helobdella stagnalis* of Glossiphoniidae family (6 %), the water louse *Asellus aquaticus*, and Chironomidae including Tanypodinae. For the statistical evaluation, the total quantity of Diptera, *Chaoborus* sp. (larvae and pupae), undetermined Chironomidae (larvae and pupae), total Chironomidae, Hirudinea, Pulmonata and Odonata were calculated individually.

Population level analysis

Eight macroinvertebrate taxa (excluding pooled taxa) fulfil the MDD criterion by Brock *et al.* (2015). For all, the MDD was below 70 % at least once during the six weeks after the first application and thus, belonged to the preferred MDD class III or IV. Four of these, i.e. the mayfly *Cloeon dipterum*, the midges *Chaoborus* sp., Tanypodinae and Chironomidae (not determined), and the water louse *Asellus aquaticus* are insects or crustaceans and thus, are considered potentially sensitive to insecticides such as acetamiprid. However, Chironomidae (not determined) should not be counted as a separate taxa due to the potential overlap with Tanypodinae. Worms of the family of Naididae were found to be potentially sensitive in a previous

mesocosm study with acetamiprid and showed sufficiently low MDDs. In conclusion, direct effects could be analysed for five potentially sensitive taxa in the macroinvertebrate data set according to the MDDs.

Furthermore, the leech *Helobdella stagnalis* and some snails (Planorbidae) fulfilled the MDD criterion by Brock *et al.* (2015), but were not considered potentially sensitive. For several other taxa, the MDDs were higher but a significant difference to the controls was found at least once after application. These MDD category 2 taxa were also analysed for treatment effects. The remaining taxa with high MDDs and without any significant findings after applications (MDD category 3) were not further considered.

Table A 35: NOEC [$\mu\text{g a.s./L}$] (calculated by Williams multiple test) and %MDD (in brackets) for the MDD category 1 and 2 taxa in the macroinvertebrate data set

Macroinvertebrates	Day after application										MDD	Min
	-8	-2	5	13	20	27	41	55	69	83	Cat	MDD
<i>Cloeon dipterum</i>	≥2.5 (54)	≥2.5 (52)	≥2.5 (54)	≥2.5 (63)	0.51- (57)	1.5- (59)	1.5- (66)	1.5- (64)	1.5- (56)	0.51- (53)	1	54
Sum Diptera	≥2.5 (58)	≥2.5 (50)	≥2.5 (34)	≥2.5 (48)	≥2.5 (37)	≥2.5 (40)	≥2.5 (44)	≥2.5 (55)	≥2.5 (58)	≥2.5 (69)	1	34
<i>Chaoborus sp.</i>	≥2.5 (63)	≥2.5 (55)	≥2.5 (39)	≥2.5 (60)	≥2.5 (70)	≥2.5 (62)	≥2.5 (85)	1.5- (89)	≥2.5 (150)	≥2.5 (96)	1	39
Sum Chironomidae	≥2.5 (67)	≥2.5 (58)	1.5- (49)	≥2.5 (62)	≥2.5 (54)	≥2.5 (54)	0.87- (46)	≥2.5 (59)	≥2.5 (57)	≥2.5 (73)	1	46
Chironomidae indet.	≥2.5 (73)	≥2.5 (59)	1.5- (47)	≥2.5 (66)	≥2.5 (59)	≥2.5 (56)	0.87- (43)	≥2.5 (66)	1.5+ (64)	≥2.5 (89)	1	43
Tanypodinae	≥2.5 (116)	≥2.5 (132)	1.5+ (n.c.)	1.5+ (n.c.)	≥2.5 (113)	0.3- (78)	≥2.5 (67)	≥2.5 (61)	1.5- (83)	≥2.5 (85)	1	67
Culicidae	≥2.5 (154)							1.5+ (n.c.)		≥2.5 (154)	2	
Coleoptera	≥2.5 (118)	≥2.5 (112)	≥2.5 (110)	≥2.5 (110)	≥2.5 (103)	0.3- (85)	≥2.5 (96)	≥2.5 (132)	1.5+ (n.c.)	1.5+ (n.c.)	2	85
Sum Odonata	≥2.5 (80)	≥2.5 (85)	≥2.5 (103)	1.5+ (129)	1.5+ (144)	≥2.5 (318)	≥2.5 (n.c.)	≥2.5 (337)	≥2.5 (94)	≥2.5 (86)	2	103
Anisoptera	≥2.5 (n.c.)	≥2.5 (n.c.)	≥2.5 (n.c.)				≥2.5 (n.c.)		1.5+ (n.c.)	1.5+ (n.c.)	2	
Zygoptera	≥2.5 (86)	≥2.5 (83)	≥2.5 (102)	1.5+ (129)	1.5+ (144)	≥2.5 (318)	≥2.5 (n.c.)	≥2.5 (337)	≥2.5 (96)	≥2.5 (87)	2	102
<i>Asellus aquaticus</i>	≥2.5 (48)	≥2.5 (55)	1.5- (42)	1.5- (31)	0.87- (42)	1.5- (36)	1.5- (51)	1.5- (41)	1.5- (39)	1.5- (46)	1	31
Acari	≥2.5 (154)	≥2.5 (252)	≥2.5 (119)			1.5+ (n.c.)	≥2.5 (331)	≥2.5 (115)	≥2.5 (75)	≥2.5 (106)	2	119
Naididae	≥2.5 (57)	≥2.5 (32)	1.5- (44)	0.87- (78)	0.87- (56)	0.87- (50)	0.87- (70)	0.87- (73)	0.87- (61)	0.51- (65)	1	44
Sum Hirudinea	≥2.5 (120)	≥2.5 (62)	≥2.5 (61)	≥2.5 (67)	≥2.5 (65)	≥2.5 (71)	≥2.5 (61)	≥2.5 (69)	≥2.5 (55)	≥2.5 (59)	1	61
<i>Helobdella stagnalis</i>	≥2.5 (160)	≥2.5 (63)	≥2.5 (75)	≥2.5 (66)	≥2.5 (67)	≥2.5 (71)	≥2.5 (62)	≥2.5 (74)	≥2.5 (58)	≥2.5 (61)	1	62
<i>Erpobdella octoculata</i>	≥2.5 (145)	≥2.5 (256)	≥2.5 (141)	≥2.5 (103)	≥2.5 (136)	≥2.5 (218)	≥2.5 (226)	≥2.5 (115)	≥2.5 (89)	1.5+ (131)	2	103
<i>Haemopsis sanguisuga</i>							≥2.5 (252)		<0.3- (72)	≥2.5 (n.c.)	2	252
Sum Pulmonata	≥2.5 (54)	≥2.5 (66)	≥2.5 (53)	≥2.5 (63)	≥2.5 (66)	≥2.5 (49)	≥2.5 (51)	≥2.5 (39)	≥2.5 (44)	≥2.5 (60)	1	49
Planorbidae	≥2.5 (60)	≥2.5 (75)	≥2.5 (57)	≥2.5 (66)	≥2.5 (68)	≥2.5 (57)	≥2.5 (56)	≥2.5 (47)	≥2.5 (51)	≥2.5 (68)	1	56
Lymnaeidae (smaller 0.5 cm)	≥2.5 (146)	≥2.5 (209)	≥2.5 (n.c.)	≥2.5 (249)	1.5+ (n.c.)	≥2.5 (n.c.)	≥2.5 (n.c.)	≥2.5 (n.c.)	≥2.5 (131)	≥2.5 (n.c.)	2	249
Physidae		≥2.5 (n.c.)	≥2.5 (252)	≥2.5 (n.c.)	≥2.5 (n.c.)	≥2.5 (154)	0.51+ (356)	≥2.5 (145)	≥2.5 (114)	≥2.5 (94)	2	154

Signs +/- indicate the direction of a significant effect and colours indicate the different NOECs. Blank fields: taxon not present.
n.c.: MDD could not be calculated because of absence in the controls.

MDD Cat. = MDD category according Brock *et al.* (2015). Min MDD = minimum MDD over the first six weeks after 1st application when exposure was still above 50 % of the nominal concentrations only. Values < 70 % indicate that medium effects could be detected and are highlighted.

Taxa set in bold represent populations of the potentially sensitive group (i.e. Arthropoda and Naididae) with sufficiently low MDDs to detect medium direct effects (MDDs < 70 % within the first 6 weeks after 1st application).

Due to their known sensitivity and thus high relevancy for the outcome of this study, abundancy figures of all macroinvertebrate taxa with an MDD category of 1 are given below. All graphs shown below use nominal concentration values and instead of relevant maximum measured values.

Cloeon dipterum

Mayflies are known to be especially sensitive to neonicotinoids and in a previous mesocosm study with acetamiprid (EFSA 2016), *Cloeon dipterum* was found to be the most sensitive species. In the present study, mayflies showed pronounced effects at 2.5 µg/L without recovery within eight weeks. However, the number of larvae started to increase again after day 41 and reached the level of the controls at the end of the study (effect class 5A). Abundance at 1.5 µg/L was often close to or slightly below the range of the controls but this was significant only on day 20 and 83. However, due to the clear recovery after pronounced effects at 2.5 µg/L the low NOEC at the end of the study is not considered treatment related. Thus, effects at 1.5 µg/L are considered class 2. The same class is used for 0.87 µg/L due to the NOEC of 0.51 µg/L on day 20 but smaller deviations from control than at 1.5 µg/L. Up to 0.51 µg/L, clearly no treatment effect was given (effect class 1).

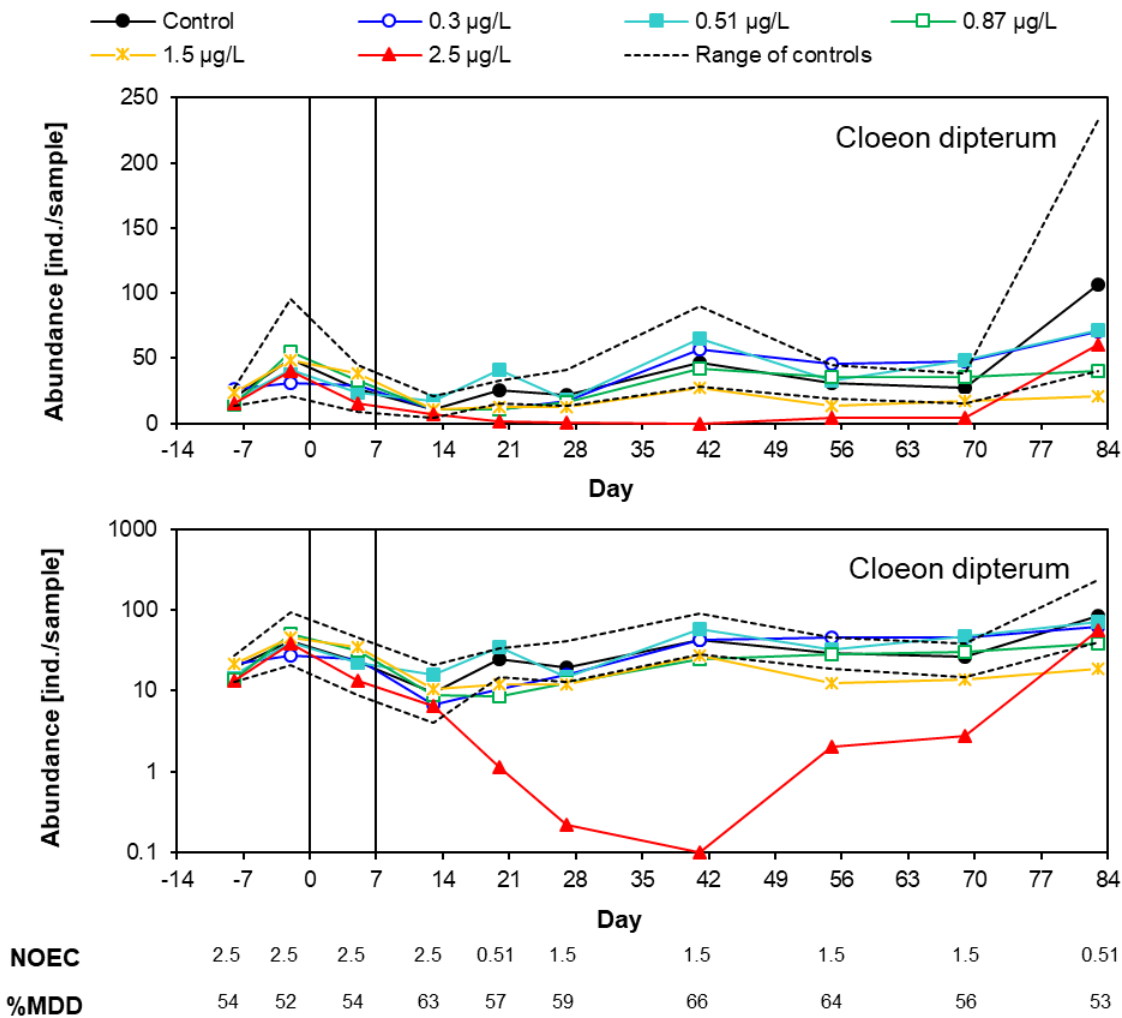


Figure A 4: *Cloeon dipterum* (Ephemeroptera) in the macroinvertebrate samples. Range of controls and arithmetic (top) and geometric (bottom) means per treatment level.

Diptera
Diptera were dominated by *Chaoborus* sp. and Chironomidae. No significant differences to controls were found for this insect order in total (effect class 1).

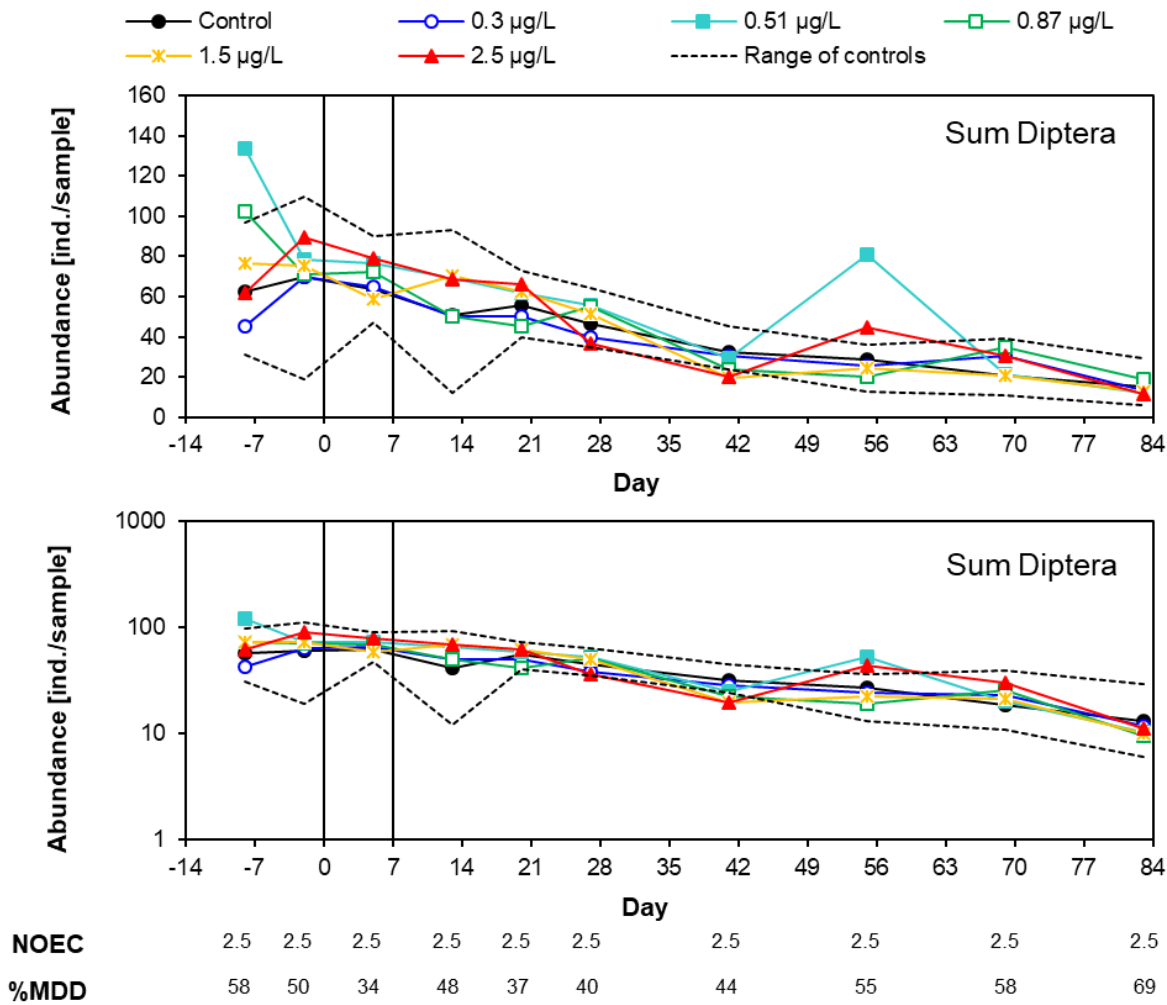


Figure A 5: Sum of Diptera in the macroinvertebrate samples.
Range of controls and arithmetic (top) and geometric (bottom) means per treatment level.

Chaoborus

In general, the total number of *Chaoborus* larvae and pupae decreased throughout the study. Nevertheless, *Chaoborus* was not affected. On one single sampling (day 55) significantly reduced abundances were found in the highest treatment 2.5 µg/L. However, until day 41, during the period of highest exposure, clearly no effect was given. Thus, this statistical finding for this single sampling date is attributed to the low numbers at that time rather than to the treatment. Thus, effect class 1 was used for *Chaoborus* up to 2.5 µg/L.

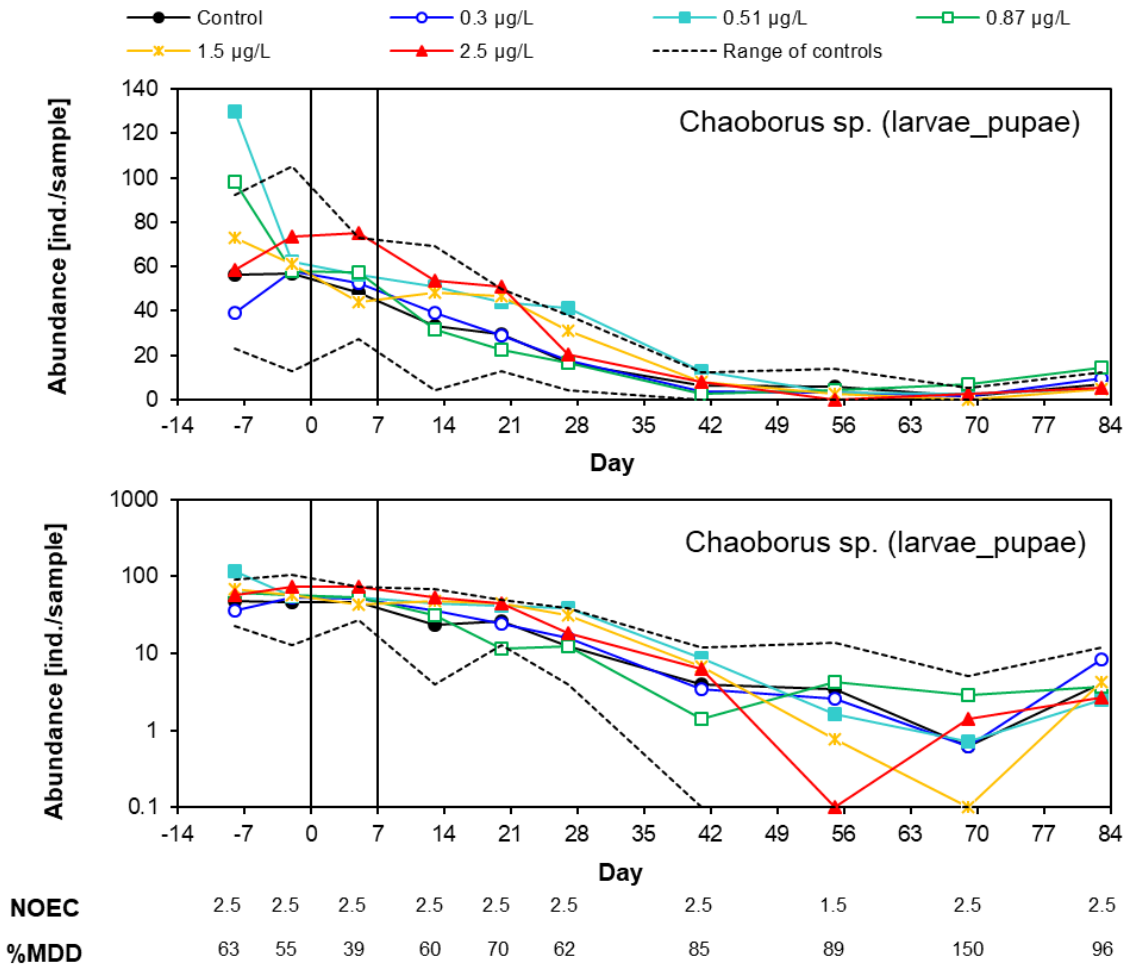


Figure A 6: *Chaoborus sp.* in the macroinvertebrate samples.
Range of controls and arithmetic (top) and geometric (bottom) means per treatment level.

Chironomidae

On three isolated sampling days, significant deviations to controls were detected but it is unlikely that these were caused by the treatment. On day 5, the number of chironomids at 2.5 µg/L was significantly lower than in the other treatments but after the second application, resulting in higher exposure, numbers increased back to the range of the controls. On day 41, the NOEC was 0.87 µg/L. On day 69, abundance at 2.5 µg/L was significantly higher than in the controls. Since the abundance at the highest test concentration showed an increase during the period with the highest exposure, effect class 1 was assumed up to 2.5 µg/L for the not determined Chironomidae.

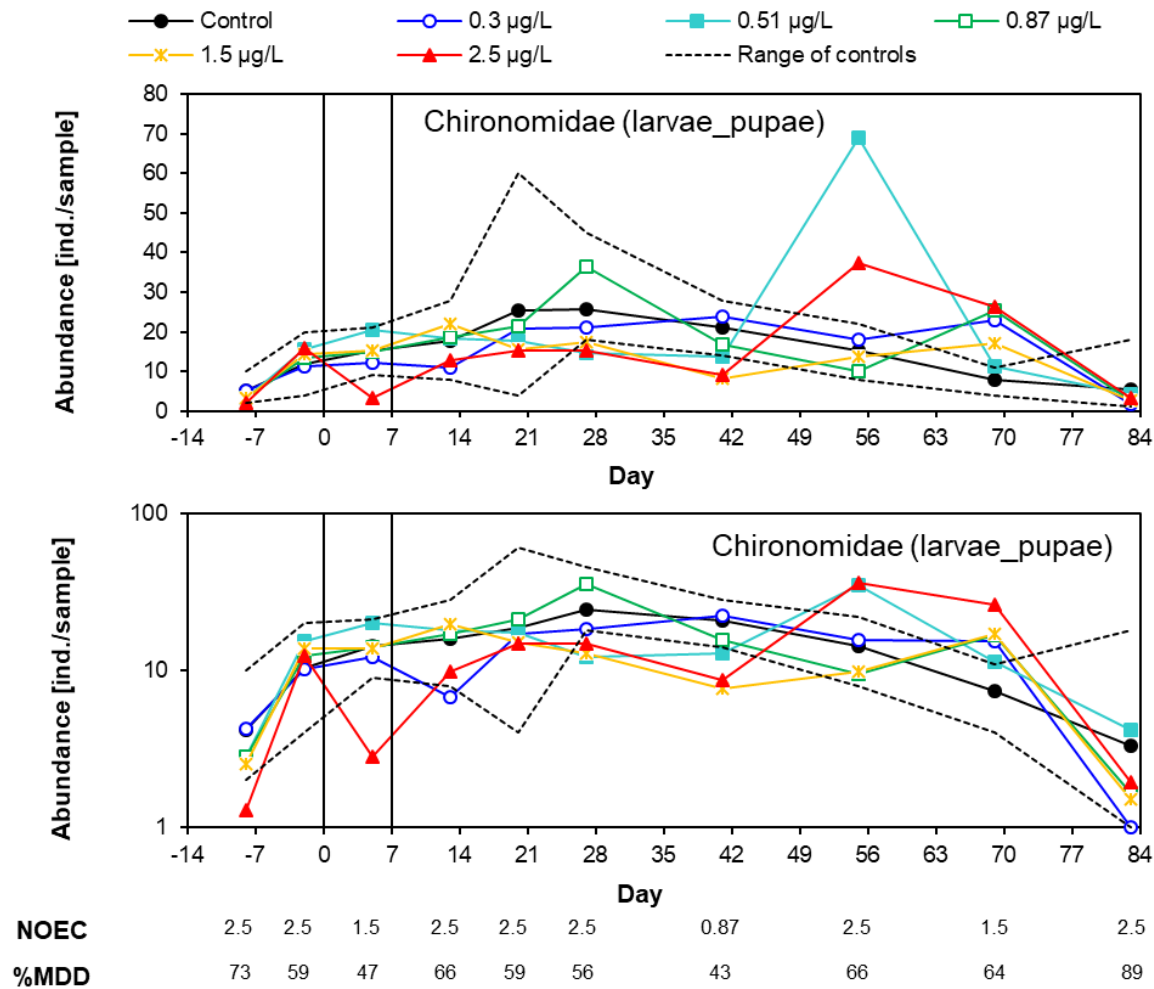


Figure A 7: Chironomidae (undetermined larvae and pupae) in the macroinvertebrate samples. Range of controls and arithmetic (top) and geometric (bottom) means per treatment level.

Tanypodinae

Tanypodinae were present in low numbers at the start of the study but on day 5 and 13 highest abundances were found at the highest treatment level. These findings are not considered as treatment related effects because they were based on only eight individuals found in the six samples at 2.4 µg/L on these two days. Significantly reduced abundances were found, on day 27 at treatment levels 0.51 µg/L and higher and on day 69 at 2.5 µg/L. However, the number of individuals per sample was relatively low and no clear concentration response was found, suggesting these effects were not treatment related. Thus, class 1 was used up to 2.5 µg/L.

Another family of Diptera, Culicidae, was very rarely found in the samples and thus, not considered for further effect evaluation.

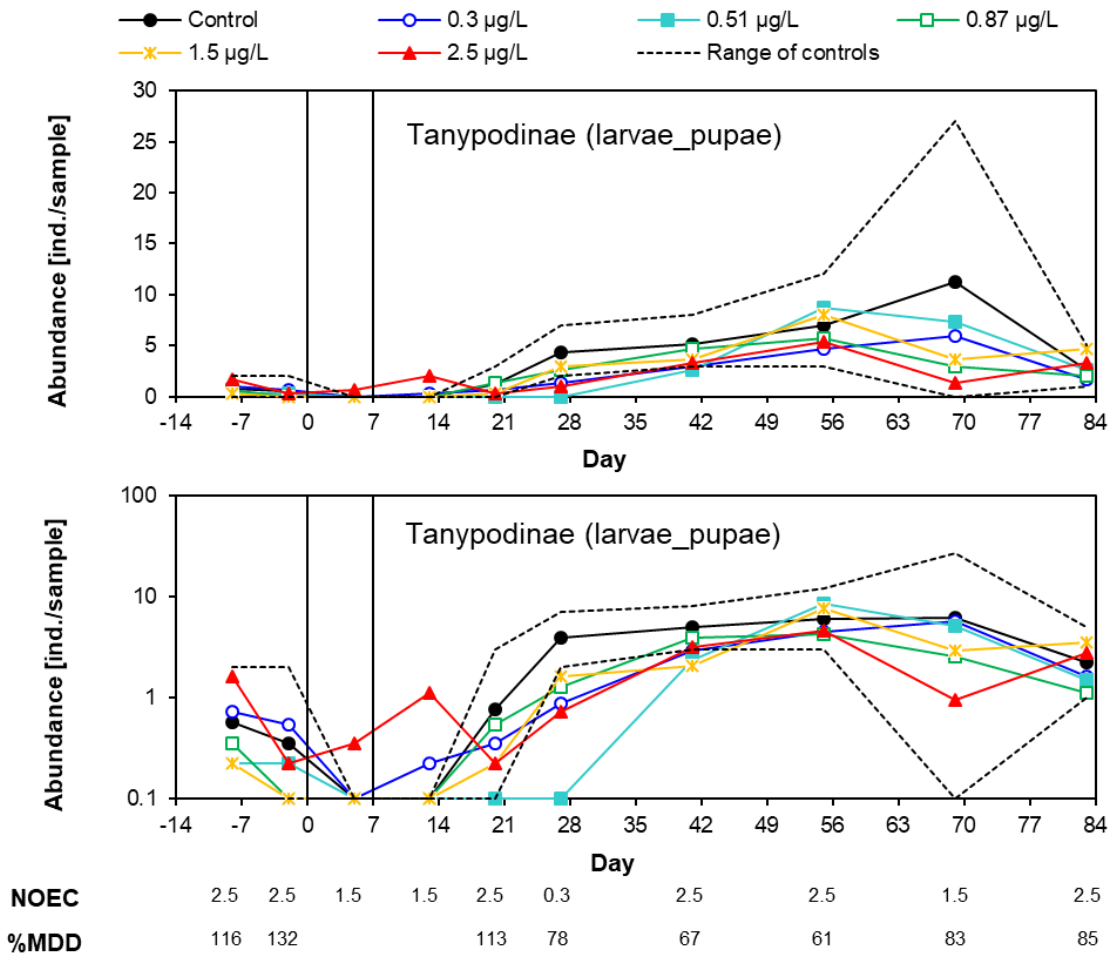


Figure A 8: Tanypodinae (larvae and pupae) in the macroinvertebrate samples. Range of controls and arithmetic (top) and geometric (bottom) means per treatment level.

Asellus aquaticus

The abundances of *Asellus aquaticus* from the order Isopoda steadily increased throughout the study. Population growth was clearly affected at the highest treatment level, 2.5 µg/L, resulting in low abundances on day 27. The population at the highest treatment level increased again but did not equal or exceed control levels within the study at 2.5 µg/L (considered class 5B). On sampling day 20, a NOEC of 0.87 µg/L was calculated but since abundance levels were close to those at 0.30 µg/L and only a single sampling event with abundance below the control level was detected, this event is considered to be unlikely a treatment effect and class 1 was used up to 1.5 µg/L.

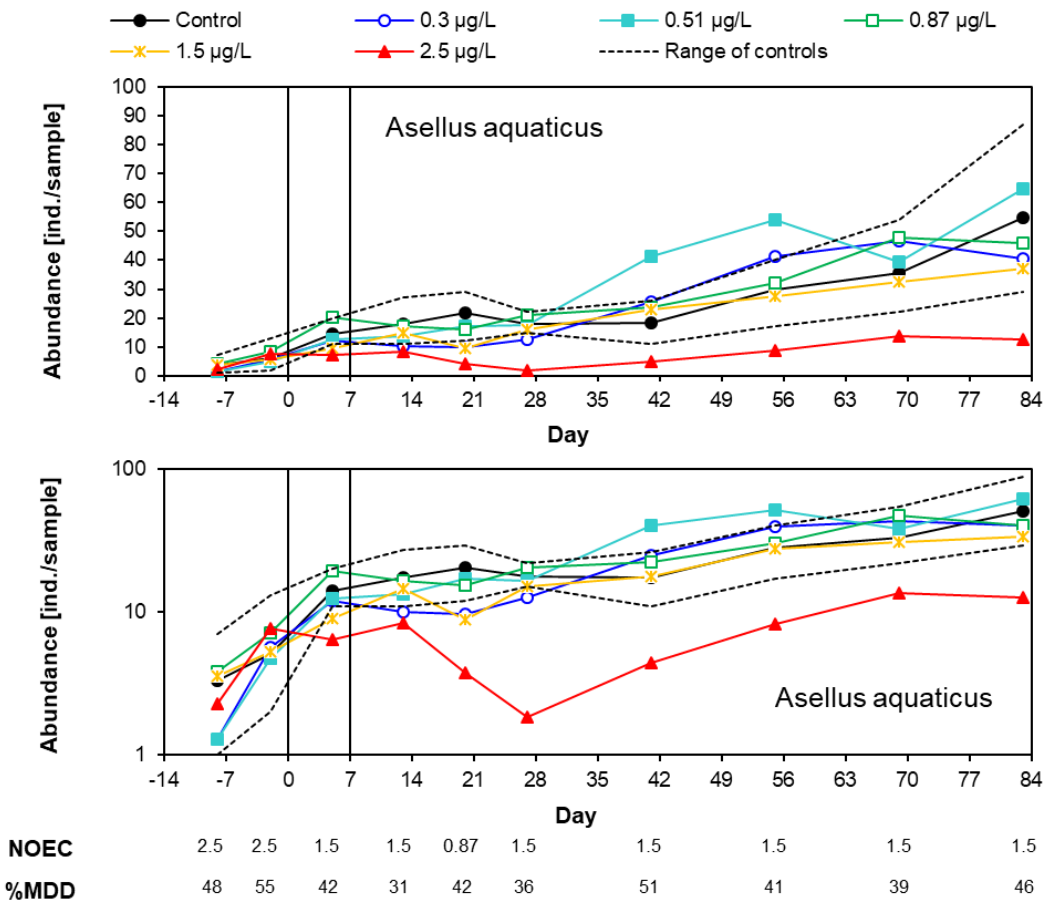


Figure A 9: *Asellus aquaticus* (Isopoda) in the macroinvertebrate samples.
Range of controls and arithmetic (top) and geometric (bottom) means per treatment level.

Naididae

In a previous mesocosm study (EFSA 2016), effects of acetamiprid on Naididae (Oligochaeta) were uncertain due to statistical findings but generally low numbers were found in the samples. Naididae (formerly known as Tubificidae) are indicators for organically polluted waters and thus, are not expected to reach high abundances in the mesocosms. Additional sediment samples were used in this study to increase sampling success, thus higher numbers in the samples were found, e.g. usually at least 10 worms in the control samples. The calculated MDDs were sufficiently low to detect medium effects below or equal to 70 % on all samplings except day 13 and day 55. The data indicate no effects up to 0.87 µg/L during the study (effect class 1) but long-term effect at 1.5 µg/L and more pronounced at 2.5 µg/L (class 5B). A significantly lower abundance was found also at 0.87 µg/L on the final sampling day, but this decline was not considered to be caused by the treatment as there were no other indications of an effect at 0.87 µg/L..

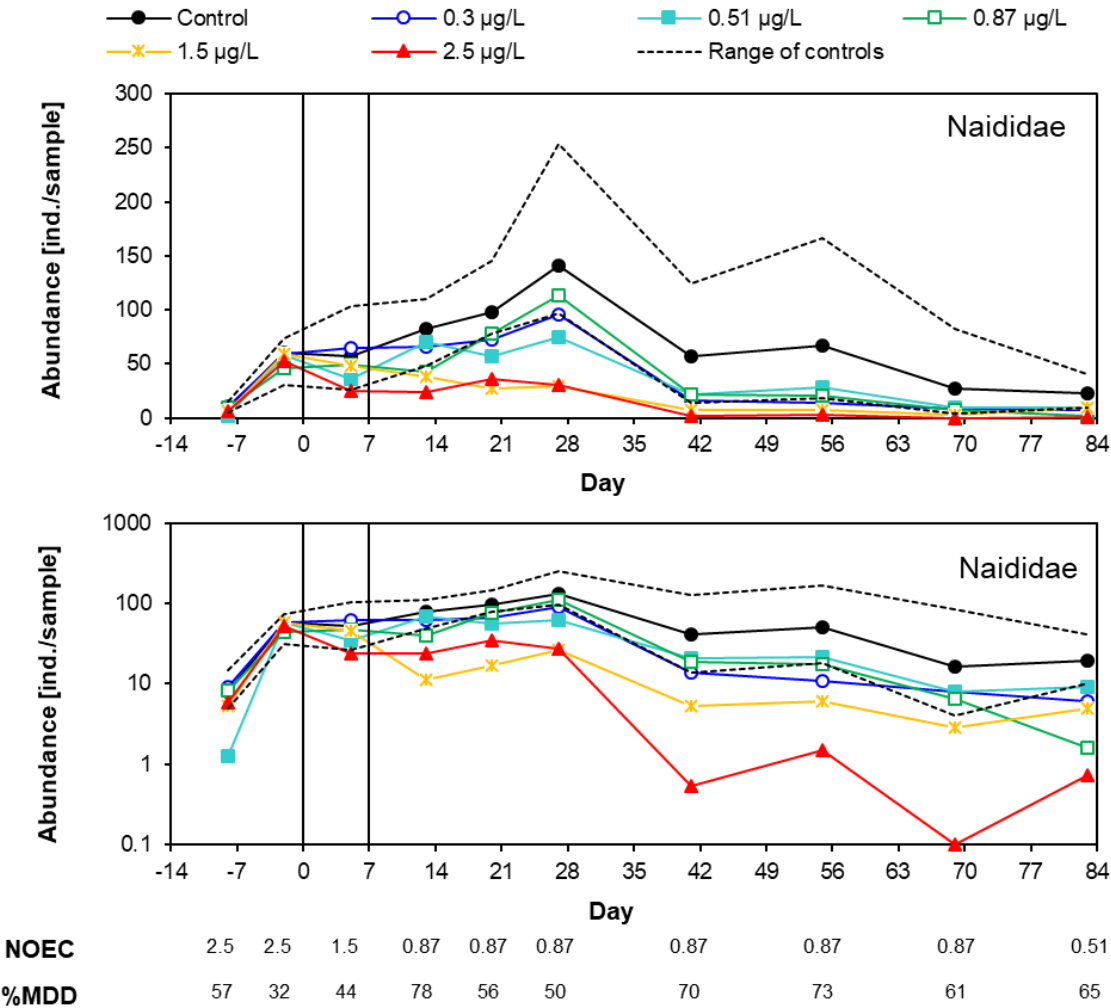


Figure A 10: Naididae (formerly Tubificidae) in the macroinvertebrate samples.
Range of controls and arithmetic (top) and geometric (bottom) means per treatment level.

Helobdella stagnalis

Helobdella stagnalis was by far the most abundant leech (Hirudinea) found in the study. No effects were observed (effect class 1). For other Hirudinea species the numbers were much lower and thus, not reliable for effect classification. For example, a total 13 individuals of *Haemopsis sanguisuga*, were found spread across 10 of all 200. The NOEC < 0.30 µg/L was caused by 5 animals found in the 5 control samples while 3 animals were found in the treated enclosures (2 of them in the highest treatment level). Thus, this NOEC does not indicate a treatment effect.

For *Erpobdella octoculata* the significant difference to control detected at 2.5 µg/L on the last sampling was considered to be a stochastic finding due to the low abundances.

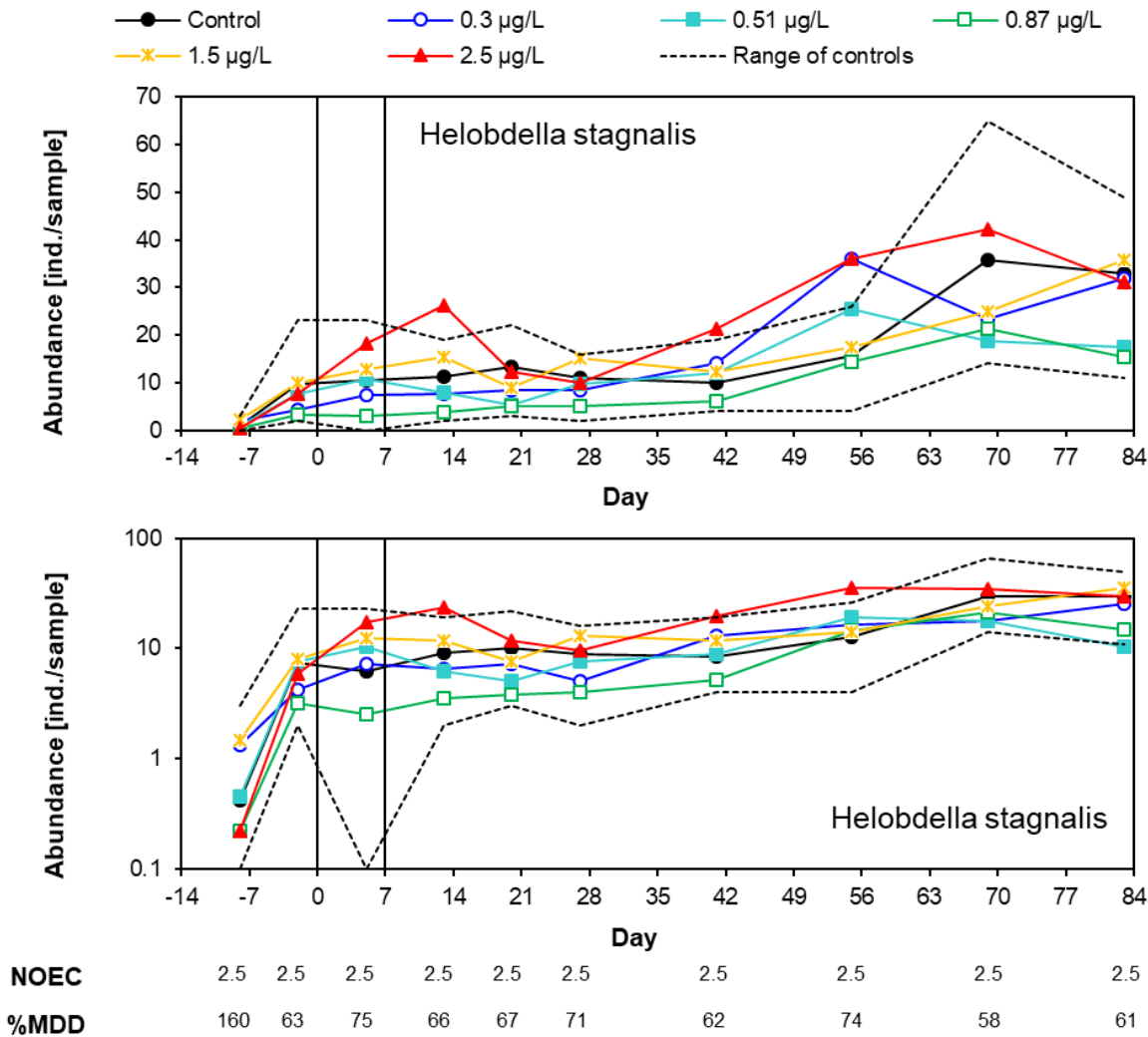


Figure A 11: *Helobdella stagnalis* (Hirudinea) in the macroinvertebrate samples. Range of controls and arithmetic (top) and geometric (bottom) means per treatment level.

Snails

Snails of the order Pulmonata, were highly abundant throughout the study. By far the most common species was *Planorbis planorbis* (Planorbidae). No effects were observed in the course of study (effect class 1). Other snail taxa, e.g. small Lymnaeidae (< 0.5 cm) were not considered further due to low numbers.

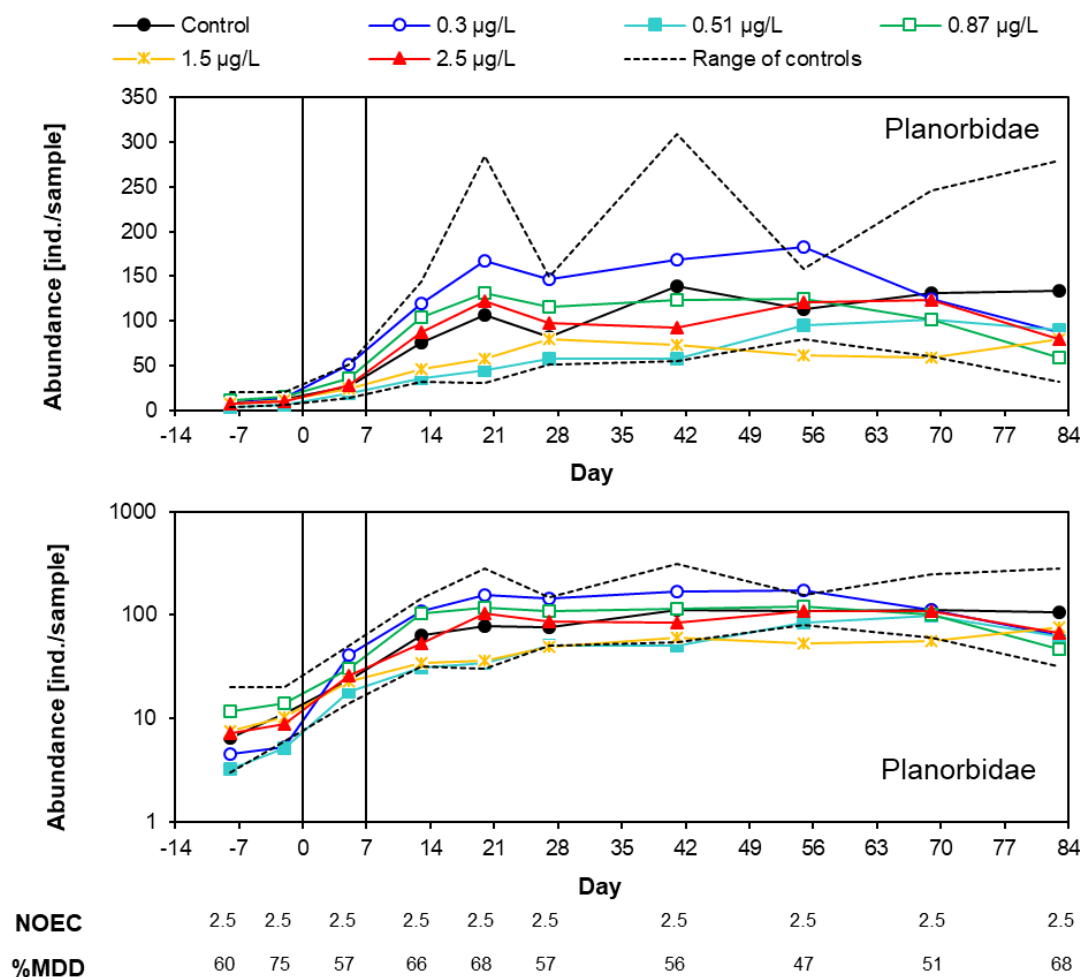


Figure A 12: Planorbidae (*Planorbis planorbis*) in the macroinvertebrate samples.
Range of controls and arithmetic (top) and geometric (bottom) means per treatment level.

Community level analysis

The effects on the macroinvertebrate community structure were analysed by ordination analysis. The PRCs indicate a pronounced response at 2.5 µg/L with recovery starting after day 41. Response at 1.5 µg/L is less pronounced but consistently stronger than at the lower treatment levels for which the PRCs were closer to zero. The community response is mainly driven by the mayflies *Cloeon* and the Naididae. The leech *Helobdella stagnalis* had the lowest negative weight which indicates that for this species the PRCs extract an inverse response, i.e. a promotion.

Redundancy analysis per sampling data revealed a significant treatment effect from day 13 until the end of the study. The Williams-test was applied to the sample scores of the first PCA axis in order to calculate community NOECs (Figure 22). On day 27, the NOEC was 0.30 µg/L indicating a significant effect starting at 0.51 µg/L. However, the PRC for 0.51 µg/L show only a slight response, smaller than before application or at 0.30 µg/L on other days.

Thus, this statistical NOEC was considered as ecotoxicologically not relevant and the community NOEC was set to 0.87 µg/L. Despite that the community NOEC was ≥ 2.5 µg/L at the end of the study, community level effects at 1.5 and 2.5 µg/L were considered class 5B (no recovery) since the absence of a statistical difference at the end of the test seems to be more related to the increasing variability than a clear recovery of the affected communities.

In summary, effects on the macroinvertebrate community are considered to be effect class 1 up to 0.87 µg/L and class 5B at 1.5 and 2.5 µg/L.

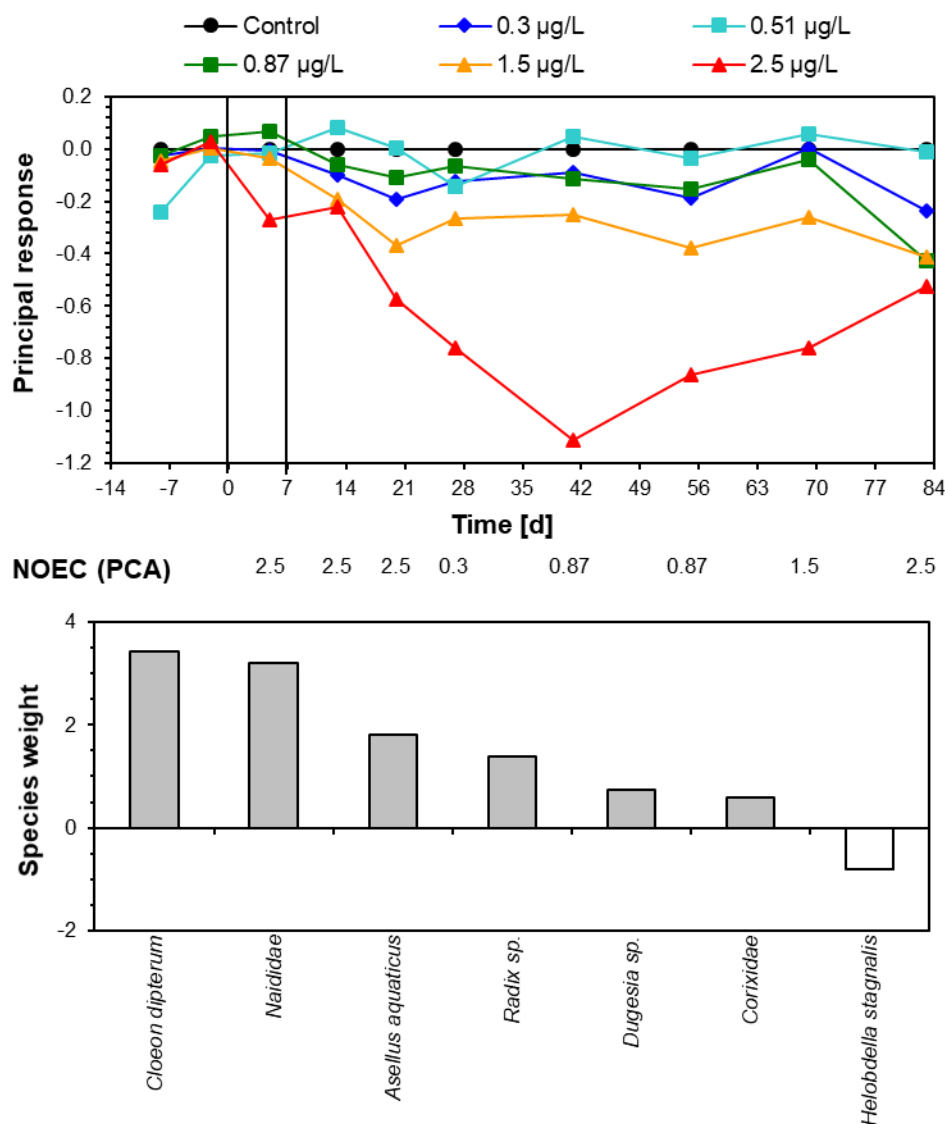


Figure A 13: PRCs for the macroinvertebrate data set.

43% of total variance explained by time, 20% of total variance explained by treatment ($p=0.002$), 31% of the variance explained by treatment are captured by the PRC ($p=0.002$).

The NOECs [µg/L] were calculated by the Williams-test applied to the sample scores of PCAs per day.

Summary macroinvertebrates

No effects on macroinvertebrates were found up to nominal 0.51 µg/L. At 0.87 µg/L slight (class 2) effects on mayfly larvae (*Cloeon* sp.) were observed. At 1.5 µg/L the worms of the Naididae family were affected and no recovery was not found throughout the study. At 2.5 µg/L, *Cloeon* and *Asellus* showed long-term (longer than 8 weeks) effects.

Response of the community level was driven by *Cloeon dipterum* and Naididae with long-term effects starting at 1.5 µg/L.

For five taxa (without pooled taxa or potential overlap) the MDDs were sufficiently low to detect medium effects (up to 70 %) within the first six weeks after the first application.

Table A 36: Effect classification for the macroinvertebrates

Exposure	nominal [$\mu\text{g a.s./L}$]	0.3	0.51	0.87	1.5	2.5
	max. measured [$\mu\text{g a.s./L}$]	0.55	0.88	1.4	2.5	4.5
	max. measured [$\mu\text{g a.s./kg dw}$]	0.61	0.71	0.77	1.9	2.3
Macroinvertebrates	<i>Cloeon dipterum</i>	1	1	2	2	5A
	Sum diptera	1	1	1	1	1
	<i>Chaoborus sp.</i>	1	1	1	1	1
	Sum Chironomidae	1	1	1	1	1
	Chironomidae n.d.	1	1	1	1	1
	Tanypodinae	1	1	1	1	1
	<i>Asellus aquaticus</i>	1	1	1	1	5B
	<i>Gammarus</i> (bioassay)	1	1	1	2	2
	Naididae	1	1	1	5B	5B
	<i>Helobdella stagnalis</i>	1	1	1	1	1
	<i>Planorbis planorbis</i>	1	1	1	1	1
	Community level	1	1	1	5B	5B

Taxa in bold represent potentially sensitive populations with sufficiently low MDDs to assess medium direct effects.

Emerging insects

Population level analysis

EFSA (2019) recommends reporting emergence per sampling date, as well as cumulative emergence of insects since emergence is a measure of production rather than population dynamics. Cumulative emergence has the advantage of being independent of variability over time and due to the increasing numbers over time the sampling error and thus, the MDDs become smaller. Therefore, this report focuses on the statistics and figures for the cumulative emergence which was calculated from day 0 -84. Emergence per week is also shown when necessary to assess the evaluation of recovery.

In the data set of emergence per sampling, six taxa fulfil the MDD criterion by Brock *et al.* (2015): *Cloeon dipterum*, *Chaoborus sp.*, Chironominae, Orthoclaadiinae, Tanypodinae and Coenagrionidae (see table below). Higher taxa or taxa with a potential overlap to other (e.g. Chironomidae undet.) were not counted

Table A 37 NOEC [$\mu\text{g a.s./L}$] and %MDD (in brackets) for emergence of insects (only MDD Category 1 and 2 taxa shown)

Emergence	Day after application									MDD	Min
	0	7	14	21	28	42	56	70	84	Cat	MDD
Sum of insects	≥ 2.5 (41)	≥ 2.5 (42)	≥ 2.5 (34)	≥ 2.5 (38)	0.87- (31)	1.5- (38)	≥ 2.5 (47)	≥ 2.5 (57)	≥ 2.5 (35)	1	31
<i>Cloeon dipterum</i>	≥ 2.5 (63)	≥ 2.5 (59)	≥ 2.5 (54)	0.87- (65)	0.87- (59)	<0.3- (65)	1.5- (61)	0.87- (70)	≥ 2.5 (74)	1	54
Sum Diptera	≥ 2.5 (40)	≥ 2.5 (46)	≥ 2.5 (39)	≥ 2.5 (43)	≥ 2.5 (34)	1.5- (42)	≥ 2.5 (53)	≥ 2.5 (60)	1.5+ (34)	1	34
<i>Chaoborus sp.</i>	≥ 2.5 (86)	≥ 2.5 (88)	≥ 2.5 (55)	≥ 2.5 (62)	≥ 2.5 (60)	0.87+ (55)	0.87+ (270)	≥ 2.5 (116)	≥ 2.5 (151)	1	55
Sum Chironomidae	≥ 2.5 (34)	≥ 2.5 (49)	≥ 2.5 (46)	≥ 2.5 (50)	≥ 2.5 (40)	1.5- (44)	≥ 2.5 (54)	1.5+ (60)	1.5+ (35)	1	35
Chironomidae indet.	≥ 2.5 (76)	≥ 2.5 (76)	≥ 2.5 (58)	0.87- (52)	0.87- (50)	≥ 2.5 (57)	≥ 2.5 (72)	≥ 2.5 (80)	≥ 2.5 (62)	1	50
Chironomidae indet. < 2 mm	≥ 2.5 (98)	≥ 2.5 (89)	≥ 2.5 (57)	≥ 2.5 (62)	1.5- (58)	1.5- (62)	≥ 2.5 (63)	1.5+ (59)	1.5+ (62)	1	57
Chironominae	≥ 2.5 (115)	≥ 2.5 (121)	1.5- (76)	≥ 2.5 (71)	1.5- (71)	1.5- (58)	≥ 2.5 (91)	≥ 2.5 (84)	≥ 2.5 (149)	1	58
Orthocladiinae	≥ 2.5 (183)	<0.3- (74)	≥ 2.5 (106)	≥ 2.5 (88)	≥ 2.5 (64)	≥ 2.5 (72)	≥ 2.5 (87)	1.5+ (82)	≥ 2.5 (82)	1	64
Tanypodinae	≥ 2.5 (48)	1.5+ (82)	≥ 2.5 (73)	1.5+ (64)	1.5+ (47)	≥ 2.5 (41)	0.3- (39)	≥ 2.5 (46)	≥ 2.5 (40)	1	39
<i>Anopheles sp.</i>	≥ 2.5 (n.c.)	≥ 2.5 (n.c.)		1.5+ (n.c.)	1.5+ (195)	≥ 2.5 (n.c.)	≥ 2.5 (149)	≥ 2.5 (97)	≥ 2.5 (n.c.)	2	97
Sum Odonata	≥ 2.5 (90)	≥ 2.5 (115)	1.5- (63)	≥ 2.5 (78)	1.5- (55)	≥ 2.5 (48)	≥ 2.5 (176)	<0.3- (95)		1	48
Coenagrionidae	≥ 2.5 (89)	≥ 2.5 (116)	1.5- (68)	≥ 2.5 (75)	1.5- (56)	≥ 2.5 (46)	≥ 2.5 (176)	<0.3- (95)		1	46
Thysanoptera	≥ 2.5 (81)	≥ 2.5 (79)	<0.3- (50)	≥ 2.5 (55)	≥ 2.5 (39)	0.3- (50)	≥ 2.5 (61)	≥ 2.5 (88)	≥ 2.5 (172)	1	39
Sum Coleoptera	≥ 2.5 (94)	≥ 2.5 (96)	1.5+ (77)	≥ 2.5 (92)	≥ 2.5 (109)	≥ 2.5 (85)	≥ 2.5 (101)	≥ 2.5 (117)	≥ 2.5 (180)	2	77
Curculionidae	≥ 2.5 (n.c.)						<0.3- (95)	≥ 2.5 (229)	≥ 2.5 (n.c.)	2	95
<i>Helophorus sp.</i>	≥ 2.5 (91)	≥ 2.5 (96)	1.5+ (81)	≥ 2.5 (87)	≥ 2.5 (112)	≥ 2.5 (108)	≥ 2.5 (n.c.)	1.5+ (n.c.)	1.5+ (n.c.)	2	81

Signs +/- indicate the direction of a significant effect and colours indicate the different NOECs. Blank fields: taxon not present. n.c.: MDD could not be calculated because of absence in the controls. Min and mean MDD are related to all MDDs after the first application. Colours indicate the MDD classes proposed by EFSA (2013). MDD Cat. = MDD category according Brock *et al.* (2015). Taxa set in bold represent populations considered to allow and assessment of medium direct effects (MDDs at least once < 70 %).

Table A 38: NOEC [µg a.s./L] and %MDD (in brackets) for cumulative emergence of insects (only taxa with MDDs < 100 % are shown)

Cumulative emergence	Day after application									Min
	0	7	14	21	28	42	56	70	84	MDD
Sum of insects	≥2.5 (41)	≥2.5 (39)	≥2.5 (35)	≥2.5 (31)	≥2.5 (26)	1.5- (26)	1.5- (27)	≥2.5 (31)	≥2.5 (30)	26
<i>Cloeon dipterum</i>	≥2.5 (63)	≥2.5 (59)	≥2.5 (55)	≥2.5 (54)	≥2.5 (52)	1.5- (42)	0.87- (27)	0.87- (33)	0.87- (36)	27
Sum Diptera	≥2.5 (40)	≥2.5 (39)	≥2.5 (37)	≥2.5 (38)	≥2.5 (33)	≥2.5 (33)	≥2.5 (33)	≥2.5 (36)	≥2.5 (35)	33
Ceratopogonidae	≥2.5 (119)	≥2.5 (98)	≥2.5 (98)	≥2.5 (118)	≥2.5 (69)	≥2.5 (75)	≥2.5 (52)	≥2.5 (51)	≥2.5 (54)	51
<i>Chaoborus</i> sp.	≥2.5 (86)	≥2.5 (85)	≥2.5 (64)	≥2.5 (59)	≥2.5 (59)	≥2.5 (56)	≥2.5 (56)	≥2.5 (56)	≥2.5 (55)	55
Sum Chironomidae	≥2.5 (34)	≥2.5 (39)	≥2.5 (40)	≥2.5 (45)	≥2.5 (40)	≥2.5 (40)	≥2.5 (37)	≥2.5 (39)	≥2.5 (37)	37
Chironomidae indet.	≥2.5 (76)	≥2.5 (76)	≥2.5 (56)	≥2.5 (49)	0.87- (44)	≥2.5 (40)	≥2.5 (46)	≥2.5 (56)	≥2.5 (55)	40
Chironomidae indet. smaller 2 mm	≥2.5 (98)	≥2.5 (61)	≥2.5 (52)	≥2.5 (55)	1.5- (54)	1.5- (57)	≥2.5 (56)	≥2.5 (54)	≥2.5 (53)	52
Chironominae	≥2.5 (115)	≥2.5 (105)	1.5- (71)	1.5- (64)	1.5- (56)	1.5- (43)	0.3- (36)	0.3- (34)	0.3- (34)	34
Orthocladiinae	≥2.5 (183)	≥2.5 (84)	≥2.5 (70)	≥2.5 (77)	≥2.5 (55)	≥2.5 (58)	≥2.5 (62)	≥2.5 (68)	≥2.5 (66)	55
Tanypodinae	≥2.5 (48)	≥2.5 (47)	≥2.5 (48)	1.5+ (52)	1.5+ (48)	≥2.5 (34)	≥2.5 (32)	≥2.5 (28)	≥2.5 (27)	27
Sum Odonata	≥2.5 (90)	0.87+ (74)	≥2.5 (45)	≥2.5 (46)	1.5- (39)	1.5- (31)	1.5- (29)	1.5- (28)	1.5- (28)	28
Coenagrionidae	≥2.5 (89)	≥2.5 (75)	≥2.5 (47)	≥2.5 (44)	1.5- (37)	1.5- (28)	1.5- (27)	1.5- (26)	1.5- (26)	26
Sum Coleoptera	≥2.5 (94)	≥2.5 (73)	1.5+ (76)	≥2.5 (78)	1.5+ (66)	1.5+ (60)	1.5+ (55)	1.5+ (56)	1.5+ (56)	55
<i>Helophorus</i> sp.	≥2.5 (91)	≥2.5 (73)	1.5+ (77)	≥2.5 (78)	1.5+ (66)	1.5+ (61)	1.5+ (59)	1.5+ (59)	1.5+ (59)	59
Thysanoptera	≥2.5 (81)	≥2.5 (53)	≥2.5 (43)	≥2.5 (44)	≥2.5 (36)	≥2.5 (38)	≥2.5 (41)	≥2.5 (41)	≥2.5 (39)	36
Mymaridae	≥2.5 (304)	≥2.5 (107)	≥2.5 (96)	≥2.5 (65)	≥2.5 (67)	≥2.5 (60)	≥2.5 (62)	≥2.5 (63)	≥2.5 (63)	60

Signs +/- indicate the direction of a significant effect and colours indicate the different NOECs. Taxa set in bold represent populations considered to allow and assessment of medium direct effects (MDDs at least once < 70 %).

Community level analysis

The community level analysis was evaluated for the emergence data per week since PRCs focus on the effect of the treatment over time.

The PRCs were dominated by the mayfly *Cloeon dipterum*, which had the highest species weight. A clear but temporary effect was found at the highest test concentration of 2.5 µg/L and less pronounced effects at 1.5 µg/L (see figure below) At the lower test concentrations, a response was found only on day 42 but without a clear concentration response.

In summary no effects are assumed up to 0.87 µg/L since the NOEC of < 0.30 µg/L on day 42 is not considered ecotoxicologically relevant due to a missing concentration response up to 0.87 µg/L. Thus, class 1 is used up to 0.87 µg/L and class 5A for 1.5 and 2.5 µg/L due to effects spanning more than 8 weeks but recovery at the end of the study.

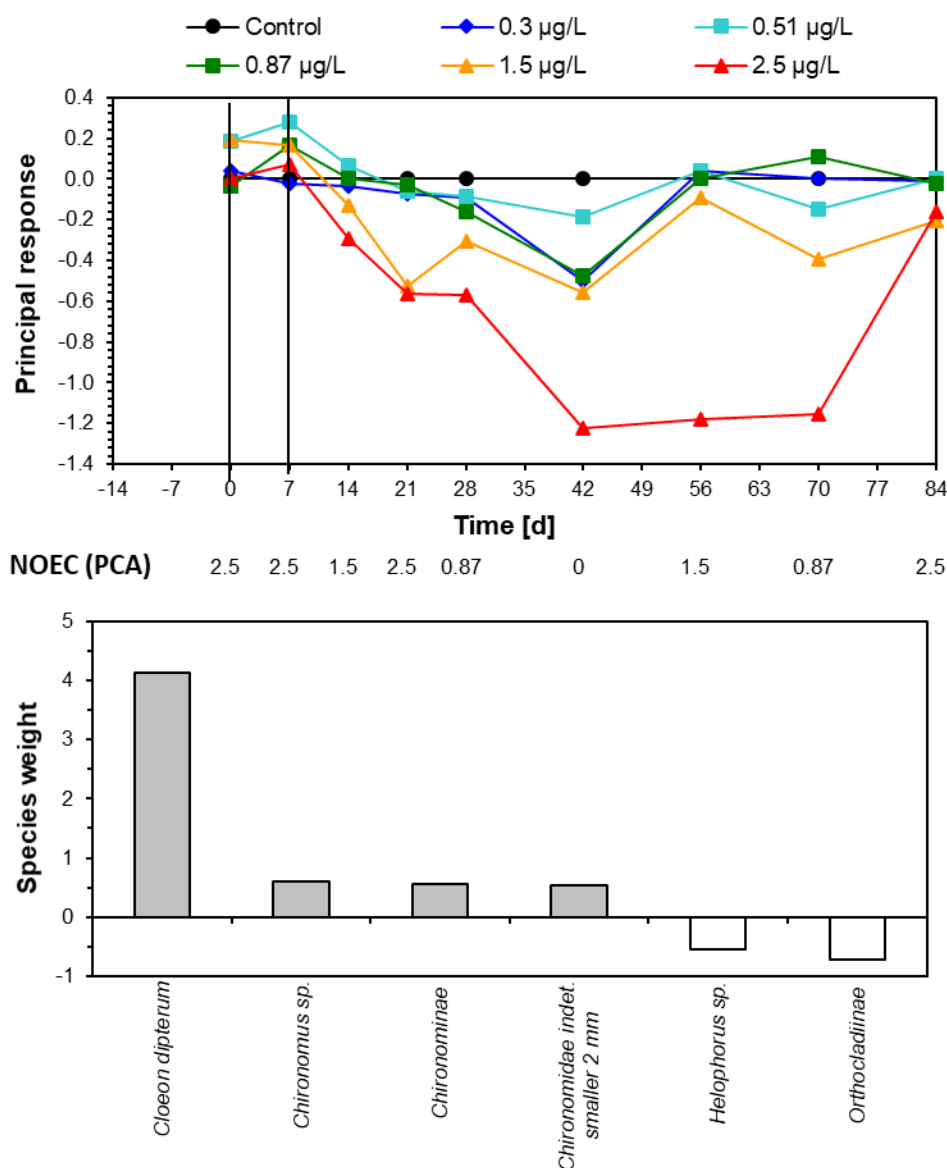


Figure A 14: PRCs for the emerged insects

56% of total variance explained by time, 18% of total variance explained by treatment ($p=0.002$), 38% of the variance explained by treatment are captured by the PRC ($p=0.002$).

The NOECs [µg/L] were calculated by the Williams-test applied to the sample scores of PCAs per day.

Summary Emergence

Insect emergence was not affected up to 0.87 µg/L. At 1.5 µg/L and especially at 2.5 µg/L emergence of *Cloeon* was clearly affected but at the end of the study, emergence was similar to that found in the controls. A few Chironomidae taxa were temporarily affected at 2.5 µg/L. Higher numbers of the water beetle *Helophorus* sp. were caught in the emergence traps at the highest treatment level the week following the second application, indicating a possible avoidance reaction. Effects on the community structure were strongly driven by the effects on *Cloeon*.

Table A 39: Effect classification for the emerged insects

Expo- sure	nominal [µg a.s./L]	0.3	0.51	0.87	1.5	2.5
	max. measured [µg a.s./L]	0.55	0.88	1.4	2.5	4.5
	max. measured [µg a.s./kg dw]	0.61	0.71	0.77	1.9	2.3
Emergence	Total insect emergence	1	1	1	1	2
	<i>Cloeon dipterum</i>	1	1	1	5A	5A
	Sum diptera	1	1	1	1	1
	Sum Chironomidae	1	1	1	1	1
	Chironomidae n.d.	1	1	1	1	1
	Chironomidae n.d. (< 2 mm)	1	1	1	1	3A
	Chironominae	1	1	1	1	3A
	Orthoclaadiinae	1	1	1	1	1
	Tanypodinae	1	1	1	1	1
	<i>Chaoborus</i> sp.	1	1	1	1	1
	Coenagrionidae	1	1	1	1	5B
	<i>Helophorus</i> sp.	1	1	1	1	2+
	Community level	1	1	1	5A	5A

Taxa in bold represent potentially sensitive populations with sufficiently low MDDs to assess medium direct effects.

Zooplankton

Forty-one taxa or life stages were differentiated in the zooplankton samples. About half of the counted individuals belonged to the rotifer *Keratella quadrata*. Another rotifer (*Polyarthra* sp.) made up 12 % of the total abundance. About 25 % of all individuals counted (per litre) were nauplius larvae of copepods which were not further taxonomically determined. All other taxa reached only up to 2 % of the total zooplankton counts, individually

Population level analysis

13 taxa fulfil the MDD criterion by Brock *et al.* (2015). *Chydorus sphaericus*, *Scapholeberis* sp., *Daphnia longispina*, *Daphnia* sp. *Simocephalus* sp., Cyclopidae and Ostracoda are considered as potentially sensitive crustaceans. Nauplius larvae are not counted since they were found only rarely. Also some rotifers belonged to MDD category 1 but were not further considered due to a lack of sensitivity to acetamiprid. For *Chydorus sphaericus*, *Daphnia* sp., *Scapholeberis* sp., Cyclopidae (and nauplii) and Ostracoda MDD were at least once < 70 % within six weeks after the first application when exposure was always above 50 % of the nominal concentration. Thus, for 5 potentially sensitive zooplankton crustaceans the MDDs are considered sufficiently small to detect medium effects.

Table A 40: NOEC [$\mu\text{g a.s./L}$] and %MDD (in brackets) for the zooplankton (only MDD Category 1 and 2 taxa are shown).

Zooplankton	Day after application												MDD	Min
	-9	-1	2	6	9	16	23	30	44	58	72	86	Cat	MDD
Sum Cladocera	≥ 2.5 (73)	≥ 2.5 (75)	1.5+ (80)	≥ 2.5 (76)	1.5+ (68)	≥ 2.5 (81)	≥ 2.5 (63)	≥ 2.5 (70)	≥ 2.5 (43)	1.5+ (48)	≥ 2.5 (62)	≥ 2.5 (83)	1	43
<i>Chydorus sphaericus</i>	≥ 2.5 (82)	≥ 2.5 (89)	≥ 2.5 (87)	≥ 2.5 (93)	≥ 2.5 (86)	≥ 2.5 (112)	≥ 2.5 (110)	≥ 2.5 (80)	1.5+ (62)	1.5+ (70)	0.87+ (60)	≥ 2.5 (98)	1	62
<i>Daphnia longispina</i>	≥ 2.5 (n.c.)	≥ 2.5 (91)	≥ 2.5 (113)	≥ 2.5 (228)	0.87+ (n.c.)	≥ 2.5 (94)	≥ 2.5 (112)	≥ 2.5 (83)	≥ 2.5 (77)	1.5+ (63)	≥ 2.5 (75)	0.87+ (115)	1	77
<i>Daphnia</i> sp.	≥ 2.5 (91)	≥ 2.5 (96)	≥ 2.5 (95)	≥ 2.5 (82)	≥ 2.5 (82)	≥ 2.5 (77)	≥ 2.5 (87)	<0.3- (66)	≥ 2.5 (65)	≥ 2.5 (53)	≥ 2.5 (77)	≥ 2.5 (87)	1	65
<i>Scapholeberis</i> sp.		1.5+ (n.c.)	<0.3- (97)	≥ 2.5 (146)	≥ 2.5 (139)	≥ 2.5 (178)	≥ 2.5 (104)	≥ 2.5 (95)	≥ 2.5 (69)	≥ 2.5 (95)	≥ 2.5 (100)	≥ 2.5 (99)	1	69
<i>Simocephalus</i> sp.	≥ 2.5 (94)	≥ 2.5 (101)	≥ 2.5 (105)	≥ 2.5 (98)	≥ 2.5 (110)	≥ 2.5 (98)	1.5+ (102)	≥ 2.5 (88)	≥ 2.5 (82)	≥ 2.5 (92)	≥ 2.5 (93)	≥ 2.5 (89)	1	82
<i>Alona</i> sp.		≥ 2.5 (n.c.)		≥ 2.5 (149)	≥ 2.5 (149)	≥ 2.5 (n.c.)	1.5+ (n.c.)	0.87+ (186)	≥ 2.5 (98)	1.5+ (119)	≥ 2.5 (256)	≥ 2.5 (n.c.)	2	98
<i>Alonella</i> sp.					≥ 2.5 (n.c.)				≥ 2.5 (n.c.)	1.5+ (n.c.)			2	
<i>Daphnia magna</i>	≥ 2.5 (97)	1.5+ (102)	1.5+ (106)	1.5+ (106)	1.5+ (101)	≥ 2.5 (100)	≥ 2.5 (120)	≥ 2.5 (126)	≥ 2.5 (142)		≥ 2.5 (n.c.)		2	100
<i>Graptoleberis</i> sp.	<0.3- (96)		≥ 2.5 (149)	≥ 2.5 (n.c.)	≥ 2.5 (149)	≥ 2.5 (n.c.)	≥ 2.5 (253)	≥ 2.5 (96)	≥ 2.5 (100)	0.87+ (n.c.)	≥ 2.5 (176)		2	96
Sum Copepoda	≥ 2.5 (77)	≥ 2.5 (72)	1.5- (79)	≥ 2.5 (57)	≥ 2.5 (40)	≥ 2.5 (50)	≥ 2.5 (49)	≥ 2.5 (59)	≥ 2.5 (50)	1.5+ (53)	≥ 2.5 (82)	≥ 2.5 (78)	1	40
Cyclopidae (adult_copepodit)	≥ 2.5 (72)	≥ 2.5 (52)	≥ 2.5 (67)	≥ 2.5 (51)	≥ 2.5 (48)	1.5+ (54)	≥ 2.5 (49)	≥ 2.5 (70)	≥ 2.5 (52)	≥ 2.5 (60)	≥ 2.5 (67)	≥ 2.5 (76)	1	48
Nauplia (larvae)	≥ 2.5 (85)	≥ 2.5 (85)	1.5- (84)	≥ 2.5 (58)	≥ 2.5 (41)	≥ 2.5 (52)	≥ 2.5 (51)	≥ 2.5 (61)	≥ 2.5 (51)	1.5+ (54)	≥ 2.5 (86)	≥ 2.5 (81)	1	41
Ostracoda	≥ 2.5 (n.c.)	≥ 2.5 (179)	≥ 2.5 (n.c.)	≥ 2.5 (238)	≥ 2.5 (151)	≥ 2.5 (101)	≥ 2.5 (84)	≥ 2.5 (89)	≥ 2.5 (68)	≥ 2.5 (79)	1.5+ (65)	≥ 2.5 (74)	1	68
Sum Diptera	≥ 2.5 (53)	≥ 2.5 (57)	≥ 2.5 (72)	≥ 2.5 (78)	≥ 2.5 (62)	≥ 2.5 (78)	≥ 2.5 (72)	≥ 2.5 (76)	1.5- (59)	≥ 2.5 (64)	≥ 2.5 (77)	≥ 2.5 (92)	1	59
Chironomidae (larvae)	≥ 2.5 (92)	≥ 2.5 (70)	≥ 2.5 (92)	1.5- (81)	≥ 2.5 (96)	≥ 2.5 (80)	≥ 2.5 (111)	≥ 2.5 (83)	1.5- (70)	≥ 2.5 (70)	≥ 2.5 (78)	≥ 2.5 (92)	1	70

Zooplankton	Day after application												MDD	Min
	-9	-1	2	6	9	16	23	30	44	58	72	86	Cat	MDD
Sum Rotatoria	≥2.5 (87)	≥2.5 (89)	1.5- (91)	0.87- (78)	≥2.5 (70)	≥2.5 (68)	≥2.5 (81)	≥2.5 (86)	≥2.5 (85)	≥2.5 (78)	≥2.5 (56)	≥2.5 (60)	1	68
<i>Keratella quadrata</i>	≥2.5 (85)	≥2.5 (89)	1.5- (88)	0.87- (73)	≥2.5 (65)	≥2.5 (87)	≥2.5 (94)	≥2.5 (91)	≥2.5 (87)	≥2.5 (78)	≥2.5 (57)	≥2.5 (59)	1	65
<i>Mytilina</i> sp.	≥2.5 (104)	≥2.5 (100)	≥2.5 (147)	≥2.5 (110)	≥2.5 (95)	1.5+ (72)	≥2.5 (72)	≥2.5 (79)	≥2.5 (88)	≥2.5 (185)	1.5- (92)	≥2.5 (87)	1	72
<i>Polyarthra</i> sp.	1.5- (93)	≥2.5 (96)	1.5- (98)	0.87- (89)	1.5- (88)	≥2.5 (86)	≥2.5 (86)	≥2.5 (88)	≥2.5 (97)	≥2.5 (97)	≥2.5 (116)	≥2.5 (109)	1	86
<i>Synchaeta</i> sp.	≥2.5 (97)	≥2.5 (92)	1.5- (92)	0.87- (74)	≥2.5 (88)	≥2.5 (98)	0.87- (95)	≥2.5 (111)	≥2.5 (188)	≥2.5 (116)	<0.3- (86)	≥2.5 (109)	1	74
<i>Testudinella</i> sp.	≥2.5 (129)	≥2.5 (98)	≥2.5 (117)	0.87- (85)	≥2.5 (92)	≥2.5 (100)	≥2.5 (142)	≥2.5 (123)	≥2.5 (91)	≥2.5 (103)	≥2.5 (91)	≥2.5 (81)	1	85
<i>Asplanchna</i> sp.		≥2.5 (138)		≥2.5 (n.c.)	≥2.5 (n.c.)	≥2.5 (n.c.)	≥2.5 (n.c.)	≥2.5 (117)	≥2.5 (207)	≥2.5 (n.c.)	≥2.5 (n.c.)	1.5+ (n.c.)	2	117
<i>Brachionus</i> sp.	≥2.5 (n.c.)		≥2.5 (127)	1.5+ (109)	≥2.5 (130)	≥2.5 (107)	≥2.5 (111)	≥2.5 (131)	≥2.5 (142)	<0.3- (96)		≥2.5 (n.c.)	2	107
<i>Cephalodella</i> sp.		≥2.5 (n.c.)		≥2.5 (96)	1.5+ (105)	≥2.5 (157)	<0.3- (86)		≥2.5 (112)	≥2.5 (105)		≥2.5 (251)	2	86
<i>Cephalodella</i> sp. cf.	≥2.5 (n.c.)		0.87- (97)					≥2.5 (105)			≥2.5 (94)		2	97
<i>Euchlanis</i> sp.		≥2.5 (135)	≥2.5 (140)	≥2.5 (102)	≥2.5 (105)	1.5+ (188)	≥2.5 (144)	≥2.5 (83)	≥2.5 (97)	≥2.5 (154)	≥2.5 (109)	≥2.5 (119)	2	83
<i>Filinia</i> sp.				≥2.5 (149)					≥2.5 (149)	≥2.5 (n.c.)	1.5+ (n.c.)		2	149
<i>Hexarthra</i> sp.	≥2.5 (203)	0.87- (82)	1.5- (90)	≥2.5 (95)	≥2.5 (97)	≥2.5 (108)	≥2.5 (104)	≥2.5 (102)	≥2.5 (110)	≥2.5 (103)	≥2.5 (102)	≥2.5 (103)	2	90
<i>Lecane</i> sp.	≥2.5 (n.c.)	≥2.5 (n.c.)		0.51- (78)	≥2.5 (228)	0.87- (72)	≥2.5 (133)	≥2.5 (110)	≥2.5 (153)	≥2.5 (128)	≥2.5 (123)	≥2.5 (279)	2	72
<i>Lepadella</i> sp.	≥2.5 (n.c.)	≥2.5 (103)	1.5- (95)	≥2.5 (113)	0.87- (78)	1.5+ (138)	≥2.5 (84)	≥2.5 (87)	≥2.5 (n.c.)	≥2.5 (144)	≥2.5 (208)	≥2.5 (112)	2	78
<i>Proales</i> sp.		≥2.5 (163)			≥2.5 (144)	0.87+ (n.c.)							2	144
<i>Rhinoglena</i> sp.			0.87+ (n.c.)	≥2.5 (143)	≥2.5 (110)	≥2.5 (153)	≥2.5 (n.c.)		≥2.5 (n.c.)				2	110
<i>Squatinella</i> sp.								1.5+ (n.c.)					2	
<i>Trichocerca</i> sp.	≥2.5 (n.c.)	≥2.5 (208)		≥2.5 (237)	≥2.5 (267)	≥2.5 (114)	≥2.5 (89)	≥2.5 (108)	≥2.5 (111)	1.5+ (138)	≥2.5 (94)	≥2.5 (111)	2	89
<i>Habrotricha</i> sp. cf.	≥2.5 (n.c.)	≥2.5 (95)	≥2.5 (242)		≥2.5 (n.c.)	≥2.5 (117)	≥2.5 (n.c.)	1.5+ (n.c.)	≥2.5 (246)		≥2.5 (149)	≥2.5 (n.c.)	2	117

Signs +/- indicate the direction of a significant effect and colours indicate the different NOECs. Blank fields: taxon not present. n.c.: MDD could not be calculated because of absence in the controls. Min and mean MDD are related to MDDs over the 6 weeks after first application only. Colours indicate the MDD classes proposed by EFSA (2013). MDD Cat. = MDD category according Brock *et al.* (2015). Taxa set in bold represent populations of the potentially sensitive group (i.e. Arthropoda) with sufficiently low MDDs to assess medium direct effects (at least once < 70 % until day 44).

Chydorus sphaericus

Chydorus sphaericus showed significantly higher abundances late in the study (day 44, 58,72) at 2.5 and 1.5 µg/L but full recovery was observed at the end of the study. This was considered as a potential indirect temporary promotion and thus as a class 2A+ and 3A+ effect for 1.5 and 2.5 µg/L, respectively.

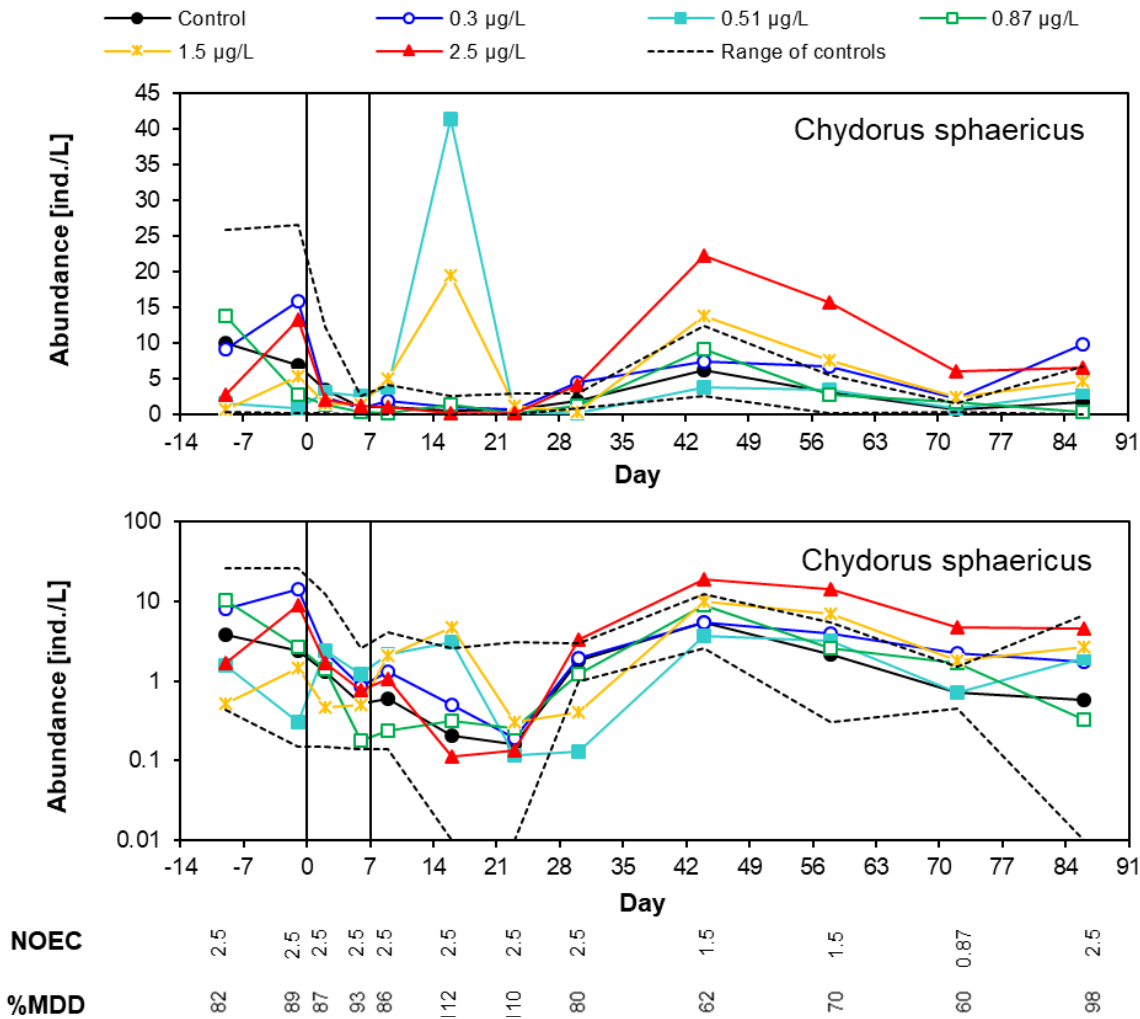


Figure A 15: *Chydorus sphaericus* in the zooplankton samples. Range of controls and arithmetic (top) and geometric (bottom) means per treatment level.

Daphnia sp.
The calculated NOEC <0.30 µg/L for Daphnia sp. on day 30 is a statistical artefact caused by very low numbers only at 0.30 µg/L and the moving average procedure included in the Williams-test. Since MDD were < 70 % within the first 6 weeks after application and no indication of reduced abundances at 2.5 µg/L was found, effect class 1 was used for Daphnia sp. up to 2.5 µg/L.

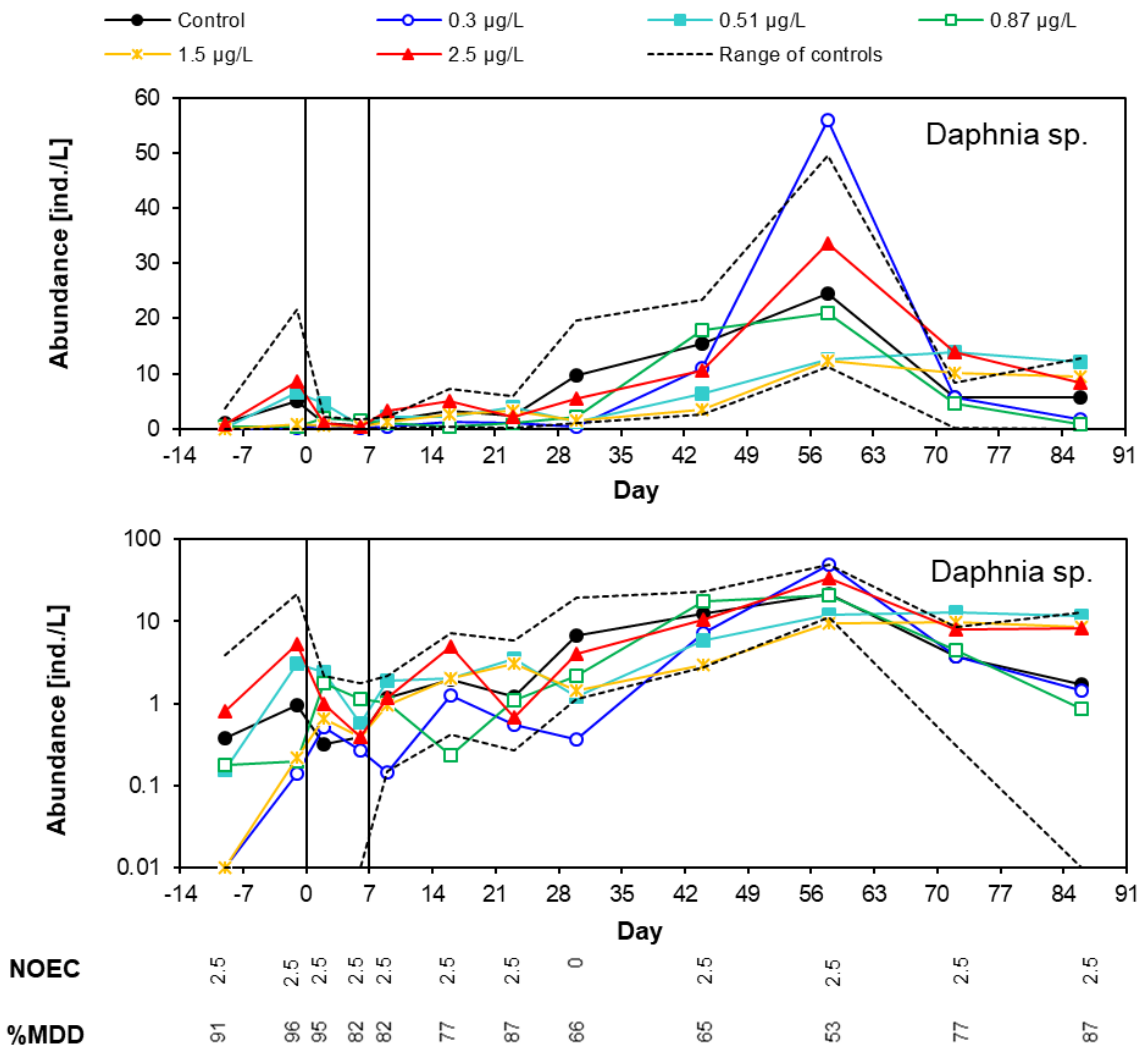


Figure A 16: *Daphnia sp.* in the zooplankton samples. Range of controls and arithmetic (top) and geometric (bottom) means per treatment level.

Scapholeberis sp.

Scapholeberis sp. is a MDD category 1 taxon and on day 44 the MDDs were just below 70 %. However, the numbers of *Scapholeberis* sp. were relatively low, especially at the beginning of the study. The calculated NOEC <0.30 mg/L on day 2 was driven by finding only a few animals in two replicates of controls and no findings in the treatments on this sampling. Therefore the species was not included in the effect classification.

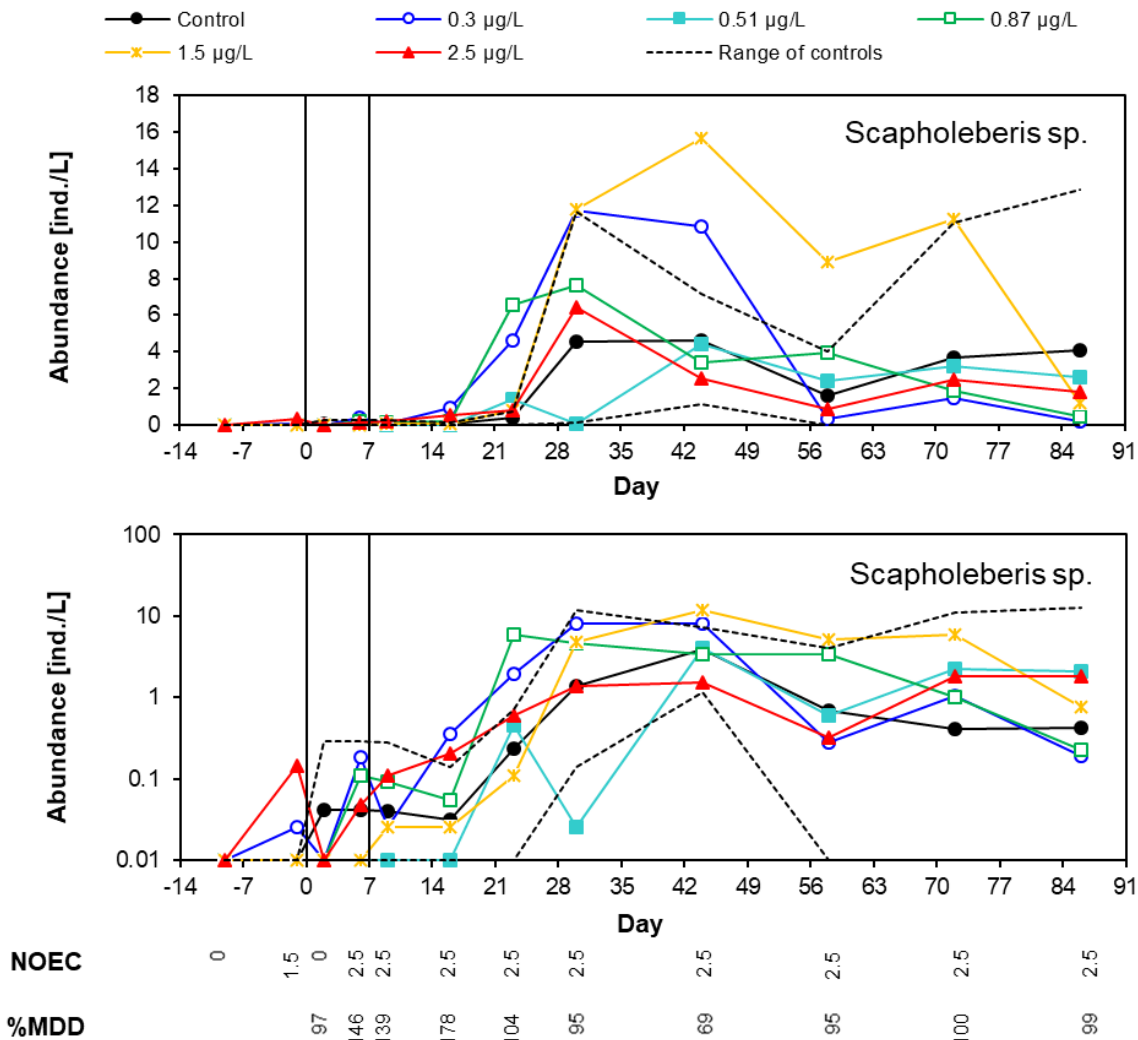


Figure A 17: *Scapholeberis* sp. in the zooplankton samples. Range of controls and arithmetic (top) and geometric (bottom) means per treatment level.

Cyclopidae
The sum of adults and copepodits of the Cyclopidae family was not affected by the treatments. The isolated NOEC calculated on day 16 suggesting a promotion at the highest treatment is not considered as treatment related since abundance at 0.30 µg/L was very similar. Effect class 1 was assigned for all treatments.

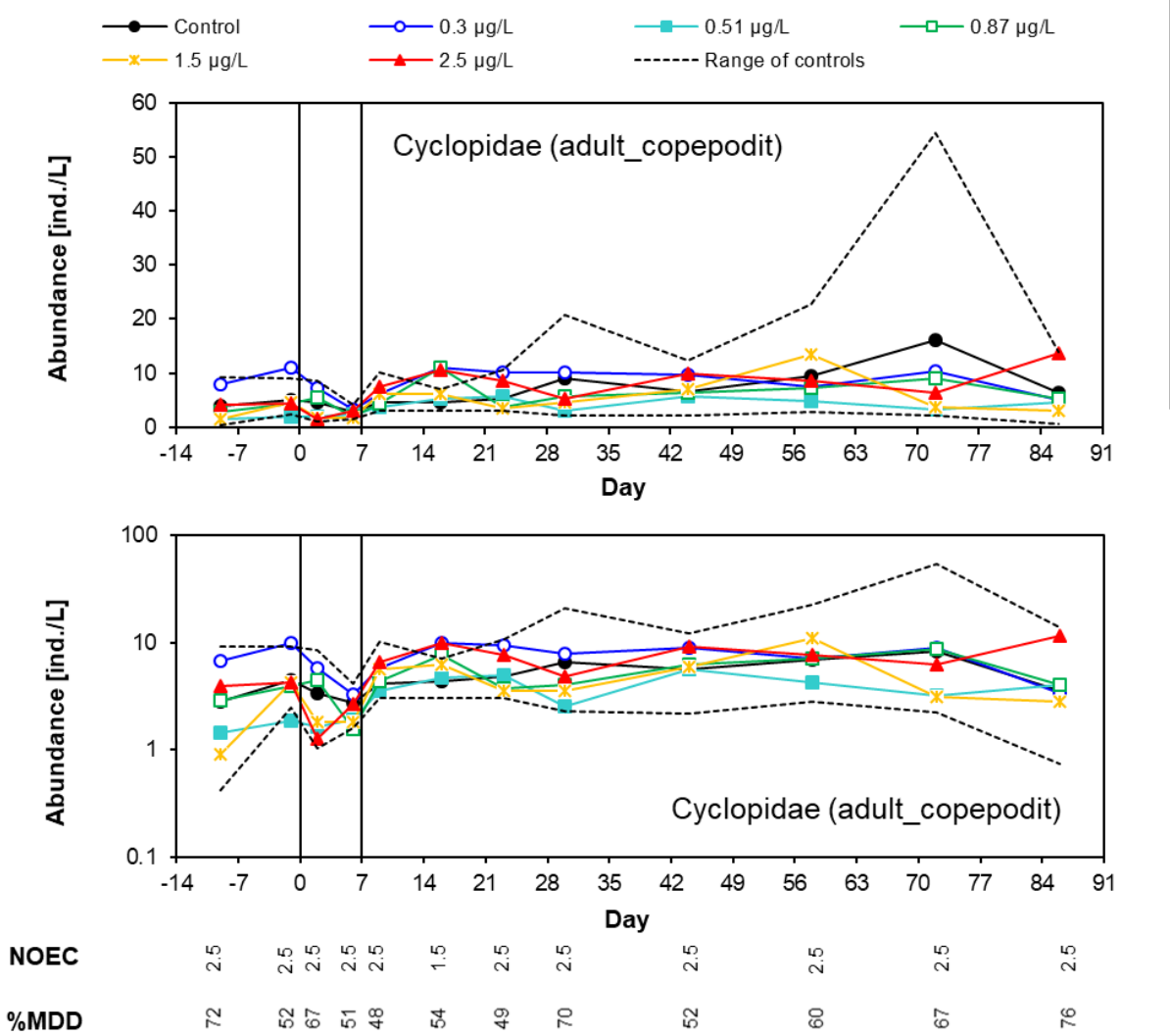


Figure A 18: Cyclopidae (sum of adults and copepodits) in the zooplankton samples. Range of controls and arithmetic (top) and geometric (bottom) means per treatment level.

Ostracoda abundances were low at the start of the study and started to increase after day 7. No direct treatment effects were found. The higher abundances at 0.30 µg/L compared to the control are considered an outlier but the slightly higher abundances at 2.5 µg/L might indicate an indirect promotion. Thus, effect class 2+ was used for 2.5 µg/L.

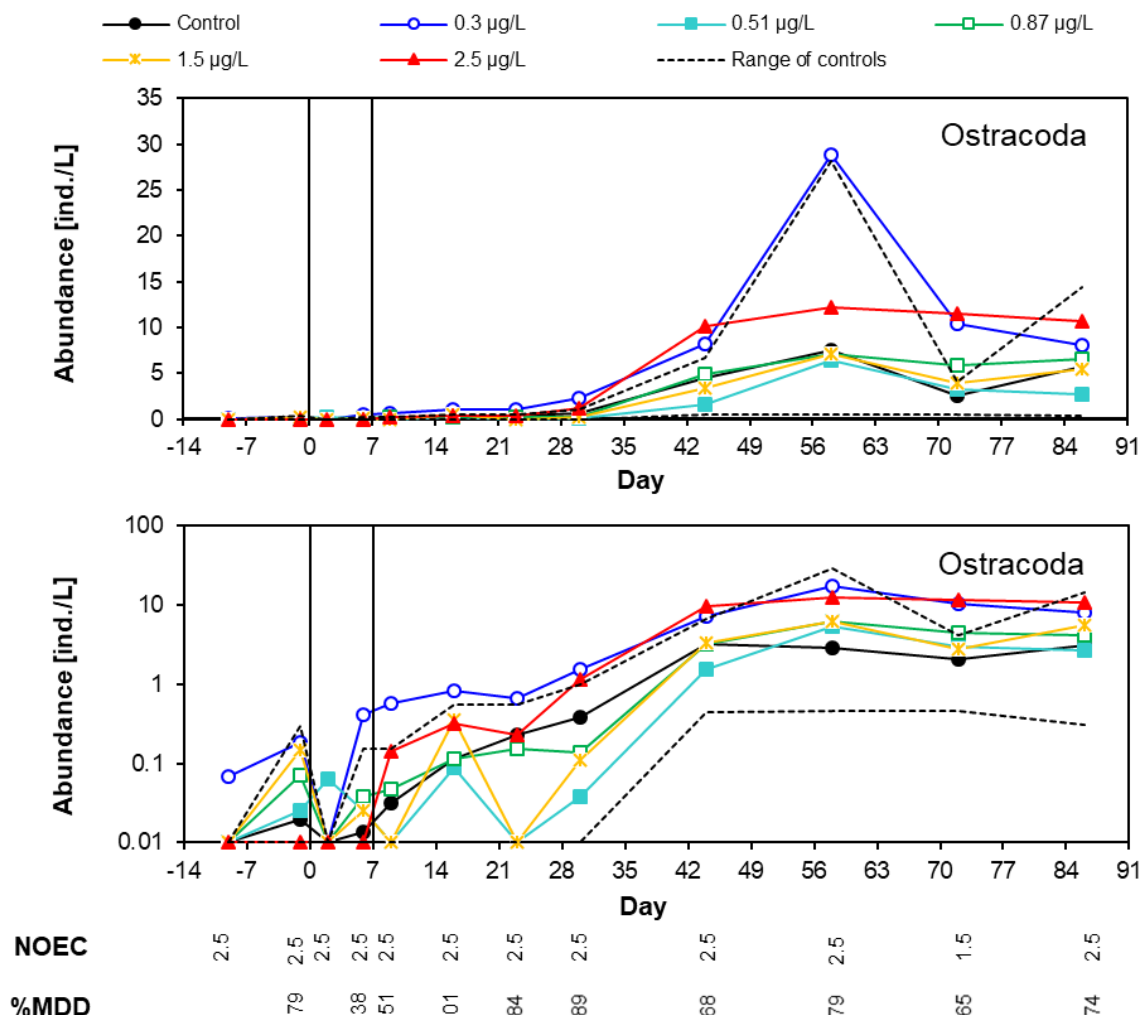


Figure A 19: Ostracoda in the zooplankton samples. Range of controls and arithmetic (top) and geometric (bottom) means per treatment level.

Chironomidae

Chironomidae do not belong to the zooplankton, but some larvae were found in the samples and the MDDs were 70 % on several samplings. However, the numbers found were very low and the NOECs of 1.5 µg/L on days 6 and 44 were not considered to indicate treatment effects at 2.5 µg/L since numbers at 2.5 µg/L increased after the second application and on day 44 the mean abundance at 0.51 µg/L was similarly low as at 2.5 µg/L. Thus, Chironomidae were not included in the zooplankton effect classification.

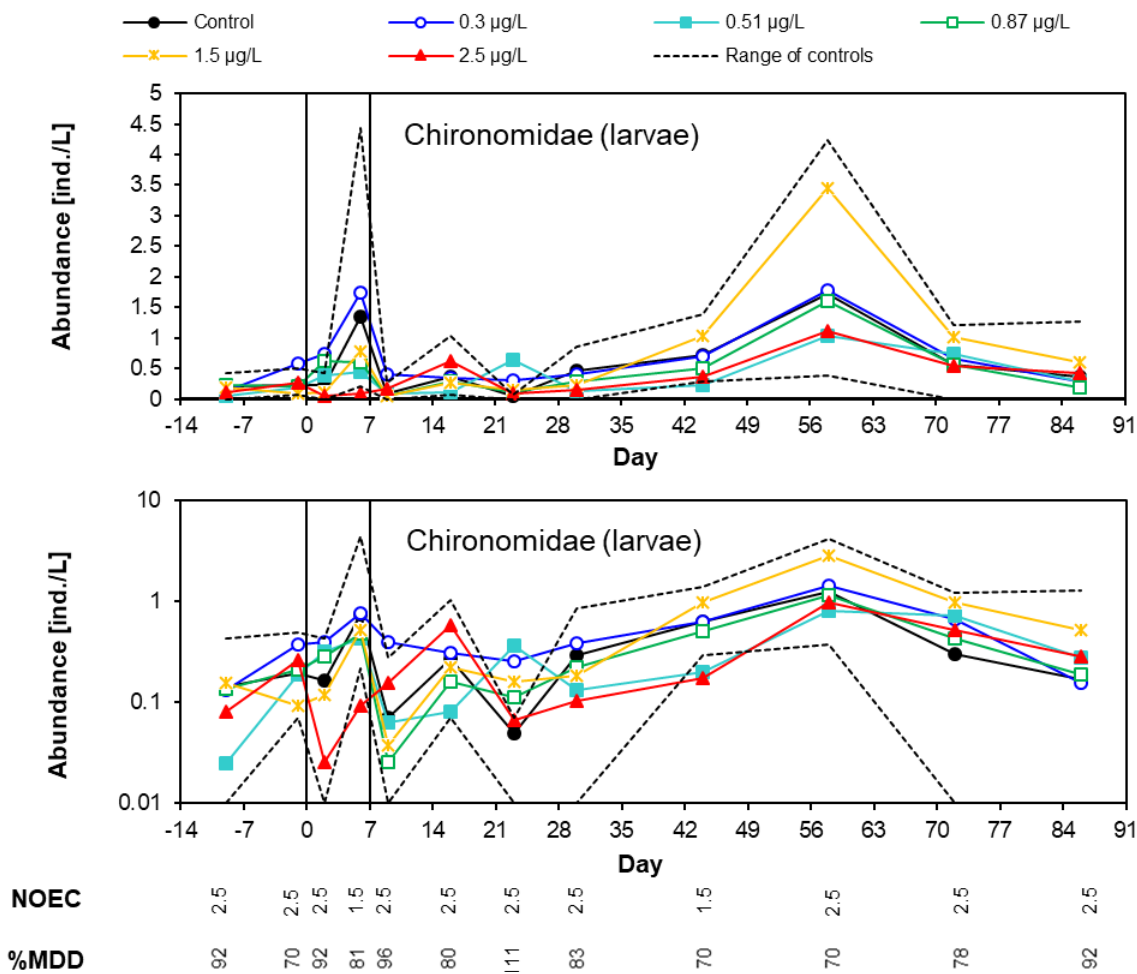


Figure A 20: Chironomidae in the zooplankton samples. Range of controls and arithmetic (top) and geometric (bottom) means per treatment level.

Community level analysis

The PRCs indicate a short-term response after the first application of 2.5 µg/L. At lower concentrations, no clear concentration response was predicted. The principal response is driven mainly by rotifers, while Daphnia taxa had inverse weights indicating short-term promotions. The PRCs do not show a significant part of the variance explained by the treatment and redundancy analysis revealed a significant treatment effect only on day 2 and 6. As discussed already for the single taxa, both findings, lower abundances of rotifers and higher abundances of Daphnia directly after the first application of 2.5 µg/L were not considered treatment related since differences to the controls were already present before application.

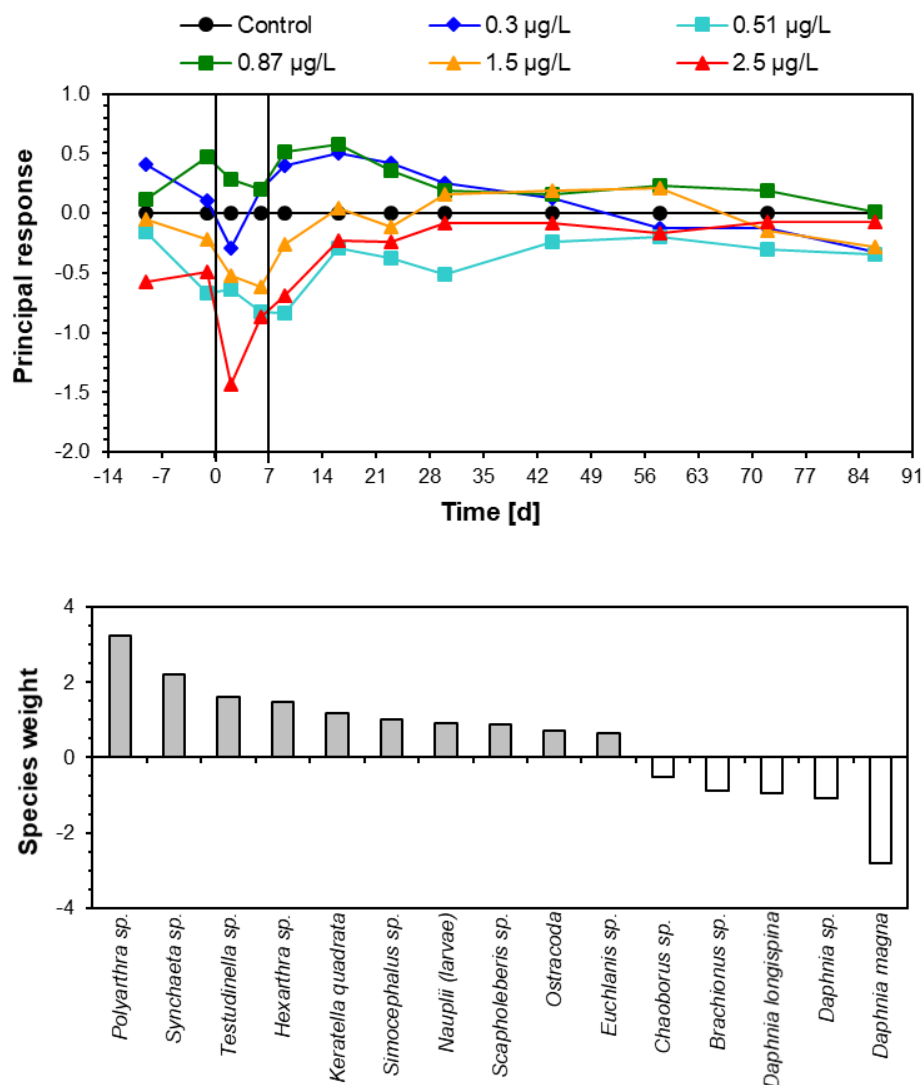


Figure A 21: PRCs for the zooplankton.

41% of total variance explained by time, 20% of total variance explained by treatment ($p=0.008$), 29% of the variance by treatment captured by the PRC ($p=0.072$).

Summary zooplankton.

There were no direct treatment effects on the zooplankton up to the highest test concentration. Later in the study, a slight promotion of *Chydorus*, Ostracoda and Copepoda cannot be excluded at 1.5 µg/L and / or 2.5 µg/L. However, no effects on the zooplankton community structure were found.

Table A 41: Effect classification for the zooplankton.

Exposure	nominal [$\mu\text{g a.s./L}$]	0.3	0.51	0.87	1.5	2.5
	max. measured [$\mu\text{g a.s./L}$]	0.55	0.88	1.4	2.5	4.5
	max. measured [$\mu\text{g a.s./kg dw}$]	0.61	0.71	0.77	1.9	2.3
Zooplankton	Cladocera	1	1	1	1	1
	<i>Chydorus sphaericus</i>	1	1	1	2A+	3A+
	<i>Daphnia sp.</i>	1	1	1	1	1
	Copepoda / nauplii	1	1	1	1	2+
	Cyclopidae (copepodits & adults)	1	1	1	1	1
	Ostracoda	1	1	1	1	2+
	Rotifera	1	1	1	1	1
	<i>Keratella quadrata</i>	1	1	1	1	1
	<i>Polyarthra sp.</i>	1	1	1	1	1
	Community level	1	1	1	1	1

Taxa in bold represent potentially sensitive populations with sufficiently low MDDs to assess medium direct effects.

Primary producers

Direct effects of acetamiprid on primary producers were not expected due to its mode of action and thus, they were monitored more as descriptors of the systems and for indirect effects. Phytoplankton and periphyton were monitored by chlorophyll measurements allowing to differentiate four major groups. The macrophytes growing in the sediment were assessed via area coverage.

Phytoplankton

Some statistically significant differences to the controls were found for the phytoplankton late in the study on days 43 and 57 (see table below), i.e. reductions of total chlorophyll a, bluegreens and diatoms on day 43 and 57 (not for bluegreens) with NOECs of 0.51 and 0.30 $\mu\text{g/L}$, respectively. An indirect promotion of the phytoplankton at the upper four test concentrations is not plausible. The data do not show a more pronounced promotion at the higher test concentration as would be expected. Chlorophyll concentrations at 0.51 up to 2.5 $\mu\text{g/L}$ were similarly low. In addition, the analysis of the macroinvertebrates and the zooplankton revealed no indication of any effects up to 0.87 $\mu\text{g/L}$ which could explain a promotion of algae. A promotion was found for blue-greens in the highest treatment on day 14.

All significant deviations of chlorophyll concentrations were within or very close to the range of the controls. In summary, no ecotoxicologically relevant effects on the phytoplankton are concluded.

Table A 42: NOEC [$\mu\text{g a.s./L}$] (calculated by Williams multiple test) and %MDD (in brackets) for the phytoplankton chlorophyll a values

Phytoplankton	Day after application											
	-9	-1	2	6	9	14	22	29	43	57	71	85
Total chlorophyll a	≥ 2.5 (34)	≥ 2.5 (48)	≥ 2.5 (60)	≥ 2.5 (60)	≥ 2.5 (66)	≥ 2.5 (73)	≥ 2.5 (67)	≥ 2.5 (56)	0.51- (65)	0.3- (64)	≥ 2.5 (82)	≥ 2.5 (82)
Bluegreens	≥ 2.5 (48)	≥ 2.5 (65)	≥ 2.5 (91)	≥ 2.5 (94)	≥ 2.5 (89)	1.5+ (71)	≥ 2.5 (72)	≥ 2.5 (77)	0.51- (64)	≥ 2.5 (75)	≥ 2.5 (78)	≥ 2.5 (72)
Greens	≥ 2.5 (39)	≥ 2.5 (49)	≥ 2.5 (62)	≥ 2.5 (70)	≥ 2.5 (80)	≥ 2.5 (85)	≥ 2.5 (84)	≥ 2.5 (71)	≥ 2.5 (93)	≥ 2.5 (95)	≥ 2.5 (97)	≥ 2.5 (97)
Diatoms	≥ 2.5 (40)	≥ 2.5 (49)	≥ 2.5 (63)	≥ 2.5 (56)	≥ 2.5 (58)	≥ 2.5 (71)	≥ 2.5 (60)	≥ 2.5 (50)	0.51- (64)	0.3- (62)	≥ 2.5 (81)	≥ 2.5 (78)
Cryptophytes	≥ 2.5 (74)	≥ 2.5 (77)	≥ 2.5 (91)	≥ 2.5 (100)	≥ 2.5 (102)	≥ 2.5 (96)	≥ 2.5 (101)	≥ 2.5 (106)	≥ 2.5 (112)	≥ 2.5 (131)	≥ 2.5 (123)	≥ 2.5 (138)

Signs +/- indicate the direction of a significant effect and colours indicate the different NOECs. Blank fields: taxon not present. n.c.: Colours indicate the NOECs.

Periphyton

The NOEC <0.30 µg/L for the periphyton chlorophyll (see table below) is triggered by low values in the 0.30 µg/L treatment and is the result of the moving average procedure of the Williams' test. The mean total chlorophyll-a value (15.6 µg/L) at 2.5 µg/L is close to those of the control (17.1 µg/L). Thus, this finding was not considered reliable for the effect evaluation.

At the end of the study significant deviations to controls were found for green algae and Cryptophyceae (higher abundances at 0.87 µg/L and higher) and diatoms (reduced abundance at 2.5 µg/L). Considering the figures, these findings likely represent increasing variability over time rather than delayed treatment effects since no plausible concentration response was observed. In summary, no ecotoxicologically relevant effects on the periphyton are concluded.

Table A 43: NOEC [µg a.s./L] (calculated by Williams multiple test) and %MDD (in brackets) for the periphyton chlorophyll a values

Periphyton	Day after application						
	-1	12	26	40	54	68	82
Total chlorophyll a	1.5- (73)	<0.3- (33)	≥2.5 (45)	≥2.5 (57)	≥2.5 (71)	≥2.5 (57)	≥2.5 (36)
Bluegreens	≥2.5 (86)	≥2.5 (46)	≥2.5 (48)	≥2.5 (63)	≥2.5 (86)	≥2.5 (67)	≥2.5 (93)
Greens	1.5- (74)	≥2.5 (45)	≥2.5 (44)	≥2.5 (56)	≥2.5 (72)	≥2.5 (59)	0.51+ (43)
Diatoms	≥2.5 (75)	≥2.5 (37)	≥2.5 (49)	≥2.5 (57)	≥2.5 (66)	≥2.5 (54)	1.5- (36)
Cryptophytes	≥2.5 (166)	≥2.5 (100)	≥2.5 (98)	≥2.5 (96)	≥2.5 (96)	≥2.5 (95)	0.51- (91)

Signs +/- indicate the direction of a significant effect and colours indicate the different NOECs. Colours indicate the NOECs.

Macrophytes

Five macrophyte species were present in the enclosures. *Chara globularis* was the most abundant species, followed by *Myriophyllum spicatum*. (see table below). Other taxa including filamentous algae were less abundant. The NOEC of <0.30 µg/L for a reduction of *Potamogeton natans* on day 15 is related to lower coverage only at 0.30 µg a.s./L but not at higher concentrations. On the last sampling day, a significantly higher coverage compared to the control was found for *Chara globularis* in all treatments. However, no treatment related effect was observed. The statistically significant higher coverage in comparison to controls was found in 0.30 and 0.51 µg/L. However this was not the case in the higher treatments and is considered to be caused by the small variability and the fact that in this dataset already small deviations from the mean became statistically significant. Therefore, the events for *Potamogeton natans* and *Chara globularis* were not considered to be a treatment related effect. *Myriophyllum spicatum* was also not affected. Thus, no effects were assumed for macrophytes and filamentous algae.

Table A 44: NOEC [$\mu\text{g a.s./L}$] (calculated by Williams multiple test) and %MDD (in brackets) for the macrophyte coverage data

Macrophytes	Day after application				
	-1	15	30	58	86
Sum Coverage [%]	≥ 2.5 (23)	≥ 2.5 (18)	≥ 2.5 (11)	≥ 2.5 (15)	≥ 2.5 (16)
<i>Chara globularis</i>	≥ 2.5 (31)	≥ 2.5 (18)	≥ 2.5 (11)	≥ 2.5 (14)	$< 0.3+$ (7)
<i>Myriophyllum spicatum</i>	≥ 2.5 (38)	≥ 2.5 (55)	≥ 2.5 (61)	≥ 2.5 (37)	≥ 2.5 (36)
<i>Potamogeton natans</i>	≥ 2.5 (40)	$< 0.3-$ (23)	≥ 2.5 (34)	≥ 2.5 (47)	≥ 2.5 (57)
<i>Zannichellia palustris</i>	≥ 2.5 (48)	≥ 2.5 (77)	≥ 2.5 (75)	≥ 2.5 (67)	≥ 2.5 (58)
Filamental algae	≥ 2.5 (39)	≥ 2.5 (53)	≥ 2.5 (69)	≥ 2.5 (52)	≥ 2.5 (69)
<i>Ceratophyllum demersum</i>				≥ 2.5 (37)	

Signs +/- indicate the direction of a significant effect and colours indicate the different NOECs. Blank fields: taxon not present.

Community metabolisms

Dissolved oxygen concentrations, pH and conductivity can be considered as indicators of community metabolisms since they are affected by primary production and in part, respiration.

Oxygen levels were temporarily (Day 29 – 63) affected in the enclosures treated with 1.5 and 2.5 $\mu\text{g/L}$ (see table below). The pH was only affected on day 56 where it was significantly higher at 2.5 $\mu\text{g/L}$ while conductivity was significantly lower at 0.51 $\mu\text{g/L}$ and higher concentrations. The increased oxygen levels (see table below) can be the result of higher primary production or lower respiration. The data for algae and macrophytes do not show higher abundances of primary producers which could explain increased oxygen production. It is also unclear whether reduced abundances of a few populations could have resulted in reduced community respiration and increased oxygen levels. Nevertheless, the slightly higher oxygen levels were considered effect class 3A+.

The reduced conductivity at the end of the study at the four higher test concentrations showed no clear concentration response and the deviations to control were less than 10 % (e.g. on average 196 $\mu\text{S/cm}$ in the controls and 184 $\mu\text{S/cm}$ at 2.5 $\mu\text{g/L}$ on day 84). Thus, they were not considered to be relevant and have no bearing on the functional performance of the mesocosms.

Table A 45: NOEC [$\mu\text{g a.s./L}$] (calculated by Williams multiple test) for dissolved oxygen concentrations, pH and conductivity as indicators for community metabolism

Phys.Chem	Day after application															
	-7	-1	2	7	9	14	21	29	35	42	49	56	63	70	77	84
Oxygen [mg/L]	≥ 2.5	≥ 2.5	≥ 2.5	≥ 2.5	≥ 2.5	≥ 2.5	≥ 2.5	1.5+	0.87+	1.5+	0.87+	1.5+	0.87+	≥ 2.5	≥ 2.5	≥ 2.5
pH	≥ 2.5	≥ 2.5	≥ 2.5	≥ 2.5	≥ 2.5	≥ 2.5	≥ 2.5	≥ 2.5	≥ 2.5	≥ 2.5	≥ 2.5	1.5+	≥ 2.5	≥ 2.5	≥ 2.5	≥ 2.5
Conductivity [$\mu\text{S/cm}$]	≥ 2.5	≥ 2.5	≥ 2.5	≥ 2.5	≥ 2.5	≥ 2.5	≥ 2.5	≥ 2.5	≥ 2.5	≥ 2.5	≥ 2.5	≥ 2.5	≥ 2.5	≥ 2.5	0.3-	0.3-

Signs +/- indicate the direction of a significant effect and colours indicate the different NOECs.

Summary

No treatment related effects were found on phytoplankton and periphyton chlorophyll. No treatment related effects were found on macrophytes. However, temporarily increased oxygen levels at 1.5 and 2.5 $\mu\text{g/L}$ might indicate increased photosynthesis activity.

Table A 46: Effect classification for primary producers

Exposure	nominal [$\mu\text{g a.s./L}$]	0.3	0.51	0.87	1.5	2.5
	max. measured [$\mu\text{g a.s./L}$]	0.55	0.88	1.4	2.5	4.5
	max. measured [$\mu\text{g a.s./kg dw}$]	0.61	0.71	0.77	1.9	2.3
Production	Phytoplankton chlorophyll a	1	1	1	1	1
	Periphyton chlorophyll a	1	1	1	1	1
	Macrophytes area coverage	1	1	1	1	1
	Community metabolism	1	1	1	3A+	3A+

Summary and conclusions

Test system

The study was conducted in outdoor model ecosystems located in Germany with a community (excluding vertebrates) representative for lentic and slow flowing water bodies. The systems included several macrophytes and many algae and invertebrate species from a large variety of taxonomic groups. Fungi, protozoa and bacteria were also present but not monitored since no specific protection goals are defined for aquatic microorganisms.

Exposure

The analysis of acetamiprid in application solutions confirmed the intended loading since the expected initial concentrations were on average 88 % of the nominal concentrations. Three hours after each application, on average, 94 % after the first and 92 % after the second application (calculated by subtraction of the measured concentrations one day before the second application) of the nominal concentrations were measured in the enclosure water.

Dissipation of acetamiprid from the water was relatively slow. DT_{50} calculated for each enclosure varied between 12 and 28 days with an average of 19 days. The DT_{50} of the single enclosures showed a slight trend of faster dissipation with increasing test concentration (about 22 d at the two lower and about 16 d at the two highest concentrations). Thus, six weeks after the first application still more than 50 % of the nominal concentrations were present in the water. Acetamiprid dissipated at least partly from the water into the sediment. Mean sediment concentrations at the highest treatment level reached a mean maximum of 2.3 $\mu\text{g/kg dw}$ four weeks after the first application and decreased down to 1.2 $\mu\text{g/kg dw}$ until the end of the study.

Thus, organisms were exposed to acetamiprid in water and sediment throughout the full study duration. No acetamiprid was found in water and sediment samples of the controls. Because the second application resulted in measured concentrations clearly above the nominal concentrations, the maximum measured concentrations are better suited for comparison with maximum PEC values than nominal concentrations.

Reliability of evaluation of direct effects

Due to the mode of action of acetamiprid, i.e. activation of nicotinic acetylcholine receptors, insects and crustaceans are expected to be the most sensitive species. Based on a previous mesocosm study, some Oligochaeta (Naididae) may also be sensitive. Following the requirements of the Aquatic Guidance Document (EFSA PPR 2013), minimum detectable differences (MDDs) were used to assess for how many potentially sensitive populations effects could be evaluated for direct effects in this study. In total 15 potentially sensitive taxa fulfil the MDD criterion proposed by Brock *et al.* (2015), including mayflies (*Cloeon dipterum*), midges (*Chaoborus crystallinus*, Chironominae, Tanypodinae, Orthocladiinae), damselflies (Zygoptera, Coenagrionidae), *Helophorus* sp., Isopoda (*Asellus aquaticus*), *Gammarus* sp. (bioassay on survival), Cladocera (three species), Copepoda (Cyclopidae), Ostracoda and Naididae.

Due to the long-term exposure of organisms in the mesocosm, the MDD criterion by Brock *et al.* (2015) based on all MDDs after the first application seems appropriate. However, if only the first six weeks are considered for macroinvertebrates and zooplankton, when exposure was on average larger than 50 % of nominal, still more than eight taxa (including *Cloeon* and Naididae) revealed at least once MDDs up to 70 % which is sufficient to detect medium effects following EFSA PPR panel (2013). Thus, a statistical analysis of direct effects was possible for at least eight potentially sensitive populations as required by EFSA PPR panel (2013).

Since the study was conducted in enclosures located in an artificial pond, typical stream taxa like stoneflies or caddisflies (Plecoptera and Trichoptera) or Amphipoda like *Gammarus* sp., were not present or rare in the test systems. However, *Gammarus* was successfully tested in an *in-situ* bioassay and there is no

indication that typical stream taxa are more sensitive than e.g. the mayflies evaluated in this study. In addition, exposure duration is expected to be much shorter in streams than in lentic or slow flowing water bodies and thus, the same maximum concentration has probably less severe effects in streams than in the test systems used in this study.

Effect classification

The following effects were observed at the different test concentrations :

- 0.55 µg a.s./L maximum measured (0.30 µg a.s./L nominal), effect class 1:
No treatment related effects were found.
- 0.88 µg a.s./L maximum measured (0.51 µg a.s./L nominal), effect class 1:
No treatment related effects were found.
- Maximum measured 1.4 µg a.s./L (0.87 µg a.s./L nominal), effect class 2:
No treatment effects were found except a slight effect on the mayfly *Cloeon dipterum*. Single statistical findings with NOECs of 1.4 µg a.s./L were found not to be ecotoxicologically relevant due to very low numbers of animals in the samples, missing concentration-response, and / or implausible timing of the statistical findings.
- 2.5 µg a.s./L maximum measured (1.5 µg a.s./L nominal) effect class 5B:
This concentration had only slight effects on mayfly larvae abundance but pronounced effects on the emergence of *Cloeon dipterum* with recovery of emergence demonstrated at the end of the study (class 5A). *Gammarus* survival was slightly affected but Naididae were affected without a clear demonstration of recovery until the end of the study (class 5B). At the community level, the macroinvertebrates and the emergence of insects were affected without recovery since *Cloeon* and Naididae dominated the community response. In the Zooplankton, *Chydorus sphaericus* abundance might have been slightly promoted. Temporarily higher oxygen levels indicate an indirect promotion of primary production but no effects on algae and macrophytes were detected.
- 4.5 µg a.s./L maximum measured (2.5 µg a.s./L nominal), effect class 5B:
Compared to 2.5 µg a.s./L (max. measured), some effects became more pronounced and for additional species slight direct or indirect effects were found. The number of mayfly larvae were clearly reduced but recovered towards the end of the study. The abundance of *Asellus* was affected but did not recover within the course of the study. Emergence of some chironomids was temporarily reduced (effect class 3A) while damselflies emerged at lower numbers until the end of the study (class 5B). The water beetle *Helophorus* sp. was found in higher numbers in the emergence traps shortly after the second application. The potential promotion of *Chydorus sphaericus* became more pronounced and slight promotion might also be given for Ostracoda and Copepoda (abundance of nauplius larvae) in the zooplankton. The promotion of the zooplankton taxa which also graze on periphyton might be an indirect effect of reduced competition by *Cloeon* larvae affected directly by the test item.

Proposal for RAC derivation

In conclusion, the maximum mean measured concentration of 1.4 µg a.s./L (0.87 µg a.s./L nominal) is the overall Class 2 concentration which can be used to derive an ETO-RAC. Uncertainty related to this concentration is considered small since clearly no pronounced effects on potentially sensitive taxa were found while only the number of mayfly larvae was slightly affected. These results are in line with the findings of a previous mesocosm study with acetamiprid (EFSA 2016).

An ERO-RAC cannot be derived from this study according to the current guidance (EFSA PPR panel 2013) since at the next higher test concentration (2.5 µg a.s./L maximum mean measured) effects on the abundance of Naididae and the emergence of mayflies lasted longer than eight weeks.

A 2.2.3 KCP 10.2.3/02 Further testing on aquatic organisms

Comments of zRMS:	<p>In support of the first zonal evaluation of CA3573 (formerly MCW-2222) the study on acute toxicity of the formulation to additional aquatic invertebrate species was submitted and accepted by the zRMS. The study was not included by the Applicant in the dRR provided following acetamiprid renewal, however the study provides useful information that may be used in order to compare toxicity of the product and the active compound. Hence, the summary has been copied from the Core Assessment, Part B, Section 6 of April 2018 and provided below for completeness.</p> <p>As the test guideline has not changed since that time, re-evaluation of the study following was not necessary and presented below conclusions were taken from the Core Assessment, Part B, Section 6 of April 2018.</p> <p>The study was performed in line with adopted OECD 202 and met all validity criteria. Following endpoints based on nominal concentrations were agreed:</p> <table> <tr> <th>Species</th><th>EC₅₀ (mg a.s./L)</th></tr> <tr> <td><i>Aeshna</i> sp</td><td>>2.13</td></tr> <tr> <td><i>Asellus aquaticus</i></td><td>0.0394</td></tr> <tr> <td><i>Chaoborus crystallinus</i></td><td>1.998</td></tr> <tr> <td><i>Cloeon dipterum</i></td><td>0.0144</td></tr> <tr> <td><i>Corixinae</i></td><td>0.0166</td></tr> <tr> <td><i>Crangonyx pseudogracilis</i></td><td>0.0307</td></tr> <tr> <td><i>Gammarus pulex</i></td><td>0.115</td></tr> <tr> <td><i>Ischnura elegans</i></td><td>1.351</td></tr> <tr> <td><i>Phryganea bipunctata</i></td><td>0.0148</td></tr> <tr> <td><i>Notonecta marmorea viridis</i></td><td>1.314</td></tr> </table>	Species	EC ₅₀ (mg a.s./L)	<i>Aeshna</i> sp	>2.13	<i>Asellus aquaticus</i>	0.0394	<i>Chaoborus crystallinus</i>	1.998	<i>Cloeon dipterum</i>	0.0144	<i>Corixinae</i>	0.0166	<i>Crangonyx pseudogracilis</i>	0.0307	<i>Gammarus pulex</i>	0.115	<i>Ischnura elegans</i>	1.351	<i>Phryganea bipunctata</i>	0.0148	<i>Notonecta marmorea viridis</i>	1.314
Species	EC ₅₀ (mg a.s./L)																						
<i>Aeshna</i> sp	>2.13																						
<i>Asellus aquaticus</i>	0.0394																						
<i>Chaoborus crystallinus</i>	1.998																						
<i>Cloeon dipterum</i>	0.0144																						
<i>Corixinae</i>	0.0166																						
<i>Crangonyx pseudogracilis</i>	0.0307																						
<i>Gammarus pulex</i>	0.115																						
<i>Ischnura elegans</i>	1.351																						
<i>Phryganea bipunctata</i>	0.0148																						
<i>Notonecta marmorea viridis</i>	1.314																						

Reference:	KCP 10.2.3/02
Report	Acetamiprid 200 SL – Acute Toxicity to Aquatic Organisms, Taylor, S. & Joyce, F., D, 2015
Report No.:	Study number: XCE2008
Document No.:	R-35057 (ADAMA reference number)
Guideline(s):	<p>The study was not conducted according to any specific regulatory guideline, but the following was consulted:</p> <p>OECD Guidelines for Testing of Chemicals, No. 202: “Daphnia sp. Acute Immobilisation Test”, adopted, 2004.</p>
Deviations:	<p>Control mortality of 12.5% was observed in the test with <i>Corixinae</i>. Given the fact that the feral instead of standard laboratory organisms were used in the present study, a mortality rate of 12.5 % is considered acceptable and the study endpoint for <i>Corixinae</i> was considered still valid due to the meaningful concentration-response.</p>
GLP:	Yes, certified laboratory
Acceptability:	Yes, study considered acceptable
Duplication (if vertebrate study)	Not applicable

Executive Summary

In a 48-hour acute toxicity study, ten invertebrate taxa namely: *Aeshna* sp., *Asellus aquaticus*, *Chaoborus crystallinus*, *Cloeon dipterum*, *Corixinae*, *Crangonyx pseudogracilis*, *Gammarus pulex*, *Ischnura elegans*, *Phryganea bipunctata* and *Notonecta marmorea viridis*. were exposed to acetamiprid 200 SL (MCW-

2222). The test item was a formulated product containing the active substance acetamiprid (17.51% w/w). The different invertebrates were exposed to a range of concentrations with nominal concentrations from 1.94 up to 2500 µg a.s./L (depending on the species) under static conditions and in accordance with the OECD guideline 202. Immobility and mortality were observed at the end of the test after 48 hours.

Analytical measurements of the test solutions confirmed that the target nominal concentrations were achieved and maintained within 20% of the nominal values for the duration of the study, with the exception of one analytical value (21% above nominal), which is not considered to have an impact on the overall results. Therefore, all toxicity results are based on the nominal concentrations of the test item.

Depending on the species, the EC₁₀ values for immobilisation based on nominal concentrations were calculated to be between 5.9 and >2130 µg test item/L, the EC₂₀ values to be between 8.4 and >2130 µg test item/L and the EC₅₀ values to be between 14.4 and >2130 µg test item/L. No LCx values were calculated as only three of the tested organisms (*Cloeon dipterum*, Corixinae and *Gammarus pulex*) displayed significant mortality.

Materials and methods

Materials

Test item Acetamiprid 200 SL (MCW-2222)

Batch # SZBC110XV

Content of active substance Acetamiprid 200 g/L

Description Clear yellow liquid

Control Pond water only

Toxic reference None

Test organism

Species Ten invertebrate taxa were used for this study, consisting of seven Insecta taxa (*Aeshnidae* (*Aeshna* sp), *Baetidae* (*Cloeon dipterum*), *Chaoboridae* (*Chaoborus crystallinus*), *Coenagrionidae* (*Ischnura elegans*), *Corixidae* (subfamily: Corixinae), *Phryganeidae* (*Phryganea bipunctata*) and *Notonectidae* (*Notonecta marmorea viridis*)) and three from the class Malacostraca (*Asellidae* (*Asellus aquaticus*), *Crangonyctidae* (*Crangonyx pseudogracilis*) and *Gammaridae* (*Gammarus pulex*))

Source In-house culture. In the case of *Gammarus pulex*, they were obtained from a commercial supplier (Blades Biological, UK)

Study design and methods

Test duration and exposure 48 hours, static exposure

Experimental dates 29 October 2014 to 05 March 2015

Test concentrations Nominal concentrations:

Aeshna sp. 150, 255, 434, 737, 1253, 2130 mg test item/L

Asellus aquaticus 1.94, 4.27, 9.39, 20.7, 45.5, 100 mg test item/L

Cloeon dipterum 1.94, 4.27, 9.39, 20.7, 45.5, 100 mg test item/L

Chaoborus crystallinus 150, 255, 434, 737, 1253, 2130 mg test item/L

Ischnura elegans 150, 255, 434, 737, 1253, 2130 mg test item/L

Corixinae 1.94, 4.27, 9.39, 20.7, 45.5, 100 mg test item/L

Crangonyx pseudogracilis 1.94, 4.27, 9.39, 20.7, 45.5, 100 mg test item/L

Gammarus pulex 79, 118, 177, 267, 400, 600 mg test item/L

Phryganea bipunctata 1.94, 4.27, 9.39, 20.7, 45.5, 100 mg test item/L

Notonecta marmorea viridis 176, 299, 509, 865, 1470, 2500 mg test item/L

Test units The organism cultures were maintained in either a 5 L glass beaker or a 12 L plastic box which contained pond water and portions of the

aquatic macrophyte *Elodea* sp. (*Chara* sp. was used for Corixinae) as a substrate.

Group size/replicates

1 (diluted control) per taxon, 6 treatment groups; replicates: 4 (group housed) or 10 (individually housed) Controls comprised twice this, i.e. 8 (group housed) or 20 (individually housed) including 5 (group housed) or 1 (individually housed) individuals per replicate organisms per concentration; 5 in each of 4 replicates

Test medium

Pond water and portions of the aquatic macrophyte *Elodea* sp. (*Chara* sp. was used for Corixinae) as a substrate. Additionally, the cultures contained portions of previously dried leaves mainly of the Alder (*Alnus* sp.) as a food source for shredding invertebrates (*Asellus aquaticus*, *Gammarus pulex* and *Crangonyx pseudogracilis*). Other invertebrates were fed *ad hoc* with various types of food during culturing: *Aeschna* sp. and *Ischnura elegans* were fed with frozen bloodworm (*Aeschna* sp. were also fed live mixed invertebrates), *Cloeon dipterum* were fed with flaked fish food, *Phryganea bipunctata* were fed with live Chaoborus and *Notonecta marmorea viridis* were fed with live mixed invertebrates. No additional food was given to *Chaoborus crystallinus* as microscopic zooplankton on which the organism feeds were considered to be present in the culture media and no additional food was given to Corixinae.

Environmental conditions

Temperature

20 ± 2°C

Photoperiod

16 hours light / 8 hours darkness

pH

6-9

Dissolved oxygen

≥ 5 mg/L

Analytical measurements

The concentrations of acetamiprid were determined in 40 mL aqueous samples collected from the fresh (0 hour) and pooled expired (48 hour) test media at each test concentration using a reversed phase-high performance liquid chromatography (RP-HPLC) with MS/MS-detection method of analysis.

Biological observations

The test organisms were observed daily at approximate 24-hour intervals for signs of mortality and immobility. For the purposes of this study, immobility was defined as the absence of free movement within 30 seconds following stimuli, i.e. gentle swirling of the media.

Statistics

For the evaluation of the 24- and 48-hour ECx values, Probit analysis with linear maximum likelihood regression was used for all taxa except *Cloeon dipterum* where Weibull analysis with linear maximum likelihood regression was used.

Results and discussion

Analytical measurements

Analytical results are given in the table below.

Table A 47: Nominal and measured concentrations of MCW-2222 in tests with the species *Aeshna* sp, *Chaoborus crystallinus* and *Ischnura elegans*

sp, <i>Chlorobacterium erythranthum</i> and <i>Ischemia elegans</i>							
	Measured concentration [mg a.s./L]						
Nominal concentration	0.0	150	255	434	737	1253	2130
Test start (0 h)							
Measured concentration	<LOQ	129	257	394	669	1176	1766
% of nominal	-	86	101	91	91	94	83
Range	86-101%						
Test end (48 h)							
Measured concentration	<LOQ	126	228	385	620	1193	1858
% of nominal	-	84	89	89	84	95	87
Range	84-95%						

LOQ = Limit of quantification (0.5 µg a.s./L)

Table A 48: Nominal and measured concentrations of MCW-2222 in tests with the species *Asellus aquaticus*, *Cloeon dipterum*, *Crangonyx pseudogracilis* and *Phryganea bipunctata*

	Measured concentration [mg a.s./L]							
Nominal concentration	0	1.94	4.27	9.39	20.7	45.5	100	
Test start (0 h)								
Measured concentration	<LOQ	1.94	5.99*	9.36	19.1	43.4	82.4	
% of nominal	-	100	140	100	92	95	82	
Range	82-140%							
Test end (48 h)								
Measured concentration	<LOQ	1.9	4.35	8.97	18.5 ^{a)}	47.8 ^{b)}	106 ^{c)}	
% of nominal	-	98	102	96	89	105	106	
Range	89-106%							

LOQ = Limit of quantification (0.5 µg a.s./L)

*Initial measured concentration 138% of nominal (5.91 µg a.s./L), considered erroneous. Test media re-analysed giving a comparable result (6.07 µg a.s./L). Presented concentration represents mean average of both measured values.

- (a) Initial measured concentration 57% of nominal (11.8 µg a.s./L), considered erroneous in comparison to measured dissipation of chemical in other samples. Test media re-analysed giving result presented. Initial value was disregarded.
(b) Initial measured concentration 61% of nominal (27.6 µg a.s./L), considered erroneous in comparison to measured dissipation of chemical in other samples. Test media re-analysed giving result presented. Initial value was disregarded.
(c) Initial measured concentration 75% of nominal (74.9 µg a.s./L), considered erroneous in comparison to measured dissipation of chemical in other samples. Test media re-analysed giving result presented. Initial value was disregarded.

Table A 49: Nominal and measured concentrations of MCW-2222 in tests with the species *Notonecta marmorea viridis*

Nonhectum maritimum viridis								
	Measured concentration [mg a.s./L]							
Nominal concentration	0	176	299	509	865	1470	2500	
Test start (0 h)								
Measured concentration	<LOQ	164	276	475	815	1444	2421	
% of nominal	-	93	92	93	94	98	97	
Range	92-98%							
Test end (48 h)								
Measured concentration	<LOQ	168	285	472	810	1406	2538 ^{a)}	
% of nominal	-	95	95	93	94	96	102	
Range	93-102%							

LOQ = Limit of quantification (0.5 µg a.s./L)

- (a) Initial measured concentration above 20% of nominal (4740 µg a.s./L), considered erroneous due to low concentration at 0 hours. Test media re-analysed giving result presented. Erroneous value was disregarded.

Table A 50: Nominal and measured concentrations of MCW-2222 in tests with the species *Gammarus pulex*

<i>Gammarus pulex</i>							
	Measured concentration [mg a.s./L]						
Nominal concentration	0	79	118	177	267	400	600
Test start (0 h)							
Measured concentration	<LOQ	75.3	114	161	251	383	581
% of nominal	-	95	97	91	94	96	97
Range	91-97%						
Test end (48 h)							
Measured concentration	<LOQ	72.9	110	171	244	385	572
% of nominal	-	92	93	97	91	96	95
Range	91-97%						

LOQ = Limit of quantification (0.5 µg a.s./L)

Table A 51: Nominal and measured concentrations of MCW-2222 in tests with the species *Corixinae*

	Measured concentration [mg a.s./L]						
Nominal concentration	0	1.94	4.27	9.39	20.7	45.5	100
	Test start (0 h)						
Measured concentration	<LOQ	1.8	3.94	8.74	21.5 ^{a)}	41.5	110 ^{b)}
% of nominal	-	93	92	93	104	91	110
Range	91-110%						
	Test end (48 h)						
Measured concentration	<LOQ	1.72	3.65	7.92	22.5 ^{a)}	45.8	91.9
% of nominal	-	89	85	84	109	101	92
Range	84-109%						

LOQ = Limit of quantification (0.5 µg a.s./L)

(a) Initial measured concentration 64% of nominal at 0 hours (13.3 µg a.s./L) and 67% of nominal at 48 hours (13.9 µg a.s./L). Re-analyses were performed giving results presented. Initial values considered erroneous in comparison to other samples and were disregarded.

(b) Initial measured concentration 75% of nominal (75.1 µg a.s./L) considered erroneous in comparison to 48-hour result. Re-analysis was performed giving result presented. Initial value was disregarded.

Biological results

Biological results are given in the tables below.

Table A 52: Percent of immobilised *Aeshna sp.* in a 48 hour acute immobilisation test exposed to MCW-2222

Nominal concentration [mg test item/L]	Control	150	255	434	737	1253	2130
Immobilisation [%]							
24 h	0	0	0	0	0	0	0
48 h	0	0	0	0	0	0	0

Table A 53: Percent of immobilised *Asellus aquaticus* in a 48 hour acute immobilisation test exposed to MCW-2222

Nominal concentration [mg test item/L]	Control	1.94	4.27	9.39	20.7	45.5	100
Immobilisation [%]							
24 h	0	0	0	0	20	40	80
48 h	5	0	0	0	30	50	90

Table A 54: Percent of immobilised *Chaoborus crystallinus* in a 48 hour acute immobilisation test exposed to MCW-2222

Nominal concentration [mg test item/L]	Control	150	255	434	737	1253	2130
Immobilisation [%]							
24 h	0	0	0	0	0	0	0
48 h	0	0	0	0	10	10	60

Table A 55: Percent of immobilised *Cloeon dipterum* in a 48 hour acute immobilisation test exposed to MCW-2222

Nominal concentration [mg test item/L]	Control	1.94	4.27	9.39	20.7	45.5	100
	Immobilisation [%]						
24 h	0	0	5	0	0	100	100
48 h	0	0	5	5	95	100	100

Table A 56: Percent of immobilised *Corixinae* in a 48 hour acute immobilisation test exposed to MCW-2222

Nominal concentration [mg test item/L]	Control	1.94	4.27	9.39	20.7	45.5	100
	Immobilisation [%]						
24 h	0	0	0	0	15	80	90
48 h	12.5	15	15	35	55	95	100

Table A 57: Percent of immobilised *Crangonyx pseudogracilis* in a 48 hour acute immobilisation test exposed to MCW-2222

Nominal concentration [mg test item/L]	Control	1.94	4.27	9.39	20.7	45.5	100
	Immobilisation [%]						
24 h	0	0	0	0	0	20	40
48 h	5	0	0	0	30	70	100

Table A 58: Percent of immobilised *Gammarus pulex* in a 48 hour acute immobilisation test exposed to MCW-2222

Nominal concentration [mg test item/L]	Control	79	118	177	267	400	600
	Immobilisation [%]						
24 h	5	10	0	80	100	100	100
48 h	5	30	30	90	100	100	100

Table A 59: Percent of immobilised *Ischnura elegans* in a 48 hour acute immobilisation test exposed to MCW-2222

Nominal concentration [mg test item/L]	Control	150	255	434	737	1253	2130
	Immobilisation [%]						
24 h	0	0	0	0	0	30	100
48 h	0	0	0	0	20	40	80

Table A 60: Percent of immobilised *Phryganea bipunctata* in a 48 hour acute immobilisation test exposed to MCW-2222

Nominal concentration [mg test item/L]	Control	1.94	4.27	9.39	20.7	45.5	100
	Immobilisation [%]						
24 h	0	0	0	0	50	80	100
48 h	0	0	0	22	70	100	100

Table A 61: Percent of immobilised *Notonecta marmorea viridis* in a 48 hour acute immobilisation test exposed to MCW-2222

Nominal concentration [mg test item/L]	Control	176	299	509	865	1470	2500
	Immobilisation [%]						
24 h	0	0	0	0	0	40	80
48 h	0	0	0	0	10	60	100

No LC_x values were calculated as only three of the tested organisms (*Cloeon dipterum*, *Corixinae* and *Gammarus pulex*) displayed significant mortality. However, statistical analyses of the available data for total immobility after 48 hours revealed that the following EC10, EC20 and EC50 values were reliably calculated:

Table A 62: Endpoints after 48 hours

Taxon	Concentration [µg a.s./L]		
	EC ₁₀ (95%-CI)	EC ₂₀ (95%-CI)	EC ₅₀ (95%-CI)
<i>Aeshna</i> sp	>2130	>2130	>2130
<i>Asellus aquaticus</i>	14.9 (5.6 - 22.8)	20.8 (10.1 - 30.2)	39.4 (26.5 - 62.0)
<i>Chaoborus crystallinus</i>	940 (304-1290)	1218 (625- 1700)	1998 (1466 - 5295)
<i>Cloeon dipterum</i>	8.7 (5.2 - 11.0)	10.7 (7.2 - 12.9)	14.4 (11.5 - 16.6)
Corixinae	5.9 (3.4 -8.2)	8.4 (5.5 -11.2)	16.6 (12.7 -21.9)
<i>Crangonyx pseudogracilis</i>	15.3 (6.7 - 21.7)	19.5 (10.4 - 26.5)	30.7 (21.6 - 43.6)
<i>Gammarus pulex</i>	67.7 (32.8 – 88.6)	81.3 (47 – 102)	115 (87.7 – 142)
<i>Ischnura elegans</i>	666 (316.5 - 900.0)	849 (504.0 - 1109)	1351 (1026 -1976)
<i>Phryganea bipunctata</i>	7.8 (3.2 - 11.0)	9.7 (5.0 - 13.2)	14.8 (10.3 - 20.6)
<i>Notonecta marmorea viridis</i>	899.2 (517 - 1105)	1024 (678 - 1233)	1314 (1054 - 1640)

Validity criteria

As shown in the following table, all validity criteria were met.

Table A 63: Validity criteria

Validity criteria according to OECD 202	Observed in study
Number of immobilised daphnids must be ≤ 10%	0-5%*
Dissolved oxygen concentration at the end of the test must be ≥ 3 mg/L in control(s) and test solutions.	> 60mg/L

* Note: although control mortality of 12.5% was observed in the test with Corixinae. Given the fact that the feral instead of standard laboratory organisms were used in the present study, a mortality rate of 12.5 % is considered acceptable and the study endpoint for Corixinae was considered still valid due to the meaningful concentration-response.

Conclusion

In a 48 hour acute toxicity study, different taxa were exposed to MCW-2222 with a range of nominal concentrations from 1.94 up to 2500 µg a.s./L (depending on the species) under static conditions and in accordance with the OECD guideline 202. Chemical analysis of the test solutions confirmed that the target nominal concentrations were achieved and maintained giving mean measured concentrations ranging from 84 to 121% of the nominal values. Therefore, the nominal concentrations were used in the calculation of the results.

Depending on the species, the EC₁₀ values for immobilisation based on nominal concentrations were calculated to be between 5.9 and >2130 µg test item/L, the EC₂₀ values to be between 8.4 and >2130 µg test item/L and the EC₅₀ values to be between 14.4 and >2130 µg test item/L. No LCx values were calculated as only three of the tested organisms (*Cloeon dipterum*, Corixinae and *Gammarus pulex*) displayed significant mortality.

A 2.2.3 KCP 10.2.3/03 Further testing on aquatic organisms

Comments of zRMS:	The following RAC values for the Tier 1, Tier 2A and Tier 2B approaches for the formulated product MCW-2222 (containing 200 g a.s./L Acetamiprid) were provided :
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	<p>RAC Tier 1: 0.093 µg a.s./L (based on an AF = 100) RAC Tier 2A (geomean Insecta): 1.49 µg a.s./L (based on an AF = 100) RAC Tier 2A (geomean Crustacea): 2.25 µg a.s./L (based on an AF = 100) RAC Tier 2B (SSD of most sensitive species): 1.11 µg a.s./L (based on an AF = 5)</p> <p>The relevant RAC for the risk assessment is the RAC Tier 2B of 1.11 µg a.s./L as based on a species sensitivity distribution (SSD) approach using the most sensitive species and an assessment factor (AF) of 5. The RAC Tier 2B of 1.1 µg a.s./L was not used in the risk assessment.</p>
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Reference:	KCP 10.2.3/03
Report	Koerner, O. (2015). MCW-2222: Evaluation of Aquatic Invertebrate Toxicity Tests to Derive a Regulatory Acceptable Concentration
Report No.:	R-36040
Document No.:	R-36040 (ADAMA reference number)
Guideline(s):	None
Deviations:	n.a.
GLP:	No
Acceptability:	Yes, considered acceptable

Executive Summary

Acute toxicity endpoints for the active substance acetamiprid obtained from 12 different tested aquatic invertebrate species were evaluated according to the EFSA Aquatic GD (2013) in order to obtain a Tier-2 RAC (Regulatory Acceptable Concentration). The estimated RAC values are:

RAC Tier 1: 0.093 µg a.s./L (based on an AF = 100)

RAC Tier 2A (geomean Insecta): 1.49 µg a.s./L (based on an AF = 100)

RAC Tier 2A (geomean Crustacea): 2.25 µg a.s./L (based on an AF = 100)

RAC Tier 2B (SSD of most sensitive species): 1.11 µg a.s./L (based on an AF = 5)

The relevant RAC for the risk assessment is the **RAC Tier 2B** of **1.11 µg a.s./L** as based on the species sensitivity distribution (SSD) approach using the most sensitive species and an assessment factor (AF) of 5.

Introduction

The expert statement evaluated several aquatic invertebrate toxicity studies performed with the active substance Acetamiprid in order to obtain a Tier-2 RAC (Regulatory Acceptable Concentration) for risk assessment purposes.

Aquatic toxicity endpoints for Acetamiprid were derived from studies conducted with the formulated product MCW-2222 (containing 200 g a.s./L Acetamiprid) and studies submitted during the EU inclusion of Acetamiprid (EU Review Report (2004)). The acute toxicity to aquatic invertebrates ranged from 9.3 to >2130 µg a.s./L for insects and from 30.7 to 17900 µg a.s./L for crustaceans.

Table A 64: Toxicity endpoints – Aquatic invertebrates

Species	Group	Test item	Endpoint [µg a.s./L]	Reference
Tier 1 studies				
<i>Daphnia magna</i>	Crustacea	Acetamiprid	49 800 (EC ₅₀) 5000 (NOEC)	EU Review Report (2004)
	Crustacea	MCW-2222	17 900 (EC ₅₀)	Juckeland (2014)
<i>Chironomus riparius</i>	Insecta	Acetamiprid	5 (NOEC)	EU Review Report (2004)
	Insecta	MCW-2222	9.3 (EC₅₀)	Juckeland (2015)
Tier 2 studies				
<i>Cloeon dipterum</i> (Baetidae)	Insecta	MCW-2222	14.4 (EC ₅₀)	Taylor and Joyce (2015)
<i>Corixinae</i> (Corixidae)	Insecta	MCW-2222	16.6 (EC ₅₀)	
<i>Phryganeae bipunctata</i> (Phryganeidae)	Insecta	MCW-2222	14.8 (EC ₅₀)	
<i>Notonecta marmoreal</i> (Notonectidae)	Insecta	MCW-2222	1314 (EC ₅₀)	

<i>Ischnura elegans</i> (Coenarionidae)	Insecta	MCW-2222	1351 (EC ₅₀)	
<i>Chaoborus crystallinus</i> (Chaoboridae)	Insecta	MCW-2222	1998 (EC ₅₀)	
<i>Aeschna</i> sp. (Aeshnidae)	Insecta	MCW-2222	> 2130 (EC ₅₀)	
<i>Crangonyx pseudogracilis</i> (Crangonyctidae)	Crustacea	MCW-2222	30.7 (EC ₅₀)	
<i>Asellus aquaticus</i> (Asellidae)	Crustacea	MCW-2222	39.4 (EC ₅₀)	
<i>Gammarus pulex</i> (Gammaridae)	Crustacea	MCW-2222	115 (EC ₅₀)	

Bold value indicates the lowest EC₅₀ endpoint out of 12 different arthropod species

MCW-2222 contains 200 g a.s./L Acetamiprid

Data evaluation

Data were evaluated according to the EFSA Aquatic GD (2013) on higher-tier effect assessment on the basis of laboratory toxicity tests with standard and additional species. The RAC for Tier 1, Tier 2A and Tier 2B are provided below.

Standard test species approach (Tier 1)

The Tier 1 data provided above indicate that *Chironomus riparius* is about four orders of magnitude more sensitive than the standard test species *Daphnia magna*, indicating that aquatic insects are the most sensitive group of organisms. Applying an AF of 100 to the lowest toxicity value of 9.3 µg a.s./L for *Chironomus riparius*, the **acute Tier 1 RAC is 0.093 µg a.s./L**.

Geomean approach (Tier 2A)

To obtain a geometric mean for the taxonomic groups *Insecta* as well as *Crustacea*, the available data were compiled and the respective geometric means were calculated. In total, data on 8 insect species and 4 crustacean species were available.

Applying an AF of 100 to the geometric means of 149 mg a.s./L for insects and 225 µg a.s./L for crustaceans, the **acute Tier 2A RAC values are 1.49 µg a.s./L and 2.25 µg a.s./L**, respectively (see table below).

According to the EFSA Aquatic GD (2013), the outcome of the geometric mean approach was tested for a biased data set, as the sensitivity of species differed in minimum of 2 orders of magnitude. The calculated difference between the lowest endpoint of insects (*Chironomus riparius*; EC₅₀ = 9.3 µg a.s./L) and Crustacea (*Crangonyx pseudogracilis*; EC₅₀ = 30.7 µg a.s./L) were less than a factor of 100 below the respective geometric means (16 and 7.3, respectively). Therefore, both geometric mean values are considered reliable and can be used in the risk assessment.

Table A 65: Geomean approach

Group	Species	48h EC ₅₀ (µg a.s./L)	Geometric mean (µg a.s./L)	AF ^A	Tier 2A RAC (µg a.s./L)	Ratio ^B
Insecta	<i>Chironomus riparius</i>	9.3	149	100	1.49	16 (=149/9.3)
	<i>Cloeon dipterum</i>	14.4				
	<i>Corixinae</i>	16.6				
	<i>Phryganeae bipunctata</i>	14.8				
	<i>Notonecta marmoreal</i>	1314				
	<i>Ischnura elegans</i>	1351				
	<i>Chaoborus crystallinus</i>	1998				
	<i>Aeschna</i> sp.	> 2130				
Crustacea	<i>Crangonyx pseudogracilis</i>	30.7	225	100	2.25	7.3 (=225/30.7)
	<i>Asellus aquaticus</i>	39.4				
	<i>Gammarus pulex</i>	115				
	<i>Daphnia magna</i>	17 900				

^AAccording to the EFSA Aquatic GD (2013)

^B geomean divided by lowest endpoint

Species Sensitivity Distribution (SSD) approach (Tier 2B)

Median HC₅ values were derived from an SSD constructed with acute EC₅₀ values using the publicly available software ETX 2.0. The calculated SSD was considered acceptable when the assumption of normal distribution was accepted at the 5%-level ($p \leq 0.05$; Anderson-Darling test for normality).

The available arthropod data were analyzed according to the recommendations provided in the EFSA Aquatic GD (2013).

First approach: As *Chironomus riparius* is about four orders of magnitude more sensitive than *Daphnia magna*, a Species Sensitivity Distribution (SSD) based on the available insect data was constructed in a first step. For hawkers (*Aeschna* sp.), where no clear EC₅₀ value could be obtained in the study by Taylor and Joyce (2015), the EC₅₀ value >2130 µg a.s./L was included. Since hawkers are less sensitive compared to the 7 remaining species, it is considered appropriate according to EFSA Aquatic GD (2013) to include this data point in the construction of an insect SSD. The toxicity data did not fit the curve very well and the Anderson-Darling goodness of fit test was not accepted at the 5% level (p = 0.05). Hence, the data set was considered as not suitable for an SSD.

Second approach: To increase the number of sensitive arthropod species, the EC₅₀ values of *Crangonyx pseudogracilis*, *Gammarus pulex* and *Asellus aquaticus* were included in the construction of the arthropod SSD. Since *Daphnia magna* appeared to be extremely insensitive to Acetamiprid compared to the other crustaceans, this species was excluded from further consideration. Still, the toxicity data did not fit the curve very well and the Anderson-Darling goodness-of-fit test was not accepted at the 5% level (p = 0.05). Hence, also this data set was not considered suitable for an SSD.

Final approach: As the relatively high EC₅₀ values for *Notonecta marmoreal*, *Ischnura elegans*, *Chaoborus crystallinus* and *Aeschna* sp. (EC₅₀ values of 1314, 1351, 1998 and >2130 µg a.s./L, respectively) were considered to be the reason for non-normal distribution of the data set, these species were excluded from further analysis. Therefore, in total 7 different species (representing the most sensitive species) were available to calculate a species sensitivity distribution.

Table A 66: Result of the test of normality of the most sensitive aquatic invertebrates (n = 7) and the calculated LL, median and HL HC₅ values

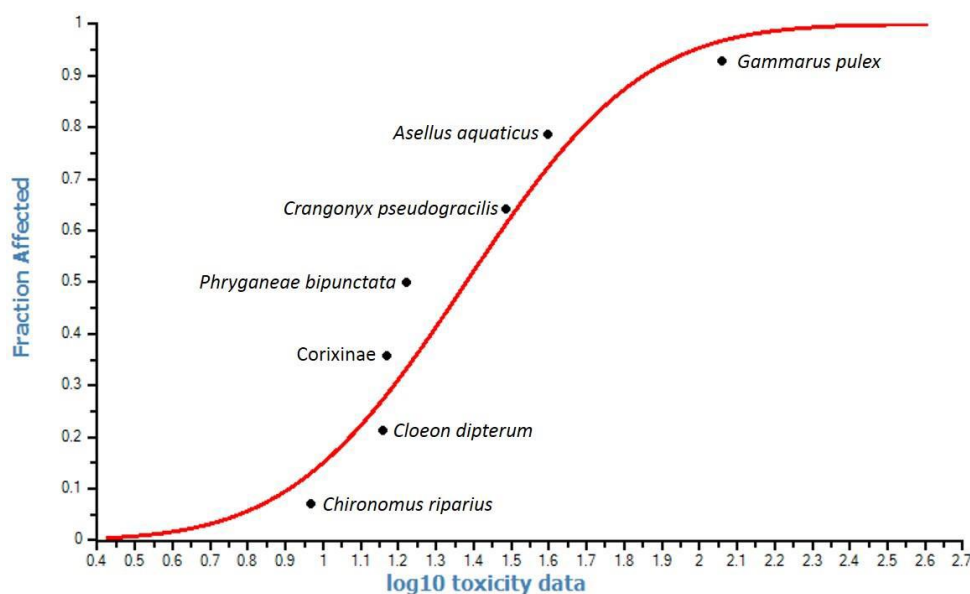
Group	Species	EC ₅₀ (µg a.s./L)	Goodness of fit
Insecta	<i>Chironomus riparius</i>	9.3	Accepted^A (AD value = 0.42)
	<i>Cloeon dipterum</i>	14.4	
	<i>Corixinae</i>	16.6	
	<i>Phryganeae bipunctata</i>	14.8	
Crustacea	<i>Crangonyx pseudogracilis</i>	30.7	
	<i>Asellus aquaticus</i>	39.4	
	<i>Gammarus pulex</i>	115	
LL HC ₅			1.35 µg a.s./L
median HC ₅			5.5 µg a.s./L
UL HC ₅			11.0 µg a.s./L

^A Normal distribution of data are tested by means of Anderson-Darling test for normality (p = 0.05; critical AD value = 0.752)

HC₅ = 5% hazardous concentration; LL = Lower limit; UL = Upper limit

Median HC₅ value is considered relevant according to EFSA Aquatic GD (2013)

According to the EFSA Aquatic GD (2013), a minimum number of 8 species is required to construct a reliable SSD. However, based on the fact that the most sensitive species were used, and the Anderson-Darling goodness-of-fit test was accepted at the 5% level (p = 0.05), the SSD based on 7 different species is considered acceptable for risk assessment. The estimated median HC₅ (hazard concentration) value was 5.55 µg a.s./L (lower limit HC₅ = 1.35 µg a.s./L and upper limit HC₅ = 11.0 µg a.s./L).



Species sensitivity distribution (SSD) based on the most sensitive aquatic invertebrates (n = 7)

For invertebrates, the EFSA Aquatic GD (2013) recommends the calculation of the Tier 2B-RAC based on acute effect data using the median HC₅ and the application of an AF of 3–6. In order to select an appropriate AF, the following aspects were considered:

- The quality of the acute toxicity data used to construct the SSD:** The large data set is comprised of 12 species from which the most sensitive species obtained from standard and additional toxicity studies were used in the SSD. Those species include the potential sensitive taxonomic groups such as *Ephemeroptera*, *Trichoptera* and *Diptera*.
- The lower limit value of the HC₅:** The lower limit HC₅ derived from the curve is less than one-third of the median HC₅. Therefore, a higher AF in the proposed range is needed.
- The lower tier RACs on the basis of standard toxicity data (tier 1), standard and additional toxicity data (geomean approach) and tier 3 data:** The Tier-1 RAC is 0.093 µg a.s./L and the lowest Tier-2A RAC is 1.49 µg a.s./L. Applying an AF of 3 is resulting in a Tier-2B RAC of 2.08 µg a.s./L (based on the selected SSD using the most sensitive species). Therefore, an AF in the lower proposed range is considered acceptable.
- The position of the toxicity data in the lower tail of the SSD (around the HC₅):** Since the toxicity data in the lower tail of the curve are on the right side, the curve is considered conservative. Hence, a lower AF in the proposed range is acceptable.
- The steepness of the SSD curve:** The factor between the lowest and highest EC₅₀ value used to construct the SSD curve is less than 100, indicating a steep SSD curve. Therefore, a higher AF in the proposed range is needed.
- Read-across information for compounds with a similar toxic mode of action:** According to the EFSA Aquatic GD (2013), insects may be more sensitive to neonicotinoid compounds than certain micro-crustacean species. Indeed, insects such as chironomids or mayflies are the most sensitive species. However, the difference between the most sensitive insect *Chironomus riparius* (EC₅₀ = 9.3 µg a.s./L) and the most sensitive crustacean species *Crangonyx pseudogracilis* (EC₅₀ = 30.7 µg a.s./L) is less than a factor of 4, indicating a certain sensitivity of crustacean species as well. Therefore, it is appropriate to combine insect and crustacean data to construct an arthropod SSD. Again, this is in line with the recommendations provided in the EFSA Aquatic GD (2013). Hence, a lower AF in the proposed range is acceptable.
- Considering information on chronic effects:** The acute to chronic ratio (ACR) is lower than a factor of 10 (ACR for *Chironomus riparius*: 9.3 µg a.s./L (EC₅₀) / 5 µg a.s./L (NOEC) = 1.9). In respect of the insensitive *Daphnia magna*, the ACR is also below the trigger value of 10 (ACR for *Daphnia magna*: 17 900 µg a.s./L (EC₅₀) / 5000 µg a.s./L (NOEC) = 3.5). Hence, a lower AF in the proposed range is acceptable.

Taking into account the aspects provided above, an AF of 5 is considered acceptable and highly conservative to derive a protective Tier-2B RAC value for the product MCW 2222 (containing 200 g a.s./L Acetamiprid). Therefore, the relevant Tier-2B RAC value based on the most sensitive species tested is estimated to be 1.11 µg a.s./L.

Conclusion

Based on the evaluation provided above, the following RAC values for the Tier 1, Tier 2A and Tier 2B approaches can be used in the risk assessment for the formulated product MCW-2222 (containing 200 g a.s./L Acetamiprid):

RAC Tier 1: 0.093 µg a.s./L (based on an AF = 100)

RAC Tier 2A (geomean Insecta): 1.49 µg a.s./L (based on an AF = 100)

RAC Tier 2A (geomean Crustacea): 2.25 µg a.s./L (based on an AF = 100)

RAC Tier 2B (SSD of most sensitive species): 1.11 µg a.s./L (based on an AF = 5)

The relevant RAC for the risk assessment is the **RAC Tier 2B** of **1.11 µg a.s./L** as based on a species sensitivity distribution (SSD) approach using the most sensitive species and an assessment factor (AF) of 5.

A 2.3 KCP 10.3 Effects on arthropods

A 2.3.1 KCP 10.3.1 Effects on bees

A 2.3.1.1 KCP 10.3.1.1 Acute toxicity to bees

A 2.3.1.1.1 KCP 10.3.1.1.1/01 Acute oral toxicity to bees

Comments of zRMS:	<p>The study has been already evaluated and accepted by the zRMS in the course of the first zonal evaluation of CA3573 (formerly MCW-2222) finalised in April 2018.</p> <p>As the test guideline has not changed since that time, re-evaluation of the study following renewal of acetamiprid was not necessary and presented below conclusions were taken from the Core Assessment, Part B, Section 6 of April 2018.</p> <p>The study was performed in line with OECD 213 and 214 and met all validity criteria. Following endpoints were agreed:</p> <p>48 h oral LD₅₀ = 51.3 µg product/bee (corresponding to 9.1 µg a.s./bee)</p> <p>48 h contact LD₅₀ = 21.2 µg product/bee (corresponding to 3.8 µg a.s./bee)</p>
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Data point: KCP 10.3.1.1.1/01 & KCP 10.3.1.1.2/01

Report: Acute toxicity of MCW-2222 to the honeybee *Apis mellifera* L. under laboratory conditions, Franke, M., 2014, R-33834, 14 10 48 076 B

Guideline(s): OECD 213/214 (1998)

Deviations: No

GLP: Yes, certified laboratory

Acceptability: Yes, study considered acceptable

Duplication: Not applicable
(if vertebrate study)

Executive Summary

In a 48 hour acute oral and contact toxicity study, adult worker honeybees (*Apis mellifera* L.) were exposed to MCW-2222 at nominal doses of 0, 2.6, 5.7, 12.4, 27.3 and 60.0 µg test item/bee for both, contact and oral tests. Mortality and unusual behaviour were observed and LD₅₀-values were determined.

Based on the effective food consumption the 48 h LD₅₀ for oral toxicity was calculated to be 21.2 µg test item/bee for the test item MCW-2222 (corresponding to 3.8 µg a.s./bee). The 48 h LD₅₀ for contact toxicity was calculated to be 51.3 µg test item/bee for the test item MCW-2222 (corresponding to 9.1 µg a.s./bee).

Materials and methods

Materials

Test item	MCW-2222
Batch #	611-280413-01
Content of active substance	Acetamiprid 200 g/L (nominal); 202.7 g/L (analysed)
Description	Yellowish liquid
Control (contact)	Deionised water
Vehicle control (contact)	Deionised water + 1.0 % v/v Tween®80
Control (oral)	50% (w/v) sucrose solution
Toxic reference	Dimethoate EC 400 (BAS 152 11 l)
Test organism	
Species	<i>Apis mellifera iberica</i> , adult worker bees
Source	Joaquin Cordero (Beekeeper), Paseo de Colón No. 19, 41370 Cazalla (Seville), Spain
Study design and methods	
Test duration	48 hours
Experimental dates	31 March to 02 April 2014
Test doses (nominal)	2.6, 5.7, 12.4, 27.3 and 60.0 µg test item/bee for both, contact and oral tests.
Test doses (actually consumed)	2.6, 5.6, 12.4, 27.3 and 55.2 µg test item/bee (oral test)
Test units	For the observation of the bees disposable cages of cardboard (95 mm x 50 mm x 65 mm) with holes in the bottom for ventilation and a glass plate in front were used.
Group size/replicates	30 bees per dose; 10 in each of 3 replicates
Experimental treatments (oral)	Oral treatment was done by administration of the test item dispersed in a 50% (w/v) sucrose solution. Bees exposed to the oral dose starved for approximately 1 h before dosing.
Experimental treatments (contact)	For contact exposure a 2 µL droplet of the test solution was applied topically to the dorsal surface of the thorax after a light anaesthesia.
Acclimatisation	The bees were transferred immediately after collection at the hive to the laboratory and acclimatised for 1 h.
Environmental conditions	
Temperature	23.4 - 27.0 °C
Photoperiod	Continuous darkness
Relative humidity	50 - 68%

Biological observations

Observations were made on mortality as well as the occurrence and type of sub-lethal effects at approximately 4, 24 and, 48 hours of exposure.

Statistics

The 48 h LD₅₀ values were calculated by probit analysis. Statistical significance was determined by Fishers's exact test with Bonferroni correction. Mortalities of the test and reference item were corrected according to Abbott.

Results and discussion

Biological results – contact toxicity

Biological results on mortality are given in the table below.

Table A 67: Honeybee mortality after contact application of MCW-2222

Treatment group	Dosage applied	Mean mortality [%]		
		4 h	24 h	48 h
Control	-	0.0	0.0	0.0
Tween control	-	0.0	0.0	0.0
Test substance [µg test item/bee]	60.0	56.7	80.0	80.0
	27.3	53.3	76.7	76.7
	12.4	13.3	23.3	23.3
	5.7	3.3	6.7	6.7
	2.6	0.0	0.0	0.0
Toxic reference	0.251	0.0	73.3	80.0

[µg a.s./bee]	0.175	0.0	30.0	40.0
	0.123	0.0	10.0	10.0
	0.086	0.0	0.0	3.3

- = not applicable

Behavioural abnormalities occurred predominantly at the 4 hour assessment and thereof at the higher dose rates. After 4 hours, honeybees treated with 60.0 and 27.3 µg test item/bee revealed abnormal behaviour that amounted to 9 out of 13 bees and 7 out of 14 bees, respectively. These effects are comprised by symptoms of moribundity and impaired locomotion. Lower dose rates revealed only slight effects on behaviour of surviving bee. After 24 h and 48 h, no or only slight behavioural abnormalities occurred at all tested dose rates.

Biological results – oral toxicity

Biological results on mortality are given in the table below.

Behavioural abnormalities occurred only at the 4 h assessment and thereof on a significant level at the highest dose rate. After 4 h, 17 out of 30 bees at the dose rates of 55.2 µg consumed test item/bee showed abnormal behaviour that comprised by a majority of moribund symptoms accounted for 15 out of 17 bees and impaired locomotion of 2 out of 17 bees. Some minor effects on behaviour occurred after oral administration of 27.3 µg test item/bee; thus, 4 out of 29 bees behaved moribund after 4 h. Lower dose rates revealed no behavioural abnormalities of bees treated with MCW-2222. Moreover, in the further progress of the oral toxicity test no abnormal occurred at all tested dose rates.

Table A 68: Cumulative honeybee mortality after oral application of MCW-2222

Treatment group	Effective dosage	Mean mortality [%]		
		4 h	24 h	48 h
Control (50 w/v sucrose)	-	0.0	0.0	0.0
Test substance [µg test item/bee]	55.2	0.0	50.0	53.3
	27.3	3.3	16.7	16.7
	12.4	0.0	0.0	0.0
	5.7	0.0	0.0	0.0
	2.6	0.0	0.0	0.0
Toxic reference [µg a.s./bee]	0.251	3.3	90.0	90.0
	0.150	3.3	50.0	53.3
	0.090	0.0	16.7	16.7
	0.054	0.0	0.0	10.0

- = not applicable

Table A 69: Endpoints for contact and oral toxicity after 48 hours

Treatment	Reference unit of endpoint	Contact toxicity 48 hours	Oral toxicity ^{a)} 48 hours
Test item	LD ₅₀ [µg test item/bee] (lower and upper 95 %-CL)	21.2 (16.8 – 26.7)	51.3 (40.6 – 65.0)
	LD ₅₀ [µg a.s./bee] (lower and upper 95 %-CL)	3.8 (3.0 – 4.8)	9.1 (7.2 – 11.6)
Reference item	LD ₅₀ [µg a.s./bee] (lower and upper 95 %-CL)	0.188 (0.169 – 0.210)	0.136 (0.116 – 0.158)

^{a)}Values refer to consumed dosages

Validity criteria

As shown in the following table, all validity criteria were met.

Table A 70: Validity criteria

Validity criteria according to OECD 213 oral (1998)	Observed in study
Mortality in control ≤ 10%	0%
The 24 h LD ₅₀ value for the reference substance should be between 0.10-0.30 µg a.s./bee	0.140 µg a.s./bee
Validity criteria according to OECD 214 contact (1998)	Observed in study
Mortality in water and vehicle controls ≤ 10%	0%
The 24 h LD ₅₀ value for the reference substance should be between 0.10-0.35 µg a.s./bee	0.203 µg a.s./bee

Conclusion

In a 48 hour acute oral and contact toxicity study, adult worker honeybees (*Apis mellifera* L.) were exposed to MCW-2222 at nominal concentrations of 0, 2.6, 5.7, 12.4, 27.3 and 60.0 µg test item/bee for both, contact and oral tests. Mortality was the observed response variable and LD₅₀-values were determined. The 48 h LD₅₀ for oral toxicity was calculated to be 21.2 µg test item/bee (for the test item MCW-2222 (corresponding to 3.8 µg a.s./bee). The 48 h LD₅₀ for oral toxicity was calculated to be 51.3 µg test item/bee for the test item MCW-2222 (corresponding to 9.1 µg a.s./bee).

A 2.3.1.1.2 KCP 10.3.1.1.2/01 Acute contact toxicity to bees

Comments of zRMS:	See KCP 10.3.1.1.1/01 above.
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Data point:	KCP 10.3.1.1.1/01 & KCP 10.3.1.1.2/01 Please see A 2.3.1.1.1 for full summary
Report:	Acute toxicity of MCW-2222 to the honeybee <i>Apis mellifera</i> L. under laboratory conditions, Franke, M., 2014, R-33834, 14 10 48 076 B
Guideline(s):	OECD 213/214 (1998)
Deviations:	No
GLP:	Yes, certified laboratory
Acceptability:	Yes, study considered acceptable
Duplication: (if vertebrate study)	Not applicable

A 2.3.1.1.3 KCP 10.3.1.2.1/01 Acute oral toxicity to bumble bees

Comments of zRMS:	<p>The study has been already evaluated and accepted by the zRMS in the course of the first zonal evaluation of CA3573 (formerly MCW-2222) finalised in April 2018.</p> <p>The test design was based on OECD 213 and 214 as well as indications of EFSA (2013), as validated method for evaluation of toxicity to bumblebees was not available at the time of the study performance. Since that time the validated guidelines OECD 246 and 247 became available and for purposes of re-evaluation of CA3573, the study by Röhlig (2014a) has been checked for compliance with the respective guideline.</p> <p>In general, the test conditions, replication, number of doses, administration of the test item, feeding etc. were in line with recommendations of OECD 246 and 247. All validity criteria of the current guidelines were met.</p> <p>Following deviations were noted:</p> <ol style="list-style-type: none"> 1. Bumblebees in the contact test were not kept individually (3 replicates with 10 bumblebees were used). In general, keeping of bumblebees in groups of 10 is not expected to have impact on the study, as the test animals were observed during the study and behaviour described in the guideline (hierarchy fights) was not observed. In addition to that, no mortality was observed in control groups. 2. Bumblebees were not individually weighed. Lack of individual weighing is also not considered to have impact on the test results.
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	<p>According to the study report, the weight of individual used for the test was in range 165-200 mg, so it was homogenous, although no information on determination of the weight is given.</p> <p>3. Acclimatisation time of 3 hours was shorter than 8 hours recommended by the guidelines. Although the acclimatisation period was shorter than recommended, only healthy bumblebees behaving normally were used for the test. Taking this into account it is not expected that this deviation would have significant impact on the test results.</p> <p>4. The feeding solutions used in oral test and chemical solutions used in contact test were not analysed during the test. Lack of chemical analyses means that the actual concentration of the test item in the solutions is not known. However, acetamiprid was confirmed to be stable in aqueous sucrose solution in chronic toxicity study performed with bees (see summary of Dressler, 2019 in KCP 10.3.1.2/01 below) and it is not expected that its behaviour would be different in study performed with bumblebees. Therefore, in opinion of the zRMS lack of analytical measurements in case of stable active substance such as acetamiprid is not a deficiency which should invalidate the test.</p> <p>5. From the description available in the study report it seems that bumblebees were collected from the single colony (3 colonies should be used according to the guidelines). In general, it is not possible to conclude how this deviation would impact the test results, as the test guideline does not specify why one colony is not sufficient to perform a dose-response design test, although from description in the test guideline it seems that single colony is sufficient to perform a limit test with more individuals (50) comparing to dose-response test (30). In opinion of the zRMS use of single colony had no impact on the test results, as the test system was demonstrated to be sufficiently sensitive (mortality in toxic reference groups in range of 50-100%, with exception of the lowest treatment group), while no lethal and sub-lethal effects were seen in the control groups.</p> <p>Overall, despite listed above deviation the study is considered acceptable with following endpoints:</p> <p>48 h oral LD₅₀ = 136.0 µg product/bee (corresponding to 24.3 µg a.s./bee) 48 h contact LD₅₀ >1122.0 µg product/bee (corresponding to >200.0 µg a.s./bee)</p>
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Data point:	KCP 10.3.1.2.1/01 & KCP 10.3.1.2.2/01
Report:	Acute toxicity of MCW-2222 to the bumblebee <i>Bombus terrestris</i> L. under laboratory conditions, Röhlig, U., 2014, R-33837, 14 10 48 024 A
Guideline(s):	OECD 213 (1998), OECD 214 (1998), EFSA (2013); 11(7):3295
Deviations:	<p>Yes, minor deviations to the testing guidance updated in 2017 OECD 246/247 (2017)</p> <p>Bumblebees were not individually weighed and the acclimatisation period was shorter (3 h) as recommended (8 h). Bumblebees (contact toxicity) were not held individually in contact test but only in the feeding test. Stock solutions were not analytically verified</p> <p>Since deviations are not considered to have an impact on the study outcome and all validity criteria were fulfilled, the study is regarded as valid.</p>
GLP:	Yes, certified laboratory
Acceptability:	Yes, study considered acceptable
Duplication: (if vertebrate study)	Not applicable

Executive Summary

The acute contact and oral toxicity of the test item MCW-2222 was tested on bumblebees under laboratory conditions for a period of 48 hours. Mortality and unusual behaviour were observed and LD₅₀ values were determined. The LD₅₀ for contact exposure (48 h) was estimated to be > 200 µg a.s./bumblebee (corresponding to > 1122 µg test item/bumblebee). The LD₅₀ for oral exposure (48 h) was calculated to be 24.3 µg a.s./bumblebee (corresponding to 136 µg test item/bumblebee).

Materials and methods

Materials

Test item	MCW-2222
Batch #	611-280413-01
Content of active substance	Acetamiprid 200 g/L (nominal); 202.7 g/L (analysed)
Description	Yellowish liquid
Control (contact)	Deionised water
Vehicle control (contact)	Acetone 1.0 % v/v Tween® 80 solution
Control (oral)	50% (w/v) sucrose solution
Toxic reference	A toxic reference study with Dimethoate EC 400 at rates of 6.7, 13.3, 26.7 and 53.4 µg reference test item/bee for the contact assessment (comprising 3 replicates of 10 bumblebees) and 0.8, 1.7, 3.3 and 6.7 µg reference test item/bee for the oral assessment (comprising 30 replicates of a single bumblebee) was evaluated in parallel.

Test organism

Species	<i>Bombus terrestris</i> L., young adult worker bees
Source	Katz Biotech AG, An der Birkenpfuhlheide 10, 15837 Baruth, Germany

Study design and methods

Test duration	48 hours
Experimental dates	05 March to 08 March 2014
Test doses (contact; nominal)	70, 140, 280, 561 and 1122 µg test item/bumblebee
Test doses (oral; nominal)	35, 70, 140, 281 and 561 µg test item/bumblebee
Test doses (oral consumed)	33, 68, 136, 267 and 532 µg test item/bumblebee
Test units contact	For the observation of the bees disposable cages of cardboard (95 mm x 50 mm x 65 mm) with holes in the bottom for ventilation and a glass plate in front were used.

Test units oral

	Nicot hair roller cages (part of the Nicot queen bee rearing system) consisting of socket, cup holder, cell cups and hair roller, a block of 15 hair roller cages was mounted on an acryl-glass plate.
Group size/replicates contact	30 bees per dose; 10 in each of 3 replicates per dose level
Group size/replicates oral	30 bees per dose; 1 in each of 30 replicates per dose level
Experimental treatments (oral)	Oral treatment was done by administration of the test item dispersed in a 50% (w/v) sucrose solution.
Experimental treatments (contact)	For contact exposure a 5 µL droplet of the test solution was applied topically to the dorsal surface of the thorax after a light anaesthesia with CO ₂ .

Acclimatisation

The bumble bees were transferred immediately after collection to the laboratory. After transfer into the test units they had time for acclimatisation to the test room conditions for about 1 hour (contact test) and an additional starving period of 3 hours in the oral toxicity test before application of the treatments.

Environmental conditions

Temperature	24 - 27 °C
Photoperiod	Continuous darkness
Relative humidity	59 -62%

Biological observations

Observations were made on mortality as well as the occurrence and type of sub-lethal effects at approximately 4, 24 and, 48 hours of exposure.

Statistics

The 48 h LD₅₀ values were calculated by probit analysis. Statistical significance was determined by Fishers's exact test with Bonferroni correction. Mortalities of the test and reference item were corrected according to Abbott.

Results and discussion

Biological results – contact toxicity

Biological results on mortality are given in the table below.

Effects on behaviour of surviving bumblebees occurred at the tested dose rates of 140, 280, 561 and 1122 µg test item/bumblebee at the 4 h assessment. No effects on behaviour of surviving bumblebees occurred at any tested dose rates at the 24 h and 48 h assessment when compared to the control.

Table A 71: Bumblebee mortality after contact application of MCW-2222

Treatment group	Dosage applied	Mean mortality [%]		
		4 h	24 h	48 h
Control	-	0.0	0.0	0.0
Tween control	-	0.0	0.0	0.0
Test substance [µg test item/bee]	1122	3.3	33.3*	36.7*
	561	0.0	13.3	16.7
	280	0.0	13.3	16.7
	140	0.0	3.3	3.3
	70	0.0	3.3	3.3
Toxic reference [µg a.s./bee]	20.0	0.0	100*	100*
	10.0	0.0	93.3*	93.3*
	5.0	0.0	46.7*	50.0*
	2.5	0.0	0.0	3.3

- = not applicable

* Significant difference in pairwise comparison between treatment and sucrose control (Fisher's Exact Binomial Test with Bonferroni Correction; $\alpha=0.05$; one sided greater)

Biological results – oral toxicity

Biological results on mortality are given in the table below.

No behavioural abnormalities of surviving bumblebees occurred in the 6.25 µg a.s./bumblebee dose rate throughout the oral toxicity test. Based on the effective uptake the LD₅₀ (48 h) was calculated to be 24.3 µg a.s./bumblebee (corresponding to 136 µg test item/bumblebee).

Table A 72: Cumulative honeybee mortality after oral application of MCW-2222

Treatment group	Effective dosage	Mean Mortality [%]	
		24 h	48 h
Control (50 w/v sucrose)	-	0.0	0.0
Test substance [µg test item/bee]	532	90.0*	90.0*
	267	73.3*	73.3*
	136	43.3*	46.7*
	68	26.7*	30.0*
	33	0.0	0.0
Toxic reference [µg a.s./bee]	2.4	83.3*	86.7*
	1.2	70.0*	73.3*
	0.6	56.7*	56.7*
	0.3	0.0	0.0

- = not applicable

* Significant difference in pairwise comparison between treatment and sucrose control (Fisher's Exact Binomial Test with Bonferroni Correction; $\alpha=0.05$; one sided greater)

Table A 73: Endpoints for contact and oral toxicity after 48 hours

Treatment	Reference unit of endpoint	Contact toxicity 48 hours	Oral toxicity ^{a)} 48 hours
Test item	LD ₅₀ [µg test item/bee] (lower and upper 95 %-CL)	> 1122	136 (103-180)
	LD ₅₀ [µg a.s./bee] (lower and upper 95 %-CL)	> 200	24.3 (18.4 - 32.0)
Reference item	LD ₅₀ [µg a.s./bee] (lower and upper 95 %-CL)	5.14 (4.44 – 5.95)	0.54 (0.30 – 0.98)

^{a)}Values refer to consumed dosages

Validity criteria

As shown in the following table, all validity criteria were met.

Table A 74: Validity criteria

Validity criteria according to OECD 246 oral (2017)	Observed in study
Mortality in control ≤ 10%	0%
Mortality in the toxic reference substance group should be ≥ 50 % at the end of the test.	(up to) 87%
Validity criteria according to OECD 247 contact (2017)	Observed in study
Mortality in water and vehicle controls ≤ 10%	0%
Mortality in the toxic reference substance group should be ≥ 50 % at the end of the test.	(up to) 100%

Conclusion

The acute contact and oral toxicity of the test item MCW-2222 was tested on bumblebees under laboratory conditions for a period of 48 hours. Mortality and unusual behaviour were observed and LD₅₀-values were determined. The LD₅₀ for contact exposure (48 h) was estimated to be > 200 µg a.s./bumblebee (corresponding to > 1122 µg test item/bumblebee). The LD₅₀ for oral exposure (48 h) was calculated to be 24.3 µg a.s./bumblebee (corresponding to 136 µg test item/bumblebee).

A 2.3.1.1.4 KCP 10.3.1.2.2/01 Acute contact toxicity to bumble bees

Comments of zRMS:	See KCP 10.3.1.2.1/01 above.
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Data point:	KCP 10.3.1.2.1/01 & KCP 10.3.1.2.2/01 Please see A 2.3.1.1.3 for full summary
Report:	Acute toxicity of MCW-2222 to the bumblebee <i>Bombus terrestris</i> L. under laboratory conditions, Röhlig, U., 2014, R-33837, 14 10 48 024 A
Guideline(s):	OECD 213 (1998), OECD 214 (1998), EFSA (2013); 11(7):3295
Deviations:	Yes: to updated testing guidance OECD 246/247 (2017) Bumblebees were not individually weighed and the acclimatisation period

was shorter (3 h) as recommended (8 h). Bumblebees (contact toxicity) were not held individually in contact test but only in the feeding test. Stock solutions were not analytically verified

Since deviations are not considered to have an impact on the study outcome and all validity criteria were fulfilled, the study is regarded as valid.

GLP: Yes, certified laboratory
Acceptability: Yes, study considered acceptable
Duplication: Not applicable
(if vertebrate study)

A 2.3.1.2 KCP 10.3.1.2/01 Chronic toxicity to bees

Comments of zRMS:	<p>The study has been already evaluated and accepted by the zRMS in the course of the first zonal evaluation of CA3573 (formerly MCW-2222) finalised in April 2018.</p> <p>It is noted that no validated guideline on chronic bee toxicity testing was available when the study has been performed, so the test protocol was based on indications available in several publications and ring-testing. Since that time the validated guideline OECD 245 became available and for purposes of re-evaluation of CA3573, the study by Kleebaum (2014a) has been checked for compliance with the respective guideline.</p> <p>In general, significant parts of the test design are in line with OECD 245, but several deviations were noted as listed in the title table below.</p> <p>Following deviations are considered to have no significant impact on the test results:</p> <ol style="list-style-type: none"> <u>20 bees in replicate instead of 10 recommended by the guideline.</u> Bees are highly social animals, so it is not expected that presence of 20 instead of 10 bees would increase the mortality rate, so risk of e.g. hierarchy fights is negligible. Furthermore, during the study bees behaviour is monitored and for this reason potential effects of the overcrowding would be captured during observations. Furthermore, validity criteria were met and with 20 bees per replicate and 3 replicates more bees were tested (60 vs. 30 recommended by the guideline). <u>The feeding solutions were not analysed during the test.</u> Lack of chemical analyses means that the actual concentration of the test item in the solutions is not known. However, acetamiprid was confirmed to be stable in aqueous sucrose solution in chronic toxicity study by Dressler, 2019 (see KCP 10.3.1.2/01 above) and it is not expected that its behaviour would be different in another chronic study, where the test item was also administered in aqueous sucrose solution. Therefore, in opinion of the zRMS lack of analytical measurements in case of stable active substance such as acetamiprid is not a deficiency which should invalidate the test. <u>The minimum RH dropped slightly below 50%.</u> As all validity criteria were met, this deviation is considered to have no impact on the test results. <p>Following deviations are considered to have potentially significant impact on the test results:</p> <ol style="list-style-type: none"> <u>Maximum age of worker bees was 3 days (2 days are recommended by the guideline).</u> In general, it is not known to conclude whether bees 1 day older would be significantly less sensitive. Nevertheless, in the course of the ring testing and validation procedure it was decided that 2 day old worker bees are most suitable at the test initiation. Therefore, use of older bees could have some impact on obtained results. <u>Evaporation of the test solution from feeders was not determined.</u>
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	<p>Although acetamiprid is stable under test conditions, evaporation is different phenomenon which may reduce the actual exposure of bees to the test item. In the study by Dressler, 2019 (see KCP 10.3.1.2/01 above) significant evaporation was observed, leading to lower test item intake and in consequence – to lower endpoints. As the extent of evaporation in the study by Kleebaum (2014a) is not known, correction of the endpoints is not possible, but based on the available information it may be expected that they would be lower. Taking this into account this deviation has significant impact on the test results.</p> <p>Overall, the study could be accepted in terms of the design and conditions, but due to lack of determination of evaporation of the test solutions, derived endpoints are considered not reliable and cannot be used in the risk assessment.</p> <p>Nevertheless, new study performed fully in line with OECD 245 has been submitted (Dresser, 2019) and its results supersede endpoints derived from Kleebaum (2014a).</p> <p>The summary below has been struck through in order to make it clear that the test is not valid.</p>
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Data point:	KCP 10.3.1.2/01
Report	Chronic toxicity of MCW-2222 to honeybee (<i>Apis mellifera</i> L.) under laboratory conditions, Kleebaum, K., 2015a; R-33835, 14 10 48 077 B
Guideline(s):	DECOURTYE <i>et al.</i> (2005), SUCHAIL <i>et al.</i> (2001), AFPP method CEB No. 230 (2012) and current ring test protocol of the AG-Bienenschutz (2013)
Deviations:	<p>Yes</p> <p>Major deviations to current guideline (OECD Guideline for the testing of chemicals No. 245):</p> <ul style="list-style-type: none"> • Maximum age of workers bees was 3 days instead of 2 days • Replicates contained 20 bees instead of 10 bees • Evaporation of test solution from feeders was not determined • No analytical verification of the test substance was conducted <p>Minor deviation:</p> <ul style="list-style-type: none"> • Relative humidity during exposure was 46.2 – 60.0 % instead of 50 – 70 %
GLP:	Yes, certified laboratory
Acceptability:	Yes, study considered acceptable
Duplication (if vertebrate study)	Not applicable

Executive Summary

In a 10 day chronic toxicity feeding study with honeybees (*Apis mellifera iberica*) were exposed to MCW-2222. The toxicity of the test item was determined at total doses of 10.000, 3.600, 1.296, 0.467 and 0.168 µg a.s./larva (corresponding to 56.1, 20.2, 7.3, 2.6 and 0.9 µg test item/larva). The concentrations of test item in the diet were 0.257, 0.092, 0.033, 0.012 and 0.004 g a.s./kg food. The LD₅₀ was determined to be 3.994 µg consumed a.s./bee/day. This corresponds to a LC₅₀ of 0.100 g a.s./kg food. The NOED was determined to be 0.546 µg consumed a.s./bee/day, and the NOEC was 0.012 g a.s./kg food, respectively.

Materials and methods

Materials

Test item	MCW-2222
Batch #	611-280413-01
Content of active substance	Acetamiprid 200 g/L (nominal); 202.7 g/L (analysed)
Description	Yellowish liquid
Control	Untreated diet

Toxic reference	Dimethoate technical (99.8%)
Test organism	
Species	<i>Apis mellifera iberica</i> , young worker bees (2 – 3 days old)
Source	Joaquin Cordero (Beekeeper), Paseo de Colón No. 19, 41370 Cazalla (Seville), Spain
Study design and methods	
Test duration	10 days of exposure
Experimental dates	09 May to 30 June 2014
Test doses	10.000, 3.600, 1.296, 0.467 and 0.168 µg a.s./bee (corresponding to 56.1, 20.2, 7.3, 2.6 and 0.9 µg test item/bee)
Test units	Aluminium cages with the dimensions: 20 cm (width) x 15 cm (height) x 10 cm (depth); with holes in the lateral walls for sufficient air supply and ventilation and two glass plates (one in front and one in the back) for observation of the bees.
Group size/replicates contact	60 bees per treatment (1 control, 5 test item dosages, 1 reference item); 20 in each of 3 replicates per treatment
Acclimatisation	Brood combs with capped cells were taken from outside hives and different colonies (D1 -3). These frames were placed without adult worker bees in a “five comb hive body” and were incubated under controlled environmental conditions in an incubator at 33 ± 2 °C and relative humidity of 70 ± 10 % at darkness for a maximum period about 24 hours (until D -2). Afterwards, the newly hatched worker bees were transferred into the test cages in groups of 20 bees/cage. For the following two days (until D 0), bees were held in the test cages at 33 ± 2 °C and 50 ± 10 % rH and provided with sugar solution and pollen food. Moribund and dead bees were rejected and replaced by healthy bees before starting the test.
Environmental conditions	
Temperature:	33.3 – 35.0 °C
Photoperiod:	Darkness (except assessments)
Relative humidity	46.2 – 60.0 %
Biological observations	
Number of dead bees per replicate was observed daily from D 0 to D 10. Number of affected bees (healthy/normal or affected e.g. differences in activity (immobile or hyperactive), moribund, cramping, or any abnormal amount/colour of excretions) per replicate was assessed from D 0 to D 10 once per day.	
Statistics	
For statistical calculation of the mortality results and of the NOEC/NOED the Fisher’s Exact Binomial test (with Bonferroni Correction) was used. The accepted significance level was $p \leq 0.05$ (one-sided greater). To calculate the LC/LD ₅₀ Probit or Weibull analysis were conducted. Mortalities of the test and reference item were corrected according to Abbott.	
Results and discussion	
Biological results	
Biological results on mortality are given in the table below.	
In the course of the study several bees were described as affected in terms of moving uncoordinated. The highest numbers of affected bees were observed in the two highest test item dosages (14.002 and 3.885 µg consumed a.s./bee/day).	
On the last day of the test the two remaining bees in the highest test item dosage (14.0 µg consumed a.s./bee/day) were described as affected, as well as 18.2 % of the remaining bees in the second highest test item dosage (3.88 µg consumed a.s./bee/day), 9.1 % in the middle test item dosage (1.4 µg consumed a.s./bee/day) and 7.3 % in the second lowest dosage (0.5 µg a.s./bee/day).	

Table A 75: Mean mortality and behaviour of bees in the chronic toxicity feeding test with MCW-2222 after 10 days

Treatment group	Dosage of a.s. [µg/bee/day]		Concentration [g a.s./ kg food]	D10		
				Mean mortality [%] ¹		Mean BA [%]
	nominal	consumed		Absolute	Corrected	
Control	-	-	-	1.7	-	0.0
Test substance [µg test item/bee]	10.000	14.002	0.257	96.7*	96.7	100.0
	3.600	3.885	0.092	26.7*	25.4	18.2
	1.296	1.412	0.033	26.7*	25.4	9.1
	0.467	0.546	0.012	8.3	6.8	7.3
	0.168	0.179	0.004	1.7*	0.1	0.0
Toxic reference [µg a.s./bee]	27.326	24.045	0.702	95.0*	94.9	0.0
	16.395	11.197	0.421	40.0*	39.0	0.0
	9.838	8.390	0.253	15.0*	13.6	0.0
	5.902	4.703	0.152	1.7	0.1	0.0

¹) Results are averages based on 3 replicates, containing 20 bees each;

corrected: corrected mortality (according to SCHNEIDER-ORELLI 1947), negative values are treated as “0”

* Statistically significant difference in pairwise comparison between treatment and untreated control (Fisher`s Exact Binominal Test with Bonferroni Correction; $\alpha=0.05$; one sided greater)

- not applicable

Table A 76: Endpoints after 96 and 120 hours of exposure

Treatment	Endpoints	Day 10
Test item doses	LD ₅₀ [µg consumed a.s./bee/day] ¹	3.994
	NOED [µg consumed a.s./bee/day] ³	0.546
Test item concentrations	LC ₅₀ [g a.s./kg food] ²	0.100
	NOEC [g a.s./kg food] ³	0.012
Reference item	LD ₅₀ [ng consumed a.s./bee/day]	12.661
	LC ₅₀ [mg a.s./kg food]	0.423

¹ Median lethal dose was calculated by using Probit analysis (linear max. likelihood regression)

² Median lethal concentration was calculated by using Weibull analysis (linear max. likelihood regression)

³ Fisher`s Exact Binominal Test with Bonferroni Correction; $\alpha=0.05$; one sided greater

Validity criteria

As shown in the following table, all validity criteria were met.

Table A 77: Validity criteria

Validity criteria according to OECD 245 (2017)	Observed in study
Mortality in control $\leq 15\%$	1.7%
Mortality in the toxic reference substance group should be $\geq 50\%$ at test end	94.9

Conclusion

In a 10 day chronic toxicity feeding study with MCW-2222, the LD₅₀ was determined to be 3.994 µg consumed a.s./bee/day. This corresponds to a LC₅₀ of 0.100 g a.s./kg food. The NOED was determined to be 0.546 µg consumed a.s./bee/day, and the NOEC was 0.012 g a.s./kg food, respectively.

A 2.3.1.3 KCP 10.3.1.2/02 Chronic toxicity to bees

Comments of zRMS:	<p>The study on chronic toxicity of CA3573 to bees (Dressler, 2019) was evaluated by zRMS in 2021 for authorisation of the product CA3573.</p> <p>The study was carried out in line with OECD 245 with no deviations.</p> <p>All validity criteria were met and the study is considered acceptable with following endpoints relevant for the risk assessment:</p> <p>LDD₅₀ = 21.8 µg product/bee/day (corresponding to 3.71 µg a.s./bee/day)</p> <p>NOEDD = 9.04 µg product/bee/day (corresponding to 1.54 µg a.s./bee/day)</p>
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Reference:	KCP 10.3.1.2/01
Report	Chronic oral toxicity of CA3573 Acetamiprid 200 SL (Carnadine) to the honey bee <i>Apis mellifera</i> L. under laboratory conditions, Dressler, K., 2019, 19 48 BAC 0028
Guideline(s):	OECD 245 (2017)
Deviations:	None
GLP:	Yes, certified laboratory
Acceptability:	Yes

Executive Summary

In a 10 day chronic toxicity feeding study with CA3573 Acetamiprid 200 SL (Carnadine), young adult honeybees (*Apis mellifera* subspecies Buckfast) were exposed to nominal doses of 47.5, 23.8, 11.9, 5.94 and 2.97 µg test item/bee/day (equivalent to 8.08, 4.04, 2.02, 1.01 and 0.505 µg a.s./bee/day) for 10 days. Feeding tubes were replaced daily and effective consumption was determined. Possible evaporation loss from the feeders was determined in similar test units but without bees. Based on the effective consumption and evaporation, effective doses were equivalent to 6.70, 3.14, 1.54, 0.833 and 0.397 µg a.s./bee/day. The LDD₅₀ was determined to be 21.8 µg test item/bee/day (equivalent to 3.71 µg a.s./bee/day) and the LC₅₀ to be 700 mg test item/kg food (equivalent to 119 mg a.s./kg food), respectively. Nominal values were corrected for evaporation and consumed amounts of food.

The NOEDD was determined to be 9.04 µg test item/bee/day (equivalent to 1.54 µg a.s./bee/day) and the NOEC to be 303 mg test item/kg food (equivalent to 51.4 mg a.s./kg food), respectively.

The LDD₅₀ was determined to be 21.8 µg test item/bee/day (equivalent to 3.71 µg a.s./bee/day) and the LC₅₀ to be 700 mg test item/kg food (equivalent to 119 mg a.s./kg food), respectively.

The NOEDD was determined to be 9.04 µg test item/bee/day (equivalent to 1.54 µg a.s./bee/day) and the NOEC to be 303 mg test item/kg food (equivalent to 51.4 mg a.s./kg food), respectively.

The recovery rate of acetamiprid ranged between 90% and 96% in samples of the highest test item concentration and between 90% and 98% in samples of the lowest test item concentration.

Materials and methods

Materials

Test item	CA3573 Acetamiprid 200 SL (Carnadine)
Batch #	981101035
Content of active substance	Acetamiprid 200 g/L (nominal); 195.5 g/L (analysed)
Description	Clear yellow-brown liquid
Control	Untreated diet
Toxic reference	Dimethoate technical 400 g/L (nominal) 429 g/L (analysed)
Test organism	
Species	<i>Apis mellifera</i> subspecies Buckfast (max 2 days old)
Source	On-site apiary maintained by BioChem agrar GmbH, Kupferstraße 6, 04827 Machern OT Gerichshain, Germany. Organisms were derived from healthy, disease free and queen-right bee colonies (colony nos.: LV201956; LV2019126; LV2019125). Prior to test start, hives had not received treatments with chemical substances for at least one month.

Study design and methods

Test duration and exposure	10 days with continuous exposure via food (spiked sucrose solution)
Experimental dates	25 June – 05 July 2019
Test doses test item	Nominal dosing: 47.5, 23.8, 11.9, 5.94 and 2.97 µg test item/bee/day equivalent to 8.08, 4.04, 2.02, 1.01 and 0.505 µg a.s./bee/day Effective dosing (based on actual daily intake): 6.70, 3.14, 1.54, 0.833 and 0.397 µg a.s./bee/day
Test doses reference item	Nominal dosing:

	0.0273 µg a.s./bee/day Effective dosing (based on actual daily intake): 0.0154 µg a.s./bee/day
Test units	Aluminium cages with the dimensions 95 mm (width) x 70 mm (height) x 60 mm (depth) with holes in the lateral walls for ventilation and two glass plates (one in front and one in the back) for observation of the bees
Group size/replicates contact	30 bees per treatment (1 control, 5 test item dosages, 1 reference treatment); 10 bees in each of 3 replicates per treatment
Acclimatisation	Brood combs with capped cells were taken from outside hives and 3 different colonies (D -2). These frames were placed without adult worker bees in a “five comb hive body” and were incubated under controlled environmental conditions in an incubator at 33 ± 2 °C and relative humidity of 70 ± 10 % at darkness for a maximum period about 24 hours (until D -2). Afterwards, the newly hatched worker bees were transferred into the test cages in groups of 10 bees/cage. For the following day (until D 0), bees were held in the test cages at 33 ± 2 °C and 50 – 70% rH and provided with sugar solution and pollen food. Moribund and dead bees were rejected and replaced by healthy bees before starting the test.

Environmental conditions

Temperature:	31.6 – 33.6 °C
Photoperiod:	Darkness (except assessments)
Relative humidity	56.5 – 63.8%

Analytical measurements

Analytical verification of test item concentrations was conducted using RP-HPLC-method with DAD-detection. Analytical samples were analysed from the highest and lowest test concentration as well as the control treatment from D0 – D9.

Biological observations

Mortality and behaviour were recorded daily at about the same time of the day (every $24 \text{ h} \pm 2 \text{ h}$), starting 24 ± 2 hours after start of the test period (initial feeding). Behaviour and occurring abnormalities were recorded according to the following categories: healthy/normal, moribund, affected in terms of uncoordinated movements, cramping, apathetic, vomiting. Any other behavioural abnormalities were noted and clearly described, if observed.

Statistics

For statistical calculation of the mortality results the Step-down RaoScott-Cochran-Armitage Test Procedure was used ($\alpha = 0.05$; one sided greater). LDD_x and LC_x values along with 95% confidence limits were determined by Probit analysis using linear max. likelihood regression. Mortalities of the test and reference item were corrected according to Abbott.

Results and discussion

Analytical results

Analytical measurements

Measured concentrations of the test item ranged from 90 and 96% of the nominal value for the lowest concentration tested and from 90 and 98% of the nominal value for the highest concentration tested. Hence, biological results are based on nominal concentrations.

Table A 78: Nominal and measured concentrations of test item

	Treatment group		
	Control	Lowest dose	Highest dose
Nominal concentration [mg a.s./L]	-	15.30	244.8
Range (D0 – D9) measured concentrations [mg a.s./L]	n.d.	13.83 – 14.78	235.0 – 219.9
Range (D0 – D9) % of nominal	-	90 – 96	90 – 98

Limit of quantification: 2.712 mg a.s./L

n.d. not detectable

Biological results

Biological results on mortality are given in the table below. In the course of the test, single bees were described as being affected in terms of uncoordinated movements in the three highest test item doses from day 2, 3 and 8 onwards, respectively. No other treatment related abnormal behaviour was observed in any other test item treatment group at any other time.

Table A 79: Mean mortality and behaviour of bees in the chronic toxicity feeding test with CA3573 Acetamiprid 200 SL (Carnadine) after 10 days

Treatment group	Daily dose [µg test item /bee/day]		Daily dose [µg a.s./bee/day]		Concentration		After 10 days		Bees showing behav. abnormalities**
	nominal	effective	nominal	effective	[mg test item/kg food]	[mg a.s./kg food]	Mean mortality [%]		
							Absolute	Corrected	-
Control	-	-	-	-	-	-	3.3	-	0 out of 29
Test item	47.5	39.4	8.08	6.70	1210	206	86.7*	86.2	1 out of 4
	23.8	18.4	4.04	3.14	605	103	46.7*	44.8	3 out of 16
	11.9	9.04	2.02	1.54	303	51.4	3.3	0.0	2 out of 29
	5.94	4.90	1.01	0.833	151	25.7	0.0	0.0	0 out of 30
	2.97	2.34	0.505	0.397	75.6	12.9	3.3	0.0	0 out of 29
	[ng ref. item/bee/day]		[ng a.s./ bee/day]		[mg ref item/ kg food]	[mg a.s./ kg food]	-	-	-
Reference item	68.5	38.6	27.3	15.4	1.745	0.696	83.3	82.7	0 out of 5

Results are averages based on 3 replicates, containing 10 bees each; nominal doses were corrected for evaporation and food uptake resulting in effective doses

mortalities of the test item and reference item group were corrected for mortality of the untreated control. Negative values are treated as “0”.

* Statistically significantly different in pairwise comparison between treatment and the untreated control (Step-down Rao-Scott-Cochran-Armitage Test Procedure; $\alpha = 0.05$; one-sided greater)

** Number of bees showing behavioural abnormalities referring to number of remaining bees

Table A 80: Endpoints

Treatment	Endpoints	Day 10
Test item doses*	LDD ₅₀ [µg test item/bee/day] ¹	21.8 (18.5 – 25.8)
	LDD ₅₀ [µg a.s./bee/day] ¹	3.71 (3.15 – 4.40)
	NOEDD [µg test item/bee/day] ²	9.04
	NOEDD [µg a.s./bee/day] ²	1.54
Test item concentrations	LC ₅₀ [mg test item/kg food] ¹	700 (601 – 821)
	LC ₅₀ [mg a.s./kg food] ¹	119 (102 – 140)
	NOEC [mg test item/kg food] ²	303
	NOEC [mg a.s./kg food] ²	51.4

¹ Median lethal dietary doses/concentrations (95%-CI lower-upper) were calculated using Probit analysis (linear max. likelihood regression)

² No observed effect dietary doses/concentrations were calculated using Step-down Rao-Scott-Cochran-Armitage Test Procedure ($\alpha = 0.05$; one-sided greater)

* endpoints based on effective doses

Validity criteria

As shown in the following table, all validity criteria were met.

Table A 81: Validity criteria

Validity criteria according to OECD 245 (2017)	Observed in study
Mortality in control $\leq 15\%$	3.3%
Mortality in the toxic reference substance group should be $\geq 50\%$ at test end	83.3%

Conclusion

The chronic oral toxicity of CA3573 Acetamiprid 200 SL (Carnadine) on young adult honey bees (*Apis mellifera*) was investigated in a 10 day chronic, dose-response feeding study under laboratory conditions. The LDD₅₀ was determined to be 21.8 µg test item/bee/day (equivalent to 3.71 µg a.s./bee/day) and the LC₅₀ to be 700 mg test item/kg food (equivalent to 119 mg a.s./kg food), respectively. The NOEDD was determined to be 9.04 µg test item/bee/day (equivalent to 1.54 µg a.s./bee/day) and the NOEC to be 303 mg test item/kg food (equivalent to 51.4 mg a.s./kg food), respectively.

A 2.3.1.4 KCP 10.3.1.3/01 Effects on honey bee development and other honey bee life stages

Comments of zRMS:	<p>The study has been already evaluated and accepted by the zRMS in the course of the first zonal evaluation of CA3573 (formerly MCW-2222) finalised in April 2018.</p> <p>The study design was based on indication of draft OECD guidelines on testing of toxicity of chemicals to bee larvae in single and repeated exposure regime. In general, the test conditions followed recommendations of the validated OECD 239, but the study was performed for 8 days and investigated effects of MCW-2222 on larvae from D3 to D8. Effects on pupation and adult emergence were not included in the test design. Taking this into account, the study is no longer suitable for purposes of the current risk assessments and was thus not re-evaluated for compliance with respective test methods, especially new study performed fully in line with OECD 239 has been submitted (Scheller, 2020) and its results supersede endpoints derived from Kleebaum (2014).</p> <p>The summary below has been struck through in order to make it clear that the test is no longer suitable for the risk assessment purposes.</p>
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Data point:	KCP 10.3.1.3/01
Report:	Chronic toxicity of MCW 2222 to honeybee larvae (<i>Apis mellifera</i> L.) under laboratory conditions (<i>in vitro</i>), Kleebaum, K., 2015b, R 33836, R 33836
Guideline(s):	OECD DRAFT Guidance Document for testing chemicals: Honey bee (<i>Apis mellifera</i>) larval toxicity test, repeated exposure (November 2013) & OECD 237 Guideline for testing chemicals: Honey bee (<i>Apis mellifera</i>) larval toxicity test, single exposure (2013)
Deviations:	<p>Yes</p> <p>Major deviations to current guidance document (OECD Environment Health and Safety Publications Series on Testing and Assessment No. 239):</p> <ul style="list-style-type: none"> • No data on pupation or emergence were recorded • The test duration was not 22 days but only 8 days
GLP:	Yes, certified laboratory
Acceptability:	Yes, study considered acceptable. No longer suitable for the risk assessment purposes, superseded by study presented under KCP 10.3.1.3/01
Duplication (if vertebrate study)	Not applicable

Executive Summary

In a chronic toxicity test, honeybee larvae (*Apis mellifera iberica*) were exposed to MCW 2222. The toxicity of the test item was determined at total doses of 37.1, 11.9, 3.8, 1.2, 0.4 and 0.1 µg a.s./larva (corresponding to 208.2, 66.6, 21.3, 6.8, 2.2 and 0.7 µg test item/larva). The concentrations of test item in the diet were 0.235, 0.075, 0.024, 0.008, 0.002 and 0.001 g a.s./kg food. Additionally, honeybee larvae were treated with Dimethoate tech. as reference item at a total concentration of 6.2 µg dimethoate/larva or with an untreated diet as control.

The LD₅₀ (96 h) was determined to be 21.1 µg a.s./larva, which is equivalent to a LC₅₀ (96 h) of 0.117 g a.s./kg food. Accordingly the NOED (96 h) was 3.8 µg a.s./larva and the corresponding NOEC (96 h) was 0.024 g a.s./kg food.

The LD₅₀ (120 h) was determined to be 10.2 µg a.s./larva, which is equivalent to a LC₅₀ (120 h) of 0.060 g a.s./kg food. Accordingly the NOED (120 h) was 3.8 µg a.s./larva and the corresponding NOEC (120 h) was 0.024 g a.s./kg food.

Materials and methods

Materials

Test item	MCW 2222
Batch #	611 280413 01
Content of active substance	Acetamiprid 200 g/L (nominal); 202.7 g/L (analysed)
Description	Yellowish liquid
Control	Untreated diet
Toxic reference	Dimethoate technical (99.8%)
Test organism	
Species	<i>Apis mellifera iberica</i> , first instar larvae
Source	Joaquín Cordero (Beekeeper), Paseo de Colón No. 19, 41370 Cazalla (Seville), Spain

Study design and methods

Test duration	11 days; 120 hours of exposure
Experimental dates	16 to 23 Jun 2014
Test doses	208.2, 66.6, 21.3, 6.8, 2.2 and 0.7 µg test item/larva corresponding to 37.1, 11.9, 3.8, 1.2, 0.4 and 0.1 µg a.s./larva
Test units	Crystal polystyrene grafting cells (CNE Nicoplast, internal diameter 9 mm) in 48 well plates. The well plates were filled up to 1/3 with a piece of dental roll. The grafting cells were placed on the wetted and disinfected dental rolls.
Group size/replicates contact	36 bees per treatment (control/test item/reference); 12 in each of 3 replicates per treatment
Environmental conditions	
Temperature:	35.2 °C—35.8 °C
Photoperiod:	Continuous darkness (except assessments)
Relative humidity	86–100%

Analytical measurements

Analytical verification of test item concentrations was conducted using an HPLC–UV detection.

Biological observations

Observations were made on mortality as well as qualitative observations as body size and remaining food after 96 hours (D7) and after 120 hours (D8) of oral exposure.

Statistics

For statistical calculation of the mortality results and of the NOEC/NOED the Fisher's Exact Binomial test (with Bonferroni Correction) was used. The accepted significance level was $p \leq 0.05$ (one-sided greater). To calculate the LC/LD₅₀ values of the test item the binomial distribution and Moving Average Computation after Thompson were used. Mortalities of the test and reference item were corrected according to Abbott.

Results and discussion

Analytical results

For the stock solution 4 samples were analysed. The recovery ranged between 94 and 97% of nominal values. For the control 4 samples were analysed. The analysed concentration of a.s. was below the level of quantification (272.1 mg/L).

Biological results

Biological results on mortality are given in the table below.

Table A 82: Toxicity of MCW-2222 to *Apis mellifera iberica* in a chronic toxicity test

Treatment group	Dosage applied [µg a.s./larvae]	Concentration [g a.s./kg food]	D7 (96h)		D8 (120h)	
			Mean mortality [%] [†]		Mean mortality [%] [†]	
			Absolute	Corrected	Absolute	Corrected
Control	-	-	11.1	0.0	11.1	0.0
Test substance [†] [µg test item/bee]	37.1	0.235	72.7 [*]	68.8	80.6 [*]	78.1
	11.9	0.075	41.7 [*]	34.4	63.9 [*]	59.4
	3.8	0.024	19.4	9.4	30.6	21.9
	1.2	0.008	13.9	3.1	22.2	12.5
	0.4	0.002	16.7	6.3	33.3	25.0
	0.1	0.001	22.2	12.5	33.3	25.0
Toxic reference [†] [µg a.s./bee]	6.2	0.039	55.6 [*]	50.0	61.1 [*]	56.3

^{††} Results are averages from 3 replicates (12 larvae each) for all treatment groups.
—= not tested
^{*} Statistically significant difference in pairwise comparison between treatment and untreated control (Fisher's Exact Binominal Test with Bonferroni Correction; α=0.05; one sided greater)

Table A 83: Endpoints after 96 and 120 hours of exposure

Treatment	Endpoints	D7 (96 h after 1 st application)	D8 (120 h after 1 st application)
Test item doses	LD50 [µg a.s./larva] (95 % CL /lower-upper)	21.1 (12.0 – 36.9)	10.2 (6.0 – 17.3)
	NOED [µg a.s./larva]	3.8	3.8
Test item concentrations	LC50 [g a.s./kg food] (95 % CL /lower-upper)	0.117 (0.070 – 0.196)	0.060 (0.037 – 0.097)
	NOEC [g a.s./kg food]	0.024	0.024

Validity criteria

As shown in the following table, all validity criteria were met.

Table A 84: Validity criteria

Validity criteria according to OECD 237 (2013)	Observed in study
Mortality in control ≤ 15%	11%
Mortality in the toxic reference substance group should be ≥ 50 % on D7.	55.6%

Conclusion

In a chronic larval toxicity study with MCW-2222, the LD₅₀ (96 h) was determined to be 21.1 µg a.s./larva, which is equivalent to a LC₅₀ (96 h) of 0.117 g a.s./kg food. Accordingly the NOED (96 h) was 3.8 µg a.s./larva and the corresponding NOEC (96 h) was 0.024 g a.s./kg food.
On D8, 120 hours after the first application, the LD₅₀ (120 h) was determined to be 10.2 µg a.s./larva, which is equivalent to a LC₅₀ (120 h) of 0.060 g a.s./kg food. Accordingly the NOED (120 h) was 3.8 µg a.s./larva and the corresponding NOEC (120 h) was 0.024 g a.s./kg food.

A 2.3.1.5 KCP 10.3.1.3/02 Effects on honey bee development and other honey bee life stages

Comments of zRMS:	<p>The study on toxicity of CA3573 to bee larvae (Scheller, 2020) has been submitted in support of the re-evaluation of CA3573 due to renewal of acetamiprid and was evaluated in 2021. The validation of the study was performed by the zRMS. No additional assessment is required for current evaluation.</p> <p>The study was carried out in line with OECD 239 with following deviations:</p> <ol style="list-style-type: none"> 1. There were some differences in larvae diet A comparing to indications of OECD 239 (slightly lower amount of royal jelly and yeast, lower amount of glucose and fructose, slightly higher amount of water). 2. On D8 bees at pre-pupal stage were transferred to new culture plates. 3. Culture plates were covered with lids throughout development between D8 and D15. <p>All these deviations were based on extensive studies on protocol for <i>in vitro</i> rearing of bee workers performed by Schmehl et al. (2016) and were demonstrated by the study authors to improve condition and health of bees during larvae testing. Therefore, based on results of the study mentioned, listed deviations are considered to have no adverse impact on results of the test performed with CA3573.</p> <p>Remaining parts of the test design as well as test conditions were fully in line with OECD 239.</p> <p>All validity criteria were met and the study is considered acceptable with following endpoints relevant for the risk assessment:</p> <p>ED₁₀ >2.861 µg product/larvae/developmental period (corresponding to >0.486 µg a.s./larvae/developmental period)</p> <p>NOED ≥2.861 µg product/larvae/developmental period (corresponding to ≥0.486 µg a.s./larvae/developmental period)</p>
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Reference:	KCP 10.3.1.3/02
Report	CA3573 Acetamiprid 200 SL (Carnadine) - Repeated exposure of honey bee larvae (<i>Apis mellifera</i> L.) under laboratory conditions, Scheller, K., 2020, 19 48 BLC 0033
Guideline(s):	Guidance Document on Honey Bee Larval Toxicity Test following Repeated Exposure, Series on Testing and Assessment, No. 239, OECD (2016)
Deviations:	<p>Yes, minor deviations to current guidance document (OECD Environment Health and Safety Publications Series on Testing and Assessment No. 239). Adaptations based on SCHMEHL et al. (2016) including:</p> <ul style="list-style-type: none"> • diet composition (more water and less royal jelly in diet A), • a pre-pupal transfer step to a new culture plate on D8, • changes to the rearing environment (a lid placed upon the culture plates throughout development between D8 and D15)
GLP:	Yes, certified laboratory
Acceptability:	Yes
Duplication (if vertebrate study)	Not applicable

Executive summary

In a toxicity study, 3-day old worker larvae of *Apis mellifera* were repeatedly orally exposed to CA3573 Acetamiprid 200 SL (Carnadine), nominally containing 200 g acetamiprid/L. The larvae were fed daily for a period of 4 days with cumulative doses of finally 0.486, 0.243, 0.122, 0.061 and 0.030 µg a.s./larva

corresponding to 2.861, 1.431, 0.715, 0.358 and 0.179 µg product/larva. The respective concentrations of the test item in the diet were 3.075, 1.537, 0.769, 0.384 and 0.192 mg a.s./kg which corresponds to 18.087, 9.043, 4.522, 2.261 and 1.130 mg product/kg food. Untreated 50 % w/w sucrose solution served as control, dimethoate was used as a toxic reference at one dose. Assessments of larval mortality were conducted on D3 to D8, pupal mortality on D15 and adult emergence on D22. Other observations such as abnormal behaviour or small body size were assessed at each mortality assessment (in comparison with controls) were recorded qualitatively. In the analytical part of the study, the test item concentration was measured in the final diets of the highest and lowest test item concentration at each feeding day. Unconsumed food was noted on D8.

No remaining food was observed at any of the remaining larvae at the end of the feeding phase and no other sublethal effects such as abnormal behaviour or small body size occurred in any of the treatments on the respective mortality assessments.

Correct dosing of the test item was verified by chemical analysis of the final diets of the highest (recoveries: 95% - 100%) and lowest (recoveries: 99%-103%) test item concentration at each feeding day. No active ingredient has been detected in the control samples.

Based on adult emergence on D22, the ED_{50/20/10} of the test item was estimated to be > 0.486 µg a.i./larva (> 2.861 µg product/larva) which is equivalent to an EC_{50/20/10} of > 3.075 mg a.i./kg food (> 18.087 mg product/kg food). The NOED was determined to be ≥ 0.485 µg a.i./larva (≥ 2.861 µg product/larva) which is equivalent to a NOEC of ≥ 3.075 mg a.i./kg food (≥ 18.087 mg product/kg food)

Materials and methods

Materials

Test item	CA3573 Acetamiprid 200 SL (Carnadine))
Batch #	981101035
Content of active substance	200 g/L acetamiprid (nominal), 195.5 g/L (analysed)
Density	1.15 g/mL
Description	Clear yellow-brown liquid
Control	Untreated diet A, B and C (see below for details on diet)
Toxic reference	Dimethoate technical, 98.8 ± 0.5 %
Test organism	
Species	Honey bee (<i>Apis mellifera</i> , hybrid line Buckfast), 3-day old worker larvae at test start
Source	Three colonies (= replicates) of the testing facility, BioChem agrar GmbH, 04827 Machern OT Gerichshain, Germany. Colonies healthy, diseases-free and with known history and physiological status. No treatment with chemicals, such as antibiotics, anti-varroa etc., was carried out within the four weeks preceding the start of test.
Food/feeding	Three different diets, adapted to the needs of the larvae at different stages of development: - diet A (feed on D1): 44.25 % weight of fresh royal jelly + 55.75 % weight of an aqueous sugar solution containing 1.61 % weight of yeast extract, 9.5 % weight glucose and 9.5 % weight of fructose - diet B (feed on D3): 50 % weight of fresh royal jelly + 50 % weight of an aqueous solution containing 3 % weight of yeast extract, 15 % weight glucose and 15 % weight of fructose - diet C (feed from D4 to D6): 50 % weight of fresh royal jelly + 50 % weight of an aqueous solution containing 4 % weight of yeast extract, 18 % weight glucose and 18 % weight of fructose. The treated diets (prepared daily), were warmed in an incubator before use. Feeding volumes using a sterile pipette: D1 & D3: 20µL/larva (no diet administered on D2) D4: 30µL/larva

D5: 40µL/larva
D6: 50µL/larva

During the feeding, care was taken to avoid touching and drowning the larvae, and the food was placed close to the larva along the wall of the grafting cell.

Study design and methods

Test duration and exposure

D3 to D8: exposure of larvae to non-spiked or spiked food for 4 days
D8 to D15: pre-pupal stage development
D15 to D22: pupal development and adult hatch

Experimental dates

12 August to 02 September 2019

Test doses/concentrations

Test item

Concentration:

18.087, 9.043, 4.522, 2.261 and 1.130 mg product/kg diet,
corresponding to
3.075, 1.537, 0.769, 0.384 and 0.192 mg a.s./kg diet,
equivalent to a total dose administered between D3 and D6*:
2.861, 1.431, 0.715, 0.358 and 0.179 µg product/larva,
corresponding to
0.486, 0.243, 0.122, 0.061 and 0.030 µg a.s./larva

Toxic reference

Concentration:

48.043 mg product/kg diet, equivalent to a total dose administered between D3 and D6*:

7.60 µg product/larva, corresponding to 7.6 µg a.s./larva

*because the administered food amounts increased with ongoing development of the larvae and the test/reference item are provided at a constant concentration the corresponding doses per larva per day increased with the diet resulting in a cumulative dose on D6

Test units

Larvae were reared in crystal polystyrene grafting cells with an internal diameter of 9 mm. Cells were sterilized by 70 % ethanol solution. Each cell was placed into a well of a 48-well plate. The top of the grafting cell was maintained at the level of the plate by placing a piece of wetted and disinfected dental roll.

From D1 to D8, the plates were placed into climatic chamber with a forced air circulation.

At D8, the tested organisms have had developed into pre-pupae. The pre-pupae were gently transferred into new 48-well plates coated with cellulose tissue and climatic conditions were adjusted (decreased relative humidity).

For adult emergence, the honey bee pupae were transferred into emergence boxes on D15 and left there until D22.

Collection of larvae

To ensure the production of synchronized larvae, the queens of three colonies were confined in their own colony in an excluder cage on D-3. The exclusion cage was placed close to combs containing brood. At D-2, approximately 24 hours after encaging, the queens were released from the excluders. The combs containing eggs were left in the excluders, near the brood, during the incubation stage and until hatching (D1). At day 1 (D1), the comb containing first instar larvae were transferred from the hive to the laboratory. A volume of 20 µL of diet A was dropped into each cell, then one larva was grafted from the comb to the cell, onto the surface of the diet, using a grafting tool.

Group size/replicates

A minimum of twelve larvae from each of three colonies were allocated on the same plate resulting in a total of 36 larvae/well plate.

Each plate corresponded to a treatment level, to the control or to the reference item.

Environmental conditions

Temperature

34.2 to 35.0°C

Relative humidity

D1 to D8: 96.0 to 100.0 %

D8 to D15: 76.0 to 84 %

D15 to D22: 57.0 to 65 %

Ventilation

By the air-conditioning equipment of the climatic chamber

Photoperiod

Constant darkness except during assessments

Analytical measurements

Analytical verification of test item concentrations was conducted using an HPLC-method with mass-spectrometric (MS-MS) detection. Analytical samples were analysed from all final diets of the highest and lowest test item concentration at each feeding day.

Biological observations

Assessments on larval mortality was performed from D4 to D8 and the pupal mortality on D15. The emergence rate of the adult bees was determined on D22. Other observations such as abnormal behaviour or small body size were assessed at each mortality assessment. Unconsumed food was noted on D8.

Statistics

Mortality was corrected according to Abbott (1925). For statistical evaluation of the mortality results of the respective test item doses on D22 and thus for determination of NOEC/NOED the Chi² 2x2 Table Test with Bonferroni Correction was used. The accepted significance level was alpha = 0.05 (one-sided greater). Prior to the Chi² 2x2 Table Test with Bonferroni Correction, descriptive statistics were performed for justification of the test procedure (Qualitative Trend Analysis by contrasts to check for monotonicity of dose/response; Tarone's Test to check for extra-binomial variance between replicates).

As the corrected mortality on D22 was increased by less than 10% in all test item doses/concentrations compared to the control (i.e. increase was between 0.0 to 3.6%) the respective ED_x/EC_x were assumed to be higher than the highest dose/concentration tested. The statistical calculations were performed with the computer program ToxRat Professional 3.3.0 (Ratte, 2018).

Results

Analytical measurements

Measured recovery of acetamiprid in the final solution samples of the highest ranged between 95% - 100% and between 99 % – 103 % of the lowest test item concentration at each feeding day. No active ingredient has been detected in the control samples.

Table A 85: Analytical recovery rates of acetamiprid in the feeding solutions

Nominal concentration	Mean recovery of the nominal values [%] on			
	Day 3	Day 4	Day 5	Day 6
Feeding solution [mg a.s./kg]				
Control	n.d.	n.d.	n.d.	n.d.
0.192 (lowest)	103	101	103	99
3.075 (highest)	100	98	95	96

Biological results

On D8 of the test, no mortality was observed in the untreated control. In the test item groups, the mean cumulative mortalities ranged between 2.8% and 5.6%. The mean mortality in the reference group was above 50 %, i.e. being 86.1%.

The mean pupal mortality between D8 and D15 was 16.7% in the untreated control and ranged between 8.6% and 14.9% in the test item group (corrected for control: 0.0% for each dose). The mean pupal mortality in the reference item group was 12.5% (corrected for control: 0.0%).

On D22, the mean adult emergence rate in the untreated control was 77.8% (cumulative mortality 22.2%). In the test item treatment group, the adult emergence rate was 75.0%, 77.8%, 80.6%, 80.6% and 83.3% (from the highest to the lowest dose/concentration). The respective mean cumulative mortality was 25.0%, 22.2%, 19.4%, 19.4% and 16.7% (corrected for control: 0.0% to 3.6%). The mean adult emergence in the

reference item group was 11.1% (cumulative mortality was 88.9%; corrected for control: 85.7%).

There were no statistically significant differences of the adult emergence rates of the respective test item doses on D22 compared to the control.

The results are summarized in the tables below.

Table A 86: Effects of CA3573 Acetamiprid 200 SL (Carnadine) to larvae, pupae and adult emergence of *Apis mellifera* L. after repeated exposure

Treatment group	Dose		Concentration		On D8			On D15		On D22		
					Mean mortality of larvae D3 to D8 [%]		Mean OO	Mean mortality of pupae D8-D15 [%]		Mean total mortality of larvae & pupae D3-D22 [%]		Mean adult emergence rate [%]
	[µg a.i./ larva]	[µg prod./ larva]	[mg a.i./ kg food]	[mg prod./ kg food]	abs.	corr.		abs.	corr.	abs.	corr.	abs.
Control	-	-	-	-	0.0	-	0.0	16.7	-	22.2	-	77.8
Test item	0.486	2.861	3.075	18.087	2.8	-	0.0	11.6	0.0	25.0	3.6	75.0
	0.243	1.431	1.537	9.043	5.6	-	0.0	14.9	0.0	22.2	0.0	77.8
	0.122	0.715	0.769	4.522	2.8	-	0.0	8.6	0.0	19.4	0.0	80.6
	0.061	0.358	0.384	2.261	5.6	-	0.0	14.6	0.0	19.4	0.0	80.6
	0.030	0.179	0.192	1.130	5.6	-	0.0	12.1	0.0	16.7	0.0	83.3
Reference item	7.600	-	48.043	-	86.1	-	0.0	12.5	0.0	88.9	85.7	11.1

Results are averages based on 3 replicates, containing 12 larvae each

abs.: mortality as derived from the results of a treatment group;

corr.: corrected mortality (according to SCHNEIDER-ORELLI 1947); test/reference item treatment groups corrected for control mortality; negative values were set to "0"

Calculations were performed with non-rounded values.

OO: Other observations (remaining food, small body size)* result significantly different to control (Step-down Cochran-Armitage tests)

Table A 87: Endpoints on D22

Endpoint	[product]	[a.s.]
Dose [µg/larva]		
ED₁₀	> 2.861	> 0.486
ED₂₀	> 2.861	> 0.486
ED₅₀	> 2.861	> 0.486
NOED	≥ 2.861	≥ 0.486
Concentration [mg/kg feeding solution]		
EC₁₀	> 18.087	> 3.075
EC₂₀	> 18.087	> 3.075
EC₅₀	> 18.087	> 3.075
NOEC	≥ 18.087	≥ 3.075

Validity criteria

As shown in the following table, all validity criteria were met.

Table A 88: Validity criteria

Validity criteria according to OECD 239 (2016)	Observed in study
Mean cumulative control mortality from D3 to D8: ≤ 15 %	0.0 % (fulfilled)
Mean control emergence rate on D22: ≥ 70 %	86.1 % (fulfilled)
Mean toxic reference mortality at D8: ≥ 50 %	70 % (fulfilled)

Conclusion

In a laboratory study, honeybee larvae (*Apis mellifera* L.) were repeatedly orally exposed for 4 days to a range of CA3573 Acetamiprid 200 SL (Carnadine) doses according to OECD 239 (2016). Based on the adult emergence at test end, the ED50 was determined to be $> 0.486 \mu\text{g a.s./larva}$, corresponding to an EC50 of $> 3.075 \text{ mg a.s./kg}$. The NOED was determined to be $\geq 0.486 \mu\text{g a.s./larva}$, corresponding to a NOEC of $\geq 3.075 \text{ mg a.s./kg}$. The analytical part proved correct dosing.

A 2.3.1.6 KCP 10.3.1.4 Sub-lethal effects

A 2.3.1.7 KCP 10.3.1.5 Cage and tunnel tests

A 2.3.1.7.1 KCP 10.3.1.5/01 Tunnel CEB study with honey bees on wheat – 1

Comments of zRMS:	<p>The study has been already evaluated and accepted by the zRMS in the course of the first zonal evaluation of CA3573 (formerly MCW-2222) finalised in April 2018. As the test guideline has not changed since that time, re-evaluation of the study following renewal of acetamiprid was not necessary and most of presented below conclusions were taken from the Core Assessment, Part B, Section 6 of April 2018. The conclusion of the evaluation are provided below:</p> <p>The study was performed in the Northern France and comprised applications to winter wheat sprayed with sugar syrup (simulating honeydew) performed at two timings:</p> <ul style="list-style-type: none"> – during bee foraging activity: water control, MCW-2222 at 100 g a.s./ha and the reference dimethoate, – out of the presence of forager bees: MCW-2222 at 100 g a.s./ha at night. <p>In these experimental conditions, MCW-2222 applied in bee presence as well as out of the bee presence triggered a statistically significant effect on daily mortality at D+2 only. Then the general daily mortality trend was similar to this seen in controls and the differences to the control mortality counts were not significant. Few signs of intoxication were recorded at D+1 in the tunnels treated with MCW-2222 during or out of the bee presence. Foraging behaviour abnormalities were also recorded on the day after the application. No signs of behavioural abnormalities were recorded after D+2. Colony strength parameters recorded in the control and in the tunnels where MCW-2222 was applied during or out the foraging activity were not significantly different. This indicates a very timely limited effect of the test item.</p> <p>Residue analysis was not performed, but the study was performed in tunnels and treated crop was the only food source for bees. Exposure of bees was thus assured.</p> <p>Although the study was correctly performed and as such is considered acceptable, it is noted that it was performed for a short time with bee mortality observations carried out during seven days after the applications and colony assessment carried out 9 days before application and 8 days after the application. Taking this into account, the design of the study is relevant to capture acute effects of application of acetamiprid on adult bees and bee brood, but is not relevant to address the chronic effects.</p>
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Data point: KCP 10.3.1.5/01

Report: Assessment of toxicity on honey bees (*Apis mellifera*) of the product MCW-2222 (acetamiprid 200 g/L) applied under insect proof tunnels on cereal crop. Mamet, O. & Molitor, C., 2015a, R-34874, 216-2014

Guideline(s): C.E.B methodology n°230, part IV

Deviations: Yes, minor deviations:
At D0, although 5 of the 12 tunnels show a daily mortality between 300 and

400 dead bees on D0 before application, daily counts were homogeneous among treatments after new distribution, with mean values from 219 to 293 dead bees within all treatments

This minor deviation did not have an impact on the reliability and the outcome of the study.

GLP: Yes, certified laboratory
Acceptability: Yes, study considered acceptable
Duplication: Not applicable
(if vertebrate study)

Executive Summary

In a semi-field study with honeybees (*Apis mellifera* L.) the potential effects of MCW-2222 on mortality, foraging activity, behaviour, colony strength, quality and quantity of brood and the amount of reserves were investigated. Flowering winter wheat served as crop (crop area: 64 m², subdivided in 4 plots and separated and surrounded by plastic lanes), which was daily sprayed with sugar syrup as artificial honeydew to make the crop attractive for foraging bees. Each tunnel was provided with a water and pollen supply. MCW-2222 was applied during bee flight (T1) and after bee flight (T2) to the crop at a rate of 0.5 L/ha (100 g a.s./ha acetamiprid). Water treated tunnels served as control (C). Tunnels, treated with Rogor plus (400 g dimethoate/ha) served as toxic reference (R). Each treatment group was three times replicated. Application in the control, the toxic reference and T1 was performed during a period of high foraging activity. T2 was applied in the evening of the same day after the end of the foraging activity.

Small honey bee colonies of approx. 10,000 bees were placed into the tunnels ten days before application (D -10) to get familiar with the new conditions. Seven days after application (D +7) and being confined within the tunnels colonies were located outside the tunnels.

Assessments on mortality (via plastic sheets) were daily conducted between D -8 to D +7; by exception, dead bees were collected twice on the application day (in the morning and in the evening) in order to observe eventual acute effect. Foraging activity was daily assessed between D -7 and D +6; on the day of application during bee flight, the foraging activity was monitored 6 times (two times before application, 30 minutes after application, followed by three other assessments). The assessment was conducted on the 4 plots of each tunnel. Potential clinical signs of intoxication were recorded during the period after application. Control of the colony (apiarist visit) with respect to colony strength, quality and quantity of brood (different stage observed) and the amount of reserves was carried out just before the introduction of the hives into the tunnels and just after the end of the study.

MCW-2222 applied during (T1) and after (T2) bee flight triggered a statistically significant effect on daily mortality at D+2 only. Then the general daily mortality trend was similar to the one met in the control and the differences to the control mortality counts were not significant. Evolution of the cumulative mortality for both test item treatments had similar trends compared to the control and data were not significantly different at the end of the experimental phase.

The foraging activity was stopped for one day after the application of MCW-2222 whatever the timing of application. From D +2 the trend was similar to the control with lower values until the end of the trial, whereas the toxic reference dimethoate clearly triggered a longer stop of the foraging activity.

Few signs of intoxication were recorded at D +1 in the tunnels treated with MCW-2222 during or out of bee presence. Foraging behavior abnormalities were also recorded on the day after the application. No signs of behavior abnormalities were recorded after D +2.

Colony strength parameters recorded in the control and in the tunnels where MCW-2222 was applied during or out the foraging activity were not significantly different. At the end of the experimental phase the adult population in the tunnels treated with MCW-2222 and the water control increased, on the contrary the population treated with the toxic reference lost 5% of its adult bees.

Materials and methods

Materials

Test item	MCW-2222
Batch #	93191024
Content of active substance	Acetamiprid 20% (nominal); 19.8% (analysed)
Description	Yellowish liquid
Control	C: Water treated crop, applied during foraging activity
Toxic reference	R: Rogor Plus (400 g a.s./ha dimethoate), applied during the foraging activity

Test organism

Species	Honey bees (<i>Apis mellifera</i> L.) of healthy colonies with sister queens and the same age (not older than 2 years), containing about 10,000 bees per colony at test start with six frames. Hives of Dadant type. All colonies at the beginning of the study - with at 2 to 4 frames containing all brood stages - with 0 to 2 storage frames - with 0 to 2 empty frames - were free of clear clinical symptoms of disease; no treatment against <i>Varroa</i> for at least 4 weeks before beginning of the study.
Source	local beekeeper, GAEC Mélibocage
Food/feeding	Each tunnel was provided with a water and pollen supply. To make the crop attractive for foraging bees the crop was daily sprayed with sugar syrup as artificial honeydew (500 g/L sucrose solution, at the dose of about 500 L/ha).

Study design and methods

Test duration	Pre-exposure phase (D -10 to D0) within the tunnels: 10 days Exposure phase (D 0 to D+7) within the tunnels: 7 days
Experimental dates	18 th May to 5 th June 2014
Test doses	Test item T1 (during bee flight): 100 g a.s./ha T2 (after bee flight): 100 g a.s./ha Toxic reference R (during bee flight): 400 g a.s./ha

Application of C, T1 and R was performed during daily bee flight, T2 on the same day after bee flight at BBCH 65 (full flowering) of the crop with a volume of 200 L water/ha. During application, the water and pollen containing supplies were removed to avoid contamination with spray residues.

All actual treatment rates were within $\pm 5\%$ from the target application rate.

Test units	Tunnels with an area of 140 m ² , containing 64 m ² (subdivided in 4 plots and separated and surrounded by plastic lanes) of flowering winter wheat (variety: Apache), each with one colony; tunnels equipped with a water and pollen supply.
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Endpoints and assessments

mortality of bees:
D -8 to D+7 via plastic lanes; assessed once a day in the morning; on the application day (D0) in the morning and evening to look for possible acute effects

foraging activity:
D -7 to D+6, on the entire 4 plots/tunnel (4 x 16 m² per tunnel) assessed once a day during the flight activity of the bees; on the day of application mortality (DAT0) was assessed
- immediately before application

- 30 minutes after application, followed by three other assessments

behaviour in the tunnels and at the entrance of the hives:

at the same time when the assessment for foraging activity took place

colony strength and colony development:

once at the beginning (D -9) and once at the end (D+8) of the study;
assessment of:

- estimated number of bees (colony strength)
- number of cells containing brood (total of cells with eggs, larvae and capped brood)
- presence of queens (e.g. presence of eggs)
- number of storage frames.

Three tunnels per treatment group

Colonies were set-up in the tunnel on 10 days before application on D -10 to get familiar with the new conditions.

Group size/replicates contact Adaptation of bees

Environmental conditions

Natural field conditions

At the beginning of the trial, weather conditions were not good as it was very cloudy with some rainfalls (from D -8 to D -3). When those conditions became appropriate (from D -3 to D0), i.e. shiny days and temperature values allowing bee activity especially in afternoon, applications could have been performed at D 0. A thermo-hygrograph placed in a weather station recorded temperature and air humidity over the whole experimentation period. Rainfall was daily recorded from the previous day.

Conditions			
	At bee flight appl.	After bee flight appl.	During exposure
Temperature:	17 °C	16 °C	8 to 22 °C
Wind speed:	0 to 2 km/h	0 to 2 km/h	not measured
Rel. humidity:	50 %	71 %	not reported
Precipitation:	none	none	D +2 (3 mm) D +3 (8 mm) D +7 (3 mm)

Biological observations

Adult mortality was recorded daily between D -8 to D +7 and foraging activity and behaviour daily between D -7 to D +6. Assessment of condition of the colony strength and colony development D -9 and D +8.

Statistics

An analysis of variance was performed to compare the control treatment to the MCW-2222 treatments following a Student-Newmans-Keuls test (average following by the same letter are not significantly different). A transformation Log (x+1) of mortality data allow reducing the heterogeneity of variance. The toxic reference treatment was excluded as it was used for validation of the trial and not for comparison.

Obtained control mortality data were related to historical control mortality data obtained by TESTAPI in previous years using absolute and relative formula in order to assess reproducibility and if the control treatment of the study is in the range of the previous data. The calculated index (relative toxicity index; itox) for historical control data amounted to 2.1 at D+1 at 95% of confidence or 2.4 at 99% of confidence. Moreover, two types of relative toxicity index (itox) were calculated, one for application during the foraging activity and another one for application outside the foraging activity.

$$\text{Itox} = (\text{Mt} \times \text{Ta}) / (\text{Ma} \times \text{Tt})$$

Mt = Mortality in the considered modality after application

Ma = Mortality in the considered modality just before application

Tt = Mortality in the water control tunnel after application

Ta = Mortality in the water control tunnel just before application

Results and discussion

Biological results

Mortality

As expected in this type of test, when the hives were introduced in the tunnels at D-8, high mortality was met in each tunnel. Then during the adaptation phase (D-7 to D0), the bee mortality tended to be stable over the time and homogeneous among tunnels and reached an acceptable level at D0 (from 219 to 293 dead bees) for performing the application.

The average mortality in the control tunnels remained low to moderate from the application date until the end of the trial. No pick of mortality was met. On the contrary the average mortality in the dimethoate tunnels increased strongly just after the application since it reached 2334 the day after application. Moreover the impact of dimethoate on bee mortality occurred 2 days after application since 816 dead bees in average were found. So the results recorded in the control and toxic tunnels allow to validate the trial.

MCW-2222 applied during the bee flight (T1) showed a slight increase of the average mortality at D+1 (from 237 dead bees at D0 to 474 the day after application) and at D+2 (390 dead bees). Nevertheless, the mortality increase observed at D+1 and D+2 was much lower than the one recorded in the toxic reference treatment (2334 dead bees at D+1 and 816 at D+2 in average). The effect was limited in time: the mortality became similar to that of the control from D+3 until the end of the experimental phase. Only mortality at D+2 was statically significantly different from that met in the control tunnels.

MCW-2222 applied after bee flight (T2) showed a significant effect on mortality only at D+2 (from 284 dead bees collected at D0 to 711 at D+2 in average). From D+3 to D+7, there was no significant difference between the control and the tunnel treated with MCW-2222.

The statistical analysis performed with historical data shows that the toxicity index at D+1 (itoxc) of the water control in this study 216-2014 reached 0.7 and was lower than the value of 2.1 calculated with Testapi's historical data at 95% confidence. This result supports the validity of the study.

Moderate short-term effects of MCW-2222 are confirmed by the relative toxicity index (itox). Indeed when the product is applied after bee flight, bees don't visit the treated crop between the application at night and the next morning when dead bees are counted at D+1, the effect is delayed by one day. Therefore for application bee flight, it is useful to compare the mortality at D0 to the one assessed at D+2.

The itox was very high for Dimethoate 400 EC (it reached 11.3 and 6.4 according to the timing of application of the test item). It was moderate for MCW-2222 applied at 0.5 L/ha during the bee flight (2.8 to 3.8) and high when MCW-2222 was applied after bee flight (5.8) This high value can also be explained by the low mortality in the control tunnels at D+2 (95 dead bees).

The average cumulative mortality after application was by far lower in the MCW-2222 tunnels than in the toxic reference tunnels. Compared to the water tunnels, it was superior in the MCW-2222 tunnels but the cumulative mortality induced by MCW-2222 was not significantly different from the control at D+7. Moreover, it has to be noted that that the application of MCW-2222 had no effect on the evolution of the mortality over the time.

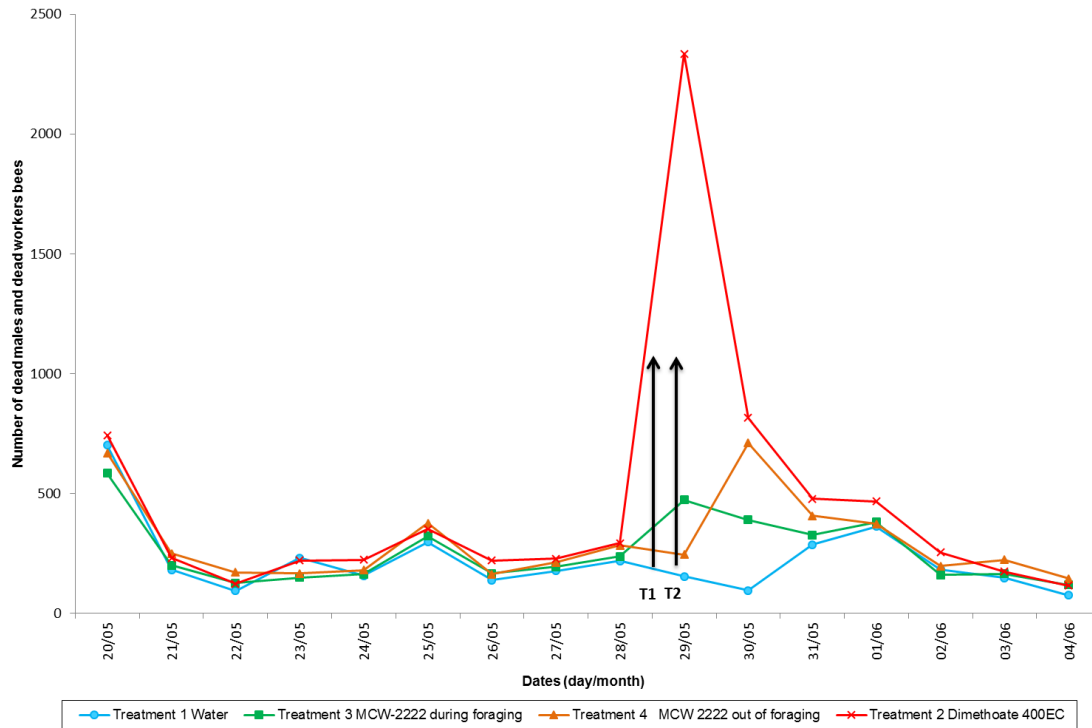


Figure A 22: Total daily mortality

Table A 89: Total daily mortality

Dates (day/month, D±x) x= delay from application day	Average number of dead bees			
	Treatment 1	Treatment 3 (T 1)	Treatment 4 (T 2)	Treatment 2
	Water	MCW-2222 during foraging	MCW-2222 out of foraging	Dimethoate 400EC
20/05 D-8	701	585	669	743
21/05 D-7	182	201	250	231
22/05 D-6	94	126	171	124
23/05 D-5	231	150	167	221
24/05 D-4	158	164	181	223
25/05 D-3	298	323	377	351
26/05 D-2	139	166	164	220
27/05 D-1	178	194	213	228
28/05 D0	219	237	284	293
29/05 D0+ +D+1	155	474	245	2334
30/05 D+2	95	390	711	816
31/05 D+3	287	327	407	478
01/06 D+4	363	381	373	468
02/06 D+5	184	161	197	255
03/06 D+6	148	165	223	174
04/06 D+7	76	119	145	115
Cumulative mortality after application date to 04/06	1308	2017	2301	4640

← Application
T1 and T2

Mortality reported on 28/05 was recorded immediately prior to the application.

Mortality reported on 29/05 is the sum of the mortality recorded on 28/05 just after the application and the mortality recorded on 29/05.

Table A 90: Relative toxicity index

Time after

Treatments \ Treatment	I tox Value*	
	I tox ₁ (D+1 versus D0) During foraging	I tox ₂ (D+2 versus D0) Out of foraging
Treatment 1 Water	1.0	1.0
Treatment 3 MCW 2222 during bee flight (T1)	2.8	3.8
Treatment 4 MCW 2222 after bee flight (T2)	Not relevant	5.8
Treatment 2 Dimethoate 400EC	11.3	6.4

* I tox value = (Mt x Ta) / (Ma x Tt)

Foraging activity

The data recorded before applications shows that foraging activity can be different the same day according to the time of the assessment (

Table A 89 and Figure A 22). This is due to weather conditions (temperature, sunshine, rainfall). For example in this study the average foraging activity moved from 11.2 on 26/05 at 10h45 to 3.4 bees/m² on 26/05 at 14h15.

On the day of application, foraging activity was high (from 5 to 11 bees/m²) and always superior to the required level (3 bees/m²).

The foraging activity in the water tunnels was good and stable from the application T1 to the end of the test (the variations were mainly due to weather conditions).

A very severe impact on foraging activity was met in the toxic reference tunnels since this activity decreased significantly just after the application at T1 and then it was stopped from D+1 until the end of the test (the repellence was so high that bees stayed in their hives).

MCW-2222 applied at 0.5 L/ha during bee flight (T1) showed an impact on the foraging activity the day of the application (D0). But just the day after (D+1), few honeybees came back on the crop plots. The following days (D+2 to D+4), this activity increased and followed the same evolution until the end of the study as that met in the control tunnels.

When MCW-2222 was applied after bee flight (T2), the foraging activity was very low at D+1. After this decrease, the same evolution as the one observed when the test item was applied during the foraging activity was observed: the activity increased until the end of the trial. From D+2 to the end of the study, the foraging activity reached a good level in the tunnels treated with MCW-2222 whatever the timing of application since it was between 4 to 6 bees/m² and was superior to the required level of 3 bees/m².

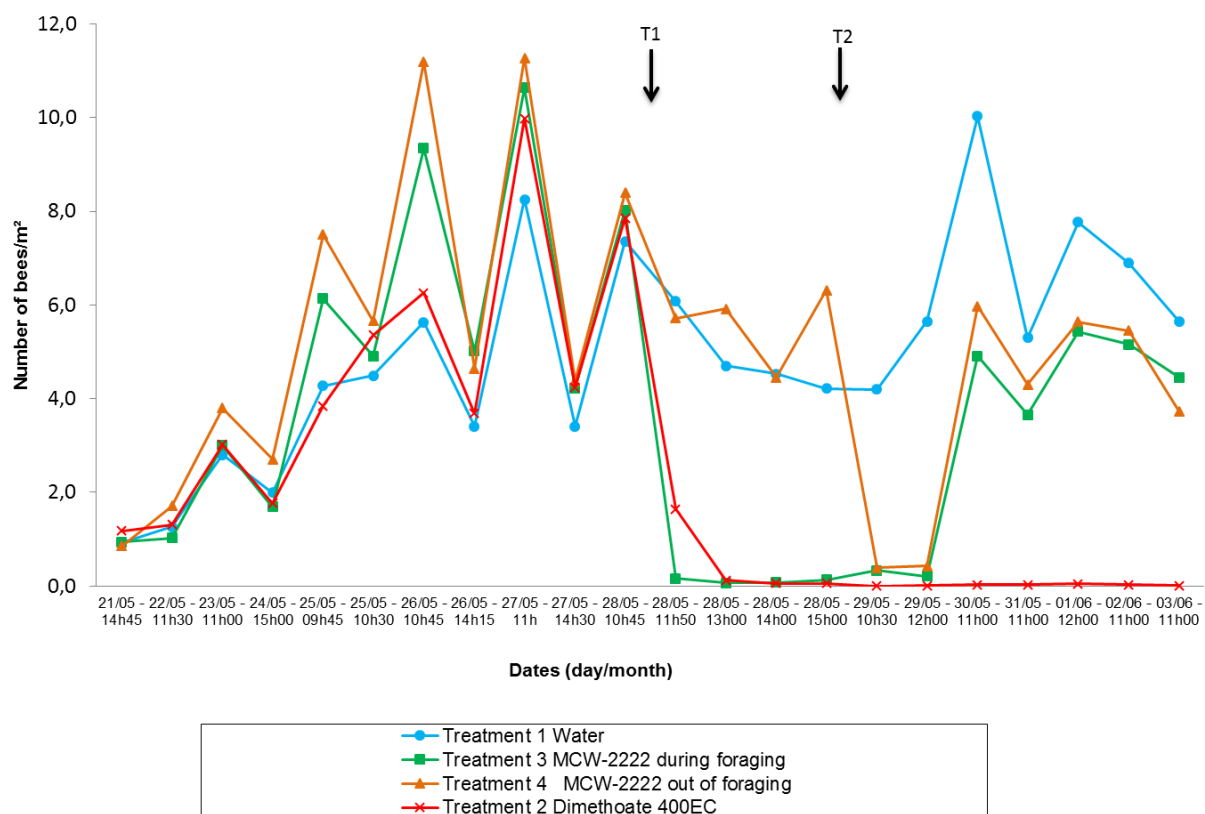


Figure A 23: Foraging activity - Average number of bees/m²

Table A 91: Foraging activity - Average number of bees/m²

Dates	Average number of bees/m ²			
(day/month-hours)	Treatment 1	Treatment 3 (T 1)	Treatment 4 (T 2)	Treatment 2
x = delay from application day	Water	MCW-2222 during foraging	MCW-2222 out of foraging	Dimethoate 400EC
D-7 21/05 - 14:45	0.9	0.9	0.9	1.2
D-6 22/05 - 11:30	1.3	1.0	1.7	1.3
D-5 23/05 - 11:00	2.8	3.0	3.8	3.0
D-4 24/05 - 11:30	N.D.	N.D.	N.D.	N.D.
D-4 24/05 - 15:00	2.0	1.7	2.7	1.8
D-3 25/05 - 09:45	4.3	6.1	7.5	3.8
D-3 25/05 - 10:30	4.5	4.9	5.7	5.4
D-2 26/05 - 10:45	5.6	9.3	11.2	6.3
D-2 26/05 - 14:15	3.4	5.0	4.6	3.7
D-1 27/05 - 11:00	8.3	10.6	11.3	10.0
D-1 27/05 - 14:30	3.4	4.2	4.4	4.2
D0 28/05 - 10:45	7.4	8.0	8.4	7.8
D0+ 28/05 - 11:50	6.1	0.2	5.7	1.6
D0+ 28/05 - 13:00	4.7	0.1	5.9	0.1
D0+ 28/05 - 14:00	4.5	0.1	4.4	0.1
D0+ 28/05 - 15:00	4.2	0.1	6.3	0.1
D+1 29/05 - 10:30	4.2	0.3	0.4	0.0
D+1 29/05 - 12:00	5.6	0.2	0.4	0.0
D+2 30/05 - 11:00	10.0	4.9	6.0	0.0
D+3 30/05 - 11:00	5.3	3.7	4.3	0.0
D+4 01/06 - 12:00	7.8	5.4	5.6	0.0
D+5 02/06 - 11:00	6.9	5.2	5.5	0.0
D+6 03/06 - 11:00	5.7	4.5	3.7	0.0

← Application T1

← Application T2

Clinic signs of intoxication were recorded in the toxic reference treatment.

In the tunnels treated with MCW-2222 during bee flight (T1), bees hesitated to forage the crop for 30 minutes after the application and a few bees presented clinic signs of intoxication in the next hours. One day later, very few bees presented those signs and behavior. Then behavior was considered normal until the end of the trial. In the tunnels treated with MCW-2222 after bee flight (T2), clinic signs of intoxication were recorded at D+1 and bees still hesitated to forage the crop at D+2. No other behavior abnormalities were recorded after D+2.

Colony strength and colony development

At the end of the experimental phase, the adult population in the tunnels treated with MCW-2222 and the water control increased (3% of increase for control, from 10% to 15% for MCW-2222 treatments). Differences in the evolution of the population of adult honeybees would be also linked to the evolution of number of brood cells: e.g. if the amount of brood decreased and the population increased during the same time, it means that brood hatched and provided new worker honeybees. This was the case in the control and the two MCW-2222 treatments.

On the contrary, the population treated with the toxic reference decreased slightly and lost 5% of its adult bees.

Concerning the number of brood cells, it decreased during the trial in all tunnels due to the experimental conditions with small colonies under tunnel (food resources in tunnels are sufficient to maintain healthy colonies for 2 to 3 weeks only). For this reason this type of test is not appropriate to study the brood evolution and no conclusion can be made from these data.

Endpoints

Whereas temporary effects on adult mortality, foraging activity and behaviour (few bees with signs of intoxication) occurred after the application MCW-2222 during (T1) and after (T2) bee flight at a rate of 100 g a.s./ha, no impact on the colony strength as well on the colony conditions was observed.

Validity criteria

As shown in the following table, all validity criteria were met.

Table A 92: Validity criteria

Validity criteria according to CEB 230 (2003), part IV	Observed in study
Before treatment:	
Daily mortality must have stabilised. In practice, this stabilisation is achieved three to six days after the hives are placed in the tunnels	Achieved
Daily mortality on the last day before treatment must not exceed 300 bees	219 to 293 dead bees/replicate in the respective treatment groups
Daily mortality must be similar in different tunnels, i.e. the deviation from average mortality on the day before application must not exceed 60%	C: - 40% to +44% T1: -28% to +42% T2: -12% to +23% R: -63% to +33%
Foraging activity must be greater than five bees / m ² on flowering plants and three bees / m ² on wheat shortly before application	C: 7.4 bees/m ² T1: 8.0 bees/m ² T2: 8.4 bees/m ² , assessed during bee flight 0 bees/m ² , assessed after bee flight R: 7.8 bees/m ²
Foraging activity in different tunnels must be comparable.	Achieved
After treatment:	
Mortality in the water control must be comparable before and after treatment, i.e. mortality the day after treatment must not exceed the average mortality figures for the day before treatment by more than 50%	Itox at D+1: 0.7 Itox at D+2: 0.4
The daily mortality in the "toxic benchmark" tunnel the day after treatment must be at least five times as high as the mortality figure for the day before treatment	Itox at D+1: 11.3 Itox at D+2: 6.4
Weather conditions must remain favourable	Achieved
All other factors regarded as abnormal in the conduct of the test	Achieved

Conclusion

In a semi-field study honeybees (*Apis mellifera* L.) were exposed to MCW-2222 (a.s. acetamiprid) applied at rate of 100 g a.s./ha during (T1) and after (T2) bee flight according to CEB 230 (2003), investigating potential effects on adult mortality, foraging activity and behaviour, conditions of the colonies (i.e. colony strength and colony development. Flowering winter wheat served as crop (crop area: 64 m², subdivided in 4 plots and separated and surrounded by plastic lanes), which was daily sprayed with sugar syrup as artificial honeydew to make the crop attractive for foraging bees.

MCW-2222 applied during bee flight (T 1) as well as after bee flight (T2), triggered a statistically significant effect on daily mortality at D+2. Then, the general daily mortality was similar to the one met in the control and the differences to the control mortality counts were not significant.

Evolution of the cumulative mortality for both MCW-2222 treatments had similar evolution compared to the control one and was not significantly different from the control one at the end of the experimental phase (D +7).

Regarding the foraging activity, there was no significant difference between the control and both test item groups at the end of the trial. A repellent effect was observed until D+1 and then, the level of the foraging activity reached a correct level of around 5 bees/m² from 2 days after application and the evolution remained comparable to the control one. The application of the toxic reference dimethoate clearly triggered a stop of this activity until the end of the trial.

The colonies strength and development were not impacted by the application of both MCW-2222 treatments.

A 2.3.1.7.2 KCP 10.3.1.5/02 Tunnel CEB study with honeybees on wheat - 2

Comments of zRMS:	<p>The study has been already evaluated and accepted by the zRMS in the course of the first zonal evaluation of CA3573 (formerly MCW-2222) finalised in April 2018. As the test guideline has not changed since that time, re-evaluation of the study following renewal of acetamiprid was not necessary and most of presented below conclusions were taken from the Core Assessment, Part B, Section 6 of April 2018.</p> <p>The study was performed in the Northern France and comprised applications to winter wheat sprayed with sugar syrup (simulating honeydew) performed at two timings:</p> <ul style="list-style-type: none"> – during bee foraging activity: water control, MCW-2222 at 100 g a.s./ha and the reference dimethoate, – out of the presence of forager bees: MCW-2222 at 100 g a.s./ha at night. <p>In these experimental conditions, MCW-2222 applied in the bee presence triggered a statistically significant effect on daily mortality at D+1 only. Then the general daily mortality trend was similar to this observed in control and the differences to the control mortality counts were not significant. When applied out of the bee presence, the product MCW-2222 showed no significant difference in any daily mortality count until the end of the trial. No signs of intoxication were recorded at D+1 in the tunnels treated with MCW-2222 during or out of the bee presence. Foraging behavior abnormalities were recorded during a short time on the day of application, just after spraying, and the day after application when treated out of the bee presence. No signs of behavioural abnormalities were recorded after D+2. The foraging activity was significantly lower just after the application of MCW-2222 during foraging and the effect lasted until D+1. When MCW-2222 was applied out of the foraging activity, a significant difference to the control was observed at D+, but the mean foraging activity stayed stable compared to the previous day in this treatment while in control it was increased. From D+3 the foraging activity increased and reached the same level as in the control tunnel at D+4 and higher level than in the control at D+5 and D+6. Nevertheless, there were no significant differences compared to the control treatment. Colony strength parameters recorded in the control and in the tunnels where MCW-2222 was applied during or out the foraging activity were not significantly different. This indicates a very timely limited effect of the test item when applied during foraging. Residue analysis was not performed, but the study was performed in tunnels and treated crop was the only food source for bees. Exposure of bees was thus assured. Although the study was correctly performed and as such is considered acceptable, it is noted that it was performed for a short time with bee mortality observations carried out during seven days after the applications and colony assessment carried out 9 days before application and 7 days after the application. Taking this into account, the design of the study is relevant to capture acute effects of application of acetamiprid on adult bees and bee brood,</p>
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	but is not relevant to address the chronic effects.
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Data point:	KCP 10.3.1.5/02
Report:	Assessment of toxicity on honey bees (<i>Apis mellifera</i>) of the product MCW-2222 on wheat crop in a tunnel trial in France. Mamet, O., 2015a, R-35845, 223-2015
Guideline(s):	C.E.B methodology n°230, part IV
Deviations:	<p>Yes, minor deviations:</p> <p>At D0 before application, honeybee foraging in one tunnel was below the trigger value of 3/m². Although the weather conditions from D-4 to D0 were good, the foraging level in this tunnel was always lower than in the other and did not increase. In order to guarantee the homogeneity among replicates, this tunnel was distributed as follow: tunnel 6 (toxic reference) in replicate 1. Thanks to that, the mean foraging level per treatment was above 3 foraging bees per meter square at D0 before the application</p> <p>This minor deviation did not have an impact on the reliability and the outcome of the study.</p>
GLP:	Yes, certified laboratory
Acceptability:	Yes, study considered acceptable
Duplication: (if vertebrate study)	Not applicable

Executive Summary

In a semi-field study with honeybees (*Apis mellifera* L.) the potential effects of MCW-2222 on mortality, foraging activity, behaviour, colony strength, quality and quantity of brood and the amount of reserves were investigated. Flowering winter wheat served as crop (crop area: 64 m², subdivided in 4 plots and separated and surrounded by plastic lanes), which was daily sprayed with sugar syrup as artificial honeydew to make the crop attractive for foraging bees. Each tunnel was provided with a water and pollen supply. MCW-2222 was applied during bee flight (T1) and after bee flight (T2) to the crop at a rate of 0.5 L/ha (100 g a.s./ha acetamiprid). Water treated tunnels served as control (C). Tunnels, treated with Rogor plus (400 g dimethoate/ha) served as toxic reference (R). Each treatment group was three times replicated. Application in the control, the toxic reference and T1 was performed during a period of high foraging activity. T2 was applied in the evening of the same day after the end of the foraging activity.

Small honey bee colonies of approx. 10,000 bees were placed into the tunnels six days before application (D -6) to get familiar with the new conditions. Seven days after application (D +7) and being confined within the tunnels colonies were located outside the tunnels.

Assessments on mortality (via plastic sheets) were daily conducted between D -4 to D +7; by exception, dead bees were collected twice on the application day (in the morning and in the evening) in order to observe eventual acute effect. Foraging activity was daily assessed between D -4 and D +7; on the day of application during bee flight, the foraging activity was monitored 5 times (once before application, 30 minutes after application, followed by three other assessments). The assessment was conducted on the 4 plots of each tunnel. Potential clinical signs of intoxication were recorded during the period after application. Control of the colony (apiarist visit) with respect to colony strength, quality and quantity of brood (different stage observed) and the amount of reserves was carried out just before the introduction of the hives into the tunnels and just after the end of the study.

MCW-2222 applied during bee flight (T 1) triggered a statistically significant effect on daily mortality from D+1 to D+3. When applied after of bee flight (T 2), the application of MCW-2222 induced a significant difference in daily mortality at D+2 and D+3. Then the general daily mortality trend was similar to the one met in the control and the differences to the control mortality counts were not significant. Evolution of the cumulative mortality for both test item treatments had similar trends compared to the control and data were not significantly different at the end of the experimental phase. This indicates a timely limited effect of the

test item.

The foraging activity was reduced after the application of MCW-2222 during bee flight (from D0+ to D+3) with a statistically significant difference from the control. When applied after bee flight, the foraging activity was significantly reduced at D+1 and D+2. From D+4 the trend was similar to the control with lower values until the end of the trial, whereas the toxic reference dimethoate clearly triggered a longer stop of the foraging activity.

Few signs of intoxication were recorded at D+1 in the tunnels treated with MCW-2222 during bee flight. No signs of behavior abnormalities were recorded after D+2 in the tunnels treated with MCW-2222.

Colony strength parameters recorded in the control and in the tunnels for both MCW-2222 treatment groups were not significantly different. At the end of the experimental phase the adult population in the tunnels treated with MCW-2222 and the water control increased or remained stable, on the contrary the population treated with the toxic reference lost 9% of its adult bees.

Materials and methods

Materials

Test item	MCW-2222
Batch #	659-030314-01
Content of active substance	Acetamiprid 200 g/L (nominal); 199.2 ± 1.3 g/L (analysed)
Description	Yellowish liquid
Control	C: Water treated crop, applied during foraging activity
Toxic reference	R: Rogor Plus (400 g a.s./ha dimethoate), applied during the foraging activity

Test organism

Species	Honey bees (<i>Apis mellifera</i> L.) of healthy colonies with sister queens and the same age (not older than 2 years), containing about 10,000 bees per colony at test start with six frames. Hives of Dadant type. All colonies at the beginning of the study - with at 2 to 4 frames containing all brood stages - with 0 to 2 storage frames - with 0 to 3 empty frames - were free of clear clinical symptoms of disease; no treatment against <i>Varroa</i> for at least 4 weeks before beginning of the study.
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Source

Food/feeding	local beekeeper, Apistory Each tunnel was provided with a water and pollen supply. To make the crop attractive for foraging bees the crop was daily sprayed with sugar syrup as artificial honeydew (500 g/L sucrose solution, at the dose of about 500 L/ha).
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Study design and methods

Test duration	Pre-exposure phase (D -6 to D0) within the tunnels: 6 days Exposure phase (D 0 to D+7) within the tunnels: 7 days
Experimental dates	16 th May to 29 th May 2015
Test doses	Test item T1 (during bee flight): 100 g a.s./ha T2 (after bee flight): 100 g a.s./ha

Toxic reference

R (during bee flight): 400 g a.s./ha

Application of C, T1 and R was performed during daily bee flight, T2 on the same day after bee flight at BBCH 66 (full flowering) of the crop with a volume of 200 L water/ha. During application, the water and pollen containing supplies were removed to avoid contamination with spray residues.

All actual treatment rates were within ± 5% from the target application rate. Tunnels with an area of 140 m², containing 64 m² (subdivided in 4 plots and separated and surrounded by plastic lanes) of flowering winter wheat

Test units

Endpoints and assessments

(variety: Euclidean), each with one colony; tunnels equipped with a water and pollen supply.

mortality of bees:

D -4 to D+7 via plastic lanes; assessed once a day in the morning; on the application day (D0) in the morning and evening to look for possible acute effects

foraging activity:

D -4 to D+7, on the entire 4 plots/tunnel (4 x 16 m² per tunnel) assessed once a day during the flight activity of the bees; on the day of application mortality (DAT0) was assessed

- immediately before application
- 30 minutes after application, followed by three other assessments

behaviour in the tunnels and at the entrance of the hives:

at the same time when the assessment for foraging activity took place

colony strength and colony development:

once at the beginning (D -4) and once at the end (D+7) of the study; assessment of:

- estimated number of bees (colony strength)
- number of cells containing brood (total of cells with eggs, larvae and capped brood)
- presence of queens (e.g. presence of eggs)
- number of storage frames.

Group size/replicates contact

Three tunnels per treatment group

Adaptation of bees

Colonies were set-up in the tunnel on six days before application on D -6 to get familiar with the new conditions.

Environmental conditions

Natural field conditions

At the beginning of the trial, weather conditions were appropriate (from D-4 to D0), i.e. shiny days and temperature values allowing bee activity especially in the end of morning and in the afternoon, applications could be performed at D0. A thermo-hygrograph placed in a weather station recorded temperature and air humidity over the whole experimentation period. Rainfall was daily recorded from the previous day

Conditions			
	At bee flight appl.	After bee flight appl.	During exposure
Temperature:	16 °C	15 °C	2 to 21 °C
Wind speed:	3 km/h	0 km/h	not measure
Rel. humidity:	50%	60%	not reported
Precipitation:	none	none	none

Biological observations

Adult mortality, foraging activity and behaviour daily between D -4 to D +7. Assessment of condition of the colony strength and colony development D -4 and D +7.

Statistics

An analysis of variance was performed to compare the control treatment to the MCW-2222 treatments following a Student-Newmans-Keuls test (average following by the same letter are not significantly different). A transformation Log (x+1) of mortality data allow reducing the heterogeneity of variance. The toxic reference treatment was excluded as it was used for validation of the trial and not for comparison. Obtained control mortality data were related to historical control mortality data obtained by TESTAPI in

previous years using absolute and relative formula in order to assess reproducibility and if the control treatment of the study is in the range of the previous data. The calculated index (relative toxicity index; itox) for historical control data amounted to 2.03 at D+1 at 95% of confidence. Moreover, two types of relative toxicity index (itox) were calculated, one for application during the foraging activity and another one for application outside the foraging activity.

$$\text{Itox} = (\text{Mt} \times \text{Ta}) / (\text{Ma} \times \text{Tt})$$

Mt = Mortality in the considered modality after application

Ma = Mortality in the considered modality just before application

Tt= Mortality in the water control tunnel after application

Ta= Mortality in the water control tunnel just before application

Results and discussion

Biological results

Mortality

No mortality was recorded just after the hives were introduced in the tunnels (D-6). During the adaptation phase (D-4 to D0), the bee mortality tended to be stable over the time and homogeneous among tunnels and reached an acceptable level at D0 (from 136 to 142 dead bees) for performing the application.

The average mortality in the control tunnels remained low from the application date until the end of the trial except at D+6 when a small pick was noted. On the contrary the average mortality in the dimethoate tunnels increased strongly just after the application performed at T1 since it reached 1316 the day after application. This mortality was still very high at D+2 with 1171 dead bees in average. The effect of dimethoate lasted until the end of the study with mean mortality value above 300. So the results recorded in the control and toxic tunnels allow to validate the trial.

MCW-2222 applied during bee flight (T1) showed a statistically significant higher average mortality from D+1 to D+3 compared to that of the control. Nevertheless, this level of mortality observed during this period was much lower than the one recorded in the toxic reference treatment (317 versus 1316 dead bees at D+1, 329 versus 1171 dead bees at D+2 and 228 versus 450 dead bees at D+3, in average). The effect of MCW-2222 was limited in time: the mortality became statically similar to that of the control from D+4 until the end of the experimental phase.

MCW-2222 applied after bee flight (T2) showed a significant effect on mortality at D+2 and D+3 (from 142 dead bees collected at D0 to 364 and 266 in average respectively at D+2 and D+3) compared to that met in the control. However the level of this effect was not so high since the maximum value was 364 dead bees. This significant difference can be explained by the low mortality recorded in the control at D+2 and D+3. From D+4 to D+7, there was no significant difference between the control and the tunnels treated with MCW-2222. The average mortality recorded with MCW-2222 applied after bee flight was even inferior to that with the control from D+5 to D+7.

Moderate short-term effects of MCW-2222 are confirmed by the relative toxicity index (itox). Two types of relative toxicity index (itox) were calculated, one for application during bee flight and another one for application after bee flight (Figure A 24). Indeed, when the product is applied after bee flight, bees don't visit the treated crop between the application at night and the next morning when dead bees are counted at D+1, the effect is delayed by one day. Therefore for application after bee flight, it is useful to compare the mortality at D0 to the one assessed at D+2.

This itox was very high for Dimethoate 400 EC (it reached 9.8 and 9.9 according to the timing of application of the test item). It was moderate for MCW-2222 during bee flight (2.4 to 2.8) and for MCW-2222 applied after bee flight (3.1). It has to be noted that the low mortality met in control tunnels at D+1 and D+2 had impacted the itox values.

The average cumulative mortality after application was by far lower in the MCW-2222 tunnels than in the toxic reference tunnels. Compared to the water tunnels, it was superior in the MCW-2222 tunnels but the cumulative mortality induced by MCW-2222 was not significantly different from the one met in the control at D+7. Moreover, it has to be noted that the application of MCW-2222 had no effect on the evolution of the mortality over the time.

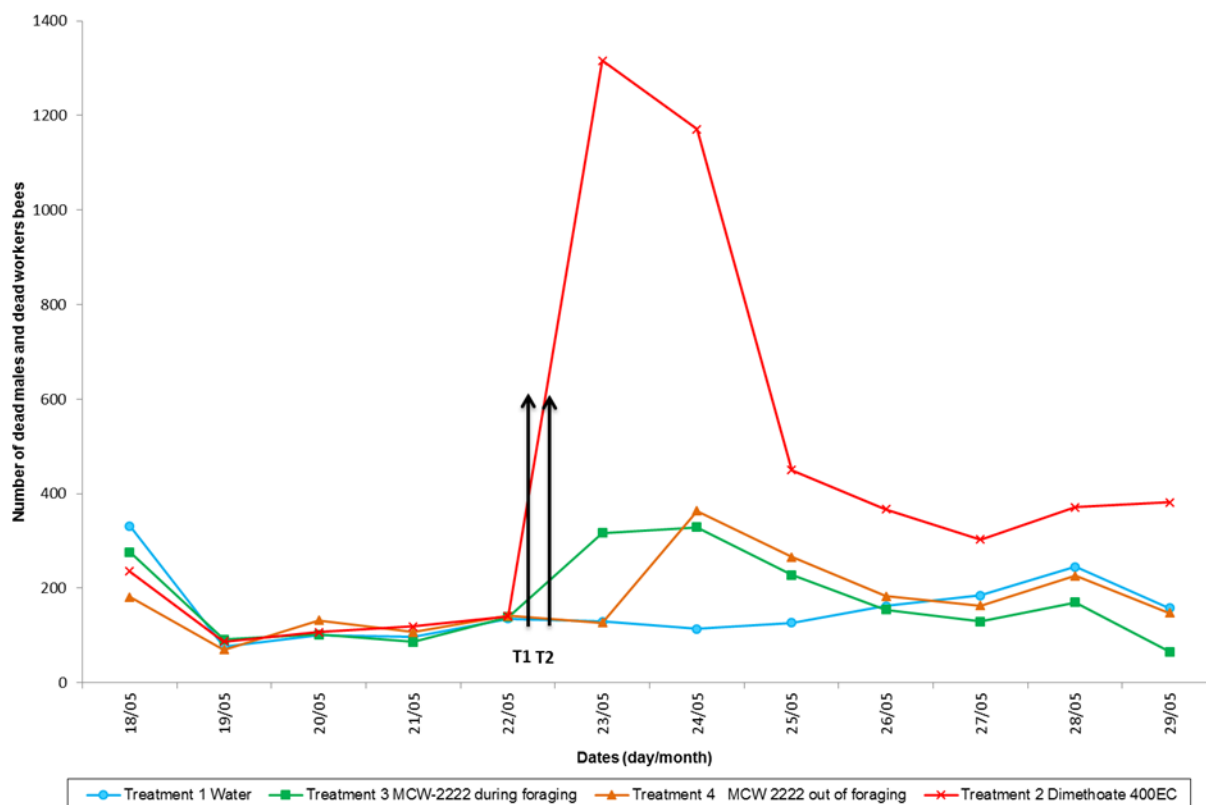


Figure A 24: Total daily mortality

Table A 93: Total daily mortality

Dates (day/month, D±x) x= delay from application day	Average number of dead bees			
	Treatment 1	Treatment 3 (T 1)	Treatment 4 (T 2)	Treatment 2
	Water	MCW-2222 during foraging	MCW-2222 out of foraging	Dimethoate 400EC
18/05 D-4	332	276	181	236
19/05 D-3	77	92	70	87
20/05 D-2	101	102	132	107
21/05 D-1	98	86	107	119
22/05 D0	136	139	142	141
23/05 D0+ +D+1	130	317	127	1316
24/05 D+2	114	329	364	1171
25/05 D+3	127	228	266	450
26/05 D+4	162	155	183	367
27/05 D+5	185	130	163	303
28/05 D+6	245	170	226	371
29/05 D+7	158	66	148	382
Cumulative mortality after application date to 04/06	1121	1395	1477	4360

← Application T1 and T2

Mortality reported on 22/05 was recorded immediately prior to the application.

Mortality reported on 23/05 is the sum of the mortality recorded on 22/05 just after the application and the mortality recorded on 23/05.

Table A 94: Relative toxicity index

Treatments	Time after Treatment	I tox Value*	
		I tox ₁ (D+1 versus D0) During foraging	I tox ₂ (D+2 versus D0) Out of foraging
Treatment 1 Water		1.0	1.0
Treatment 3 MCW 2222 during bee flight (T1)		2.4	2.8
Treatment 4 MCW 2222 after bee flight (T2)		Not relevant	3.1
Treatment 2 Dimethoate 400EC		9.8	9.9

$$* I_{tox} \text{ value} = (M_t \times T_a) / (M_a \times T_t)$$

Foraging activity

On the day of application, the bee activity was high (from 5.2 to 7.2 bees/m²) and always superior to the required level (3 bees/m²).

The foraging activity in the control tunnels declined after the application T1 from 6.8 up to 1.9 bees/m² in average. This activity was higher the following days and the number of bees/m² was above 3 up to 9.8 bees/m².

A very severe impact on foraging activity was met in the toxic reference tunnels since this activity decreased significantly just after the application at T1 and then it was stopped from D+1 until the end of the test (the repellence was so high that bees stayed in their hives).

MCW-2222 applied at 0.5 L/ha during bee flight (T1) showed an impact on the foraging activity the day of the application (D0). But just the day after (D+1), few honeybees came back on the crop plots notably at the end of the morning when the temperature was higher. The foraging activity reached a correct level from D+4. Statistical analysis showed significant differences between control and MCW-2222 treatments during foraging at D0+, D+1, D+2 and D+3.

The foraging activity in the tunnel when MCW-2222 was applied after bee flight (T 2) decreased on 22/05 afternoon to reach 2.1 bees/me² due to climate conditions, which was similarly observed in the control. Two days after the application, the foraging activity was very low in the morning and was higher in the afternoon. The foraging activity reached an acceptable level from D+4 (above 3 bees/m²). Significant differences between control and MCW-2222 applied out of the bee presence were found at D+1 and D+2.

At D+7 (29/05) the foraging activity decreased in the control and MCW-2222 tunnels.

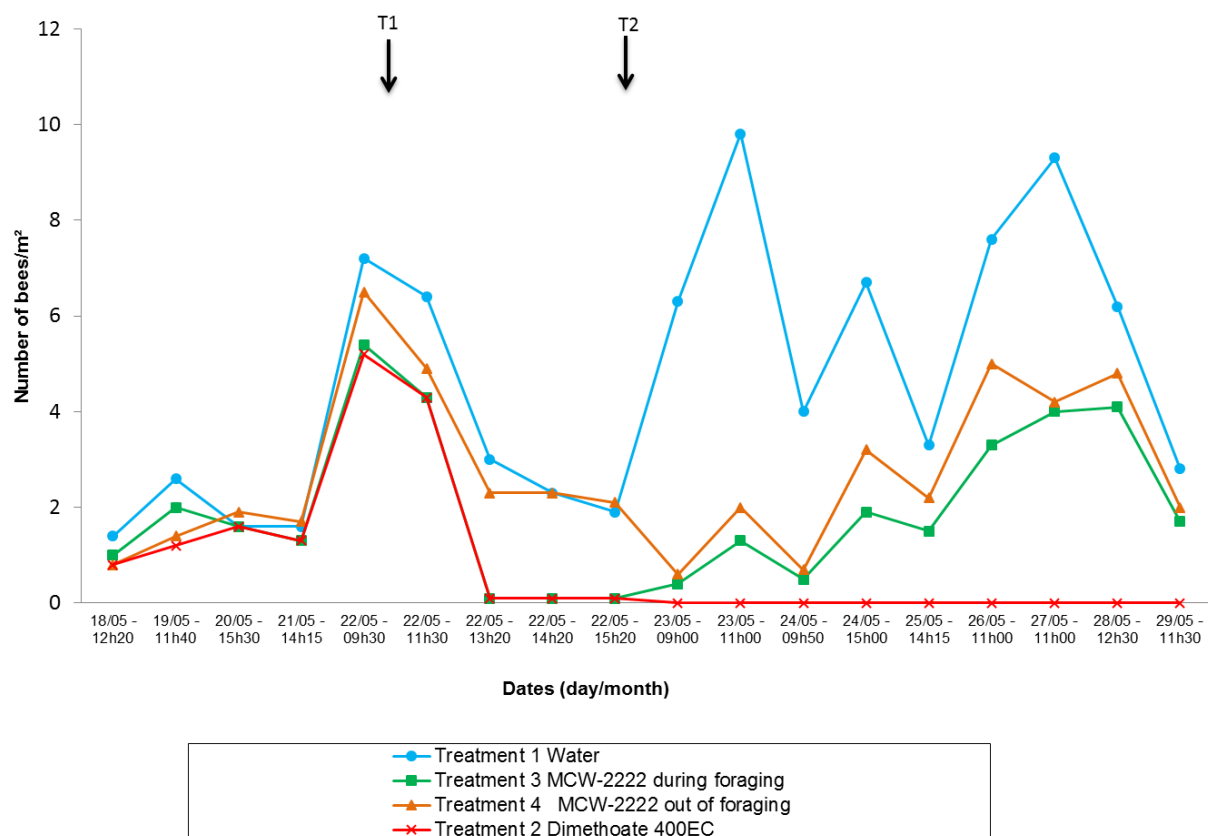


Figure A 25: Foraging activity - Average number of bees/m²
Table A 95: Foraging activity - average number of bees/m²

Dates (day/month-hours) x= delay from application day	Average number of bees/m ²			
	Treatment 1	Treatment 3 (T 1)	Treatment 4 (T 2)	Treatment 2
	Water	MCW-2222 during foraging	MCW-2222 out of foraging	Dimethoate 400EC
D-4 18/05 - 12:20	1.4	1.0	0.8	0.8
D-3 19/05 - 11:40	2.6	2.0	1.4	1.2
D-2 20/05 - 15:30	1.6	1.6	1.9	1.6
D-1 21/05 - 10:30	3.7	3.1	3.2	3.1
D-1 21/05 - 14:15	1.6	1.3	1.7	1.3
D0 22/05 - 09:30	7.2	5.4	6.5	5.2
D0+ 22/05 - 11:30	6.4	4.3	4.9	4.3
D0+ 22/05 - 13:20	3.0	0.1	2.3	0.1
D0+ 22/05 - 14:20	2.3	0.1	2.3	0.1
D0+ 22/05 - 15:20	1.9	0.1	2.1	0.1
D+1 23/05 - 09:00	6.3	0.4	0.6	0.0
D+1 23/05 - 11:00	9.8	1.3	2.0	0.0
D+2 24/05 - 09:50	4.0	0.5	0.7	0.0
D+2 24/05 - 15:00	6.7	1.9	3.2	0.0
D+3 25/05 - 14:15	3.3	1.5	2.2	0.0
D+4 26/05 - 11:00	7.6	3.3	5.0	0.0
D+5 27/05 - 11:00	9.3	4.0	4.2	0.0
D+6 28/05 - 12:30	6.2	4.1	4.8	0.0
D+7 29/05 - 11:30	2.8	1.7	2.0	0.0
D-4 18/05 - 12:20	1.4	1.0	0.8	0.8

← Application T1

← Application T2

Behaviour

Clinic signs of intoxication were recorded in the toxic reference treatment.

In the tunnels treated with MCW-2222 during bee flight, bees hesitated to forage the crop for 30 minutes after the application and a few bees presented clinic signs of intoxication in the next hours. One day later, very few bees presented those signs and behaviour. Then the behaviour was considered normal until the end of the trial. In the tunnels treated with MCW-2222 after bee flight, clinic signs of intoxication were recorded at D+1 and bees still hesitated to forage the crop at D+2. No other behaviour abnormalities were recorded after D+2.

Colony strength and colony development

At the end of the experimental phase, the adult population in the tunnels treated with MCW-2222 after bee flight and in the water control tunnels increased (5% of increase for control, 36% MCW-2222 treatment) and was stable in the tunnels treated with MCW-2222 during bee flight. Differences in the evolution of the population of adult honeybees would be also linked to the evolution of number of brood cells: e.g. if the amount of brood decreased and the population increased at the same time, it means that brood hatched and provided new worker honeybees. This was the case in the control and the MCW-2222 tunnels where the item product was applied after bee flight.

On the contrary, the population treated with the toxic reference decreased slightly and lost 9% of its adult bees.

Concerning the number of brood cells, it decreased during the trial period in all tunnels due to the experimental conditions with small colonies under tunnel (food resources in tunnels are sufficient to maintain healthy colonies for 2 to 3 weeks only). For this reason this type of test is not appropriate to study the brood evolution and no conclusion can be made from these data.

Endpoints

Whereas temporary effects on adult mortality, foraging activity and behaviour (few bees with signs of intoxication on D+1) occurred after the application MCW-2222 during (T1) and after (T2) bee flight at a

rate of 100 g a.s./ha, no impact on the colony strength as well on the colony conditions was observed.

Validity criteria

As shown in the following table, all validity criteria were met.

Table A 96: Validity criteria

Validity criteria according to CEB 230 (2012), part IV	Observed in study
Before treatment:	
Daily mortality must have stabilised. In practice, this stabilisation is achieved three to six days after the hives are placed in the tunnels	Achieved
Daily mortality on the last day before treatment must not exceed 300 bees	136 to 142 dead bees/replicate in the respective treatment groups
Daily mortality must be similar in different tunnels, i.e. the deviation from average mortality on the day before application must not exceed 60%	C: - 12% to +7% T1: -36% to +40% T2: -30% to +46% R: -21% to +22%
Foraging activity must be greater than five bees / m ² on flowering plants and three bees / m ² on wheat shortly before application	C: 6.8 bees/m ² T1: 4.8 bees/m ² T2: 5.7 bees/m ² , assessed during bee flight 0 bees/m ² , assessed after bee flight R: 4.8 bees/m ²
Foraging activity in different tunnels must be comparable.	Achieved
After treatment:	
Mortality in the water control must be comparable before and after treatment, i.e. mortality the day after treatment must not exceed the average mortality figures for the day before treatment by more than 50%	Itox at D+1: 1.0 Itox at D+2: 0.8
The daily mortality in the "toxic benchmark" tunnel the day after treatment must be at least five times as high as the mortality figure for the day before treatment	Itox at D+1: 9.8 Itox at D+2: 9.9
Weather conditions must remain favourable	Achieved
All other factors regarded as abnormal in the conduct of the test	Achieved

Conclusion

In a semi-field study honeybees (*Apis mellifera* L.) were exposed to MCW-2222 (a.s. acetamiprid) applied at rate of 100 g a.s./ha during (T1) and after (T2) bee flight according to CEB 230 (2003), investigating potential effects on adult mortality, foraging activity and behaviour, conditions of the colonies (i.e. colony strength and colony development). Flowering winter wheat served as crop (crop area: 64 m², subdivided in 4 plots and separated and surrounded by plastic lanes), which was daily sprayed with sugar syrup as artificial honeydew to make the crop attractive for foraging bees.

MCW-2222 applied during the foraging activity (T 1), triggered a statistically significant effect on daily mortality from D+1 to D+3. When applied after bee flight activity (T 2) MCW-2222 triggered a statically significant effect on daily mortality at D+2 and D+3. Then, the general daily mortality was similar to the one met in the control and the differences to the control mortality counts were not significant.

Evolution of the cumulative mortality for both MCW-2222 treatments had similar evolution compared to the control one and was not significantly different at the end of the experimental phase (D+7).

The toxicity index was moderate for both MCW-2222 treatments whereas it was high for the reference dimethoate.

Regarding the foraging activity, in the tunnels where MCW-2222 was applied during the foraging activity a light repellent effect was observed until D+3. By comparison it was recorded until D+2 when MCW-2222 was applied out of the presence of bees. Afterwards the level of the foraging activity reached a correct level of around 3 bees/m² from 4 days after application and the trend remained comparable to the control. On the contrary the application of the toxic reference dimethoate clearly triggered a stop of this foraging activity until the end of the trial.

The colonies strength and development were not impacted by the application of MCW-2222 treatments.

A 2.3.1.7.3 KCP 10.3.1.5/03 Tunnel CEB study with honey bees on wheat – 3

Comments of zRMS:	<p>The study has been already evaluated and accepted by the zRMS in the course of the first zonal evaluation of CA3573 (formerly MCW-2222) finalised in April 2018. As the test guideline has not changed since that time, re-evaluation of the study following renewal of acetamiprid was not necessary and most of presented below conclusions were taken from the Core Assessment, Part B, Section 6 of April 2018.</p> <p>The study was performed in the Northern France and comprised applications to winter wheat sprayed with sugar syrup (simulating honeydew) performed at two timings:</p> <ul style="list-style-type: none"> – during bee foraging activity: water control, MCW-2222 at 100 g a.s./ha and the reference dimethoate, – out of the presence of forager bees: MCW-2222 at 100 g a.s./ha at night. <p>In these experimental conditions, MCW-2222 applied in the bee presence triggered a statistically significant effect on daily mortality at D+1 only. Then the general daily mortality trend was similar to this observed in control and the differences to the control mortality counts were not significant. When applied out of the bee presence, the product MCW-2222 showed no significant difference in any daily mortality count until the end of the trial. No signs of intoxication were recorded at D+1 in the tunnels treated with MCW-2222 during or out of the bee presence. Foraging behavior abnormalities were recorded during a short time on the day of application, just after spraying, and the day after application when treated out of the bee presence. No signs of behavioural abnormalities were recorded after D+2. The foraging activity was significantly lower just after the application of MCW-2222 during foraging and the effect lasted until D+1. When MCW-2222 was applied out of the foraging activity, a significant difference to the control was observed at D+, but the mean foraging activity stayed stable compared to the previous day in this treatment while in control it was increased. From D+3 the foraging activity increased and reached the same level as in the control tunnel at D+4 and higher level than in the control at D+5 and D+6. Nevertheless, there were no significant differences compared to the control treatment. Colony strength parameters recorded in the control and in the tunnels where MCW-2222 was applied during or out the foraging activity were not significantly different. This indicates a very timely limited effect of the test item when applied during foraging. Residue analysis was not performed, but the study was performed in tunnels and treated crop was the only food source for bees. Exposure of bees was thus assured.</p> <p>Although the study was correctly performed and as such is considered acceptable, it is noted that it was performed for a short time with bee mortality observations carried out during seven days after the applications and colony assessment carried out 9 days before application and 7 days after the application. Taking this into account, the design of the study is relevant to capture acute effects of application of acetamiprid on adult bees and bee brood, but is not relevant to address the chronic effects.</p>
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Data point:	KCP 10.3.1.5/03
Report:	Assessment of toxicity on honey bees (<i>Apis mellifera</i>) of the product MCW-2222 on wheat crop in a tunnel trial in France. Mamet, O., 2015b, R-35846, 224-2015, 217-2014
Guideline(s):	C.E.B methodology n°230, part IV
Deviations:	None
GLP:	Yes, certified laboratory
Acceptability:	Yes, study considered acceptable
Duplication: (if vertebrate study)	Not applicable

Executive Summary

In a semi-field study with honeybees (*Apis mellifera* L.) the potential effects of MCW-2222 on mortality, foraging activity, behaviour, colony strength, quality and quantity of brood and the amount of reserves were

investigated. Flowering winter wheat served as crop (crop area: 64 m², subdivided in 4 plots and separated and surrounded by plastic lanes), which was daily sprayed with sugar syrup as artificial honeydew to make the crop attractive for foraging bees. Each tunnel was provided with a water and pollen supply. MCW-2222 was applied during bee flight (T1) and after bee flight (T2) to the crop at a rate of 0.5 L/ha (100 g a.s./ha acetamiprid). Water treated tunnels served as control (C). Tunnels, treated with Rogor plus (400 g dimethoate/ha) served as toxic reference (R). Each treatment group was three times replicated. Application in the control, the toxic reference and T1 was performed during a period of high foraging activity. T2 was applied in the evening of the same day after the end of the foraging activity.

Small honey bee colonies of approx. 10,000 bees were placed into the tunnels nine days before application (D -9) to get familiar with the new conditions. Seven days after application (D +7) and being confined within the tunnels colonies were located outside the tunnels.

Assessments on mortality (via plastic sheets) were daily conducted between D -8 to D +7; by exception, dead bees were collected twice on the application day (in the morning and in the evening) in order to observe eventual acute effect. Foraging activity was daily assessed between D -7 and D +7; on the day of application during bee flight, the foraging activity was monitored 5 times (once before application, 30 minutes after application, followed by three other assessments). The assessment was conducted on the 4 plots of each tunnel. Potential clinical signs of intoxication were recorded during the period after application. Control of the colony (apiarist visit) with respect to colony strength, quality and quantity of brood (different stage observed) and the amount of reserves was carried out just before the introduction of the hives into the tunnels and just after the end of the study.

MCW-2222 applied during bee flight (T 1) triggered a statistically significant effect on daily mortality at D+1 only. Then the general daily mortality trend was similar to the one met in the control and the differences to the control mortality counts were not significant. When applied after of bee flight (T 2), the product MCW-2222 showed no significant difference in any daily mortality count during until the end of the trial. Evolution of the cumulative mortality for both test item treatments had similar trends compared to the control and data were not significantly different at the end of the experimental phase. This indicates a very timely limited effect of the test item when applied during bee flight.

The foraging activity was significantly lower just after the application of MCW-2222 during bee flight and the effect lasted until D+1. When MCW-2222 was applied after bee flight, a significant difference to the control was observed at D+1 as the mean foraging activity stayed stable compared to the previous day in this treatment but was lower than the one recorded in the control one which increased. From D+3 this foraging activity increased and reached the same level as in the control tunnel at D+4 and even a higher level than in the control at D+5 and D+6, nevertheless there were no significant differences compared to the control treatment. The toxic reference dimethoate clearly triggered a longer stop of the foraging activity. No signs of intoxication were recorded at D+1 in the tunnels treated with MCW-2222 during or after bee flight. Foraging behavior abnormalities were recorded on the day of application, just after spraying during a short time and the day after application when treated out of the bee presence. No signs of behaviour abnormalities were recorded after D+2.

Colony strength parameters recorded in the control and in the tunnels where MCW-2222 was applied during or after bee flight were not significantly different. At the end of the experimental phase the adult population in the tunnels treated with MCW-2222 and the water control increased, on the contrary the populations treated with the toxic reference decrease.

Materials and methods

Materials

Test item	MCW-2222
Batch #	659-030314-01
Content of active substance	Acetamiprid 200 g/L (nominal); 199.2 ± 1.3 g/L (analysed)
Description	Yellowish liquid
Control	C: Water treated crop, applied during foraging activity
Toxic reference	R: Rogor Plus (400 g a.s./ha dimethoate), applied during the foraging activity

Test organism

Species	Honey bees (<i>Apis mellifera</i> L.) of healthy colonies with sister queens and the same age (not older than 2 years), containing about 10,000 bees per colony at test start with six frames. Hives of Dadant type. All colonies at the beginning of the study
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	<ul style="list-style-type: none">- with at 2 to 4 frames containing all brood stages- with 0 to 2 storage frames- with 0 to 3 empty frames- were free of clear clinical symptoms of disease; no treatment against <i>Varroa</i> for at least 4 weeks before beginning of the study.
Source	local beekeeper, Apistroy
Food/feeding	Each tunnel was provided with a water and pollen supply. To make the crop attractive for foraging bees the crop was daily sprayed with sugar syrup as artificial honeydew (500 g/L sucrose solution, at the dose of about 500 L/ha).
Study design and methods	
Test duration	Pre-exposure phase (D -8 to D0) within the tunnels: 8 days Exposure phase (D 0 to D+7) within the tunnels: 7 days
Experimental dates	9 th June to 25 th June 2015
Test doses	Test item T1 (during bee flight): 100 g a.s./ha T2 (after bee flight): 100 g a.s./ha Toxic reference R (during bee flight): 400 g a.s./ha Application of C, T1 and R was performed during daily bee flight, T2 on the same day after bee flight at BBCH 73 (early milk stage) of the crop with a volume of 200 L water/ha. During application, the water and pollen containing supplies were removed to avoid contamination with spray residues. All actual treatment rates were within $\pm 5\%$ from the target application rate.
Test units	Tunnels with an area of 140 m ² , containing 64 m ² (subdivided in 4 plots and separated and surrounded by plastic lanes) of flowering winter wheat (variety: Canabro), each with one colony; tunnels equipped with a water and pollen supply.
Endpoints and assessments	<i>mortality of bees:</i> D -8 to D+7 via plastic lanes; assessed once a day in the morning; on the application day (D0) in the morning and evening to look for possible acute effects <i>foraging activity:</i> D -7 to D+7, on the entire 4 plots/tunnel (4 x 16 m ² per tunnel) assessed once a day during the flight activity of the bees; on the day of application mortality (DAT0) was assessed <ul style="list-style-type: none">- immediately before application- 30 minutes after application, followed by three other assessments <i>behaviour in the tunnels and at the entrance of the hives:</i> at the same time when the assessment for foraging activity took place <i>colony strength and colony development:</i> once at the beginning (D -9) and once at the end (D+7) of the study; assessment of: <ul style="list-style-type: none">- estimated number of bees (colony strength)- number of cells containing brood (total of cells with eggs, larvae and capped brood)- presence of queens (e.g. presence of eggs)- number of storage frames.
Group size/replicates contact	Three tunnels per treatment group

Adaptation of bees

Colonies were set-up in the tunnel on nine days before application on D -9 to get familiar with the new conditions.

Environmental conditions

Natural field conditions

At the beginning of the trial, weather conditions were bad with cloudy and rainy days. Then, from June 16th (D-2) and until the end of the trial, dry weather and sufficient temperature values allowed the bee activity and permitted to perform the applications on June 18th. Meteorological data were collected from the nearest weather station recording daily minimal and maximal temperature and rainfall

Conditions			
	At bee flight appl.	After bee flight appl.	During exposure
Temperature:	21 °C	18 °C	9 to 27 °C
Wind speed:	1 to 2 km/h	0 km/h	not measured
Rel. humidity:	68 %	70 %	not reported
Precipitation:	none	none	none

Biological observations

Adult mortality was recorded daily between D -8 to D +7 and foraging activity and behaviour daily between D -7 to D +7. Assessment of condition of the colony strength and colony development D -9 and D +7.

Statistics

An analysis of variance was performed to compare the control treatment to the MCW-2222 treatments following a Student-Newmans-Keuls test (average following by the same letter are not significantly different). A transformation Log (x+1) of mortality data allow reducing the heterogeneity of variance. The toxic reference treatment was excluded as it was used for validation of the trial and not for comparison. Obtained control mortality data were related to historical control mortality data obtained by TESTAPI in previous years using absolute and relative formula in order to assess reproducibility and if the control treatment of the study is in the range of the previous data. The calculated index (relative toxicity index; itox) for historical control data amounted to 2.03 at D+1 at 95% of confidence. Moreover, two types of relative toxicity index (itox) were calculated, one for application during the foraging activity and another one for application outside the foraging activity.

$$\text{Itox} = (\text{Mt} \times \text{Ta}) / (\text{Ma} \times \text{Tt})$$

Mt = Mortality in the considered modality after application

Ma = Mortality in the considered modality just before application

Tt= Mortality in the water control tunnel after application

Ta= Mortality in the water control tunnel just before application

Results and discussion

Biological results

Mortality

During the adaptation phase (D-8 to D0), bee mortality tended to be stable over the time and homogeneous among tunnels and reached an acceptable level at D0 (from 148 to 225 dead bees) for performing the application.

The average mortality in the control tunnels remained moderate and regular from the application date until the end of the trial.

On the contrary the average mortality in the dimethoate tunnels increased strongly just after the application performed at T1 since it reached 1986 the day after application. This mortality was still high at D+2 and D+3 with respectively 626 and 508 dead bees in average. So the results recorded in the control and toxic tunnels allow to validate the trial.

MCW-2222 applied during bee flight (T1) showed a statistically significant higher average mortality only at D+1 compared to that of the control. Nevertheless, this level of mortality observed during this period was much lower than the one recorded in the toxic reference treatment (364 versus 1986 dead bees at D+1). The effect of MCW-2222 was limited in time: the mortality became statically similar to that of the control

from D+2 until the end of the experimental phase.

MCW-2222 after bee flight (T2) showed a slight increase of the mortality only at D+2. Compared to the control there was no statically significant difference at all the assessment timings.

Moderate short-term effects of MCW-2222 are confirmed by the relative toxicity index (itox). Two types of relative toxicity index (itox) were calculated, one for application during bee flight and another one for application after bee flight (Table A 97) Indeed when the product is applied after bee flight, bees don't visit the treated crop between the application at night and the next morning when dead bees are counted at D+1, the effect is delayed by one day. Therefore for application after bee flight, it is useful to compare the mortality at D0 to the one assessed at D+2.

The itox value was very high for Dimethoate 400 EC at D+1 because it reached 11.3. It was moderate at D+1 for MCW-2222 applied during bee flight (2.4) and equal to the control one at D+2. The itox value was low for MCW-2222 applied after bee flight (1.2).

The average cumulative mortality after application of MCW-2222 was by far lower in the MCW-2222 tunnels than in the toxic reference tunnels. Compared to the water tunnels, the cumulative mortality induced by MCW-2222 was not significantly different from the control at D+7. Moreover, it was inferior in the MCW-2222 tunnels when the product was applied after bee flight than in the control tunnels. The curves of MCW-2222 after bee flight and control are superimposed over the time proving the no effect of this treatment on bee mortality.

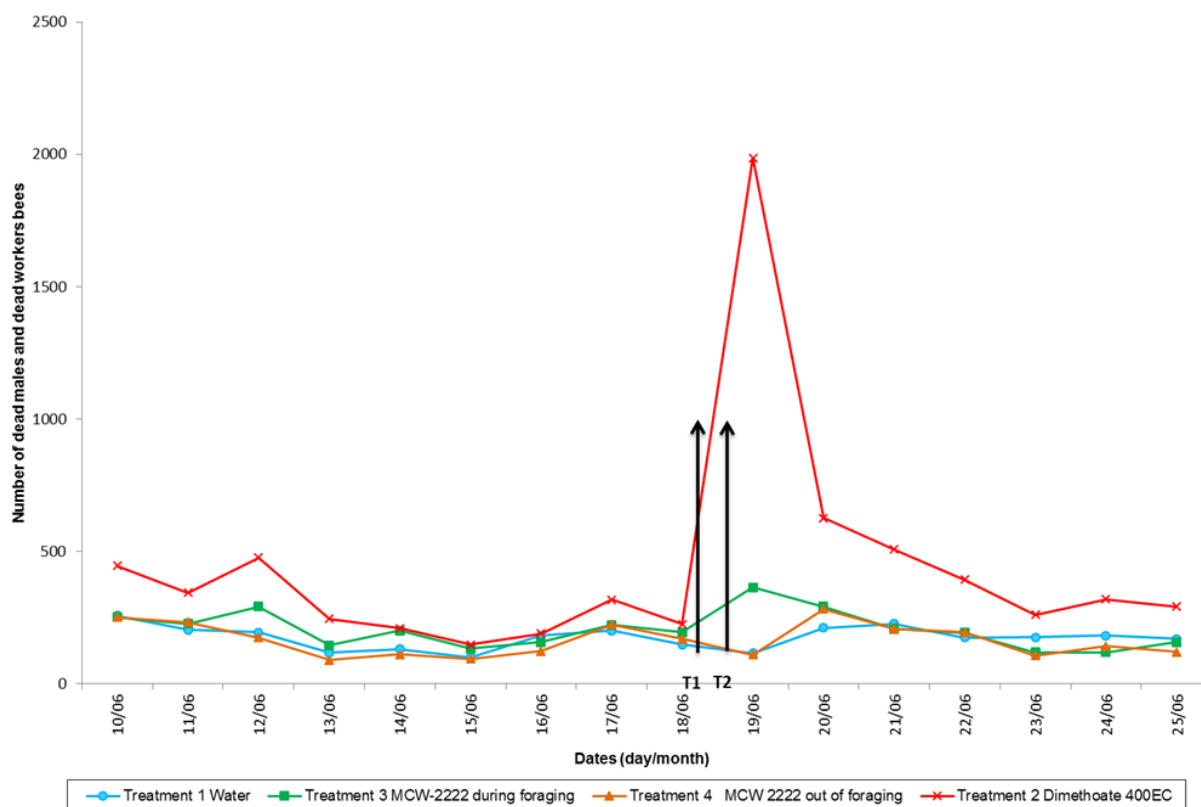


Figure A 26: Total daily mortality

Table A 97: Total daily mortality

Dates (day/month, D±x) x= delay from application day	Average number of dead bees			
	Treatment 1	Treatment 3 (T 1)	Treatment 4 (T 2)	Treatment 2
	Water	MCW-2222 during foraging	MCW-2222 out of foraging	Dimethoate 400EC
10/06 D-8	258	251	252	446
11/06 D-7	204	227	233	344
12/06 D-6	194	292	174	476
13/06 D-5	117	145	90	246
14/06 D-4	131	201	111	210
15/06 D-3	101	133	94	148

Dates (day/month, D±x) x= delay from application day	Average number of dead bees			
	Treatment 1	Treatment 3 (T 1)	Treatment 4 (T 2)	Treatment 2
	Water	MCW-2222 during foraging	MCW-2222 out of foraging	Dimethoate 400EC
16/06 D-2	182	158	124	190
17/06 D-1	201	222	224	318
18/06 D0	148	195	169	225
19/06 D0+ +D+1	116	364	109	1986
20/06 D+2	212	291	282	626
21/06 D+3	227	209	207	508
22/06 D+4	174	193	195	394
23/06 D+5	176	119	107	260
24/06 D+6	182	119	142	320
25/06 D+7	169	158	120	291
Cumulative mortality after application date to 19/06	1256	1453	1162	4385

← Application T1
and T2

Mortality reported on 18/06 was recorded immediately prior to the application.

Mortality reported on 19/06 is the sum of the mortality recorded on 18/06 just after the application and the mortality recorded on 19/06.

Table A 98: Relative toxicity index

Treatments	Time after Treatment	I tox Value*	
		I tox ₁ (D+1 versus D0) During foraging	I tox ₂ (D+2 versus D0) Out of foraging
Treatment 1 Water		1.0	1.0
Treatment 3 MCW 2222 during bee flight (T1)		2.4	1.0
Treatment 4 MCW 2222 after bee flight (T2)		Not relevant	1.2
Treatment 2 Dimethoate 400EC		11.3	1.9

* I tox value = (Mt x Ta) / (Ma x Tt)

Foraging activity

On the day of application, the bee activity was high (from 4.3 to 6.3 bees/m² in average) and always superior to the required level (3 bees/m²).

The foraging activity in the control tunnels declined after the application of T1 from 5.5 up to 2.1 bees/m². This activity was higher the following days and the number of bees/m² was above 3 up to 5.3 bees/m². This activity in the control and the MCW-2222 tunnels decreased at the end of the study (D+6 and D+7) due to the attractiveness of the sugar syrup that dropped as the syrup became dried quickly during these warm days.

A very severe impact on foraging activity was met in the toxic reference tunnels since it was close to 0 just after the application at T1 and then it was stopped from D+1 until the end of the test (the repellence was so high that bees stayed in their hives).

MCW-2222 during the bee flight (T1) showed an impact on the foraging activity just few hours after the application at T1. This activity increased afterward the day of application in the afternoon. The following days the foraging activity continued to increase and was respectively and equal and superior to that in the control at D+2 and from D+3. The difference between the foraging activity in those MCW-2222 tunnels and that in the control ones were statically significant just after the application and at D+1.

The foraging activity in the tunnel when MCW-2222 applied after bee flight was at the same level after the application at T2 (D+1) as before (D0+), whereas it increased in the control tunnels leading to a statistically significant difference between both treatments only at this date of assessment. From D+3 this activity increased and reached the same level as in the control tunnel from D+4 and a higher level than in the control ones from D+5.

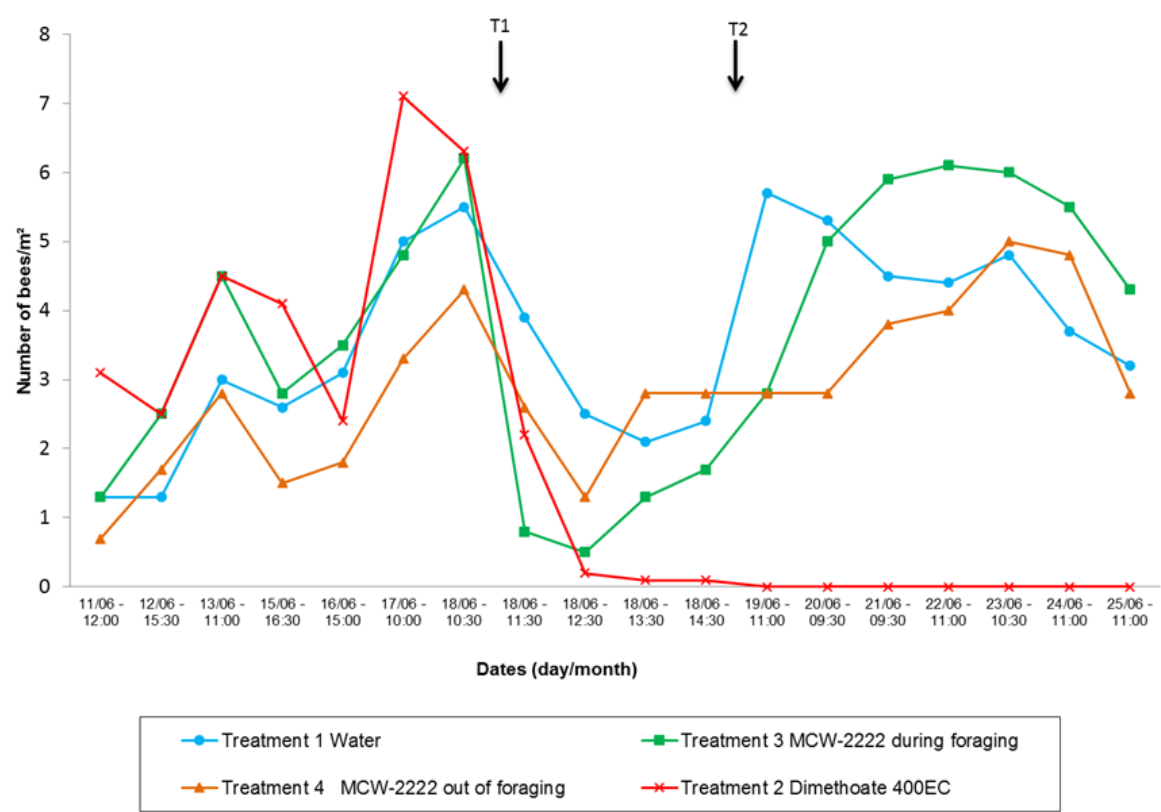


Figure A 27: Foraging activity - Average number of bees/m²

Table A 99: Foraging activity - average number of bees/m²

Dates	Average number of bees/m ²			
(day/month-hours) x= delay from application day	Treatment 1	Treatment 3 (T 1)	Treatment 4 (T 2)	Treatment 2
	Water	MCW-2222 during foraging	MCW-2222 out of foraging	Dimethoate 400EC
D-7 11/06 - 12:00	1.3	1.3	0.7	3.1
D-6 12/06 - 15:30	1.3	2.5	1.7	2.5
D-5 13/06 - 11:00	3.0	4.5	2.8	4.5
D-4 14/06 -	No assessment due to rain			
D-3 15/06 - 16:30	2.6	2.8	1.5	4.1
D-2 16/06 - 15:00	3.1	3.5	1.8	2.4
D-1 17/06 - 10:00	5.0	4.8	3.3	7.1
D0 18/06 - 10:30	5.5	6.2	4.3	6.3
D0+ 18/06 - 11:30	3.9	0.8	2.6	2.2
D0+ 18/06 - 12:30	2.5	0.5	1.3	0.2
D0+ 18/06 - 13:30	2.1	1.3	2.8	0.1
D0+ 18/06 - 14:30	2.4	1.7	2.8	0.1
D+1 19/06 - 11:00	5.7	2.8	2.8	0.0
D+2 20/06 - 09:30	5.3	5.0	2.8	0.0
D+3 21/06 - 09:30	4.5	5.9	3.8	0.0
D+4 22/06 - 11:00	4.4	6.1	4.0	0.0
D+5 23/06 - 10:30	4.8	6.0	5.0	0.0
D+6 24/06 - 11:00	3.7	5.5	4.8	0.0
D+7 25/06 - 11:00	3.2	4.3	2.8	0.0

← Application T1

← Application T2

Behaviour

Clinic signs of intoxication were recorded in the toxic reference treatment.

In the tunnels treated with MCW-2222 during bee flight, bees hesitated to forage the crop for 30 minutes after the application and no bees presented clinic signs of intoxication in the next hours. One day later, behavior was considered normal until the end of the trial. In the tunnels treated with MCW-2222 after bee flight, no clinic signs of intoxication was noted and bees still hesitated to forage the crop at D+2. No other behavior abnormalities were recorded after D+2.

Colony strength and colony development

At the end of the experimental phase, the adult population in the tunnels treated with MCW-2222 and the water control increased (23% of increase for control, 8% for MCW-2222 when the product was applied during bee flight, 45% for MCW-2222 treatment when the product was applied outside the foraging activity). On the contrary, the population treated with the toxic reference decreased and lost 37% of its adult bees.

Concerning the number of brood cells, it decreased significantly during the trial period in all tunnels due to experimental conditions with small colonies under tunnels (food resources in tunnels are sufficient to maintain healthy colonies for 2 to 3 weeks only). For this reason this type of test is not appropriate to study the brood evolution and no conclusion can be made from these data.

Endpoints

Whereas temporary effects on adult mortality, foraging activity and behaviour occurred after the application MCW-2222 during (T1) and after (T2) bee flight at a rate of 100 g a.s./ha, no impact on the colony strength as well on the colony conditions was observed.

Validity criteria

As shown in the following table, all validity criteria were met.

Table A 100: Validity criteria

Validity criteria according to CEB 230 (2012), part IV	Observed in study
Before treatment:	
Daily mortality must have stabilised. In practice, this stabilisation is achieved three to six days after the hives are placed in the tunnels	Achieved
Daily mortality on the last day before treatment must not exceed 300 bees	148 to 225 dead bees/replicate in the respective treatment groups
Daily mortality must be similar in different tunnels, i.e. the deviation from average mortality on the day before application must not exceed 60%	C: - 15% to +46% T1: -22% to +33% T2: -5% to +6% R: -24% to +27%
Foraging activity must be greater than five bees / m ² on flowering plants and three bees / m ² on wheat shortly before application	C: 5.5 bees/m ² T1: 6.2 bees/m ² T2: 4.3 bees/m ² , assessed during bee flight 0 bees/m ² , assessed after bee flight R: 6.3 bees/m ²
Foraging activity in different tunnels must be comparable.	Achieved
After treatment:	
Mortality in the water control must be comparable before and after treatment, i.e. mortality the day after treatment must not exceed the average mortality figures for the day before treatment by more than 50%	Itox at D+1: 0.8 Itox at D+2: 1.4
The daily mortality in the "toxic benchmark" tunnel the day after treatment must be at least five times as high as the mortality figure for the day before treatment	Itox at D+1: 11.3 Itox at D+2: 1.9
Weather conditions must remain favourable	Achieved
All other factors regarded as abnormal in the conduct of the test	Achieved

Conclusion

In a semi-field study honeybees (*Apis mellifera* L.) were exposed to MCW-2222 (a.s. acetamiprid) applied at rate of 100 g a.s./ha during (T1) and after (T2) bee flight according to CEB 230 (2003), investigating potential effects on adult mortality, foraging activity and behaviour, conditions of the colonies (i.e. colony strength and colony development). Flowering winter wheat served as crop (crop area: 64 m², subdivided in 4 plots and separated and surrounded by plastic lanes), which was daily sprayed with sugar syrup as artificial honeydew to make the crop attractive for foraging bees.

MCW-2222 applied during bee flight (T 1), triggered a statistically significant effect on daily mortality only at D+1. From D+2 the mortality level was statically equivalent to that met with the control treatment. When applied after bee flight activity (T 2) MCW-2222 presented the same level of mortality as the control whatever the timing of assessment.

Cumulative mortality for both MCW-2222 treatments had similar evolution compared to the control and was not significantly different at the end of the experimental phase (D+7). Moreover, it was lower in the MCW-2222 tunnels when the product was applied after bee flight than in the control tunnels. The curves of MCW-2222 applied after bee flight and control are superimposed over the time proving the no effect of this treatment on bee mortality.

The toxicity index was moderate at D+1 for MCW-2222 during the bee flight and equal to the control one at D+2. The itox value was low for MCW-2222 applied after bee flight.

Regarding the foraging activity, MCW-2222 applied during bee flight (T1) showed an impact on the foraging activity just few hours after the application at T1. The activity increased afterwards on the day of application in the afternoon. On the following days the foraging activity continued to increase and was respectively equal and superior to that in the control from D+3. The difference between the foraging activity in those MCW-2222 tunnels and that in the control ones are statically significant just after the application and at D+1.

The foraging activity in the tunnel when MCW-2222 was applied after bee flight (T 2) was at the same level after the application as before, whereas it increased in the control tunnels leading to a statically significant difference between both treatments only at this date of assessment. From D+3 this activity increased and reached the same level as in the control tunnel from D+4 and a higher level than in the control ones from D+5

The colonies strength and development were not impacted by the application of MCW-2222 treatments.

A 2.3.1.7.4 KCP 10.3.1.5/04 Tunnel CEB study with honey bees on *Phacelia* - 1

Comments of zRMS:	<p>The study has been already evaluated and accepted by the zRMS in the course of the first zonal evaluation of CA3573 (formerly MCW-2222) finalised in April 2018. Although the test guideline has not changed since that time, the study has been re-evaluated by the zRMS in 2021 .</p> <p>The study was performed in the Northern France and comprised applications to flowering <i>Phacelia</i> performed at two timings:</p> <ul style="list-style-type: none"> – during bee foraging activity: water control, MCW-2222 at 100 g a.s./ha and the reference dimethoate, – out of the presence of forager bees: MCW-2222 at 100 g a.s./ha at night. <p>In these experimental conditions, MCW-2222 applied at 0.5 L/ha during the foraging activity induced a slight effect on daily mortality at D+1. On remaining days of the study, the differences to the control were not significant. When applied out of the bee presence, there was no significant difference compared to the control throughout the whole trial period. From D+2 to the end of the experimental period, the general daily mortality trend recorded in the two MCW-2222 treatments was similar to the one observed in control. No signs of intoxication were recorded at D+1 in the tunnels treated with MCW-2222 during or out of the bee presence.</p> <p>No impact on the foraging activity was observed in both treatment groups when compared to the activity observed in the control treatment.</p> <p>Colony strength parameters recorded in the control and in the tunnels where MCW-2222 was applied during or out the foraging activity were not significantly different.</p> <p>Residue analysis was not performed, but the study was performed in tunnels and treated crop was the only food source for bees. Exposure of bees was thus assured.</p> <p>Although the study was correctly performed and as such is considered acceptable, it is noted that it was performed for a short time with bee mortality observations carried out during six days after the applications and colony assessment carried out 12 days before application and 7 days after the application. Taking this into account, the design of the study is relevant to capture acute effects of application of acetamiprid on adult bees and bee brood, but is not relevant to address the chronic effects.</p>
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Data point: KCP 10.3.1.5/04

Report: Assessment of toxicity on honey bees (*Apis mellifera*) of the product MCW-2222 (acetamiprid 200 g/L) applied under insect proof tunnels on a phacelia crop in Northern France. Mamet, O. & Molitor, C., 2015b, R-34875, 217-2014

Guideline(s): C.E.B methodology n°230, part IV

Deviations: Yes, minor deviations:
At D0 before application, honeybee mortality in one tunnel was above the trigger value of 300 individuals. As the weather conditions at D-1 were improved after a period of rainy days (D-6 to D-4 and D-2), a high foraging activity was recorded (over 10 bees/m²) explaining the fact that more dead bees than expected could be found at D0 (i.e. 337 dead bees in this tunnel). Whatever the distribution of the tunnels in the several treatments could be, mean mortality level per treatment is always below 300 dead bees at D0 before the application,

This minor deviation did not have an impact on the reliability and the outcome of the study.

GLP: Yes, certified laboratory

Acceptability: Yes, study considered acceptable

Duplication: Not applicable
(if vertebrate study)

Executive Summary

In a semi-field study with honeybees (*Apis mellifera* L.) the potential effects of MCW-2222 on mortality, foraging activity, behaviour, colony strength, quality and quantity of brood and the amount of reserves were investigated. Flowering *Phacelia* served as crop (crop area: 64 m², subdivided in 4 plots and separated and surrounded by plastic lanes). Each tunnel was provided with a water and pollen supply. MCW-2222 was applied during bee flight (T1) and after bee flight (T2) to the crop at a rate of 0.5 L/ha (100 g a.s./ha acetamiprid). Water treated tunnels served as control (C). Tunnels, treated with Rogor plus (400 g dimethoate/ha) served as toxic reference (R). Each treatment group was three times replicated. Application in the control, the toxic reference and T1 was performed during a period of high foraging activity. T2 was applied in the evening of the same day after the end of the foraging activity.

Small honey bee colonies of approx. 10,000 bees were placed into the tunnels nine days before application (D -9) to get familiar with the new conditions. Six days after application (D +6) and being confined within the tunnels colonies were located outside the tunnels.

Assessments on mortality (via plastic sheets) were daily conducted between D -7 to D +6; by exception, dead bees were collected twice on the application day (in the morning and in the evening) in order to observe eventual acute effect. Foraging activity was daily assessed between D -7 and D +5; on the day of application during bee flight, the foraging activity was monitored 5 times (two times before application, 30 minutes after application, followed by two other assessments). The assessment was conducted on the 4 plots of each tunnel. Potential clinical signs of intoxication were recorded during the period after application. Control of the colony (apiarist visit) with respect to colony strength, quality and quantity of brood (different stage observed) and the amount of reserves was carried out before the introduction of the hives into the tunnels and just after the end of the study.

MCW-2222 applied during bee flight (T 1) induced a small effect on daily mortality at D+1. All the other days, the differences to the control were not significant. When applied after of bee flight (T 2), there was no significant difference compared to the control all along the trial. From D+2 to the end of the experimental period, the general daily mortality trend recorded in the two MCW-2222 treatments was similar to the one met in the control.

Evolution of the cumulative mortality for both test item treatments had similar trends compared to the control and data were not significantly different at the end of the experimental phase.

The toxicity index is a value expressed relatively to the control mortality data. The difference between MCW-2222 applied during foraging activity or out foraging and the control treatment at D+1 was not significant.

The application of MCW-2222 during and after bee flight had no impact on the foraging activity when compared to the activity observed in the control treatment. From D0 the trend was similar to the control until the end of the trial with no significant differences, whereas the toxic reference dimethoate clearly triggered a stop of the foraging activity.

Behaviour and colony strength parameters recorded in the control and in both MCW-2222 treatment groups were not different. At the end of the experimental phase populations grew in all treatments and were therefore able to enlarge their further development.

Materials and methods

Materials

Test item	MCW-2222
Batch #	93191024
Content of active substance	Acetamiprid 20% (nominal); 19.8% (analysed)
Description	Yellowish liquid
Control	C: Water treated crop, applied during foraging activity
Toxic reference	R: Rogor Plus (400 g a.s./ha dimethoate), applied during the foraging activity

Test organism

Species Honey bees (*Apis mellifera* L.) of healthy colonies with sister queens and the same age (not older than 2 years), containing about 10,000 bees per colony at test start with six frames. Hives of Dadant type.
All colonies at the beginning of the study

	<ul style="list-style-type: none"> - with at 2 to 4 frames containing all brood stages - with 0 to 2 storage frames - with 0 to 2 empty frames - were free of clear clinical symptoms of disease; no treatment against <i>Varroa</i> for at least 4 weeks before beginning of the study.
Source	local beekeeper, GAEC Mélibocage
Food/feeding	Full flowering <i>Phacelia tanacetifolia</i> served as food supply, no additional feeding throughout the study. Each tunnel was provided with a water supply.
Study design and methods	
Test duration	Pre-exposure phase (D -10 to D0) within the tunnels: 10 days Exposure phase (D 0 to D+7) within the tunnels: 7 days
Experimental dates	23 rd June to 9 th July 2014
Test doses	Test item T1 (during bee flight): 100 g a.s./ha T2 (after bee flight): 100 g a.s./ha
	Toxic reference R (during bee flight): 400 g a.s./ha
	Application of C, T1 and R was performed during daily bee flight, T2 on the same day after bee flight at BBCH 65 (full flowering of <i>Phacelia</i>) of the crop with a volume of 200 L water/ha. During application, the water supplies were removed to avoid contamination with spray residues. All actual treatment rates were within $\pm 5\%$ from the target application rate.
Test units	Tunnels with an area of 140 m ² , containing 64 m ² (subdivided in 4 plots and separated and surrounded by plastic lanes) of flowering <i>Phacelia tanacetifolia</i> (variety: Meva), each with one colony; tunnels equipped with a water supply.
Endpoints and assessments	<i>mortality of bees:</i> D -7 to D+6 via plastic lanes; assessed once a day in the morning; on the application day (D0) in the morning and evening to look for possible acute effects <i>foraging activity:</i> D -7 to D+5, on the entire 4 plots/tunnel (4 x 16 m ² per tunnel) assessed once a day during the flight activity of the bees; on the day of application mortality (DAT0) was assessed <ul style="list-style-type: none"> - approx. 1.5 hours before application - immediately before application - 30 minutes after application, followed by two other assessments <i>behaviour in the tunnels and at the entrance of the hives:</i> at the same time when the assessment for foraging activity took place <i>colony strength and colony development:</i> once at the beginning (D -12) and once at the end (D+7) of the study; assessment of: <ul style="list-style-type: none"> - estimated number of bees (colony strength) - number of cells containing brood (total of cells with eggs, larvae and capped brood) - presence of queens (e.g. presence of eggs) - number of storage frames.
Group size/replicates contact	Three tunnels per treatment group

Adaptation of bees

Colonies were set-up in the tunnel on ten days before application on D -10 to get familiar with the new conditions.

Environmental conditions

Natural field conditions

At the beginning of the trial, weather conditions were inclement as it was often cloudy with some storms. Applications were performed when conditions were appropriate, with shiny days and sufficient temperature values to allow bee activity. A thermo-hygrograph placed in a weather station recorded temperature and air humidity over the whole experimentation period. Rainfall was daily recorded from the previous day.

Conditions			
	At bee flight appl.	After bee flight appl.	During exposure
Temperature	23 °C	21 °C	11 to 32 °C
Wind speed	0 km/h	0 km/h	not measured
Rel. humidity	49 %	70 %	not reported
Precipitation	none	none	D+2 (3 mm) D+3 (6 mm) D+4 (1 mm) D+5 (13 mm) D+7 (2 mm)

Biological observations

Adult mortality was recorded daily between D -7 to D +6 and foraging activity and behaviour daily between D -7 to D +5. Assessment of condition of the colony strength and colony development D -12 and D +7.

Statistics

An analysis of variance was performed to compare the control treatment to the MCW-2222 treatments following a Student-Newmans-Keuls test (average following by the same letter are not significantly different). A transformation Log (x+1) of mortality data allow reducing the heterogeneity of variance. The toxic reference treatment was excluded as it was used for validation of the trial and not for comparison.

Obtained control mortality data were related to historical control mortality data obtained by TESTAPI in previous years using absolute and relative formula in order to assess reproducibility and if the control treatment of the study is in the range of the previous data. The calculated index (relative toxicity index; itox) for historical control data amounted to 2.1 at D+1 at 95% of confidence or 2.4 at 99% confidence. Moreover, two types of relative toxicity index (itox) were calculated, one for application during the foraging activity and another one for application outside the foraging activity.

$$\text{Itox} = (\text{Mt} \times \text{Ta}) / (\text{Ma} \times \text{Tt})$$

Mt = Mortality in the considered modality after application

Ma = Mortality in the considered modality just before application

Tt= Mortality in the water control tunnel after application

Ta= Mortality in the water control tunnel just before application

Results and discussion

Biological results

Mortality

During the adaptation phase (D-7 to D0), bee mortality tended to be stable over the time and homogeneous among tunnels and reached an acceptable level at D0 (from 186 to 256 dead bees) for performing the application.

The average mortality in the control tunnels remained low to moderate from the application date until the end of the trial (159 to 236).

On the contrary the average mortality in the dimethoate tunnel increased strongly just after the application performed at T1 since it reached 2520. Moreover the impact of dimethoate on bee mortality was in average still high until D+3 with respectively 919 at D+2 and 487 at D+3. So the results recorded in the control and toxic tunnels allow to validate the trial.

MCW-2222 applied during bee flight (T1) showed a very slight increase of the mortality at D+1 (from 256

dead bees at D0 to 312 the day after application). Despite this small increase, the average mortality at D+1 was significantly different from that met in the control tunnels because in those last tunnels the average mortality decreased from D0 to D+1. The level of mortality found at D+1 with MCW-2222 applied during bee flight can also be considered as low when we consider the high foraging activity assessed at D0 (19.5 bees/m² in average). Then, the mortality decreased already at D+2 (mean of 193 dead bees) to a regular level of mortality comparable to the one met in the control treatment (until the end of the trial).

MCW-2222 after bee flight (T2) induced no effect on mortality (from 186 dead bees at D+1 to 162 at D+2 in average). Compared to the control, there was no significant difference during the whole experimental phase.

The lack of effect of MCW-2222 on mortality is also confirmed by the low values of the relative toxicity index (itox). Two types of relative toxicity index (itox) were calculated, one for application during the foraging activity and another one for application outside the foraging activity. Indeed when the product is applied after bee flight, bees don't visit the treated crop between the application at night and the next morning when dead bees are counted at D+1, the effect is delayed by one day.

Therefore for application after bee flight, it is useful to compare the mortality at D0 to the one assessed at D+2.

Although this itox was very high for Dimethoate 400 EC since it reached 14.4 and 5.1 according to the timing of application of the test item, it was very low for MCW-2222 (1.4 and 0.9), compared to the control (1.0). The main information resulting from this index calculation is the very limited toxicity of the test item MCW-2222 applied during bee flight and the absence of impact of this test item when it was outside of bee flight.

The average cumulative mortality after application was by far lower in the MCW-2222 tunnels than in the toxic reference tunnel. The cumulative mortality induced by MCW-2222 whatever the timing of application was closed to that of the control tunnels was not significantly different from that of the control at D+6 after application.

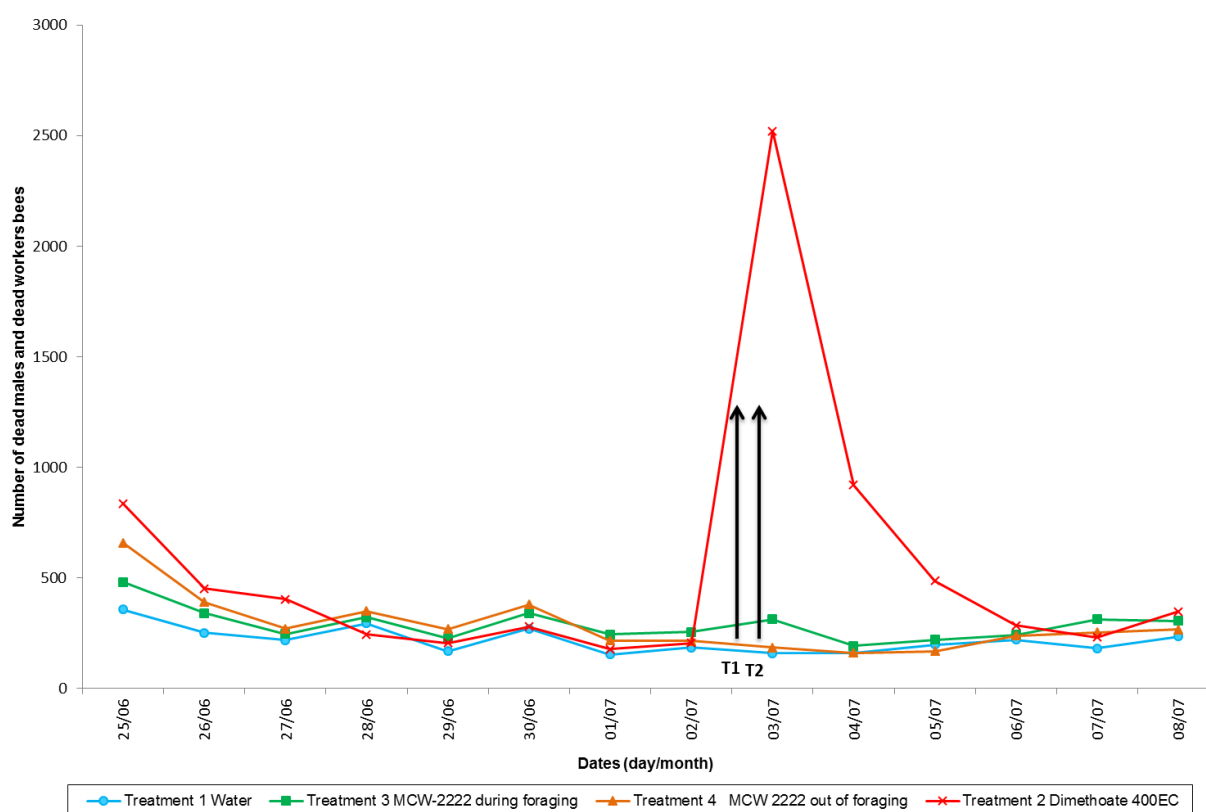


Figure A 28: Total daily mortality

Table A 101: Total daily mortality

Dates (day/month, D±x) x= delay from application day	Average number of dead bees			
	Treatment 1	Treatment 3 (T 1)	Treatment 4 (T 2)	Treatment 2
	Water	MCW-2222 during foraging	MCW-2222 out of foraging	Dimethoate 400EC
25/06 D-7	358	482	659	837
26/06 D-6	252	341	391	452
27/06 D-5	219	247	271	404
28/06 D-4	295	322	349	245
29/06 D-3	169	226	268	206
30/06 D-2	270	340	380	279
01/07 D-1	154	244	216	177
02/07 D0	186	256	215	205
03/07 D0+ +D+1	159	312	186	2520
04/07 D+2	162	193	162	919
05/07 D+3	199	220	168	487
06/07 D+4	221	243	238	286
07/07 D+5	182	313	253	232
08/07 D+6	236	306	266	348
Cumulative mortality after application date to 08/07	1159	1587	1273	4792

← Application T1
and T2

Mortality reported on 02/07 was recorded immediately prior to the application.

Mortality reported on 03/07 is the sum of the mortality recorded on 02/07 just after the application and the mortality recorded on 03/07.

Table A 102: Relative toxicity index

Treatments	Time after Treatment	I tox Value*	
		I tox ₁ (D+1 versus D0) During foraging	I tox ₂ (D+2 versus D0) Out of foraging
Treatment 1 Water		1.0	1.0
Treatment 3 MCW 2222 during bee flight (T1)		1.4	0.9
Treatment 4 MCW 2222 after bee flight (T2)		Non relevant	0.9
Treatment 2 Dimethoate 400EC		14.4	5.1

* I tox value = (Mt x Ta) / (Ma x Tt)

Foraging activity

The day of the application, the bee activity was high (from 16 to 23 bees/m² at D0) and always superior to the required level (5 bees/m²).

The foraging activity in the water tunnel was good and stable from the application T1 to the end of the test (the variations are mainly due to weather conditions).

A very severe impact on foraging activity was met in the toxic reference tunnels since this activity decreased significantly just after the application at T1 and then it was stopped from D+1 until the end of the test (the repellence was so high that bees stayed in their hives).

All along the trial, the foraging activity was similar between the tunnels sprayed with the test item and the water control. The decrease met in all the tunnels from D+2 to D+4 was due to weather conditions. No effect was observed on the foraging activity after application of MCW-2222 at 0.5 L/ha on the crop neither during the foraging activity nor outside the foraging activity.

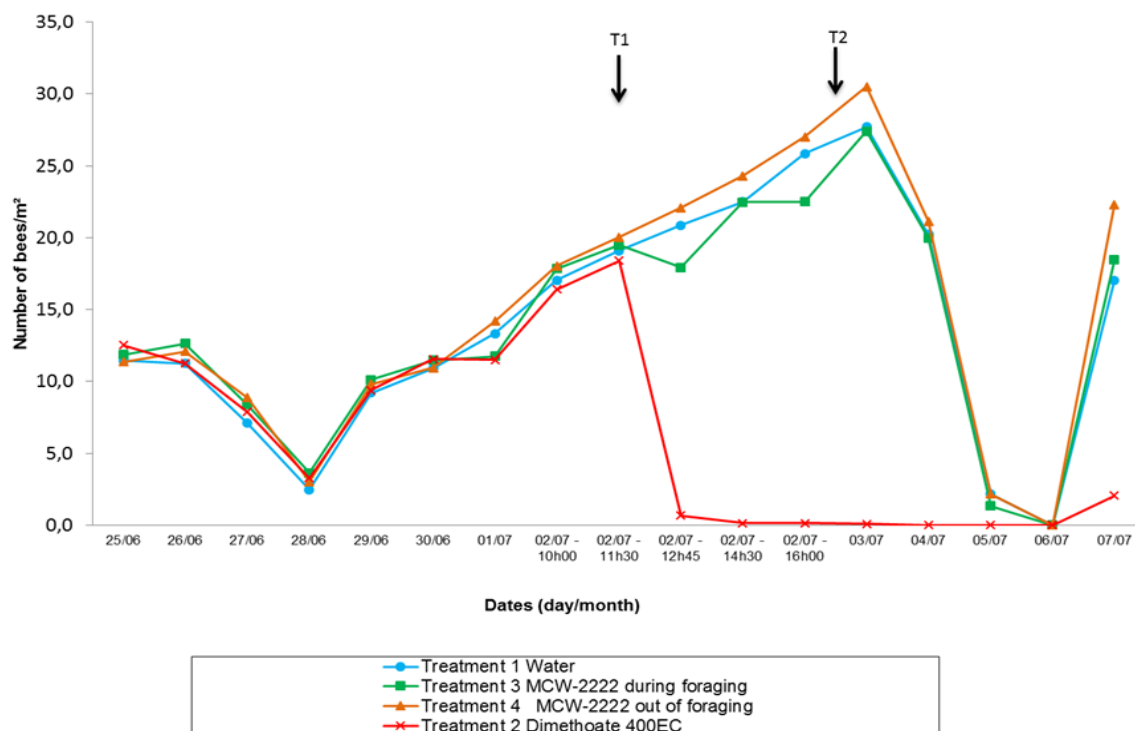


Figure A 29: Foraging activity - Average number of bees/m²
Table A 103: Foraging activity - average number of bees/m²

Dates	Average number of bees/m ²			
(day/month-hours)	Treatment 1	Treatment 3 (T 1)	Treatment 4 (T 2)	Treatment 2
x= delay from application day	Water	MCW-2222 during foraging	MCW-2222 out of foraging	Dimethoate 400EC
D-7 25/06 - 14:30	11.4	11.9	11.4	12.5
D-6 26/06 - 11:00	11.3	12.7	12.1	11.3
D-5 27/06 - 10:30	7.1	8.4	8.9	7.9
D-4 28/06 - 10:00	2.5	3.6	3.0	3.3
D-3 29/06 - 12:00	9.2	10.1	9.8	9.4
D-2 30/06 - 10:30	10.9	11.5	10.9	11.6
D-1 01/07 - 10:00	13.3	11.8	14.2	11.5
D0 02/07 - 10:00	17.1	17.8	18.0	16.4
D0 02/07 - 11:30	19.1	19.5	20.0	18.4
D0+ 02/07 - 12:45	20.9	17.9	22.1	0.7
D0+ 02/07 - 14:30	22.5	22.5	24.3	0.2
D0+ 02/07 - 16:00	25.9	22.5	27.0	0.2
D+1 03/07 - 10:30	24.6	26.0	26.8	0.2
D+1 03/07 - 14:30	30.8	28.9	34.3	0.0
D+2 04/07 - 12:00	20.2	20.0	21.2	0.0
D+3 05/07 - 10:00	2.2	1.3	2.2	0.0
D+4 06/07	No assessment due to rain			
D+5 07/07 - 15:00	17.0	18.5	22.3	2.1

← Application T1

← Application T2

Behaviour

No clinic signs of intoxication were recorded in the control and MCW-2222 tunnels whereas some were recorded in the toxic reference tunnels.

Colony strength and colony development

All the colonies were well provided with brood in early trial. As usual in this kind of test with small colonies under tunnels, the confinement didn't induce enough eggs lying from the queens so the number of brood cells decreased drastically in all treatments including the water control.

In all hives, the adult bee population grew from about 32% (toxic reference treatment) to 90% in the control treatment. This population evolution of adult honeybees was linked to the evolution of the number of brood cells: e.g. if the amount of brood decreased and the adult population increased during the same time, this means that brood hatched and provided new worker honeybees. This is the case in all treatments in this study.

Endpoints

Whereas a slight and temporary effect on adult mortality was observed when MCW-2222 was applied at a rate of 100 g a.s./ha during bee flight (T1), no effects on foraging activity, behaviour, the colony strength as well on the colony conditions were observed. When MCW-2222 was applied after bee flight (T 2) at a rate of 100 g a.s./ha, no effects were observed at all.

Validity criteria

As shown in the following table, all validity criteria were met.

Table A 104: Validity criteria

Validity criteria according to CEB 230 (2012), part IV	Observed in study
Before treatment:	
Daily mortality must have stabilised. In practice, this stabilisation is achieved three to six days after the hives are placed in the tunnels	Achieved
Daily mortality on the last day before treatment must not exceed 300 bees	186 to 215 dead bees/replicate in the respective treatment groups
Daily mortality must be similar in different tunnels, i.e. the deviation from average mortality on the day before application must not exceed 60%	C: - 46% to +26% T1: -19% to +32% T2: -31% to +22% R: -30% to +21%
Foraging activity must be greater than five bees / m ² on flowering plants and three bees / m ² on wheat shortly before application	C: 18.1 bees/m ² T1: 18.7 bees/m ² T2: 19.0 bees/m ² , assessed during bee flight 0 bees/m ² , assessed after bee flight R: 17.4 bees/m ²
Foraging activity in different tunnels must be comparable.	Achieved
After treatment:	
Mortality in the water control must be comparable before and after treatment, i.e. mortality the day after treatment must not exceed the average mortality figures for the day before treatment by more than 50%	Itox at D+1: 0.85 Itox at D+2: 0.87
The daily mortality in the "toxic benchmark" tunnel the day after treatment must be at least five times as high as the mortality figure for the day before treatment	Itox at D+1: 14.4 Itox at D+2: 5.1
Weather conditions must remain favourable	Achieved, except on D+3 and D+4
All other factors regarded as abnormal in the conduct of the test	Achieved

Conclusion

In a semi-field study honeybees (*Apis mellifera* L.) were exposed to MCW-2222 (a.s. acetamiprid) applied at rate of 100 g a.s./ha during (T1) and after (T2) bee flight according to CEB 230 (2003), investigating potential effects on adult mortality, foraging activity and behaviour, conditions of the colonies (i.e. colony strength and colony development). Flowering *Phacelia tanacetifolia* served as crop (crop area: 64 m², subdivided in 4 plots and separated and surrounded by plastic lanes), which was daily sprayed with sugar syrup as artificial honeydew to make the crop attractive for foraging bees.

MCW-2222 applied during bee flight (1) induced a slight but significant increase in the number of dead bees at D+1. At all other assessment days, the mortality was not significantly different from that met in the control.

When MCW-2222 was applied after bee flight (T2), no significant difference in mortality counts with those met in the control treatment was found from D0 to the end of the trial.

MCW-2222 whatever the timing of application had no significant effect on cumulative mortality, toxicity index, and foraging activity.

The colonies strength and development were not impacted by MCW-2222 applied during or after bee flight.

A 2.3.1.7.5 KCP 10.3.1.5/05 Tunnel CEB study with honey bees on *Phacelia* - 2

Comments of zRMS:	<p>The study has been already evaluated and accepted by the zRMS in the course of the first zonal evaluation of CA3573 (formerly MCW-2222) finalised in April 2018. Although the test guideline has not changed since that time, the study has been re-evaluated by the zRMS in 2021.</p> <p>The study was performed in the Northern France and comprised applications to flowering <i>Phacelia</i> performed at two timings:</p> <ul style="list-style-type: none"> <input type="checkbox"/> during bee foraging activity: water control, MCW-2222 at 100 g a.s./ha and the reference dimethoate, <input type="checkbox"/> out of the presence of forager bees: MCW-2222 at 100 g a.s./ha at night. <p>In these experimental conditions, when MCW-2222 was applied at 0.5 L/ha during the foraging activity or out of the bee presence, the general daily mortality trend was similar to this observed in control and there was no significant difference in the daily number of dead bees recorded compared to the control. No signs of intoxication were recorded at D+1 in the tunnels treated with MCW-2222 during or out of the bee presence. Application of MCW-2222 had no impact on the foraging activity in both treatment groups when compared to the activity observed in the control treatment.</p> <p>Colony strength parameters recorded in the control and in the tunnels where MCW-2222 was applied during or out the foraging activity were not significantly different.</p> <p>Residue analysis was not performed, but the study was performed in tunnels and treated crop was the only food source for bees. Exposure of bees was thus assured.</p> <p>Although the study was correctly performed and as such is considered acceptable, it is noted that it was performed for a short time with bee mortality observations carried out during six days after the applications and colony assessment carried out 10 days before application and 5 days after the application. Taking this into account, the design of the study is relevant to capture acute effects of application of acetamiprid on adult bees and bee brood, but is not relevant to address the chronic effects.</p>
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Data point:	KCP 10.3.1.5/05
Report:	Assessment of toxicity on honey bees (<i>Apis mellifera</i>) of the product MCW-2222 (acetamiprid 200 g/L) applied under insect proof tunnels on a phacelia crop during summer in France. Mamet, O. & Molitor, C., 2015c, R-34876, 218-2014
Guideline(s):	C.E.B methodology n°230, part IV
Deviations:	<p>Yes, minor deviations:</p> <p>At D0 before application, honeybee mortality under one tunnel was above the trigger value of 300 individuals fixed by the CEB method number 230, part IV. The colony under tunnel No.1 was stronger than other ones except the hive in the tunnel No. 7 (higher bee population, higher number of brood cells) and therefore the daily mortality in this tunnel was higher than the other ones, from the first mortality assessment at D-6 to D0. The concerned tunnel (with 330 dead bees collected at D0) was chosen to be one of the three, replicates of the control treatment and the mean mortality was below 300 dead bees at D0 before the application (mean of 235 dead bees).</p> <p>This minor deviation did not have an impact on the reliability and the outcome of the study.</p>
GLP:	Yes, certified laboratory
Acceptability:	Yes, study considered acceptable
Duplication:	Not applicable

(if vertebrate study)

Executive Summary

In a semi-field study with honeybees (*Apis mellifera* L.) the potential effects of MCW-2222 on mortality, foraging activity, behaviour, colony strength, quality and quantity of brood and the amount of reserves were investigated. Flowering *Phacelia* served as crop (crop area: 64 m², subdivided in 4 plots and separated and surrounded by plastic lanes). Each tunnel was provided with a water and pollen supply. MCW-2222 was applied during bee flight (T1) and after bee flight (T2) to the crop at a rate of 0.5 L/ha (100 g a.s./ha acetamiprid). Water treated tunnels served as control (C). Tunnels, treated with Rogor plus (400 g dimethoate/ha) served as toxic reference (R). Each treatment group was three times replicated. Application in the control, the toxic reference and T1 was performed during a period of high foraging activity. T2 was applied in the evening of the same day after the end of the foraging activity.

Small honey bee colonies of approx. 10,000 bees were placed into the tunnels nine days before application (D -9) to get familiar with the new conditions. Seven days after application (D +6) and being confined within the tunnels colonies were located outside the tunnels.

Assessments on mortality (via plastic sheets) were daily conducted between D -8 to D +6; by exception, dead bees were collected twice on the application day (in the morning and in the evening) in order to observe eventual acute effect. Foraging activity was daily assessed between D -8 and D +5; on the day of application during bee flight, the foraging activity was monitored 5 times (two times before application, 30 minutes after application, followed by two other assessments). The assessment was conducted on the 4 plots of each tunnel. Potential clinical signs of intoxication were recorded during the period after application. Control of the colony (apiarist visit) with respect to colony strength, quality and quantity of brood (different stage observed) and the amount of reserves was carried out before the introduction of the hives into the tunnels and just after the end of the study.

When MCW-2222 was applied during (T1) or after bee flight (T2), the general daily mortality trend was similar to the one met in the control and there was no any significant difference in the daily number of dead bees recorded compared to the control one all along the trial.

Evolution of the cumulative mortality for both test item treatments had similar trends compared to the control and data were not significantly different at the end of the experimental phase.

The toxicity index is a value expressed relatively to the control mortality data. The indexes of the MCW-2222 treatment groups were not significantly different from the control at D+1 and D+2.

The application of MCW-2222 during (T1) or after bee flight (T2), had no impact on the foraging activity when compared to the activity observed in the control treatment. From D0 the trend was similar to the control until the end of the trial with no significant differences, whereas the toxic reference dimethoate clearly triggered a stop of the foraging activity.

Behavior and colony strength parameters recorded in the control and in both MCW-2222 treatment groups were not different. At the end of the experimental phase populations grew in the test item treatments and were therefore able to enlarge their further development.

Materials and methods

Materials

Test item	MCW-2222
Batch #	93191024
Content of active substance	Acetamiprid 20% (nominal); 19.8% (analysed)
Description	Yellowish liquid
Control	C: Water treated crop, applied during foraging activity
Toxic reference	R: Rogor Plus (400 g a.s./ha dimethoate), applied during the foraging activity

Test organism

Species	Honey bees (<i>Apis mellifera</i> L.) of healthy colonies with sister queens and the same age (not older than 2 years), containing about 10,000 bees per colony at test start with six frames. Hives of Dadant type. All colonies at the beginning of the study - with at 2 to 4 frames containing all brood stages - with 0 to 2 storage frames - with 0 to 2 empty frames
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	<p>- were free of clear clinical symptoms of disease; no treatment against <i>Varroa</i> for at least 4 weeks before beginning of the study.</p>
Source	local beekeeper, M. Coueron
Food/feeding	Full flowering <i>Phacelia tanacetifolia</i> served as food supply, no additional feeding throughout the study. Each tunnel was provided with a water supply.
Study design and methods	
Test duration	Pre-exposure phase (D -9 to D0) within the tunnels: 9 days Exposure phase (D 0 to D+6) within the tunnels: 6 days
Experimental dates	19 th August to 4 th September 2014
Test doses	Test item T1 (during bee flight): 100 g a.s./ha T2 (after bee flight): 100 g a.s./ha
	Toxic reference R (during bee flight): 400 g a.s./ha
	<p>Application of C, T1 and R was performed during daily bee flight, T2 on the same day after bee flight at BBCH 65 (full flowering of <i>Phacelia</i>) of the crop with a volume of 200 L water/ha. During application, the water supplies were removed to avoid contamination with spray residues.</p> <p>All actual treatment rates were within $\pm 5\%$ from the target application rate.</p>
Test units	Tunnels with an area of 140 m ² , containing 64 m ² (subdivided in 4 plots and separated and surrounded by plastic lanes) of flowering <i>Phacelia tanacetifolia</i> (variety: Meva), each with one colony; tunnels equipped with a water supply.
Endpoints and assessments	<p><i>mortality of bees:</i></p> <p>D -8 to D+6 via plastic lanes; assessed once a day in the morning; on the application day (D0) in the morning and evening to look for possible acute effects</p> <p><i>foraging activity:</i></p> <p>D -8 to D+5, on the entire 4 plots/tunnel (4 x 16 m² per tunnel) assessed once a day during the flight activity of the bees; on the day of application mortality (DAT0) was assessed</p> <ul style="list-style-type: none">- approx. 1 hour before application- immediately before application- 30 minutes after application, followed by two other assessments <p><i>behaviour in the tunnels and at the entrance of the hives:</i></p> <p>at the same time when the assessment for foraging activity took place</p> <p><i>colony strength and colony development:</i></p> <p>once at the beginning (D -10) and once at the almost end (D+5) of the study; assessment of:</p> <ul style="list-style-type: none">- estimated number of bees (colony strength)- number of cells containing brood (total of cells with eggs, larvae and capped brood)- presence of queens (e.g. presence of eggs)- number of storage frames.
Group size/replicates contact	Three tunnels per treatment group
Adaptation of bees	Colonies were set-up in the tunnel on ten days before application on D -10 to get familiar with the new conditions.
Environmental conditions	

Natural field conditions

Except one day of rainfall (D-3), weather conditions were appropriate. Applications were performed during a period of shiny day with sufficient temperature values to allow bee activity. A thermo-hygrograph placed in a weather station recorded temperature and air humidity over the whole experimentation period. Rainfall was daily recorded from the previous day.

Conditions			
	At bee flight appl.	After bee flight appl.	During exposure
Temperature:	20 °C	18 °C	9 to 25 °C
Wind speed:	0 km/h	0 km/h	not measured
Rel. humidity:	49 %	62 %	not reported
Precipitation:	none	none	none

Biological observations

Adult mortality was recorded daily between D -8 to D +6 and foraging activity and behaviour daily between D -8 to D +5. Assessment of condition of the colony strength and colony development D -10 and D +5.

Statistics

An analysis of variance was performed to compare the control treatment to the MCW-2222 treatments following a Student-Newmans-Keuls test (average following by the same letter are not significantly different). A transformation Log (x+1) of mortality data allow reducing the heterogeneity of variance. The toxic reference treatment was excluded as it was used for validation of the trial and not for comparison.

Obtained control mortality data were related to historical control mortality data obtained by TESTAPI in previous years using absolute and relative formula in order to assess reproducibility and if the control treatment of the study is in the range of the previous data. The calculated index (relative toxicity index; itox) for historical control data amounted to 2.1 at D+1 at 95% of confidence or 2.4 at 99% confidence. Moreover, two types of relative toxicity index (itox) were calculated, one for application during the foraging activity and another one for application outside the foraging activity.

$$\text{Itox} = (\text{Mt} \times \text{Ta}) / (\text{Ma} \times \text{Tt})$$

Mt = Mortality in the considered modality after application

Ma = Mortality in the considered modality just before application

Tt= Mortality in the water control tunnel after application

Ta= Mortality in the water control tunnel just before application

Results and discussion

Biological results

Mortality

During the adaptation phase, bee mortality was moderate in all tunnels. The day of application, this mortality was quite homogeneous among tunnels before application (from 225 to 253 dead bees in average at D0).

The mortality in the control tunnel remained stable and moderate from the application date until the end of the trial.

On the contrary the mortality in the dimethoate tunnel increased strongly just after the application performed at T1 since it reached 2199 in average. Moreover the impact of dimethoate on bee mortality was in average still high until D+4 with respectively 721 at D+2 and 685 at D+3 and 588 at D+4. So the results recorded in the control and toxic tunnels allow to validate the trial.

MCW-2222 applied during bee flight (T1) showed a slight increase of the average mortality at D+1 (from 227 dead bees at D0 to 407 the day after application). This level of increase was slightly higher than in the control treatment (from 235 at D0 to 300 at D+1). Then, the average mortality decreased already at D+2 (mean of 232 dead bees) to a regular level of mortality comparable to the one met in the control treatment (until the end of the trial). No statistical difference was met between this treatment and the control water.

MCW-2222 applied after bee flight (T2) induced no effect on mortality (from 366 dead bees at D+1 to 206 at D+2 in average). Compared to the control, there was no significant difference during the whole experimental phase.

The lack of effect of MCW-2222 on mortality is also confirmed by the low values of the relative toxicity

index (itox). Two types of relative toxicity index (itox) were calculated, one for application during the foraging activity and another one for application outside the foraging activity.

Indeed when the product is applied after bee flight, bees don't visit the treated crop between the application at night and the next morning when dead bees are counted at D+1, the effect is delayed by one day. Therefore for application outside the foraging activity, it is useful to compare the mortality at D0 to the one assessed at D+2.

Although this itox was very high for Dimethoate 400 EC since it reached 6.8 and 3.2 according to the timing of application of the test item, it was very low for MCW-2222 (1.4 and 1.2), compared to the control (1.0). The main information resulting from this index calculation is the very limited toxicity of the test item MCW-2222 applied during bee flight and the absence of impact when the product was applied after bee flight.

The average cumulative mortality after application was by far lower in the MCW-2222 tunnels than in the toxic reference tunnel. The average cumulative mortality induced by MCW-2222 was very close to that recorded in water control and was not significantly different from that of the control at D+6 after application.

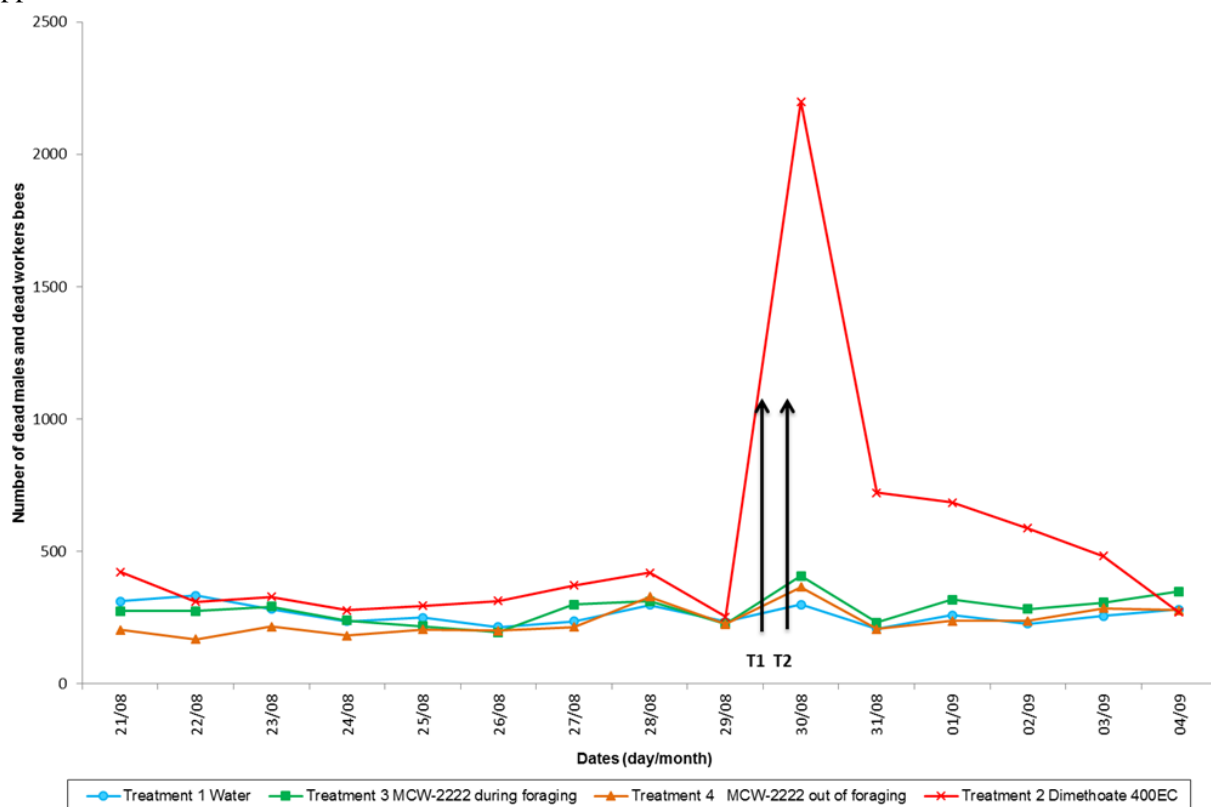


Figure A 30: Total daily mortality
Table A 105: Total daily mortality

Dates (day/month, D±x) x= delay from application day	Average number of dead bees			
	Treatment 1	Treatment 3 (T 1)	Treatment 4 (T 2)	Treatment 2
	Water	MCW-2222 during foraging	MCW-2222 out of foraging	Dimethoate 400EC
21/08 D-8	312	275	204	422
22/08 D-7	333	274	168	310
23/08 D-6	282	291	216	329
24/08 D-5	236	239	182	278
25/08 D-4	250	217	205	294
26/08 D-3	215	195	200	313
27/08 D-2	236	299	214	372
28/08 D-1	297	312	328	420
29/08 D0	235	227	225	253
30/08 D0+ +D+1	300	407	366	2199
31/08 D+2	208	232	206	721

← Application T1
and T2

Dates (day/month, D±x) x= delay from application day	Average number of dead bees			
	Treatment 1	Treatment 3 (T 1)	Treatment 4 (T 2)	Treatment 2
	Water	MCW-2222 during foraging	MCW-2222 out of foraging	Dimethoate 400EC
01/09 D+3	259	318	237	685
02/09 D+4	227	283	237	588
03/09 D+5	256	307	286	483
04/09 D+6	280	349	277	270
Cumulative mortality after application date to 04/09	1530	1896	1609	4946

Mortality reported on 29/08 was recorded immediately prior to the application.

Mortality reported on 30/08 is the sum of the mortality recorded on 29/08 just after the application and the mortality recorded on 30/08.

Table A 106: Relative toxicity index

Treatments	Time after Treatment	I tox Value*	
		I tox ₁ (D+1 versus D0) During foraging	I tox ₂ (D+2 versus D0) Out of foraging
Treatment 1 Water		1.0	1.0
Treatment 3 MCW 2222 during bee flight (T1)		1.4	1.2
Treatment 4 MCW 2222 after bee flight (T2)		Non relevant	1.0
Treatment 2 Dimethoate 400EC		6.8	3.2

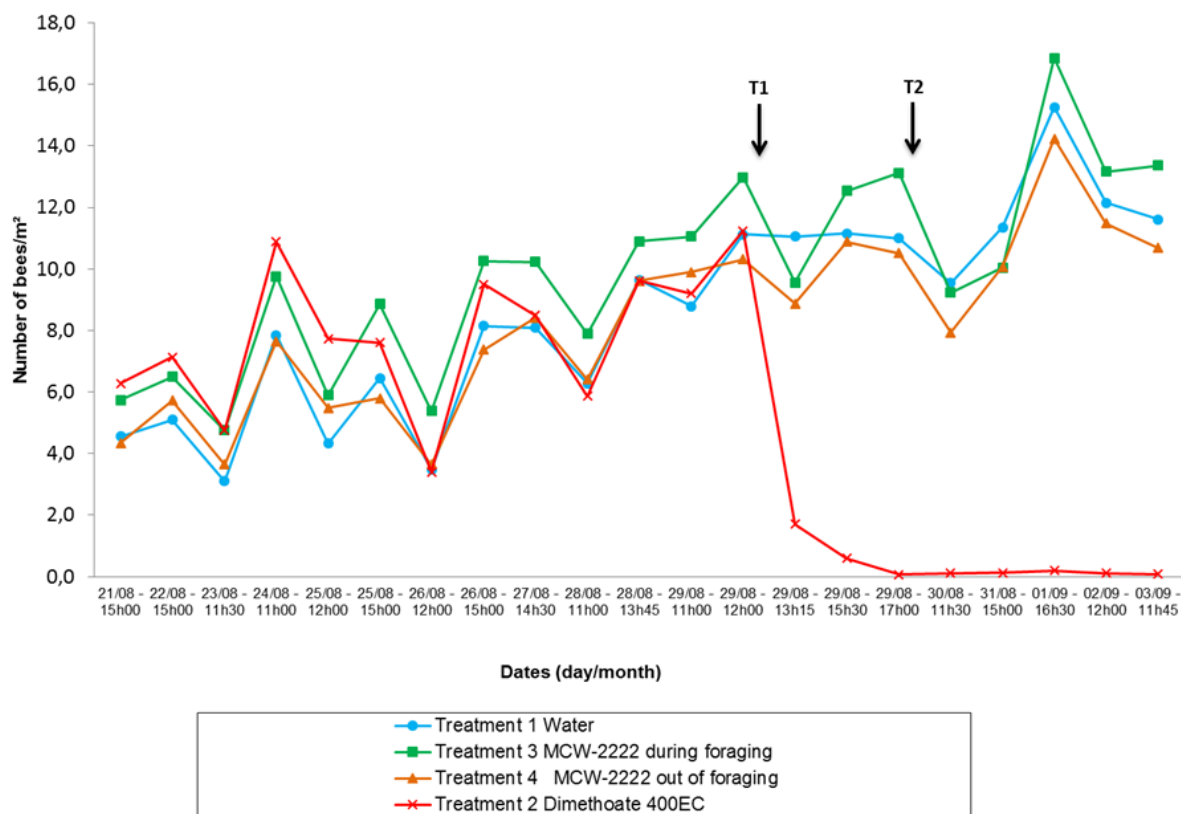
* I tox value = (Mt x Ta) / (Ma x Tt)

Foraging activity

The day of the application, the average bee activity was high (from 9 to 13 bees/m² at D0) and always superior to the required level (5 bees/m²). The foraging activity in the water tunnel was good and stable from the application T1 to the end of the test.

A very severe impact on foraging activity was met in the toxic reference tunnels since this activity decreased significantly just after the application at T1 and then it was stopped from D+1 until the end of the test (the repellence was so high that bees stayed in their hives).

After application of MCW-2222 during bee flight, no effect on foraging activity was observed. At D+1 and D+2, this activity was slightly lower than the control one but stayed over 9 bees/m². At D+3, the foraging activity increased drastically to more than 16 bees/m² (higher than in the control) and stayed higher than that recorded in the control treatment until D+5. When MCW-2222 was applied after bee flight, the recorded value after D+1 was slightly lower than the control one with no statistically significant difference. At D+2 this level of activity was in average above 10 bees/m². At D+3, the foraging activity increased drastically to more than 14 bees/m² and stayed higher than that recorded in the control treatment until D+5. All along the trial, the foraging activity was similar between the tunnels sprayed with the test item and those with water; not any significant difference in foraging activity between both MCW-2222 and control treatments was observed.



← Application T1

← Application T2

Behaviour

No clinic signs of intoxication were recorded in the control and MCW-2222 tunnels whereas some were recorded in the toxic reference tunnels.

Colony strength and colony development

All the colonies were well provided with brood in early trial. As usual in this kind of test with small colonies under tunnels, the confinement didn't induce enough eggs lying from the queens so the number of brood cells decreased drastically in all treatments.

For the MCW-2222 treatments, the adult bee population grew by about 8% (during the foraging activity) and 22% (outside the foraging activity) whereas the one in the water control decreased by about 8%. In the toxic reference treatment, the population decreased by 18%. This population evolution of adult honeybees was linked to the evolution of number of brood cells: e.g. if the amount of brood decreased and the population increased during the same time, it means that brood hatched and provides new worker honeybees.

This is the case in all MCW-2222 treatments. However, the difference between control and MCW-2222 population estimation is biologically not relevant.

Endpoints

No effects on adult mortality, foraging activity, behaviour, colony strength as well on the colony conditions were observed when MCW-2222 was applied during (T1) or after bee flight (T 2) at a rate of 100 g a.s./ha.

Validity criteria

As shown in the following table, all validity criteria were met.

Table A 108: Validity criteria

Validity criteria according to CEB 230 (2012), part IV	Observed in study
Before treatment:	
Daily mortality must have stabilised. In practice, this stabilisation is achieved three to six days after the hives are placed in the tunnels	Achieved
Daily mortality on the last day before treatment must not exceed 300 bees	225 to 253 dead bees/replicate in the respective treatment groups
Daily mortality must be similar in different tunnels, i.e. the deviation from average mortality on the day before application must not exceed 60%	C: - 47% to +40% T1: -29% to +21% T2: -22% to +13% R: -12% to +18%
Foraging activity must be greater than five bees / m ² on flowering plants and three bees / m ² on wheat shortly before application	C: 10.0 bees/m ² T1: 12.0 bees/m ² T2: 10.1 bees/m ² , assessed during bee flight 0 bees/m ² , assessed after bee flight R: 10.2 bees/m ²
Foraging activity in different tunnels must be comparable.	Achieved
After treatment:	
Mortality in the water control must be comparable before and after treatment, i.e. mortality the day after treatment must not exceed the average mortality figures for the day before treatment by more than 50%	Itox at D+1: 1.28 Itox at D+2: 0.89
The daily mortality in the "toxic benchmark" tunnel the day after treatment must be at least five times as high as the mortality figure for the day before treatment	Itox at D+1: 6.8 Itox at D+2: 3.2
Weather conditions must remain favourable	Achieved
All other factors regarded as abnormal in the conduct of the test	Achieved

Conclusion

In a semi-field study honeybees (*Apis mellifera* L.) were exposed to MCW-2222 (a.s. acetamiprid) applied at rate of 100 g a.s./ha during (T1) and after (T2) bee flight according to CEB 230 (2003), investigating potential effects on adult mortality, foraging activity and behaviour, conditions of the colonies (i.e. colony strength and colony development). Flowering *Phacelia tanacetifolia* served as crop (crop area: 64 m², subdivided in 4 plots and separated and surrounded by plastic lanes), which was daily sprayed with sugar syrup as artificial honeydew to make the crop attractive for foraging bees.

MCW-2222 applied during (T1) or after (T2) bee flight did not significantly impact mortality, foraging activity, behaviour as well as colonies strength and development.

A 2.3.1.7.6 KCP 10.3.1.5/01 Tunnel CEB study test with honey bees on *Phacelia* - 3

Comments of zRMS:	<p>The study has been already evaluated and accepted by the zRMS in the course of the first zonal evaluation of CA3573 (formerly MCW-2222) finalised in April 2018. Although the test guideline has not changed since that time, the study has been re-evaluated by the zRMS-PL in 2021.</p> <p>The study was performed in the Northern France and comprised applications to flowering <i>Phacelia</i> performed at two timings:</p> <ul style="list-style-type: none"> – during bee foraging activity: water control, MCW-2222 at 100 g a.s./ha and the reference dimethoate, – out of the presence of forager bees: MCW-2222 at 100 g a.s./ha at night. <p>In these experimental conditions, when MCW-2222 was applied at 0.5 L/ha during the foraging activity or out of the bee presence, the general daily mortality trend was similar to this observed control and there was no significant difference in the daily number of dead bees recorded compared to the control. No signs of intoxication were recorded at D+1 in the tunnels treated with MCW-2222 during or out of bee presence. Application of MCW-2222 had no impact on the foraging activity in both treatment groups when compared to the activity observed in the control treatment.</p> <p>Colony strength parameters recorded in the control and in the tunnels where MCW-2222 was applied during or out the foraging activity were not significantly different.</p> <p>Residue analysis was not performed, but the study was performed in tunnels and treated crop was the only food source for bees. Exposure of bees was thus assured.</p> <p>Although the study was correctly performed and as such is considered acceptable, it is noted that it was performed for a short time with bee mortality observations carried out during seven days after the applications and colony assessment carried out 4 days before application and 7 days after the application. Taking this into account, the design of the study is relevant to capture acute effects of application of acetamiprid on adult bees and bee brood, but is not relevant to address the chronic effects.</p>
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Data point:	KCP 10.3.1.5/06
Report:	Assessment of toxicity on honey bees (<i>Apis mellifera</i>) of the product MCW-2222 (acetamiprid 200 g/L) on phacelia crop in a tunnel trial. Molitor, C., 2015a, R-35847, 225-2015
Guideline(s):	C.E.B methodology n°230, part IV
Deviations:	<p>Yes, minor deviations:</p> <p>Under the tunnels Nos. 9 to 12, phacelia plants were affected by a heat wave occurring in July. It was decided to assess the number of foraging bees on the real area covered by flowers (32 m²). In order to guarantee the homogeneity among the test item treated replicates, those tunnels were distributed in the control and toxic reference treatments as follow: tunnels Nos. 9 and 10 in the water control treatment and tunnels Nos. 11 and 12 in the toxic reference treatment. The mean foraging level per treatment in those tunnels was above 5 foraging bees per meter square at D0 before the application in compliance with the CEB guideline n°230.</p> <p>This minor deviation did not have an impact on the reliability and the outcome of the study.</p>
GLP:	Yes, certified laboratory
Acceptability:	Yes, study considered acceptable
Duplication: (if vertebrate study)	Not applicable

Executive Summary

In a semi-field study with honeybees (*Apis mellifera* L.) the potential effects of MCW-2222 on mortality, foraging activity, behaviour, colony strength, quality and quantity of brood and the amount of reserves were investigated. Flowering *Phacelia* served as crop (crop area: 64 m², subdivided in 4 plots and separated and surrounded by plastic lanes). Each tunnel was provided with a water and pollen supply. MCW-2222 was applied during bee flight (T1) and after bee flight (T2) to the crop at a rate of 0.5 L/ha (100 g a.s./ha acetamiprid). Water treated tunnels served as control (C). Tunnels, treated with Rogor plus (400 g dimethoate/ha) served as toxic reference (R). Each treatment group was three times replicated. Application in the control, the toxic reference and T1 was performed during a period of high foraging activity. T2 was applied in the evening of the same day after the end of the foraging activity.

Small honey bee colonies of approx. 10,000 bees were placed into the tunnels three days before application (D -3) to get familiar with the new conditions. Seven days after application (D +7) and being confined within the tunnels colonies were located outside the tunnels.

Assessments on mortality (via plastic sheets) were daily conducted between D -2 to D +7; by exception, dead bees were collected twice on the application day (in the morning and in the evening) in order to observe eventual acute effect. Foraging activity was daily assessed between D -2 and D +7; on the day of application during bee flight, the foraging activity was monitored 5 times (once before application, 30 minutes after application, followed by three other assessments). The assessment was conducted on the 4 plots of each tunnel. Potential clinical signs of intoxication were recorded during the period after application. Control of the colony (apiarist visit) with respect to colony strength, quality and quantity of brood (different stage observed) and the amount of reserves was carried out before the introduction of the hives into the tunnels and just after the end of the study.

When MCW-2222 was applied during (T1) or after bee flight (T2), the general daily mortality trend was similar to the one met in the control and there was no any significant difference in the daily number of dead bees recorded compared to the control one all along the trial. Evolution of the cumulative mortality for both test item treatments had similar trends compared to the control one and data were not significantly different at the end of the experimental phase. The toxicity index is a value expressed relatively to the control mortality data. The indexes of MCW-2222 treatments applied during foraging activity and out of the foraging activity were not significantly different from the control at D+1 and D+2.

The application of MCW-2222 during or after bee flight had no impact on the foraging activity when compared to the activity observed in the control treatment. From D0 the trend was similar to the control until the end of the trial with no significant differences, whereas the toxic reference dimethoate clearly triggered a stop of the foraging activity.

Behaviour and colony strength parameters recorded in the control and in the two MCW-2222 treatment groups were not different. At the end of the experimental phase populations grew in the test item treatments and were therefore able to enlarge their further development.

Materials and methods

Materials

Test item	MCW-2222
Batch #	659-030314-01
Content of active substance	Acetamiprid 200 g/L (nominal); 199.2 ± 1.3 g/L (analysed)
Description	Yellowish liquid
Control	C: Water treated crop, applied during foraging activity
Toxic reference	R: Rogor Plus (400 g a.s./ha dimethoate), applied during the foraging activity

Test organism

Species	Honey bees (<i>Apis mellifera</i> L.) of healthy colonies with sister queens and the same age (not older than 2 years), containing about 10,000 bees per colony at test start with six frames. Hives of Dadant type. All colonies at the beginning of the study - with at 2 to 4 frames containing all brood stages - with 0 to 2 storage frames - with 0 to 3 empty frames - were free of clear clinical symptoms of disease; no treatment against <i>Varroa</i> for at least 4 weeks before beginning of the study.
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Source	local beekeeper, Apistory
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Food/feeding	Full flowering <i>Phacelia tanacetifolia</i> served as food supply, no additional feeding throughout the study. Each tunnel was provided with a water supply.
Study design and methods	
Test duration	Pre-exposure phase (D -3 to D0) within the tunnels: 3 days Exposure phase (D 0 to D+7) within the tunnels: 7 days
Experimental dates	14 th July to 24 th July 2015
Test doses	Test item T1 (during bee flight): 100 g a.s./ha T2 (after bee flight): 100 g a.s./ha Toxic reference R (during bee flight): 400 g a.s./ha Application of C, T1 and R was performed during daily bee flight, T2 on the same day after bee flight at BBCH 65 (full flowering of <i>Phacelia</i>) of the crop with a volume of 200 L water/ha. During application, the water supplies were removed to avoid contamination with spray residues. All actual treatment rates were within $\pm 5\%$ from the target application rate.
Test units	Tunnels with an area of 140 m ² , containing 64 m ² (subdivided in 4 plots and separated and surrounded by plastic lanes) of flowering <i>Phacelia tanacetifolia</i> (variety: Meva), each with one colony; tunnels equipped with a water supply.
Endpoints and assessments	<i>mortality of bees:</i> D -2 to D+7 via plastic lanes; assessed once a day in the morning; on the application day (D0) in the morning and evening to look for possible acute effects <i>foraging activity:</i> D -2 to D+7, on the entire 4 plots/tunnel (4 x 16 m ² per tunnel) assessed once a day during the flight activity of the bees; on the day of application mortality (DAT0) was assessed - approx. 1 hour before application - immediately before application - 30 minutes after application, followed by two other assessments <i>behaviour in the tunnels and at the entrance of the hives:</i> at the same time when the assessment for foraging activity took place <i>colony strength and colony development:</i> once at the beginning (D -4) and once at the end (D+7) of the study; assessment of: - estimated number of bees (colony strength) - number of cells containing brood (total of cells with eggs, larvae and capped brood) - presence of queens (e.g. presence of eggs) - number of storage frames.
Group size/replicates contact	Three tunnels per treatment group
Adaptation of bees	Colonies were set-up in the tunnel on ten days before application on D -3 to get familiar with the new conditions.
Environmental conditions	

Natural field conditions

Except one day of rainfall (D-3), weather conditions were appropriate. Applications were performed during a period of shiny day with sufficient temperature values to allow bee activity. A thermo-hygrograph placed in a weather station recorded temperature and air humidity over the whole experimentation period. Rainfall was daily recorded from the previous day.

Conditions			
	At bee flight appl.	After bee flight appl.	During exposure
Temperature:	22 °C	22 °C	14 to 32 °C
Wind speed:	0 to 5 km/h	0 km/h	not measured
Rel. humidity:	44 %	60 %	not reported
Precipitation:	none	none	D+7 (2 mm)

Biological observations

Adult mortality, foraging activity and behaviour was daily recorded between D -2 to D +7. Assessment of condition of the colony strength and colony development D -4 and D +7.

Statistics

An analysis of variance was performed to compare the control treatment to the MCW-2222 treatments following a Student-Newmans-Keuls test (average following by the same letter are not significantly different). A transformation Log (x+1) of mortality data allow reducing the heterogeneity of variance. The toxic reference treatment was excluded as it was used for validation of the trial and not for comparison.

Obtained control mortality data were related to historical control mortality data obtained by TESTAPI in previous years using absolute and relative formula in order to assess reproducibility and if the control treatment of the study is in the range of the previous data. The calculated index (relative toxicity index; itox) for historical control data amounted to 2.03 at D+1 at 95% of confidence or 2.35 at 99% confidence. Moreover, two types of relative toxicity index (itox) were calculated, one for application during the foraging activity and another one for application outside the foraging activity.

$$\text{Itox} = (\text{Mt} \times \text{Ta}) / (\text{Ma} \times \text{Tt})$$

Mt = Mortality in the considered modality after application

Ma = Mortality in the considered modality just before application

Tt= Mortality in the water control tunnel after application

Ta= Mortality in the water control tunnel just before application

Results and discussion

Biological results

Mortality

During the adaptation phase, bee mortality was low in all tunnels. The day of application, this mortality was still low among the tunnels before application.

The mortality in the control tunnel remained stable and low from the application date until the end of the trial.

On the contrary the mortality in the dimethoate tunnel increased strongly just after the application performed at T1 since it reached 951 in average. So the results recorded in the control and toxic tunnels allow to validate the trial.

Whatever the timing of application, MCW-2222 didn't show any effect on bee mortality compared to the control. No statistical difference was met between the MCW-2222 treatments and the control one.

The lack of effect of MCW-2222 on mortality is also confirmed by the low values of the relative toxicity index (itox). Two types of relative toxicity index (itox) were calculated, one for application during the foraging activity and another one for application outside the foraging activity.

Indeed when the product is applied out of foraging activity, bees don't visit the treated crop between the application at night and the next morning when dead bees are counted at D+1, the effect is delayed by one day. Therefore for application outside the foraging activity, it is useful to compare the mortality at D0 to the one assessed at D+2.

Although this itox was very high for Dimethoate 400 EC since it reached 8.1 at D+1, it was very low for MCW-2222 since it was inferior to the reference value for the control. The main information resulting from

this index calculation is the absence of impact of the test item MCW-2222 application at both timings on the bee mortality.

The average cumulative mortality after application was by far lower in the MCW-2222 tunnels than in the toxic reference tunnel. The average cumulative mortality induced by MCW-2222 was very close to that recorded in water control and was not significantly different from that of the control at D+7 after application.

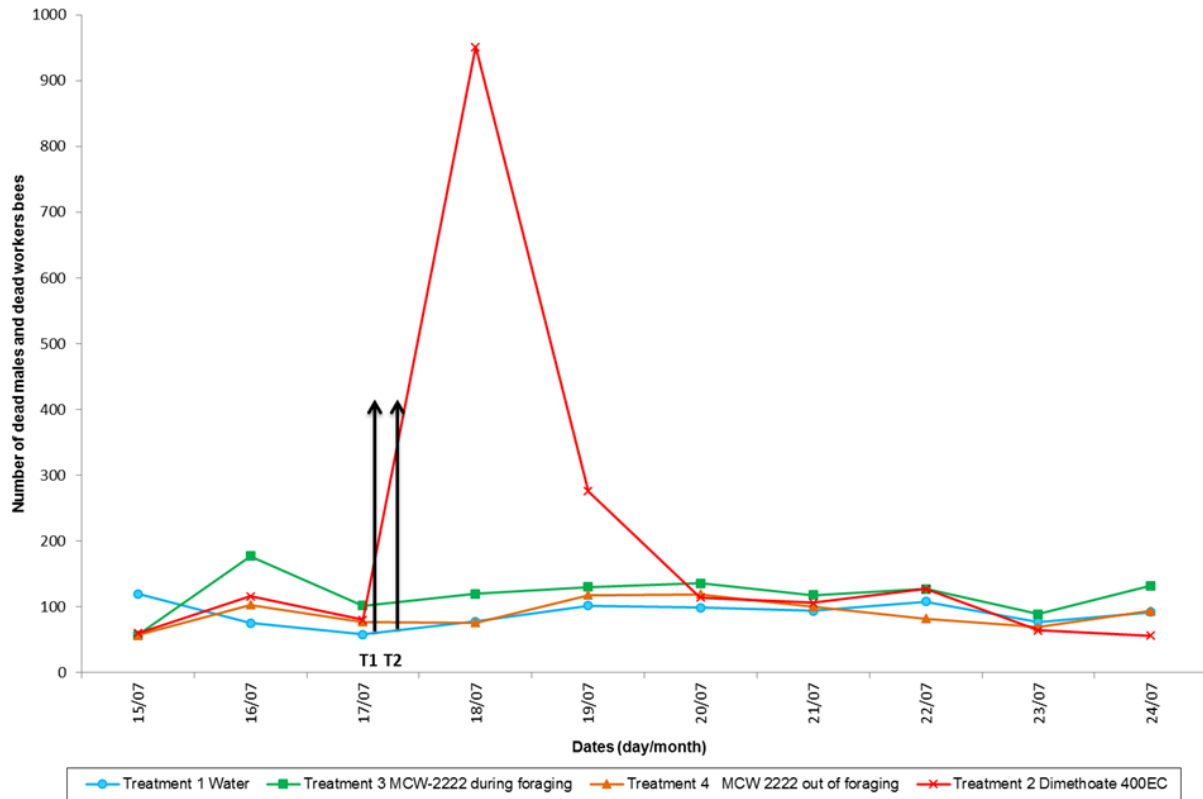


Figure A 32: Total daily mortality

Table A 109: Total daily mortality

Dates (day/month, D±x) x= delay from application day	Average number of dead bees			
	Treatment 1	Treatment 3 (T 1)	Treatment 4 (T 2)	Treatment 2
	Water	MCW-2222 during foraging	MCW-2222 out of foraging	Dimethoate 400EC
15/07 D-2	120	57	57	60
16/07 D-1	75	177	103	116
17/07 D0	58	102	77	80
18/07 D0+ +D+1	78	120	76	951
19/07 D+2	102	130	118	276
20/07 D+3	99	136	119	114
21/07 D+4	94	118	100	106
22/07 D+5	108	127	82	127
23/07 D+6	77	89	69	64
24/07 D+7	92	132	94	56
Cumulative mortality after application date to 17/07	650	852	658	1694

← Application T1
and T2

Mortality reported on 17/07 was recorded immediately prior to the application.

Mortality reported on 18/07 is the sum of the mortality recorded on 17/07 just after the application and the mortality recorded on 30/08.

Table A 110: Relative toxicity index

Treatments	Time after Treatment	I tox Value*	
		I tox ₁ (D+1 versus D0) During foraging	I tox ₂ (D+2 versus D0) Out of foraging
Treatment 1 Water		1.0	1.0
Treatment 3 MCW 2222 during bee flight (T1)		0.9	0.7
Treatment 4 MCW 2222 after bee flight (T2)		Non relevant	0.9
Treatment 2 Dimethoate 400EC		8.8	2.0

* I tox value = (Mt x Ta) / (Ma x Tt)

Foraging activity

In the tunnels 9 to 12 (9 and 10 for water control and 11 and 12 for the toxic reference), phacelia was affected by heat wave. In consequence the foraging activity was assessed on 32 m² instead of 64 m². Phacelia in the other test tunnels were not affected and the assessments were carried out on 64 m².

This deviation was taken into account in the calculation of number of bees/m².

The day of the application, the average bee activity was high (from 7.1 to 8.6 bees/m² at D0) and always superior to the required level (5 bees/m²).

The foraging activity started to decrease in the control tunnel and in the test items tunnels from D+5 (22/07) because the crop became less attractive to bees.

A very severe impact on foraging activity was met in the toxic reference tunnels since this activity decreased significantly just after the application at T1 and then was very low until the end of the test.

After application of MCW-2222 during bee flight (T1), the foraging activity was slightly lower in the item tunnels than in the control ones, which was already the case on D-2 and shortly before application, but stayed on a high level (over 5 bees/m²). Then the evolution of this activity followed the same evolution as for the control but with a slightly lower level until D+4, which was already observed before application. From D+4, it reached the same level as in the control.

When MCW-2222 after bee flight (T2), the foraging activity was lower than in the control tunnels before the application at T2 (6.8 versus 8 in average), but which was already observed before application. Then the evolution of this activity followed the same evolution as for the control but with a slightly lower level while being above 5 bees/m² until D+5.

All along the trial, the foraging activity was similar between the tunnels sprayed with the test item and those with water; not any significant difference in foraging activity between both MCW-2222 and control treatments was observed. Differences in the numbers between the control and both test item groups were already present before application and thus not test item related.

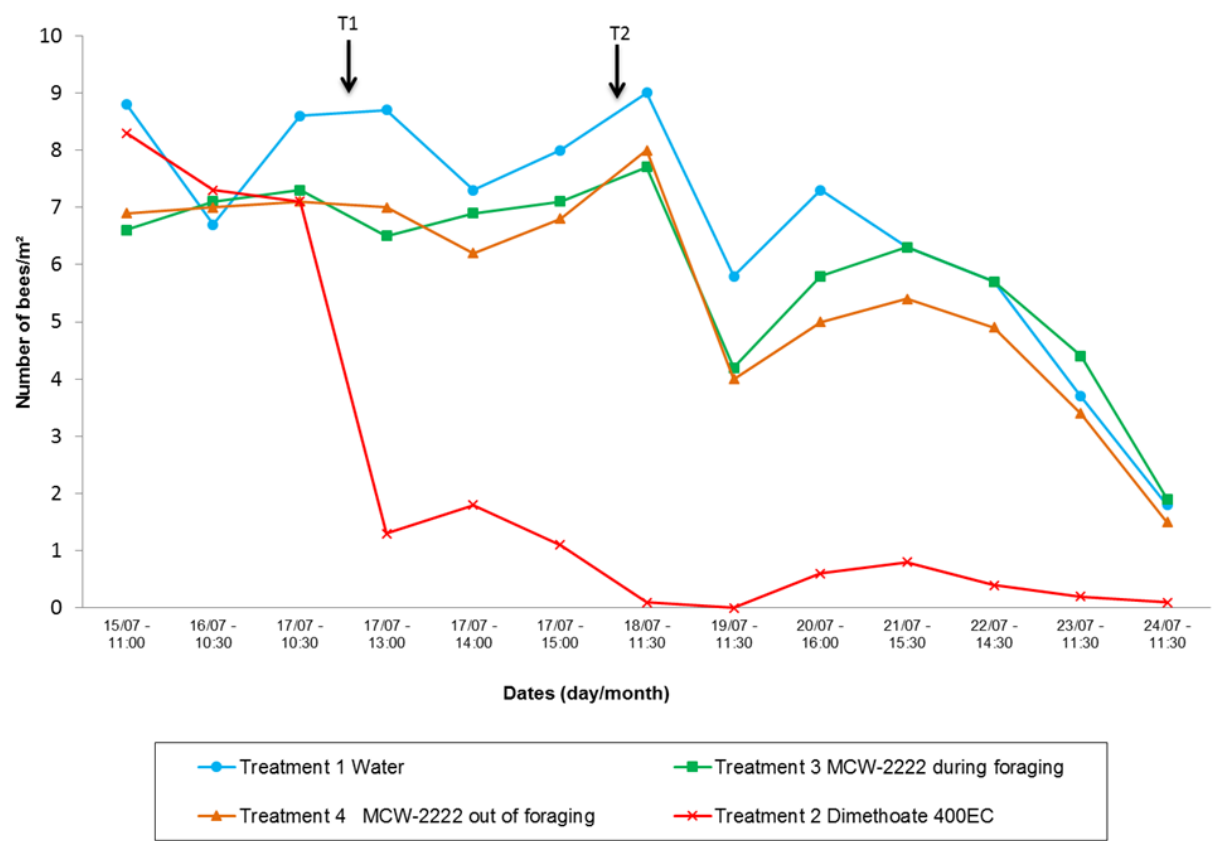


Figure A 33: Foraging activity - Average number of bees/m²

Table A 111: Foraging activity - average number of bees/m²

Dates (day/month-hours) x= delay from application day	Average number of bees/m ²			
	Treatment 1	Treatment 3 (T 1)	Treatment 4 (T 2)	Treatment 2
	Water	MCW-2222 during foraging	MCW-2222 out of foraging	Dimethoate 400EC
D-2 15/07 - 11:00	8.8	6.6	6.9	8.3
D-1 16/07 - 10:30	6.7	7.1	7.0	7.3
D0 17/07 - 10:30	8.6	7.3	7.1	7.1
D0+ 17/07 - 12:00	9.1	5.0	7.4	3.2
D0+ 17/07 - 13:00	8.7	6.5	7.0	1.3
D0+ 17/07 - 14:00	7.3	6.9	6.2	1.8
D0+ 17/07 - 15:00	8.0	7.1	6.8	1.1
D+1 18/07 - 11:30	9.0	7.7	8.0	0.1
D+2 19/07 - 11:30	5.8	4.2	4.0	0.0
D+3 20/07 - 16:00	7.3	5.8	5.0	0.6
D+4 21/07 - 15:30	6.3	6.3	5.4	0.8
D+5 22/07 - 14:30	5.7	5.7	4.9	0.4
D+6 23/07 - 11:30	3.7	4.4	3.4	0.2
D+7 24/07 - 11:30	1.8	1.9	1.5	0.1

← Application T1

← Application T2

Behaviour

No clinic signs of intoxication were recorded in the control and MCW-2222 tunnels whereas some were recorded in the toxic reference tunnels.

Colony strength and colony development

All the colonies were well provided with brood in early trial. As usual in this kind of test with small colonies under tunnels, the confinement didn't induce enough eggs laying from the queens so the number of brood cells decreased drastically in all treatments.

For the MCW-2222 treatments, the adult bee population grew by about 14% in the water control, 10% in the tunnels where MCW-2222 was applied after bee flight, 4% in the tunnels where MCW-2222 was applied during bee flight. This population evolution of adult honeybees was linked to the evolution of number of brood cells: e.g. if the amount of brood decreased and the population increased during the same time, it means that brood hatched and provides new worker honeybees.

As main information regarding the bee population and the brood cell number evolution during the trial is that there was no impact from the two MCW-2222 items compared to the control treatment.

Endpoints

No effects on adult mortality, foraging activity, behaviour, colony strength as well on the colony conditions were observed when MCW-2222 was applied during (T1) or after bee flight (T 2) at a rate of 100 g a.s./ha.

Validity criteria

As shown in the following table, all validity criteria were met.

Table A 112: Validity criteria

Validity criteria according to CEB 230 (2012), part IV	Observed in study
Before treatment:	
Daily mortality must have stabilised. In practice, this stabilisation is achieved three to six days after the hives are placed in the tunnels	Achieved
Daily mortality on the last day before treatment must not exceed 300 bees	58 to 102 dead bees/replicate in the respective treatment groups
Daily mortality must be similar in different tunnels, i.e. the deviation from average mortality on the day before application must not exceed 60%	C: - 28% to +52% T1: -23% to +22% T2: -45% to +60% R: -39% to +76%
Foraging activity must be greater than five bees / m ² on flowering plants and three bees / m ² on wheat shortly before application	C: 8.6 bees/m ² T1: 7.3 bees/m ² T2: 7.1 bees/m ² , assessed during bee flight 0 bees/m ² , assessed after bee flight R: 7.1 bees/m ²
Foraging activity in different tunnels must be comparable.	Achieved
After treatment:	
Mortality in the water control must be comparable before and after treatment, i.e. mortality the day after treatment must not exceed the average mortality figures for the day before treatment by more than 50%	Itox at D+1: 1.34 Itox at D+2: 1.76
The daily mortality in the "toxic benchmark" tunnel the day after treatment must be at least five times as high as the mortality figure for the day before treatment	Itox at D+1: 8.8 Itox at D+2: 2.0
Weather conditions must remain favourable	Achieved
All other factors regarded as abnormal in the conduct of the test	Achieved

Conclusion

In a semi-field study honeybees (*Apis mellifera* L.) were exposed to MCW-2222 (a.s. acetamiprid) applied at rate of 100 g a.s./ha during (T1) and after (T2) bee flight according to CEB 230 (2003), investigating potential effects on adult mortality, foraging activity and behaviour, conditions of the colonies (i.e. colony strength and colony development). Flowering *Phacelia tanacetifolia* served as crop (crop area: 64 m², subdivided in 4 plots and separated and surrounded by plastic lanes), which was daily sprayed with sugar syrup as artificial honeydew to make the crop attractive for foraging bees.

MCW-2222 applied during (T1) or after (T2) bee flight did not impact mortality, foraging activity, behaviour, colonies strength and development.

A 2.3.1.7.7 KCP 10.3.1.5/07 Tunnel OECD GD 75 honey bee brood development study

Comments of zRMS:	<p>The semi-field study on effects of CA3573 (formerly MCW-2222) on honeybee brood has been submitted in support of the re-evaluation of CA3573 in 2021 due to renewal of acetamiprid. The validation of the study was thus performed by the zRMS.</p> <p>The study was carried out in line with OECD 75 with no major deviations.</p> <p>The study was conducted in tunnels assembled on a field of <i>Phacelia tanacetifolia</i> in Germany between the municipalities of Ladenburg and Heddesheim in Baden-Württemberg. During the study the product was applied twice at rate of 80 g a.s./ha - 1st application was performed at the beginning of flowering (BBCH 59-61), 4 days before bees were introduced to the tunnels. Second application was performed in the evening after bee flight during full flowering (BBCH 60-65), 7 days later (4 days after bees introduction). Hives and the water supply were covered with plastic sheets to avoid direct overspray.</p> <p>The investigated parameters and timing of observations were in line with recommendations of OECD 75. The study duration was 28 days (8 days exposure in the tunnels followed by 20 days observation at the monitoring site).</p> <p>It is noted that during the exposure phase in the tunnels, rainfall occurred on days DALA 1, DALA 2 and to DALA 3 at 1, 1 and 0.5 mm, respectively. However, precipitation was too low to have significant impact on exposure and residue analyses confirmed that acetamiprid was present in flowers and pollen.</p> <p>After 8 days of exposure in tunnels, bees were further observed for 20 days at the monitoring site.</p> <p>No effects of the treatment were observed on adult mortality, pupae mortality, foraging activity, bees behaviour, colony strength and the bee brood development.</p>
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	<p>Significant effects seen on the bee brood in the toxic standard group demonstrated sufficient sensitivity of the test system.</p> <p>Based on obtained results it may be concluded that CA3573 is not expected to have adverse impact on bees and bee brood when is applied up to 80 g a.s./ha to flowering crop outside the bee activity.</p> <p>However, potential effects on overwintering success cannot be addressed based on results of this study.</p>
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Data point:	KCP 10.3.1.5/07
Report:	Semi-field brood study to evaluate potential effects of MCW-2222 on brood development of honeybees (<i>Apis mellifera</i> L.). Hecht-Rost, S. & Mayer, O., 2018, R-37336, R1640035
Guideline(s):	OECD GD 75 (2007)
Deviations:	None
GLP:	Yes, certified laboratory
Acceptability:	Yes, study considered acceptable
Duplication: (if vertebrate study)	Not applicable

Executive Summary

In a semi-field tunnel study with honeybees (*Apis mellifera* L.) the potential effects of MCW-2222 on adult and pupal mortality, foraging activity, behaviour, colony strength, quality and quantity of brood and the amount of reserves were investigated. Special attention was laid on the detailed brood assessment of marked cells containing eggs.

Phacelia tanacetifolia served as crop (crop area: 84 m²). Each tunnel was provided with a water supply. MCW-2222 was applied in the evening once before flowering (BBCH 59-61, single plants with open flowers) without hives present in the tunnels, and once during flowering (BBCH 60-65) with hives being placed in the tunnels after bee flight. The application rate was 0.4 kg/ha (80 g a.s./ha acetamiprid) at both applications. Water treated tunnels served as control (C). Tunnels, treated with Insegar 25 WG (250 g fenoxycarb/kg) served as toxic reference (R) and were applied at a rate of 300 g a.s./ha. Each treatment group was four times replicated. Application of the control and the toxic reference was performed once at the time of the 2nd test item application after bee flight (C) or on the subsequent day during bee flight (R). Small honey bee colonies of approx. 8,000 bees were placed into the tunnels four days before the 2nd application (DAA -5) to get familiar with the new conditions. Eight days after application (DAA 7) the tunnels colonies were moved to the monitoring phase and placed there until DAA 28.

Assessments on adult and pupal mortality were daily conducted on DAA -6 & -5 (two before set-up of the colonies in the tunnels at the pre-exposure monitoring site) and DAA 8 to DAA 28 (post-exposure) via dead bee traps; assessments between DAA -5 to DAA 7 (inside the tunnels) were conducted via sheets and dead bee traps; by exception, mortality was additionally also assessed on DAA -5 shortly before transport of colonies to the tunnels, the day of the 2nd T application (DAA -1) shortly before application, and the day after the 2nd T application (DAA 0) in the morning, 2h after the application of R and in the evening.

Foraging activity was daily assessed between DAA-4 to DAA 7 counting the number of foraging bees on three 1m² plots per tunnel for 15 seconds; additional assessments were conducted shortly before the 2nd T application to ensure no bees were actively foraging, shortly before application of R to ensure that enough bees were actively foraging (only in the reference item tunnels), and seven times after the application of R in all tunnels (four times within the first hour after, and 2, 4 and 6 hours after the application of R. Potential effects on the behaviour were recorded during the assessment on the foraging activity.

Assessments of the condition of the colonies (colony strength, development of the brood and food area) were performed on Brood Area Fixing Day 0 (BFD 0) which was one day before the 2nd T application (DAA -1), BFD 5, BFD 10, BFD 16, BFD 22 and BFD 29 (= DAA 28), covering one complete brood cycle (21 days for worker bees) and the beginning of a second one.

Detailed assessments of the bee brood development was carried out by marking individual brood cells containing eggs BFD 0 (DAA -1). At this day > 200 cells of each development stage were selected in each

hive and followed until BFD22, which covered one brood cycle. Next to the assessment on BFD 22 the development of each individually marked cell was assessed at BFD 5, BFD 10, BFD 16 and BFD 22. Each brood comb was photographed at each assessment time.

Two additional tunnels (C & T) were set-up for the generation of pollen (via pollen traps), nectar (via honey stomach), larvae and beeswax on DAA-4 (pollen additionally on DAA-3), DAA 0aa, DAA 3 and DAA 7 for residue analysis; honey samples were taken on DAA -5 and DAA 18. Flower samples were taken in all C and T tunnels as well as in the additional residue tunnels on DAA -4 and DAA 0aa; on DAA 3 and DAA 7 only the samples of the residue tunnels were analysed.

The application of MCW-2222 did not cause an effect on adult honey bee mortality. In fact, the mortalities of all treatment groups were at low and comparable levels during the exposure and post-exposure period and within the normal expected biological variability in all treatment groups. Moreover, only a few dead pupae were recorded in the control and test item group during the entire Field Phase. In contrast, the reference item colonies showed an increased daily pupal mortality from DAA 10 until DAA 28, indicating the sensitivity of the test system to detect adverse effect on the pupal development.

The application of MCW-2222 did not cause an effect on the foraging activity of the honey bees. In fact, the mean and overall foraging activities of all treatment groups before application and after the 2nd T application were on comparable levels. However, due to rainy weather conditions during the assessment on DAA 2, no foraging activity was recorded. Moreover, no test item related effect on honey bee behaviour was noted.

The pre-exposure colony condition assessment indicated that the honey bee colonies were healthy, all brood stages were present and colony strengths were comparable and a sufficient amount of nectar and pollen was available in all colonies. During the entire course of the study, no considerable differences in numbers of bees, brood and food cells were observed between the colonies of all treatment groups.

The detailed brood assessment resulted in comparable BTRs in the control and test item group. In fact, the mean BTR at the end of the brood cycle in the control amounted to 33.4% compared to 27.7% in the test item group, being not statistically different. As BI is inverse related to the BTR, meaning that the lower the BTR the higher the BI, the corresponding BI in the control amounted to 3.3 compared to 3.6 in the test item group. The BCI as an indicator for recovery of the brood and indicating that terminated cells were re-filled with eggs, displayed slightly higher values in the control and the test item group, i.e. 4.2 and 4.3, respectively. No significant differences between the C and T were detected.

The determined residues of acetamiprid in the treated flowers ranged between 3.7 mg a.s./kg and 7.5 mg a.s./kg on DAA -4 and between 18 mg a.s./kg and 25 mg a.s./kg on DAA 0 in all treated tunnels. On DAA 3, the flowers in the treated residue tunnel showed acetamiprid residues of 6.4 mg a.s./kg and 1.0 mg a.s./kg on DAA 7. The determined residues of acetamiprid in pollen were 0.60 mg a.s./kg on DAA -4 and 8.5 mg a.s./kg on DAA 0. The residue analysis for DAA 3 and DAA 7 were reported to be 0.46 mg a.s./kg and 0.61 mg a.s./kg on DAA 3 and 0.51 mg a.s./kg and 0.61 mg a.s./kg on DAA 7.

Materials and methods

Materials

Test item	MCW-2222
Batch #	811-021115-01
Content of active substance	Acetamiprid 200 g/L (nominal); 205.1 ± 1.1 g/L (analysed)
Description	Liquid / clear yellow to brown
Control	C: Tap water
Toxic reference	R: Insegar 25 WG (250 g fenoxycarb/kg)
Test organism	
Species	Honey bees (<i>Apis mellifera</i> L.) of healthy colonies with sister queens from the previous year, containing about 8,000 bees per colony, 14,000 to 20,800 brood cells and 4,200 to 13,200 food cells at test start with ten frames. Hives of Zander type.

All colonies at the beginning of the study

- with at 3 to 5 frames containing all brood stages
- with a sufficient food supply
- were free of visible clinical symptoms of disease (e.g. varroaosis, noseiosis, amoebiasis, chalkbrood, sacbrood, American or European foulbrood) or pests (e. g. Varroa destructor), as far as possible;
- were free of unusual occurrences (e.g. presence of dark "bald" bees,

	"crawlers" or flightless bees, unusual brood patterns or brood age structure).
Source	Company's own apiary
Food/feeding	Full flowering <i>Phacelia tanacetifolia</i> served as food supply, no additional feeding throughout the study. Each tunnel was provided with a water supply.
Study design and methods	
Test duration	Exposure phase before the 2 nd application (DAA -5 to DAA -1): 4 days in the tunnels Exposure phase after the 2 nd application (DAA 0 to DAA 7): 8 days in the tunnels Post-Exposure phase (DAA 8 to DAA 28): 20 days at the monitoring site
Experimental dates	3 rd July to 8 th August 2016
Test doses	Test item (T): 2 x 80 g a.s./ha 1 st application on 3 rd July 206 (DAA -8; BBCH 59-61, single plants with open flowers) without hives present in the tunnels 2 nd application on 10 th July 2016 (DAA -1; BBCH 60-65) with hives present in the tunnels but applied after bee flight Toxic reference (R): 1 x 300 g a.s./ha, applied on 11 th July (DAA 0), during bee flight First application of T was performed to <i>Phacelia</i> before flowering (BBCH 59-61) after daily bee flight, the second application during full flowering of the crop (BBCH 65) after bee flight at BBCH 65 (full flowering of) but with hives present in the tunnels; C was applied in the evening of the 2 nd test item application, R in the morning of the subsequent day. All applications were carried out with a spray volume of 400 L water/ha. During the applications the water supply was removed from the respective tunnels and the bee colonies were covered with a plastic sheet until the end of application to avoid direct contamination. All actual treatment rates were within $\pm 5\%$ from the target application rate.
Test units	Tunnels with an area of 108 m ² , containing 84 m ² of <i>Phacelia tanacetifolia</i> , each with one colony; tunnels equipped with a water supply.
Group size/replicates contact	Four tunnels per treatment group, two additional tunnels for the generation of samples for residue analysis.
Endpoints and assessments	<i>mortality of worker bees, drones and pupae/larvae:</i> DAA -6 & -5 (two days before set-up of the colonies in the tunnels at the pre-exposure monitoring site) and DAA 8 to DAA 28 (post-exposure) via dead bee traps; DAA -5 to DAA 7 (inside the tunnels) via sheets spread out at the front, middle and back of the tunnels and dead bee traps attached to the entrances of the hives; assessed once a day in the morning; additional assessments on: - DAA -5 shortly before transport of colonies to the tunnels - the day of the 2 nd T application (DAA -1) shortly before application; - the day after the 2 nd T application (DAA 0) in the morning, 2h after R application and in the evening. <i>foraging activity:</i> DAA -4 to DAA 7, counting the number of foraging bees on three 1m ² plots per tunnel for 15 seconds; assessed once a day during the flight

activity of the bees; additional assessments on:

- the day of 2nd T application (=DAA -1) shortly before treatment to ensure no bees were actively foraging
- the day after the 2nd T application (DAA 0)
 - shortly before application of R to ensure that enough bees were actively foraging (only in the reference item tunnels),
 - 4 times within the first hour after application of R (in all tunnels)
 - 2 hours after application
 - 4 hours after application
 - 6 hours after application

behaviour in the tunnels and at the entrance of the hives:

at the same time when the assessment for foraging activity took place

condition of the colonies:

Assessments were performed on BFD 0 (= DAA -1), BFD 5, BFD 10, BFD 16, BFD 22 and BFD 29 (= DAA 28), covering one complete brood cycle (21 days for worker bees) and the beginning of a second one. Assessment of the:

- estimated number of bees (colony strength)
- presence of queens (e.g. presence of eggs)
- comb area containing eggs, larvae and capped cells
- comb area containing pollen and nectar.

detailed bee brood development:

The development of the bee brood in individual marked cells was observed with the aid of the digital image processing software "HiveAnalyzer" (Höferlin & Höferlin, 2014). At the assessment before the application of the reference item (-1DAA = brood area fixing day (BFD) 0), one to three sides of brood combs from each colony were selected and digitally photographed. Afterwards the pictures were evaluated with the digital image processing software. 207 – 399 cells filled with eggs were marked per colony.

On every following BFD assessment the software recovered exactly the cells which were marked on BFD 0. For the assessments at the following BFDs, the contents of single cells were identified and marked individually for the different cell contents with the aid of the software. In this way, the development of each individually marked cell throughout the duration of the Field Phase of the study was determined (the pre-imaginal developmental period of worker honeybees is normally 21 days). A successful brood development is assumed at the last assessment date when cells are empty due to hatching of adult bees or again filled with eggs, young larvae, pollen or nectar. In contrast, a termination of the brood in the marked cells can be presumed if a cell is empty during BFD 5 to BFD 16 or if the cell contains an earlier brood stage than expected, or if the cell is filled with pollen or nectar.

After the BFD assessments the determined brood stages of the marked cells were classified and the brood termination rates (BTR, proportion of eggs which failed to develop successfully until adult hatch), the brood indices (BI, indicator of bee brood development and facilitates a comparison between different treatments) and the brood compensation indices (BCI, indicator for the recovery of a colony) were calculated with the software "HiveAnalyzer".

Assessments were performed on BFD 0 (= DAA -1), BFD 5, BFD 10, BFD 16, BFD 22 (= DAA 21), covering one complete brood cycle (21

days for worker bees).

Specimens sampling for residue analysis

Flower samples were taken in all control and test item tunnels as well as in the additional residue tunnels on DAA -4 and DAA 0; on DAA 3 and DAA 7 only the samples of the residue tunnels were analysed. Samples of pollen of *P. tanacetifolia*, nectar from forager bees (via honey stomach extraction, larvae and beeswax were collected in two additional assembled test item and control residue tunnels on DAA-4 (pollen additionally on DAA-3), DAA 0, DAA 3 and DAA 7; honey samples were taken on DAA -5 and DAA 18.

Half of the collected samples were transported to the analytical laboratory Eurofins Agroscience Services Chem GmbH, Hamburg, Germany for residue analysis of acetamiprid.

Specimen extraction and determination of residues were performed according to an analytical procedure that is based on the multi-residue QuEChERS. For pollen and wax an additional homogenisation step with a miniaturized cell disruption system (FastPrep) was included to the extraction procedure. Quantification was performed by use of LC-MS/MS detection.

The limit of quantification (LOQ) of the analytical method was 0.01 mg a.s./kg for each matrix with a limit of detection (LOD) set at 0.003 mg a.s./kg (30 % of the LOQ).

DAA = days the application (DAA 0 = 1st day on which the bees were exposed to the water treated control, the 2nd test item and the reference item application)

BFD = brood area fixing day

Adaptation of bees

Colonies were set-up in the tunnel on DAA -5, four days before the 2nd test item application to get familiar with the new conditions.

Environmental conditions **Natural field conditions**

The daily min., max. and mean temperature and humidity were recorded with a data logger, rainfall with a rain gauge. During the application the weather data were recorded with portable devices. No rainfall was recorded at the monitoring site before the colonies were set up. The daily temperature there were favourable for bee activity on most days.

Slight rainfall during the period inside the tunnels (DAA -4 to DAA 7) was recorded on DAA 1 to DAA 3 (DAA 1 and DAA 2: 1.0 mm each; DAA 3: 0.5 mm). The daily temperatures ranged from 8.2 (DAA 4) to 36.4 °C (DAA 7). The weather conditions were thus suitable for good foraging activity during the exposure period inside the tunnels.

At the monitoring site, after the exposure period inside the tunnels, rainfall occurred on eleven out of 21 days (DAA 10 to DAA 14: 7.0 mm, 3.0 mm, 15.5 mm, 0.5 mm, 0.5 mm; DAA 18: 0.5 mm; DAA 22 to DAA 26: 1.5 mm, 12.0 mm, 1.0 mm, 12.0 mm, 0.5 mm). The daily temperatures ranged from 13.0 °C (DAA 25) to 30.3 °C (DAA 9).

Biological observations

Mortality was recorded daily between DAA -6 to DAA 28 and foraging activity and behaviour daily between DAA -4 to DAA 7. Assessment of condition of the colonies was performed on BFD 0 (= DAA -1), BFD 5, BFD 10, BFD 16, BFD 22 and BFD 29 (= DAA 28), detailed brood assessments were carried out on BFD 0 (= DAA -1), BFD 5, BFD 10, BFD 16, BFD 22 (= DAA 21), covering one complete brood cycle (21 days for worker bees).

Statistics

The data for mortality, foraging activity and bee brood development (except replicate R1 which was excluded) were tested for normal distribution and homogeneous variances; if both were positive, this was followed by an ANOVA and a Dunnett test. If there was no normal distribution or homogeneous variances, a test for equal distribution (or median test) and a Kruskal-Wallis test were carried out. If both tests were positive, they were followed by a U test. If the test for equal distribution is negative, the test result shows the differences between the medians of the data. Test directions: For all pre-application data two-sided; for post-application data one-sided greater for mortality and brood termination rate and one-sided less for foraging activity, brood and compensation indices. Significance level was $\alpha = 0.05$. The statistical analysis was performed with the software R (version 3.0.3).

For time periods, linear mixed effect models or generalised linear mixed effect models (depending on the data distribution) were established for each treatment group and tested for overdispersion. Afterwards the treatment models were compared with an ANOVA.

Results and discussion

Biological results

Mortality, adult honeybees

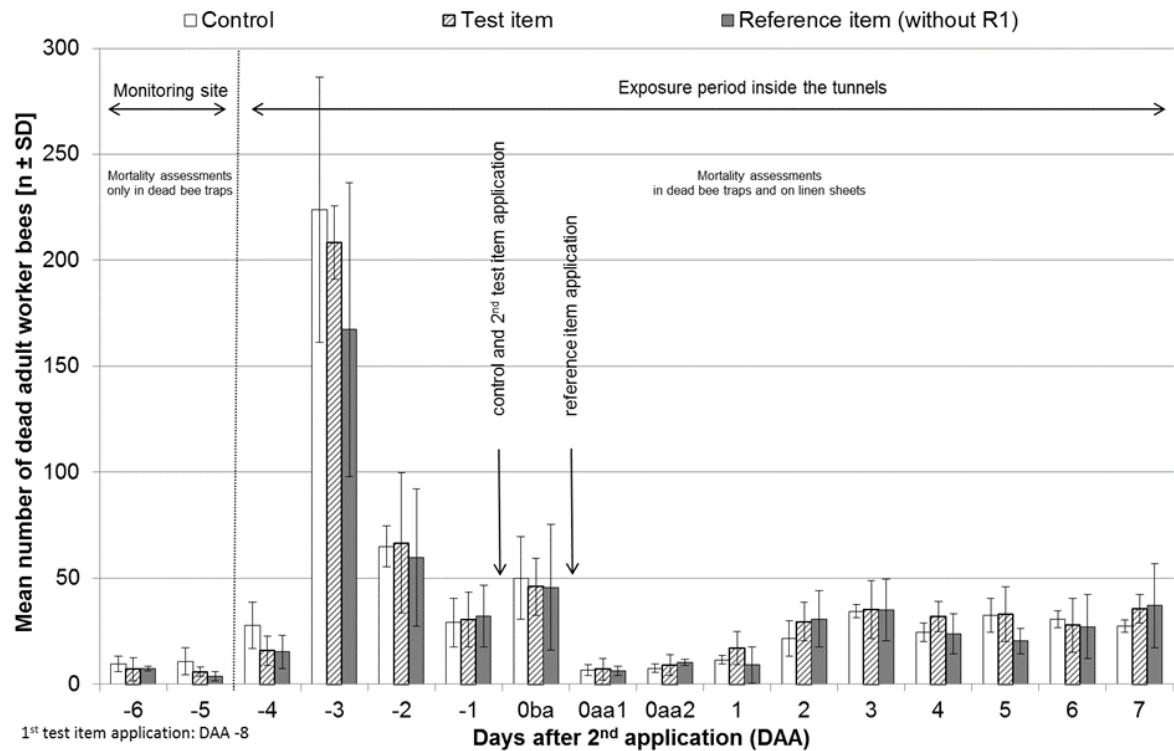
The daily mean mortalities of worker bees during the pre-exposure period at the monitoring site (DAA -6 to -5) were at a similar and low level in all treatment groups (< 11 dead bees/day).

During the period before the control and the second test item application (DAA -4 to DAA -1), conspicuously large numbers of dead bees were observed on DAA -3, in all treatment groups. The highest mortality was recorded in the control, with 223.8 dead bees. On the following assessment day (DAA -2), reduced (by the factor 3), but still slightly increased numbers of dead bees were recorded. As these increased numbers of dead bees were only observed on the sheets (not in the dead bee traps) and in all treatment groups, it can be assumed that the colonies had short-term problems with acclimatising to the new environmental conditions inside the tunnels. On all other assessment days during the exposure period inside the tunnels, i.e. also after the second test item application, the mortalities of all treatment groups were again at low and comparable levels.

Regarding the post-exposure period at the monitoring site (DAA 8 to 28) and the post-application periods from DAA -4 to DAA 28 and DAA 0aa to 28, the mean mortalities were in general comparable and within the range of the normal expected biological variability in all treatment groups. Thus, a test item related adverse effect on the adult bee mortality can be excluded.

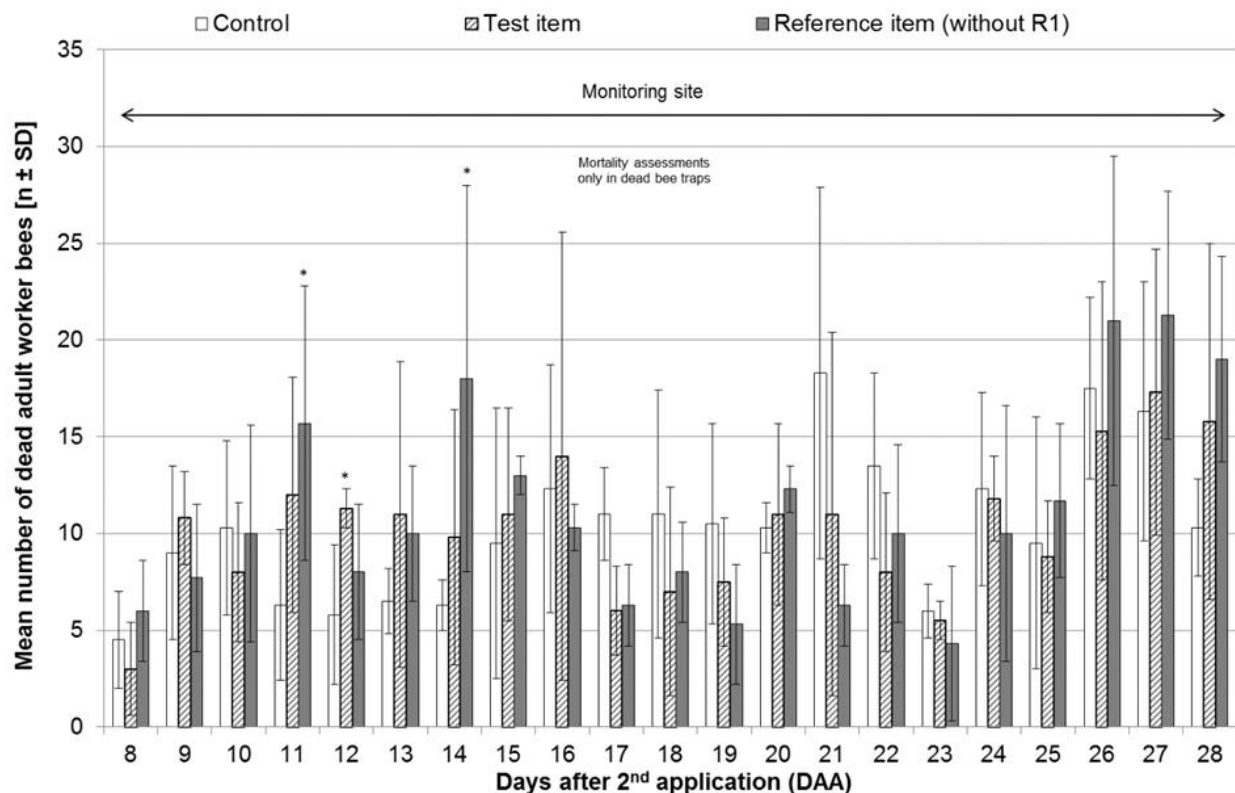
Mortality, pupae

During the entire Field Phase (DAA -6 to 28) only a few dead pupae were recorded in the control and test item group. In contrast, the reference item colonies showed an increase in daily mean pupal mortality from DAA 10 until DAA 28. Thus the sensitivity of the test system was confirmed and a test item related adverse effect on pupal development can be excluded.



DAA 0 = 11.07.2016 (1st day on which the bees were exposed to the water treated control, the 2nd test item and the reference item application);
ba = before application; aa = after application (1: assessment 2 hours after application; 2: assessment in the evening after daily foraging activity);
SD = standard deviation

Figure A 34: Adult worker bee mortality during the pre-exposure and exposure periods



DAA 0 = 11.07.2016 (1st day on which the bees were exposed to the water treated control, the 2nd test item and the reference item application);
SD

Figure A 35: Adult worker bee mortality during the post-exposure period at the monitoring site

Table A 113: Adult worker bee mortality during the entire period of the study

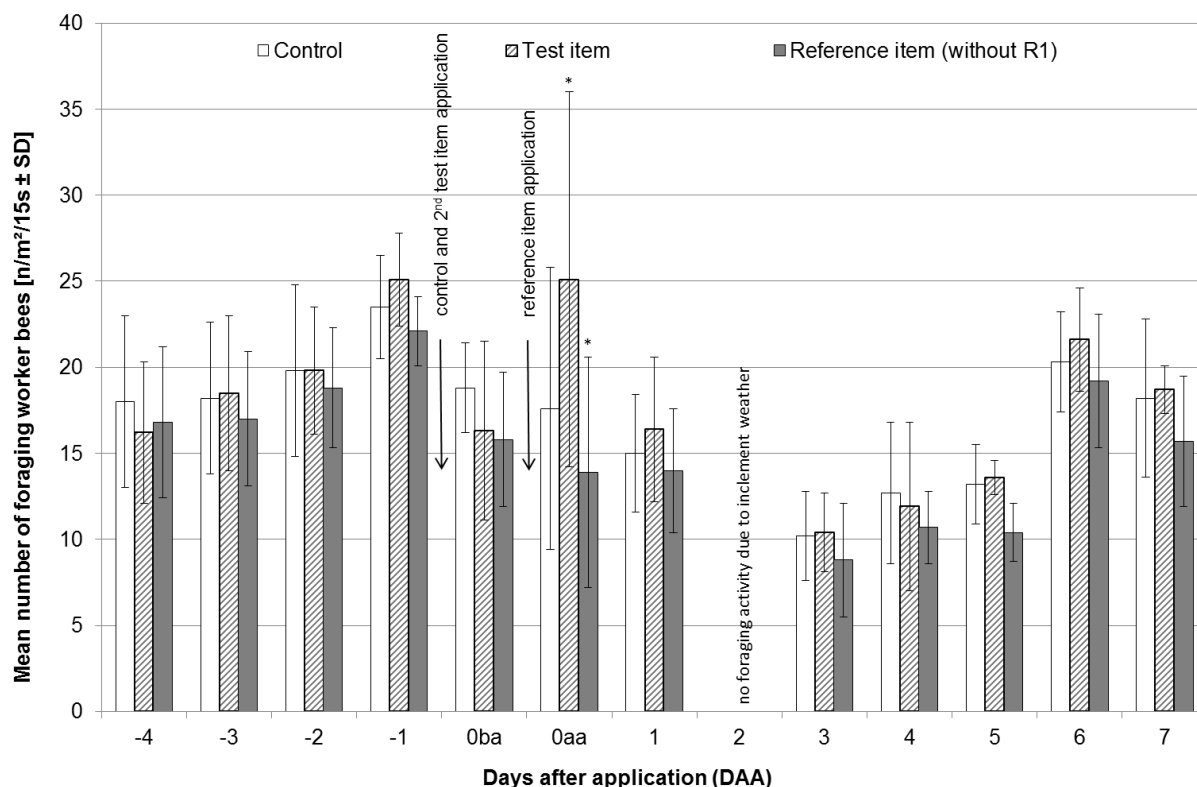
Date [dd.mm.yyyy]	DAA	Control [n]		Test item [n]			Reference item [n] (without R1)		
		Mean [n]	SD	Mean [n]	SD	Stat.	Mean [n]	SD	Stat.
05.07.2016	-6	9.5	3.7	9.5	3.7	n.s.	7.3	1.2	n.s.
06.07.2016	-5	10.8	6.4	10.8	6.4	n.s.	3.7	2.1	n.s.
Mean (-6 to -5)¹⁾		10.1	4.9³⁾	6.4	3.9³⁾	n.s.	5.5	2.5³⁾	n.s.
07.07.2016	-4	27.8	11.0	15.8	7.0	n.s.	15.3	7.8	n.s.
08.07.2016	-3	223.8	62.5	208.5	17.3	n.s.	167.3	69.2	n.s.
09.07.2016	-2	65.0	9.6	66.5	33.1	n.s.	59.7	32.5	n.s.
10.07.2016	-1	29.0	11.3	30.5	12.9	n.s.	32.0	14.5	n.s.
Mean (-4 to -1)²⁾		86.4	88.3³⁾	80.3	80.8³⁾	n.s.	68.6	70.2³⁾	n.s.
11.07.2016	0ba	50.0	19.4	46.0	13.5	n.s.	45.7	29.7	n.s.
11.07.2016	0aa1	6.5	2.5	7.0	5.2	n.s.	6.3	2.1	n.s.
11.07.2016	0aa2	7.5	2.1	9.0	5.0	n.s.	10.3	1.5	n.s.
Σ 0aa²⁾		14.0	3.6³⁾	16.0	10.0³⁾	n.s.	16.7	3.5³⁾	n.s.
12.07.2016	1	11.5	2.1	17.0	7.7	n.s.	9.0	8.7	n.s.
Σ 0aa+1²⁾		25.5	4.2	33.0	17.1	n.s.	25.7	5.7	n.s.
13.07.2016	2	21.5	8.3	29.5	9.2	n.s.	30.7	13.3	n.s.
14.07.2016	3	34.3	3.1	35.3	13.6	n.s.	35.0	14.4	n.s.
15.07.2016	4	24.5	4.4	31.8	7.1	n.s.	23.7	9.5	n.s.
16.07.2016	5	32.3	8.0	33.0	13.0	n.s.	20.3	5.9	n.s.
17.07.2016	6	30.8	4.0	27.8	12.8	n.s.	27.0	15.1	n.s.
18.07.2016	7	27.5	2.9	35.5	6.6	n.s.	37.0	19.7	n.s.
Mean (Σ 0aa+1 to 7)²⁾		28.0	6.4³⁾	32.3	10.8³⁾	n.s.	28.5	12.2³⁾	n.s.
Mean (-4 to 7)²⁾		49.3	57.2³⁾	49.4	51.6³⁾	n.s.	43.3	45.1³⁾	n.s.
19.07.2016	8	4.5	2.5	3.0	2.4	n.s.	6.0	2.6	n.s.
20.07.2016	9	9.0	4.5	10.8	2.4	n.s.	7.7	3.8	n.s.
21.07.2016	10	10.3	4.5	8.0	3.6	n.s.	10.0	5.6	n.s.

Date [dd.mm.yyyy]	DAA	Control [n]		Test item [n]			Reference item [n] (without R1)		
		Mean [n]	SD	Mean [n]	SD	Stat.	Mean [n]	SD	Stat.
22.07.2016	11	6.3	3.9	12.0	6.1	n.s.	15.7	7.1	* ^D
23.07.2016	12	5.8	3.6	11.3	1.0	* ^M	8.0	3.5	n.s.
24.07.2016	13	6.5	1.7	11.0	7.9	n.s.	10.0	3.5	n.s.
25.07.2016	14	6.3	1.3	9.8	6.6	n.s.	18.0	10.0	* ^U
26.07.2016	15	9.5	7.0	11.0	5.5	n.s.	13.0	1.0	n.s.
27.07.2016	16	12.3	6.4	14.0	11.6	n.s.	10.3	1.2	n.s.
28.07.2016	17	11.0	2.4	6.0	2.3	n.s.	6.3	2.1	n.s.
29.07.2016	18	11.0	6.4	7.0	5.4	n.s.	8.0	2.6	n.s.
30.07.2016	19	10.5	5.2	7.5	3.3	n.s.	5.3	3.1	n.s.
31.07.2016	20	10.3	1.3	11.0	4.7	n.s.	12.3	1.2	n.s.
01.08.2016	21	18.3	9.6	11.0	9.4	n.s.	6.3	2.1	n.s.
02.08.2016	22	13.5	4.8	8.0	4.1	n.s.	10.0	4.6	n.s.
03.08.2016	23	6.0	1.4	5.5	1.0	n.s.	4.3	4.0	n.s.
04.08.2016	24	12.3	5.0	11.8	2.2	n.s.	10.0	6.6	n.s.
05.08.2016	25	9.5	6.5	8.8	2.9	n.s.	11.7	4.0	n.s.
06.08.2016	26	17.5	4.7	15.3	7.7	n.s.	21.0	8.5	n.s.
07.08.2016	27	16.3	6.7	17.3	7.4	n.s.	21.3	6.4	n.s.
08.08.2016	28	10.3	2.5	15.8	9.2	n.s.	19.0	5.3	n.s.
Mean (8 to 28) ¹⁾		10.3	5.7 ³⁾	10.3	6.2 ³⁾	n.s.	11.2	6.4 ³⁾	n.s.
Mean (Σ 0aa+1 to 28) ^{1) & 2)}		14.7	9.7 ³⁾	15.8	12.2 ³⁾	n.s.	15.5	11.1 ³⁾	n.s.
Mean (-4 to 28) ^{1) & 2)}		24.5	39.4 ³⁾	24.5	36.6 ³⁾	n.s.	22.8	31.5 ³⁾	n.s.

For all calculations (means, SDs) DAA 0ba and DAA Σ 0aa1+2 were considered as daily values although covering less than 24 hours. DAA = days after application (DAA 0 = 11.07.2016 (1st day on which the bees were exposed to the water treated control, the 2nd test item and the reference item application)); ba = before application; aa = after application; SD = standard deviation; Stat. = Statistics; n.s. = not statistically significantly different; * statistically significantly different compared to the control ($p < 0.05$); ^D Dunnett test; ^U U-test; ^M Median test; ¹⁾ mortality in dead bee traps; ²⁾ mortality in dead bee traps and on sheets; ³⁾ standard deviation calculated for the individual values of the respective group

Foraging activity

The mean and overall foraging activities of all treatment groups before application were at comparable levels. On the day after the second test item application (DAA 0aa), the foraging activity was highest in the test item group. From DAA 1 on, the mean and overall foraging activities of all treatment groups were again at comparable levels. However, due to rainy weather conditions during the assessment on DAA 2, no foraging activity was recorded. Thus, a test item related adverse effect of the test item on the foraging activity can be excluded.



DAA 0 = 11.07.2016 (1st day on which the bees were exposed to the water treated control, the 2nd test item and the reference item application); ba = before application; aa = after application; SD = standard deviation

Figure A 36: Foraging activity - Average number of bees/m²

Table A 114: Foraging activity - average number of bees/m²

Date [dd.mm.yyyy]	DAA	Control		Test item			Reference item (without R1)		
		Mean [n/m²/15s] ¹⁾	SD	Mean [n/m²/15s] ¹⁾	SD	Stat.	Mean [n/m²/15s] ¹⁾	SD	Stat.
07.07.2016	-4	18.0	5.0	16.2	4.1	n.s.	16.8	4.4	n.s.
08.07.2016	-3	18.2	4.4	18.5	4.5	n.s.	17.0	3.9	n.s.
09.07.2016	-2	19.8	5.0	19.8	3.7	n.s.	18.8	3.5	n.s.
10.07.2016	-1	23.5	3.0	25.1	2.7	n.s.	22.1	2.0	n.s.
Mean (-4 to -1)		19.9	4.0	19.9	4.4	n.s.	18.7	3.0	n.s.
11.07.2016	0ba	18.8	2.6	16.3	5.2	n.s.	15.8	3.9	n.s.
	0aa	17.6	8.2	25.1	10.9	* ^M	13.9	6.7	* ^U
12.07.2016	1	15.0	3.4	16.4	4.2	n.s.	14.0	3.6	n.s.
13.07.2016	2 ²⁾	0.0	0.0	0.0	0.0	n.s.	0.0	0.0	n.s.
14.07.2016	3	10.2	2.6	10.4	2.3	n.s.	8.8	3.3	n.s.
15.07.2016	4	12.7	4.1	11.9	4.9	n.s.	10.7	2.1	n.s.
16.07.2016	5	13.2	2.3	13.6	1.0	n.s.	10.4	1.7	n.s.
17.07.2016	6	20.3	2.9	21.6	3.0	n.s.	19.2	3.9	n.s.
18.07.2016	7	18.2	4.6	18.7	1.4	n.s.	15.7	3.8	n.s.
Mean (0aa to 7)		13.4	6.6	14.7	7.6	n.s.	11.6	5.9	n.s.
Mean (-4 to 7)		15.8	6.4	16.4	6.9	n.s.	14.1	5.9	n.s.

DAA = days after application (DAA 0 = 11.07.2016 (1st day on which the bees were exposed to the water treated control, the 2nd test item and the reference item application)); ba = before application; aa = after application; SD = standard deviation (calculated for the mean values per tunnel and assessment (three locations/tunnel)/date); Stat. = Statistics; n.s. = not statistically significantly different; * statistically significantly different compared to the control ($p < 0.05$); ^M Median test; ^U U-test; ¹⁾ mean foraging activity on DAA -4, DAA -3, DAA -2 and DAA 1 (3 assessments) and on DAA 0aa (7 assessments); ²⁾ foraging activity was low due to bad weather conditions

Behaviour

There was no test item related effect on honey bee behaviour.

Condition of the colonies

The pre-exposure colony condition assessment indicated that the honey bee colonies were healthy, all brood stages were present and colony strengths were comparable. A sufficient amount of nectar and pollen was available in all colonies.

During the entire course of the Field Phase no considerable differences in numbers of bees, brood and food cells were observed between the colonies of all treatment groups.

Detailed brood development

Brood Termination Rate (BTR)

The mean BTRs at the final BFD assessment at BFD 22 were $33.4 \pm 3.8\%$ for the control group, $27.7 \pm 3.7\%$ for the test item group, and $47.8 \pm 14.8\%$ for the reference item group. The mean BTR of the test item group was thus lower than the mean BTR of the control group. Thus, a test item related adverse effect on the detailed brood development can therefore be excluded.

Brood index (BI)

At the final BFD assessment at BFD 22, the determined mean BI of the test item group was 3.6 ± 0.2 and therefore slightly higher than that of the control group with 3.3 ± 0.2 . Thus, a test item related adverse effect on the bee brood development can be excluded. The mean brood index of the reference item group was 2.6 ± 0.7 .

Compensation index (CI)

Generally the mean CI values of all treatment groups were slightly higher than the corresponding mean brood-indices, indicating that cells with terminated brood were at least partially refilled with new eggs, which shows the recovery of the colonies. Hence, at the final BFD assessment (BFD 22) the mean CI values of the control, the test item and the reference item group were with 4.2 ± 0.2 , 4.3 ± 0.2 and 3.9 ± 0.3 at slightly higher levels than their corresponding brood indices, indicating that none of the treatments had an adverse effect on the recovery of bee brood development.

Residue analysis

The analytical method used in the current study was previously validated in this study (see Section 5.1.2). In order to confirm the analytical method was functioning correctly when used in the current study, procedural recovery samples were processed and analysed concurrently with the test samples and are summarised in the table below.

Table A 115: Procedural recovery data for acetamiprid in flowers, nectar, pollen, larvae, honey and wax

Matrix	Fortification level [mg/kg]	Recovery (%)		Overall RSD (%)	n
		Range	Overall Mean		
Flowers	0.01	84 – 97	91	5.9	1
	0.1				1
	1				1
	30				1
Nectar	0.01	104 – 115	110	3.1	1
	0.1				1
Pollen	0.01	71 – 100	86	15	1
	0.1				1
	1				1
	10				1
Larvae	0.01	106 – 110	108	2.6	1
	0.1				1
Honey	0.01	106 – 109	108	2.0	1
	0.1				1
Wax	0.01	100 – 105	103	3.5	1
	0.1				1

Residues of acetamiprid in the control flower, pollen, nectar, larvae, honey and beeswax specimens were

below the limit of detection (LOD).

The determined residues of acetamiprid in the treated flowers ranged between 3.7 mg a.s./kg and 7.5 mg a.s./kg on DAA -4 and between 18 mg a.s./kg and 25 mg a.s./kg on DAA 0 in all treated tunnels. On DAA 3, the flowers in the treated residue tunnel showed acetamiprid residues of 6.4 mg a.s./kg and 1.0 mg a.s./kg on DAA 7.

The determined residues of acetamiprid in pollen sampled only from the treated residue tunnel were 0.60 mg a.s./kg on DAA -4 and 8.5 mg a.s./kg on DAA 0. The residue analysis for DAA 3 and DAA 7 (see Attachment 1) were repeated and finally reported to be 0.46 mg a.s./kg and 0.61 mg a.s./kg on DAA 3 and 0.51 mg a.s./kg and 0.61 mg a.s./kg on DAA 7.

Endpoints

Two applications of MCW-2222 after bee flight to *Phacelia tanacetifolia* at a rate of 80 g a.s./ha did not cause any adverse effects on the survival of adult worker bees and bee pupae, foraging activity, behaviour, colony condition (colony strength, brood and food). Furthermore, the specific evaluation of the detailed bee brood development showed no impact of the test item on the development on honeybee brood.

Validity criteria

As shown in the following table, all validity criteria were met.

Table A 116: Validity criteria

No validity criteria are given by OECD GD 75 (2007) but by the study plan	Observed in study
Before treatment:	
Mean foraging activity shortly before the water treated control and the second test item application (BBCH 63-65) should have been stopped (0 bees/m ²)	Foraging activity shortly before the water treated control and the second test item application had stopped and no more bees foraging on the crop were observed. Criterion was achieved
Mean foraging activity shortly before the application of the reference item should be >10 bees/m ² in the respective reference item tunnels	Foraging activity before the reference item application was: C: 18.8 bees/m ² T: 16.3 bees/m ² R: 15.8 bees/m ² Criterion was achieved
After treatment:	
A detectable effect of the reference item should be given, i.e. the brood termination rate or the pupae mortality is increased compared to the control group.	An increased pupal mortality was observed between DAA 10 to DAA 28 (except for DAA 16, DAA 19 and DAA 21 to DAA 23). The maximum mortality was reached on DAA 13 (20.0 ± 9.5 dead pupae). Criterion was achieved

Conclusion

To assess the potential effects of MCW-2222 on the honeybee (*Apis mellifera* L.), MCW-2222 was applied under semi-field conditions at a nominal rate of 444.3 g product/ha (80 g a.s./ha acetamiprid) once before (BBCH 59 – 61) and once during flowering of *Phacelia tanacetifolia* (BBCH 60 – 65). The second application took place seven days after the first application and after set-up of the bee hives and was conducted in the evening, after daily bee flight activity, under semi-field conditions in Germany in summer 2016. Potential effects on bee mortality, foraging activity, behaviour and colony condition (i.e. colony strength, brood and food amount) were investigated. Special attention was laid on the assessment of the detailed bee brood development.

Residues of acetamiprid in flowers and in pollen between DAA -4 to DAA 7 confirmed the exposure of the bees. No residues were found in nectar, beeswax, honey and larvae during the entire study.

The application of MCW-2222 did not cause any adverse effects on the survival of adult worker bees and bee pupae, foraging activity, behaviour, colony condition (colony strength, brood and food). Furthermore, the specific evaluation of the detailed bee brood development showed no impact of the test item on the development on honeybee brood.

In conclusion the study clearly demonstrated that two applications of MCW-2222, at a nominal rate of 2 x 444.3 g product/ha MCW-2222 (corresponding to 2 x 80 g a.s./ha acetamiprid), did not adversely affect the survival and fitness of honeybee brood or colonies.

A 2.3.1.8 KCP 10.3.1.6 Field tests with honey bees

A 2.3.1.8.1 KCP 10.3.1.6/01 Field study with honey bees on *Phacelia*

Comments of zRMS:	<p>The study has been already evaluated and accepted by the zRMS in the course of the first zonal evaluation of CA3573 (formerly MCW-2222) finalised in April 2018. Although the test guideline has not changed since that time, the study has been re-evaluated by the zRMS in 2021.</p> <p>The study was conducted in line with methodology described in OEPP/EPPO Bulletin 40, 313-319 on a field of <i>Phacelia tanacetifolia</i> in the Northern France. Application of MCW-2222 (100 g a.s./ha) was performed at BBCH 64, in the evening without presence of foraging bees, 7 days after hives settlement. Untreated <i>Phacelia</i> field served as control.</p> <p>The distance between control and treatment fields was approximately 6 km (at least 4 km are currently required). No bee attractive crops were present at the test site during the experimental phase.</p> <p>Observations of bee mortality, behaviour and effects on bee brood were performed up to 41 days after the treatment (41 DAA). Observation of bee brood covered full brood cycle and beginning of a new one (from BFD 0 to BFD 28 but statistical analyses were performed for results up to BFD 22). No brood measurements were taken at the test termination (41 DAA).</p> <p>During the exposure phase rainfall occurred on DAA 2, DAA 3, DAA 4 and DAA 5 at 3, 6, 1 and 13 mm, respectively. Although residues of acetamiprid were detected in chemical analyses in nectar and bee bread up to 8 DAA (in pollen low levels were detected) and in honey at 20 DAA, exposure could be reduced to some extent. However, precipitation was low and residue analyses confirmed that despite rainfall acetamiprid was present in flowers and pollen.</p> <p>Additionally, due to the rainfall and bad weather, at DAA 2 to DAA 4 foraging activity decreased in both treatments. At DAA 5 the activity increased in both treatments (around 8 bees/m²). Then, a continuous decrease was recorded from DAA 6 until the end of the trial, with daily variability due to the weather. This further decrease was a result of a slow falloff of the phacelia fields attractiveness.</p> <p>During two days (DAA 18 and DAA 19), a higher mortality was recorded for all hives in the test item treatment compared to the control. However, no acetamiprid residues were found in the dead bees and most probably this higher mortality late in the study was due some other biological reasons. This is further confirmed by increase of mortality in some treatment group hives on DAA 5, DAA 9, DAA 17 and DAA 35 (i.e. not in every hives of the MCW-2222 treated field), which was also seen in control hives. Overall, the mortality pattern in control and treatment groups was comparable with exception of increased mortality in treatment groups at 18 DAA and 19 DAA (see Figure A 18).</p> <p>Elevated pupae mortality was observed in treatment groups comparing to controls on DAA 4, DAA 5 and DAA 6, but it was still at low level comparable with mortality in treatment groups before application. Difference between test item and control groups was more pronounced due to very low pupae mortality in controls, lower than observed before the treatment.</p> <p>The test item had no effect on investigated bee brood parameters.</p> <p>It is noted that 3 test item colonies lost their queens, while one queen was lost in the control group. In neither of those hives the queen cells were observed till the end of the study, meaning that recovery at the end of the season was unlikely. In addition to that, especially</p>
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	<p>at 2nd but also on 3rd colony strength assessment very low number of brood cells was observed in two control hives, indicating weak reproductive performance of the queens. Better reproductive performance was observed in test item groups. It is also noted that already at the beginning of the study the colonies were not particularly strong and the number of bees in most of hives (nursery bees) was too low in relation to the amount of brood to assure successful development of all brood cells. This was also seen at next colony assessments, but was less pronounced. In general, at the test termination the colonies were not stronger comparing to the study initiation and some colonies in both, control and test item groups, were actually weaker. Nevertheless, this pattern could be observed in control and test item hives, so it is not considered to be treatment related.</p> <p>Effects of the test item on the overwintering success were not investigated and the trial was terminated 41 days after application.</p> <p>Overall, application of MCW-2222 to flowering <i>Phacelia tanacetifolia</i> at 100 g a.s./ha had no adverse effects on bees mortality, foraging activity and bee brood. However, the zRMS is of the opinion that bad weather and decreased foraging activity might affected the actual exposure of the bees and results of the study should be treated with caution.</p>
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Data point: KCP 10.3.1.6/01

Report: Field Study to Evaluate Potential Side Effects of the product MCW-2222 (acetamiprid 200 g/L) on Brood Development, Foraging Activity, Mortality and Behaviour of Adult Honeybees *Apis mellifera* L. (Hymenoptera: Apidae) Following Application after Bee-Flight on *Phacelia tanacetifolia*. Molitor, C., 2015b, R-34877, 215-2014; including Final Report Amendment N°1

Guideline(s): EPPO 1/170 (4) (2010), C.E.B methodology n°230, part IV (2003), OECD GD75 (2007)

Deviations: None

GLP: Yes, certified laboratory

Acceptability: Yes, study considered acceptable

Duplication: Not applicable
(if vertebrate study)

Executive Summary

In a field study with honeybees (*Apis mellifera* L.) the potential effects of MCW-2222 on mortality, foraging activity, behaviour, colony strength and colony development (i.e. quality and quantity of brood and the amount of reserves) were investigated. Special attention was laid on the detailed brood assessment of marked cells containing eggs, young and old larvae.

Two fields (2 ha each, separated from each other by a distance of around 6 km) with flowering *Phacelia* served as plots. One was used for the application of MCW-2222 at a rate of 0.5 L/ha (100 g a.s./ha acetamiprid) (T). The second was left untreated field and served as control (C). Application of the test item to the crop was performed after bee flight.

Seven honey bee colonies, each about 20,000 bees were placed at each field 7 days before the application (7DBA) to get familiar with the new conditions with a crop being at BBCH 62. They were placed at a sufficient distance from the crop to avoid any spray drift. All colonies were used to record mortality. Moreover, four of the seven hives were used for the brood development assessments, whereas the three remaining ones were used for sampling of pollen (via pollen traps fixed at the entrance of the hives), nectar, bee bread and honey for residue analysis. Exposure phase lasted from the day of application (0DAA, BBCH 64) to the end of flowering (37DAA, BBCH 69). 38 days after application (38DAA), the colonies were located to the monitoring site where no further pesticide exposure was expected. They were returned to the beekeeper's apiary on 54DAA.

In order to ensure that bees were expose to the test item, observations on the foraging activity were scheduled daily from 1DBA to 14DAA. One extra assessment per day was performed at 0DBA and at 1DAA, meaning that there were two counts on these days. The foraging activity in each field was recorded

by counting the number of forager bees on two areas of 10 m² per field.

Assessments on adult and pupal mortality (via dead bee traps) were daily conducted between 1BDA to 21DAA and then on 27, 35 and 41DAA. Moreover, mortality was assessed once more on the day of application (0DBA) and the day after (1DAA). Dead adults and pupae were sampled in a plastic jars (one per treatment per day) and kept frozen for potential residue analysis.

The behaviour or possible behavioural anomalies of the bees were observed and recorded on the crop and at the entrance of the hives, at the same time as the observation on foraging activity. Possible clinic signs of poisoning were recorded too.

Detailed assessments of the bee brood development was carried out by marking individual brood cells containing either eggs, young or old larvae at the Brood Area Fixing Day 00 (BFD00), which was one day before application (1DBA). At this day one hundred cells of each development stage were selected in each hive and followed until 28 days after BFD (BFD28) which covered one brood cycle and the beginning of the expected second one. Next to the assessment on BFD 28 the development of each individually marked cell was assessed at BFD05, BFD10, BFD16 and BFD22. Each brood comb was photographed at each assessment time.

Three apiarist visits were scheduled on the day of Brood Fixing Day (BFD00 = 1DBA), at BFD 28 and BFD 42, in order to assess the colony development. Parameter taken into account was the adult bee population recorded according to the adapted Liebfeld method. The estimated quantity and quality of the brood (different stages observed) and amount of reserves were also recorded.

For residue analysis, flowers were gathered on 1DAA from 12 different points in each field. Additionally, specimen for residue analysis were sampled in each of the three dedicated hives per treatment group. I.e., samples of pollen were collected 3DAA and 8DAA via pollen taps, samples of nectar were taken 8DAA from newly filled reserve combs, samples of bee bread and honey were respectively collected 8DAA and 20DAA. Some adult bees were also collected from bee traps when the recorded mortality was significantly higher than the other days.

On the day of the evening application (0DBA), the foraging activity was around 6 bees/m² in the control and 8 bees/m² in the MCW-2222 treated field, which is considered as a good level. This foraging activity level was even higher the day after application since it reached around 9 bees/m² in both fields, which confirmed the exposure of foraging bees just after the application. Few days after application (2DAA to 4DAA), foraging activity decreased in both treatments due to rainfalls and bad weather, with a density below the validity criteria of 3 bees/m² at 3DAA and 4DAA. At 5DAA, the activity again increased drastically in both treatments (around 8 bees/m²). Then, a continuous decrease was recorded from 6DAA until the end of the trial, with daily variability due to the weather. This decrease resulted of a slow falloff of the phacelia fields' attractiveness. But nevertheless, foraging activity was above 3 bees/m² at almost all days. Overall, no adverse effects on the foraging activity, no abnormal behaviour of the bees and no symptoms of intoxication were recorded after the application of MCW-2222.

Daily mortality of adult bees recorded in the two treatments were stable and comparable from 0DBA to 4DAA. Then, differences to the control were recorded on single days which were significantly different at 5, 7, 9, 17, 18, 19, 27 and 35DAA. At 5, 9, 17 and 35 DAA, this difference was due to an increase of mortality in some hives (i.e. not in every hive of the MCW-2222 treated field), which variability between hives was also seen in the untreated control. Moreover, as no residues of acetamiprid were quantified in the dead honeybees (samples of 9, 17 and 19DAA) it can be assumed that differences in mortality data were not linked to an intoxication but to some biological reasons.

Regarding dead pupae, the number of dead pupae found each day was low (up to 8 daily dead pupae only). However, statistically significant differences were observed on 2, 10 and 12 DAA, which were regarded as biological not relevant with respect to the thousands of pupae being in a colony.

Apiarist visits at the beginning of the experimental phase, during the trial after the last brood assessments, and at the end of the trial did not indicate any impact of the test item on the colony strength as well as on the quantity and quality of the brood. Observed differences were mostly due to experimental manipulation (loss of queens is not rare because of the high frequency of hive opening in order to conduct the brood assessments) and seasonal conditions (less resources in the late summer).

The detailed assessment of single brood stages resulted in low and comparable BTRs in the control and test item group. In fact, mean BTRs at the end of the brood cycle for eggs, young and old larvae in the control amounted to 11.67%, 8.67% and 8.0% compared to 10.25%, 7.5% and 6.25% in the test item group, respectively. No statistical difference was met between both treatment groups. Due to the low and similar

BTRs, Brood and Compensation Indexes were high and almost equal in both treatment groups without any significant differences between being detected. Overall, no effect of the test item on the pre-imaginal development of eggs, young larvae or old larvae could be detected.

No acetamiprid was detected in the flower specimens sampled in the untreated field, while 5.0 mg/kg of acetamiprid and 0.017 mg/kg of acetamiprid-N-desmethyl were measured in the flower sample from the treated field; it verified the exposure of the honeybees foraging in the phacelia and thus validates the trial design.

In the specimens of flowers sampled in the untreated field as well as in the specimens of pollen sampled from hives placed in this control plot, no acetamiprid was detected. In-hive nectar, bee bread and honey specimens were free from residues in one hive of the untreated control whereas in the two other hives, levels of acetamiprid were quantified (0.018±0.004 mg/kg in in-hive nectar at 8DAA, 0.066±0.035 mg/kg in bee bread at 8DAA and 0.020±0.005 mg/kg in honey at 20DAA). Residue level of 0.012 mg/kg of acetamiprid-N-desmethyl metabolite has been measured in bee bread specimen of one hive. The origin of these residues was not characterized due to the design of the study as it is an open field study and honeybees were not confined to the untreated control field.

From the treated field, residue levels of acetamiprid were detected in all specimens collected in the hives attesting exposition of hives to the test item. Residue level was 0.111±0.086 mg/kg at 3DAA in pollen, 0.033±0.013 mg/kg at 8DAA in nectar, 0.109±0.048 mg/kg at 8DAA in bee bread and 0.031±0.008 mg/kg at 20 DAA in honey specimens. Pollen specimens collected at 8DAA showed a much lower level (<LOQ to 0.015 mg/kg). No residue of acetamiprid-N-desmethyl metabolite has been measured in any specimen. Validity of the study was given, because in both fields the recorded foraging activity was about 8 bees/m² (trigger: 3 bees/m²) and daily mortality the day before application was less than 50 bees/hive (trigger value fixed in the CEB methodology n°230). Moreover, the BTR in the control was below the trigger value of 30% for the respective brood stages at the end of the observed bee brood cycle, i.e. 11.67% for eggs, 8.67% for young larvae and 8.00% for old larvae.

Materials and methods

Materials

Test item	MCW-2222
Batch #	93191024
Content of active substance	Acetamiprid 200 g/L (nominal); 198 g/L (analysed)
Description	Yellowish liquid
Control	C: Untreated crop
Toxic reference	none
Test organism	
Species	Honey bees (<i>Apis mellifera</i> L.) of healthy colonies with sister queens and the same age (not older than 2 years), containing about 20,000 bees per colony at test start with ten frames. Hives of Dadant type. All colonies at the beginning of the study - with at 4 to 9 frames containing all brood stages - with 1 to 5 storage frames - with 0 to 2 empty frames - were free of clear clinical symptoms of disease; no treatment against <i>Varroa</i> for at least 4 weeks before beginning of the study.
Source	local beekeeper, Alban Couëron
Food/feeding	Full flowering <i>Phacelia tanacetifolia</i> served as food supply, no additional feeding throughout the study. Watering was available in the near surroundings of the fields.

Study design and methods

Test duration	Pre-exposure phase (6DBA to 0DBA): 7 days at the study fields Exposure phase (0DAA to 37DAA): 37 days at the study fields Post-Exposure phase (38DAA to 40DAA): 3 days at the monitoring site
Experimental dates	1 th July to 12 th August 2014
Test doses	T: 100 g a.s./ha, applied after bee flight

Application was performed after bee flight (from 22:30 to 22:45) at

Test units

Group size/replicates

Endpoints and assessments

BBCH 64 (full flowering of *Phacelia*) of the crop with a volume of 200 L water/ha.

The actual treatment rate was 98% of the target application rate.

Study fields with flowering *Phacelia tanacetifolia* (variety: Meva), each with an area of 2 ha, and separated from each other by a distance of around 6 km; both study fields were surrounded by woods (few flowering plants were met at the considered period), cereals and sunflowers. The sunflower fields started to bloom at the end of the exposure phase of the study. Each study field with 7 colonies.

One study field per treatment group, each with each with 7 colonies; 4 colonies were used for biological assessments, 3 colonies for residue sampling; moreover, all colonies were used for recording of mortality.

mortality of adult bees and pupae:

Recording via dead bees traps; daily between 1 DBA to 21 DAA and then on 27, 35 and 41DAA. Moreover, mortality was assessed once more on the day of application (0DAA) and the day after (1DAA). Dead adults and pupae were sampled in a plastic jars (one per treatment per day) and kept frozen for potential residue analysis.

foraging activity:

Daily recording of the number of forager bees daily on two areas of 10 m² between 1DBA to 14DAA. One extra assessment per day was performed at 0DBA and at 1DAA, meaning that there were two counts on these days.

behaviour on the crop and at the entrance of the hives:

at the same time when the assessment for foraging activity took place

colony strength and colony development:

once at the beginning on the day of Brood Fixing Day (BFD00 = 1DBA), on BFD 28 and on BFD 42 (end of the study); assessment of:

- estimated number of bees (colony strength) acc. to Liebefeld method
- number of cells containing brood (total of cells with eggs, larvae and capped brood) to Liebefeld method
- presence of queens (e.g. presence of eggs)
- number of reserve, empty and foundation combs.

detailed bee brood development:

Marking of individual brood cells containing eggs, young and old larvae at BFD00 (= 1 DBA); 100 brood cells of each selected brood stage and hive. Monitoring the subsequent development until adult hatch using a digital image analysis.

Assessments on BFD00 (= 1DBA), BFD 5, BFD 10, BFD 16, BFD 22 and BFD 28, covering one complete brood cycle (21 days for worker bees) and the beginning of a new one.

The time schedule of assessment days was chosen in order to check the bee brood at different expected stages during the development. Each brood comb was photographed at each assessment time.

Based on number of cells with eggs marked at BFD 0 and number of eggs which failed to develop successfully until adult hatch the Brood Termination Rates (BTR) were determined for each replicate at each assessment day. Moreover, attributing values from 1 (egg stage) to 4 (pupae/capped cell) and 0 (empty after hatch) to the respective brood stages, the brood indices (BI) were calculated. As an recovery indicator for recovery of the bee brood the brood compensation indices (BCI) were calculated

Bee brood categories:

Value	Corresponding contents	Value	Corresponding contents
0	Empty	5	Nectar
1	Egg	6	Pollen
2	Young larvae (L1-L2)	7	Dead
3	Old larvae (L3-L5)	8*	Not characterized
4	Pupae (capped cell)		

*if the cell is noted 8, this cell is not included in any calculations

Expected brood development in case of marked eggs (a), young larvae (b) or old larvae (c) at BFD00

(a)

Assessment day	Expected brood stage in cell	Brood category for index calculation
BFD00	Egg	1
5 days \pm 1 after BFD00	Young larvae or old larvae	2 or 3
10 days \pm 1 after BFD00	Capped cells	4
16 days \pm 2 after BFD	Capped cells shortly before hatch	4
22 days \pm 2 after BFD00	Empty or reserve cells after hatch or new egg laid	5

(b)

Assessment day	Expected brood stage in cell	Brood category for index calculation
BFD00	Young larvae	2
5 days \pm 1 after BFD00	Old larvae or capped cells	3 or 4
10 days \pm 1 after BFD00	Capped cells	4
16 days \pm 2 after BFD00	Capped cells or empty or reserve cells after hatch or new egg laid	4 or 5
22 days \pm 2 after BFD00	Empty, reserve, egg or larvae after hatch	5

(c)

Assessment day	Expected brood stage in cell	Brood category for index calculation
BFD00	Old larvae	3
5 days \pm 1 after BFD00	Capped cells	4
10 days \pm 1 after BFD00	Capped cells or empty or reserve cells after hatch or new egg laid	4 or 5
16 days \pm 2 after BFD00	Empty, reserve, egg or larvae after hatch	5
22 days \pm 2 after BFD00	Empty, reserve, egg or larvae after hatch	5

The Brood Termination Rate (BTR) expresses the quantity of cell's failure in percentage for each brood comb at each assessment day. BTR was calculated by dividing the number of cells that do not reach the expected growth stage at a specific assessment day by the total number of cells observed. If no failure occurred during the brood development,

the BTR would be equal to 0%. Otherwise this rate increases with the number of terminated cells (dead larvae, nymph or significant delay in the development process, or food stored in cells at BFD05, 10 or 16). Cells noted 0 (empty), 5 (nectar) or 6 (pollen) before hatch (BFD22) or 7 (dead) or with any unexpected value at a specific BFD were considered to be failures in the brood development; value of these cells were equal to 0 for the calculation of BTR and the following index BI.

The Brood Index (BI) is an indicator of bee brood development and was calculated for each brood comb at each assessment day. As it is inverse related to the BTR, means that the lower the BTR the higher the BI. If brood cell contents reach the expected brood stage at the specific assessment day (see above), the cells are classified using the brood category number as defined above. On the opposite, if the expected brood stage is not reached or occurred with big delay or if food is stored in the cells at the respective assessments dates, the cells were valued with 0 at the assessment date and also the following dates, disregarding if cells were again filled with brood. The BI of a colony was obtained by summing up the value of all cells assessed the same day and divided by the number of observed cells. If all cells present a successful development (expected pattern), BI is equal to 5 which is the maximal value for this index.

The Compensation Index (CI) indicates the recovery of a colony and was calculated for each brood comb at each assessment day. Cells containing a brood stage were classified according to categories (from 0 to 8). Then values were converted to brood categories as described. If a cell was empty, contained nectar, pollen before hatch (BFD22) or contained dead larvae or pupae, its value became 0, meaning that the cell was empty from any brood stage. Only values of category at each date of assessment were taken into account, without considering the expected brood stage. Therefore this index does not penalize the development value of the brood after termination, suspension or delay. *Important note: At BFD05, honeybees of hive R011 (untreated control) did not take care of the eggs laid by the queen on the chosen comb at BFD00. The consequence was a high BTR calculated at BFD05 which was not representative compared to the other hives and a normal development. This hive R011 was excluded from mean calculations and graphical overviews of Brood Termination Rate (BTR) and Brood and Compensation Indexes (BI and CI) when eggs were selected at BFD00 and was replaced by the hive R014. However the hive R011 was kept when larvae were selected at BFD00.*

Specimens sampling for residue analysis

Samples of pollen from traps in front of three hives were collected 3DAA and 8DAA (24 specimens).

Samples nectar from newly filled reserve combs were put in plastic jars 8DAA (12 specimens).

Samples of bee bread and honey were respectively collected 8DAA (12 specimens) and 20 DAA (12 specimens).

Flowers were gathered from 12 different points in each field plot 1DAA (4 specimens).

Half of collected specimen were transported to the analytical laboratory GIRPA for residue analysis of acetamiprid and acetamiprid-N-desmethyl.

Some adult bees were also collected from bee traps when the recorded mortality was significantly higher than the other days.
Residues of acetamiprid and acetamiprid-N-desmethyl were extracted from the pollen with ethyl acetate using an automatic extractor, and from the other samples (flowers, nectar, honey, bee bread) by agitation in acetonitrile and ultra-pure water and purification by dispersive solid phase extraction. The quantification was performed by liquid chromatography with tandem mass spectrometry detection (LC-MS-MS).

Extra samples have been collected by the way of an amendment. Indeed, a high mortality was sometimes recorded in the dead bee trap and led to a sampling of these adult bees.
Colonies were set-up at the fields ten days before application on 7DBA to get familiar with the new conditions.

Adaptation of bees

Environmental conditions
Natural field conditions

There were changes of weather conditions with rainfalls during the experimental field phase. However, there were mostly dry days allowing bees to forage the crops during day light and sufficiently high temperatures (mean temperature around 18°C) to allow bee activity throughout. Exception occurred between 2DAA and 4DAA and at 10DAA and 11DAA when rain and wind did not allow a good bee foraging activity.

Conditions during application						
Temperature:	21 °C					
Wind speed:	0 km/h					
Rel. humidity:	54 %					
Precipitation:	none					
Conditions between						
DAA	0 to 7	8 to 15	16 to 21	22-28	29 to 35	36 to 40
Min. to max.						
Temp. [°C]:	11 to 30	11 to 30	14 to 34	8 to 33	7 to 24	7 to 25
Precip. [Σ mm]:	25	17	15	7	1	35
Days with rain [n]:	2	3	3	2	1	3

Biological observations

Foraging activity and behaviour was daily recorded between 1DBA to 14DAA, adult and pupal mortality was daily recored between 1BDA to 21DAA and on 27, 35 and 41DAA. For the detailed assessments of the bee brood development, 100 individual brood cells per hive containing either eggs, young or old larvae were marked at the Brood Area Fixing Day 00 (BFD00). The development of each marked cell was assessed at BFD05, BFD10, BFD16, BFD22 and BFD 28. The assessment of condition of the colony strength and colony development was performed on BFD00, BFD 28 and BFD 42.

Statistics

A statistical analysis was performed on the brood development results (BTR, BI and CI). ARM 6 Software was used to analyse the variance of treatments that are compared by a Student-Newmans-Keuls test (average followed by the same letter are not significantly different). This test gave an observed computed probability to be compared with a significance level which was defined at 5%. In order to perform statistical analysis, 8 hives (4 in the untreated control and 4 in the test item treatment) were used, the number of groups was 2 (both control and MCW2222 treatments) and there were five assessment days (BFD00, BFD05, BFD10, BFD16 and BFD22).

Moreover, a statistical analysis was performed on the mortality data of adult bees as well as pupae. The same procedure as the one describe above was used with a transformation $\text{Log}(x+1)$ of the data in order to reduce the heterogeneity of variance.

Results and discussion

Biological results

Foraging activity

The foraging activity was assessed from 1DBA to 14DAA on two areas in each plot. On the day of the evening application (0DBA), the foraging activity was around 6 bees/m² in the control field and 8 bees/m² in the MCW-2222 field, which is considered as a good level. This foraging activity level was even higher the day after application since it reached around 9 bees/m² in both plots.

Those above data confirms the exposure of foraging bees just after the application.

Few days after application (2DAA to 4DAA), foraging activity decreased in both treatments because of rainfalls and bad weather, with a density below the validity criteria of 3 bees/m² at 3DAA and 4DAA. At 5DAA, the activity increased drastically in both treatments (around 8 bees/m²). Then, a continuous decrease was recorded from 6DAA until the end of the trial, with daily variability due to the weather. This decrease resulted of a slow falloff of the phacelia fields' attractiveness. But nevertheless, foraging activity was above 3 bees/m² at almost all days.

No adverse effect on the foraging activity was observed further to the application of MCW-2222 on the phacelia field.

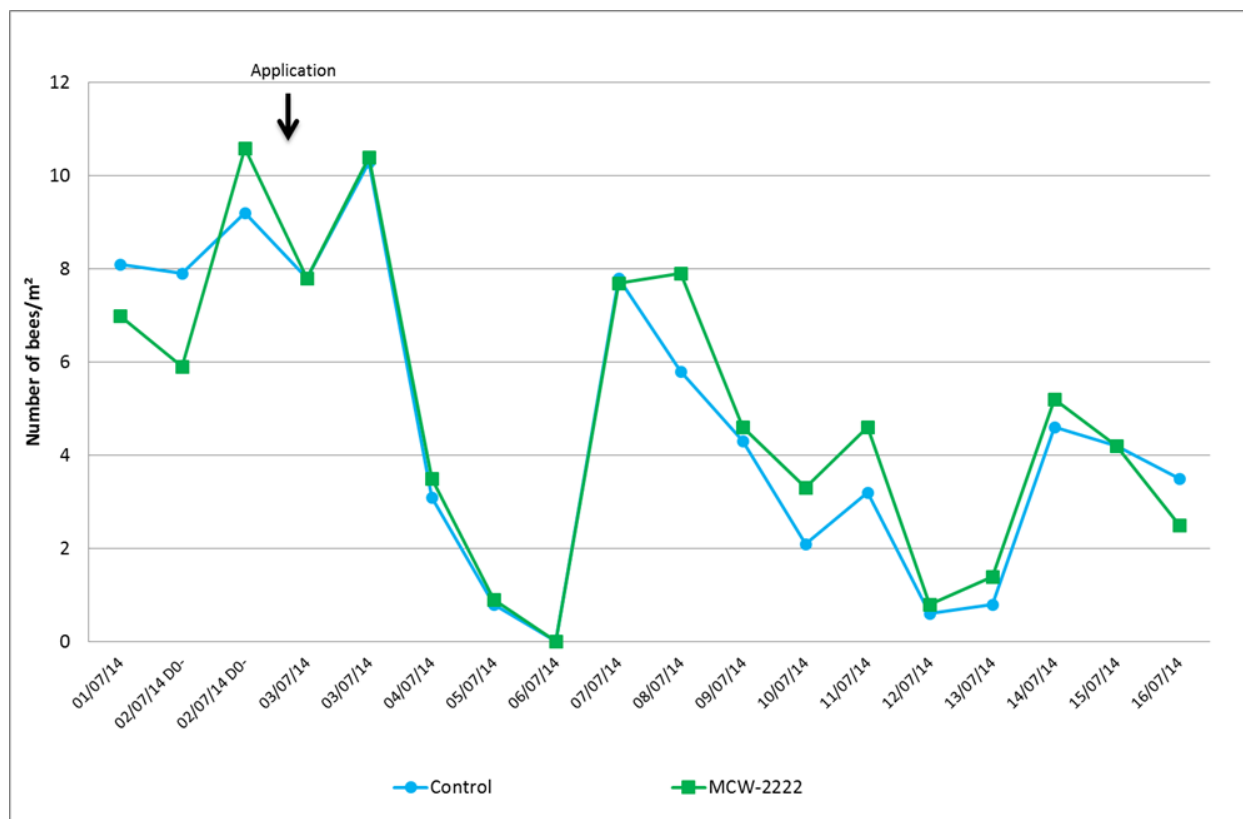


Figure A 37: Daily mean foraging activity

Table A 117: Daily mean foraging activity

Date	Timing	Number of bees/m ²	
		Control	MCW-2222
01/07/14	1DBA	8.1	7
02/07/14	0DBA	7.9	5.9
02/07/14	0DBA	9.2	10.6
03/07/14	1DAA	7.8	7.8
03/07/14	1DAA	10.3	10.4
04/07/14	2DAA	3.1	3.5
05/07/14	3DAA	0.8	0.9
06/07/14	4DAA	0	0
07/07/14	5DAA	7.8	7.7
08/07/14	6DAA	5.8	7.9
09/07/14	7DAA	4.3	4.6
10/07/14	8DAA	2.1	3.3
11/07/14	9DAA	3.2	4.6
12/07/14	10DAA	0.6	0.8
13/07/14	11DAA	0.8	1.4
14/07/14	12DAA	4.6	5.2
15/07/14	13DAA	4.2	4.2
16/07/14	14DAA	3.5	2.5

← Application after
bee flight

DBA = days before application; DAA = days after application

Behaviour

No abnormal behaviour of the bees and no symptoms of intoxication were recorded after the application of MCW-2222.

Mortality

Daily mortality of adult bees recorded in the two treatments were stable and comparable from 0DBA to 4DAA. Then, some daily differences were recorded from 5 to 9DAA, at 13DAA and from 17 to 41DAA with higher mean mortality in the hives of the test item treated plot.

Statistical analysis revealed that mortality data between both treatments were significantly different at 5 DAA, 7 DAA, 9 DAA, 17 DAA, 18 DAA, 19 DAA, 27 DAA and 35DAA.

During two days (18 DAA & 19DAA), a higher mortality was recorded for all hives in the test item treatment compared to the control one. A peak of mortality in hive R017 was observed and the dead bodies found in the catch trap were sampled. A multi-residue analysis was performed on the 19DAAs' sample and no quantifiable level (<LOQ) of acetamiprid nor any other usual active substance used in agriculture was found in the sample.

At 5 DAA, 9 DAA, 17 DAA and 35DAA, an increase of mortality in some hives was recorded (i.e. not in every hives of the MCW-2222 treated field), this variability between hives was also seen in the untreated control. Moreover, as no residue was quantified in the dead honeybees (samples of 9DAA and 17DAA) it can be assumed that differences in mortality data were not linked to an intoxication but to some biological reasons.

At 7DAA, the number of dead adult honeybees found in the dead bee traps of the hives set in the test item stayed relatively low (8 to 31 dead bees per hive) and comparable to the control (5 to 12 dead bees per hive); the statistically significant difference is not biologically relevant as the number of dead bees recorded was low. For the same reason, the difference at 27DAA is not relevant because the maximum value of mortality recorded among hives was only 43 dead bees (hive R021).

Concerning the dead pupae found in the dead bee trap, there were mostly more dead bodies found in the traps set in the test item treated field than in the control one. Nevertheless, statistical analysis demonstrate that significant difference was met only at 2 DAA, 10 DAA and 12DAA. However, the number of dead pupae found each day was low (up to 8 daily dead pupae only) and therefore are not biologically relevant. Indeed, those recorded data of dead pupae found each day were very low compared to the thousands of pupae that you can have in one hive

Based on the mortality assessed on adult worker honeybees and the results of the residue analysis of adult honeybee samples, the application of MCW-2222 outside the foraging activity did not induce any

significant adult mortality during the field phase.

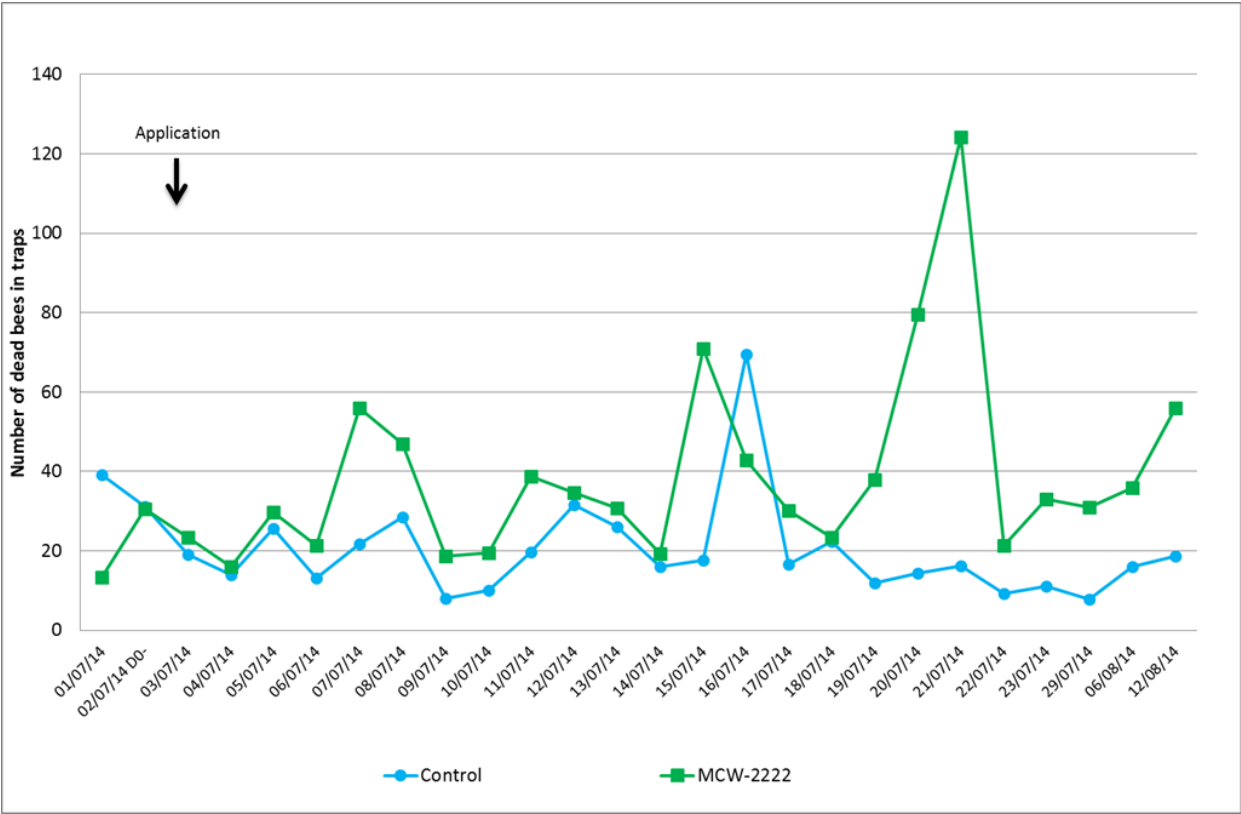


Figure A 38: Daily mean mortality of adult bees

Table A 118: Daily mean mortality of adult bees

Date	Timing	Average number of dead bees	
		Control	MCW-2222
01/07/14	1DBA	39.1	13.4
02/07/14	0DBA	31.1	30.6
03/07/14	1DAA	19	23.3
04/07/14	2DAA	14	16.1
05/07/14	3DAA	25.7	29.7
06/07/14	4DAA	13.1	21.4
07/07/14	5DAA	21.7	56
08/07/14	6DAA	28.6	46.9
09/07/14	7DAA	8.1	18.7
10/07/14	8DAA	10.1	19.4
11/07/14	9DAA	19.7	38.7
12/07/14	10DAA	31.6	34.6
13/07/14	11DAA	26.1	30.7
14/07/14	12DAA	16.1	19.3
15/07/14	13DAA	17.7	70.9
16/07/14	14DAA	69.4	42.9
17/07/14	15DAA	16.7	30.1
18/07/14	16DAA	22.3	23.3
19/07/14	17DAA	11.9	38
20/07/14	18DAA	14.3	79.6
21/07/14	19DAA	16.3	124.1
22/07/14	20DAA	9.3	21.3
23/07/14	21DAA	11	33
29/07/14	27DAA	7.9	30.9
06/08/14	35DAA	15.9	35.9
12/08/14	41DAA	18.6	56

DBA = days before application; DAA = days after application

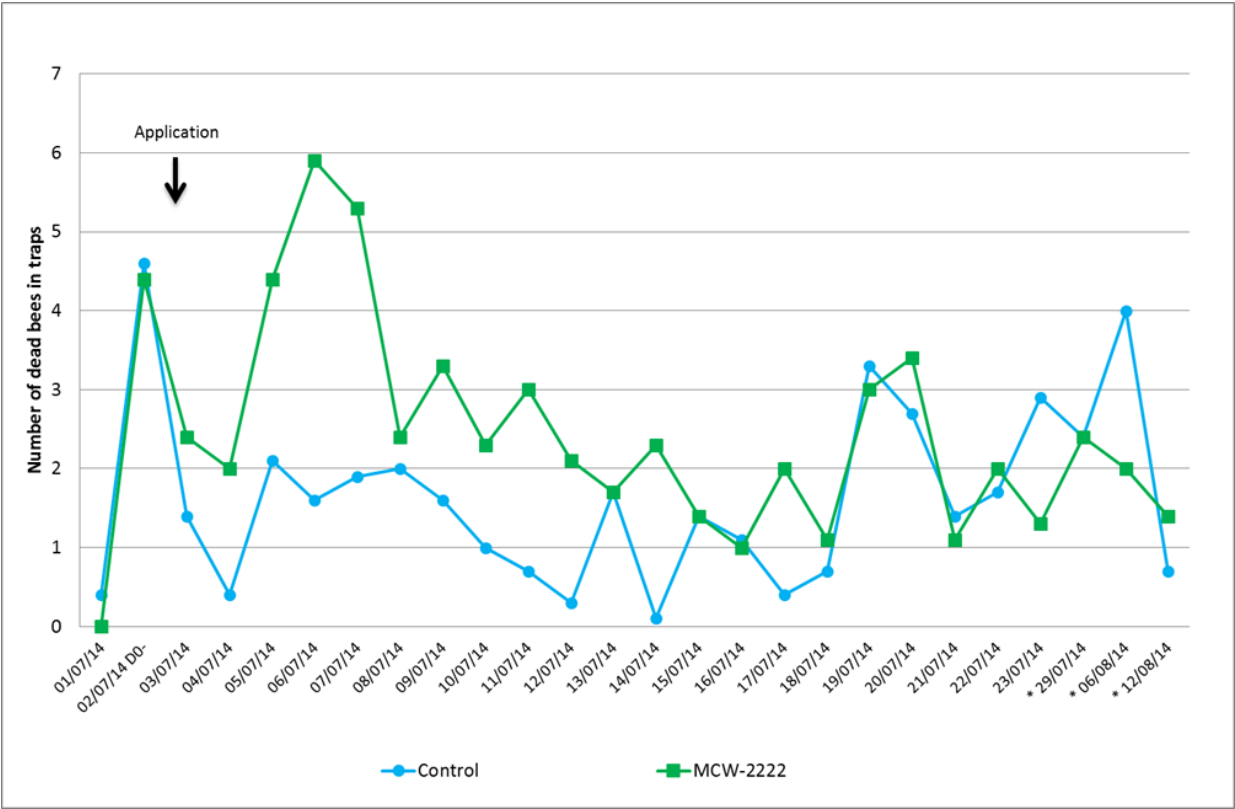


Figure A 39: Daily mean mortality of bee pupae
Table A 119: Daily mean mortality of bee pupae

Date	Timing	Average number of dead bee pupae	
		Control	MCW-2222
01/07/14	1DBA	0.4	0
02/07/14	0DBA	4.6	4.4
03/07/14	1DAA	1.4	2.4
04/07/14	2DAA	0.4	2
05/07/14	3DAA	2.1	4.4
06/07/14	4DAA	1.6	5.9
07/07/14	5DAA	1.9	5.3
08/07/14	6DAA	2	2.4
09/07/14	7DAA	1.6	3.3
10/07/14	8DAA	1	2.3
11/07/14	9DAA	0.7	3
12/07/14	10DAA	0.3	2.1
13/07/14	11DAA	1.7	1.7
14/07/14	12DAA	0.1	2.3
15/07/14	13DAA	1.4	1.4
16/07/14	14DAA	1.1	1
17/07/14	15DAA	0.4	2
18/07/14	16DAA	0.7	1.1
19/07/14	17DAA	3.3	3
20/07/14	18DAA	2.7	3.4
21/07/14	19DAA	1.4	1.1
22/07/14	20DAA	1.7	2
23/07/14	21DAA	2.9	1.3
29/07/14	27DAA	2.4	2.4
06/08/14	35DAA	4	2
12/08/14	41DAA	0.7	1.4

DBA = days before application; DAA = days after application

Colony strength and colony development

Apiarist visits at the beginning of the experimental phase, during the trial after the last brood assessments, and at the end of the trial did not indicate any impact of the test item on the colony strength as well as on the quantity and quality of the brood. Observed differences were mostly due to experimental manipulation (loss of queens is not rare because of the high frequency of hive opening in order to conduct the brood assessments) and seasonal conditions (less resources in the late summer).

Detailed bee brood development

For both treatment groups, the BTR was very low at BFD22 since it was respectively below 12% for eggs selected at BFD00 and below 9% for larvae (young and old). No statistical difference was met between both treatments. The BTR was even slightly below with MCW-2222 than with the control whatever the development stage selected at BFD00.

BI generally correlates with the brood termination rate: the higher the brood termination rate the lower the brood index and vice versa. Whatever the development stage selected at BFD00, from BFD00 to BFD22 the BI curves were similar for both treatments.

The value of 5 (successful development) was reached at BFD22 in most cells. Mean BIs at the end of the experimental phase were very close to 5 whatever the development stage selected at BFD00:

- 4.42 in the control treatment and 4.49 in the MCW-2222 treatment for eggs selected at BFD00;
- 4.57 in the control treatment and 4.63 in the MCW-2222 treatment for young larvae selected at BFD00;
- 4.6 in the control treatment and 4.69 in the MCW-2222 treatment for young larvae selected at BFD00.

No statistical difference between both treatment and between both treatments over the day was found.

This excellent result proves that the brood development was not impacted in both treatments. The compensation index CI, which indicates the compensation level of the colony has low impact in this study whatever the development stage selected at BFD00, because the brood index were high for both treatments and only very few cells were terminated. In consequence the CI and BI had similar values for both treatments. No statistical difference between both treatment and between both treatments over the day was found

In conclusion, independently of the brood stage chosen at BFD00 (eggs, young or old larvae), the test item MCW-2222 applied after bee flight presented very similar BTR and indices (BI and CI) to the ones reached in the untreated control (no significant difference was highlighted for any indexes). The BTR values were very low, whereas other indexes reach values close to the possible maximum one (i.e. 5). This shows that the tests item didn't have any effect on the brood development.

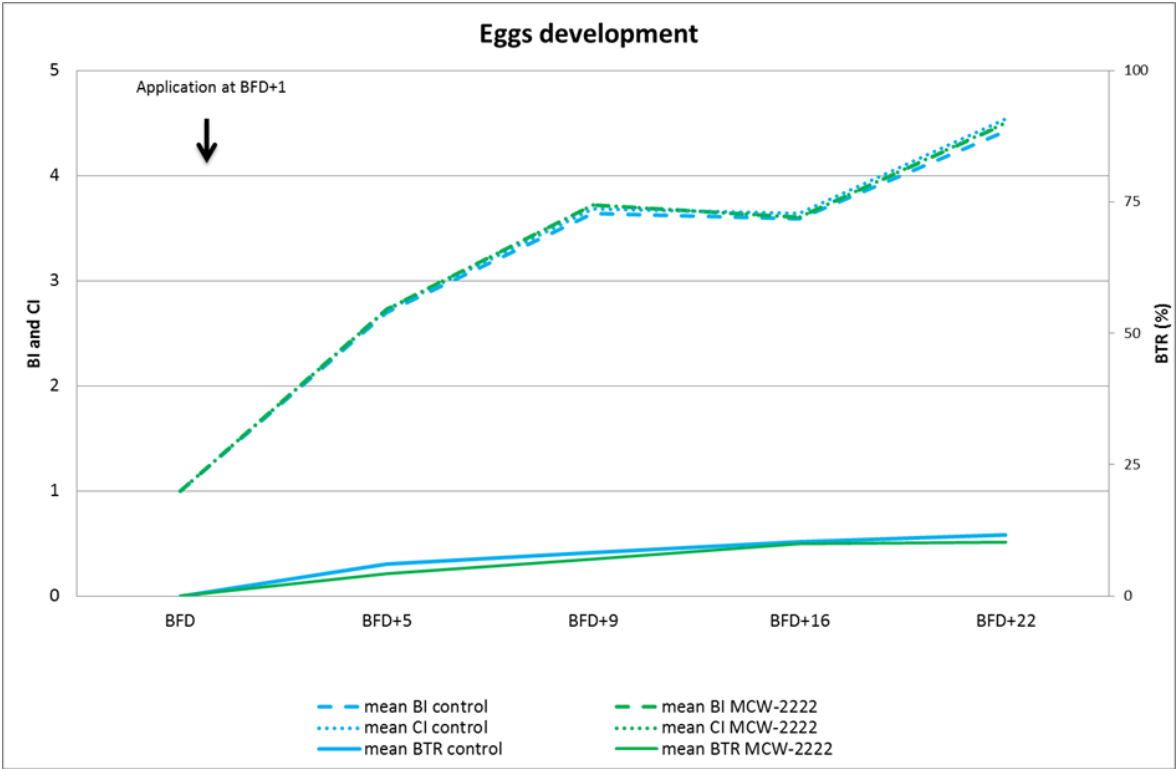


Figure A 40: Development of eggs (BTR, BI, BCI)

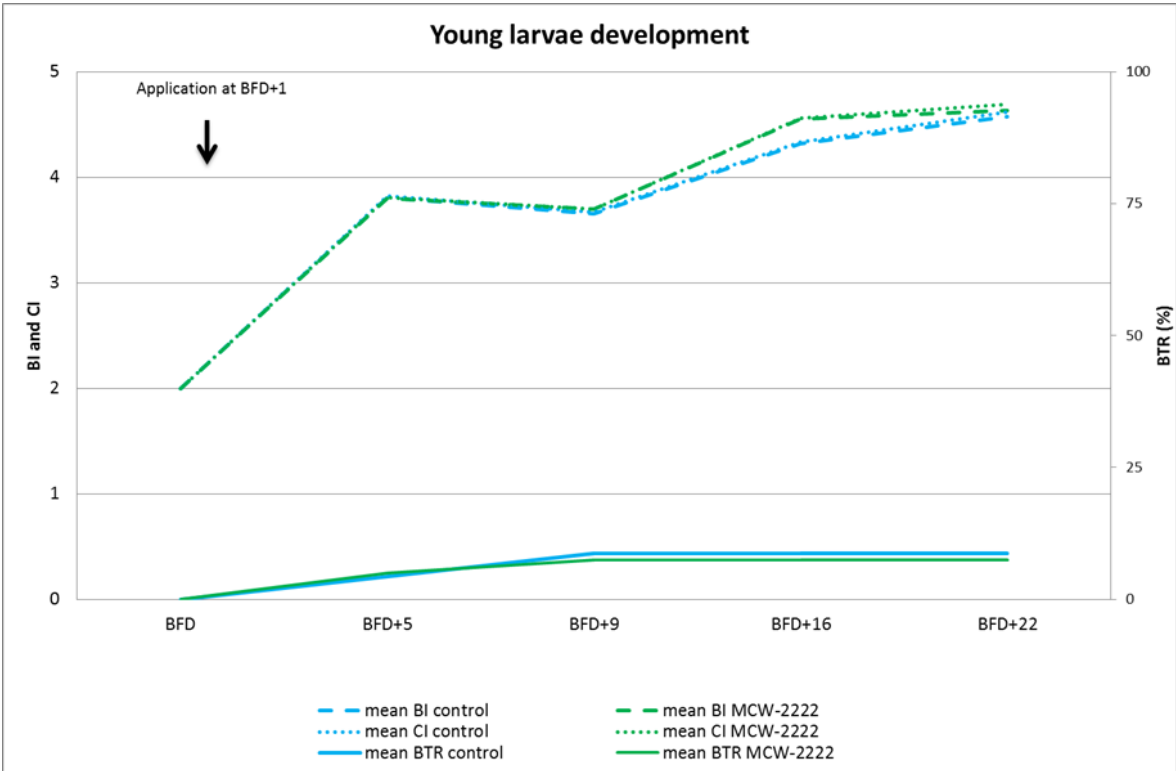


Figure A 41: Development of young larvae (BTR, BI, BCI)

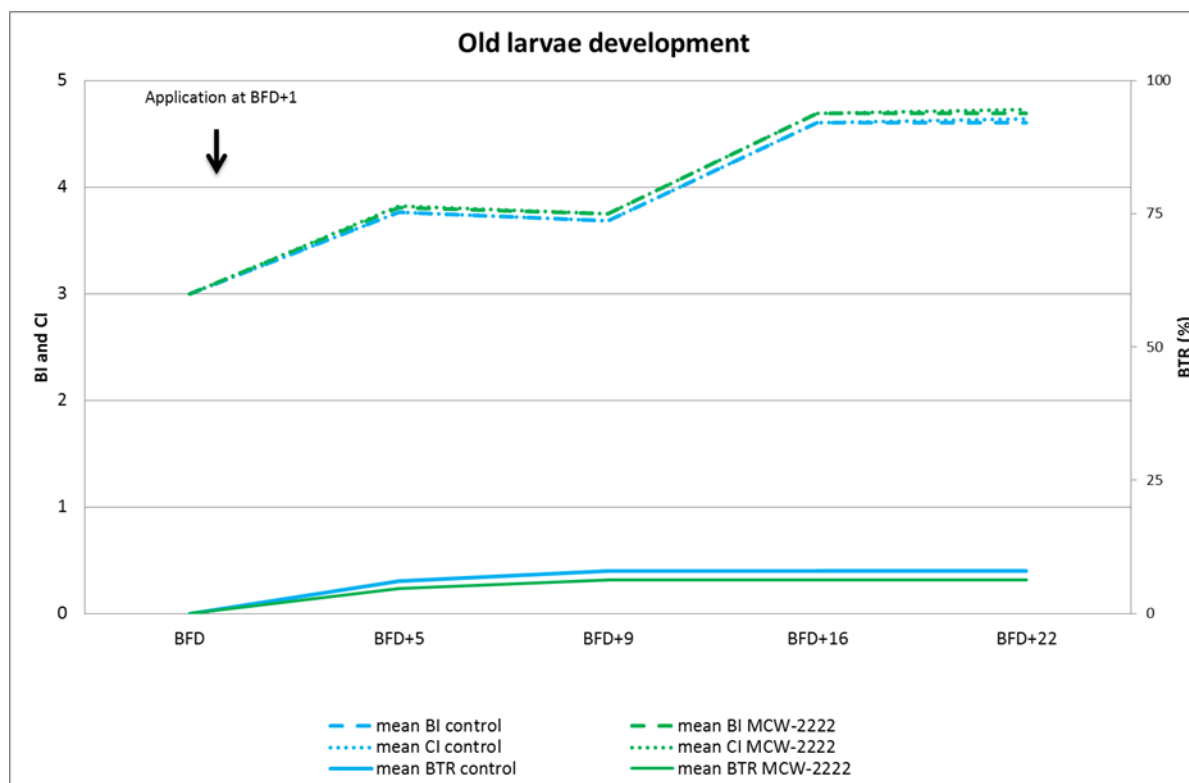


Figure A 42: Development of old larvae (BTR, BI, BCI)

Table A 120: Brood termination rate (%) per hive and per treatment over the time

Date	BFDxx days	Treatment	Control				MCW-2222				
		Hive N°	R009	R012	R014/R011 *	Mean	R017	R019	R020	R021	Mean
Eggs											
01/07/14	BFD00	1 day before exposure	0	0	0	0	0	0	0	0	0
06/07/14	BFD05	4 days after exposure	2	11	5	6	5	5	3	4	4.25
11/07/14	BFD10	9 days after exposure	3	12	10	8.33	11	7	6	4	7
17/07/14	BFD16	15 after exposure	3	13	15	10.33	17	8	10	5	10
23/07/14	BFD22	21 after exposure	4	16	15	11.67	17	9	10	5	10.25
Young larvae											
01/07/14	BFD00	1 day before exposure	0	0	0	0	0	0	0	0	0
06/07/14	BFD05	4 days after exposure	2	4	7	4.33	8	1	9	2	5
11/07/14	BFD10	9 days after exposure	11	5	10	8.67	12	2	11	5	7.5
17/07/14	BFD16	15 after exposure	11	5	10	8.67	12	2	11	5	7.5
23/07/14	BFD22	21 after exposure	11	5	10	8.67	12	2	11	5	7.5
Old larvae											
01/07/14	BFD	1 day before exposure	0	0	0	0	0	0	0	0	0
06/07/14	BFD05	4 days after exposure	6	7	5	6	14	1	2	2	4.75
11/07/14	BFD10	9 days after exposure	12	7	5	8	17	1	4	3	6.25
17/07/14	BFD16	15 after exposure	12	7	5	8	17	1	4	3	6.25
23/07/14	BFD22	21 after exposure	12	7	5	8	17	1	4	3	6.25

* The hive R014 was used when eggs were selected at BFD00. The hive R011 was used when larvae (young and old) were selected at BFD00

Table A 121: Brood index per hive and per treatment over the time

Date	BFDxx days	Treatment	Control				MCW-2222				
		Hive N°	R009	R012	R014/R011 *	Mean	R017	R019	R020	R021	Mean
Eggs											
01/07/14	BFD00	1 day before exposure	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
06/07/14	BFD05	4 days after exposure	2.56	2.68	2.87	2.7	2.92	2.82	2.27	2.89	2.73
11/07/14	BFD10	9 days after exposure	3.86	3.50	3.57	3.64	3.55	3.71	3.76	3.84	3.72
17/07/14	BFD16	15 after exposure	3.88	3.48	3.40	3.59	3.32	3.68	3.60	3.80	3.6
23/07/14	BFD22	21 after exposure	4.80	4.20	4.25	4.42	4.15	4.55	4.50	4.75	4.49
Young larvae											
01/07/14	BFD00	1 day before exposure	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
06/07/14	BFD05	4 days after exposure	3.87	3.84	3.72	3.81	3.68	3.96	3.64	3.92	3.8
11/07/14	BFD10	9 days after exposure	3.56	3.80	3.60	3.65	3.52	3.92	3.56	3.80	3.7
17/07/14	BFD16	15 after exposure	3.91	4.58	4.47	4.32	4.40	4.88	4.39	4.54	4.55
23/07/14	BFD22	21 after exposure	4.45	4.75	4.50	4.57	4.40	4.90	4.45	4.75	4.63
Old larvae											
01/07/14	BFD00	1 day before exposure	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00
06/07/14	BFD05	4 days after exposure	3.76	3.72	3.80	3.76	3.44	3.96	3.92	3.92	3.81
11/07/14	BFD10	9 days after exposure	3.52	3.72	3.80	3.68	3.32	3.96	3.84	3.88	3.75
17/07/14	BFD16	15 after exposure	4.40	4.65	4.75	4.6	4.15	4.95	4.80	4.85	4.69
23/07/14	BFD22	21 after exposure	4.40	4.65	4.75	4.6	4.15	4.95	4.80	4.84	4.69

* The hive R014 was used when eggs were selected at BFD00. The hive R011 was used when larvae (young and old) were selected at BFD00

Table A 122: Brood compensation index per hive and per treatment over the time

Date	BFDxx days	Treatment	Control				MCW-2222				
		Hive N°	R009	R012	R014/R011 *	Mean	R017	R019	R020	R021	Mean
Eggs											
01/07/14	BFD00	1 day before exposure	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
06/07/14	BFD05	4 days after exposure	2.57	2.73	2.87	2.72	2.92	2.82	2.27	2.89	2.73
11/07/14	BFD10	9 days after exposure	3.92	3.54	3.57	3.68	3.55	3.71	3.78	3.84	3.72
17/07/14	BFD16	15 after exposure	3.96	3.56	3.40	3.64	3.32	3.68	3.60	3.80	3.6
23/07/14	BFD22	21 after exposure	4.93	4.43	4.26	4.54	4.15	4.61	4.50	4.75	4.5
Young larvae											
01/07/14	BFD00	1 day before exposure	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
06/07/14	BFD05	4 days after exposure	3.87	3.86	3.86	3.82	3.70	3.96	3.65	3.92	3.81
11/07/14	BFD10	9 days after exposure	3.56	3.84	3.84	3.67	3.52	3.92	3.56	3.80	3.7
17/07/14	BFD16	15 after exposure	3.93	4.58	4.58	4.33	4.40	4.88	4.42	4.54	4.56
23/07/14	BFD22	21 after exposure	4.60	4.75	4.75	4.62	4.40	4.94	4.67	4.75	4.69
Old larvae											
01/07/14	BFD00	1 day before exposure	3.00	3.00	2.00	3.00	3.00	3.00	3.00	3.00	3.00
06/07/14	BFD05	4 days after exposure	3.76	3.72	3.86	3.76	3.47	3.96	3.92	3.92	3.82
11/07/14	BFD10	9 days after exposure	3.52	3.72	3.84	3.68	3.32	3.96	3.84	3.88	3.75
17/07/14	BFD16	15 after exposure	4.40	4.65	4.58	4.6	4.15	4.97	4.80	4.86	4.69
23/07/14	BFD22	21 after exposure	4.51	4.65	4.75	4.64	4.15	4.99	4.89	4.91	4.73

* The hive R014 was used when eggs were selected at BFD00. The hive R011 was used when larvae (young and old) were selected at BFD00

Residue analysis

The analytical method used in the current study was validated in this (see Section 5.1.2). In order to confirm the analytical method was functioning correctly when used in the current study, procedural recovery samples were processed and analysed concurrently with the test samples and are summarised in the tables below.

Table A 123: Procedural recovery data for acetamiprid in honey, flowers pollen and bee bread

Matrix	Fortification level [mg/kg]	Recovery (%)		RSD (%)	n
		Range	Mean		
Honey	0.01	-	94*	-	1
	0.10	-	99*	-	1
Flowers	10	-	106*	-	1
Pollen	0.01	-	95	14.9	2
	0.250	-	70*	-	1
Bee Bread	0.01	-	101*	-	1
	0.50	-	108*	-	1

* Single replicate

Table A 124: Procedural recovery data for acetamiprid-N-desmethyl in honey, pollen and bee bread

Matrix	Fortification level [mg/kg]	Recovery (%)		RSD (%)	n
		Range	Mean		
Honey	0.01	-	99*	-	1
Pollen	0.01	-	91*	-	1
Bee Bread	0.01	-	101*	-	1
	0.50	-	92*	-	1

* Single replicate

In flower (collected 1DAA) and pollen specimens (3DAA and 8 DAA) sampled in untreated plots, no residue of acetamiprid and its metabolite was found. This result validates the trial design since no acetamiprid and its metabolites were found in the control plot. On the other hand 5.0 mg/kg of acetamiprid and 0.017 mg/kg of acetamiprid-N-desmethyl were measured in the flowers sampled in the MCW-2222 treated plot. This value shows clearly that foraging bees were exposed to MCW-2222.

Residue levels of acetamiprid were detected in the three pollen specimens of total pollen contained in the pollen traps of hives settled on the MCW-2222 treated field at 3DAA (0.039 to 0.24 mg/kg) and in one sample at 8DAA (from hive R018 with a much lower value of 0.015 mg/kg). This proves the exposure of the colonies to pollen contaminated with the test item (no contaminated pollen in the untreated field) and shows that the residues decrease consequently 8 days after application.

In-hive nectar, bee bread and honey specimens were free from residue in the hive R008 of the untreated control. In the two other hives, levels of acetamiprid were quantified, i.e. 0.014 to 0.021 mg.kg⁻¹ (mean of 0.018 ± 0.004 mg.kg⁻¹) in in-hive nectar at 8DAA, 0.031 to 0.10 mg/kg (mean of 0.066 ± 0.035 mg/kg) in bee bread at 8DAA (and even 0.012 mg/kg of acetamiprid-N-desmethyl in hive R013) and 0.015 to 0.024 mg/kg (mean of 0.020 ± 0.005 mg/kg) in honey at 20DAA. The origin of these residues was not characterized due to the design of the study: open field study, honeybees are not confined to the trial fields. In-hive nectar specimens collected at 8DAA from the hives set in the test item treated field show levels of residue of acetamiprid close to the control (from 0.014 to 0.046 mg/kg, mean of 0.033 ± 0.013 mg/kg). Then honey specimens sampled at 20DAA show slightly higher level than in the control with values from 0.019 to 0.039 mg/kg (mean of 0.031 ± 0.008 mg/kg). On the other hand, quantified residue of acetamiprid in bee bread sampled at 8DAA in the hives settled in the MCW-2222 plot were higher than in the hives settled in the untreated plots, with values from 0.050 to 0.18 mg/kg (mean of 0.109 ± 0.048 mg/kg) attesting the in-hive presence of the test item at higher level than in the control hives and that resources used to feed young larvae was contaminated with acetamiprid.

No residue of acetamiprid-N-desmethyl metabolite has been measured in any pollen, nectar, honey or bee bread specimen in the hives set on the test item treated field. From the untreated control, a level of 0.012 mg/kg was quantified in the specimen of bee bread of hive R013 at 8DAA. No acetamiprid-N-desmethyl was quantified in all other specimens sampled in the untreated control.

Endpoints

No effects on adult and pupal bee mortality, foraging activity, behaviour, colony strength, colony conditions as well as on the specific evaluation of the detailed bee brood development were observed when MCW-2222 was applied after bee flight at a rate of 100 g a.s./ha to flowering *Phacelia*.

Validity criteria

As shown in the following table, all validity criteria were met.

Table A 125: Validity criteria

Validity criteria according to CEB 230 (2012), part IV, adapted from semi-field studies and regarded relevant for field studies	Observed in study
Before treatment:	
Daily mortality must be similar between the treatments. The difference between the average adult mortality on the day before application must not exceed 60%	On the day of application, the average mortality in T was 30.6 dead bees/colony and 31.1 dead bees/colony in C, resulting in a difference of 2% compared to T. Criterion was achieved
After treatment:	
Mortality in the control must be comparable before and after the treatment, i.e. mortality the day after treatment must not exceed the average mortality figures for the day before treatment by more than 50%	Average mortality on 0DBA: 31.1 dead bees/colony Average mortality on 1DAA: 19.0 dead bees/colony Difference: -39% Criterion was achieved
Additional validity criteria according to study plan	Observed in study
Before treatment:	
The foraging activity should be significant in each field (over 3 bee/m ²) and comparable between treatments	C: 7.9 to 9.2 bees/m ² T: 5.9 to 10.6 bees/m ² Criterion was achieved
After brood fixing day:	
Assuming a normal brood development, mean brood indexes should increase at further assessments: from eggs (1) to larvae (2-3), then pupae stage (4) and finally empty cells after hatch or new eggs (5).	BI of marked eggs in C: 1.00 – 2.7 – 3.64 – 3.59 – 4.42 BI of marked young larvae in C: 2.00 – 3.81 – 3.65 – 4.32 – 4.57 BI of marked old larvae in C: 3.00 – 3.76 – 3.68 – 4.6 – 4.6 Criterion was achieved
The termination rate in the control should be below 30%	BTR of eggs at BFD 22: 11.67% BTR of young larvae at BFD 22: 8.67% BTR of old larvae at BFD 22: 8.00% Criterion was achieved
Weather conditions must remain favourable (mean temperature between 15°C and 30°C)	Criterion was achieved at most days
Any other phenomena that have been considered as abnormal in the course of the study will be reported	None observed Criterion was achieved

Conclusion

In a field study based on EPPO 1/170 (4) (2010), C.E.B methodology n°230, part IV (2003) and OECD GD 75 (2007), honeybees (*Apis mellifera* L.) were exposed to MCW-2222 (a.s. acetamiprid) applied at rate of 100 g a.s./ha after bee flight to flowering *Phacelia tanacetifolia*, investigating potential effects on adult and pupal mortality, foraging activity and behaviour, conditions of the colonies (i.e. colony strength and colony development). Special attention was laid on the assessment of the detailed bee brood development. Residues levels of acetamiprid were quantified in flowers (1DAA), pollen (at 3DAA & 8DAA), in-hive nectar and bee bread (at 8DAA) and honey specimens (at 20DAA) of the test item treatment, which confirmed the exposure of the foraging bees, larvae and the colonies to the test item.

The results showed, that MCW-2222 (a.s. acetamiprid) didn't have any impact on foraging activity, behaviour, adult bee and pupal mortality. When significant differences appeared in mortality, the non-GLP quantification of acetamiprid on the dead bees sampled in front of the hives proved that this mortality was not due to a chemical intoxication.

Furthermore, the assessment of the colony strength and colony development as well as the specific evaluation of the detailed bee brood development showed no impact of the test item on the development on honeybee brood.

A 2.3.1.8.2 KCP 10.3.1.6/02 Field study with honey bees on oil seed rape

Comments of zRMS:	<p>The study has been already evaluated and considered not fully reliable by the zRMS in the course of the first zonal evaluation of CA3573 (formerly MCW-2222) finalised in April 2018. Although the test guideline has not changed since that time, the study has been re-evaluated by the zRMS in 2021.</p> <p>The study was conducted in line with methodology described in OEPP/EPPO Bulletin 40, 313-319 on a field of winter oilseed rape in the Northern France. Application of MCW-2222 was performed twice at 100 g a.s./ha: first time just before the flowering (BBCH 59) and second time in the evening without presence of foraging bees at BBCH 64, 7 days after hives settlement.</p> <p>The distance between control and treatment fields was approximately 13 km (at least 4 km are currently required). Both study fields were surrounded by woods (few flowering plants were met at the considered period) and cereals. However, at a distance of at least 1 km oilseed rape fields were present (accurate distance not specified). According to information provided in the study report, owners of those oilseed rape fields were asked to avoid applications with any product containing acetamiprid during the experimental phase of the study, but it cannot be confirmed if acetamiprid was not applied. Furthermore, application of other insecticides and resulting cross-contamination of the pollen and nectar supplies in the field with other substances potentially used on other fields cannot be ruled out. In case no insecticides were used by the farmers, bees could collect uncontaminated pollen and nectar which might have led to dilution of acetamiprid residues in the hives. Although chemical analyses confirmed presence of acetamiprid in flowers, pollen, nectar and bee bread, it cannot be excluded that the in-hive exposure was reduced due to access of bees to uncontaminated food supplies. It should be noted that according to OEPP/EPPO methods, the distance of at least 2-3 km from other bee attractive crops is required, while according to EFSA (2013) this distance should be at least 4 km. This issue was further consulted with the zRMS apiary expert, who indicated that bees are not likely to risk the energy losses to fly even only 1 km to forage on the same crop which is present just next to hives, so they will forage first on the nearest crop. Flying on longer distances would be highly probable in case different flowering bee attractive crop was present so close to the test site, as bees would fly there to collect different type of pollen. Choosing of another OSR field over the field next to hives would be possible rather when for some reason bees were incapable of foraging on flowers on the nearest field due to e.g. repellent effect of the applied pesticide. However, the foraging activity in control and test item plots was comparable, so no repellent effect of MCW-2222 was observed. Taking this into account, flying of bees to neighbouring OSR fields could not be fully excluded, but was not likely.</p> <p>Observations of bee mortality, behaviour and effects on bee brood were performed up to 41 days after the treatment (41 DALA). Observation of bee brood covered full brood cycle and beginning of a new one (from BFD 0 to BFD 28 but statistical analyses were performed for results up to BFD 22). No brood measurements were taken at the test termination (41 DALA).</p> <p>No precipitation was observed during application and for the most of the study period. Only at 4 DALA slight rainfall occurred at only 1 mm. Then, first rainfall was observed 18 DALA and then on 22 to 25 DALA. Overall, favourable conditions for bees were observed during the study.</p>
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	<p>Similar pattern of foraging activity was observed on both fields.</p> <p>Mortality of worker bees in test item and control fields was low after the applications and up to 19 DALA. On 20 DALA mortality suddenly increased in both groups, however was higher in controls. Therefore, this effects is considered not to be treatment related.</p> <p>Pupae mortality was low over the entire study period and similar in test item and control groups, but slightly increased on both test fields on 20 DALA. Although on Figure A 25 it looks like pupae mortality was higher in test item group from 10 to 15 DALA, it has to be noted that in both fields it was around and below 1 dead pupae, so even one more dead pupae leads to elevation of the graph.</p> <p>The test item had no effect on brood and compensation indices as well as termination rates of young and old larvae, but BTR of eggs was higher in the test item group (not statistically significant). Analysis of the raw data shows, however, that this increased mean BTR in test groups was due to clearly higher BTR in one hive (R098), while in other test item hives BTR was at level comparable with controls (see Table A 96). When results from hive R098 are removed, the mean egg BTR is even slightly lower than in controls. Overall, the zRMS is of the opinion that this effect was not treatment related, as it was observed in only one hive and not in remaining 3 hives.</p> <p>One control colony lost the queen, but queen cells were present at test termination, so recovery of the hive was likely. In test item groups queens were present during the whole study period.</p> <p>The brood cells number at test initiation was rather low, but sufficient and was gradually increasing during the test duration to high number at test end. The number of bees in some hives (nursery bees) was too low in relation to the amount of brood to assure successful development of all brood cells. However, this pattern could be observed in control and test item hives, so it is not considered to be treatment related. Overall bee colonies at test termination were clearly stronger comparing to the test start, which indicates correct development.</p> <p>Effects of the test item on the overwintering success were not investigated and the trial was terminated 41 days after application.</p> <p>Overall, application of MCW-2222 to flowering oilseed rape at 2x100 g a.s./ha (with first application just before flowering) had no adverse effects on bees mortality, foraging activity and bee brood. However, presence of other flowering oilseed rape fields too close to the test site could lead to decrease in acetamiprid residues due to collection of uncontaminated pollen and nectar (or contaminated with other pesticides). Taking this into account, results of this study must be treated with caution.</p>
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Data point: KCP 10.3.1.6/02

Report: Field Study to Evaluate Potential Side Effects of MCW-2222 on Brood Development, Foraging Activity, Mortality and Behaviour of Adult Honeybees (*Apis mellifera* L.) on Oilseed Rape. Molitor, C., 2015c, R-35844, 230-2015

Guideline(s): EPPO 1/170 (4) (2010), C.E.B methodology n°230, part IV (2003), OECD GD75 (2007)

Deviations: None

GLP: Yes, certified laboratory

Acceptability: Yes, study considered acceptable

Duplication: Not applicable
(if vertebrate study)

Executive Summary

In a field study with honeybees (*Apis mellifera* L.) the potential effects of MCW-2222 on mortality,

foraging activity, behaviour, colony strength and colony development (i.e. quality and quantity of brood and the amount of reserves) were investigated. Special attention was laid on the detailed brood assessment of marked cells containing eggs, young and old larvae.

Two fields (3 ha each, separated from each other by a distance of around 13 km) with flowering oil seed rape (*Brassica napus*) as plots. One was used for the two-times application of MCW-2222 at a rate of 0.5 L/ha (100 g a.s./ha acetamiprid) (T). The second was left untreated field and served as control (C). The first application of the test item to the crop was performed in the evening just before crop flowering (i.e. 25th of March, 2015, BBCH 59) without having the colonies placed to the fields and the second one when the crop was flowering but after bee flight (i.e. 9th April, 2015, BBCH 63).

Seven honey bee colonies, each about 20,000 bees were placed at each field 7 days before the last application (7DBLA) to get familiar with the new conditions with a crop being at BBCH 61. They were placed at a sufficient distance from the crop to avoid any spray drift. All colonies were used to record mortality. Moreover, four of the seven hives were used for the brood development assessments, whereas the three remaining ones were used for sampling of pollen (via pollen traps fixed at the entrance of the hives), nectar, bee bread, honey and wax for residue analysis. The exposure phase lasted from the day of the last (2nd) application (0DALA, BBCH 63) to the end of flowering (35DALA, BBCH 69). On that day, the colonies were located to the monitoring site where no further pesticide exposure was expected. They were returned to the beekeeper's apiary on 43DALA (Day After Last Application).

In order to ensure that bees were exposed to the test item, observations on the foraging activity were scheduled daily from 1DBLA to 14DALA. The foraging activity in each field was recorded by counting the number of forager bees on two areas of 10 m² per field.

Assessments on adult and pupal mortality (via dead bee traps) were daily conducted between 1DBLA to 20DALA and then on 27, 35 and 41DALA, meaning, that the data of the last three samplings display a cumulative mortality from the previous assessment timing.

The behaviour or possible behavioural anomalies of the bees were observed and recorded on the crop and at the entrance of the hives, at the same time as the observation on foraging activity. Possible clinic signs of poisoning were recorded, too.

Three apiarist visits were scheduled on the day of Brood Area Fixing Day (BFD00 = 2DBLA), at BFD 29 and BFD 43, in order to assess the colony development. Parameter taking into account was the adult bee population recorded according to the adapted Liebfeld method. Estimated the quantity and quality of the brood (different stages observed) and amount of reserves were also recorded.

Detailed assessments of the bee brood development was carried out by marking individual brood cells containing either eggs, young or old larvae at the Brood Area Fixing Day 00 (BFD00), which was two days before the last (2nd) application (2DBLA). At this day one hundred cells of each development stage were selected in each hive and followed until 28 days after BFD (BFD28) which covered one brood cycle and the beginning of the expected second one. Next to the assessment on BFD 28 the development of each individually marked cell was assessed at BFD05, BFD10, BFD16 and BFD22. Each brood comb was photographed at each assessment time.

For residue analysis, flowers were gathered on 1DALA from 12 different points in each field. Additionally, specimen for residue analysis were sampled in each of the three dedicated hives per treatment group. I.e., samples of pollen were collected 3DALA and 8DALA via pollen taps, samples of nectar were taken 3DALA and 8DALA from newly filled reserve combs, samples of bee bread and honey were collected 8DALA and 20DALA, respectively and wax samples were taken 2DBLA and 20DALA.

On the day of the evening application (0DBLA), the foraging activity was around 7 bees/10 m² in the control field and 4.5 bees/10 m² in the MCW-2222 field, which is considered as a good level on oilseed rape at this time of the year (beginning of spring with fresh temperatures). This foraging activity level was even higher from 3DALA to 7DALA (day after the last application (second one)) since it reached between 9.5 to 17 bees/10m² in the control plot and 6 and 13 bees/10m² in the MCW-2222 treated study field. Those data confirm the exposure of foraging bees just after the application. Then, the foraging activity decreased at 8 and 9 DALA in both treatments due to low maximum temperatures and increased afterwards. 13DALA and 14DALA the foraging activity was lower in the MCW-2222 plot than in the control plot due to stronger wind in the MCW-2222 plot than in the control one. Overall, no adverse effects on the foraging activity were observed and no symptoms of intoxication were recorded during the study.

Daily mortality of adult bees recorded in the two treatments were stable, very low and comparable from 1DBLA to 20 DALA. Statistical analysis revealed that mortality data between both treatments were

significantly different at 3DALA, 4DALA and 15 DALA. However this static difference is regarded not relevant due to the very low level of mortality recorded in the MCW-2222 treatments (7 bees at 3DALA, 5 at 4DALA and 11 at 15 DALA). Concerning the dead pupae found in the dead bee trap, there were mostly no dead bodies found in the traps set in the test item treated field and in the control one. Based on these findings, the applications of MCW-2222 did not induce any effect on bee mortality during the field phase.

Regarding the strength and development of the colonies, there was no difference between the two treatment groups between the start and the end of the study. Between the first and the last apiarist visit, almost all hives showed an increase of their brood cell number in both treatments. This result is logic as the spring was started and population grew thanks to increasing food resources that stimulated the queen to lay eggs. The detailed assessment of single brood stages resulted in low and comparable BTRs in the control and test item group, which amounted to 6.00%, 3.25% and 0.25% for eggs, young and old larvae in the test item group compared to 2.75%, 2.50% and 1.25% in the control, respectively. No statistical difference was met between both treatment groups. Due to the low and similar BTRs, Brood and Compensation Indexes were high and almost equal in both treatment groups without any significant differences between being detected. Overall, no effect of the test item on the pre-imaginal development of eggs, young larvae or old larvae could be detected.

In flower (collected 1DALA) and pollen specimens (collected 3DALA and 8DALA) sampled in untreated field, no residues of acetamiprid and its metabolite were found. This result validates the trial design and attests that the colonies settled in the untreated control plot were not exposed to the active ingredient and its metabolite. On the other hand 6.8 mg/kg of acetamiprid and 0.093 mg/kg of acetamiprid-N-desmethyl were measured in the flowers sampled in the MCW-2222 treated field 1DALA. This value shows clearly that foraging bees were exposed to MCW-2222.

Residue levels of acetamiprid were detected in the three pollen specimens of total pollen contained in the pollen traps of hives settled on the MCW-2222 treated field at 3 DALA (0.063 to 0.17 mg/kg, mean 0.128 mg/kg) and 8 DALA (0.14 to 0.22 mg/kg, mean 0.170 mg/kg). This proves the exposure of the colonies to the pollen contaminated with the test item (no contaminated pollen in the untreated field) and that this exposure lasted more than one week.

In-hive nectar bee bread and honey specimens were free from residue in the untreated control at both sampling dates. In-hive nectar specimens collected respectively at 3 DALA and 8DALA from the hives set in the test item treated field show levels of residue of acetamiprid from 0.013 to 0.16 mg/kg (mean of 0.062 mg/kg) and from 0.039 to 0.17 mg/kg (mean of 0.070 mg/kg).

Then honey specimens sampled at 20DAA show a residue level with values from 0.023 to 0.041 mg/kg (mean of 0.030 mg/kg) whereas no residues were found in the control samples. On the other hand, quantified residue of acetamiprid in bee bread sampled at 8DAA in the hives settled in the MCW-2222 plot were from 0.082 to 0.20 mg/kg (mean of 0.131 mg/kg) attesting that resources used to feed young larvae was contaminated with acetamiprid.

Regarding wax collected in the MCW-2222 treatment, no residue of acetamiprid was found 2 DBLA and residue of it was met at 20 DALA in all the 3 hives from 0.016 mg/kg to 0.031 mg/kg.

No residue of acetamiprid-N-desmethyl metabolite has been measured in any nectar, wax, honey or bee bread specimen in the hives set on the test item treated field. This compound was found in only one pollen sample (0.013 mg/kg at 8 DALA).

Materials and methods

Materials

Test item	MCW-2222
Batch #	659-030314-01
Content of active substance	Acetamiprid 200 g/L (nominal); 199.2 ± 1.3 g/L (analysed)
Description	Not given
Control	C: Untreated crop
Toxic reference	none
Test organism	
Species	Honey bees (<i>Apis mellifera</i> L.) of healthy colonies with sister queens and the same age (not older than 2 years), containing about 20,000 bees per colony at test start with ten frames. Hives of Dadant type. All colonies at the beginning of the study - with at 3 to 6 frames containing all brood stages

	<ul style="list-style-type: none"> - with 1 to 5 storage frames - with 0 to 2 empty frames - were free of clear clinical symptoms of disease; no treatment against <i>Varroa</i> for at least 4 weeks before beginning of the study.
Source	local beekeeper, Bernard Bru
Food/feeding	Full flowering oilseed rape (<i>Brassica napus</i>) served as food supply, no additional feeding throughout the study. Watering was available in the near surroundings of the fields.
Study design and methods	
Test duration	<p>Pre-exposure phase (6DBLA to 0DBLA): 7 days at the study fields</p> <p>Exposure phase (0DALA to 35DALA): 35 days at the study fields</p> <p>Post-Exposure phase (36DALA to 41DALA): 6 days at the monitoring site</p>
Experimental dates	25 th March to 20 th May 2015
Test doses	<p>2 x 100 g a.s./ha</p> <p>1st application on 25th March 2015 (15DBLA) without hives present at the field</p> <p>2nd application on 9th April 2015 (0DBLA), with hives present at the field, applied after bee flight</p> <p>The 2nd application was performed after bee flight (from 21:10 to 21:35) at BBCH 63 (full flowering of oilseed rape) of the crop with a volume of 200 L water/ha.</p> <p>At both applications, the actual treatment rate was 100% of the target rate.</p>
Test units	Study fields with flowering <i>Brassica napus</i> (variety: Hybrirock), each with an area of 3 ha, and separated from each other by a distance of around 13 km; both study fields were surrounded by woods (few flowering plants were met at the considered period), cereals and oilseed rape field (at least at 1km away from the study plots). Owner of those oilseed rape fields were asked to avoid applications with any product containing acetamiprid during the experimental phase of this study. Each study field with 7 colonies.
Group size/replicates	One study field per treatment group, each with each with 7 colonies; 4 colonies were used for biological assessments, 3 colonies for residue sampling; moreover, all colonies were used for recording of mortality.
Endpoints and assessments	<p><i>mortality of adult bees and pupae:</i></p> <p>Recording via dead bees traps; daily between 1DBLA to 20DALA and then on 27, 35 and 41DAA; thus, the data of the last three samplings display a cumulative mortality from the previous assessment timing</p> <p><i>foraging activity:</i></p> <p>Daily recording of the number of forager bees daily on two areas of 10 m² between 1DBLA to 14DALA.</p> <p><i>behaviour on the crop and at the entrance of the hives:</i></p> <p>at the same time when the assessment for foraging activity took place</p> <p><i>colony strength and colony development:</i></p> <p>once at the beginning on the day of Brood Fixing Day (BFD00 = 2DBLA), on BFD 29 (= 27DALA) and on BFD 43 (= 41 DALA; end of the study); assessment of:</p> <ul style="list-style-type: none"> - estimated number of bees (colony strength) acc. to Liebefeld method - number of cells containing brood (total of cells with eggs, larvae and capped brood) to Liebefeld method - presence of queens (e.g. presence of eggs)

- number of reserve, empty and foundation combs.

detailed bee brood development:

Marking of individual brood cells containing eggs, young and old larvae at BFD00 (= 2DBLA); 100 brood cells of each selected brood stage and hive. Monitoring the subsequent development until adult hatch using a digital image analysis.

Assessments on BFD00 (= 2DBLA), BFD 5, BFD 10, BFD 16, BFD 22 and BFD 28, covering one complete brood cycle (21 days for worker bees) and the beginning of a new one.

The time schedule of assessment days was chosen in order to check the bee brood at different expected stages during the development. Each brood comb was photographed at each assessment time.

Based on number of cells with eggs marked at BFD 0 and number of eggs which failed to develop successfully until adult hatch the Brood Termination Rates (BTR) were determined for each replicate at each assessment day. Moreover, attributing values from 1 (egg stage) to 4 (pupae/capped cell) and 0 (empty after hatch) to the respective brood stages, the brood indices (BI) were calculated. As an recovery indicator for recovery of the bee brood the brood compensation indices (BCI) were calculated

Bee brood categories:

Value	Corresponding contents	Value	Corresponding contents
0	Empty	5	Nectar
1	Egg	6	Pollen
2	Young larvae (L1-L2)	7	Dead
3	Old larvae (L3-L5)	8*	Not characterized
4	Pupae (capped cell)		

*if the cell is noted 8, this cell is not included in any calculations

Expected brood development in case of marked eggs (a), young larvae (b) or old larvae (c) at BFD00

(a)

Assessment day	Expected brood stage in cell	Brood category for index calculation
BFD00	Egg	1
5 days \pm 1 after BFD00	Young larvae or old larvae	2 or 3
10 days \pm 1 after BFD00	Capped cells	4
16 days \pm 2 after BFD	Capped cells shortly before hatch	4
22 days \pm 2 after BFD00	Empty or reserve cells after hatch or new egg laid	5

(b)

Assessment day	Expected brood stage in cell	Brood category for index calculation
BFD00	Young larvae	2
5 days \pm 1 after BFD00	Old larvae or capped cells	3 or 4
10 days \pm 1 after BFD00	Capped cells	4

16 days \pm 2 after BFD00	Capped cells or empty or reserve cells after hatch or new egg laid	4 or 5
22 days \pm 2 after BFD00	Empty, reserve, egg or larvae after hatch	5

(c)

Assessment day	Expected brood stage in cell	Brood category for index calculation
BFD00	Old larvae	3
5 days \pm 1 after BFD00	Capped cells	4
10 days \pm 1 after BFD00	Capped cells or empty or reserve cells after hatch or new egg laid	4 or 5
16 days \pm 2 after BFD00	Empty, reserve, egg or larvae after hatch	5
22 days \pm 2 after BFD00	Empty, reserve, egg or larvae after hatch	5

The BTR expresses the quantity of cell's failure in percentage for each brood comb at each assessment day. BTR was calculated by dividing the number of cells that do not reach the expected growth stage at a specific assessment day by the total number of cells observed. If no failure occurred during the brood development, the BTR would be equal to 0%. Otherwise this rate increases with the number of terminated cells (dead larvae, nymph or significant delay in the development process, or food stored in cells at BFD05, 10 or 16). Cells noted 0 (empty), 5 (nectar) or 6 (pollen) before hatch (BFD22) or 7 (dead) or with any unexpected value at a specific BFD were considered to be failures in the brood development; value of these cells were equal to 0 for the calculation of BTR and the following index BI.

The Brood Index (BI) is an indicator of bee brood development and was calculated for each brood comb at each assessment day. As it is inverse related to the BTR, means that the lower the BTR the higher the BI. If brood cell contents reach the expected brood stage at the specific assessment day (see above), the cells are classified using the brood category number as defined above. On the opposite, if the expected brood stage is not reached or occurred with big delay or if food is stored in the cells at the respective assessments dates, the cells were valued with 0 at the assessment date and also the following dates, disregarding if cells were again filled with brood. The BI of a colony was obtained by summing up the value of all cells assessed the same day and divided by the number of observed cells. If all cells present a successful development (expected pattern), BI is equal to 5 which is the maximal value for this index.

The Compensation Index (CI) indicates the recovery of a colony and was calculated for each brood comb at each assessment day. Cells containing a brood stage were classified according to categories (from 0 to 8). Then values were converted to brood categories as described. If a cell was empty, contained nectar, pollen before hatch (BFD22) or contained dead larvae or pupae, its value became 0, meaning that the cell was empty from any brood stage. Only values of category at each date of assessment were taken into account, without considering the expected brood stage. Therefore this index does not penalize the development value of the brood after termination, suspension or delay.

Important note: Even if the colony of the hive R094 (MCW-2222 treatment) was considered as healthy on the first apiarist visit (BFD00), a heterogeneity of the brood was noted in the course of the experimental phase. It is frequently due to a mycosis that slowly become visible while the colony is growing and causes the death of the young stages of larvae. Consequently this hive could not be used for the brood development and was replaced by the hive R099.

Specimens sampling for residue analysis

Flowers were gathered from 12 different points in each field plot on 1DALA (4 specimens).
Samples of pollen from traps in front of three hives were collected 3DALA and 8 DALA (24 specimens).
Samples of nectar from newly filled reserve combs were put in plastic jars 3DALA and 8DALA (12 specimens).
Samples of bee bread and honey were collected 8DALA (12 specimens) and 20DALA (12 specimens), respectively.
Wax specimens from the hives (main part of the hive where the colony lives) and the super (where honey is stored) of the hives were sampled 2DBLA and 20DALA.
Half of collected specimen were transported to the analytical laboratory GIRPA for residue analysis of acetamiprid and acetamiprid-N-desmethyl.

Residues of acetamiprid and acetamiprid-N-desmethyl were extracted from the pollen with ethyl acetate using an automatic extractor, and from the other samples (flowers, nectar, honey, bee bread) by agitation in acetonitrile and ultra-pure water and purification by dispersive solid phase extraction. The quantification was performed by liquid chromatography with tandem mass spectrometry detection (LC-MS-MS).

DBLA = days before last application
DALA = day after last application
BFD = brood fixing day

Adaptation of bees

Colonies were set-up at the fields seven days before the 2nd application on 7DBLA to get familiar with the new conditions.

Environmental conditions
Natural field conditions

During the experimental field phase, weather conditions were good most of the time with very few rainfalls. Temperatures increased day by day allowing bees to forage the oilseed rape. Nevertheless, there were windy days (specially on 1DALA, 8DALA, 13 & 14 DALA) and at each time its effect was stronger on the area of the treated plot

	Conditions at the	
	1 st application	2 nd application (after bee flight)
Temperature:	6 °C	13 °C
Wind speed:	0 km/h	0 km/h
Rel. humidity:	49 %	45 %
Precipitation:	none	none
BBCH:	59	63

Conditions between

DALA	0 to 7	8 to 14	15 to 21	22-28	29 to 35	36 to 41
Min. to max.						
Temp. [°C]:	2 to 29	4 to 24	0 to 34	4 to 19	5 to 30	4 to 20
Precip. [Σ mm]:	1	1	10	67	0	7
Days with rain [n]:	1	1	1	5	0	3

Biological observations

Foraging activity and behaviour was daily recorded between 1DBLA to 14DALA, adult and pupal mortality was daily recored between 1BDLA to 20DALA and on 27, 35 and 41DALA. For the detailed assessments of the bee brood development, 100 individual brood cells per hive containing either eggs, young or old larvae were marked at the Brood Area Fixing Day 00 (BFD00). The development of each marked cell was assessed at BFD05, BFD10, BFD16, BFD22 and BFD 28. The assessment of condition of the colony strength and colony development was performed on BFD00, BFD 29 and BFD 43.

Statistics

A statistical analysis was performed on the brood development results (BTR, BI and CI) and mortality results. ARM 6 Software was used to analyse the variance of treatments that are compared by a Student-Newmans-Keuls test (average followed by the same letter are not significantly different, see appendix 8). This test gave an observed computed probability to be compared with a significance level which was defined at 5%. In order to perform statistical analysis on the brood development, 8 hives (4 in the untreated control and 4 in the test item treatment) were used, the number of groups was 2 (both control and MCW-2222 treatment) and five assessment days (BFD00, BFD05, BFD10, BFD16 and BFD22) were considered.

Results and discussion

Biological results

Foraging activity

On the day of the evening application (0DBLA), the foraging activity was around 7 bees/10 m² in the control field and 4.5 bees/10 m² in the MCW-2222 field, which is considered as a good level on oilseed rape at this time of the year (beginning of spring with fresh temperatures). This foraging activity level was even higher from 3DALA to 7DALA (day after the last application (second one)) since it reached between 9.5 to 17 bees/10m² in the control plot and 6 and 13 bees/10m² in the MCW-2222 treated study field. Those above data confirms the exposure of foraging bees just after the application. Then the foraging activity decreased at 8 and 9 DALA in both treatments due to low maximum temperatures and increased afterwards. 13DALA and 14DALA the foraging activity was lower in the MCW-2222 plot than in the control plot due to stronger wind in the MCW-2222 plot than in the control one. Overall, no adverse effects on the foraging activity were observed.

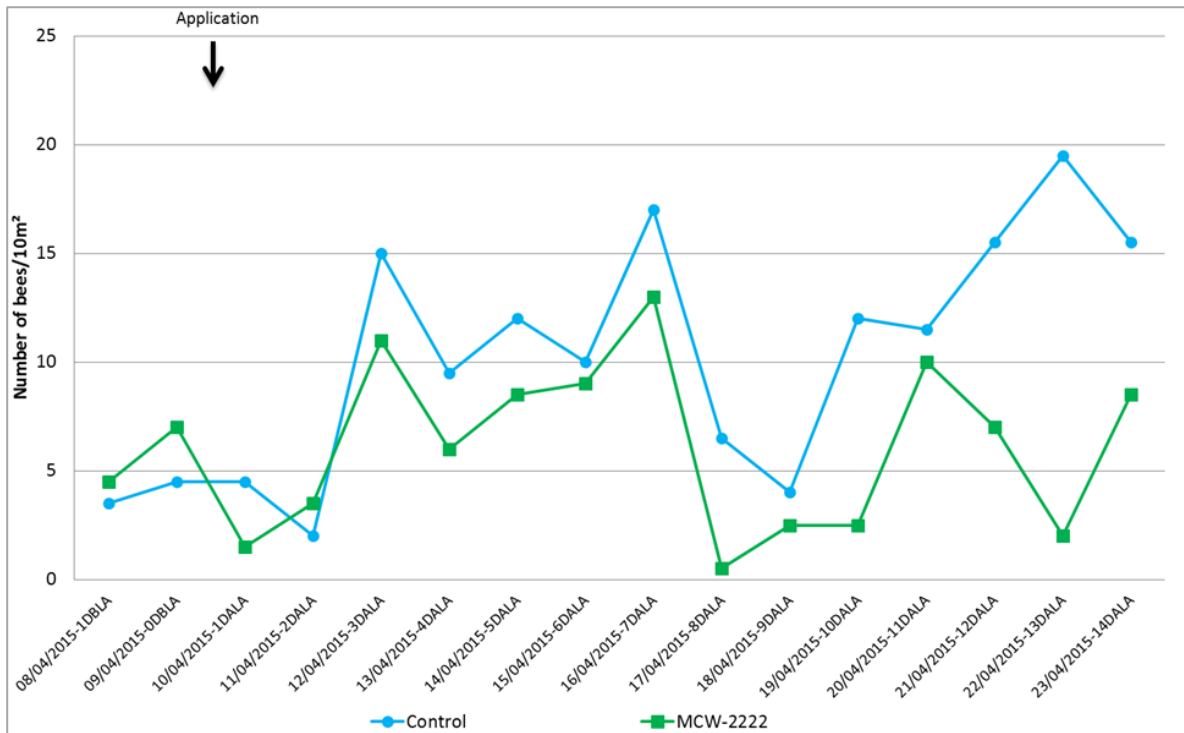


Figure A 43: Daily mean foraging activity

Table A 126: Daily mean foraging activity

Date	Timing	Number of bees/m²	
		Control	MCW-2222
08/04/15	1DBLA	3.5	4.5
09/04/15	0DBLA	4.5	7
10/04/15	1DALA	4.5	1.5
11/04/15	2DALA	2	3.5
12/04/15	3DALA	15	11
13/04/15	4DALA	9.5	6
14/04/15	5DALA	12	8.5
15/04/15	6DALA	10	9
16/04/15	7DALA	17	13
17/04/15	8DALA	6.5	0.5
18/04/15	9DALA	4	2.5
19/04/15	10DALA	12	2.5
20/04/15	11DALA	11.5	10
21/04/15	12DALA	15.5	7
22/04/15	13DALA	19.5	2
23/04/15	14DALA	15.5	8.5

← 2nd Application
after bee flight

DBLA = days before last (second) application; DALA = days after last (second) application

Behaviour

No symptoms of intoxication were recorded during the study.

Mortality

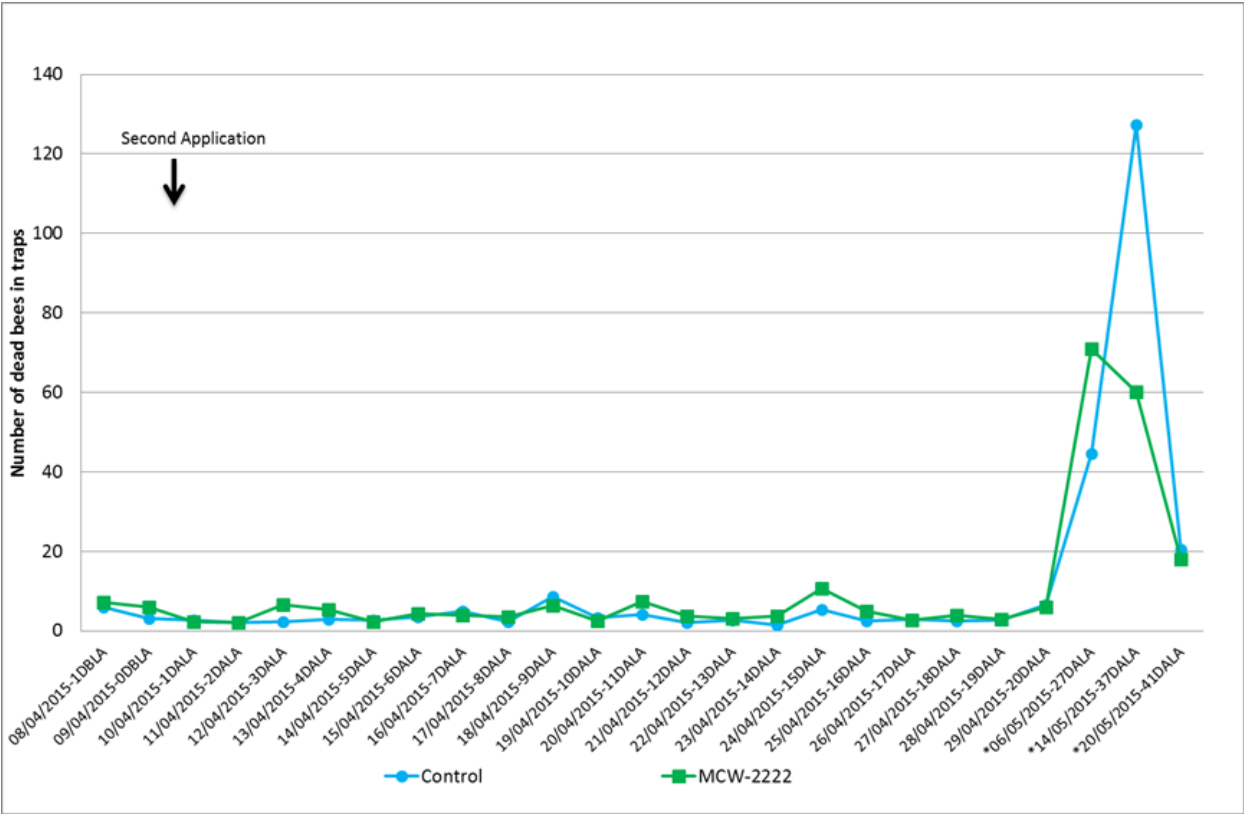
Daily mortality of adult bees recorded in the two treatments were stable, very low and comparable from 1DBLA to 20 DALA.

Statistical analysis revealed that mortality data between both treatments were significantly different at 3DALA, 4DALA and 15 DALA. However this statically difference is not relevant due to the very low level of mortality recorded in the MCW-2222 treatments (7 at 3DALA, 5 at 4DALA and 11 at 15 DALA).

Concerning the dead pupae found in the dead bee trap, there were mostly no dead bodies found in the traps set in the test item treated field and in the control one.

Based on the data, the applications of MCW-2222 according to the conditions described in material and

method chapter did not induce any effect on bee mortality during the field phase.



* The mortality at 27, 35 and 41DALA is a cumulative mortality from the previous assessment timing

Figure A 44: Daily mean mortality of adult bees

Table A 127: Daily mean mortality of adult bees

Date	Timing	Average number of dead bees	
		Control	MCW-2222
08/04/15	1DBLA	5.9	7.1
09/04/15	0DBLA	3	5.9
10/04/15	1DALA	2.7	2.3
11/04/15	2DALA	2	2.1
12/04/15	3DALA	2.3	6.6
13/04/15	4DALA	2.9	5.4
14/04/15	5DALA	2.7	2.3
15/04/15	6DALA	3.4	4.3
16/04/15	7DALA	4.9	3.9
17/04/15	8DALA	2.3	3.4
18/04/15	9DALA	8.6	6.3
19/04/15	10DALA	3.3	2.4
20/04/15	11DALA	4.1	7.3
21/04/15	12DALA	2	3.6
22/04/15	13DALA	2.6	3
23/04/15	14DALA	1.4	3.7
24/04/15	15DALA	5.3	10.6
25/04/15	16DALA	2.4	4.9
26/04/15	17DALA	2.9	2.6
27/04/15	18DALA	2.4	3.9
28/04/15	19DALA	2.6	2.9
29/04/15	20DALA	6.6	6
06/05/15*	27DALA	44.6	70.9
14/05/15*	35DALA	127.3	60.1
20/05/15*	41DALA	20.4	17.9

DBLA = days before last (second) application; DALA = days after last (second) application
* The mortality at 27, 3 and 41DALA is a cumulative mortality from the previous assessment timing

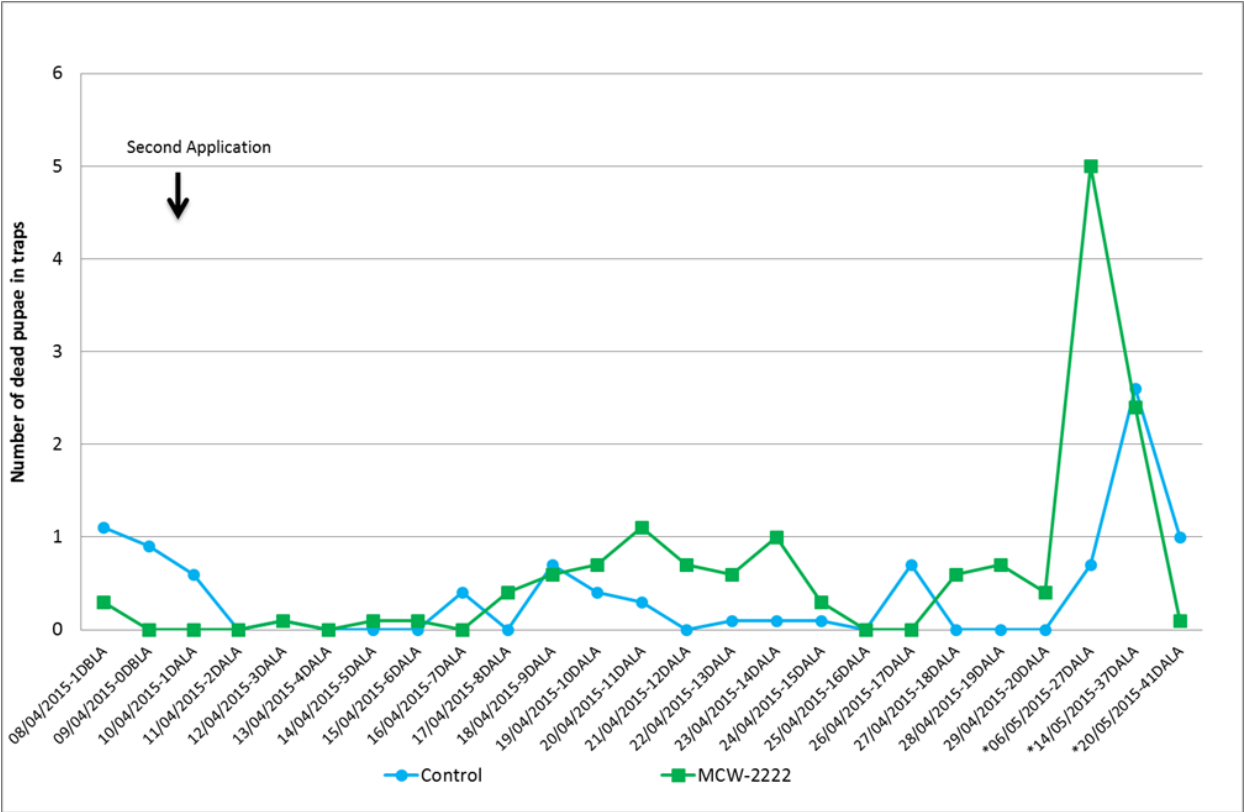


Figure A 45: Daily mean mortality of bee pupae

Table A 128: Daily mean mortality of bee pupae

Date	Timing	Average number of dead bee pupae	
		Control	MCW-2222
08/04/15	1DBLA	1.1	0.3
09/04/15	0DBLA	0.9	0.0
10/04/15	1DALA	0.6	0.0
11/04/15	2DALA	0.0	0.0
12/04/15	3DALA	0.1	0.1
13/04/15	4DALA	0.0	0.0
14/04/15	5DALA	0.0	0.1
15/04/15	6DALA	0.0	0.1
16/04/15	7DALA	0.4	0.0
17/04/15	8DALA	0.0	0.4
18/04/15	9DALA	0.7	0.6
19/04/15	10DALA	0.4	0.7
20/04/15	11DALA	0.3	1.1
21/04/15	12DALA	0.0	0.7
22/04/15	13DALA	0.1	0.6
23/04/15	14DALA	0.1	1.0
24/04/15	15DALA	0.1	0.3
25/04/15	16DALA	0.0	0.0
26/04/15	17DALA	0.7	0.0
27/04/15	18DALA	0.0	0.6
28/04/15	19DALA	0.0	0.7
29/04/15	20DALA	0.0	0.4
06/05/15*	27DALA	0.7	5.0
14/05/15*	35DALA	2.6	2.4
20/05/15*	41DALA	1.0	0.1

DBLA = days before last (second) application; DALA = days after last (second) application

* The mortality at 27, 35 and 41DALA is a cumulative mortality from the previous assessment timing

Colony strength and colony development

There was no difference within the two treatments according to the population evolution between the start and the end of the study.

Between the first and the last apiarist visit, almost all hives showed an increase of their brood cell number in both treatments. This result is logic as the spring was started and population grew thanks to increasing food resources that stimulated the queen to lay eggs.

Detailed bee brood development

For both treatments, the BTR was very low at BFD22 for all stages selected which amounted to 6.00%, 3.25% and 0.25% for eggs, young and old larvae in the test item group compared to 2.75%, 2.50% and 1.25% in the control, respectively. No statistical difference was met between both treatments.

BI generally correlates with the brood termination rate: the higher the brood termination rate the lower the brood index and vice versa. Whatever the development stage selected at BFD00 was, from BFD00 to BFD22 the brood index curves were almost superimposed for both treatments.

The value of 5 (successful development) was reached at BFD22 in most cells. Mean BIs at the end of the experimental phase were very close to 5 whatever the development stage selected at BFD00:

- 4.86 in the control treatment and 4.70 in the MCW-2222 treatment for eggs selected at BFD00;
- 4.88 in the control treatment and 4.84 in the MCW-2222 treatment for young larvae selected at BFD00;
- 4.94 in the control treatment and 4.99 in the MCW-2222 treatment for young larvae selected at BFD00.

No statistical difference between both treatment and between both treatments over the day was found. This excellent result proves that the brood development was not impacted in both treatments.

The compensation index CI, which indicates that the compensation level of the colony was low in this study whatever the development stage selected at BFD00 was, because the brood indices were already high for both treatments and only very few cells were terminated. This proves that a very small amount of cells got his development cycle terminated. In consequence the CI and BI had similar values for both treatments. No statistical difference between both treatment and between both treatments over the day was found.

In conclusion, independently of the brood stage chosen at BFD00 (eggs, young or old larvae), the test item MCW-2222 applied twice, first application just before flowering and the second one during the flowering period but outside the foraging activity of honeybees, resulted in very similar BTR and indices (BI and CI) to the ones reached in the untreated control (no significant difference was highlighted for any index). The BTR values were very low, therefore the indices reach values close to the possible maximum one (i.e. 5). This shows that the tests item didn't have any effect on the brood development.

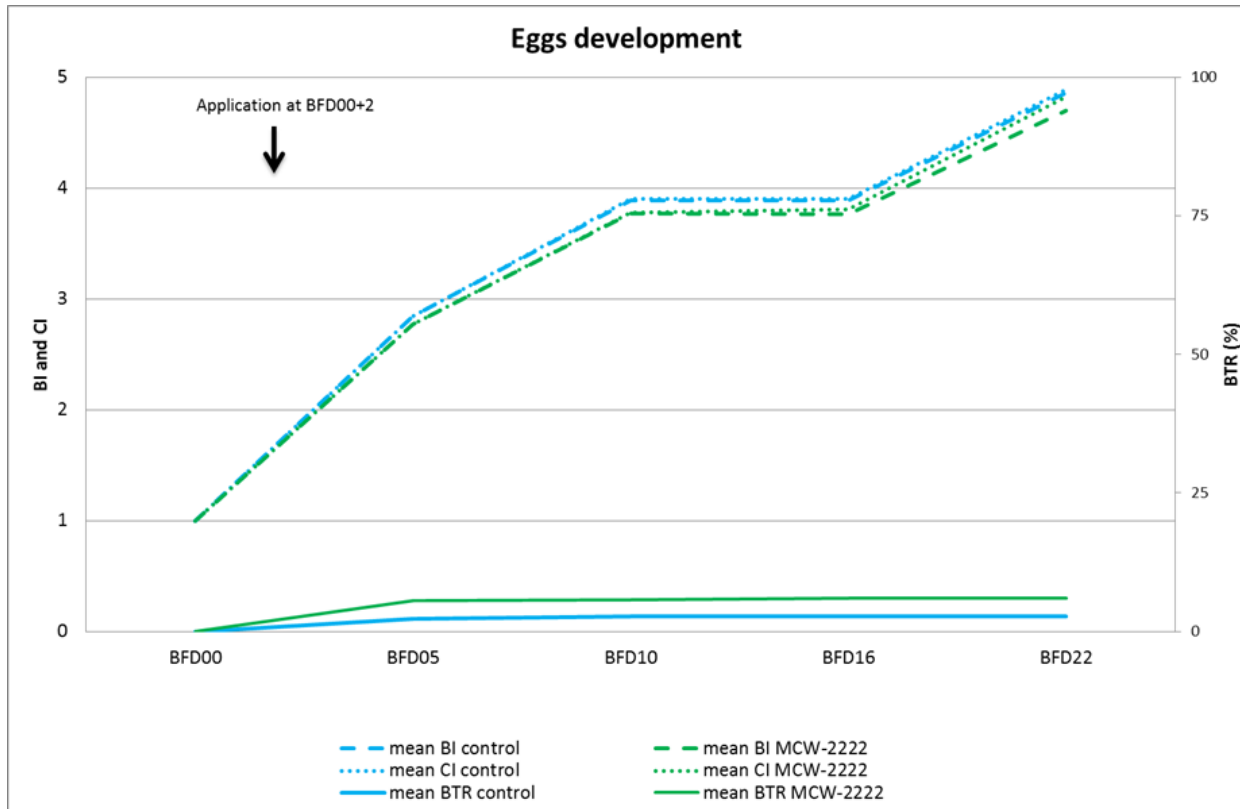


Figure A 46: Development of eggs (BTR, BI, BCI)

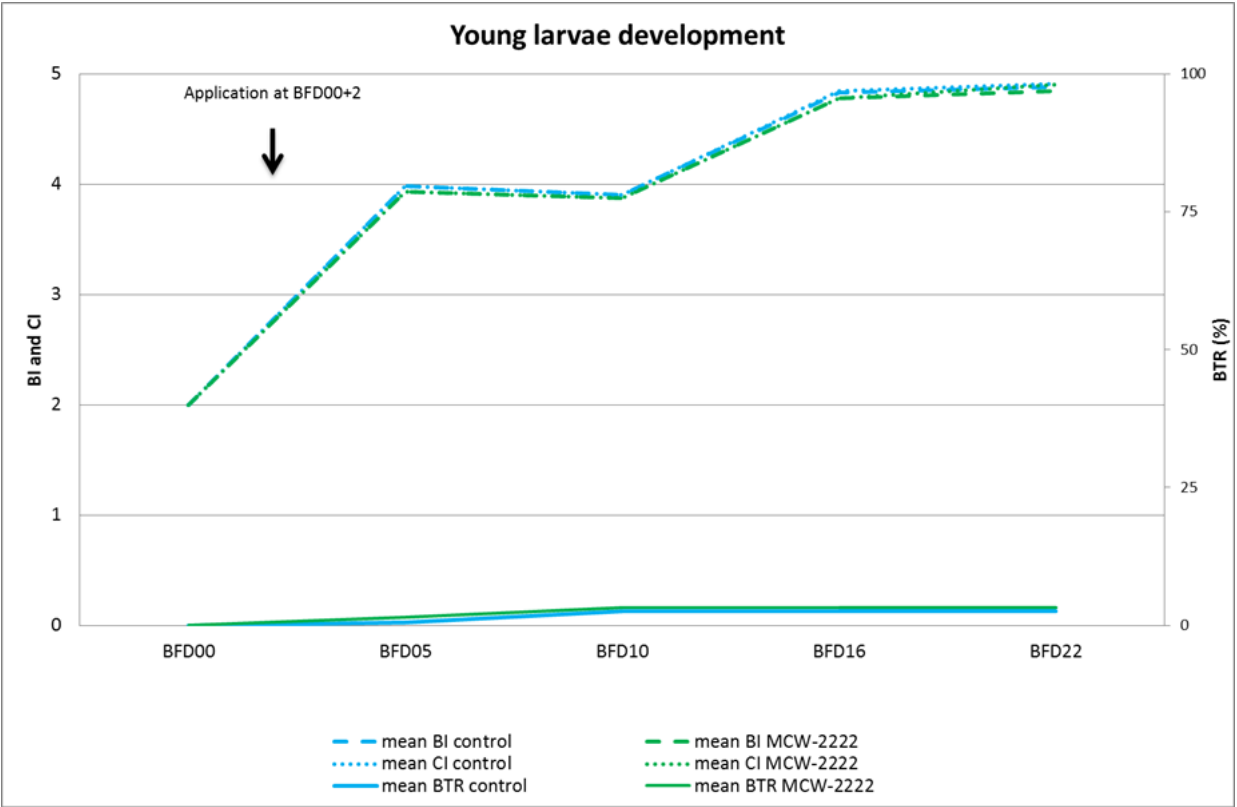


Figure A 47: Development of young larvae (BTR, BI, BCI)

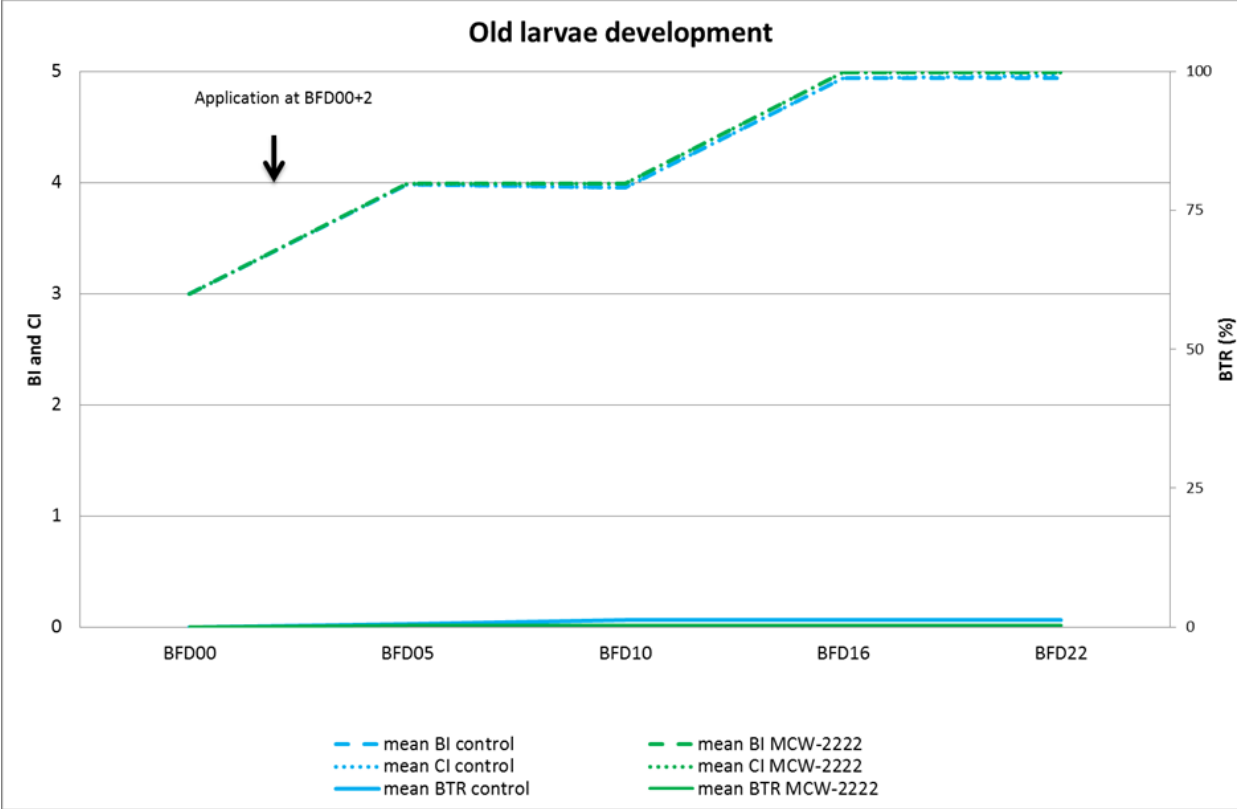


Figure A 48: Development of old larvae (BTR, BI, BCI)

Table A 129: Brood termination rate (%) per hive and per treatment over the time

Date	BFDxx days	Treatment	Control					MCW-2222				
		Hive N°	R087	R089	R091	R092	Mean	R095	R096	R098	R099	Mean
	Eggs											
07/04/15	BFD00	2 DBLA	0	0	0	0	0	0	0	0	0	0
12/04/15	BFD05	3 DALA	0	3	4	2	2.25	3	0	16	3	5.50
17/04/15	BFD10	8 DALA	0	3	5	3	2.75	3	1	16	3	5.75
23/04/15	BFD16	14 DALA	0	3	5	3	2.75	4	1	16	3	6.00
29/04/15	BFD22	20 DALA	0	3	5	3	2.75	4	1	16	3	6.00
	Young Larvae											
07/04/15	BFD00	2 DBLA	0	0	0	0	0	0	0	0	0	0
12/04/15	BFD05	3 DALA	0	1	0	1	0.50	1	1	4	0	1.50
17/04/15	BFD10	8 DALA	0	3	3	4	2.50	7	1	5	0	3.25
23/04/15	BFD16	14 DALA	0	3	3	4	2.50	7	1	5	0	3.25
29/04/15	BFD22	20 DALA	0	3	3	4	2.50	7	1	5	0	3.25
	Old Larvae											
07/04/15	BFD00	2 DBLA	0	0	0	0	0	0	0	0	0	0
12/04/15	BFD05	3 DALA	0	2	0	0	0.50	1	0	0	0	0.25
17/04/15	BFD10	8 DALA	0	3	0	2	1.25	1	0	0	0	0.25
23/04/15	BFD16	14 DALA	0	3	0	2	1.25	1	0	0	0	0.25
29/04/15	BFD22	20 DALA	0	3	0	2	1.25	1	0	0	0	0.25

Table A 130: Brood index per hive and per treatment over the time

Date	BFDxx days	Treatment	Control					MCW-2222				
		Hive N°	R087	R089	R091	R092	Mean	R095	R096	R098	R099	Mean
		Eggs										
07/04/15	BFD00	2 DBLA	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
12/04/15	BFD05	3 DALA	2.94	2.72	3.10	2.60	2.84	3.11	2.41	2.39	3.15	2.77
17/04/15	BFD10	8 DALA	4.00	3.88	3.80	3.88	3.89	3.88	3.96	3.36	3.88	3.77
23/04/15	BFD16	14 DALA	4.00	3.88	3.80	3.88	3.89	3.84	3.96	3.36	3.88	3.76
29/04/15	BFD22	20 DALA	5.00	4.85	4.75	4.85	4.86	4.80	4.95	4.20	4.85	4.70
		Young Larvae										
07/04/15	BFD00	2 DBLA	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
12/04/15	BFD05	3 DALA	4.00	3.96	4.00	3.96	3.98	3.96	3.95	3.81	4.00	3.93
17/04/15	BFD10	8 DALA	4.00	3.88	3.88	3.84	3.90	3.72	3.96	3.80	4.00	3.87
23/04/15	BFD16	14 DALA	4.88	4.81	4.84	4.80	4.83	4.53	4.92	4.70	4.95	4.78
29/04/15	BFD22	20 DALA	5.00	4.85	4.85	4.80	4.88	4.65	4.95	4.75	5.00	4.84
		Old Larvae										
07/04/15	BFD00	2 DBLA	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00
12/04/15	BFD05	3 DALA	4.00	3.92	4.00	4.00	3.98	3.96	4.00	4.00	4.00	3.99
17/04/15	BFD10	8 DALA	4.00	3.88	4.00	3.92	3.95	3.96	4.00	4.00	4.00	3.99
23/04/15	BFD16	14 DALA	5.00	4.85	5.00	4.90	4.94	4.95	5.00	5.00	5.00	4.99
29/04/15	BFD22	20 DALA	5.00	4.85	5.00	4.90	4.94	4.95	5.00	5.00	5.00	4.99

* The hive R014 was used when eggs were selected at BFD00. The hive R011 was used when larvae (young and old) were selected at BFD00

Table A 131: Brood compensation index per hive and per treatment over the time

Date	BFDxx days	Treatment	Control					MCW-2222				
		Hive N°	R087	R089	R091	R092	Mean	R095	R096	R098	R099	Mean
			Eggs									
07/04/15	BFD00	2 DBLA	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
12/04/15	BFD05	3 DALA	2.94	2.72	3.10	2.60	2.84	3.11	2.41	2.40	3.16	2.77
17/04/15	BFD10	8 DALA	4.00	3.88	3.80	3.90	3.90	3.88	3.96	3.39	3.89	3.78
23/04/15	BFD16	14 DALA	4.00	3.88	3.80	3.92	3.90	3.87	3.96	3.45	3.96	3.81
29/04/15	BFD22	20 DALA	5.00	4.85	4.76	4.93	4.89	4.93	4.95	4.48	4.93	4.82
			Young Larvae									
07/04/15	BFD00	2 DBLA	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
12/04/15	BFD05	3 DALA	4.00	3.96	4.00	3.96	3.98	3.96	3.95	3.81	4.00	3.93
17/04/15	BFD10	8 DALA	4.00	3.88	3.88	3.84	3.90	3.72	3.96	3.80	4.00	3.87
23/04/15	BFD16	14 DALA	4.88	4.81	4.84	4.84	4.84	4.53	4.92	4.71	4.95	4.78
29/04/15	BFD22	20 DALA	5.00	4.85	4.86	4.91	4.91	4.80	4.97	4.82	5.00	4.90
			Old Larvae									
07/04/15	BFD00	2 DBLA	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00
12/04/15	BFD05	3 DALA	4.00	3.92	4.00	4.00	3.98	3.96	4.00	4.00	4.00	3.99
17/04/15	BFD10	8 DALA	4.00	3.88	4.00	3.92	3.95	3.96	4.00	4.00	4.00	3.99
23/04/15	BFD16	14 DALA	5.00	4.85	5.00	4.90	4.94	4.95	5.00	5.00	5.00	4.99
29/04/15	BFD22	20 DALA	5.00	4.85	5.00	4.92	4.96	4.95	5.00	5.00	5.00	4.99

Residue analysis

The analytical method used in the current study was previously validated in this study (See Section 5.1.2). In order to confirm the analytical method was functioning correctly when used in the current study, procedural recovery samples were processed and analysed concurrently with the test samples and are summarised in the tables below.

Table A 132: Procedural recovery data for acetamiprid in nectar, honey, flowers, pollen and bee bread

Matrix	Fortification level [mg/kg]	Recovery (%)		RSD (%)	n
		Range	Mean		
Nectar	0.01	-	88*	-	1
	0.50	-	95*	-	1
Honey	0.01	-	97*	-	1
Flowers	0.01	91 – 99	95	6	2
Pollen	0.01	-	70*	-	1
	0.50	-	82*	-	1
Bee Bread	0.01	-	78*	-	1

* Single replicate

Table A 133: Procedural recovery data for acetamiprid-N-desmethyl in nectar, honey, flowers, bee bread and pollen

Matrix	Fortification level [mg/kg]	Recovery (%)		RSD (%)	n
		Range	Mean		
Nectar	0.01	-	102*	-	1
	0.50	-	88*	-	
Honey	0.01	-	110*	-	1
Flowers	0.01	-	85*	-	1
Bee Bread	0.01	-	70*	-	1
Pollen	0.01	-	70*	-	1
	0.50	-	79*	-	1

* Single replicate

In flower (collected 1DALA) and pollen specimens (collected 3DALA and 8DALA) sampled in untreated plots, no residues of acetamiprid and its metabolite were found. This result validates the trial design and attests that the colonies settled in the untreated control plot were not exposed to the active ingredient and its metabolite. On the other hand 6.8 mg/kg of acetamiprid and 0.093 mg/kg of acetamiprid-N-desmethyl were measured in the flowers sampled in the MCW-2222 treated plot 1DALA. This value shows clearly that foraging bees were exposed to MCW-2222.

Residue levels of acetamiprid were detected in the three pollen specimens of total pollen contained in the pollen traps of hives settled on the MCW-2222 treated field at 3 DALA (0.063 to 0.17 mg/kg, mean 0.128 mg/kg) and 8 DALA (0.14 to 0.22 mg/kg, mean 0.170 mg/kg). This proves the exposure of the colonies to the pollen contaminated with the test item (no contaminated pollen in the untreated field) and that this exposure lasted more than one week.

In-hive nectar bee bread and honey specimens were free from residue in the untreated control at both sampling dates. In-hive nectar specimens collected respectively at 3 DALA and 8DALA from the hives set in the test item treated field show levels of residue of acetamiprid from 0.013 to 0.16 mg/kg (mean of 0.062 mg/kg) and from 0.039 to 0.17 mg/kg (mean of 0.070 mg/kg).

Then honey specimens sampled at 20DAA show a residue level with values from 0.023 to 0.041 mg/kg (mean of 0.030 mg/kg) whereas no residues were found in the control samples. On the other hand, quantified residue of acetamiprid in bee bread sampled at 8DAA in the hives settled in the MCW-2222 plot were from 0.082 to 0.20 mg/kg (mean of 0.131 mg/kg) attesting that resources used to feed young larvae was contaminated with acetamiprid.

Regarding wax collected in the MCW-2222 treatment, no residue of acetamiprid was found 2 DBLA and residue of it was met at 20 DALA in all the 3 hives from 0.016 m/kg to 0.031 mg/kg.

No residue of acetamiprid-N-desmethyl metabolite has been measured in any nectar, wax, honey or bee bread specimen in the hives set on the test item treated field. This compound was found in only one pollen sample (0.013 mg/kg at 8 DALA).

Endpoints

No effects on adult and pupal bee mortality, foraging activity, behaviour, colony strength, colony conditions as well as on the specific evaluation of the detailed bee brood development were observed when MCW-2222 was applied two times (the first shortly before flowering at BBCH 59, the second during full flowering of the crop at BBCH 63 with hives present in the orchard but after bee flight) at a rate of 100 g a.s./ha to flowering *Phacelia*.

Validity criteria

As shown in the following table, all validity criteria were met.

Table A 134: Validity criteria

Validity criteria according to CEB 230 (2012), part IV, adapted from semi-field studies and regarded relevant for field studies	Observed in study
Before treatment:	
Daily mortality must be similar between the treatments. The difference between the average adult mortality on the day before application must not exceed 60%	On the day of application, the average mortality in T was 7.1 dead bees/colony and 5.1 dead bees/colony in C, resulting in a difference of 28% compared to T. Criterion was achieved
The foraging activity should be significant in each field and comparable between treatments	C: 4.5 bees/10m ² T: 7 bees/10m ² Criterion was achieved
After treatment:	
Mortality in the control must be comparable before and after the treatment, i.e. mortality the day after treatment must not exceed the average mortality figures for the day before treatment by more than 50%	Average mortality on 0DBLA: 3 dead bees/colony Average mortality on 1DALA: 2.7 dead bees/colony Difference: -10% Criterion was achieved
Additional validity criteria according to study plan	
After brood fixing day:	
Assuming a normal brood development, mean brood indexes should increase at further assessments: from eggs (1) to larvae (2-3), then pupae stage (4) and finally empty cells after hatch or new eggs (5).	BI of marked eggs in C: 1.00 – 2.84 – 3.89 – 3.89 – 4.82 BI of marked young larvae in C: 2.00 – 3.98 – 3.90 – 4.83 – 4.88 BI of marked old larvae in C: 3.00 – 3.98 – 3.95 – 4.94 – 4.94 Criterion was achieved

The termination rate in the control should be below 30%	BTR of eggs at BFD 22: 2.75% BTR of young larvae at BFD 22: 2.5% BTR of old larvae at BFD 22: 1.25% Criterion was achieved
Weather conditions must remain favourable	Criterion was achieved
Any other phenomena that have been considered as abnormal in the course of the study will be reported	None observed Criterion was achieved

Conclusion

In a field study based on EPPO 1/170 (4) (2010), C.E.B methodology n°230, part IV (2003) and OECD GD 75 (2007), honeybees (*Apis mellifera* L.) were exposed to MCW-2222 (a.s. acetamiprid), two times applied at rate of 100 g a.s./ha to oilseed rape (*Brassica napus*), investigating potential effects on adult and pupal mortality, foraging activity and behaviour, conditions of the colonies (i.e. colony strength and colony development). Special attention was laid on the assessment of the detailed bee brood development. The first application was performed just before flowering of the crop, the second application during its flowering but after bee flight. Residues levels of acetamiprid were quantified in flowers (1DALA), pollen (at 3DALA & 8DALA), in-hive nectar (at 3DALA & 8DALA), bee bread (at 8DALA) and honey specimen (at 20DAA) of the test item treatment, which confirmed the exposure of the foraging bees, larvae and the colonies to the test item. No residues in wax were found.

The results showed, that MCW-2222 (a.s. acetamiprid) didn't have any impact on foraging activity, behaviour, adult bee and pupal mortality.

Furthermore, the assessment of the colony strength and colony development as well as the specific evaluation of the detailed bee brood development showed no impact of the test item on the development on honeybee brood.

A 2.3.1.8.3 KCP 10.3.1.6/03 Field study with honey bee brood in apple

Comments of zRMS:	<p>The study has been already evaluated and accepted by the zRMS in the course of the first zonal evaluation of CA3573 (formerly MCW-2222) finalised in April 2018. Although the test guideline has not changed since that time, the study has been re-evaluated by the zRMS in 2021.</p> <p>The study was conducted in line with methodology described in OEPP/EPPO Bulletin 40, 313-319 in apple orchards in the Northern Italy. Application of MCW-2222 was performed twice at 100 g a.s./ha: first time just before the flowering (BBCH 57) and second time at BBCH 64, 8 days later in the evening without presence of foraging bees (3 days after hives settlement). Two replicate fields for test item were used and one for the water treated control.</p> <p>The distance between control and particular treatment fields ranged from 4.7 to 6.2 km (at least 4 km are currently required). In the study fields surroundings trees (<i>Castanea sativa</i>, <i>Prunus avium</i>, <i>Robinia pseudoacacia</i>, <i>Juglans regia</i>, <i>Quercus</i> spp., <i>Populus</i> spp., <i>Ailanthus altissima</i> and <i>Salix</i> spp.) and weeds (<i>Salvia pratensis</i> and <i>Trifolium</i> spp.) were present. No information on the flowering status of those trees is provided, while some of them are attractive to bees and could be in flowering during the test period (from mid-April to 7th of May). No clear information on other flowering orchard crops is presented in the study report, but according to information provided in Appendix 2 of the study report peach orchards were present just next to the test plots. Their flowering status is not provided.</p> <p>The colonies comprising of approximately 6000 young bees, >3000 brood and 2 frames of honey, were housed in 6 frame Dadant hives. It is noted that according to OEPP/EPPO colonies containing at least 10000 bees, covering at least 10-12 frames (including 5-6 brood frames) should be used, so colonies used in the study were too small.</p> <p>Observations of bee mortality, behaviour and effects on bee brood were performed up to 21 days after the treatment (21 DALA). Observation of bee brood covered full brood cycle and beginning of a new one (from BFD 0 to BFD 22). Additionally at BFD 28 colony assessment (adults and brood) was carried out. The study duration in general followed indications of OEPP/EPPO, however in opinion of the zRMS it should have been prolonged as some effects on brood termination rates in test item groups were seen. According to EFSA (2013)</p>
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	<p>the study duration should be at least 42 days in order to cover 2 brood cycles.</p> <p>No precipitation was observed during application, but rain occurred on 1 DALA, 2 DALA and 3 DALA at 0.8, 7.0 and 10.6 mm, respectively. Especially on 2 and 3 DALA precipitation was high enough to reduce the exposure of bees to the test item. Some showers were observed later in the study on 25 and 26 DALA (0.2 and 0.8 mm, respectively) and significant precipitation was on 27 DALA (26 mm). This, however, was not as important for exposure as rain during first days after the application.</p> <p>The foraging activity in the treatment plots was statistically significantly lower comparing to controls.</p> <p>Number of dead bees found in dead bee traps in control and test item fields was variable and no clear dose-response pattern could be observed (see graph in the study summary). Number of dead bees on the collecting sheets was in general low, but clearly higher in the second test item plot (TB) comparing to control (U) and first test item plot (TA).</p> <p>Pupae mortality was low over the entire study period and similar in test item and control groups.</p> <p>Obtained results indicate that the brood termination rates in test item groups were clearly higher than in controls, while brood indices in test item groups were lower. It is noted that although at some dates the BTR in test item groups was several times higher than in controls, no statistically significant differences were detected in performed analyses. Taking this into account, the statistical power of the study to detect effects may be questioned. Overall, the zRMS is of the opinion that observed effects could be of biological relevance and the study should have been prolonged in order to cover at least 2 brood cycles to investigate duration of these effects.</p> <p>Compensation indices were not determined, so potential recovery of affected brood could not be confirmed.</p> <p>Effects of the test item on the overwintering success were not investigated and the trial was terminated 25 days after second application (BFD 28).</p> <p>Overall, due to deficiencies mentioned above (rainfall during 3 days after the second application, no information on flowering weeds and trees in the field surroundings, no information on flowering orchard crops in field surroundings) results of the study cannot be considered fully reliable. Furthermore, the bee colonies used in the study were too small when compared with indications of OEPP/EPPO.</p> <p>Nevertheless, despite potentially reduced exposure clear effects on brood termination rates were seen in the treatment fields, which could be of biological relevance, even if statistically not significant. Mortality of adult bees was variable and no clear dose-response pattern could be observed. Pupae mortality was low in both test item and control fields.</p>
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Data point:	KCP 10.3.1.6/03
Report:	Effects and Determination of Residues of Acetamiprid 200 SL on the Honeybee (<i>Apis mellifera</i> L.) Brood in Apple, under Field Conditions, in Italy 2015. Aucejo, C., 2015, R-35961 307SRES15C01; including Final Report Amendment N°1
Guideline(s):	OECD GD75 (2007), adapted to field situations
Deviations:	None
GLP:	Yes, certified laboratory
Acceptability:	Yes, study considered acceptable

Duplication: Not applicable
(if vertebrate study)

Executive Summary

In a field study with honeybees (*Apis mellifera* L.) the potential effects of MCW-2222 on mortality, foraging and flight activity, colony strength and brood amount were investigated. Special attention was laid on the detailed brood assessment of marked cells containing eggs.

Three study orchards (plots) with flowering apple (*Malus domestica*) in Italy, one was treated with water and served as control (U) (area: 16 ha), two were treated with the test item (TA: 3.6 ha; TB: 9 ha) served as plots. The test item plots were separated from control plots approx. 13.3 km (TA) and 15.6 km (TB), the distance between TA and TU was 2.6 km. The test item was two times applied at a rate of 100 g a.s./ha. The first application (application A) was performed before crop flowering (i.e. 9th of April, 2015, BBCH 57) without having the colonies placed to the fields and the second one (application B) when the apples were full flowering, with hives present in the plots but after bee flight (i.e. 16th April, 2015, BBCH 65).

Eight honey bee colonies, each with at least 6,000 young honey bees, more than 3,000 cells of brood and 2 frames of honey, were placed at each plot 4 days before the last application (-4 DAB) to get familiar with the new conditions. All colonies were used for the biological assessments; three of the eight colonies were randomly selected at each sampling event for in-hive residue sampling (nectar, young larvae). Hives, where pollen sampling was conducted were not used at this time for mortality assessments. The exposure phase lasted from the day of the last (2nd) application (0 DAB, BBCH 65) to the end of flowering (11 DAB). On the next day, the colonies were moved to the monitoring site where no further pesticide exposure was expected.

In order to ensure that bees were exposed to the test item, observations on the foraging and flight activity were scheduled daily from -3 DAB until 7 DAB. On 1 DAB, bee flight assessments were performed in the morning, midday and evening. Foraging bees, as well as bees in flight, were counted by observing canopies from the same 9 trees distributed in 3 rows (3 trees per row) for 30 seconds each. Trees were about 10 m apart in each row. Recordings were done at the observer's height.

Assessments on adult, larval and pupal mortality (via dead bee traps and collecting sheets of 4.5m² per hive) were daily conducted between -3 DAB until 21 DAB.

The colony status, i.e. colony strength (number of bees per colony) and amount of brood the colonies was inspected on the day of Brood Fixing Day (BFD 0 = -3 DAB) and on BFD 3, BFD 5, BFD 10, BFD 16, BFD 22 and BFD 28 (= 25 DAB).

Detailed assessments of the bee brood development was carried out by marking individual brood cells containing eggs at the Brood Area Fixing Day 0 (BFD 0), which was three days before the last (2nd) application (-3 DAB). At this day, at least one hundred cells were selected in each hive and followed until 22 days after BFD (BFD22), which covered one brood cycle. Next to the assessment on BFD 22 the development of each individually marked cell was assessed at BFD 5, BFD 9 and BFD 15, using acetate sheets.

In order to confirm the concentration of the test item active substance in the applied solutions, two samples of 100 ml of spray solution were taken from the nozzles for each plot at the time of applications A and B. To determine possible residues of Acetamiprid 200 SL in the relevant matrices, samples of pollen, nectar and young larvae were taken from 3 (randomly selected at each sampling event) of the 8 hives on -1, 1, 7 and 13 DAB (12 DAB for pollen).

The number of bees flying per 30 seconds ranged from 0 to 1.67 in the untreated plot, 0 to 1.0 in both treated plots with no statistically significant differences on any of the assessment dates. Significant differences occurred between the control and the two Acetamiprid 200 SL treated plots two days before the second application. 7 days after the second application there were fewer flying bees in the control and treated plot A than in treated plot B. For the foraging activity, there were significantly fewer bees foraging in the Acetamiprid treated plots than in the control on all samplings before the second application and on five of the seven sampling occasions after the second application of treatments.

No dead larvae and a very low number of dead pupae were found in the dead bee traps and on the collecting sheets at each of the assessment events. In fact, on most assessments days, no dead pupae were found in the bee traps and only once on the collecting sheets (TB at BFD+5: 0.14 dead pupae/hive). Daily pupal mortality in the control varied between 0.00 and 2.50 dead pupae/hive, 0.00 to 0.63 dead pupae/hive in TA and 0.00 to 0.85 dead pupae/hive in TB with significant differences at any assessment day.

Daily mortality of adult bees in the control and both the Acetamiprid 200SL treated plots, TA and TB, was generally low and showed no signs of a peak in response to a toxic effect of treatment. The number of dead individuals was below 30 per hive per day on all except five dates in the control hives and on all except 9 days in the TA plot and 7 days in TB plot. Maximum mortality in dead bee traps were recorded after movement of the hives to wild forest areas (12 DAB). In the untreated plot a mean of 47.50 dead bees/hive at 19 DAB, in the TA plot it was 52.25 dead bees/hive at 19 DAB and in the TB plot it was 42.77 dead bees/hive at 14 DAB.

Regarding the assessments on the collecting sheets, the maximum adult mortality values were recorded during the presence of the hives in the orchards. In the untreated plot maximum mortality was 7.75 dead bees/hive at 3 DAB, in the TA plot it was 5.50 dead bees/hive at 1 DAB and in the TB plot it was 8.05 dead bees/hive at 7 DAB. These results indicate that the test item Acetamiprid 200 SL applied twice at the rate of 100 g a.s./ha did not cause any adverse effect on the adult worker bee population.

The colony strength on BFD 0 in the colonies of the control plot displayed an average of 3472 adult worker bees and colonies on TA and TB plots held on average 3859 and 3672 bees, respectively. These values increased with the growth of all colonies until BFD+16 (average numbers of adult worker bees/4 frames/hive of 6566.40 in the water control, 7175.78 in TA and 6394.53 in TB). After their movement to wild forest areas (12 DAB), the total number of bees declined slightly in all plots, which amounted to average numbers of adult worker bees/4 frames/hive of 5152.34 in the control, 6261.72 in TA and 5222.66 in TB on BFD +28.

Regarding the brood presence at BFD 0, the control plot (14570.63 cells containing food and immatures/4 frames/hive) was significantly different from TA (9333.75 cells containing food and immatures/4 frames/hive) but not from TB (11898.75 cells containing food and immatures/4 frames/hive).

No significant differences in terms of brood and food presence were detected between the untreated and the treated plots at the end of the period in the orchards (BFD+16). And also on the last assessment on BFD+28, no statistical differences were observed between the treatments, i.e. 25341.25 cells/4 frames/hive in the control, 26861.25 cells/4 frames/hive in TA and 24153.75 cells/4 frames/hive in TB.

The detailed brood assessment displayed no statistical differences of the BTR at any assessment date between the treatments. In healthy hives, a number of eggs are removed by workers so they can enter the cells to control temperature. This means that a control BTR of 20% is quite normal. Almost all detected values remain within this percentage, with the exception of the hives in Acetamiprid 200 SL Plot B where it reached 31% at BFD+15, on 28 April 2015.

The mean BI show a normal development from eggs to larvae, pupae and subsequent emergence in both, the control and the treated plots. There were no statistically significant differences on any assessment events.

The mean acetamiprid residues in the spray solutions was 72.4 mg/L for plot A and 96.4 mg/L for plot B at the first application (equivalent to 76.2% and 105.9% of the nominal application rate) and 98.7 mg/L and 74.3 mg/L on the second application (equivalent to 103.9% and 81.6% of the nominal application rate respectively).

The residues of acetamiprid in samples of larvae taken on .1 DAB and 1, 7 and 14 DAB were all found to be below the LOQ of 0.01 mg/kg.

Materials and methods

Materials

Test item	MCW-2222
Batch #	659-030314-01
Content of active substance	Acetamiprid 200 g/L (nominal); 199.2 L (analysed)
Description	Clear yellowish liquid
Control	Untreated crop
Toxic reference	none
Test organism	
Species	Queen right colonies of the honeybee, <i>Apis mellifera</i> , with at least 6,000 young honey bees, more than 3,000 cells of brood and 2 frames of honey, were used. All development stages of brood (eggs, young and old larvae, pupae) were present. Test colonies were housed in 5 frame Dadant hives and were placed elevated 20 cm, avoiding the contact with the ground. They were visually assessed prior to trial initiation to be disease free and have low

	<p>mite (<i>Varroa destructor</i>) populations.</p> <p>Two days before the BFD (and six days after the second application of treatments), eight bee colonies were randomly assigned to control and test item treatments. They were distributed in the central part of each plot, so that foraging activity outside the experimental area was minimized.</p>
Source	Commercial farm, in 12100 Cuneo (CN), Italy
Food/feeding	Full flowering apple (<i>Malus domestica</i> , variety Gala, red group) served as food supply, no additional feeding throughout the study.
Study design and methods	
Test duration	<p>Pre-exposure phase (-4 DAB to 0 DAB): 4 days at the orchards</p> <p>Exposure phase (0 DAB to 11 DAB): 11 days at the orchards</p> <p>Post-Exposure phase (12 DAB to 25 DAB): 13 days at the monitoring site (wild forest flowering areas)</p>
Experimental dates	9 th April to 11 th May 2015
Test doses	<p>2 x 100 g a.s./ha</p> <p>1st application on 9th April 2015 (-7 DAB; BBCH 57) without hives present in the plots</p> <p>2nd application on 16th April 2015 (0 DAB; BBCH 65) with hives present in the plots but applied after bee flight</p>
	<p>The applications in the control were performed with a water volume of 1250 L/ha, in TA with 1050 L/ha and in TB with 1100 L/ha.</p> <p>The deviation from the target rate was < 5% in test item plot TA at both applications in the test item plot TB < 0.6% at the both applications.</p>
Test units	<p>Three study orchards (plots) with flowering apple (<i>Malus domestica</i>) (variety: Hybrirock), one was treated with water and served as control (U) (area: 16 ha, 2080 trees/ha), two were treated with the test item (TA: 3.6 ha, 1630 trees/ha; TB: 9 ha, 2500 trees/ha). The test item plots were separated from control plots approx. 13.3 km (TA) and 15.6 km (TB); distance between TA and TU: 2.6 km.</p> <p>Each study field with 8 colonies.</p>
Group size/replicates	<p>One orchard for the control, two for the test item group, each with 8 colonies; all colonies were used for the biological assessments; three of the eight colonies were randomly selected at each sampling event for in-hive residue sampling. Hives, where pollen sampling was conducted were not used at this time for mortality assessments.</p>
Endpoints and assessments	<p><i>foraging and flight activity:</i></p> <p>Foraging bees, as well as bees in flight, were counted by observing canopies from the same 9 trees distributed in 3 rows (3 trees per row) for 30 seconds each. Trees were about 10 m apart in each row. Recordings were done at the observer's height and the assessments were conducted from - 3 DAB (= BFD 0) until 7 DAB (= BFD 10). On 1 DAB, bee flight assessments were performed in the morning, midday and evening.</p> <p><i>mortality of adult bees, larvae and pupae:</i></p> <p>For the mortality assessments, a dead bee trap was placed at the entrance of each hive, as well as a collecting sheet of 4.5 m² (3 m x 1.5 m) and the number of dead adults, larvae and pupae was recorded at each assessment event. After each recording (performed at approx. the same time of the day) all the dead individuals were removed.</p> <p>The number of dead bees was recorded daily from - 3 DAB (= BFD 0) until 21 DAB (Days after Application B) (= BFD 24).</p>

colony status

At each assessment the approximate area of 4 frames containing adults and brood was recorded. For doing so, an acetate sheet divided in 25 cm² squares was placed on both sides of the combs and an estimation of the number of squares with bees or brood was made.

To obtain the number of bees per dm² (colony strength) the number of squares was multiplied by a conversion factor of 125 bees/dm², while the estimated area containing brood was multiplied by a conversion factor of 380 cells/dm².

Assessments were conducted on the day of Brood Fixing Day (BFD 0 = -3 DAB), BFD 3, BFD 5, BFD 10, BFD 16, BFD 22 and BFD 28 (= 25 DAB); assessment of:

- estimated number of bees (colony strength),
- number of cells containing brood,
- number of cells containing food.

detailed bee brood development:

One suitable frame, containing sufficient food and eggs, was selected for brood analysis in each hive of both plots. This frame was not used for residue sample collection. On the BFD 0 (-3 DAB), an area of more than 100 eggs was selected in these frames and was marked on an acetate sheet, which was fixed on the wooden frames and the position on the frame was marked. This allowed placing subsequent sheets exactly in the same position in each of the following observing days. In these new acetate sheets, the eggs area was copied and growth stage of the brood in each cell was noted.

This procedure allowed an evaluation of the development of each individually marked cell (noting if they were eggs, young and old larvae, pupae or capped brood) throughout the duration of the study and the calculation of Brood Termination Rate (BTR) and Brood Index (BI) for control and test item hives throughout the study.

Assessments on BFD 0 (= -3 DAB), BFD 5, BFD 9, BFD 15, BFD 22 (= 19 DAB), covering one complete brood cycle (21 days for worker bees).

The time schedule of assessment days was chosen in order to check the bee brood at different expected stages during the development.

Based on number of cells with eggs marked at BFD 0 and number of eggs which failed to develop successfully until adult hatch the BTR were determined for each replicate at each assessment day.

Moreover, attributing values from 0 (termination of development), 1 (egg stage) to 4 (capped brood) and 5 (empty after hatch) to the respective brood stages, the brood indices (BI) were calculated. If the cell was empty or the individual was dead, the cell was counted as 0 (that day and the following assessment days).

Specimens sampling for residue analysis

Spray solution: two samples of 100 ml of spray solution were taken from the nozzles for each plot (U, TA, TB) at each of the two applications.

Residue samples were taken from 3 (randomly selected at each sampling event) of the 8 hives on -1, 1, 7 and 13 DAB (12 DAB for

pollen):

- samples of pollen from traps in front of three hives
- samples of nectar from uncapped cells
- samples of young larvae.

Half of the collected samples were transported to the analytical laboratory GIRPA/FREDON Pays de la Loire, France for residue analysis of acetamiprid.

Residues of acetamiprid were extracted from specimens in frozen conditions by agitation in acetonitrile and ultra-pure water. Then extracts were purified by dispersive solid phase extraction (SPE). The quantification was performed by liquid chromatography with tandem mass spectrometry detection (LCMS-MS).

DAB = days application B (= last/2nd application)

BFD = brood area fixing day

Adaptation of bees

Colonies were set-up at the fields seven days before the 2nd application (application B) to get familiar with the new conditions.

Environmental conditions

Natural field conditions

Environmental conditions were provided from two weather stations. The first one was located in Fossano (CN) and (max.: 29.1°C, min.: 3.4°C), the second one in Centallo (CN) (max.: 27.3°C, min.: 3.8°C)

Conditions at TA		
	1 st application	2 nd application (after bee flight)
Temperature:	18.2 °C	15.8 °C
Wind speed:	0 km/h	0 km/h
Rel. humidity:	45 %	69 %
Precipitation°:	none	none
BBCH:	57	65

Conditions at TB		
	1 st application	2 nd application (after bee flight)
Temperature:	22.2 °C	15.9 °C
Wind speed:	0 km/h	0 km/h
Rel. humidity:	41 %	70 %
Precipitation°:	none	none
BBCH:	57	65

° within 24 h after application

Biological observations

Foraging activity and behaviour was daily recorded between -3 DAB to 7 DAB, adult larval and pupal mortality was daily recorded between -3 DAB to 21 DAB. For the detailed assessments of the bee brood development, at least 100 individual brood cells per hive containing eggs were marked at the Brood Area Fixing Day 0 (BFD 0). The development of each marked cell was assessed on BFD 5, BFD 9, BFD 15 and BFD 22. The assessment of condition of the colony strength and colony development was performed on BFD 3, BFD 5, BFD 10, BFD 16, BFD 22 and BFD 28.

Statistics

The commercial statistics programme "Agricultural Research Manager 9.2014.7 (ARM)" was used to determine whether there were significant differences between the treatments. An ANOVA and a Student-Newman-Keuls (SNK) test was done to determine if there was a significant difference between the control treatment and the test item Acetamiprid 200 SL. Analysis was performed on untransformed data and on transformed data (using LOG(X+1), arcsine square root percent or square root transformations) when ARM software recommended to transform data according to the Bartlett's test used to verify the homogeneity of variance. If data transformation could not solve the invalidity of ANOVA assumptions (including the homogeneity of variance), Friedman's non-parametric test was used to check for significant differences

between treatments. The probability of no significant differences occurring between treatment means was calculated as the F probability value (Treatment Prob(F)). Results obtained were indicated by a letter - treatment means with no letters in common are significantly different in accordance with a SNK conducted at a 95% confidence level.

Results and discussion

Biological results

Flight and foraging activity

The number of bees flying per 30 seconds ranged from 0 to 1.67 in the untreated plot, 0 to 1.0 in both treated plots with no statistically significant differences on any of the assessment dates. Significant differences occurred between the control and the two Acetamiprid 200 SL treated plots two days before the second application. 7 days after the second application there were fewer flying bees in the control and treated plot A than in treated plot B.

For the foraging activity, there were significantly fewer bees foraging in the Acetamiprid treated plots than in the control on all samplings before the second application and on five of the seven sampling occasions after the second application of treatments.

Figure A 49: Daily mean flying activity

Date	Timing (DAB)	Mean number of flying bees		
		Control	Acetamiprid 200 SL Plot A	Acetamiprid 200 SL Plot B
13/04/2015	-3	1.11 a	0.78 a	0.44 a
14/04/2015	-2	1.67 a	0.78 b	0.44 b
15/04/2015	-1	0.89 a	0.78 a	0.89 a
16/04/2015	0	1.00 a	1.00 a	0.78 a
17/04/2015	1, a.m.	0.00 a	0.00 a	0.00 a
	1, p.m.	0.11 a	0.00 a	0.89 a
	1, evening	0.33 a	0.33 a	0.22 a
18/04/2015	2	0.67 a	0.33 a	1.00 a
19/04/2015	3	0.56 a	0.89 a	0.67 a
20/04/2015	4	0.89 a	0.22 a	0.33 a
21/04/2015	5	0.11 a	0.00 a	0.44 a
22/04/2015	6	0.11 a	0.33 a	0.11 a
23/04/2015	7	0.11 b	0.11 b	0.78 a

DAB = days after application B (= second application)

Means followed by same letter do not differ significantly in a Student-Newman-Keuls (S-N-K) test conducted at a 95% confidence level. DAB is the number of days after the second application of treatments.

Table A 135: Daily mean foraging activity

Date	Timing (DAB)	Mean number of foraging bees		
		Control	Acetamiprid 200 SL Plot A	Acetamiprid 200 SL Plot B
13/04/2015	-3	7.56 a	3.78 b	0.78 c
14/04/2015	-2	10.00 a	4.33 b	2.89 b
15/04/2015	-1	9.89 a	3.11 b	3.67 b
16/04/2015	0	6.33 a	3.22 b	3.00 b
17/04/2015	1	0.00 a	0.00 a	0.00 a
18/04/2015	2	12.56 a	5.00 b	2.00 c
19/04/2015	3	0.22 a	0.44 a	0.78 a
20/04/2015	4	13.33 a	4.78 b	3.78 b
21/04/2015	5	10.00 a	4.44 b	3.67 b
22/04/2015	6	9.78 a	2.89 b	4.89 b
23/04/2015	7	8.00 a	3.11 b	3.89 b

DAB = days after application B (= second application)

Means followed by same letter do not differ significantly in a Student-Newman-Keuls (S-N-K) test conducted at a 95% confidence level.

Mortality

No dead larvae and a very low number of dead pupae were found in the dead bee traps and on the collecting sheets at each of the assessment events. In fact, on most assessments days, no dead pupae were found in

the bee traps and only once on the collecting sheets. Daily pupal mortality in the control varied between 0.00 and 2.50 dead pupae/hive, 0.00 to 0.63 dead pupae/hive in TA and 0.00 to 0.85 dead pupae/hive in TB with significant differences at any assessment day.

Daily mortality of adult bees in the control and both the Acetamiprid 200SL treated plots, TA and TB, was generally low and showed no signs of a peak in response to a toxic effect of treatment. The number of dead individuals was below 30 per hive per day on all except five dates in the control hives and on all except 9 days in the TA plot and 7 days in TB plot. Maximum mortality in dead bee traps were recorded after movement of the hives to wild forest areas (12 DAB). In the untreated plot a mean of 47.50 dead bees/hive at 19 DAB, in the TA plot it was 52.25 dead bees/hive at 19 DAB and in the TB plot it was 42.77 dead bees/hive at 14 DAB.

Regarding the assessments on the collecting sheets, the maximum adult mortality values were recorded during the presence of the hives in the orchards. In the untreated plot maximum mortality was 7.75 dead bees/hive at 3 DAB, in the TA plot it was 5.50 dead bees/hive at 1 DAB and in the TB plot it was 8.05 dead bees/hive at 7 DAB. These results indicate that the test item Acetamiprid 200 SL applied twice at the rate of 100 g a.s./ha did not cause any adverse effect on the adult worker bee population.

Table A 136: Daily mean mortality of adult bees

Date	Timing (DAB)	Mean adult bee mortality [n]recorded by					
		bee traps			collecting sheets		
		Control	Acetamiprid 200 SL Plot A	Acetamiprid 200 SL Plot B	Control	Acetamiprid 200 SL Plot A	Acetamiprid 200 SL Plot B
13/04/2015	-3	10.75 b	27.88 a	25.60 a	0.38 a	0.00 a	0.04 a
14/04/2015	-2	7.13 b	13.38 ab	17.75 a	0.00 a	0.00 a	0.00 a
15/04/2015	-1	33.50 a	12.25 b	16.02 b	4.25 a	1.63 b	2.08 b
16/04/2015	0	29.75 a	30.13 a	31.72 a	4.75 a	3.50 a	5.27 a
17/04/2015	1	20.50 a	21.25 a	28.09 a	5.00 a	5.50 a	8.04 a
18/04/2015	2	28.00 a	23.75 a	22.80 a	6.63 a	3.88 a	7.89 a
19/04/2015	3	14.38 b	19.38 b	41.16 a	7.75 a	4.63 a	7.97 a
20/04/2015	4	40.63 a	31.25 a	25.51 a	2.13 a	1.75 a	2.22 a
21/04/2015	5	14.88 a	29.75 a	22.88 a	1.13 a	0.50 a	1.17 a
22/04/2015	6	21.00 a	18.63 a	15.46 a	1.63 a	2.88 a	4.54 a
23/04/2015	7	24.50 a	20.25 a	19.73 a	2.88 ab	1.38 b	8.05 a
24/04/2015	8	17.88 a	20.75 a	29.03 a	1.00 b	1.25 b	4.27 a
25/04/2015	9	24.50 a	19.13 a	29.74 a	0.63 a	0.50 a	1.63 a
26/04/2015	10	22.00 a	18.63 a	23.67 a	0.75 a	1.00 a	0.73 a
27/04/2015	11	20.38 a	16.25 a	19.38 a	0.38 a	0.50 a	0.94 a
28/04/2015	12	11.00 b	15.13 ab	26.99 a	0.38 a	0.75 a	1.13 a
29/04/2015	13	23.88 a	30.50 a	24.90 a	0.25 a	0.38 a	0.10 a
30/04/2015	14	29.13 a	36.13 a	42.77 a	1.13 a	0.75 a	1.15 a
01/05/2015	15	22.75 a	30.50 a	25.63 a	0.13 a	0.50 a	0.67 a
02/05/2015	16	33.00 a	32.50 a	34.32 a	0.63 a	1.00 a	1.03 a
03/05/2015	17	30.13 a	24.25 a	38.12 a	1.13 a	0.63 a	1.52 a
04/05/2015	18	22.13 a	19.13 a	30.91 a	0.63 a	0.50 a	0.92 a
05/05/2015	19	47.50 a	52.25 a	40.23 a	1.50 a	0.63 a	1.49 a
06/05/2015	20	25.13 a	33.38 a	29.39 a	0.50 a	0.75 a	0.34 a
07/05/2015	21	29.75 a	30.25 a	26.21 a	0.38 a	0.00 a	0.33 a

DAB = days after application B (= second application)

Means followed by same letter do not differ significantly in a Student-Newman-Keuls (S-N-K) test conducted at a 95% confidence level.

Colony status

On BFD 0 the colonies in the control plots held an average of 3472 adult worker bees and colonies on TA and TB plots held on average 3859 and 3672 bees, respectively. These values increased with the growth of all colonies until BFD+16 (average numbers of adult worker bees/4 frames/hive of 6566.40 in the water control, 7175.78 in TA and 6394.53 in TB). After their movement to wild forest areas (12 DAB), the total number of bees declined slightly in all plots, which amounted to average numbers of adult worker bees/4

frames/hive of 5152.34 in the control, 6261.72 in TA and 5222.66 in TB on BFD +28.

Regarding the brood presence at BFD 0, the control plot (14570.63 cells containing food and immatures/4 frames/hive) was significantly different from TA (9333.75 cells containing food and immatures/4 frames/hive) but not from TB (11898.75 cells containing food and immatures/4 frames/hive).

No significant differences in terms of brood and food presence were detected between the untreated and the treated plots at the end of the period in the orchards (BFD+16). And also on the last assessment on BFD+28, no statistical differences were observed between the treatments, i.e. 25341.25 cells/4 frames/hive in the control, 26861.25 cells/4 frames/hive in TA and 24153.75 cells/4 frames/hive in TB.

Table A 137: Mean estimated number of adults in the colony in the water treated control and in the Acetamiprid 200 SL treated plots

	-3 DAB	0 DAB	2 DAB	7 DAB	13 DAB	19 DAB	25 DAB
Treatment	BFD 0	BFD +3	BFD +5	BFD +10	BFD +16	BFD +22	BFD +28
Control	3472.66 a	4523.44 a	5117.19 a	4695.31 a	6566.41 a	6054.69 a	5152.34 a
Acetamiprid 200 SL Plot A	3859.38 a	3960.94 a	4039.06 a	4414.06 a	7175.78 a	6878.91 a	6261.72 a
Acetamiprid 200 SL Plot B	3671.88 a	4117.19 a	3984.38 a	4230.47 a	6394.53 a	6000.00 a	5222.66 a

DAB = days after application B (= second application); BFD = Brood area fixing day

Means followed by same letter do not differ significantly in a Student-Newman-Keuls (S-N-K) test conducted at a 95% confidence level.

Table A 138: Mean estimated number of cells with brood in the water treated control and in the Acetamiprid 200 SL treated plots

	-3 DAB	0 DAB	2 DAB	7 DAB	13 DAB	19 DAB	25 DAB
Treatment	BFD 0	BFD +3	BFD +5	BFD +10	BFD +16	BFD +22	BFD +28
Control	14570.63 a	21161.25 a	21576.88 a	20555.63 b	22170.63 a	21660.00 a	25341.25 a
Acetamiprid 200 SL Plot A	9333.75 b	22681.25 a	24130.00 a	25163.13 a	26457.50 a	25685.63 a	26861.25 a
Acetamiprid 200 SL Plot B	11898.75 ab	23037.50 a	24082.50 a	23310.63 ab	24367.50 a	23738.13 a	24153.75 a

DAB = days after application B (= second application); BFD = Brood area fixing day

Means followed by same letter do not differ significantly in a Student-Newman-Keuls (S-N-K) test conducted at a 95% confidence level.

Detailed bee brood development

Regarding the brood termination rate (BTR), no statistical differences were detected at any assessment date between the treatments. In healthy hives, a number of eggs are removed by workers so they can enter the cells to control temperature. This means that a control BTR of 20% is quite normal. Almost all detected values remain within this percentage, with the exception of the hives in Acetamiprid 200 SL Plot B where it reached 31% at BFD+15, on 28 April 2015.

Table A 139: Mean Brood Termination Rate (%age) on each assessment date after BFD 0

	-3 DAB	2 DAB	6 DAB	12 DAB	19 DAB
Treatment	BFD 0	BFD +5	BFD +9	BFD +15	BFD +22
Control	0.0 a	1.71 a	8.00 a	17.67 a	17.78 a
Acetamiprid 200 SL Plot A	0.0 a	15.55 a	18.76 a	17.01 a	20.17 a
Acetamiprid 200 SL Plot B	0.0 a	12.39 a	24.17 a	31.08 a	31.08 a

DAB = days after application B (= second application); BFD = Brood area fixing day

Means followed by same letter do not differ significantly in a Student-Newman-Keuls (S-N-K) test conducted at a 95% confidence level.

The mean Brood Indices (BI) show a normal development from eggs to larvae, pupae and subsequent emergence in both, the control and the treated plots. There were no statistically significant differences on any assessment events.

Table A 140: Mean Brood Index on each assessment date after BFD 0

	-3 DAB	2 DAB	6 DAB	12 DAB	19 DAB
Treatment	BFD 0	BFD +5	BFD +9	BFD +15	BFD +22
Control	1.00	1.96 a	3.59 a	3.29 a	4.05 a
Acetamiprid 200 SL Plot A	1.00	1.68 a	2.96 a	3.17 a	3.96 a
Acetamiprid 200 SL Plot B	1.00	1.73 a	2.92 a	2.76 a	3.42 a

DAB = days after application B (= second application); BFD = Brood area fixing day

Means followed by same letter do not differ significantly in a Student-Newman-Keuls (S-N-K) test conducted at a 95% confidence level.

Residue analysis

The analytical method used in the current study was previously validated in study GLP-study 215-2014 (R-34877), (Molitor, 2015b). See Section 5.1.2. In order to confirm the analytical method was functioning correctly when used in the current study, procedural recovery samples were processed and analysed concurrently with the test samples and are summarised in the table below.

Table A 141: Procedural recovery data for acetamiprid in nectar, pollen and larvae

Matrix	Fortification level [mg/kg]	Recovery (%)		RSD (%)	n
		Range	Mean		
Nectar	0.01	92 - 97	97	12	3
	0.5	-	92*	-	1
Larvae	0.01	-	76*	-	1
Pollen	0.01	62 - 73	70	9.1	3
	2.0	-	77*	-	1

* Single replicate

The mean acetamiprid residues in the spray solutions was 72.4 mg/L for plot A and 96.4 mg/L for plot B at the first application (equivalent to 76.2% and 105.9% of the nominal application rate) and 98.7 mg/L and 74.3 mg/L on the second application (equivalent to 103.9% and 81.6% of the nominal application rate respectively).

The residues of acetamiprid in samples of larvae taken one day before the second application of treatments and one, seven and 14 days after the second application were all found to be below the LOQ of 0.01 mg/kg.

Table A 142: Mean residues (mg/kg) of acetamiprid in fresh nectar taken from hives in the water treated control and in the two Acetamiprid 200 SL treated plots

	-1 DAB	1 DAB	7 DAB	14 DAB
Treatment	BFD+2	BFD +4	BFD +10	BFD +17
Control	<LOQ	<LOQ	<LOQ	<LOQ
Acetamiprid 200 SL Plot A	<LOQ	<LOQ	<LOQ	0.011
Acetamiprid 200 SL Plot B	0.023	0.019	0.085	0.099

LOQ (Limit of quantification): 0.010 mg.kg⁻¹

DAB = Days After the second application of treatments

Residues of acetamiprid in samples of pollen taken one day before the second application of treatments were all found to be below the LOQ of 0.01 mg/kg. One day after the second treatment mean residues of 1.13 and 0.75 mg/kg were found in pollen from the two Acetamiprid 200SL treated plots respectively. Residues in pollen declined rapidly and were lower than the LOQ in one plot and just higher than the LOQ (0.012 mg/kg) in the second plot 7 days after the second treatment.

Table A 143: Mean residues (mg/kg) of acetamiprid in pollen taken from hives in the water treated control and in the two Acetamiprid 200 SL treated plots

	-1 DAB	1 DAB	7 DAB	14 DAB
Treatment	BFD+2	BFD +4	BFD +10	BFD +17
Control	<LOQ	<LOQ	<LOQ	<LOQ
Acetamiprid 200 SL Plot A	<LOQ	1.13	<LOQ	<LOQ
Acetamiprid 200 SL Plot B	<LOQ	0.75	0.012	<LOQ

LOQ (Limit of quantification): 0.010 mg.kg⁻¹

DAB = Days After the second application of treatments

Endpoints

No effects on adult and pupal bee mortality, foraging activity, colony strength, brood amount as well as on the specific evaluation of the detailed bee brood development were observed when MCW-2222 was applied two times at a 7 day interval (the first during pre-flowering at BBCH 57, the second during full flowering of the crop at BBCH 65 with hives present in the orchard but after bee flight) at a rate of 100 g a.s./ha to flowering apple (*Malus domestica*).

Validity criteria

As shown in the following table, all validity criteria were met.

Table A 144: Validity criteria

No validity criteria are given by OECD GD 75 (2007) but by the study plan	Observed in study
before treatment	
The average mortality has to be checked just before the BFD to demonstrate stable background mortality and to show that bees have acclimatized to the test conditions.	The mean mortality before the BFD was low both in the dead bee traps and on the collecting sheets in front of the hives. Criterion was achieved
After treatment	
The control mortality cannot be excessively high after applications (no more than 20 bees per day per hive).	Although the daily mortality was above 20 bees/day/hive at several days after application the study can be considered valid because: <ul style="list-style-type: none"> the mortality values of adults, pupae and larvae in dead bee traps and in

	<p>collecting sheets recorded in both the water treated control and the two Acetamiprid 200 SL treated plots TA and TB did not significantly differ among them throughout the period of observations,</p> <ul style="list-style-type: none"> the recorded mortality of adults, pupae and larvae could be considered at normal level in the area where the study was performed, taking into account the weather conditions and manipulation the hives underwent during the assessment period <p>Criterion was achieved</p>
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Conclusion

In a field study based on OECD GD 75 (2007), honeybees (*Apis mellifera* L.) were exposed to MCW-2222 (a.s. acetamiprid), two times applied at rate of 100 g a.s./ha to apple orchards (*Malus domestica*) in Italy, investigating potential effects on bee mortality, flight and foraging activity, colony status (i.e. colony strength and brood amount). Special attention was laid on the assessment of the detailed bee brood development. The first application was performed before flowering of the apple at BBCH 57, the second application 7 days later during its flowering period at BBCH 65 but after bee flight. Residues levels of acetamiprid were quantified in the spray solutions at each of the two applications, as well as in pollen (obtained via pollen traps), nectar and young larvae on -1 DAB, 1 DAB, 7 DAB and 14 DAB. Residues of the test item in the spray solution samples and in pollen confirmed the exposure of the bees. No residues were found in pollen 14 DAB and in larvae during the entire study.

The results showed, that MCW-2222 (a.s. acetamiprid) didn't have any impact on mortality, flight and foraging activity and bee mortality.

Furthermore, the assessment of the colony strength and brood amount as well as the specific evaluation of the detailed bee brood development showed no impact of the test item on the development on honeybee brood.

A 2.3.2 KCP 10.3.2 Effects on non-target arthropods other than bees

A 2.3.2.1 KCP 10.3.2.1 Standard laboratory testing for non-target arthropods

Comments of zRMS:	<p>The study has been already evaluated and accepted by the zRMS in the course of the first zonal evaluation of CA3573 (formerly MCW-2222) finalised in April 2018. As the test guideline has not changed since that time, re-evaluation of the study following renewal of acetamiprid was not necessary and presented below conclusions were taken from the Core Assessment, Part B, Section 6 of April 2018.</p> <p>The study was performed in line with the respective guideline and met all validity criteria. Following endpoints were agreed:</p> <p>LR₅₀ = 9.13 g a.s./ha ER₅₀ >6.17 g a.s./ha</p>
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A 2.3.2.1.1 KCP 10.3.2.1/01 Laboratory test with *Typhlodromus pyri*

Data point	KCP 10.3.2.1/01
Report	Effects of MCW-2222 on the predatory mite <i>Typhlodromus pyri</i> SCHEUTEN in a laboratory test - Rate-Response-Test (LR50) -, Röhlig, U., 2014
Report No.:	R-33838
Document No.:	
Guideline(s):	Blümel <i>et al.</i> (2000). Laboratory residual contact test with the predatory mite <i>Typhlodromus pyri</i> Scheuten (Acari: Phytoseiidae) for regulatory testing of plant protection products.
Deviations from current test guideline:	No
Previous evaluation	No, not previously submitted
GLP:	Yes, certified laboratory
Acceptability:	Yes, study considered acceptable
Duplication (if vertebrate study)	Not applicable

Executive Summary

A 14 day worst-case laboratory study was carried out to determine the effects of the MCW-2222 on the predatory mite *Typhlodromus pyri*. The LR₅₀ for MCW-2222 was calculated to be 9.13 g a.s./ha. The ER₅₀ for MCW-2222 was estimated to be > 6.17 g a.s./ha in 200 L water/ha.

Materials and methods

Materials

Test item	MCW-2222
Batch #	611-280413-01
Content of active substance	Acetamiprid 200 g/L (nominal); 202.7 g/L (analysed)
Description	Yellowish liquid
Control	Deionised water
Toxic reference	Dimethoate (content: 400 g/L)
Test organism	
Species	<i>Typhlodromus pyri</i> , protonymphs (< 24 hours old)
Source	In-house culture, originally obtained from Dr. Peter Katz, PK-Nützlingszuchten, Industriestraße 38, D-73642 Welzheim
Acclimatisation	The test organisms were and kept in the test arenas with conditions similar to the test conditions

Reanalysis/Expiry date

Study design and methods

Test duration and exposure	14 days of exposure on a dried glass plate prior sprayed with respective test rate.
Experimental dates	18 February to 04 March 2014.
Test concentrations	1.91, 3.43, 6.17, 11.1 and 20 g a.s./ha
Test units	2 glass plates (cover glasses: 50 mm x 22 mm stuck together along their longitudinal sides) with a barrier of sticky material on moistened filter paper on a sponge placed in a plastic tray - Bellaplast (inside dimensions: 165 mm x 120 mm x 60 mm) filled with tap water up to a height of approx. 15 mm
Food	Pollen, <i>Pinus nigra</i> and <i>Betula pendula</i> (each assessment day)
Group size/replicates	100 organisms per treatment; 20 in each of 5 replicates per treatment group
Environmental conditions	
Temperature	23 – 27 °C
Photoperiod	16 h light/8 h dark; 1950 lx
Relative humidity	65 - 72%

Biological observations

The numbers of dead and surviving mites were assessed after 1 and 7 days. The reproduction rate of the

surviving mites was evaluated in a further fertility test. Therefore, the number of offspring (eggs and larvae) was counted on day 9, 11 and 14.

Statistics

The LR₅₀ were calculated by probit analysis. Mortality was analysed for statistical significance using Fishers's Exact Binomial test. Reproductive capacity was analysed for statistical significance using Williams t-test.

Results and discussion

Biological results – mortality

Results are given in the table below.

Table A 145: Pre-imaginal mortality in predatory mites after 7 days of exposure

	Test substance [g a.s./ha]					Control	Toxic reference
	1.91	3.43	6.17	11.1	20.0		
Mortality (%) ¹	7.0	8.0	20.0*	60.0*	96.0*	3.0	81.0*
Corrected mortality (%) ²	4.1	5.2	17.5	58.8	95.9	-	80.4

¹ Mortality after 7 days of exposure to the test item on treated glass plates. The results for mortality in individual treatments were compared to that in the control using FISHER's Exact Binomial test with Bonferroni Correction ($\alpha = 0.05$).

² Mortality corrected for any control treatment deaths using Abbott's formula.

*Statistically significantly different compared to the control

Biological results – reproduction

Results are given in the table below.

Table A 146: Reproduction of female mites during the 7 day egg laying period

Mites	Control	Test substance [g a.s./ha]				
		1.91	3.43	6.17	11.1	20.0
Reproduction rate (mean no. of eggs/female) ¹	6.41	6.45	5.03*	4.67*	-	-
Effect on reproduction (%) ²	-	-0.6	21.5	27.1	-	-

¹ Results for reproduction compared by WILLIAMS t-test ($\alpha > 0.05$).

² Negative values indicate an increase in reproduction

*Statistically significantly different compared to the control

Table A 147: Endpoints

	Endpoints
LR₅₀ (95% CI)	9.13 g a.s./ha 5.72 - 14.56 g a.s./ha
ER₅₀	> 6.17 g a.s./ha

Validity criteria

As shown in the following table, all validity criteria were met.

Table A 148: Validity criteria

Validity criteria according to Blümel <i>et al.</i>	Observed in study
Control mortality < 20%	3%
Mortality in the reference item treatment should be > 50 %	81%
Number of eggs produced per female in the control should be > 4	6.41/female

Conclusion

A 14 day worst-case laboratory study was carried out to determine the effects of the MCW-2222 on the predatory mite *Typhlodromus pyri*. The LR₅₀ for MCW-2222 was calculated to be 9.13 g a.s./ha. The ER₅₀ for MCW-2222 was estimated to be > 6.17 g a.s./ha in 200 L water/ha.

A 2.3.2.1.2 KCP 10.3.2.1/02 Laboratory test with *Aphidius rhopalosiphi*

Comments of zRMS:	<p>The study has been already evaluated and accepted by the zRMS in the course of the first zonal evaluation of CA3573 (formerly MCW-2222) finalised in April 2018. As the test guideline has not changed since that time, re-evaluation of the study following renewal of acetamiprid was not necessary and presented below conclusions were taken from the Core Assessment, Part B, Section 6 of April 2018.</p> <p>The study was performed in line with the respective guideline and met all validity criteria. Following endpoints was agreed:</p> <p>LR₅₀ = 0.0243 g a.s./ha</p> <p>Effects on reproduction were not investigated.</p>
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Data point	KCP 10.3.2.1/02
Report	Effects of MCW-2222 on the parasitic wasp <i>Aphidius rhopalosiphi</i> (DESTEFANI-PEREZ) in a laboratory test - Rate-Response-Test (LR50) - Röhlig, U., 2014
Report No.:	R-33839
Document No.:	
Guideline(s):	Mead-Briggs <i>et al.</i> (2000). A laboratory test for evaluating the effects of plant protection products on the parasitic wasp, <i>Aphidius rhopalosiphi</i> (Hymenoptera, Braconidae)
Previous evaluation	No, not previously submitted
GLP:	Yes, certified laboratory
Acceptability:	Yes, study considered acceptable
Duplication (if vertebrate study)	Not applicable

Executive Summary

A 48 hour laboratory study was carried out to determine the effects of the MCW-2222 on the parasitic wasp

Aphidius rhopalosiphi. The LR₅₀ for MCW-2222 was calculated to 0.02430 g a.s./ha.

Materials and methods

Materials

Test item	MCW-2222
Batch #	611-280413-01
Content of active substance	Acetamiprid 200 g/L (nominal); 202.7 g/L (analysed)
Description	Yellowish liquid
Control	Deionised water
Toxic reference	Dimethoate (content: 400 g/L)
Test organism	
Species	<i>Aphidius rhopalosiphi</i> , less than 48 hours old
Source	In-house culture, originally obtained from Katz Biotech AG, an der Birkenpfuhlheide 10, 15837 Baruth, Germany
Rearing	Pupae of the parasitic wasp (i.e. aphid mummies) were placed in glass bottles for hatching. A cotton wool pad soaked with aqueous fructose solution as food supply was fixed at one opening of the hatching bottle. The wasps were not fed, but only provided with water for approx. 18 hours prior to exposure initiation.

Study design and methods

Test duration and exposure	48 hours of exposure on a dried glass plate prior sprayed with respective test rate.
Experimental treatments	
Experimental dates	17 - 19 February 2014
Test rates	0.0194, 0.0427, 0.0939, 0.207, 0.455, 1 mL test item/ha corresponding to 0.00388, 0.00854, 0.0188, 0.0413, 0.0909 and 0.2 g a.s./ha
Test units	2 square glass plates (13 cm x 13 cm), held apart by an aluminium frame (13 cm x 13 cm x 1.4 cm) with gauze covered holes for forced air ventilation (blowing air; flow rate: 2.5 L/min)
Group size/replicates	40 organisms (28 females, 12 males) per treatment; 10 in each of 4 replicates per treatment group
Environmental conditions	
Temperature	19-22 °C
Photoperiod	light / dark 16 / 8 h, 1020 lx
Relative humidity	67 – 72%

Biological observations

The behaviour of each wasp in each chamber was recorded after 2, 24 and 48 hours after treatment. Observations included the numbers of wasps alive, affected, moribund or dead.

Statistics

The 48 h EC₅₀ were calculated by probit analysis. Mortality was analysed for statistical significance using Fishers's Exact Binomial test.

Results and discussion

Biological results

Biological results are given in the table below.

Table A 149: Mortality of *Aphidius rhopalosiphi* after 48 h of exposure to MCW-2222

	Control	Test rates [g a.s./ha]						Toxic standard
		0.00388	0.00854	0.0188	0.0413	0.0909	0.2	
Alive (individuals)	40	40	34	21	9	7	1	0
Moribund (individuals)	0	0	0	0	0	0	0	0
Dead (individuals)	0	0	6	19	31	33	39	40
Mortality (%) ¹	0	0	15*	47.5*	77.5*	82.5*	97.5*	100

¹ Mortality after 48 hours of exposure to the test item on treated glass plates. The results for mortality in individual treatments were compared to that in the control using FISHER's Exact Binomial test with Bonferroni Correction ($\alpha = 0.05$).

* Statistically significantly different compared to the control

Table A 150: Acute toxicity of test item to *Aphidius rhopalosiphi*

	Endpoints
LR₅₀ 48 h	0.02430 g a.s./ha
(95% CI)	0.01966-0.03005 g a.s./ha

Validity criteria

As shown in the following table, all validity criteria were met.

Table A 151: Validity criteria

Validity criteria according to Mead-Briggs <i>et al.</i>	Observed in study
Control mortality < 13%	0%
Corrected mortality in the reference item treatment should be > 50 % and preferably < 100 % (48 hours)	100%

Conclusion

A 48 hour laboratory study was carried out to determine the effects of the MCW-2222 on the parasitic wasp *Aphidius rhopalosiphi*. The LR₅₀ for MCW-2222 was calculated to 0.02430 g a.s./ha.

A 2.3.2.2 KCP 10.3.2.2 Extended laboratory studies

A 2.3.2.2.1 KCP 10.3.2.2/01 Extended laboratory test with *Typhlodromus pyri*

Comments of zRMS:	<p>The study has been already evaluated and accepted by the zRMS in the course of the first zonal evaluation of CA3573 (formerly MCW-2222) finalised in April 2018. As the test guideline has not changed since that time, re-evaluation of the study following renewal of acetamiprid was not necessary and presented below conclusions were taken from the Core Assessment, Part B, Section 6 of April 2018.</p> <p>The study was performed in line with the respective guideline and met all validity criteria. Following endpoints were agreed:</p> <p>LR₅₀ = 31.9 g a.s./ha ER₅₀ >12.5 g a.s./ha</p>
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Data point	KCP 10.3.2.2/01
Report	Effects of MCW-2222 on the predatory mite <i>Typhlodromus pyri</i> SCHEUTEN in an extended laboratory test - Rate-Response-Test (LR50) -, Röhlig, U., 2014
Report No.:	R-34780
Document No.:	
Guideline(s):	Blümel <i>et al.</i> (2000). Laboratory residual contact test with the predatory mite <i>Typhlodromus pyri</i> Scheuten (Acari: Phytoseiidae) for regulatory testing of

	plant protection products.
Previous evaluation	No, not previously submitted
GLP:	Yes, certified laboratory
Acceptability:	Yes, study considered acceptable
Duplication (if vertebrate study)	Not applicable

Executive Summary

An extended laboratory study was carried out to determine the effects of the test item MCW-2222 on the predatory mite *Typhlodromus pyri*. Based on nominal concentrations, the LR₅₀ value for freshly dried spray residues of MCW-2222 to *Typhlodromus pyri* mites on leaf discs taken from French bean plants was calculated to be 31.9 g/ha. The ER₅₀ for MCW-2222 was estimated to be > 12.5 g a.s./ha.

Materials and methods

Materials

Test item	MCW-2222
Batch #	611-280413-01
Content of active substance	Acetamiprid 200 g/L (nominal); 202.7 g/L (analysed)
Description	Yellowish liquid
Control	Deionised water
Toxic reference	Dimethoate (content: 400 g/L)
Test organism	
Species	<i>Typhlodromus pyri</i> , protonymphs (< 24 hours old)
Source	In-house culture, originally obtained from Dr. Peter Katz, PK-Nützlingszuchten, Industriestraße 38, D-73642 Welzheim
Acclimatisation	The test organisms were and kept in the test arenas with conditions similar to the test conditions

Study design and methods

Test duration and exposure	14 days, 7 days of exposure on French bean leaves prior sprayed with respective test rates and a subsequent reproduction phase of 7 days
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Experimental treatments

Experimental dates	13 - 27 May 2014
Test rates	6.25, 12.5, 25, 50 and 100 g a.s./ha
Test units	Bean leaf disc (<i>Phaseolus vulgaris</i> , variety: "Jutta", 4 cm diameter) surrounded with insect glue (TEM MEN Insektenleim) on cotton wool moistened with tap water in a Petri dish (9 cm diameter)
Food	Pollen, <i>Pinus nigra</i> and <i>Betula pendula</i> (each assessment day)
Group size/replicates	100 organisms per treatment; 20 in each of 5 replicates per treatment group
Environmental conditions	
Temperature	23 – 26 °C
Photoperiod	Photoperiod: 16 h light, 8 h dark; 2130 lx
Relative humidity	65 - 72%

Biological observations

On day 3, 7, 9, 11 and 14 after the application, the number of surviving predatory mites were counted (from day 7 onward differentiated according to the sex), dead mites were recorded and removed; mites that were missing or trapped were separately recorded. The males were differentiated from the females by their smaller and flatter phenotype. At each observation time the condition of the mites was recorded as: alive, dead and escaped. The number of laid eggs and hatched juveniles present was determined on days 9, 11 and 14, these were removed after counting. Any eggs found on day 7 were removed and not counted in the reproduction assessment.

Statistics

The LR₅₀ were calculated by probit analysis. Mortality was analysed for statistical significance using Fishers's Exact Binomial test. Reproductive capacity was analysed for statistical significance using Williams t-test.

Results and discussion

Biological results

Results are given in the table below.

Table A 152: Mortality and effect on the reproductive capacity of *Typhlodromus pyri* following Haseven days exposure to MCW-2222

Treatment	Rate ^a [g a.s./ha]	Mortality ^b [%]	Corrected Mortality ^c [%]	Reproduction ^d [eggs/female]	Effect on reproduction ^e [%]
Control	0	1.0	-	6.38	-
MCW-2222	6.25	1.0	0	6.56	-2.8
MCW-2222	12.5	3.0	2.0	6.14	3.8
MCW-2222	25.0	54.0*	53.5	-	-
MCW-2222	50.0	65.0*	67.7	-	-
MCW-2222	100.0	86.0*	85.9	-	-
Toxic standard	30 mL test item/ha	82.0	81.8	-	-

^a Application rate in 200 L water/ha

^b 7 day mortality rate (Fisher Exact Binomial Test with BONFERRONI correction ($\alpha = 0.05$))

^c Corrected mortality according to Abbott (1925)

^d 14 day reproduction rate (WILLIAMS-t-test ($\alpha = 0.05$))

^e Calculated on the exact raw data; negative values mean increased reproduction compared to control

*Statistically significantly different compared to the control

Table A 153: Endpoints

	Endpoints
LR ₅₀ (95% CI)	31.9 g a.s./ha 16.3 – 62.4 g a.s./ha
ER ₅₀	> 12.5 g a.s./ha

Validity criteria

As shown in the following table, all validity criteria were met.

Table A 154: Validity criteria

Validity criteria according to Blümel <i>et al.</i> (2000)	Observed in study
Control mortality < 20%	1%
Mortality in the reference item treatment should be > 50 %	81%
Number of eggs produced per female in the control should be > 4	6.38/female

Conclusion

In this extended laboratory study, based on nominal concentrations, the LR₅₀ value for freshly dried spray residues of MCW-2222 to *Typhlodromus pyri* mites on leaf discs taken from French bean plants was calculated to be 31.9 g/ha (95% confidence limit: 16.3-62.4 g a.s./ha) in 200 L water/ha. The ER₅₀ for MCW-2222 was estimated to be >12.5 g a.s./ha.

A 2.3.2.2.2 KCP 10.3.2.2/02 Extended laboratory test with *Aphidius rhopalosiph*

Comments of zRMS:	The study has been already evaluated and accepted by the zRMS in the course of the first zonal evaluation of CA3573 (formerly MCW-2222) finalised in April 2018. As the test guideline has not changed since that time, re-evaluation of the study following renewal of acetamiprid was not necessary and presented below conclusions were taken from the Core Assessment, Part B, Section 6 of April 2018.
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	<p>The study was performed in line with the respective guideline and met all validity criteria. Following endpoints were agreed:</p> <p>LR₅₀ = 0.555 mL product/ha (corresponding to 0.111 g a.s./ha) ER₅₀ > 0.502 mL product/ha (corresponding to 0.100 g a.s./ha)</p>
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Data point	KCP 10.3.2.2/02
Report	MCW-2222 – A rate-response extended laboratory bioassay of the effects of fresh residues on the parasitic wasp <i>Aphidius rhopalosiphi</i> (Hymenoptera, Braconidae), Stevens, J., 2015
Report No.:	R-35026
Document No.:	
Guideline(s):	Mead-Briggs <i>et al.</i> 2009
Previous evaluation	No, not previously submitted
GLP:	Yes, certified laboratory
Acceptability:	Yes, study considered acceptable
Duplication (if vertebrate study)	Not applicable

Executive Summary

In an extended laboratory test to determine the effects of MCW-2222 on the parasitoid wasp *Aphidius rhopalosiphi*, the 48-h median lethal rate (LR₅₀) was 0.555 mL test item/ha. Based on statistical comparison with the control, the no-observed-effect rate (NOER) with respect to wasp survival was 0.126 mL test item/ha (corresponding to 0.025 g a.s./ha). In terms of effects on the reproductive performance of surviving wasps, the median effect rate (ER₅₀) for MCW-2222 was > 0.502 corresponding to 0.1 g a.s./ha) mL test item/ha corresponding to 0.1 g a.s./ha) and the NOER was 0.502 mL test item/ha .

Materials and methods

Materials

Test item	MCW-2222
Batch #	659-030314-01
Content of active substance	Acetamiprid 200 g/L (nominal); 199.2 g/L (analysed)
Description	Yellowish liquid
Control	Purified water
Toxic reference	Dimethoate (content: 400 g/L)
Test organism	
Species	<i>Aphidius rhopalosiphi</i> , less than 48 hours old
Source	Katz Biotech AG, an der Birkenpfehlheide 10, 15837 Baruth, Germany

Study design and methods

Test duration and exposure	<p>14 days, exposure phase (48 h) followed by a reproduction phase (10 days).</p> <p>Exposure phase: wasps were introduced to test arenas where floor and ceiling consisted of French bean leaves sprayed at the respective rate. Reproduction phase: surviving females from the respective treatment group were introduced into a cylinder containing approx. 15 untreated barley seedling infested with > 100 adult and nymphal aphids (<i>M. dirhodum</i> and <i>R. padi</i>)</p>
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Experimental dates	01 October - 17 November 2014
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Test rates	2.008, 1.004, 0.502, 0.251 and 0.126 mL test item/ha, corresponding to 0.4, 0.2, 0.1, 0.05 and 0.025 g a.s./ha
Test units	Exposure phase: Test arenas comprised circular frames made from clear acrylic tubing (these were of approx. 5.1 cm internal diameter and 15 mm deep). Reproduction phase: Acrylic cylinder (about 9 cm Ø, 20 cm high) with potted barley (mortality phase) or wheat (reproduction phase) plants and covered at the top of the cylinder nylon netting
Group size/replicates	Mortality phase: 40 females per treatment; 10 in each of 4 replicates per treatment group. Reproduction phase: 15 females per treatment; 1 replicate in 15 replicates/treatment group
Environmental conditions	
Temperature	21 -22 °C
Photoperiod/Intensity	light / dark 16 / 8 h, 1664 lx
Relative humidity	65 – 73%

Biological observations

Assessments of mortality were made over 48 hours. To determine any sub-lethal effects on the reproductive capacity of the surviving wasps, assessments were then carried out for the control and for the three highest treatment rates of the test item that had resulted in $\leq 60\%$ corrected mortality. Fifteen female wasps from each treatment were confined individually for 24 hours over untreated barley plants that had previously been infested with cereal aphids (*Metopolophium dirhodum* and *Rhopalosiphum padi*). The wasps were then removed and the plants left for a further 10 days before the number of ‘mummies’ (parasitised aphids containing wasp pupae) that had developed was recorded.

Statistics

The 48 hour LR₅₀ were calculated by probit analysis. The mortality was analysed for statistical significance using Fishers’s Exact Binomial test. The reproductive capacity was analysed for statistical significance using the Dunnetts t-test after passing a Shapiro-Wilk’s test on normal distribution and Levene’s test procedure on variance homogeneity.

Results and discussion

Biological results

Biological results are given in the table below.

Table A 155: Mortality of *Aphidius rhopalosiphi* after exposure to MCW-2222

Treatment	Rate [mL test item/ha]	Rate [g a.s./ha]	Mortality ^a [%]	Corrected mortality ^b [%]
Control	0	0	5.0	-
MCW-2222	0.126	0.025	12.5	7.9
MCW-2222	0.251	0.05	32.5*	28.9
MCW-2222	0.502	0.1	45.0*	42.1
MCW-2222	1.004	0.2	72.5*	71.1
MCW-2222	2.008	0.4	87.5*	86.8

^a Individual treatments were compared to the control by Fisher’s Exact Test ($\alpha = 0.05$) and an asterisk (*) indicates where they differed significantly.

^b Corrected mortality according to Abbott.

Table A 156: Reproduction of *Aphidius rhopalosiphi* after exposure to MCW-2222

Treatment	Rate [mL test item/ha]	Rate [g a.s./ha]	Reproduction [mean number of mummies/female] ^a	Effects on reproduction [%] ^b
Control	0	0	14.1	-
MCW-2222	0.126	0.025	17.1	-21.3
MCW-2222	0.251	0.05	15.0	-6.6
MCW-2222	0.502	0.1	14.3	-1.9
MCW-2222	1.004	0.2	n.d.	-
MCW-2222	2.008	0.4	n.d.	-

^a Reproduction: mean number of parasitised aphids (mummies)/surviving female. The results for the test item

^b Change in mean number of mummies per female, relative to control. A positive value indicates a decrease and a negative value indicates an increase relative to the control.

Treatments were compared to the control by one-way ANOVA and Dunnett's t-test ($\alpha = 0.05$), but there were no significant differences.

n.d. not determined (corrected mortality > 50 %, compared to the control)

The reference item caused a mortality of 100 % of exposed wasps, resulting in a corrected mortality of 100 %.

Table A 157: Acute toxicity of test item to *Aphidius rhopalosiphi*

	Endpoints
LR₅₀ 48 h (95% CI)	0.555 mL test item/ha 0.408-0.733 mL test item/ha
ER₅₀	> 0.502 mL test item/ha (effects)
NOER	0.126 mL test item/ha, corresponding to 0.025 g a.s./ha (mortality) 0.502 mL test item/ha, corresponding to 0.1 g a.s./ha (reproduction)

Validity criteria

As shown in the following table, all validity criteria were met.

Table A 158: Validity criteria

Validity criteria according Mead-Briggs <i>et al.</i> (2009)	Observed in study
Control mortality (48 hours) < 10%	5%
Reproduction in the control group should be ≥ 5 mummies per female	14.1
No more than 2 wasps in control producing 0 mummies	All control replicates produced mummies
Corrected mortality (48 hours) in the reference item treatment should be > 50 % and preferably < 100 % (48 hours)	100%

Conclusion

In an extended laboratory test to determine the effects of MCW-2222 on the parasitoid wasp *Aphidius rhopalosiphi*, the 48 hour median lethal rate (LR₅₀) was 0.555 mL test item/ha. Based on statistical comparison with the control, the no-observed-effect rate (NOER) with respect to wasp survival was 0.126 mL test item/ha (corresponding to 0.025 g a.s./ha). In terms of effects on the reproductive performance of surviving wasps, the median effect rate (ER₅₀) for MCW-2222 was > 0.502 mL test item/ha (corresponding to 0.1 g a.s./ha) and the NOER was 0.502 mL test item/ha (corresponding to 0.1 g a.s./ha).

A 2.3.2.2.3 KCP 10.3.2.2/03 Extended laboratory test with *Aphidius rhopalosiphi*

Comments of zRMS:	<p>The study has been already evaluated and accepted by the zRMS in the course of the first zonal evaluation of CA3573 (formerly MCW-2222) finalised in April 2018. As the test guideline has not changed since that time, re-evaluation of the study following renewal of acetamiprid was not necessary and presented below conclusions were taken from the Core Assessment, Part B, Section 6 of April 2018.</p> <p>The study was performed in line with the respective guideline and met all validity criteria. Following endpoints were agreed:</p> <p>LR₅₀ = 3.56 g a.s./ha ER₅₀ could not be determined (effects >50% at 0.64 g a.s./ha, the lowest rate tested)</p>
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Data point	KCP 10.3.2.2/03
Report	Effects of MCW-2222 on the parasitic wasp <i>Aphidius rhopalosiphi</i> (DESTEFANI-PEREZ) in an extended laboratory test - Rate-Response-Test (LR50) -, Röhlig, U., 2014
Report No.:	R-33839A
Document No.:	14 10 48 037 A
Guideline(s):	IOBC (Mead-Briggs <i>et al.</i> 2009)
Previous evaluation	No, not previously submitted
GLP:	Yes, certified laboratory
Acceptability:	Yes, study considered acceptable
Duplication (if vertebrate study)	Not applicable

Executive Summary

An extended laboratory study on potted barley plants was carried out to determine the effects of the test item MCW-2222 on *Aphidius rhopalosiphi*. Based on nominal concentrations, the LR₅₀ for *Aphidius rhopalosiphi* was estimated to be 3.56 g a.s./ha. Statistically significant effects on the reproductive capacity of *Aphidius rhopalosiphi* were determined at treatment rates up to and including 3.1 g a.s./ha.

Materials and methods

Materials

Test item	MCW-2222
Batch #	611-280413-01
Content of active substance	Acetamiprid 200 g/L (nominal); 202.7 g/L (analysed)
Description	Yellowish liquid
Control	Deionised water
Toxic reference	Dimethoate (content: 400 g/L)
Test organism	
Species	<i>Aphidius rhopalosiphi</i> , less than 48 hours old
Source	Katz Biotech AG, an der Birkenpfehlheide 10, 15837 Baruth, Germany
Rearing	Pupae of the parasitic wasp (i.e. aphid mummies) were placed in glass bottles for hatching. A cotton wool pad soaked with aqueous fructose solution as food supply was fixed at one opening of the hatching bottle. The wasps were not fed, but only provided with water for approx. 18 hours prior to exposure initiation.

Study design and methods

Test duration and exposure	14 days, exposure phase (48 h) followed by a reproduction phase (12 days). Exposure phase: wasps were introduced to acrylic glass cylinders containing a barley plants previously sprayed at respective test rates. Reproduction phase: surviving females from the respective treatment group were introduced into a cylinder containing an untreated potted wheat plant infested with > 100 adult and nymphal aphids (<i>Rhopalosiphum padi</i>)
Experimental dates	07 - 21 April 2014
Test rates	0.64, 1.4, 3.1, 6.8 and 15 g a.s./ha
Test units	Acrylic cylinder (about 11 cm Ø, 20 cm high) with potted barley (mortality phase) or wheat (reproduction phase) plants and covered at the top of the cylinder with gauze.
Group size/replicates	Mortality phase: 30 females per treatment; 5 in each of 6 replicates per treatment group. Reproduction phase: 15 females per treatment; 1 replicate in 15 replicates/treatment group.
Environmental conditions	
Temperature	19 – 22 °C
Photoperiod/Intensity	light / dark 16 / 8 h, Mortality phase: 1020 lx Paratisation phase: 4210 lx Reproduction phase: 6250 lx
Relative humidity	67 – 72%

Biological observations

Effects on reproduction were assessed by the number of parasitised aphids (mummies) produced per female. Endpoints of the study were the mortality (including calculation of the LR₅₀, if possible) and additionally effects on reproduction. Mortality assessments were carried out 2, 24 and 48 hours after exposure of the wasps. At 48 hours, surviving wasps (15 females per treatment) were removed and their reproductive capacity was assessed by confining them individually over untreated wheat plants infested with adult and nymphal aphids (*Rhopalosiphum padi*). Assessment of reproductive capacity i.e. number of mummies per female was made for the control and all test item rates in which the corrected mortality was ≤ 50%. (1 assessment, 14 days after application).

Statistics

The 48 h LR₅₀ were calculated by probit analysis. The mortality was analysed for statistical significance using Fishers's Exact Binomial test. The repellence (position) was analysed for statistical significance using the Williams-t-test following Shapiro-Wilk's test on normal distribution and Bartlett's test procedure on variance homogeneity.

The reproductive capacity was analysed for statistical significance using the Welch-t-test, following Shapiro-Wilk's test on normal distribution and Levene's test procedure on variance homogeneity.

Results and discussion

Biological results

Biological results are given in the table below.

Table A 159: Mortality of *Aphidius rhopalosiphi* after exposure to MCW-2222

Treatment	Rate ^a [g a.s./ha]	Mortality ^b [%]	Corrected mortality ^c [%]
Control	0	3.3	-
MCW-2222	0.64	0	-3.4
MCW-2222	1.4	6.7	3.4
MCW-2222	3.1	36.7*	34.5
MCW-2222	6.8	93.3*	93.1
MCW-2222	15	100*	100

^a) Application rate in 400 L water/ha.

^b) Mortality after 48 hours of exposure to the test item on treated barley plants. The results for mortality in individual treatments were compared to that in the control using Fisher's Exact Binomial test with Bonferroni Correction ($\alpha = 0.05$).

^c) Corrected mortality according to Abbott (1925).

* statistically significantly different compared to the control.

Table A 160: Reproduction of *Aphidius rhopalosiphi* after exposure to MCW-2222

Treatment	Rate ^a [g a.s./ha]	Reproduction [mean number of mummies/female] ^b	Effects on reproduction [%] ^c
Control	0	20.9	-
MCW-2222	0.64	9.9*	52.6
MCW-2222	1.4	4.5*	78.5
MCW-2222	3.1	2.5*	88.5
MCW-2222	6.8	n.d.	-
MCW-2222	15	n.d.	-

^a) Application rate in 400 L water/ha.

^b) Reproduction: mean number of parasitised aphids (mummies)/surviving female. The results for the test item

^c) Change in mean number of mummies per female, relative to control. A positive value indicates a decrease and a negative value indicates an increase relative to the control.

* statistically significantly different compared to the control.

n.d. not determined (corrected mortality > 50 %, compared to the control)

The reference item caused a mortality of 100 % of exposed wasps, resulting in a corrected mortality of 100 %.

Table A 161: Acute toxicity of test item to *Aphidius rhopalosiphi*

	Endpoints
LR₅₀ 48 h (95% CI)	3.56 g a.s./ha 3.02-4.20 g a.s./ha

Validity criteria

As shown in the following table, all validity criteria were met.

Table A 162: Validity criteria

Validity criteria according Mead-Briggs <i>et al.</i> (2009)	Observed in study
Control mortality (48 hours) < 10%	3.3%
Reproduction in the control group should be ≥ 5 mummies per female	20.9
No more than 2 wasps in control producing 0 mummies	1 control replicate produced 0 mummies
Corrected mortality (48 hours) in the reference item treatment should be > 50 % and preferably < 100 % (48 hours)	100%

Conclusion

An extended laboratory study on potted barley plants was carried out to determine the effects of the test item MCW-2222 on *Aphidius rhopalosiphi*. Based on nominal concentrations, the LR₅₀ for *Aphidius rhopalosiphi* was estimated to be 3.56 g a.s./ha. Statistically significant effects on the reproductive capacity of *Aphidius rhopalosiphi* were determined at treatment rates up to and including 3.1 g a.s./ha.

A 2.3.2.2.4 KCP 10.3.2.2/04 Extended laboratory test with *Chrysoperla carnea*

Comments of zRMS:	<p>The study has been already evaluated and accepted by the zRMS in the course of the first zonal evaluation of CA3573 (formerly MCW-2222) finalised in April 2018. As the test guideline has not changed since that time, re-evaluation of the study following renewal of acetamiprid was not necessary and presented below conclusions were taken from the Core Assessment, Part B, Section 6 of April 2018.</p> <p>The study was performed in line with the respective guideline and met all validity criteria. Following endpoints were agreed:</p> <p>LR₅₀ = 106.0 g a.s./ha ER₅₀ >116.0 g a.s./ha</p>
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Data point	KCP 10.3.2.2/04
Report	Effects of MCW-2222 on the green lacewing <i>Chrysoperla carnea</i> STEPH. under extended laboratory conditions - Rate-Response-Test (LR50) -, Röhlig, U., 2014
Report No.:	R-34781
Document No.:	
Guideline(s):	IOCB (Vogt <i>et al.</i> , 2000)
Previous evaluation	No, not previously submitted
GLP:	Yes, certified laboratory
Acceptability:	Yes, study considered acceptable
Duplication (if vertebrate study)	Not applicable

Executive Summary

An extended laboratory study was carried out to determine the effects of the test item MCW-2222 on the green lacewing *Chrysoperla carnea*. Based on nominal concentrations, the LR₅₀ value for freshly dried spray residues of MCW-2222 to *Chrysoperla carnea* on leaf discs taken from French bean plants was calculated to be 106 g a.s./ha (95% confidence limit: 89-125 g a.s./ha) in 200 L water/ha. The ER₅₀ for MCW-2222 was estimated to be >116 g a.s./ha in 200 L water/ha.

Materials and methods

Materials

Test item	MCW-2222
Batch #	611-280413-01
Content of active substance	Acetamiprid 200 g/L (nominal); 202.7 g/L (analysed)
Description	Yellowish liquid
Control	Deionised water
Toxic reference	Dimethoate (content: 400 g/L)
Test organism	
Species	<i>Chrysoperla carnea</i> , larvae (2 – 3 days old)
Source	In-house culture, originally obtained from Neudorff GmbH, 31860 Emmerthal, Germany
Rearing	Before test initiation the eggs from a single cohort were incubated in a Bellaplast cage (inside dimensions about 16.5 cm x 12 cm x 6 cm) with a small stripe of a thin layer of Fluon on the walls (to prevent escape of the hatched larvae); the hatched larvae were fed ad libitum with <i>S. cerealella</i> before test initiation

Study design and methods

Test duration and exposure	Up to 20 days depending on date of pupation and hatching of adults. Exposure: until 5 days after pupation (actually 10-13 days)f
Experimental treatments	Lacewing larvae were exposed to the test item on bean leaves. Exposure lasted until pupae (at least 5 days after formation) were transferred to oviposition units for development of adults.
Experimental dates	12 June to 18 July 2008
Test rates	11, 24, 53, 116 and 255 g a.s./ha
Test units	Bean leafs <i>Phaseolus vulgaris</i> (variety: “Jutta”) Glass cylinder (4 cm Ø, 4 cm high) with gauze cover; with a treated bean leaf on moistened filter paper as bottom, fixed to a glass plate and an acrylic plate (both 25 cm x 25 cm and untreated)
Food	Larvae: ad libitum 3 times a week, <i>Sitotroga cerealella</i> eggs Adults: each day of assessment, synthetic diet (according to the guideline) placed in small amounts on the inner wall
Group size/replicates	40 organisms per treatment; 1 larvae in each of 40 replicates per treatment group
Environmental conditions	
Temperature	23 – 27 °C
Photoperiod	16 h light, 8 h dark; 1260 lx
Relative humidity	67 – 75%

Biological observations

The condition of the larvae during the exposure phase was assessed daily until they pupated. Observations included abnormal behaviour, mortality and pupation. The number of lacewings that had emerged successfully was also recorded every 2-3 days.

The reproductive performance of the lacewings was assessed for the test groups, in which a sufficient number of test organisms survived the exposure phase and successfully completed their metamorphosis. The reproduction phase started with adults from a treatment hatched within a period of up to seven days and without deformations. These adults were sexed and put together in oviposition units. The oviposition started about one week after the first egg laying had been observed. For assessment of sublethal effects two egg samples were taken within one week. Each sample covered an egg laying period of 24 hours. Eggs, which were laid on the walls of the oviposition unit, were counted as well. The number of eggs was counted after renewal of the gauze. After 2-3 days of incubation of the eggs on the gauze in a hatching box, The larvae hatched from the eggs on the gauze only were counted after 4 days.

Statistics

The LR₅₀ were calculated by probit analysis. Mortality was analysed for statistical significance using Fishers’s Exact Binomial test.

Results and discussion

Biological results

Results on mortality and reproduction are given in the tables below.

Table A 163: Mortality of *Chrysoperla carnea* after exposure to MCW-2222

Treatment	Rate ^a [g a.s./ha]	Pre-imaginal mortality ^b [%]	Corrected pre-imaginal mortality ^c [%]
Control	0	7.5	-
MCW-2222	11	0	-8.1
MCW-2222	24	5.0	-2.7
MCW-2222	53	25.0	18.9
MCW-2222	116	55.0*	51.4
MCW-2222	255	92.5*	91.9
Toxic standard Dimethoate EC 400	40 mL/ha	72.5	70.3

^a Application rate in 200 L water/ha

^b FISHER's Exact Binomial test with Bonferroni Correction ($\alpha = 0.05$)

^c Corrected pre-imaginal mortality according to Abott (1925)

*Statistically significantly different compared to the control

Table A 164: Reproduction of *Chrysoperla carnea* after exposure to MCW-2222

Treatment	Rate ^a [g a.s./ha]	Reproduction [eggs/female/day] ^b	Hatching rate [%]
Control	0	19.2	74.4
MCW-2222	11	19.1	74.5
MCW-2222	24	18.4	74.4
MCW-2222	53	18.8	74.2.
MCW-2222	116	19.5	74.9
MCW-2222	255	-	-

^a Application rate in 200 L water/ha

^b Based on all eggs laid on the fibrous tissue sheet

Table A 165: Endpoints

	Endpoints
LR₅₀ (95% CI)	106 g a.s./ha 89 - 125 g a.s./ha
ER₅₀	> 116 g a.s./ha

Validity criteria

As shown in the following table, all validity criteria were met.

Table A 166: Validity criteria

Validity criteria according to Vogt <i>et al.</i> (2000)	Observed in study
Control pre-imaginal mortality should be $\leq 20\%$	7.5%
Control mean egg production should be ≥ 15 eggs/female/day	19.2
Control mean viability (hatching rate) of the eggs should be $\geq 70\%$	74.4
Mortality in toxic reference should be $\geq 50\%$	70.3%

Conclusion

An extended laboratory study was carried out to determine the effects of the test item MCW-2222 on the green lacewing *Chrysoperla carnea*. Based on nominal concentrations, the LR₅₀ value for freshly dried spray residues of MCW-2222 to *Chrysoperla carnea* on leaf discs taken from French bean plants was calculated to be 106 g a.s./ha (95% confidence limit: 89-125 g a.s./ha) in 200 L water/ha. The ER₅₀ for MCW-2222 was estimated to be >116 g a.s./ha in 200 L water/ha.

A 2.3.2.2.5 KCP 10.3.2.2/05 Extended laboratory test with *Coccinella septempunctata*

Comments of zRMS:	<p>The study has been already evaluated and accepted by the zRMS in the course of the first zonal evaluation of CA3573 (formerly MCW-2222) finalised in April 2018. As the test guideline has not changed since that time, re-evaluation of the study following renewal of acetamiprid was not necessary and presented below conclusions were taken from the Core Assessment, Part B, Section 6 of April 2018.</p> <p>The study was performed in line with the respective guideline and met all validity criteria. Following endpoints were agreed:</p> <p>LR₅₀ = 22.1 g a.s./ha ER₅₀ >20.7 g a.s./ha</p>
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Data point	KCP 10.3.2.2/05
Report	Effects of MCW-2222 on the ladybird <i>Coccinella septempunctata</i> L. in an extended laboratory test - Rate-Response-Test (LR50) -, Röhlig, U., 2014
Report No.:	R-34782
Document No.:	
Guideline(s):	IOBC (Schmuck <i>et al.</i> 2000) modified for the exposure on natural substrate (extended laboratory test)
Previous evaluation	No, not previously submitted
GLP:	Yes, certified laboratory
Acceptability:	Yes, study considered acceptable
Duplication (if vertebrate study)	Not applicable

Executive Summary

An extended laboratory study was carried out to determine the effects of the test item MCW-2222 on the ladybird *Coccinella septempunctata* (Coleoptera: Coccinellidae). For determination of the mortality larvae were exposed to fresh, dry residues of MCW-2222 on detached bean leaves (*Phaseolus vulgaris*). Survival of the larvae and pupae (pre-imaginal mortality) was determined. Effects on reproduction were assessed by the number of eggs produced per female and the hatching rate. The LR₅₀ for *Coccinella septempunctata* was estimated to be 22.1 g a.s./ha in 200 L water/ha. No adverse effects on mortality of *Coccinella septempunctata* occurred, when MCW-2222 was applied up to and including an application rate of 9.4 g a.s./ha in 200 L water/ha. No adverse effects on reproduction of *Coccinella septempunctata* occurred, when MCW-2222 was applied up to and including an application rate of 20.7 g a.s./ha in 200 L water/ha.

Materials and methods

Materials

Test item	MCW-2222
Batch #	611-280413-01
Content of active substance	Acetamiprid 200 g/L (nominal); 202.7 g/L (analysed)
Description	Yellowish liquid
Control	Deionised water
Toxic reference	Dimethoate (content: 400 g/L)
Test organism	
Species	Larvae, 3-5 days old of seven pointed ladybird <i>Coccinella septempunctata</i> L. (Coleoptera: Coccinellidae)
Source	Katz Biotech AG, an der Birkenpfuhlheide 10, 15837 Baruth, Germany
Food	Aphids <i>ad libitum</i> (daily, except Saturdays)

Study design and methods

Test duration and exposure

For determination of the mortality ladybird larvae were exposed to fresh, dry residues of MCW-2222 on detached bean leaves (*Phaseolus vulgaris*) for up to 18 days. Survival of the larvae and pupae (pre-imaginal mortality) was determined. Effects on reproduction (oviposition and fertility) were assessed by the number of eggs produced per female and the hatching rate for a duration of up to 56 days after application.

Experimental dates

02 July - 26 August 2014

Test rates

4.3, 9.4, 20.7, 45.5 and 100 g a.s./ha (nominally equivalent to 21.3, 47, 103, 227 and 500 mL test item/ha) with a water volume corresponding to 200 L/ha.

Test units

Glass cylinder (4 cm Ø, 4 cm high) with gauze cover; with a treated bean leaf on moistened filter paper as bottom, fixed to a glass plate and an acrylic plate (both 25 cm x 25 cm and untreated)

Group size/replicates

Mortality phase: 40 larvae per treatment; 1 in each of 40 replicates per treatment group.

Reproduction phase: ≥ 23 individuals (males and females) in 1 replicate/treatment group.

Environmental conditions

Temperature

23 – 27 °C

Photoperiod/Intensity

light / dark 16 / 8 h, 2030 lx

Relative humidity

60 – 74%

Biological observations

Mortality assessments were carried out on a daily basis until hatching of the adult beetles. The reproduction was assessed on a daily basis over 2 weeks and additionally 4 days for larval hatch.

Statistics

The 48 h LR₅₀ was calculated by probit analysis. The mortality was analysed for statistical significance using Fishers's Exact Binomial test.

Results and discussion

Biological results

Biological results are given in the table below.

Table A 167: Mortality of *Coccinella septempunctata* after exposure to MCW-2222

Treatment	Rate ^a [g a.s./ha]	Mortality ^b [%]	Corrected mortality ^c [%]
Control	0	15.0	-
MCW-2222	4.3	17.5	2.9
MCW-2222	9.4	25.0	11.8
MCW-2222	20.7	42.5*	32.4
MCW-2222	45.5	92.5*	91.2
MCW-2222	100	100*	100

^a Application rate in 200 L water/ha.

^b Mortality: percentage of individuals which did not reach maturity. The results for mortality in individual test item treatments were compared to that in the control using Fisher's Exact Binomial test with Bonferroni correction ($\alpha = 0.05$)

^c Corrected mortality according to Abbott (1925) * statistically significantly different compared to the control.

Table A 168: Reproduction of *Coccinella septempunctata* after exposure to MCW-2222

Treatment	Rate ^a [g a.s./ha]	Reproduction [fertile eggs/female/day] ^b	Hatching rate [%] ^c
Control	0	3.1	73.4
MCW-2222	4.3	2.9	73.2
MCW-2222	9.4	3.0	72.8
MCW-2222	20.7	3.2	73.7
MCW-2222	45.5	n.d.	-
MCW-2222	100	n.d.	-

^a Application rate in 200 L water/ha.

n.d. not determined (corrected mortality > 50 %, compared to the control)

The reference item caused a mortality of 77.5 % of exposed ladybirds, resulting in a corrected mortality of 73.5 %.

Table A 169: Acute toxicity of test item to *Coccinella septempunctata*

	Endpoints
LR ₅₀ (95% CI)	22.1 14.5 – 33.7 g a.s./ha

Validity criteria

As shown in the following table, all validity criteria were met.

Table A 170: Validity criteria

Validity criteria according Schmuck <i>et al.</i> (2009)	Observed in study
Pre-imaginal mortality in the control group should be ≤ 30%	15%
Corrected pre-imaginal mortality in the reference item group should be > 40%	100%
Average number of fertile eggs per viable female per day in the control group should be ≥ 2	3.1

Conclusion

In an extended laboratory study with MCW-2222 the LR₅₀ for *Coccinella septempunctata* was estimated to be 22.1 g a.s./ha in 200 L water/ha. No adverse effects on mortality of *Coccinella septempunctata* occurred, when MCW-2222 was applied up to and including an application rate of 9.4 g a.s./ha in 200 L water/ha. No adverse effects on reproduction of *Coccinella septempunctata* occurred, when MCW-2222 was applied up to and including an application rate of 20.7 g a.s./ha in 200 L water/ha.

A 2.3.2.3 KCP 10.3.2.3 Aged residue studies

A 2.3.2.3.1 KCP 10.3.2.3/01 Aged residue study with *Typhlodromus pyri*

Comments of zRMS:	<p>The aged residue study on effects to <i>A. rhopalosiphi</i> has been submitted in support of the re-evaluation of CA3573 (formerly MCW-2222) in 2021 due to renewal of acetamiprid and The validation of the study was performed by the zRMS.</p> <p>The study was carried out in line with the respective guideline with no major deviations.</p> <p>It is noted that the maximum relative humidity (90.7-91.3%) was slightly above the maximum 90% recommended by the guideline. However, as the maximum 90% was just slightly exceeded and all validity criteria were met, this deviation is considered to have no impact on the test results.</p> <p>The study was performed as a limit test at application rate of 45 g a.s./ha with 4 replicates per test group with 10 individuals each (5 males and 5 females), while in line with the test guideline the limit test should comprise 6 replicates with minimum 5 females each. Nevertheless, two other limit tests with the same design but at different application rates (70 and 102 g a.s./ha) were run in parallel, so all together sufficient range of rates was tested on</p>
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	<p>bean leaves. Taking this into account, the lower number of replicates is agreed by the zRMS in this case.</p> <p>Overall, the study is considered acceptable.</p> <p>Obtained results demonstrated that lethal and sub-lethal effects on <i>A. rhopalosiphi</i> following application of CA3573 at 45 g a.s./ha are <50% after 28 days of aging.</p>
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Data point	KCP 10.3.2.3/01
Report	Aged residue test with the formulation “MCW-2222” on the predatory mite <i>Typhlodromus pyri</i> (Acari: Phytoseiidae), Luna, F., 2017b
Report No.:	TRC16-074BA,
Document No.:	R-37335
Guideline(s):	Aged residue test with the formulation “MCW-2222” on the predatory mite <i>Typhlodromus pyri</i> (Acari: Phytoseiidae), Luna, F., 2017b, TRC16-074BA, R-37335
Previous evaluation	No, not previously submitted
GLP:	Yes, certified laboratory
Acceptability:	Yes, study considered acceptable
Duplication (if vertebrate study)	Not applicable

Executive Summary

To determine the extent and persistence of effects on mortality and fecundity on the predatory mite *Typhlodromus pyri* after the application of 102 and 170 g a.s./ha (equivalent to 0.4973 and 0.8289 L test item/ha, respectively) an aged residue study was performed. Potted apple plants were treated and maintained under outdoor conditions to provide natural aging conditions, except washing-off by rain. Assessments were performed with residues aged for 0, 35 and 42 days.

Lethal or sub-lethal effects less than the threshold of 50% (50% effect compared to the control) were observed after exposure to 0, 35 and 42 days old residues with the tested rates of the test item, 102 and 170 g a.s./ha.

Significant differences compared to control (T-Test, $\alpha=0.05$) with mortality and fecundity results were observed in the exposure of 0 day old residues (fresh and dry residues) at the maximum tested rate of 170 g a.s./ha, and no lethal or sub-lethal effects were recorded after exposure to residues aged for 35 and 42 days. No significant differences were observed in mortality nor fecundity with the rate of 102 g a.s./ha from the exposure of 0 day old residues.

Based on the results of the present study it can be concluded that residues of the test item “MCW-2222” (Acetamiprid 20 % w/v SL) applied up to the rate of 170 g a.s./ha causes mortality less than 50% compared to the control and has less than 50% reduction on the reproduction of *Typhlodromus pyri* from the day of the application with fresh and dry residues.

Materials and methods

Materials

Test item	MCW-2222 (Acetamiprid 20% w/v SL)
Batch #	811-021115-01
Content of active substance	acetamiprid: 205.1 ± 1.1 g/L, (nominal: 200 g/L)
Control	Tap water
Toxic reference	Deltamethrin 2.5% EC at 1.15 L test item/ha = 29.325 g a.s./ha
Test organism	
Species	Predatory mite <i>Typhlodromus pyri</i> Protonymphs not later than 24 hours from moulting

Source	Katz Biotech AG”, An der Birkenpfehlheide 10, 15837 Baruth, Germany
Study design and methods	
Ageing periods	0, 32 and 45 days
Exposure duration	7 days
Experimental dates	12 Jul 2016 to 6 Sep 2016
Test doses (nominal)	102 and 170 g acetamiprid/ha, equivalent to 0.4973 and 0.8289 L/ha of formulated test item
Test units	Apple plants (<i>Malus domestica</i>) of the variety GOLDEN were used for trials purpose. Three plots with 17 potted plants per plot were used: One plot for the water treated control, one plot for the test item and one plot for the toxic reference. Plot size was 20 m ² (10 m × 2 m) for the treatments and they were arranged in two rows (0.5 m to each other).
Group size/replicates	20 protonymphs per replicate, 5 replicates per treatment
Experimental treatments	Application was performed using a backpack mist blower simulating a commercial field application at a volume of 2000 L/ha in order to spray to the point of runoff (“thoroughly wet”). After application, plants were maintained under outdoor conditions in an opened greenhouse, equipped with a polycarbonate roof closed only when it rains and opened laterals to provide natural aging conditions, except washing-off by rain. The reference product was applied once at the same time as the test item.
Environmental conditions	
Temperature	0 DAA: 24.5–40.2 °C 35 DAA: 24.8–25.3 °C 42 DAA: 24.8–25.3 °C
Photoperiod	16 h light (1699–2184 lx mortality phase, 1164–3213 lx fecundity phase) : 8 h dark
Relative humidity	0 DAA: 35.7–89.6% 35 DAA: 72.9–95.6% 42 DAA: 72.9–95.6%

Biological observations

Assessments of mortality: After each ageing period, 5–6 leaves were sampled per plot from different plants and transported to the laboratory to prepare the test arenas. After being collected and cut at fragments 1.5 × 5 cm approximately, the test units were mounted and then 20 protonymphs were placed in each arena, with 5 replicates per treatment.

Exposures to the residues (bioassays) were performed 0, 35 and 42 days after application (DAA). The test units were placed into an environmental chamber between 25 ± 2 °C (actual between 24.5 and 40.2 °C), 60–90% RH (actual between 35.7 and 95.6%), and with a 16:8h L:D photoperiod. Temperature was registered with values above 27 °C and humidity with values below 60% during more than 2 hours continuously (16 Jul to 18 Jul 2016 at the mortality period of the exposure at 0 DAA) although without negative effects in the study.

Mortality assessments were carried out after 1 and 7 days of each exposure.

Assessments of fecundity: The corrected mortality after 7 days was ≤ 50% in the test item group in all the assayed ageing periods and therefore, the fecundity was assessed in the control and test item groups between 7 and 14 days after each exposure (9, 11 and 14 days after each exposure). The test units were placed into an environmental chamber with same climatic conditions as in the mortality period (actual temperature between 24.5 and 25.7 °C and relative humidity between 72.6 and 95.6%).

Statistics

Results of 7 d mortality and 7-14 d fecundity (eggs per female) were analysed with the Shapiro-Wilk test for normality of data distribution and with the Levene’s test for homoscedasticity (Annex IV). The non-parametric Mann-Whitney test (exact sig., 1-tailed, α=0.05) or the parametric T-test with Levene’s test for

equality of variances ($\alpha=0.05$) were performed in order to study significant differences between the test item treatments and control according to the normality or not of data. No statistical analysis was performed with results in the test reference treatment.

Results and discussion

Based on mortalities being less than 20% at the end of all exposure periods (actual maximum value was 7.0% in the exposure of 35 DDA), reproductive performances above 4 eggs per female at the fecundity assessment 0, 35 and 42 DAA in the control (actual minimum value was 8.90 eggs per female in the exposure of 35 DDA) and a corrected mortality greater than 50% in the toxic reference until the exposure of 35 DAA (56.99% corrected mortality), the sensitivity of the test species and the suitability of the test system was confirmed and the study can be regarded to be valid.

Biological results – mortality

With fresh and dry residue (exposure of 0 DAA) and after the ageing periods of 35 and 42 days, corrected mortality was less than 50%, i.e. 42.55, 0.0 and 3.19%, respectively.

Mortality in the test item group of the rate 170 g a.s./ha was statistically significant higher than control as the assessment started on the same day of the application, i.e. 0 DAA (T-test, $\alpha=0.05$). This mortality (rate of 170 g a.s./ha with the exposure of 0 DAA) was mainly due to a repellency effect; 36% of individuals tried to escape (glued in the barrier of the test units or escaped) and only 10% died.

The observed lethal effect of the test item at the assayed rate of 102 g a.s./ha was not significant different compared to the control (T-test, $\alpha=0.05$) in the bioassays started 0, 35 and 42 DAA.

The mortality results are presented in the following table.

Table A 171: Effects of MCW-2222 (Acetamiprid 20% w/v SL) on mortality of the predatory mite, *Typhlodromus pyri*

	Rate [g a.s./ha]	Exposure					
		0 DAA ¹⁾		35 DAA		42 DAA	
		M (%) ²⁾	C _m (%)	M (%)	C _m (%) ³⁾	M (%)	C _m (%)
Control	0	6.00	-	7.00	-	6.00	-
MCW-2222 (Acetamiprid 20% w/v SL)	102	7.00	1.06	3.00	-4.30	8.00	2.13
	170	46.00 ^{SD}	42.55	7.00	0.00	9.00	3.19
Reference Item (Deltamethrin 2.5% EC)	29.325	100	100	60.00	56.99	17.00	11.70

¹⁾ DAA = Days after application; M [%] = Mortality [%]; C_m [%] = Corrected mortality [%].

²⁾ SD = statistically significant different compared to the control (T-test, $\alpha=0.05$).

³⁾ Negative value indicates less effect relative to the control.

The reference treatment was not statistically analysed.

Biological results – fecundity

The reduction of number of eggs/female was below the ESCORT 2 trigger value of 50% in the bioassays performed from 0 DAA at the tested rates of 102 and 170 g a.s./ha (maximum reduction relative to control was 27.65% in the treatment of the rate 170 g a.s./ha with fresh and dry residues).

The fecundity results (mummies per female and progeny reduction) are presented in the following table.

Table A 172: Effects of MCW-2222 (Acetamiprid 20% w/v SL) on fecundity of the predatory mite, *Typhlodromus pyri*

	Rate [g a.s./ha]	Exposure					
		0 DAA ¹⁾		35 DAA		42 DAA	
		e/f ²⁾	R [%]	e/f	R [%] ³⁾	e/f	R [%] ³⁾
Control	0	11.02	-	8.90	-	9.33	-
MCW-2222 (Acetamiprid 20% w/v SL)	102	10.20	7.41	10.37	-16.61	9.86	-5.66
	170	7.97 ^{SD}	27.65	9.20	-3.37	8.47	9.24

¹⁾ DAA = Days after application; ; F [m/f]= Fecundity [mummies per female]; R [%]= Reduction [%].

²⁾ SD = statistically significant different compared the control (T-test, $\alpha=0.05$).

³⁾ Negative value indicates an increase relative to the control.

Reproduction performance with the rate of 102 g a.s./ha was not statistically significant affected (T-test, $\alpha=0.05$) by 0, 35 and 42-day old residues; reproduction amounted to be less than 10% reduction by 0 day old residues and even higher than in control by 35 and 42 days old residues. Reduction on reproduction with the rate of 170 g a.s./ha was 27.65% compared to control with 0 day old residues (less than 50%) and significantly different to control (T-test, $\alpha=0.05$). No significant differences compared to control were observed by 35 and 42 days old residues.

Validity criteria

As shown in the following table, all validity criteria were met.

Table A 173: Validity criteria

Validity criteria according to Blümel <i>et al.</i>	Observed in study
Mortality in the control should not exceed 20%	$\leq 7\%$
Mortality in the reference should range between 50% - 100%	100% at 0 DAA, 56.99% at 35 DAA, 11.7% at 42 DAA ¹⁾
More than 4 eggs per female should be achieved	≥ 8.90

¹⁾ Validity criterion regarding mortality in toxic standard group relevant only for 0 DAA (test guideline does not provide validity criteria for particular aging periods)

Conclusion

Based on the results of the present study it can be concluded that residues of the test item “MCW- 2222” (Acetamiprid 20 % w/v SL) applied up to the rate of 170 g a.s./ha causes mortality less than 50% compared to the control and has less than 50% reduction on the reproduction of *Typhlodromus pyri* from the day of the application with fresh and dry residues.

A 2.3.2.3.2 KCP 10.3.2.3/02 Aged residue study with *Aphidius rhopalosiphi*

Comments of zRMS:	<p>The aged residue study on effects to <i>A. rhopalosiphi</i> has been submitted in support of the re-evaluation of CA3573 (formerly MCW-2222) in 2021 due to renewal of acetamiprid . The validation of the study was performed by the zRMS.</p> <p>The study was carried out in line with the respective guideline with no major deviations.</p> <p>It is noted that the maximum relative humidity (90.7-91.3%) was slightly above the maximum 90% recommended by the guideline. However, as the maximum 90% was just slightly exceeded and all validity criteria were met, this deviation is considered to have no impact on the test results.</p> <p>The study was performed as a limit test at application rate of 70 g a.s./ha with 4 replicates per test group with 10 individuals each (5 males and 5 females), while in line with the test guideline the limit test should comprise 6 replicates with minimum 5 females each. Nevertheless, two other limit tests with the same design but at different application rates (45 and 102 g a.s./ha) were run in parallel, so all together sufficient range of rates was tested on bean leaves. Taking this into account, the lower number of replicates is agreed by the zRMS in this case.</p> <p>Overall, the study is considered acceptable.</p> <p>Obtained results demonstrated that lethal and sub-lethal effects on <i>A. rhopalosiphi</i> following application of CA3573 at 70 g a.s./ha are <50% after 28 days of aging.</p>
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Data point

KCP 10.3.2.3/02

Report

Aged residue test with the formulation “MCW-2222” (Acetamiprid 20% w/v SL) at 45 g a.s./ha on the parasitic wasp *Aphidius rhopalosiphi* (Hymenoptera: Braconidae), Luna, F., 2016a

Report No.:	TRC15-242BA
Document No.:	R-36938A
Guideline(s):	Mead-Briggs <i>et al.</i> 2010, and an unpublished draft guideline by Mead-Briggs and Longley 1997
Previous evaluation	Yes submitted
GLP:	Yes, certified laboratory
Acceptability:	Yes, study considered acceptable
Duplication (if vertebrate study)	Not applicable

Executive Summary

To determine the extent and persistence of effects on mortality and fecundity on the parasitoid *Aphidius rhopalosiphi* after the application of 45 g acetamiprid/ha (equivalent to 0.2259 L test item/ha) an aged residue study was performed. Potted bean plants were treated and maintained under field conditions in a tunnel, equipped with an UV-permeable plastic roof and opened laterals to provide natural aging conditions, except washing-off by rain. Assessments were performed with residues aged for 1, 28 and 36 days.

Whereas the tested rate exceeded the mortality threshold of 50% (50% corrected mortality compared to the control) after the exposure to 1 d old residues, no lethal or sub-lethal effects were observed after exposure to 28 and 36 days old residues.

Based on the results of this study performed on *Aphidius rhopalosiphi* after the application of “MCW-2222” (Acetamiprid 20% w/v SL) at a rate of 45 g acetamiprid/ha it can be concluded that 28 days old residues will not adversely affect mortality or reproduction.

Materials and methods

Materials

Test item	MCW-2222 (Acetamiprid 20% w/v SL)
Batch #	659-030314-01
Content of active substance	acetamiprid: 199.2 ± 1.3 g/L, (nominal: 200 g/L)
Control	Tap water
Toxic reference	Deltamethrin 2.5% EC at 0.05% = 7.65 g a.s./ha
Test organism	
Species	Parasitic wasp <i>Aphidius rhopalosiphi</i> Adults, < 48 h old
Source	Katz Biotech AG”, An der Birkenpfuhlheide 10, 15837 Baruth, Germany

Study design and methods

Aging periods	1, 28 and 36 days
Exposure duration	48 hours
Experimental dates	30 Nov 2015 to 18 Jan 2016
Test doses (nominal)	45 g acetamiprid/ha, equivalent to 0.2259 L/ha of formulated test item
Test units	Three plots with approximately 68 potted plants per plot (2 bean plants per pot, <i>Phaseolus vulgaris</i> , variety ROMA) were selected: One plot for the water treated control, one plot for the test item and one plot for the toxic reference. Plot size was 5 m ² (5 m × 1 m) for the treatments and they were arranged in two crop rows (0.5 m to each other).
Group size/replicates	5 females and 5 males per replicate, 4 replicates (40 adults) per treatment
Experimental treatments	The dose of the test item (45 g a.s./ha) was applied once in the field using a compressed air knapsack sprayer equipped with a spray bar and 2 nozzles (Black Hardy 4110-14 Flat fan) with 50 cm distance, simulating a commercial field application in field (volume 600 L/ha). After application, plants were maintained under field conditions in a tunnel,

equipped with a UV-permeable plastic roof and opened laterals to provide natural aging conditions, except washing-off by rain.

Environmental conditions

Temperature

1 DAA: 19.6–20.5 °C
28 DAA: 19.0–21.0 °C
36 DAA: 18.4–20.7 °C

Photoperiod

16 h light (4000–20000 lx): 8 h dark

Relative humidity

1 DAA: 64.5–90.7%
28 DAA: 56.7–91.3%
36 DAA: 50.8–91.3%

Biological observations

Assessments of mortality: After each ageing period, at least 8 leaves per plot were sampled at random and transported to the laboratory to prepare the test arenas. These were built by transparent plastic tubes which sides were closed by Petri-dishes with punched out leaf discs facing towards each other. Then, 10 adult wasps (at least 5 females) were placed in each arena (excised leaf test units) with 4 replicates per treatment. Arenas were ventilated with an air pump and wasps were sufficiently provided with food and water. Mortality assessments (bioassays) were performed 1, 28 and 36 days after application (DAA). The test units were placed into an environmental chamber between 19.0–21.0°C and 56.7–91.3% RH, with a 16:8 h L:D photoperiod. Mortality assessments were carried out after 24 and 48 hours of exposure. Adult mortality after an exposure of 48 hours (lethal effect) to residues on leaves aged for 1, 28 and 36 days after application (DAA).

Assessments of fecundity: If after 48 hours the corrected mortality was $\leq 50\%$ in the test item group, which was the case after 28 and 36 days of ageing, the reproductive capacity was assessed in the control and test item group confining 15 females individually over untreated barley plants infested with the host cereal aphids, *Rhopalosiphum padi*. The females were removed after 24 hours and the aphid-infested plants were left for a further 10–11 days before the numbers of aphid mummies that had developed were assessed. The test units were placed into an environmental chamber between 18.4–20.7 °C and with a 16:8 h L:D photoperiod.

Fecundity of 15 surviving females during 24 hours in presence of their host aphids for the aforementioned ageing periods when mean mortality in the test item group was $\leq 50\%$.

Statistics

Results of mortality and mummies per female were analysed with the Shapiro-Wilk test for normality of data distribution and with the Levene's test for homoscedasticity (Annex IV). The parametric T-test with Levene's test for equality of variances (sig. 2-tailed, $\alpha=0.05$) or the non-parametric Mann-Whitney test (exact sig., 1-tailed, $\alpha=0.05$) were performed in order to study significant differences between the test item treatment and control according to the normality or not of data. No statistical analysis was performed with results in the test reference treatment.

Results and discussion

Biological results – mortality

Based on mortalities being less than 13% at the end of all exposure periods, reproductive performances above 5 mummies per female at the fecundity assessments 28 and 36 DAA in the control and a corrected mortality greater than 50% in the toxic reference, the sensitivity of the test species and the suitability of the test system was confirmed and the study can be regarded to be valid.

The mortality results are presented in the following table.

Table A 174: Effects of MCW-2222 (Acetamiprid 20% w/v SL) on mortality of the parasitoid, *Aphidius rhopalosiphi*

	Rate ¹⁾ [g a.s./ha]	Bioassay					
		1 DAA ²⁾		28 DAA		36 DAA	
		M (%)	C _m (%)	M (%)	C _m (%)	M (%)	C _m (%)
Control	0	0.0	-	0.0	-	0.0	-
MCW-2222 (Acetamiprid 20% w/v SL)	45	100 ^{SD}	100	10.0 ^{NS}	10.0	5.0 ^{NS}	5.0
Reference Item (Deltamethrin 2.5% EC)	7.65	100	100	70	70	85	85

¹⁾ Application rate in 600 L water/ha

²⁾ DAA = Days after application; M [%] = Mortality [%]; C_m [%] = Corrected mortality [%].

SD = statistically significant different compared to the control (Mann-Whitney test, exact sig., 1-tailed greater, $\alpha=0.05$).

NS = not statistically significant different compared to the control (Mann-Whitney test, exact sig., 1-tailed greater, $\alpha=0.05$).

The reference treatment was not statistically analysed.

Biological results – fecundity

After an ageing period of 28 and 36 days corrected mortalities less than 50% and not statistically different compared to the control (Mann-Whitney test, exact sig., 1-tailed greater, $\alpha=0.05$), i.e. 10.0% and 5.0% were observed, respectively. A lethal effect higher than 50% was observed in the exposure assessment started at 1 DAA with 100% corrected mortality compared to the control.

The fecundity results (mummies per female and progeny reduction) are presented in the following table.

Table A 175: Effects of MCW-2222 (Acetamiprid 20% w/v SL) on fecundity of the parasitoid, *Aphidius rhopalosiphi*

	Rate ¹⁾ [g a.s./ha]	Bioassay					
		1 DAA ²⁾		28 DAA		36 DAA	
		F [m/f]	R [%] ⁴⁾	F [m/f]	R [%]	F [m/f]	R [%]
Control	0	N/S ³⁾		24.4	-	42.7	-
MCW-2222 (Acetamiprid 20% w/v SL)	45	N/S ³⁾		32.9 ^{NS}	-35.0	37.6 ^{NS}	12.0

¹⁾ Application rate in 600 L water/ha

²⁾ DAA = Days after application; ; F [m/f]= Fecundity [mummies per female]; R [%]= Reduction [%].

³⁾ N/S = Reproduction was not studied as mortality was > 50% in T.

⁴⁾ Negative value indicates an increase in number of mummies compared to the control.

NS = fecundity was not statistically significant different compared to the control (T-Test, $\alpha=0.05$).

Reproduction performance was not affected by 28 and 36-day old residues. In fact, it was on the control level (36 DAA) or even higher (28 DAA). Thus, no statistically significant differences were observed compared to the control (T-test, sig. 2-tailed, $\alpha=0.05$). The reduction of the reproduction relative to the control was below the ESCORT 2 trigger value of 50% and amounted to be -35.0% for the bioassay started on 28 DAA, meaning a higher reproduction compared to the control and +12.0% for the bioassay started on 36 DAA.

Validity criteria

As shown in the following table, all validity criteria were met.

Table A 176: Validity criteria

Validity criteria according to Mead-Briggs <i>et al.</i>	Observed in study
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Mortality in the control should not exceed 13%	0%
Mortality in the reference should range between 50% - 100%	≥70%
Wasps in the control should produce ≥ 5 mummies per female	≥22.4
Not more than two wasps should produce no mummies	≤ 1

Conclusion

Based on the results of this study performed on *Aphidius rhopalosiphi* after the application of “MCW-2222” (Acetamiprid 20% w/v SL) at a rate of 45 g acetamiprid /ha it can be concluded that 28 days old residues will not adversely affect mortality or reproduction.

A 2.3.2.3.3 KCP 10.3.2.3/03 Aged residue study with *Aphidius rhopalosiphi*

Comments of zRMS:	<p>The aged residue study on effects to <i>A. rhopalosiphi</i> has been submitted in support of the re-evaluation of CA3573 (formerly MCW-2222) in 2021 due to renewal of acetamiprid . The validation of the study was performed by the zRMS.</p> <p>The study was carried out in line with the respective guideline with no major deviations.</p> <p>It is noted that the maximum relative humidity (90.4-91.3%) was slightly above the maximum 90% recommended by the guideline. However, as the maximum 90% was just slightly exceeded and all validity criteria were met, this deviation is considered to have no impact on the test results.</p> <p>The study was performed as a limit test at application rate of 102 g a.s./ha with 4 replicates per test group with 10 individuals each (5 males and 5 females), while in line with the test guideline the limit test should comprise 6 replicates with minimum 5 females each. Nevertheless, two other limit tests with the same design but at different application rates (45 and 70 g a.s./ha) were run in parallel, so all together sufficient range of rates was tested on bean leaves. Taking this into account, the lower number of replicates is agreed by the zRMS in this case.</p> <p>Overall, the study is considered acceptable.</p> <p>Obtained results demonstrated that lethal effects on <i>A. rhopalosiphi</i> following application of CA3573 at 102 g a.s./ha are <50% after 36 days of aging. Effects on fecundity were <50% after 42 days of aging (no fecundity assessment carried out after 36 days of aging).</p>
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Data point	KCP 10.3.2.3/03
Report	Aged residue test with the formulation “MCW-2222” (Acetamiprid 20% w/v SL) at 70 g a.s./ha on the parasitic wasp <i>Aphidius rhopalosiphi</i> (Hymenoptera: Braconidae), Luna, F., 2016b
Report No.:	TRC15-243BA
Document No.:	R-36938A
Guideline(s):	Mead-Briggs <i>et al.</i> 2000, and an unpublished draft guideline by Mead-Briggs and Longley 1997
Previous evaluation	No, not previously submitted
GLP:	Yes, certified laboratory
Acceptability:	Yes, study considered acceptable
Duplication (if vertebrate study)	Not applicable

Executive Summary

To determine the extent and persistence of effects on mortality and fecundity on the parasitoid *Aphidius rhopalosiphi* after the application of 70 g acetamiprid/ha (equivalent to 0.3514 L test item/ha) an aged residue study was performed. Potted bean plants were treated and maintained under field conditions in a tunnel, equipped with an UV-permeable plastic roof and opened laterals to provide natural aging conditions, except washing-off by rain. Assessments were performed with residues aged for 1, 28 and 36 days.

Whereas the tested rate exceeded the mortality threshold of 50% (50% corrected mortality compared to the control) after the exposure to 1 day old residues, no lethal or sub-lethal effects were observed after exposure to 28 and 36 days old residues.

Based on the results of this study performed on *Aphidius rhopalosiphi* after the application of “MCW-2222” (Acetamiprid 20% w/v SL) at a rate of 70 g acetamiprid/ha it can be concluded that 28 days old residues will not adversely affect mortality or reproduction.

Materials and methods

Materials

Test item	MCW-2222 (Acetamiprid 20% w/v SL)
Batch #	659-030314-01
Content of active substance	Acetamiprid: 199.2 ± 1.3 g/L, (nominal: 200 g/L)
Control	Tap water
Toxic reference	Deltamethrin 2.5% EC at 0.05% = 7.65 g a.s./ha
Test organism	
Species	Parasitic wasp <i>Aphidius rhopalosiphi</i> Adults, < 48 h old
Source	Katz Biotech AG”, An der Birkenpfuhlheide 10, 15837 Baruth, Germany

Study design and methods

Ageing periods	1, 28 and 36 days
Exposure duration	48 hours
Experimental dates	30 Nov 2015 to 18 Jan 2016
Test doses (nominal)	70 g acetamiprid/ha, equivalent to 0.3514 L/ha of formulated test item
Test units	Three plots with approximately 68 potted plants per plot (2 bean plants per pot, <i>Phaseolus vulgaris</i> , variety ROMA) were selected: One plot for the water treated control, one plot for the test item and one plot for the toxic reference. Plot size was 5 m ² (5 m × 1 m) for the treatments and they were arranged in two crop rows (0.5 m to each other).
Group size/replicates	10 adults (≥ 5 females) per replicate, 4 replicates (40 adults) per treatment
Experimental treatments	The dose of the test item (70 g a.s./ha) was applied once in the field using a compressed air knapsack sprayer equipped with a spray bar and 2 nozzles (Black Hardi 4110-14 Flat fan) with 50 cm distance, simulating a commercial field application in field (volume 600 L/ha). After application, plants were maintained under field conditions in a tunnel, equipped with a UV-permeable plastic roof and opened laterals to provide natural aging conditions, except washing-off by rain.
Environmental conditions	
Temperature	1DAA: 19.6–20.5 °C 28 DAA: 19.0–21.0 °C 36 DAA: 18.4–20.7 °C
Photoperiod	16 h light (4000–20000 lx) : 8 h dark
Relative humidity	1DAA: 64.5–90.7% 28 DAA: 56.7–91.3% 36 DAA: 50.8–91.3%

Biological observations

Assessments of mortality: After each ageing period, at least 8 leaves per plot were sampled at random and transported to the laboratory to prepare the test arenas. These were built by transparent plastic tubes which sides were closed by Petri-dishes with punched out leaf discs facing towards each other. Then, 10 adult wasps (at least 5 females) were placed in each arena (excised leaf test units) with 4 replicates per treatment. Arenas were ventilated with an air pump and wasps were sufficiently provided with food and water. Mortality assessments (bioassays) were performed 1, 28 and 36 days after application (DAA). The test units were placed into an environmental chamber between 19.0–21.0°C and 56.7–91.3% RH, with a 16:8 h L:D photoperiod. Mortality assessments were carried out after 24 and 48 hours of exposure. Adult mortality after an exposure of 48 hours (lethal effect) to residues on leaves aged for 1, 28 and 36 days after application (DAA).

Assessments of fecundity: If after 48 hours the corrected mortality was $\leq 50\%$ in the test item group, which was the case after 28 and 36 days of ageing, the reproductive capacity was assessed in the control and test item group confining 15 females individually over untreated barley plants infested with the host cereal aphids, *Rhopalosiphum padi*. The females were removed after 24 hours and the aphid-infested plants were left for a further 10–11 days before the numbers of aphid mummies that had developed were assessed. The test units were placed into an environmental chamber between 18.4–20.7 °C and with a 16:8 h L:D photoperiod.

Fecundity of 15 surviving females during 24 hours in presence of their host aphids for the aforementioned ageing periods when mean mortality in the test item group was $\leq 50\%$.

Statistics

The statistical management of data was conducted according to the OECD guideline number 54 (OECD series on testing and assessment) and the appropriate Trialcamp SOP. All the statistical analysis were performed using the software IBM® SPSS Statistics 19.0.

Results and discussion

Biological results – mortality

Based on mortalities being less than 13% at the end of all exposure periods, reproductive performances above 5 mummies per female at the fecundity assessments 28 and 36 DAA in the control and a corrected mortality greater than 50% in the toxic reference, the sensitivity of the test species and the suitability of the test system was confirmed and the study can be regarded to be valid.

The mortality results are presented in the following table.

Table A 177: Effects of MCW-2222 (Acetamiprid 20% w/v SL) on mortality of the parasitoid, *Aphidius rhopalosiphii*

	Rate ¹⁾ [g a.s./ha]	Bioassay					
		1 DAA ²⁾		28 DAA		36 DAA	
		M (%)	C _m (%)	M (%)	C _m (%)	M (%)	C _m (%)
Control	0	0.0	-	0.0	-	0.0	-
MCW-2222 (Acetamiprid 20% w/v SL)	70	100 ^{SD}	100	27.5 ^{SD 3)}	27.5	20.0 ^{SD}	20.0
Reference Item (Deltamethrin 2.5% EC)	7.65	100	100	70	70	85	85

¹⁾ Application rate in 600 L water/ha

²⁾ DAA = Days after application; M [%] = Mortality [%]; C_m [%] = Corrected mortality [%].

³⁾ Signs of intoxication (lack of coordination) were observed on 17.2% of survivors in the test treatment at 28 DAA.

SD = statistically significant different compared to the control (Mann-Whitney test, exact sig., 1-tailed greater, $\alpha=0.05$).

NS = not statistically significant different compared to the control (Mann-Whitney test, exact sig., 1-tailed greater, $\alpha=0.05$).

The reference treatment was not statistically analysed.

Biological results – fecundity

After an ageing period of 28 and 36 days corrected mortalities less than 50%, i.e. 27.5% and 20.0% were observed, respectively. The mortalities at these exposures were statistically different compared to the control (Mann-Whitney test, exact sig., 1-tailed, $\alpha=0.05$),

A lethal effect higher than 50% was observed in the exposure assessment started at 1 DAA with 100% corrected mortality compared to the control.

The fecundity results (mummies per female and progeny reduction) are presented in the following table.

Table A 178: Effects of MCW-2222 (Acetamiprid 20% w/v SL) on fecundity of the parasitoid, *Aphidius rhopalosiphi*

	Rate ¹⁾ [g a.s./ha]	Bioassay					
		1 DAA ²⁾		28 DAA		36 DAA	
		F [m/f]	R [%] ⁴⁾	F [m/f]	R [%]	F [m/f]	R [%]
Control	0	N/S ³⁾		24.4	-	42.7	-
MCW-2222 (Acetamiprid 20% w/v SL)	70	N/S ³⁾		26.0 ^{NS}	-6.6	35.9 ^{NS}	15.9

¹⁾ Application rate in 600 L water/ha

²⁾ DAA = Days after application; ; F [m/f]= Fecundity [mummies per female]; R [%]= Reduction [%].

³⁾ N/S = Reproduction was not studied as mortality was > 50% in T.

⁴⁾ Negative value indicates an increase in number of mummies compared to the control.

NS = fecundity was not statistically significant different compared to the control (T-Test, $\alpha=0.05$).

Reproduction performance was not affected by 28 and 36-day old residues. In fact, it was on the control level (36 DAA) or even higher (28 DAA). Thus, no statistically significant differences were observed compared to the control (T-test, sig. 2-tailed, $\alpha=0.05$). The reduction of the reproduction relative to the control was below the ESCORT 2 trigger value of 50% and amounted to be -6.6% for the bioassay started on 28 DAA, meaning a higher reproduction compared to the control and +15.9% for the bioassay started on 36 DAA.

Validity criteria

As shown in the following table, all validity criteria were met.

Table A 179: Validity criteria

Validity criteria according to Mead-Briggs <i>et al.</i>	Observed in study
Mortality in the control should not exceed 13%	0%
Mortality in the reference should range between 50% - 100%	$\geq 70\%$
Wasps in the control should produce ≥ 5 mummies per female	≥ 22.4
Not more than two wasps should produce no mummies	≤ 1

Conclusion

Based on the results of this study performed on *Aphidius rhopalosiphi* after the application of “MCW-2222” (Acetamiprid 20% w/v SL) at a rate of 70 g acetamiprid /ha it can be concluded that 28 days old residues will not adversely affect mortality or reproduction.

A 2.3.2.3.4 KCP 10.3.2.3/04 Aged residue study with *Aphidius rhopalosiphi*

Comments of zRMS:	<p>The aged residue study on effects to <i>A. rhopalosiphi</i> has been submitted in support of the re-evaluation of CA3573 (formerly MCW-2222) in 2021. The validation of the study was performed by the zRMS.</p> <p>The study was carried out in line with the respective guideline with no major deviations.</p> <p>It is noted that the maximum relative humidity (90.4-91.3%) was slightly above the maximum 90% recommended by the guideline. However, as the maximum 90% was just slightly exceeded and all validity criteria were met, this deviation is considered to have no impact on the test results.</p> <p>The study was performed as a limit test at application rate of 102 g a.s./ha with 4 replicates per test group with 10 individuals each (5 males and 5 females), while in line with the test guideline the limit test should comprise 6 replicates with minimum 5 females each. Nevertheless, two other limit tests with the same design but at different application rates (45 and 70 g a.s./ha) were run in parallel, so all together sufficient range of rates was tested on</p>
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	<p>bean leaves. Taking this into account, the lower number of replicates is agreed by the zRMS in this case.</p> <p>Overall, the study is considered acceptable.</p> <p>Obtained results demonstrated that lethal effects on <i>A. rhopalosiphi</i> following application of CA3573 at 102 g a.s./ha are <50% after 36 days of aging. Effects on fecundity were <50% after 42 days of aging (no fecundity assessment carried out after 36 days of aging).</p>
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Data point	KCP 10.3.2.3/04
Report	Aged residue test with the formulation “MCW-2222” (Acetamiprid 20% w/v SL) at 102 g a.s./ha on the parasitic wasp <i>Aphidius rhopalosiphi</i> (Hymenoptera: Braconidae), Luna, F., 2016c
Report No.:	TRC15-244BA
Document No.:	R-36938A
Guideline(s):	Mead-Briggs <i>et al.</i> 2000, and an unpublished draft guideline by Mead-Briggs and Longley 1997
Previous evaluation	Yes previously submitted
GLP:	Yes, certified laboratory
Acceptability:	Yes, study considered acceptable
Duplication (if vertebrate study)	Not applicable

Executive Summary

To determine the extent and persistence of effects on mortality and fecundity on the parasitoid *Aphidius rhopalosiphi* after the application of 102 g acetamiprid/ha (equivalent to 0.5120 L test item/ha) an aged residue study was performed. Potted bean plants were treated and maintained under field conditions in a tunnel, equipped with an UV-permeable plastic roof and opened laterals to provide natural aging conditions, except washing-off by rain. Assessments were performed with residues aged for 1, 28, 36 and 42 days. Whereas the tested rate exceeded the mortality threshold of 50% (50% corrected mortality compared to the control) after the exposure to 1 day and 28 days old residues, lethal effects less than 50% were observed after exposure to 36 and 42 days old residues, and no sub-lethal effects, i.e. fecundity were recorded after exposure to residues aged for 42 days.

Based on the results of this study performed on *Aphidius rhopalosiphi* after the application of “MCW-2222” (Acetamiprid 20% w/v SL) at a rate of 102 g acetamiprid/ha it can be concluded that 36 days old residues will not adversely affect mortality and 42 days old residues will not impact reproduction.

Materials and methods

Materials

Test item	MCW-2222 (Acetamiprid 20% w/v SL)
Batch #	659-030314-01
Content of active substance	acetamiprid: 199.2 ± 1.3 g/L, (nominal: 200 g/L)
Control	Tap water
Toxic reference	Deltamethrin 2.5% EC at 0.05% = 7.65 g a.s./ha
Test organism	
Species	Parasitic wasp <i>Aphidius rhopalosiphi</i> Adults, < 48 h old
Source	Katz Biotech AG”, An der Birkenpfuhlheide 10, 15837 Baruth, Germany

Study design and methods

Ageing periods	1, 28, 36 and 42 days
Exposure duration	48 hours
Experimental dates	30 Nov 2015 to 25 Jan 2016
Test doses (nominal)	102 g acetamiprid/ha, equivalent to 0.5120 L/ha of formulated test item
Test units	Three plots with approximately 68 potted plants per plot (2 bean plants per pot, <i>Phaseolus vulgaris</i> , variety ROMA) were selected: One plot for the water treated control, one plot for the test item and one plot for the toxic reference. Plot size was 5 m ² (5 m × 1 m) for the treatments and they were arranged in two crop rows (0.5 m to each other).
Group size/replicates	10 adults (≥ 5 females) per replicate, 4 replicates (40 adults) per treatment
Experimental treatments	The dose of the test item (102 g a.s./ha) was applied once in the field using a compressed air knapsack sprayer equipped with a spray bar and 2 nozzles (Black Hardi 4110-14 Flat fan) with 50 cm distance, simulating a commercial field application in field (volume 600 L/ha). After application, plants were maintained under field conditions in a tunnel, equipped with a UV-permeable plastic roof and opened laterals to provide natural aging conditions, except washing-off by rain.
Environmental conditions	
Temperature	1DAA: 19.6–20.5 °C 28 DAA: 19.6–21.0 °C 36 DAA: 19.0–20.6 °C 42 DAA: 18.4–20.7 °C
Photoperiod	16 h light (4000–20000 lx) : 8 h dark
Relative humidity	1DAA: 64.5–90.7% 28 DAA: 62.9–90.7% 36 DAA: 56.7–91.3% 42 DAA: 50.8–90.4%

Biological observations

Assessments of mortality: After each ageing period, at least 8 leaves per plot were sampled at random and transported to the laboratory to prepare the test arenas. These were built by transparent plastic tubes which sides were closed by Petri-dishes with punched out leaf discs facing towards each other. Then, 10 adult wasps (at least 5 females) were placed in each arena (excised leaf test units) with 4 replicates per treatment. Arenas were ventilated with an air pump and wasps were sufficiently provided with food and water. Mortality assessments (bioassays) were performed 1, 28, 36 and 42 days after application (DAA). The test units were placed into an environmental chamber between 19.0–21.0°C and 56.7–91.3% RH, with a 16:8 h L:D photoperiod. Mortality assessments were carried out after 24 and 48 hours of exposure. Adult mortality after an exposure of 48 hours (lethal effect) to residues on leaves aged for 1, 28, 36 and 42 days after application (DAA).

Assessments of fecundity: If after 48 hours the corrected mortality was ≤ 50% and at least 15 females were survived in the test item group, which was the case after 42 days of ageing, the reproductive capacity was assessed in the control and test item group confining 15 females individually over untreated barley plants infested with the host cereal aphids, *Rhopalosiphum padi*. The females were removed after 24 hours and the aphid-infested plants were left for a further 10–11 days before the numbers of aphid mummies that had developed were assessed. The test units were placed into an environmental chamber between 18.4–20.5 °C and with a 16:8 h L:D photoperiod. It was not considered necessary to regulate humidity during the reproduction phases.

Fecundity of 15 surviving females during 24 hours in presence of their host aphids for the aforementioned ageing periods when mean mortality in the test item group was ≤ 50%.

Statistics

The statistical management of data was conducted according to the OECD guideline number 54 (OECD series on testing and assessment) and the appropriate Trialcamp SOP. All the statistical analysis were performed using the software IBM© SPSS Statistics 19.0.

Results and discussion

Biological results – mortality

Based on mortalities being less than 13% at the end of all exposure periods, reproductive performances above 5 mummies per female at the fecundity assessments 42 DAA in the control and a corrected mortality greater than 50% in the toxic reference, the sensitivity of the test species and the suitability of the test system was confirmed and the study can be regarded to be valid.

The mortality results are presented in the following table.

Table A 180: Effects of MCW-2222 (Acetamiprid 20% w/v SL) on mortality of the parasitoid, *Aphidius rhopalosiphi*

	Rate ¹⁾ [g a.s./ha]	Bioassay							
		1 DAA ²⁾		28 DAA		36 DAA		42 DAA	
		M (%)	C _m (%)	M (%)	C _m (%)	M (%)	C _m (%)	M (%) ³⁾	C _m (%)
Control	0	0.0	-	0.0	-	0.0	-	2.5	-
MCW-2222 (Acetamiprid 20% w/v SL)	102	100 ^{SD}	100	75.0 ^{SD}	75.0	42.5 ^{SD}	42.5	25.0 ^{NS}	23.1
Reference Item (Deltamethrin 2.5% EC)	7.65	100	100	70.0	70.0	85.0	85.0	70.0	69.2

¹⁾ Application rate in 600 L water/ha

²⁾ DAA = Days after application; M [%] = Mortality [%]; C_m [%] = Corrected mortality [%].

³⁾ Signs of intoxication (lack of coordination) were observed on 6.7% of survivors in the test treatment at 42 DAA.

SD = statistically significant different compared to the control (Mann-Whitney test, exact sig., 1-tailed greater, $\alpha=0.05$).

NS = not statistically significant different compared to the control (Mann-Whitney test, exact sig., 1-tailed greater, $\alpha=0.05$).

The reference treatment was not statistically analysed.

Biological results – fecundity

After an ageing period of 36 and 42 days corrected mortality was less than 50%, i.e. 42.5% and 23.1%. A lethal effect higher than 50 % was observed in the exposures assessment started at 1 and 28 DAA with 100% and 75.0% corrected mortality, respectively.

Mortality in the test group was statistically significant higher in the test substance group at the assessment started on 1, 28 and 36DAA (Mann-Whitney test, exact sig., 1-tailed, $\alpha=0.05$) but not on 42 DAA (Mann-Whitney test, exact sig., 1-tailed, $\alpha=0.05$).

The fecundity results (mummies per female and progeny reduction) are presented in the following table.

Table A 181: Effects of MCW-2222 (Acetamiprid 20% w/v SL) on fecundity of the parasitoid, *Aphidius rhopalosiphi*

	Rate ¹⁾ [g a.s./ha]	Bioassay							
		1 DAA ²⁾		28 DAA		36 DAA		42 DAA	
		F [m/f]	R [%]	F [m/f]	R [%]	F [m/f]	R [%]	F [m/f]	R [%]
Control	0	N/S		N/S		N/S		69.5	-
MCW-2222 (Acetamiprid 20% w/v SL)	102	N/S		N/S		N/S		61.4 ^{NS}	11.6

¹⁾ Application rate in 600 L water/ha

²⁾ DAA = Days after application; ; F [m/f]= Fecundity [mummies per female]; R [%]= Reduction [%].

N/S = Reproduction was not studied as mortality was > 50% in T.

NS = fecundity was not statistically significant different compared to the control (T-Test, $\alpha=0.05$).

Reproduction performance was not statistically significant affected (T-test, $\alpha=0.05$) by 42-day old residues; reduction of reproduction relative amounted to be 11.6% and was therefore below the ESCORT 2 trigger value of 50%.

Validity criteria

As shown in the following table, all validity criteria were met.

Table A 182: Validity criteria

Validity criteria according to Mead-Briggs <i>et al.</i>	Observed in study
Mortality in the control should not exceed 13%	0%
Mortality in the reference should range between 50% - 100%	$\geq 70\%$
Wasps in the control should produce ≥ 5 mummies per female	≥ 69.5
Not more than two wasps should produce no mummies	0.0 %

Conclusion

Based on the results of this study performed on *Aphidius rhopalosiphi* after the application of “MCW-2222” (Acetamiprid 20% w/v SL) at a rate of 102 g acetamiprid /ha it can be concluded that 36 days old residues will not adversely affect mortality and 42 days old residues will not impact reproduction.

A 2.3.2.3.5 KCP 10.3.2.3/05 Aged residue study with *Aphidius rhopalosiphi*

Comments of zRMS:	<p>The aged residue study on effects to <i>A. rhopalosiphi</i> has been submitted in support of the re-evaluation of CA3573 (formerly MCW-2222) due to renewal of acetamiprid in 2021. . The validation of the study was thus performed by the zRMS.</p> <p>The study was carried out in line with the respective guideline with no major deviations.</p> <p>It is noted that the maximum relative humidity (99.8-99.7%) was above the maximum 90% recommended by the guideline. However, as all validity criteria were met, this deviation is considered to have no impact on the test results.</p> <p>In this particular study, potted apple branches were used (3-D system), whereas bean leaves were used in remaining aged residue studies. Nevertheless, the study was performed with 6 replicates containing 5 females each (i.e. relevant for limit test), so results of this study may be considered as independent from remaining studies and representative for uses in apples.</p> <p>Overall, the study is considered acceptable.</p> <p>Obtained results demonstrated that lethal and sub-lethal effects on <i>A. rhopalosiphi</i> following application of CA3573 at 170 g a.s./ha are <50% after 42 days of aging.</p>
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Data point	KCP 10.3.2.3/05
Report	Aged residue test with the formulation “MCW-2222” at 170 g a.s./ha on the parasitic wasp <i>Aphidius rhopalosiphi</i> (Hymenoptera: Braconidae), Luna, F., 2017a
Report No.:	TRC16-073BA
Document No.:	R-37333
Guideline(s):	Mead-Briggs <i>et al.</i> 2010
Previous evaluation	Yes ,previously submitted
GLP:	Yes, certified laboratory
Acceptability:	Yes, study considered acceptable
Duplication	Not applicable

(if vertebrate study)

Executive Summary

To determine the extent and persistence of effects on mortality and fecundity on the parasitoid *Aphidius rhopalosiphi* after the application of 170 g acetamiprid/ha (equivalent to 0.8289 L test item/ha) an aged residue study was performed. Potted apple plants were treated and maintained under outdoor conditions to provide natural aging conditions, except washing-off by rain. Assessments were performed with residues aged for 0, 42 and 49 days.

Whereas the tested rate exceeded the mortality threshold of 50% (50% corrected mortality compared to the control) after the exposure to fresh and dry residues (0 days old residues), lethal and sub-lethal effects, i.e. fecundity, less than 50% were observed after exposure to residues aged for 42 and 49 days.

Based on the results of this study performed on *Aphidius rhopalosiphi* after the application of “MCW-2222” (Acetamiprid 20% w/v SL) at a rate of 170 g acetamiprid/ha it can be concluded that 42 days old residues will not adversely affect mortality and will not impact reproduction (less than 50% reduction).

Materials and methods

Materials

Test item	MCW-2222 (Acetamiprid 20% w/v SL)
Batch #	811-021115-01
Content of active substance	acetamiprid: 205.1 ± 1.1 g/L, (nominal: 200 g/L)
Control	Tap water
Toxic reference	Deltamethrin 2.5% EC at 1.15 L test item/ha = 29.325 g a.s./ha
Test organism	
Species	Parasitic wasp <i>Aphidius rhopalosiphi</i> Adults, < 48 h old
Source	Katz Biotech AG”, An der Birkenpfehlheide 10, 15837 Baruth, Germany

Study design and methods

Ageing periods	0, 42 and 49 days
Exposure duration	48 hours
Experimental dates	12 Jul 2016 to 12 Sep 2016
Test doses (nominal)	170 g acetamiprid/ha, equivalent to 0.8289 L/ha of formulated test item
Test units	Apple plants (<i>Malus domestica</i>) of the variety GOLDEN were used for trials purpose. Three plots with 17 potted plants per plot were used: One plot for the water treated control, one plot for the test item and one plot for the toxic reference. Plot size was 20 m ² (10 m × 2 m) for the treatments and they were arranged in two rows (0.5 m to each other).
Group size/replicates	5 females per replicate, 6 replicates (30 adults) per treatment
Experimental treatments	The dose of the test item (170 g a.s./ha) was applied once in the field using a backpack mist blower simulating a commercial field application at a volume of 2000 L/ha in order to spray to the point of runoff (“thoroughly wet”). After application, plants were maintained under outdoor conditions in an opened greenhouse, equipped with a polycarbonate roof closed only when it rains and opened laterals to provide natural aging conditions, except washing-off by rain. The reference product was applied once at the same time as the test item.

Environmental conditions

Temperature	0DAA: 19.4–21.2 °C 42 DAA: 19.6–21.6 °C 49 DAA: 19.7–21.6 °C
Photoperiod	16 h light (432–699 lx mortality phase, 4582–5591 lx parasitisation phase, 9309–10982 lx reproduction phase) : 8 h dark
Relative humidity	1DAA: 74.9–98.9%

42 DAA: 74.1–98.8%
49 DAA: 77.0–99.7%

Biological observations

Assessments of mortality: After each ageing period, 6 small branches with 2–3 leaves were sampled per plot from different plants and transported to the laboratory to prepare the test arenas. The branches were enclosed within clear acrylic cylinders (9 cm in diameter by 20 cm high) with the top covered with wasp-proof netting. Six replicates per treatment were used and 5 adult females were placed in each arena.

Exposures to the residues (bioassays) were performed 0, 42 and 49 days after application (DAA). The test units were placed into an environmental chamber between 20 ± 2 °C (actual between 19.4 and 21.2 °C), 60–90% RH (actual between 74.1 and 99.7%), and with a 16:8 h L:D photoperiod.

Mortality assessments were carried out after 2, 24 and 48 hours of exposure. Repellency assessments were also carried out during the initial 3 h after the release of adults on each exposure with 5 separate sets of observations.

Assessments of fecundity: If after 48 hours the corrected mortality was $\leq 50\%$ and at least 15 females were survived in the test item group, which was the case after 42 and 49 days of ageing, the reproductive capacity was assessed in the control and test item group confining 15 females individually over untreated barley plants infested with the host cereal aphids, *Rhopalosiphum padi*. The females were removed after 24 hours and the aphid-infested plants were left for a further 10 days before the numbers of aphid mummies that had developed were assessed. The test units were placed into an environmental chamber between 19.6 and 21.6 °C and with a 16:8 h L:D photoperiod. It was not considered necessary to regulate humidity during the reproduction phases.

Statistics

Results of mortality, repellency and mummies per female were analysed with the Shapiro-Wilk test for normality of data distribution and with the Levene's test for homoscedasticity (Annex IV). The non-parametric Mann-Whitney test (exact sig., 1-tailed, $\alpha=0.05$) or the parametric T-test with Levene's test for equality of variances ($\alpha=0.05$) were performed in order to study significant differences between the test item treatment and control according to the normality or not of data. No statistical analysis was performed with results in the test reference treatment.

Results and discussion

Biological results – mortality

Based on mortalities being less than 10% at the end of all exposure periods, reproductive performances above 5 mummies per female at the fecundity assessments 42 and 49 DAA in the control and a corrected mortality greater than 50% in the toxic reference until the exposure of 42 DAA, the sensitivity of the test species and the suitability of the test system was confirmed and the study can be regarded to be valid.

The mortality results are presented in the following table.

Table A 183: Effects of MCW-2222 (Acetamiprid 20% w/v SL) on mortality of the parasitoid, *Aphidius rhopalosiphii*

	Rate [g a.s./ha]	Bioassay					
		0 DAA ¹⁾		42 DAA		49 DAA	
		M (%) ²⁾	C _m (%)	M (%) ³⁾	C _m (%)	M (%) ²⁾	C _m (%)
Control	0	3.33	-	6.67	-	6.67	-
MCW-2222 (Acetamiprid 20% w/v SL)	170	100 ^{SD}	100	33.33 ND	28.57	20.00 ^{NS}	14.29
Reference Item (Deltamethrin 2.5% EC)	29.325	100	100	56.67	53.57	20.00	14.29

¹⁾ DAA = Days after application; M [%] = Mortality [%]; C_m [%] = Corrected mortality [%].

²⁾ SD = statistically significant different compared to the control (Mann-Whitney test, exact sig., 1-tailed greater, $\alpha=0.05$).

³⁾ NS = not statistically significant different compared to the control (Mann-Whitney test, exact sig., 1-tailed greater, $\alpha=0.05$).

The reference treatment was not statistically analysed.

Biological results – fecundity

After an ageing period of 42 and 49 days, corrected mortality was less than 50%, i.e. 28.57% and 14.29%, respectively. A lethal effect higher than 50 % was observed in the exposures assessment started at 0 DAA (fresh and dry residues) with 100%.

Mortality in the test group was statistically significant higher in the test substance group at the assessment started on 42 and 49 DAA (Mann-Whitney test, exact sig., 1-tailed, $\alpha=0.05$).

The fecundity results (mummies per female and progeny reduction) are presented in the following table.

Table A 184: Effects of MCW-2222 (Acetamiprid 20% w/v SL) on fecundity of the parasitoid, *Aphidius rhopalosiphi*

	Rate [g a.s./ha]	Bioassay					
		0 DAA1)		42 DAA		49 DAA	
		F [m/f]	R [%]	F [m/f] 2)	R [%]	F [m/f]	R [%] 3)
Control	0	N/S		16.43	-	29.33	-
MCW-2222 (Acetamiprid 20% w/v SL)	170	N/S		9.53SD	41.97	31.80	-8.41

N/S = Reproduction was not studied as mortality was > 50% in T.

1) DAA = Days after application; ; F [m/f]= Fecundity [mummies per female]; R [%]= Reduction [%].

2) SD = statistically significant different compared the control (T-Test, $\alpha=0.05$).

3) Negative value indicates an increase relative to the control.

Reproduction performance was below the ESCORT 2 trigger value of 50% with 42 and 49 days old residues. Reduction on reproduction was 41.97% compared to control with 42 days old residues (less than 50%) and significantly different to control (T-test, $\alpha=0.05$). Reproduction, i.e. fecundity, was not statistically significant affected (T-test, $\alpha=0.05$) by 49 days old residues; reproduction amounted to be higher than in the control treatment (-8.41% reduction).

Validity criteria

As shown in the following table, all validity criteria were met.

Table A 185: Validity criteria

Validity criteria according to Mead-Briggs <i>et al.</i>	Observed in study
Mortality in the control should not exceed 10%	6.67 %
Mortality in the reference should range between 50% - 100%	$\geq 53.57\%$
Wasps in the control should produce ≥ 5 mummies per female	≥ 16.43
Not more than two wasps should produce no mummies	0

Conclusion

Based on the results of this study performed on *Aphidius rhopalosiphi* after the application of “MCW-2222” (Acetamiprid 20% w/v SL) at a rate of 170 g acetamiprid/ha it can be concluded that 42 days old residues will not adversely affect mortality and will not impact reproduction (less than 50% reduction).

A 2.3.2.3.6 KCP 10.3.2.3/06 Aged residue study with *Coccinella septempunctata*

Comments of zRMS:	<p>The aged residue study on effects to <i>C. septempunctata</i> has been submitted in support of the re-evaluation of CA3573 (formerly MCW-2222) in 2021 due to renewal of acetamiprid . The validation of the study was thus performed by the zRMS.</p> <p>The study was carried out in line with the respective guideline. Following deviations were noted:</p> <ol style="list-style-type: none"> 1. In groups exposed to fresh and aged residues the maximum temperature (40.2 and 31.3°C) during mortality phase was clearly above the maximum of 27°C recommended by the guideline, while the minimum temperature (21.5 and 22.5°C, respectively) was below the recommended 25 °C. 2. In group exposed to fresh residues (0 DAA) the minimum relative humidity (35.7%) during mortality phase was clearly below the minimum of 60% recommended by the guideline. 3. In groups exposed to fresh and aged residues the maximum relative humidity (95.6%) was above the maximum of 90% recommended by the guideline. <p>All deviations lasted for more than 2 hours, but all validity criteria were met and for this reason all mentioned deviations are considered to have no impact on test results.</p> <p>It is noted that the mean number of eggs per female per day and mean number of viable eggs per female per day were reduced by more than 50% comparing to control in test groups exposed to residues aged for 42 days. However, according to Haskell & McEwen (1998)⁹ the high variability of reproductive performance of ladybird beetles is observed in laboratory tests. Differences between individuals or subgroups in the control treatments are greater than 30%. The available data show that in glass-plates tests groups of ladybird females produce between 2 and 10 fertile eggs per female per day over a 5-week period following metamorphosis. The same number was obtained in extended laboratory studies performed on bean leaves. Therefore, it is proposed that for regulatory purposes the effect is considered as treatment related when it falls below the lower limit of these ranges (i.e. below 2). The same is proposed in guideline of Schmuck et al. (2000), which states that due to the high variability, the reproductive performance of this species may be evaluated only qualitatively. Furthermore, it should be also noted that >50% reduction in reproductive capacity was observed only in groups exposed to residues aged for 42 days and no such a reduction was observed in groups exposed to residues aged for shorter period of time or exposed to fresh residues at 102 g a.s./ha. Taking this into account it seems to be highly unlikely that residues aged for longer period of time would have more pronounced adverse effects than fresh residues and the observed reduction seems to be rather due to unexpectedly high production of eggs in controls.</p> <p>Overall, the study is considered acceptable.</p> <p>Obtained results demonstrated that lethal and sub-lethal effects on <i>C. septempunctata</i> following application of CA3573 at 102 g a.s./ha are <50% for both aging periods and after exposure to fresh residues. In case of higher application rate (170 g a.s./ha) effects were at acceptable level after 35 days of aging.</p>
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⁹ Haskell P.T., McEwen P. (1998): Pesticides and beneficial organisms. Springer-Science+Business media B.V.

Data point	KCP 10.3.2.3/06
Report	Aged residue test with the formulation “MCW-2222” on <i>Coccinella septempunctata</i> L (Coleoptera:Coccinellidae), Luna, F., 2017c
Report No.:	TRC16-075BA
Document No.:	R-37334
Guideline(s):	Schmuck <i>et al.</i> 2000
Previous evaluation	Yes, previously submitted
GLP:	Yes, certified laboratory
Acceptability:	Yes, study considered acceptable
Duplication (if vertebrate study)	Not applicable

Executive Summary

To determine the extent and persistence of effects on mortality and reproductive capacity on the aphid predatory *Coccinella septempunctata* L. after the application of 102 and 170 g a.s./ha (equivalent to 0.4973 and 0.8289 L test item/ha respectively) an aged residue study was performed. Potted apple plants were treated and maintained under outdoor conditions to provide natural aging conditions, except washing-off by rain. Assessments were performed with residues aged for 0, 35 and 42 days.

Lethal effects less than the threshold of 50% (50% effect compared to the control) were observed after exposure to 0, 35 and 42-day old residues with the tested rate of 102 g a.s./ha of the test item. Mortality in the test item group of the rate 170 g a.s./ha was higher than 50% (61.54% corrected mortality) at the assessment started with fresh and dry residues (exposure at 0 DDA).

Significant differences compared to control (Fisher's exact Test) with mortality results were observed in the exposure of 0 day old residues (fresh and dry residues) at the tested rates of 102 and 170 g a.s./ha, and no significant lethal effects were recorded after exposure to residues aged for 35 and 42 days.

Based on the results of the present study it can be concluded that residues of the test item “MCW-2222” (Acetamiprid 20 % w/v SL) applied up to the rate of 170 g a.s./ha causes mortality less than 50% compared to the control and has less than 50% reduction on the reproduction of *Coccinella septempunctata* from 42 day of the application.

Materials and methods

Materials

Test item	MCW-2222 (Acetamiprid 20% w/v SL)
Batch #	811-021115-01
Content of active substance	acetamiprid: 205.1 ± 1.1 g/L, (nominal: 200 g/L)
Control	Tap water
Toxic reference	Deltamethrin 2.5% EC at 1.15 L test item/ha = 29.325 g a.s./ha
Test organism	
Species	Aphid predatory <i>Coccinella septempunctata</i> Larvae, 3–4 days old
Source	Katz Biotech AG”, An der Birkenpfuhlheide 10, 15837 Baruth, Germany

Study design and methods

Ageing periods	0, 35 and 42 days
Experimental dates	12 Jul 2016 to 4 Oct 2016
Test doses (nominal)	102 and 170 g acetamiprid/ha, equivalent to 0.4973 and 0.8289 L/ha of formulated test item
Test units	Apple plants (<i>Malus domestica</i>) of the variety GOLDEN were used for trials purpose. Three plots with 17 potted plants per plot were used: One plot for the water treated control, one plot for the test item and one plot for the toxic reference. Potted apple plants between 1.5–1.8 m height and 0.4–0.5 m canopy were used for trial purpose. Plot size was

	20 m ² (10 m × 2 m) for the treatments and they were arranged in one row (0.5 m distance between plants and 2 m row spacing).
Group size/replicates	40 larvae per treatment
Experimental treatments	Application was performed using a backpack mist blower simulating a commercial field application at a volume of 2000 L/ha in order to spray to the point of runoff (“thoroughly wet”). After application, plants were maintained under outdoor conditions in an opened greenhouse, equipped with a polycarbonate roof closed only when it rains and opened laterals to provide natural aging conditions, except washing-off by rain. The reference product was applied once at the same time as the test item.
Environmental conditions	
Temperature	0DAA: 21.5–40.2 °C 35 DAA: 22.5–31.3 °C 42 DAA: 22.5–31.3 °C
Photoperiod	16 h light (1467–3224 lx mortality phase, 1391–4751 lx reproduction phase) : 8 h dark
Relative humidity	0DAA: 35.7–95.6% 35 DAA: 72.9–95.6% 42 DAA: 72.6–95.6%

Biological observations

Assessments of mortality: After each ageing period, at least 40 leaves were sampled per plot from different plants and transported to the laboratory to prepare the test arenas. Larvae of *Coccinella septempunctata* L. (3–5 days old) were isolated and exposed to the differently aged residues on leaves. The larvae were continuously exposed to the residue on the leaves until they moulted to adults. Forty larvae per treatment were individually confined within test units.

Exposures to the residues (bioassays) were performed 0, 35 and 42 days after application (DAA). The test units were placed into an environmental chamber between 25 ± 2 °C (actual between 24.5 and 40.2 °C), 60–90% RH (actual between 35.7 and 95.6%), and with a 16:8 h L:D photoperiod. Temperature was registered with values greater than 27 °C and humidity with values below 60% during more than 2 hours continuously (16 Jul to 18 Jul 2016 at the mortality period of the exposure at 0 DAA) although without negative effects in the study.

Mortality assessments were carried out daily except weekends and the number of dead larvae/pupae was recorded together. Pupation and hatching of the adults were recorded. The number of dead larvae and the number of pupae that fail to develop into adults were combined and the value used to calculate the total juvenile mortality

Assessments of fecundity: The sub-lethal effects on the reproductive performance of the emerging adults was evaluated when possible (corrected mortality < 50 %), with 8 synchronizations of egg laying (24 h periods) in two weeks to calculate the eggs per female and day (fecundity rate) and the larvae emerging from eggs to calculate the percentage of viable eggs (fertility rate). It was not possible with the test item group of the rate 170 g a.s./ha in the exposure to fresh and dry residues (0 DAA).

Statistics

Statistical analysis was performed with data mortality in order to study any significant differences compared to control with the statistic Fisher’s exact test (Crosstabs, $\alpha=0.05$). The reproductive performance data were not analysed; the obtained value with fecundity and fertility were compared to the threshold values for control: 2 viable (or fertile) eggs/female/day.

No statistical analysis was performed with results in the test reference treatment.

Results and discussion

Based on mortalities being less than 30% at the end of all exposure periods (actual maximum value was 5.0% in the exposure of 42 DDA), reproductive performances above 2 fertile eggs per female per day at the

fecundity assessment 0, 35 and 42 DAA in the control (actual minimum value was 10.32 fertile eggs per female in the exposure of 35 DDA) and a corrected mortality greater than 40% in the toxic reference in the exposures of 0 and 35 DAA (100 and 42.5% corrected mortality, respectively), the sensitivity of the test species and the suitability of the test system was confirmed and the study can be regarded to be valid.

Biological results – mortality

With fresh and dry residues (exposure of 0 DAA) and after the ageing periods of 35 and 42 days of the test item at the rate of 102 g a.s./ha, corrected mortality was less than 50% i.e. 48.72, 5.26 and 2.83%, respectively. Statistically significant different to control was the mortality obtained with fresh and dry residues (Fisher's exact Test, 1-sided, $\alpha=0.05$).

Mortality in the test item group of the rate 170 g a.s./ha was higher than 50% (61.54% corrected mortality) at the assessment started with fresh and dry residues (exposure of 0 DAA) and statistically significant higher than control (Fisher's exact Test, 1-sided, $\alpha=0.05$). No lethal effects were observed in the exposures of 35 and 42 DAA; 5.13 and 3.05% corrected mortality, respectively.

The mortality results are presented in the following table.

Table A 186: Effects of MCW-2222 (Acetamiprid 20% w/v SL) on mortality of *Coccinella septempunctata*

	Rate [g a.s./ha]	Exposure					
		0 DAA ¹⁾		35 DAA		42 DAA	
		M (%) ²⁾	C _m (%)	M (%)	C _m (%)	M (%)	C _m (%)
Control	0	0.00	-	0.00	-	5.00	-
MCW-2222 (Acetamiprid 20% w/v SL)	102	48.72 ^{SD}	48.72	5.26	5.26	7.69	2.83
	170	61.54 ^{SD}	61.54	5.13	5.13	7.89	3.05
Reference Item (Deltamethrin 2.5% EC)	29.325	100	100	42.50	42.50	21.62	17.50

¹⁾ DAA = Days after application; M [%] = Mortality [%]; C_m [%] = Corrected mortality [%].

²⁾ SD = statistically significant different compared to the control (T-test, $\alpha=0.05$).

Biological results – fecundity

Reproduction performance was studied for the rate of 102 g a.s./ha with 0, 35 and 42 days old residues and for the rate of 170 g a.s./ha with 35 and 42 days old residues. As the reproductive output was above 2 fertile eggs per female per day when it was possible to study this parameter, no effect on the reproduction capacity is considered to have had the test item with the tested rates when mortality was less than 50%.

The fecundity results (mummies per female and progeny reduction) are presented in the following table.

Table A 187: Effects of MCW-2222 (Acetamiprid 20% w/v SL) on fecundity of *Coccinella septempunctata*

	Rate [g a.s./ha]	Exposure		
		0 DAA ¹⁾	35 DAA	42 DAA
		[Fertile.eggs per female per day]	[Fertile.eggs per female per day]	[Fertile.eggs per female per day]
Control	0	15.33	10.32	58.67
MCW-2222 (Acetamiprid 20% w/v SL)	102	25.77	10.43	24.20
	170	Not assayed ²⁾	11.98	19.94

¹⁾ DAA = Days after application.

²⁾ Reproduction capacity was not assessed when corrected juvenile mortality with the test item was higher than 50%.

Effects of MCW-2222 (Acetamiprid 20% w/v SL) on fertility of *Coccinella septempunctata*

	Rate [g a.s./ha]	Exposure		
		0 DAA ¹⁾	35 DAA	42 DAA
		Mean eggs viability [%]		
Control	0	91.76	97.93	98.08
MCW-2222 (Acetamiprid 20% w/v SL)	102	96.62	89.43	97.24
	170	Not assayed ²⁾	93.93	95.71

¹⁾ DAA = Days after application.
²⁾ Reproduction capacity was not assessed when corrected juvenile mortality with the test item was higher than 50%.

More than 2 fertile eggs per female per day is considered a normal reproductive output for the control treatment, so the test item is considered harmless in reproduction when these results are obtained.

Validity criteria

As shown in the following table, all validity criteria were met.

Table A 188: Validity criteria

Validity criteria according to Schmuck <i>et al.</i>	Observed in study
Cumulative mortality in the control should not exceed 30%	5%
Mean number of eggs per viable female per day should be ≥ 2	≥ 10.32
Mortality in the reference treatment should be $\geq 40\%$	100% at 0 DAA, 42.50% at 35 DAA, 17.5% at 42 DAA ¹⁾

¹⁾ Validity criterion regarding mortality in toxic standard group relevant only for 0 DAA (test guideline does not provide validity criteria for particular aging periods)

Conclusion

Based on the results of this study performed on *Coccinella septempunctata* after the application of “MCW-2222” (Acetamiprid 20 % w/v SL) it can be concluded that at a rate of 102 g a.s./ha with fresh and dry residues (0 day old residues) will not cause mortality greater than 50% and will not impact reproduction, and a rate of 170 g a.s./ha will not adversely affect mortality and will not impact reproduction after 35 days old residues.

A 2.3.2.4 KCP 10.3.2.4 Higher tier testing

Comments of zRMS:	<p>The study has been already evaluated and accepted by the zRMS in the course of the first zonal evaluation of CA3573 (formerly MCW-2222) finalised in April 2018. As the test guideline has not changed since that time, re-evaluation of the study following renewal of acetamiprid was not necessary and presented below conclusions were taken from the Core Assessment, Part B, Section 6 of April 2018. Comments received from particular cMS during the commenting period were also considered.</p> <p>The study was conducted on a meadow in Bisingen, Germany. A meadow was selected because it is representative for off-crop areas. Meadows also have good species composition of plant-(foliage) dwelling arthropods and ground living arthropods.</p> <p>Application scheme in this study included two applications with 6 days interval (only single applications are currently proposed in the Central Zone GAP to all intended crops). Application rates considered in the study covered drift rates for most of crops indicated in the GAP, with exception of the application to apples at 60 g a.s./ha, for which drift rate calculated in line with indications of ESCORT 2 is higher comparing to tested rates. Nevertheless, in case the drift rate exceeds the NOEAER value the risk may be mitigated with buffer zones or drift reducing techniques.</p> <p>Effects classes were determined on the basis of indications of de Jong <i>et al.</i> (2010), with effects class 2 described as “slight and transient effects observed on one occasion only”. Based on indications of the document mentioned, NOER value is usually set as rate at which only effects class 1 and 2 are observed (regardless if effects class 2 are statistically significant), whereas in determination of the NOEAER value effects class 3 (i.e. including recovery) are taken into account.</p> <p><u>Determination of NOER</u></p> <p>In the two lowest treatment levels T4 (0.7 g a.s./ha) and T3 (1.4 g a.s./ha) only effects class 1 and 2 were observed, most of which were considered to be not treatment related due to lack of effects at higher rates.</p> <p>The only exception were statistically significant effects class 3b on <i>Xysticus cochi</i> (Thomisidae) observed at the last sampling point in the lowest treatment group (0.7 g a.s./ha). These effects were, however, considered to be random and not treatment related as effects class 2 were observed on this species in the next higher treatment group (1.4 g a.s./ha) and no effects on this species were seen at the two highest treatment rates.</p> <p>Based on these findings, the NOER from the study was set to 1.4 g a.s./ha, i.e. highest rate tested with only effects class 1 and 2, relevant for determination of NOER.</p> <p><u>Determination of NOEAER</u></p> <p>In line with available guidance documents, NOEAER from a field study is based on application rates at which potential for recovery within ecologically relevant time frame was observed. Hence, in determination of this endpoint effects class 3 are taken into account. In</p>
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	<p>this study, at two highest treatment levels of 3.4 g a.s./ha (T2) and 7.2 g a.s./ha (T1) effects class 2 were observed on majority of species, however treatment related effects class 3a and 3b were seen on <i>Aphidoidea</i> in T2 and T1, respectively. Furthermore, effects class 8 were seen on <i>Thysanoptera</i> juveniles in the highest treatment group T1. Based on these findings the NOEAER from this study was set to 3.4 g a.s./ha, i.e. the highest rate with significant effects lasting more than 2 weeks followed by recovery observed in the course of the trial.</p> <p>The Applicants' summary below has been supplemented with more extent information taken from the Core Assessment, Part B, Section 6 of April 2018.</p> <p>The zRMS would like also to point out that summary Table A 154 presented below is limited to species/families/subfamilies for which any effects were seen in the course of the trial (treatment and non-treatment related) as due to very high number of arthropods caught in this study it was not possible to provide tables with abundance of all species caught at particular sampling points using different techniques, e.g. in the study report taxonomic and statistical data only for Vortis Suction Sampling are presented on 17 pages and graphs presenting total caught for each taxonomic group/species are presented on 35 pages. Therefore, in order to check results of the study in more detail, the cMS must request the study report from the Applicant.</p> <p>It is noted that in the laboratory studies <i>Aphidius rhopalosphi</i> was particularly sensitive to acetamiprid in CA3573. However, during the field study family <i>Braconidae</i> (parasitoid wasps) was present on the study plots and no effects of the treatment with CA3573 were observed. The only statistically significant and treatment-related effects were seen in the toxic standard group, confirming that the design the study was sufficient to detect effects on these sensitive insects.</p>
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Data point	KCP 10.3.2.4/01
Report	A Field Study Assessing the Impact of Drift Rates of Acetamiprid on the Non-Target Arthropod Fauna on a Meadow in Germany, Appeltauer, A., 2016
Report No.:	S15-01184
Document No.:	R-35848
Guideline(s):	Candolfi et al. (2000); De Jong <i>et al.</i> (2010); Alix et al., 2011
Previous evaluation	No, not previously submitted
GLP:	Yes, certified laboratory
Acceptability:	Yes, study considered acceptable
Duplication (if vertebrate study)	Not applicable

Executive Summary

Overall based on statistical analyses, the effects of acetamiprid (formulated as MCW- 2222) applied twice to an off-crop meadow arthropod fauna are classified as follows:

At the population level several taxa were considered adversely affected by treatment with acetamiprid at the rates T1 (7.2 g a.s./ha), T2 (3.4 g a.s./ha) and T3 (1.4 g a.s./ha). For the rate T1 one taxon (juvenile specimens of the order Thysanoptera) did not recover within the assessed sampling period of 27 days after the 2nd application. Therefore the rate T1 (7.2 g a.s./ha) is the population LOEAER (Lowest Observed Ecologically Adverse Effect Rate). For all other test item treatments statistically significant adverse population effects of single taxa were observed to be transient with recovery until the end of the study period. Therefore, the rate T2 (3.6 g a.s./ha) is classified as the population NOEAER (No Observed Ecologically Adverse Effect Rate). Based on the multivariate analysis of the community the PRC did not display statistically significant adverse effects up to and including the highest test item rate T1 (7.2 g a.s./ha) until the end of the study period. Thus, this rate is classified as the community NOER (No Observed Effect

Rate).

Materials and methods

Materials

Test item	MCW-2222
Batch #	659-030314-01
Content of active substance	Acetamiprid 200 g/L (nominal); 199.2 g/L (analysed)
Description	Liquid / clear yellow to brown
Control	Water
Toxic reference	Dimethoate (content: 400 g/L)
Test organism	
Species	Naturally occurring populations of arthropods in the meadow at the field site
Source	Not applicable
Study design and methods	The field study was carried out on a meadow in Bisingen, Germany, and was in compliance with the 'Principles for regulatory testing and interpretation of the semi-field and field studies with non-target arthropods' (CANDOLFI et al., 2000) and the 'Guidance for summarizing and evaluating field studies with nontarget arthropods' (DE JONG et al., 2010). The study consisted of one field trial, (S15-01184-01), and one taxonomic phase, (S15-01184-02). The first sampling was 2 to 3 days before the 1 st application and the final sampling was 27 days after the 2 nd application.

Four different sampling methods were used: Pitfall traps, Photoelectors, ground Foliage/Litter sampling (extracted using a high temperature gradient extractor) and Vortis suction sampling. Pitfall trap, Photoelector and Vortis suction samplings were performed six times, each.

Foliage/Litter sampling was performed four times. On 14 June 2015, a visual assessment of vegetation was performed to assess the effect of plant species distribution on the arthropod distribution.

The trial included four test item groups with MCW-2222 (T1, T2, T3, T4), a water treated control and a reference item treatment (Danadim Progress) with two applications each to assess the sensitivity of the test system. All treatments comprised four plots (replicates) of about 900 m² each.

Test duration and exposure	27 days after 2 nd application (6 days between 1 st and 2 nd application)
Experimental dates	15 May to 24 June 2015
Test rates	C = tap water treated control T1 = MCW-2222 (36 mL product/ha; 7.2 g a.s./ha nominal) T2 = MCW-2222 (17 mL product/ha; 3.4 g a.s./ha nominal) T3 = MCW-2222 (7 mL product/ha; 1.4 g a.s./ha nominal) T4 = MCW-2222 (3.5 mL product/ha; 0.7 g a.s./ha nominal) R = Danadim Progress (dimethoate 400 g/L) (4 L product/ha; 1600 g a.s./ha nominal)
	Both applications were conducted with a spray volume of 100 L water/ha at an interval of 6 days

Group size/replicates	Four plots (replicates) per treatment each replicate of about 900 m ²
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Test medium	Not applicable
Environmental conditions	The climatic conditions during the trial compared to the long term average (1961-1990) revealed slightly higher average temperatures for May and June 2015. The rainfall at the field site was about 112 % and 52 % of the long-term average in May and June, recorded at a weather station approximately 11 km distance from the field site. According to SCHUBERT et al. (2001) the field site was classified as a cultivated pasture (Molinio-Arrhenatheretum).

Endpoints

The study was designed to determine a NOER (No observed effect rate) and NOEAER (No observed ecologically adverse effect rate)/LOEAER (Lowest observed ecologically adverse effect rate) value for the arthropod community and populations of individual taxa. The NOER is the highest test rate where no statistically significant differences to the control occurred. The NOEAER is defined as the highest test rate where at least 1 taxon with effect class 2 (i.e. clear response to treatment, but with recovery within 2 weeks after last application) is observed.

The LOEAER is defined as the lowest test rate for which at least 1 taxon had a statistically significant adverse response to treatment, lasting more than 2 weeks after last application.

Evaluation

For the evaluations of results univariate statistics (two sided Dunnett's t-test for the test item treatments; pooled t-test, Satterthwaite t-test or two sided Wilcoxon test) and multivariate analysis (Principle response curves PRC) were used. Univariate analysis was applied to abundances on individual taxon level, higher taxonomic groups and total abundance for each sampling occasion and sampling type.

The multivariate analysis was applied on individual taxon level and higher taxonomic groups for each sampling type and sampling date.

Prior to multivariate analyses of the entire arthropod datasets of each sampling method, the relation between arthropod distributions before treatment and vegetation structure was examined to decide whether vegetation data should be included as a covariable in the final model.

Based on the detected statistically significant differences, density graphs and expert judgment, effect classes according to DE JONG et al. (2010) are assigned to all taxa evaluated statistically, and summarised in a taxon classification table.

For this report, regarding a post-application sampling period of 27 days, only effect classes 2 (effects observed on one occasion only), 3a (clear response of taxa, recovery within one month after application; no effects observed on the last two sampling occasions) and 3b (clear response of taxa, recovery within one month after application; no effects observed on the last sampling occasion) and 8 (effects observed at two sampling occasions, no recovery within the study period) are applicable.

Results and discussion

In total 1,205,510 arthropods were caught in this study and identified. Data were analysed by multivariate (i.e. Principal Response Curves (PRCs), to evaluate effects on the community level) and univariate analysis. The total number of arthropods caught by different sampling methods in the different treatment during the study period is given in table below.

	Number of arthropods						Total
	C	T1	T2	T3	T4	R	
Pitfall traps	3462	3760	3410	3353	3527	1869	19381
Photoeclector	2197	2119	1927	2909	2196	1335	12683
Foliage/Litter	7257	5508	6718	7317	8546	5915	41261
Vortis	207420	235228	216956	226004	202440	44137	1132185
Total	220336	246615	229011	239583	216709	53256	1205510

The applied reference item (applied on the same days as the test item; Danadim Progress (dimethoate 400 g/L) at a rate of 4 L/ha, equivalent to 1600 g a.i./ha) gave a reduction for a number of arthropods and a change in diversity of the community for all sampling types. In the Pitfall traps of the 32 taxa analysed (including the total catch) 27 showed a statistically significant reduction of > 50%, including the total number of arthropods caught. In the Photoeclector assessments, of the 42 analysed (including the total catch) 24 taxa were statistically significantly reduced for > 50%. In the Vortis suction samples of 72 analysed taxa (including the total catch) 56 showed a statistically significant reduction of abundances of > 50%. For Foliage/Litter sampling, of 25 taxa analysed seven taxa showed statistically significant reductions of > 50% for the reference item treatment.

The PRCs confirmed a statistically significant influence of the reference item treatment for the Pitfall traps, the Photoeclectors and the Vortis suction sampling on the arthropod community. Therefore, the reference substance proved that the test system was sensitive to the application of an insecticide.

According to the multivariate analysis (PRC) the four test item treatments had no statistically significant impact on the ground- and plant-living arthropod communities within the Pitfall traps, Photoeclectors, Vortis suction sampling and Foliage/Litter sampling. Most of the variation was based on population dynamics due to seasonal changes, causing fluctuations in species composition of communities.

The summary of the Principal Response Curve (PRC) for the four sampling methods evaluated by multivariate analysis revealed that 81.7 – 87.5 % of the total variation was not related to treatment but was either due to time (seasonal changes) or can be classified as random. 12.5 – 18.3 % of the variation was treatment related.

The results for the univariate statistics are discussed further in the following parts, since the results are much more detailed.

Pitfall Traps

The number of individuals caught with this kind of trap depends on the activity of the animals, as well as on the abundance. There were six samplings performed during the study period. The 1st sampling was taken 2 days before the 1st application and the succeeding samplings 5, 10, 15, 20 and 27 days after the 2nd application, respectively. Abundances of total arthropods collected with Pitfall traps showed no statistically significant effects of the four test item treatments when compared to the control.

Of the 32 taxa analysed eight showed a statistically significant reduction ($p \leq 0.05$) between at least one test item treatment and the control (for details see below). Statistically significantly higher numbers were found for three taxa.

An important group of the ground-dwelling arthropod community caught in Pitfall traps are the spiders (Araneae) with their ecological function as predators on many other soil surface inhabiting species. Abundances of total spiders were statistically significantly increased in test item treatment T1 at the 3rd sampling (10DAA2). Numbers were already higher at the 2nd sampling (5DAA2) though not to a statistically significant extent. However, it was only a short-term effect at a single sampling date and with comparable numbers to the control from the following sampling on. This single short-term effect in test item treatment T1 is possibly an indirect treatment related effect, but can also be due to natural population dynamics.

The Lycosidae subfamily Lycosinae showed a statistically significantly lower number in test item treatment T3 compared to the control at the 5th sampling (20DAA2). As no statistically significant differences occurred in former samplings or in the higher test item rates, this was most likely due to chance or normal population dynamics.

Further, the adult specimens of two spider species of the family Lycosidae (wolf spiders) showed statistically significant differences to the control: Abundances of adult specimens of the spider species *Pardosa palustris* were statistically significantly lower compared to the control in test item treatment T3 at the 2nd sampling (5DAA2). No statistically significant differences occurred in the two higher test item rates T1 and T2, though abundances decreased from the 1st to the 2nd sampling (8DBA2 to 5DAA2) in all test

item treatments and were below control level. This could be a slight and transient effect (effect class 2, DE JONG *et al.*, 2010) of the test item treatment as the decline was observed in all four test item rates. However, a recovery was found in the subsequent samplings.

Adult specimens of the Lycosidae species *Pardosa pullata* were statistically significantly higher in test item treatment T4 at the 3rd sampling (10DAA2). No further statistically significant differences occurred in former or later samplings or in the higher test item rates. Further abundances developed similarly to the control in all test item treatments, though on a higher level. The effect was rather due to chance than test item related.

Abundances of the adult specimens of the Thomisidae species *Xysticus kochi* were statistically significantly lower compared to the control in test item treatment T3 at the 5th sampling (20DAA2) and in test item treatment T4 at the 2nd and 5th sampling (5 and 20DAA2). The effect in test item treatment 3 was not likely to be test item related as abundances in former samplings, directly after applications, were comparable to the control and no statistically significant differences occurred in the higher test item rates T1 and T2. In test item treatment T4 abundances were clearly below those observed in the control from the 1st sampling (8DBA2) on followed by numbers around zero until the last sampling (27DAA2). Therefore the statistically significant differences to the control are not supposed to be treatment related, but might be due to normal population dynamics.

The order Coleoptera (beetles) accounted for 54.4 % of the class Insecta caught by Pitfall traps in the control. Abundances of total Insecta and total Coleoptera were decreased to a statistically significant extent in the highest test rate T1 at the 2nd sampling (5DAA2). From the 3rd sampling (10DAA2) on numbers were comparable to the control again. This effect could be test item related as abundances in the other test item treatments were also below control level at the 2nd sampling.

The Coleoptera suborder Polyphaga was decreased to a statistically significant extent in test item treatment T2 at the 2nd sampling (5DAA2) and in test item treatment T1 at the 2nd, 3rd and 5th sampling (5, 10 and 20DAA2). Abundances in the test item treatments followed a dose response pattern and effects are therefore classified as test item related. A recovery was observed until the end of the study (27DAA2).

For the Chrysomelidae (leaf beetles) subfamily Alticinae (flea beetles) a statistically significant reduction of numbers was observed for test item treatment T1 at the 2nd and 4th sampling (5 and 15DAA2) and for the lower test rates T2, T3 and T4 at the 2nd sampling (5DAA2). For test item treatment T1 a recovery was observed at the last sampling (27DAA2) and for test rates T2, T3 and T4 at the 3rd sampling (10DAA2). No further statistically significant differences to the control occurred until the end of the study (27DAA2). These effects are test item related. However, the Alticinae comprise a lot of pest beetles and are therefore a target taxonomical group for the test item.

The beetle family Hydrophilidae (water scavenger beetles) showed statistically significantly higher numbers compared to the control in test item treatment T2 at the 2nd sampling (5DAA2). No specimens were caught in the control and all test item treatments at the 1st sampling (8DBA2). Abundances increased at the 2nd sampling (5DAA2), with a slightly higher increase in test item treatment T2 compared to the control. Therefore, and as no effects occurred in the highest test rate T1, this effect might be rather due to chance or normal population dynamics than related to the test item.

Abundances of the juvenile specimens of the family Cicadellidae (cicadas) were statistically significantly lower compared to the control in test item treatment T3 at the 6th sampling (27 DAA2). In former samplings and in the higher test item rates T1 and T2 abundances developed similar to the control. Therefore it is unlikely that this effect is test item related. All other taxa analyzed were not affected.

Photoelectors

The arthropods collected with Photoelectors are specimens emerging from the soil, as well as plant- and ground-dwelling arthropods enclosed at trap set-up.

There were six samplings performed during the study period. The 1st sampling was taken 2 days before the 1st application and the succeeding samplings 5, 10, 15, 20 and 27 days after the 2nd application, respectively. Abundances of total arthropods collected with Photoelectors showed no statistically significant effects of the four test item treatments when compared to the control.

Of the 42 taxa analysed seven showed a statistically significant reduction ($p \leq 0.05$) between at least one test item treatment and the control (for details see below). Statistically significantly higher numbers were observed for two taxa.

The spider species *Pardosa pullata* (adult) was reduced to a statistically significant extent in test item treatments T1 and T4 at the 4th sampling. Abundances were on control level at former samplings and at later samplings without further statistically significant differences. This was most likely due to normal population dynamics and is not related to the test item treatment.

The beetle suborder Polyphaga accounted for 85.6 % of all beetles caught by Photoelectors in the control. Abundances were statistically significantly higher when compared to the control in test item treatment T3 at the 4th sampling (15DAA2). No effects occurred in the higher test item rates T1 and T2 and no statistically significant differences were observed in former samplings. Therefore these single short-term effects are not supposed to be test item related.

The Coleoptera family Staphylinidae (rove beetles) showed statistically significantly lower numbers compared to the control in test item treatments T2 and T4 at the 2nd sampling (5DAA2). Abundances in all test item treatments were below control level at this sampling. However, in the highest test item rate T1 and in test item rate T3 no statistically significant effects occurred.

Staphylinidae numbers in Photoelectors were generally low and this group is not the main target group of this trapping type, therefore this effect is not clearly related to the test item. Further results of Vortis suction sampling showed no test item related effects directly after application between test item treatments and the control.

The Diptera family Chloropidae (grass flies) was reduced to a statistically significant extent in test item treatment T1 at the 2nd sampling (5DAA2). No statistically significantly adverse effects were observed in the three lower test item rates or at the following samplings. As abundances were generally low for the family Chloropidae and numbers in test item treatment T1 were already below control level at the 1st sampling (8DAA2) this short-term effect is not clearly related to the test item.

For the Diptera superfamily Empidoidea statistically significantly higher numbers were observed for test item treatments T1 and T4 at the 3rd sampling (10DAA2). Until the 4th sampling (15DAA2) only single specimens were observed in the control and in all test item treatments. Further abundances were on control level directly after application and in later samplings. Therefore the effects in test item rates T1 and T4 are supposed to be due to chance or normal population dynamics.

Abundances of the Sternorrhyncha superfamily Aphidoidea (aphids) were statistically significantly lower compared to the control in test item treatment T1 at the 4th and 5th sampling (15 and 20DAA2). However, abundances were clearly below those in the control from the 1st sampling on and at the last sampling. Therefore this effect is supposed to be caused by normal population dynamics rather than test item related.

The Hymenoptera family Platygasteridae was decreased to a statistically significant extent in test item treatment T2 at the 2nd sampling (5DAA2). At the following samplings abundances increased and were at control level again. In the other test item treatments abundances were below those observed in the control at the 2nd sampling, though not on a statistically significant level. The single short-term effect in test item treatment T2 might be test item related. All other taxa analyzed were not affected.

Vortis Suction Sampling

The individuals caught with Vortis suction sampler are active and passive specimens. Therefore, the sampling method gives an estimate of the arthropod community within a defined area. Vortis suction sampling is biased towards smaller arthropods. Large beetles or larger spiders are under-represented.

There were six samplings performed during the study period. The 1st sampling was taken 3 days before the 1st application and the succeeding samplings 3, 8, 14, 20 and 27 days after the 2nd application, respectively. Abundances of total arthropods collected with Vortis suction sampling showed no statistically significant effects of the four test item treatments when compared to the control.

Of the 72 taxa analysed 18 showed a statistically significant reduction ($p \leq 0.05$) between at least one test item treatment and the control (for details see below). Statistically significantly higher numbers were observed for seven taxa.

Abundances of total spiders (Araneae) showed statistically significantly lower numbers compared to the control in test item treatment T1 and T2 at the 5th sampling (20DAA2). It is not clear, if this was related to the test item, as abundances were on control level at the samplings directly after application (3, 8 and 14DAA2) and developed similar to the control. In test item treatment T3 abundances were statistically significantly higher compared to the control at the 6th sampling (27DAA2). It is unlikely that this effect was caused by the test item as abundances were on control level in all samplings before and developed similar to the control with exception of the last sampling (27DAA2). Therefore the statistically significantly higher number is most likely due to normal population dynamics and seasonal changes.

The spider family Linyphiidae (money spiders) was represented by 40.3 % by the subfamily Erigoninae. Both taxa developed similarly and were present at statistically significantly higher numbers in test item treatment T1 at the 2nd sampling (3DAA2). However, from the 3rd sampling (8DAA2) onwards abundances were at control level again. Abundances in test item treatments T2, T3 and T4 developed similarly, with higher numbers compared to the control at the 2nd sampling (3DAA2), though not statistically significant. This single short-term effect in test item treatment T1 is possibly an indirect treatment related effect, but can also be due to natural population dynamics.

For the family Lycosidae (wolf spiders) statistically significantly lower numbers compared to the control were observed in test item treatment T1 at the 3rd sampling (8DAA2). At the 4th sampling (14DAA2) numbers were on control level again, without further statistically significant differences. This single short-term effect is supposed to be treatment related.

Abundances of juvenile specimens of the genus *Pardosa* (sp.) were statistically significantly lower in the lowest test item rate T4 compared to the control at the 4th sampling (14DAA2). This was most likely due to normal population dynamics as no specimens were caught before in the control or in any of the test item treatments. Further no effects occurred in the higher test item rates T1, T2 and T3.

The Collembola species *Lepidocyrtus lanuginosus* (all) showed decreasing numbers in the highest test item treatments T1 and T2 from the 1st to the 3rd sampling (9DBA2 to 8DAA2), statistically significantly lower numbers were observed in test item treatment T2 at the 3rd sampling (8DAA2); in test item treatment T1 abundances were not statistically significantly lower at the 3rd sampling (8DAA2) at $\alpha=0.05$, but at $\alpha=0.1$. In test item treatment T1 and T2 abundances were around control level in the following samplings until 27DAA2. This single short-term effect (effect class 2, DE JONG *et al.*, 2010) could be treatment related but may also be caused by normal seasonal changes.

Abundances of the species *Lepidocyrtus ruber* were statistically significantly lower compared to the control in test item treatment T2 at the 6th sampling (27DAA2). This was most likely due to chance or normal population dynamics as specimens of this species were only caught from the 4th sampling (14DAA2) onwards. Further abundances in the highest test item rate T1 developed similar to the control without statistically significant differences to the control.

The Collembola suborder Symphypleona was represented for 41.5 % by the species *Sminthurinus aureus*. Therefore the species was the main trigger for the effect observed for the suborder Symphypleona. Abundances decreased from the 1st to the 3rd sampling (9DBA2 to 8DAA2) resulting in statistically significantly lower numbers compared to the control in test item treatment T1 at the 3rd sampling (8DAA2). From the 4th sampling (14DAA2) on abundances were comparable to the control again without further statistically significant differences. This single short-term effect (effect class 2, DE JONG *et al.*, 2010) is possibly treatment related but may also be related to normal seasonal changes as abundances were below

control level from the 1st sampling (9DBA2) on for the species *Sminthurinus aureus*, though not on a statistically significant level. Moreover, no statistically significant effects occurred for total Symphypleona or *Sminthurinus aureus* in the three lower test item rates.

The Coleoptera suborder Polyphaga was represented for 69.0 % by the family Staphylinidae. Therefore this family was the main driver for changes of Polyphaga. Abundances of both taxa were statistically significantly lower compared to the control in test item treatment T3 at the 4th sampling (14DAA2).

For the family Staphylinidae abundances in test item treatment T1 were statistically significantly lower at the 4th sampling (14DAA2). Further in test item treatment T2 lower numbers were observed at this sampling, too, though only at $\alpha=0.1$. At the following samplings no further statistically significant differences were observed. These effects might be related to the test item, but could also be caused by seasonal changes.

The order Diptera (true flies) showed a similar development of abundances in the control and the test item treatments. However abundances in test item treatments T1, T2 and T3 developed on a lower level leading to a statistically significantly lower number compared to the control in test item treatment T2 at the 3rd sampling (8DAA2). From the following sampling on abundances were on control level again, without further statistically significant differences. This single short-term effect was mainly caused by the lower variance in the control plots compared to the other samplings. As test item treatment numbers generally showed an increasing tendency and no statistically significant differences were observed in the highest test item rate T1 the effect in test item treatment T2 was rather due to natural variability than treatment related.

The family Chloropidae (grass flies) was present at statistically significantly higher numbers in test item treatment T3 compared to the control at the 5th sampling (20DAA2) after lower numbers were observed at the 2nd and 3rd sampling (3 and 8DAA2), though only at $\alpha=0.1$. However, this was most likely due to normal population dynamics as no effects occurred in the higher test item rates T1 and T2.

The suborder Nematocera (long-horned flies) was represented for 90.5 % by the superfamily Sciaroidea (fungus gnats) in the control. Abundances of superfamily Sciaroidea were statistically significantly lower compared to the control in test item treatment T2 at the 2nd sampling (3DAA2). In test item treatment T1 both taxa showed statistically significantly lower abundances compared to the control at the 3rd sampling (8DAA2). Further at the 3rd sampling in T2 abundances of the superfamily Sciaroidea were statistically significantly lower compared to the control, though only at $\alpha=0.1$. At the following samplings a recovery of abundances was observed. These effects could be related to the test item.

Adult specimens of the family Cecidomyiidae (gall midges) were present at statistically significantly higher numbers in test item treatment T3 at the 4th sampling (14DAA2). As abundances were on control level in former and later samplings and no effects were observed in the higher test item rates T1 and T2, this effect is supposed to be caused by normal population dynamics.

Abundances of the family Sciaridae (dark-winged fungus gnats) showed a lower increase compared to the control in test item treatment T1 from the 2nd to the 3rd sampling (3 to 8DAA2), resulting in statistically significantly lower numbers at the 3rd sampling (8DAA2). Further, in test item treatment T2 statistically significantly lower numbers were observed at this sampling, though only at $\alpha=0.1$. Abundances in the highest test item rate T1 were already below those observed in the control at the 2nd sampling (3DAA2) and were still lower at the 5th and 6th sampling (20 and 27DAA2). Therefore, this effect could be classified as test item related.

The order Hemiptera (true bugs) showed statistically significantly higher numbers compared to the control in test item treatment T3 at the 5th sampling (20DAA2). This was rather due to chance than test item related as no statistically significant effects were observed in former samplings or in the higher test item rates T1 and T2.

The family Cicadellidae (cicadas) was mainly represented by juvenile specimens (86.2 % in the control) which were the main trigger for the development of the total family. At the 4th sampling (14DAA2) abundances in test item treatment T4 were statistically significantly lower compared to the control. This

effect was caused by normal population dynamics and is not supposed to be test item related, as abundances in the higher test item rates T1, T2 and T3 were comparable to the control. Further in former samplings abundances of test item treatment T4, too, were on control level.

Abundances of the Hemiptera superfamily Aphidoidea (aphids) were lower compared to the control at the 2nd sampling (3DAA2) and were decreased to a statistically significant extent in test item treatments T1, T2 and T3 at the 3rd sampling (8DAA2). In test item treatment T4 abundances were statistically significantly lower, too, at the 3rd sampling (8DAA2), though only at $\alpha=0.1$. At the following samplings abundances were on control level again in all test item treatments. The effects show a dose response pattern and can therefore be classified as test item related.

For the Hymenoptera family Mymaridae (fairy flies) decreasing abundances were observed in the control and all test item treatments. However, in test item treatment T1 numbers decreased to a higher extent with a statistically significantly lower number of individuals observed at the 2nd sampling (3DAA2) when compared to the control. At the 3rd sampling abundances recovered again without further statistically significant differences to the control until the end of the study. This effect is most likely test item related. In the three lower test item rates T2, T3 and T4 no statistically significant effects were observed.

Abundances of adult Thysanoptera (thrips) were higher compared to the control in test item treatment T3 from the 1st sampling (9DBA2) on. A steep increase from the 3rd to the 4th sampling (8 to 14DAA2) resulted in statistically significantly higher numbers compared to the control at 14DAA2. At the 5th sampling numbers were still higher compared to the control, though not to a statistically significant extent. This single effect was most likely caused by normal population dynamics, as no significant effects were observed in the higher test item rates or in former samplings.

Juvenile specimens of the order Thysanoptera (thrips) were statistically significantly lower compared to the control in test item treatment T1 at the 5th and 6th sampling (20 and 27DAA2). Lower numbers were already observed directly after the 2nd application (3 and 8 DAA2), though not on a statistically significant level, and at the 4th sampling (14DAA2), though only at $\alpha=0.1$. This effect in the highest test item rate T1 can be classified as test item related.

All other taxa analyzed were not affected.

Foliage/Litter Sampling

Arthropods collected with Foliage/Litter samplings are mainly ground-dwelling Acari (Gamasina, Oribatida, Prostigmata).

There were four samplings performed during the study period. The 1st sampling was taken 3 days before the 1st application and the succeeding samplings 4, 14 and 27 days after the 2nd application, respectively. Abundances of total Acari (mites) collected with Foliage/Litter sampling showed no statistically significant effects of the four test item treatments when compared to the control.

Of the 25 taxa analysed two showed a statistically significant reduction ($p \leq 0.05$) between at least one test item treatment and the control (for details see below). Statistically significantly higher numbers were observed for one taxon.

Abundances of the family Scheloribatidae were statistically significantly lower compared to the control in test item treatment T1 at the 1st and the 4th sampling (9DBA2 and 27DAA2). As abundances were already statistically significantly lower compared to the control at the pre-sampling, this was most likely due to chance or normal population dynamics and not related to the test item.

Abundances of the cohort Heterostigmata were statistically significantly higher in test item treatment T3 at the 2nd sampling (4DAA2). An effect of the test item is unlikely as abundances in test item treatments T1 and T2 showed no statistically significant differences to the control.

For the family Tarsonemidae abundances in test item treatments T3 and T4 first increased at the 2nd sampling (4DAA2), with approx. twofold higher numbers compared to the control and decreased in the following sampling (14DAA2) to statistically significantly lower numbers compared to the control. At the last sampling (27DAA2) abundances in all test item treatments were comparable to the control. This

development was most likely due to natural population dynamics or time and random as no statistically significant effects occurred in former samplings or in the two higher test item rates T1 and T2.

All other taxa analysed were not affected.

Table A 189: Summary of effect classification (according to multivariate analyses)

Effect classification (based on DE JONG <i>et al.</i> , 2010):				Effect class
	No consistent statistically significant adverse effect observed			-
Community level effects	Treatment			
	T1	T2	T3	T4
(PRC/Monte-Carlo; 5% alpha level)	Effect class			
Pitfall traps	-	-	-	-
Photoeclector sampling	-	-	-	-
Vortis suction sampling	-	-	-	-
Foliage/Litter sampling	-	-	-	-
Conclusion	Community NOER			

- No consistent statistically significant adverse effect observed

NOER: No Observed Effect Rate (highest test rate where no statistically significant differences to the control occurred)

Test item treatments (each with 2 applications): T1 = 7.2 g a.s./ha; T2 = 3.4 g a.s./ha; T3 = 1.4 g a.s./ha; T4 = 0.7 g a.s./ha

Table A 190: Summary of effect classification (according to univariate analyses)

Sampling type	Taxon	Lifestage	Effect class			
			T1	T2	T3	T4
PT	Araneae total		2↑	-	-	-
PT	Lycosinae total		-	-	2↓*	-
PT	<i>Pardosa palustris</i>	adult	-	-	2↓	-
PT	<i>Pardosa pullata</i>	adult	-	-	-	2↑*
PT	<i>Xysticus kochi</i>	adult	-	-	2↓*	3b↓*
PT	Insecta total		2↓	-	-	-
PT	Coleoptera total		2↓	-	-	-
PT	Polyphaga total		3b↓	2↓	-	-
PT	Alticinae	adult	3a↓	2↓	2↓	2↓
PT	Hydrophilidae	adult	-	2↑*	-	-
PT	Cicadellidae	juvenile	-	-	2↓*	-
PE	<i>Pardosa pullata</i>	adult	2↓*	-	-	2↓*
PE	Polyphaga total		-	-	2↑*	-
PE	Staphylinidae total		-	2↓*	-	2↓*
PE	Chloropidae	adult	2↓	-	-	-
PE	Empidoidea total		2↑*	-	-	2↑*
PE	Aphidoidea	all	3a↓*	-	-	-
PE	Platygastridae total		-	2↓	-	-
V	Araneae total		2↓	2↓	2↑*	-
V	Linyphiidae total		2↑	-	-	-
V	Erigoninae total		2↑	-	-	-
V	Lycosidae total		2↓	-	-	-
V	<i>Pardosa sp.</i>	juvenile	-	-	-	2↓*
V	<i>Lepidocyrtus lanuginosus</i>	all	-	2↓	-	-
V	<i>Lepidocyrtus ruber</i>	all	-	2↓*	-	-
V	Symphyleona total		2↓	-	-	-
V	<i>Sminthurinus aureus</i>	all	2↓	-	-	-
V	Polyphaga total		-	-	2↓	-
V	Staphylinidae total		2↓	-	2↓	-
V	Diptera total		-	2↓	-	-
V	Chloropidae	adult	-	-	2↑*	-
V	Nematocera total		2↓	-	-	-
V	Sciaroidea total		2↓	2↓	-	-
V	Cecidomyiidae	adult	-	-	2↑*	-
V	Sciaridae	adult	2↓	-	-	-
V	Hemiptera total		-	-	2↑	-
V	Cicadellidae total		-	-	-	2↓*
V	Cicadellidae	juvenile	-	-	-	2↓*
V	Aphidoidea	all	3b↓	3a↓	2↓	-
V	Mymaridae	adult	2↓	-	-	-
V	Thysanoptera	adult	-	-	2↑*	-
V	Thysanoptera	juvenile	8↓	-	-	-
LS	Scheloribatidae total		2↓*	-	-	-
LS	Heterostigmata total		-	-	2↑*	-
LS	Tarsonemidae		-	-	2↓*	2↓*
Conclusion			Population LOEAE	Population NOEAE		

* = Effects not treatment related, see discussion

Effects treatment related are highlighted in **bold** and yellow

- No consistent statistically significant adverse effect observed

2 = One occasion Slight and transient effects observed on one occasion only

3a = < 1 months (a) Effects no longer statistically significant on the last two sampling dates

3b = < 1 months (b) Effects no longer statistically significant on the last sampling date

8 = 1 months Pronounced effects; no recovery within the study period

↓ = Numbers lower than control; ↑ = numbers higher than control

Test item treatments (each with 2 applications at a rate of): T1 = 7.2 g a.s./ha; T2 = 3.4 g a.s./ha; T3 = 1.4 g a.s./ha; T4 = 0.7 g a.s./ha

NOEAER = No Observed Ecologically Adverse Effect Rate (highest test rate where at least 1 taxon with effect class 3, i.e. clear response to treatment occurred, but with recovery within 1 month after last application)

LOEAER = Lowest Observed Ecologically Adverse Effect Rate (lowest test rate for which at least 1 taxon had a statistically significant adverse response to treatment, lasting more than 1 month after last application)

PT = Pitfall trap sampling, PE = Photoeclector sampling, V = Vortis suction sampling, LS = Foliage/Litter sampling

Conclusion

Acetamiprid (formulated as MCW-2222) was applied twice to a meadow at nominal rates of 36 mL/ha, 17 mL/ha, 7 mL/ha and 3.5 mL/ha (nominally 7.2 g a.s./ha, 3.4 g a.s./ha, 1.4 g a.s./ha and 0.7 g a.s./ha) for test item treatments T1, T2, T3 and T4, respectively.

Overall based on statistical analyses effects of acetamiprid (formulated as MCW-2222) applied twice to an off-crop meadow arthropod fauna are classified as follows:

At the population level several taxa were considered adversely affected by treatment with acetamiprid at the rates T1 (7.2 g a.s./ha), T2 (3.4 g a.s./ha) and T3 (1.4 g a.s./ha). For the rate T1 one taxon (juvenile specimens of the order Thysanoptera) did not recover within the assessed sampling period of 27 days after the 2nd application. Therefore the rate T1 (7.2 g a.s./ha) is the population LOEAER (Lowest Observed Ecologically Adverse Effect Rate). For all other test item treatments statistically significant adverse population effects of single taxa were observed to be transient with recovery until the end of the study period. Therefore, the rate T2 (3.4 g a.s./ha) is classified as the population NOEAER (No Observed Ecologically Adverse Effect Rate). Based on the multivariate analysis of the community the PRC did not display statistically significant adverse effects up to and including the highest test item rate T1 (7.2 g a.s./ha) until the end of the study period. Thus, this rate is classified as the community NOER (No Observed Effect Rate).

A 2.4 KCP 10.4 Effects on non-target soil meso- and macrofauna

A 2.4.1 KCP 10.4.1 Earthworms

A 2.4.1.1 KCP 10.4.1.1 Earthworms - sub-lethal effects

Comments of zRMS:	<p>The study has been already evaluated and accepted by the zRMS in the course of the first zonal evaluation of CA3573 (formerly MCW-2222) finalised in April 2018. As the test guideline has not changed since that time, re-evaluation of the study following renewal of acetamiprid was in general not necessary and presented below conclusions were taken from the Core Assessment, Part B, Section 6 of April 2018.</p> <p>The study was performed in line with the OECD 222 with no deviations and met all validity criteria. Following endpoints were agreed:</p> <p>NOEC_{mortality} = 4.10 mg a.s./kg dws NOEC_{biomass} = 1.44 mg a.s./kg dws NOEC_{reproduction} = 0.85 mg a.s./kg dws EC₁₀ = 0.90 mg a.s./kg dws</p> <p>According the conclusions presented in EFSA Supporting publication 2019:EN-1673, reliability of the derived EC₁₀ value should be evaluated, which was not required before. Taking this into account, reliability assessment was carried out by the zRMS based on indications of Appendix E of the document mentioned:</p> <ul style="list-style-type: none"> • NW (normalised width) of 0.16 was calculated, which results with rating “excellent” in line with Table E9, • median EC₁₀ is lower than EC_{20,low}, • the dose-response curve is medium with steepness of 0.60 (i.e. in range of 0.33-0.66). <p>Based on above indications, the calculated EC₁₀ is considered to be sufficiently reliable.</p>
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Reference	KCP 10.4.1.1/01
Report	MCW-2222 - Sublethal toxicity to the earthworm <i>Eisenia fetida</i> in artificial soil, Friedrich, S. 2014, R-33840
Guideline(s):	OECD 222 (2004)
Deviations:	No
GLP:	Yes, certified laboratory
Acceptability:	Yes
Duplication (if vertebrate study)	Not applicable

Executive Summary

Effects of MCW-2222 on mortality, biomass and the reproductive potential of the earthworm species *Eisenia fetida* were determined. The 8-week study was conducted with six different nominal application rates (0.50, 0.85, 1.44, 2.43, 4.10 mg a.s./kg soil dry weight, nominally equivalent to 2.86, 4.83, 8.16, 13.8, 23.3 mg test item/kg soil dry weight test item/ha) applied on the surface of an artificial soil containing 10% peat. Four replicates with each ten worms were set up per treatment group. After 28 days, no statistically significant mortality compared to the control was observed at any test item concentration. After 28 days of exposure, the test item caused statistically significant changes in biomass (change in fresh weight after 4 weeks relative to initial fresh weight) compared to the control treatment at concentrations of 2.43 and 4.10 mg a.s./kg soil dry weight. Statistically significant effects on the number of juveniles compared to the control group were recorded at concentrations of 1.44, 2.43 and 4.10 mg a.s./kg soil dry weight. The EC₁₀, EC₂₀ and EC₅₀ values for reproduction were estimated to be 0.90, 1.07 and 1.50 mg a.s./kg soil dry weight, respectively. The NOEC for biomass and reproduction were determined to be 1.44 and 0.85 mg a.s./kg soil

dry weight, respectively.

Materials and methods

Materials

Test item	MCW-2222
Batch #	611-280413-01
Purity	Acetamiprid 200 g/L (nominal); 202.7 g/L (analysed)
Description	Yellowish liquid
Control	Deionised water
Toxic reference	Nutdazim 50 FLOW (Carbendazim, SC 500), tested in a separate study (BioChem project No. R 13 10 48 005 S, (November 2013).
Test organism	
Species	<i>Eisenia fetida</i> (Earthworm), about 3-month old (with clitellum), weight: 280 – 469 mg/worm
Source	In-house culture, originally obtained from W. Neudorff GmbH KG", An der Mühle 3, 31860 Emmerthal, Germany
Food	5 g of dried horse manure per replicate and week

Study design and methods

Test duration and exposure	8 weeks (overall) 4 weeks mortality and sublethal observations 4 weeks for reproductive success The test item was applied on the surface of the artificial soil containing 10% peat
Experimental dates	18 February - 15 April 2015
Test rates	0.50, 0.85, 1.44, 2.43, 4.10 mg a.s./kg soil dry weight, nominally corresponding to 2.86, 4.83, 8.16, 13.8, 23.3 mg test item/kg soil dry weight test item/ha
Test units	Plastic vessel of Bellaplast with inside dimensions: about 16.5 cm x 12 cm x 6 cm and a lid pervious to air and light filled with 600 g dw of artificial substrate
Group size/replicates	Test rates: 40 organisms per concentration; 10 in each of 4 replicates Control: 80 organisms per concentration; 10 in each of 8 replicates
Test substrate	Artificial substrate (10% peat) according to OECD 222
Max Water holding capacity	62.8 g/100 g dw
Environmental conditions	
Temperature	18.2 – 21.9 °C
Photoperiod	16 hours light / 8 hours darkness 510 lx
pH	Test start: 6.07 – 6.10 Test end: 5.76 – 5.82
Water content	Test start: 55.7 – 55.9 % of WHC Test end: 55.1 – 55.6 % of WHC.

Biological observations

The body weight of the adult earthworms was determined on day 0 and on day 28, individually. After the first four weeks adult worms were removed and mortality and morphological as well as behavioural changes were recorded. Four weeks thereafter the numbers of offspring hatched from the cocoons were counted. At the start and end of the test, pH-value and moisture content of the test substrate were determined in every treatment and control.

Statistics

The EC₁₀, EC₂₀ and EC₅₀ values (number of juveniles) were calculated by Probit analysis using the maximum likelihood method (Finney 1971). For identifying the NOEC values Fisher's Exact Binomial Test with Bonferroni Correction and the Williams-t-test were used to compare the control with the independent test item groups. For statistical evaluation of the biomass change, the changed mean fresh weight of surviving worms per replicate was used.

Results and discussion

Biological results

Biological results are given in the table below.

Table A 191: Effect of MCW-2222 on *Eisenia fetida* mortality and body weight after an exposure period of 28 days and reproduction after 56 days

Endpoint	Treatment rate [mg a.s./kg soil dry weight]					
	Control	0.5	0.85	1.44	2.43	4.10
Mortality [%]	1.3	0.0	2.5	0.0	2.5	7.5
Mean biomass change [%]	24.1	27.5	25.8	19.2	11.7*	-18.6*
Mean number of juveniles after 8 weeks	125.5	130.0	114.0	69.0*	12.3*	0.0*
Change of reproduction compared to control (%)	-	-3.6	9.2	45.0	90.2	100

* statistically significant different compared to the control for biomass and reproduction (Williams-t-test; ≥ 0.05 , one-sided smaller)

Negative values indicate an increase, relative to control

Table A 192: Endpoints

	Endpoints
NOEC (mortality)	4.10 mg a.s./kg dw
NOEC (biomass)	1.44 mg a.s./kg dw
NOEC (reproduction)	0.85 mg a.s./kg dw
LC ₅₀	> 4.10 mg a.s./kg dw
EC ₁₀ (95% CI)	0.90 mg a.s./kg dw (0.84 – 0.98) mg a.s./kg dw
EC ₂₀ (95% CI)	1.07 mg a.s./kg dw (1.01 – 1.14) mg a.s./kg dw
EC ₅₀ (95% CI)	1.50 mg a.s./kg dw (1.44 – 1.55) mg a.s./kg dw

Validity criteria

As shown in the following table, all validity criteria were met.

Table A 193: Validity criteria

Validity criteria according to OECD 222 (2016)	Observed in study
The mortality in the control group should be below 10%	1.3%
The number of juveniles in the control group was ≥ 30	≥ 84
The coefficient of variance (CV %) of reproduction should be ≤ 30	16.7%

Conclusion

In a 56-day earthworm reproduction study with MCW-2222 no statistically significantly adverse effects on mortality of the earthworm *Eisenia fetida* in artificial soil were determined up to and including 4.10 mg a.s./kg soil dry weight, i.e. the highest concentration tested. The EC₁₀, EC₂₀ and EC₅₀ values for reproduction were estimated to be 0.90, 1.07 and 1.50 mg a.s./kg soil dry weight, respectively. The NOEC for biomass and reproduction were determined to be 1.4

A 2.4.1.2	KCP 10.4.1.2	Earthworms - field studies
A 2.4.2	KCP 10.4.2	Effects on non-target soil meso- and macrofauna (other than earthworms)
A 2.4.2.1	KCP 10.4.2.1	Species level testing
A 2.4.2.1.1	KCP 10.4.2.1/01	<i>Folsomia candida</i>

Comments of zRMS:	<p>The study has been already evaluated and accepted by the zRMS in the course of the first zonal evaluation of CA3573 (formerly MCW-2222) finalised in April 2018. As the test guideline has not changed since that time, re-evaluation of the study following renewal of acetamiprid was in general not necessary and presented below conclusions were taken from the Core Assessment, Part B, Section 6 of April 2018.</p> <p>The study was performed in line with the OECD 223 with no deviations and met all validity criteria. Following endpoints were agreed:</p> <p>NOEC_{mortality} = 0.30 mg a.s./kg dws NOEC_{reproduction} = 0.18 mg a.s./kg dws EC₁₀ = 0.41 mg a.s./kg dws</p> <p>According the conclusions presented in EFSA Supporting publication 2019:EN-1673, reliability of the derived EC₁₀ value should be evaluated, which was not required before. Taking this into account, reliability assessment was carried out by the zRMS based on indications of Appendix E of the document mentioned:</p> <ul style="list-style-type: none"> • NW (normalised width) of 1.23 was calculated, which results with rating “poor” in line with Table E9, • median EC₁₀ is higher than EC_{20,low}, • the dose-response curve is shallow with steepness of 0.28 (i.e. in range of 0.33-0.66). <p>Based on above indications, the calculated EC₁₀ is considered to be not sufficiently reliable, but potentially the lower limit EC₁₀ of 0.22 mg a.s./kg dws could be considered. For selection of endpoints for the risk assessment, please refer to point 9.8.1 of this document.</p>
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Reference:	KCP 10.4.2.1/01
Report	MCW-2222 - Effects on the reproduction of the collembolan <i>Folsomia candida</i> , Friedrich, S. 2014, R-33841
Guideline(s):	OECD 223 (2009), ISO 11267 (1999)
Deviations:	No
GLP:	Yes, certified laboratory
Acceptability:	Yes
Duplication (if vertebrate study)	Not applicable

Executive Summary

Effects of MCW-2222 on mortality and reproduction of the collembolan species *Folsomia candida* were determined. The study was conducted under static conditions over 28 days with a control group and eight test item concentrations ranging from 0.1 to 4.1 mg test item/kg dry soil incorporated once into artificial soil containing 5% peat. The EC₁₀, EC₂₀ and EC₅₀ values for reproduction were estimated to be 0.41, 0.64 and 1.48 mg a.s./kg soil dry weight, respectively. The NOEC for mortality and reproduction was determined to be 0.30 and 0.18 mg a.s./kg soil dry weight, respectively.

Materials and methods

Materials

Test item	MCW-2222
Batch #	611-280413-01
Purity	Acetamiprid 200 g/L (nominal); 202.7 g/L (analysed)
Description	Yellowish liquid
Control	Deionised water
Toxic reference	The reference item boric acid (100% analysed) was tested in a separate study (BioChem project No. R 13 10 48 004 S (July 2013).
Test organism	
Species	<i>Folsomia candida</i> (Collembola), juveniles 9 – 12 days old
Source	In-house culture, originally obtained from the Biologische Bundesanstalt BBA, Berlin-Dahlem, Germany
Food	Granulated dry yeast, ~2 mg at test start and after 14 days
Study design and methods	
Test duration and exposure	4 weeks (28 days) The test item was mixed into the substrate containing 5 % peat
Experimental dates	04 March - 01 April 2014
Test rates	0.10, 0.18, 0.30, 0.50, 0.85, 1.44, 2.43, 4.10 mg a.s./kg soil dry weight nominally corresponding to 0.59, 1.00, 1.69, 2.86, 4.83, 8.16, 13.8, 23.3 mg test item/kg soil dry weight
Test units	Glass container (approximately 150 mL) covered with a glass lid; surface area of soil: 18.9 cm ²
Group size/replicates	Test rates: 40 organisms per concentration; 10 in each of 4 replicates Control: 80 organisms per concentration; 10 in each of 8 replicates
Test substrate	Artificial substrate (5% peat) according to OECD 223, crumbly structured in test vessel
Max Water holding capacity	43.1 g/100 g dw
Environmental conditions	
Temperature	18.2 – 21.0 °C
Photoperiod	16 hours light / 8 hours darkness 530 lx
pH	Test start: 6.06 – 6.10 Test end: 5.80 – 5.84
Water content	Test start: 57.8 – 58.2% of WHC Test end: 56.8 – 57.3 % of WHC

Biological observations

After 28 days potential effects of the test item on the mortality the reproduction of collembolan were estimated by determination of numbers of offspring and surviving parental collembolans.

Statistics

Fisher's Exact Binomial Test with Bonferroni Correction and the Williams-t-test were used to compare the control with the independent test item groups

Results and discussion

Biological results

Biological results are given in the table below.

Table A 194: **Effect of MCW-2222 on *Folsomia candida* mortality and reproduction after an exposure period of 28 days**

	Treatment rate [mg as.s/kg soil dry weight]								
	Control	0.10	0.18	0.30	0.50	0.85	1.44	2.43	4.10
Mortality of parental collembolans in [%] ^a	2.5	2.5	2.5	12.5	40*	47.5*	50.0*	42.5*	50.0*
Mean number of juveniles ^b	749	775	783	557*	517*	387*	361*	301*	229*
Difference to control for reproduction [%]	-	-4	-4	26	31	48	52	60	69

* statistically significant different compared to the control (^a Fisher-exact test for mortality, $\alpha = 0.05$, one-sided greater;

^b Williams-t-test for reproduction; $\alpha = 0.05$, one-sided smaller)

Negative values = increase, relative to control

Table A 195: Endpoints

	Endpoints
NOEC (mortality)	0.30 mg a.s./kg dw
NOEC (reproduction)	0.18 mg a.s./kg dw
LC ₅₀ (95% CI)	2.30 mg a.s./kg dw (0.7 – 5.86) mg a.s./kg dw
EC ₁₀ (95% CI)	0.41 mg a.s./kg dw (0.22 – 0.79) mg a.s./kg dw
EC ₂₀ (95% CI)	0.64 mg a.s./kg dw (0.35 – 1.17) mg a.s./kg dw
EC ₅₀ (95% CI)	1.48 mg a.s./kg dw (0.71 – 3.08) mg a.s./kg dw

Validity criteria

As shown in the following table, all validity criteria were met.

Table A 196: Validity criteria

Validity criteria according to OECD 223 (2009)	Observed in study
The mortality in the control group should be below 20%	2.5%
The number of juveniles in the control group was ≥ 100	749
The coefficient of variance (CV %) of reproduction should be ≤ 30	12.4%

Conclusion

In a 28-day *Folsomia candida* reproduction study, in which collembolans were exposed to MCW-2222, the LC₅₀ value was calculated to be 2.03 mg a.s./kg soil dry weight. The EC₁₀, EC₂₀ and EC₅₀ values for reproduction were estimated to be 0.41, 0.64 and 1.48 mg a.s./kg soil dry weight, respectively. The NOEC for mortality and reproduction was determined to be 0.30 and 0.18 mg a.s./kg soil dry weight, respectively.

A 2.4.2.1.2 KCP 10.4.2.1/02 *Hypoaspis aculeifer*

Comments of zRMS:	<p>The study has been already evaluated and accepted by the zRMS in the course of the first zonal evaluation of CA3573 (formerly MCW-2222) finalised in April 2018. As the test guideline has not changed since that time, re-evaluation of the study following renewal of acetamiprid was in general not necessary.</p> <p>The study was performed in line with the OECD 226 with no deviations and met all validity criteria. Overview of the endpoint agreed in the course of the first zonal evaluation revealed, however, that 16% reduction of reproduction and 12.5% mortality were observed at concentration set as NOEC, which could be of biological relevance, even if statistically not significant. Furthermore, no reliable EC₁₀ or LC₁₀ could be calculated based on the study results, as effects >10% were observed only at the highest concentration tested. Taking this into account, for precautionary reasons the reproduction NOEC was set by the zRMS to the maximum concentration at which effects <10% were observed. Following endpoints were thus agreed for purposes of re-evaluation of CA3753:</p> <p>NOEC_{mortality} = 100 mg a.s./kg dws NOEC_{reproduction} = 100 mg a.s./kg dws</p> <p>Reliable EC₁₀ could not be determined</p>
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Reference:	KCP 10.4.2.1/02
Report	Effects of MCW-2222 on the reproduction of the predatory mite <i>Hypoaspis aculeifer</i> , Schulz, L., 2014, R-33842
Guideline(s):	OECD 226 (2008)
Deviations:	Yes
GLP:	Yes, certified laboratory
Acceptability:	Yes
Duplication (if vertebrate study)	Not applicable

Executive Summary

In a 14-day study the effects of MCW-2222 on mortality and reproduction of the soil mite species *Hypoaspis aculeifer* were investigated. The LC₅₀ for mortality and the EC₅₀ for reproduction could not be calculated due to an absence of adverse effects. Hence it can be concluded that the LC₅₀ and the EC₅₀ are greater than 200 mg a.s./kg soil dry weight, the highest concentration tested. The NOEC for mortality and the NOEC for reproduction were determined to be 200 mg a.s./kg soil dry weight.

Materials and methods

Materials

Test item	MCW-2222
Batch #	611-280413-01
Purity	Acetamiprid 200 g/L (nominal); 202.7 g/L (analysed)
Description	Yellowish liquid
Control	Deionised water
Toxic reference	The reference item Dimethoate was tested in a separate study; BioChem project No. R 14 10 48 001 S14 10 48 001 S (June 2014)

Test organism

Species	<i>Hypoaspis aculeifer</i> (Canestrini), adult age synchronised (\leq 3-days) females
Source	In-house culture, originally obtained from Bayer CropScience AG, Mohnheim, Germany
Food	Before and during the test, the predatory mites were fed every 2 - 3 days with <i>Tyrophagus putrescentiae</i> (Schränk)

Study design and methods

Test duration and exposure	14 days. The test item was mixed into the substrate.
Experimental dates	25 Jul – 13 Aug 2014
Test rates	6.25, 12.5, 25, 50, 100, 200 mg a.s./kg soil dry weight, nominally corresponding to 36, 71, 142, 284, 569, 1137 mg test item/kg soil dry weight
Test units	100 mL SCHOTT-bottles with screw cap (inside dimensions: 4 cm in diameter, 11 cm high). Bottle contained 20 g soil dry weight
Group size/replicates	Test rates: 40 organisms per concentration; 10 in each of 4 replicates Control: 80 organisms per concentration; 10 in each of 8 replicates
Test substrate	Artificial substrate containing 5% peat
Max Water holding capacity	36-09 g/100 g dw
Environmental conditions	
Temperature	19.7 - 21.2 °C
Photoperiod	16 hours light / 8 hours darkness 513 lx
pH	Test start: 5.6 – 5.7 Test end: 5.6 – 5.7
Water content	Test start: 50.34 - 52.82% of WHC Test end: 49.32 - 52.36 % of WHC

Biological observations

For the main measured variable, the number of juveniles per test vessel and additionally the mortality of the adult female mites were determined. The reproductive output of the mites exposed to the test substance was compared to that of the control in order to determine the no observed effect concentration (NOEC). Assessment of adult mortality and reproduction effects was carried out after 14 days.

Statistics

Fisher's Exact Binomial Test with Bonferroni Correction and the Williams-t-test were used to compare the control with the independent test item groups

Results and discussion

Biological results

Biological results are given in the table below.

Table A 197: Effect of MCW-2222 on *Hypoaspis aculeifer* mortality and reproduction after an exposure period of 14 days

	Treatment rate [mg a.s./kg dry soil]						
	Control	6.2	12.5	25	50	100	200
Adult mortality [%]	3.8	2.5	2.5	10.0	7.5	2.5	12.5
Mean number of juveniles (day 14)	201.1	222.0	218.0	200.5	200.5	199.0	169.5
Reproduction in [%] of control (day 14)	100	110	108	100	104	99	84

Table A 198: Endpoints

Endpoint	[mg a.s./kg dry weight]
NOEC (mortality)	200
NOEC (reproduction)	>200
LC ₅₀	>200
EC ₁₀	>200
EC ₂₀	>200
EC ₅₀	>200

Validity criteria

As shown in the following table, all validity criteria were met.

Table A 199: Validity criteria

Validity criteria according to OECD 226 (2008)	Observed in study
The mortality in the control group should be below 20%	3.8%
The number of juveniles in the control group was ≥ 50	201.1
The coefficient of variance (CV %) of reproduction should be ≤ 30	12.4%

Conclusion

In a 14-day *Hypoaspis aculeifer* reproduction study with MCW-2222, the LC₅₀ for mortality and the EC₅₀ for reproduction could not be calculated due to an absence of adverse effects. Hence it can be concluded that the LC₅₀ and the EC₅₀ are greater than 200 mg a.s./kg soil dry weight, the highest concentration tested. The NOEC for mortality and the NOEC for reproduction were determined to be 200 mg a.s./kg soil dry weight.

A 2.4.2.2 KCP 10.4.2.2 Higher tier testing

<p>Comments of zRMS:</p>	<p>A long-term field study was conducted to investigate the possible effects and potential recovery of field populations of Collembola after application of Acetamiprid 200 SL (a.s.: 200 g acetamiprid/L) in an agricultural scenario.</p> <p>On the study field, 30 study plots each with a size of 10 m x 10 m (100 m²) were established. Defined areas were sampled to assess the Collembola population before and five times after test item application. The treatments were assigned to the plots in a randomized block design with six replicates for the test item treatment group (Acetamiprid 200 SL (a.s.: 200 g acetamiprid/L). Acetamiprid 200 SL was applied twice (8 days apart) on bare soil at application rates of 0.15 L/ha, corresponding to 30 g a.s./ha (nominal), 0.25 L/ha, corresponding to 50 g a.s./ha (nominal) and 0.40 L/ha, corresponding to 80 g a.s./ha (nominal).</p> <p>As reference test item, Clarnet (chlorpyrifos 48% w/v (nominal)) was applied once to the reference plots at a rate of 2.5 L/ha, corresponding to 1200 g chlorpyrifos/ha, in parallel to the 1st test item application. The control plots were left untreated.</p> <p>Over the test period of 12 month, six Collembola samplings were evaluated: Pre-sampling (DAT -4), 1st sampling, (DAT 28), 2nd sampling (DAT 56), 3rd sampling (DAT 182), 4th sampling (DAT 331) and 5th sampling (DAT 365). Collembola were captured from the soil surface with pitfall traps (4 pitfall traps per plot) and were extracted from soil cores taken in the upper 5 cm of the soil (6 soil cores per plot). The caught and extracted test organisms of the 4th sampling conducted 331 days after 1st application were not counted and not identified.</p> <p>In total, Collembola of 2 orders, 2 suborders, 12 families, 5 subfamilies, 24 genera, 2 species groups and 11 species were determined.</p> <p><u>Collembola of the upper 5 cm of the soil (soil cores)</u></p> <p>The test item caused no statistically significant reductions in abundance of the large majority of the Collembola taxa of the upper 5 cm of the soil of all test item treatment groups throughout the whole test period. Only a few taxa showed transient statistically significant reductions in the low, middle and high test item treatment groups (2 x 0.15 L and 2 x 0.25 L test item/ha and 2 x 0.40 L test item/ha) shortly after application (day 28 after 1st application, 1st sampling) and only one taxon showed statistically significant reductions in the low and high test item treatment group (2 x 0.15 L and 2 x 0.40 L test item/ha) on day 182 after 1st application.</p> <p>No statistically significant reductions could be observed for all dominant Collembola taxa at the end of the test on day 365 after 1st application (5th sampling).</p> <p><u>Collembola of the soil surface (pitfall traps)</u></p> <p>The test item caused no statistically significant reductions in abundance of the majority of the Collembola taxa of the soil surface of all test item treatment groups throughout the whole test period. Only a few taxa showed transient statistically significant reductions in the low, middle and high test item treatment groups shortly after application (day 28 after 1st application, 1st sampling) and only one taxon showed statistically significant reductions in the middle and high test item treatment group (2 x 0.25 L and 2 x 0.40 L test item/ha) on day 182 after 1st application. No statistically significant reductions could be observed for all dominant Collembola taxa at the end of the test on day 365 after 1st application (5th sampling).</p> <p>It can be concluded that the application of Acetamiprid 200 SL tested at application rates of 2 x 0.15 L/ha (corresponding to 2 x 30 g a.s./ha), 2 x 0.25 L/ha (corresponding to 2 x 50 g a.s./ha) and 2 x 0.40 L/ha (corresponding to 2 x 80 g a.s./ha) had no adverse effects on single Collembola taxa and total Collembola as well as on the community structure of Collembola of the upper 5 cm of the soil and the soil surface one year after application.</p>
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Data point:	KCP 10.4.2.2/01
Report author	Schulz, L.
Report year	2022
Report title	Effects of Acetamiprid 200 SL on Collembola under field conditions
Report No	21 48 FCM 0002
Guidelines followed in study	ISO 23611-2 (2006) Technical Recommendations to ISO 11268-3 (KULA et al. 2006)
Deviations from current test guideline	None
Previous evaluation	Not previously submitted
GLP/Officially recognised testing facilities	Yes, conducted under GLP (certified laboratory)
Acceptability/Reliability:	Yes

Executive summary

A long-term field study was conducted to investigate the possible effects and potential recovery of field populations of Collembola after application of Acetamiprid 200 SL (a.s.: 200 g acetamiprid/L) in an agricultural scenario. On the study field, 30 study plots each with a size of 10 m x 10 m (100 m²) were established. Defined areas were sampled to assess the Collembola population before and five times after test item application. The treatments were assigned to the plots in a randomized block design with six replicates for the test item treatment group (Acetamiprid 200 SL (a.s.: 200 g acetamiprid/L), the reference item (Clarnet, containing nominally 48.0 % w/v chlorpyrifos) and the control group (tap water), respectively.

Acetamiprid 200 SL was applied twice (8 days apart) on bare soil at application rates of 0.15 L/ha, corresponding to 30 g a.s./ha (nominal), 0.25 L/ha, corresponding to 50 g a.s./ha (nominal) and 0.40 L/ha, corresponding to 80 g a.s./ha (nominal). As reference test item, Clarnet (chlorpyrifos 48% w/v (nominal)) was applied once to the reference plots at a rate of 2.5 L/ha, corresponding to 1200 g chlorpyrifos/ha, in parallel to the 1st test item application. The control plots were left untreated.

Since the average residue levels of the active substance in the soil samples taken immediately after 1st and 2nd application as well as in the soil of the spray targets were within the recommended range of 50% to 150% of the nominal values, the correct applications of the test item were verified.

Over the test period of 12 month, six Collembola samplings were evaluated: Pre-sampling (DAT -4), 1st sampling, (DAT 28), 2nd sampling (DAT 56), 3rd sampling (DAT 182), 4th sampling (DAT 331) and 5th sampling (DAT 365). Collembola were captured from the soil surface with pitfall traps (4 pitfall traps per plot) and were extracted from soil cores taken in the upper 5 cm of the soil (6 soil cores per plot). The caught and extracted test organisms of the 4th sampling conducted 331 days after 1st application were not counted and not identified.

In total, Collembola of 2 orders, 2 suborders, 12 families, 5 subfamilies, 24 genera, 2 species groups and 11 species were determined.

The test item caused no statistically significant reductions in abundance of the large majority of the Collembola taxa of the upper 5 cm of the soil of all test item treatment groups throughout the whole test period. Only a few taxa showed transient statistically significant reductions in the low, middle and high test item treatment groups (2 x 0.15, 2 x 0.25 and 2 x 0.40 L test item/ha) shortly after application (day 28 after 1st application, 1st sampling) and only one taxon showed statistically significant reductions in the low and high test item treatment group (2 x 0.15 and 2 x 0.40 L test item/ha) on day 182 after 1st application. Clear recovery took place in the course of the test so that no statistically significant reductions could be observed for all dominant Collembola taxa at the end of the test on day 365 after 1st application (5th sampling).

The test item caused no statistically significant reductions in abundance of the majority of the Collembola taxa of the soil surface of all test item treatment groups throughout the whole test period. Only a few taxa showed transient statistically significant reductions in the low, middle and high test item treatment groups shortly after application (day 28 after 1st application, 1st sampling) and only one taxon showed statistically significant reductions in the middle and high test item treatment group (2 x 0.25 and 2 x 0.40 L test item/ha) on day 182 after 1st application. Clear recovery took place in the course of the test so that no statistically

significant reductions could be observed for all dominant Collembola taxa at the end of the test on day 365 after 1st application (5th sampling).

The PRCs indicate short-term effects of the test item on the Collembola community of the upper 5 cm of the soil and the soil surface at all tested application rates shortly after application (1st sampling at day 28 after 1st application) but clear recovery was observed with no effects at all following samplings (56, 182, and 365 days after 1st application).

The toxic reference item reduced total Collembola abundance in the upper 5 cm of the soil of the test field by 91.4% at 1st sampling (28 days after 1st application), 89.7% at 2nd sampling (56 days after 1st application) and 39.2% at 3rd sampling (182 days after 1st application). The abundance of Collembola of the most common Collembola order Arthropleona (85.8% of the total Collembola abundance at pre-sampling) was reduced by 89.6% at 1st sampling, 91.4% at 2nd sampling and 41.9% at 3rd sampling. The abundance of Collembola of the second dominant Collembola order Symphypleona (14.0% of the total Collembola abundance at pre-sampling) was reduced by 98.6% at 1st sampling and 50.0% at 2nd sampling.

The toxic reference item reduced total Collembola numbers on the soil surface of the test field by 89.8% at 1st sampling (28 days after 1st application), 53.2% at 2nd sampling (56 days after 1st application) and 14.4% at 3rd sampling (182 days after 1st application). The abundance of Collembola of the most common order Arthropleona (91.0% of the total Collembola numbers at pre-sampling) was reduced by 85.0% at 1st sampling, 47.3% at 2nd sampling and 13.6% at 3rd sampling. Most of the dominant Collembola taxa showed reductions of > 50% in the course of the test with statistically significant reductions compared to the control determined for numerous taxa. The clear effects of the toxic reference on Collembola of the soil surface confirmed the sensitivity of the test system.

It can be concluded that the application of Acetamiprid 200 SL tested at application rates of 2 x 0.15 L/ha (corresponding to 2 x 30 g a.s./ha), 2 x 0.25 L/ha (corresponding to 2 x 50 g a.s./ha) and 2 x 0.40 L/ha (corresponding to 2 x 80 g a.s./ha) had no adverse effects on single Collembola taxa and total Collembola as well as on the community structure of Collembola of the upper 5 cm of the soil and the soil surface one year after application.

Materials and methods

Materials

Test item	Acetamiprid 200 SL (200 g/L acetamiprid; SL), ADM.00150.I.2.A, MCW-2222
Batch #	1242-050520-01
Purity	200 g/L (nominal), 203.4 ± 1.4 g/L (analysed)
Description	Clear yellow to brown liquid
Control	Untreated
Toxic reference	Clarnet (chlorpyrifos, 48% w/v (nominal))
Test organism	
Species	Naturally occurring field population of Collembola of 2 orders, 2 suborders, 12 families, 5 subfamilies, 24 genera, 2 species groups and 11 species

Study design and methods

Test duration and exposure	One year
Experimental dates	23. April 2021 – 27. April 2022 (field phase) 27. April 2021 & 05. May 2021 (application dates)
Test concentrations	Test item Low rate: 0.15 L test item/ha (nominally equivalent to 30 g a.s./ha) Middle rate: 0.25 L test item/ha (nominally equivalent to 50 g a.s./ha) High rate: 0.40 L test item/ha (nominally equivalent to 80 g a.s./ha) <u>Reference item</u> 2.5 L reference item/ha (nominally equivalent to 1200 g chlorpyrifos/ha)
Sampling areas/Replicates	Six replicates per plot; the central areas of the plots (6 m x 6 m) were sampled.
Test design	Thirty study plots each with a size of 10 m x 10 m (100 m ²), were each

Application method and exposure	<p>plot was surrounded by at least 2 m guard row between the plots and at least 5 m distance to the outer boarder of the field site. The test consisted of 5 treatment groups arranged in a randomised block design. The selection of the test field was based on a preliminary sampling (non-GLP) of <i>Collembola</i> before test start.</p> <p>The treatments were applied with plot sprayers from Schachtner, Ludwigsburg-Oßweil (PSG-F5.3 B 01.25.19 with TEEJET DG80015VS nozzles) and agrotop GmbH, Obertraubling (PL 2 with Lechler IDK 90-015c nozzles). The spray width of the spraying booms was 2.5 m with a distance between nozzles of 0.25 m and a total of 10 nozzles. The spray pressure at the spray booms was 3 bar. The sprayers were also calibrated before application by assessing the application volume, targeting 600 L/ha.</p>
Field location Field history	<p>Arable land situated near Machern, Saxony, Germany</p> <p>Cultural practices performed on the test field during 2018 till 2020 followed the usual agricultural practice. The only cultivated crop within this time span was <i>Phacelia tanacetifolia</i>.</p> <p>No further plant protection products were applied on the test field. Furthermore, no mineral or organic fertilisers were applied to the test field.</p>
Sampling method:	<p>Soil core sampling and extraction of the test organisms and sampling of the test organisms of the soil surface with pitfall traps</p>
Sampling dates	<p>Pre-sampling: -4 DAT, 23.04.2021</p> <p>1st: 28 DAT, 25.05.2021</p> <p>2nd: 56 DAT, 22.06.2021</p> <p>3rd: 182 DAT, 26.10.2021</p> <p>4th: 331 DAT, 24.03.2023 (not counted and identified)</p> <p>5th: 365 DAT, 27.04.2022</p>
Weather conditions at application	<p>Air temperature 2.8–10.2 °C</p> <p>Soil temperature 4.8–8.9 °C</p> <p>Relative humidity 42.4–80.4%</p> <p>Wind speed max 1.0–3.0 m/s</p>
Natural field conditions	<p>Soil type Silty loamy sand</p> <p>pH 5.5</p> <p>Total organic carbon 1.03%</p> <p>Humus content 1.77</p> <p>Maximum water holding capacity 31.5g/100g soil d.w.</p>

Residue analysis

The application rate in the field was verified by residue analysis of soil core samples and spray target samples. Spray target samples were taken at the application in each control plots and test item plot. Before the applications, open plastic vessels (16.8 cm x 12 cm x 5 cm) filled with field soil (500 g soil dry weight) were placed across each test item treatment plot. The four vessels per plot were pooled, placed in plastic bags and stored deep frozen at ≤ -18 °C until sample homogenisation.

Soil core samples were taken after the application in each control and test item plot. A mixed bulk sample of 10 soil cores of each individual plot was produced. Each individual soil core had a diameter of 5 cm and was taken from the 0-10 cm soil layer. Samples were pooled in plastic bags and stored deep frozen at ≤ -18 °C until sample homogenisation.

The analytical method for the determination of acetamiprid in soil samples was fully validated in the analytical phase of this study according to the requirements of SANTE/2020/12830 rev.1. Control

specimens of soil samples were analysed each at least in duplicate and fortified specimens were analysed at least in quintuple for each fortification level.

Mean recovery values obtained by HPLC-MS/MS for Acetamiprid ranged from 70% to 97% with RSD's of 3.6% to 7.3% at the respective fortification levels and were thus well within the accepted recovery range of 70 to 120%. In the control soil sample(s) used for the fortification experiments no residues of Acetamiprid could be detected.

Table A 200: Recoveries in the soil spray target samples

Matrix	Forti- fication level in mg/kg	Recoveries			No of analyses	Overall recovery	
		Single values in %	Mean in %	RSD in %		Mean in %	RSD in %
Acetamiprid with m/z = 223 → 126 (quantifier)							
Soil (S01)	0.005	68 – 74	72	3.6	5	75	7.4
	1.00	73 – 86	79	6.9	5		
Acetamiprid with m/z = 223 → 90 (qualifier)							
Soil (S01)	0.005	66 – 74	70	4.0	5	75	9.1
	1.00	74 – 89	80	7.1	5		

Table A 201: Recoveries in the soil core samples

Matrix	Forti- fication level in mg/kg	Recoveries			No of analyses	Overall recovery	
		Single values	Mean	RSD		Mean	RSD
		in %	in %	in %		in %	in %
Acetamiprid with m/z = 223 → 126 (quantifier)							
Soil Spray Target (ST01)	0.005	74	74	-	1	88	10.1
	2.00	79 – 95	90	7.3	5		
Acetamiprid with m/z = 223 → 90 (qualifier)							
Soil Spray Target (ST01)	0.005	82	82	-	1	94	8.8
	2.00	86 – 101	97	6.6	5		

Biological observations

At each sampling occasion, 6 soil cores were sampled per plot from the central area in the plot (6 m x 6 m), taken with stainless tubes (sampling area per tube = 19.6 cm²) with a diameter of 5 cm and covering a depth of 0 - 5 cm. Immediately after sampling, the tubes were sealed with caps, labelled and stored in cool boxes for transportation to the laboratory. The test organisms were extracted from the soil cores on day of sampling using a MacFadyen high-gradient extractor (heat/light extraction method). After extraction, the extracted samples were stored cool until identification of the test organisms.

Additionally, pitfall traps (4 per plot) were used to capture Collembola of the soil surface. The traps (plastic funnels with collecting flasks and fixing liquid) were dug into the ground of the test field in the central area of the plot so that the tops of the traps were at the level of the soil surface. The funnels (15 cm in diameter, with an outlet of 2 cm in diameter) were connected with collecting flasks containing about 50 mL of a fixing liquid (70% ethanol solution). When the traps were not in use for collecting Collembola, the openings of the traps (i.e. the funnels) were covered with lids. The lids were removed on the day of the soil cores sampling. The traps were opened for 3 - 7 days depending on weather and soil conditions. Afterwards, the labelled collecting flasks were transferred to the laboratory and stored cool until identification of the captured Collembola.

For determination of the extracted Collembola, the content of each sample vessel was separately poured into a Petri dish and examined using a pipette and a stereomicroscope. The Collembola were identified to species level if possible, otherwise to genus, subfamily, family, suborder or order level.

Statistics

Statistical analysis was carried out with the statistical software package ToxRat Professional 3.3.0 (2018). Totals for all parameters per plot were calculated prior to statistical analysis, so that all calculations are based on six replicates.

Univariate analysis

Only taxa with a minimum total abundance of 10 individuals of at least one control plot on at least two sampling dates were taken into account. Pre-treatment sample data were analysed for normal distribution (Shapiro-Wilk's-test or Kolmogorov-Smirnoff-test). Afterwards, data were analysed with a two-factorial analysis of variance (ANOVA) with treatment as fixed factor and block as random factor. Post-treatment sample data for the test item and reference item were analysed separately. Both data sets were analysed for normal distribution (Shapiro-Wilk's-test or Kolmogorov-Smirnoff-test) and for homogeneity in variance (Levene's test). If necessary, the data were transformed ($y' = \ln(y)$) to achieve normal distribution and homogeneity of variance. Afterwards test item data were analysed by a one-sided Dunnett's Multiple t-test Procedure, Multiple Sequentially-rejective Welch-t-test after Bonferroni-Holm or Williams Multiple Sequential t-test Procedure with $p < 0.05$. Reference item data were analysed by a one-sided Student-t-test or Welch-t-test with $p < 0.05$.

Multivariate analysis

Effects on the community structure were analysed for the control and the test item treatment group using multivariate analysis (non-GLP). The reference item treatment group was not analysed. PRCs are calculated via the ordination technique Redundancy Analysis (RDA), which can be seen as a canonical (or constrained) form of a Principal Component Analysis (PCA) because RDA uses only the variance, which can be attributed to the explanatory variables. For PRCs, the combination of time and treatment level is used as an explanatory variable while the time is considered as a co-variable.

The calculations were done using the software Community Analysis (CA) Version 4.3 and CANOCO 4.5 (Ter Braak & Smilauer 2002).

Results

Residue analysis

No measurable residues ($< \text{LOD}$) of acetamiprid were determined in the untreated soil samples used for the spray targets. The mean residue values corresponded to 97.4%, 86.6 % and 85.7% of the application rates of 0.15 L test item/ha, 0.25 L test item/ha and 0.40 L test item/ha, respectively, immediately after 1st application and 95.8%, 99.8% and 85.4% of the application rates of 0.15 L test item/ha, 0.25 L test item/ha and 0.40 L test item/ha after the 2nd application.

Table A 202: Residues of acetamiprid in the soil spray targets after applications

Treatment group	Repl.	1 st Application			2 nd Application		
		Acetamiprid concentration [mg a.s./kg soil d.w.]		Recovery [%]	Acetamiprid concentration [mg a.s./kg soil d.w.]		Recovery [%]
		theoretical ¹⁾	measured		theoretical ¹⁾	measured	
Control	-	-	< LOD	-	-	< LOD	-
Acetamiprid 200 SL (0.15 L/ha)	1	0.123	0.108	88.0	0.122	0.128	104.9
	2	0.118	0.125	105.8	0.122	0.113	92.5
	3	0.119	0.113	94.7	0.123	0.129	105.1
	4	0.123	0.137	111.4	0.123	0.123	100.0
	5	0.120	0.122	101.8	0.123	0.115	93.8
	6	0.123	0.102	82.7	0.124	0.097	78.5
	Mean	-	0.118	97.4	-	0.118	95.8
Acetamiprid 200 SL (0.25 L/ha)	1	0.205	0.193	94.2	0.205	0.202	98.6
	2	0.199	0.187	94.2	0.204	0.204	100.1
	3	0.207	0.161	77.7	0.205	0.177	86.4
	4	0.208	0.160	77.1	0.204	0.253	123.9
	5	0.206	0.215	104.6	0.204	0.187	91.6

	6	0.206	0.148	71.7	0.202	0.198	98.1
	Mean	-	0.177	86.6	-	0.204	99.8
Acetamiprid 200 SL (0.40 L/ha)	1	0.330	0.335	101.43	0.328	0.270	82.3
	2	0.332	0.248	74.79	0.328	0.309	94.1
	3	0.332	0.280	84.44	0.329	0.286	87.0
	4	0.333	0.244	73.36	0.329	0.255	77.4
	5	0.329	0.310	94.25	0.328	0.267	81.3
	6	0.329	0.282	85.74	0.329	0.297	90.4
	Mean	-	0.283	85.7	-	0.281	85.4

¹⁾ Theoretical concentration of a.s. = theoretical amount of a.s. applied on the sampled area per plot (4 vessels of Bellaplast = 806.4 cm²) / mass of the sampled soil (Mass of dry soil sample) per plot (sum of 4 vessels of Bellaplast)

LOD: 0.0015 mg/kg; LOQ: 0.005 mg/kg

No measurable residues (< LOD) of acetamiprid were determined in any of the soil samples of the control plots taken immediately after 1st and 2nd application. The mean recoveries of acetamiprid in the soil samples (sampling depth: 0 - 10 cm) from the treated plots of the three test item treatment groups, taken immediately after 1st application, were 70.7% at an application rate of 0.15 L test item/ha, 73.1% at an application rate of 0.25 L test item/ha and 80.8% at an application rate of 0.40 L test item/ha.

Assuming that 100% of the active ingredient of the 1st application was already degraded, the mean recoveries of acetamiprid in the soil samples (sampling depth: 0 - 10 cm) from the treated plots of the three test item treatment groups, taken immediately after 2nd application, were 88.6% at an application rate of 0.15 L test item/ha, 80.6% at an application rate of 0.25 L test item/ha and 81.7% at an application rate of 0.40 L test item/ha.

Table A 203: Residues of acetamiprid in the soil cores after applications (0 - 10 cm)

Treatment group	Repl.	1 st Application			2 nd Application		
		Acetamiprid concentration [mg a.s./kg soil d.w.]		Recovery [%]	Acetamiprid concentration [mg a.s./kg soil d.w.]		Recovery [%]
		theoretical ¹⁾	measured		theoretical ¹⁾	measured	
Control	1	-	< LOD	-	-	< LOD	-
	2	-	< LOD	-	-	< LOD	-
	3	-	< LOD	-	-	< LOD	-
	4	-	< LOD	-	-	< LOD	-
	5	-	< LOD	-	-	< LOD	-
	6	-	< LOD	-	-	< LOD	-
Acetamiprid 200 SL (0.15 L/ha)	1	0.017	0.012	72.6	0.017	0.020	116.2
	2	0.017	0.012	71.9	0.019	0.017	89.0
	3	0.017	0.009	52.6	0.018	0.014	78.9
	4	0.018	0.010	55.7	0.020	0.015	75.5
	5	0.018	0.013	71.3	0.018	0.012	65.1
	6	0.017	0.017	100.2	0.022	0.024	106.8
	Mean	-	0.012	70.7	-	0.017	88.6
Acetamiprid 200 SL (0.25 L/ha)	1	0.035	0.023	65.9	0.035	0.024	69.3
	2	0.038	0.027	71.1	0.036	0.035	97.4
	3	0.031	0.025	80.5	0.040	0.029	72.5
	4	0.036	0.026	73.1	0.034	0.030	88.6

	5	0.030	0.025	84.4	0.036	0.023	63.5
	6	0.030	0.019	63.5	0.034	0.031	92.2
	Mean	-	0.024	73.1	-	0.029	80.6
Acetamiprid 200 SL (0.40 L/ha)	1	0.056	0.063	112.3	0.056	0.040	71.60
	2	0.053	0.048	91.2	0.056	0.046	81.80
	3	0.052	0.041	78.1	0.056	0.057	101.22
	4	0.048	0.031	63.9	0.053	0.045	85.01
	5	0.051	0.041	80.3	0.057	0.044	77.39
	6	0.051	0.030	58.9	0.061	0.045	73.41
	Mean	-	0.042	80.8	-	0.046	81.7

¹⁾ Theoretical concentration of a.s. = theoretical amount of a.s. applied on the sampled area per plot (10 soil cores with a diameter of 5 cm) / mass of the sampled soil (Mass of dry soil sample) per plot (sum of 10 soil cores)

LOD: 0.0015 mg/kg; LOQ: 0.005 mg/kg

The average residue levels of the active substance in the samples were within the recommended range of 50% to 150% of the nominal values, thus, the correct application of the test item was verified.

Biological results

In total, Collembola of 2 orders, 2 suborders, 12 families, 5 subfamilies, 24 genera, 2 species groups and 11 species were determined.

The test item caused no statistically significant reductions in abundance of the large majority of the Collembola taxa of the upper 5 cm of the soil of all test item treatment groups throughout the test period. Only a few taxa showed transient statistically significant reductions in the low, middle and high test item treatment groups (2 x 0.15, 2 x 0.25 L and 2 x 0.40 L test item/ha) shortly after application (day 28 after 1st application, 1st sampling) and only one taxon showed statistically significant reductions in the low and high test item treatment group (2 x 0.15 and 2 x 0.40 L test item/ha) on day 182 after 1st application.

Clear recovery took place during the test so that no statistically significant reductions could be observed for all dominant Collembola taxa at the end of the test on day 365 after 1st application (5th sampling).

Table A 204: Mean abundance of the Collembola population of the upper 5 cm of the soil (soil cores)

	Treatment group		Abundance (ind./m ²)				
			Sampling (days after 1 st application)				
			Pre	1 st	2 nd	3 rd	5 th
			(-4)	(28)	(56)	(182)	(365)
Total Collembola	Control	Mean	12760.7	14500.8	551.7	8771.2	15618.4
		SD	2629.8	6454.5	488.4	3362.3	5762.8
		% of control	100.0	100.0	100.0	100.0	100.0
	Acetamiprid 200 SL (0.15 L/ha)	Mean	11176.2	12619.2	481.0	6649.1	17287.8
		SD	3584.7	6235.4	411.2	3299.6	9411.0
		% of control	87.6	87.0	87.2	75.8	110.7
	Acetamiprid 200 SL (0.25 L/ha)	Mean	8078.0	5955.9	396.1	6719.9	14359.3
		SD	2747.6	3323.4	272.0	2591.0	3005.1
		% of control	63.3	41.1	71.8	76.6	91.9
	Acetamiprid 200 SL (0.40 L/ha)	Mean	8162.9	14670.5	933.7	7582.8	14939.3
		SD	2122.7	9369.0	632.9	1568.5	2129.2
		% of control	64.0	101.2	169.2	86.5	95.7
	Reference item	Mean	12958.7	1244.9	56.6	5333.5	18221.5
		SD	6006.6	519.6	69.3	2928.3	8692.9
		% of control	101.6	8.6	10.3	60.8	116.7

	Treatment group		Abundance (ind./m ²)				
			Sampling (days after 1 st application)				
			Pre	1 st	2 nd	3 rd	5 th
			(-4)	(28)	(56)	(182)	(365)
Arthropleona	Control	Mean	11091.3	11473.3	495.1	8247.8	15165.7
		SD	2777.4	5844.6	444.0	3349.8	5643.9
		% of control	100.0	100.0	100.0	100.0	100.0
	Acetamiprid 200 SL (0.15 L/ha)	Mean	9690.8	10299.1	466.9	6097.4	16792.6
		SD	3145.2	5684.9	382.4	3155.3	9140.3
		% of control	87.4	89.8	94.3	73.9	110.7
	Acetamiprid 200 SL (0.25 L/ha)	Mean	6974.5	4640.3	339.5	6097.4	13722.7
		SD	2359.3	2919.1	289.1	2372.7	3017.2
		% of control	62.9	40.4	68.6	73.9	90.5
	Acetamiprid 200 SL (0.40 L/ha)	Mean	6903.8	11841.1	820.5	7087.7	14458.3
		SD	2188.4	8064.3	611.3	1399.7	2347.2
		% of control	62.2	103.2	165.7	85.9	95.3
	Reference item	Mean	10964.0	1188.4	42.4	4795.9	17584.9
		SD	5567.5	552.7	71.0	2613.9	8726.4
		% of control	98.9	10.4	8.6	58.1	116.0
Entomobryomorpha	Control	Mean	8035.6	10624.5	438.6	7964.8	14911.0
		SD	1784.3	5392.7	463.1	3377.2	5642.9
		% of control	100.0	100.0	100.0	100.0	100.0
	Acetamiprid 200 SL (0.15 L/ha)	Mean	6932.1	9846.4	438.6	5984.2	16651.1
		SD	2132.3	5434.1	345.5	3165.5	9186.2
		% of control	86.3	92.7	100.0	75.1	111.7
	Acetamiprid 200 SL (0.25 L/ha)	Mean	5107.1	4230.0	339.5	5814.5	13326.6
		SD	2304.3	2807.8	289.1	2369.9	2975.5
		% of control	63.6	39.8	77.4	73.0	89.4
	Acetamiprid 200 SL (0.40 L/ha)	Mean	4979.8	11515.7	749.8	6889.6	14274.4
		SD	1156.0	7860.1	591.5	1417.7	2418.4
		% of control	62.0	108.4	171.0	86.5	95.7
	Reference item	Mean	8134.6	565.9	42.4	4583.7	17089.7
		SD	4466.3	421.6	71.0	2571.3	8772.6
		% of control	101.2	5.3	9.7	57.5	114.6
Entomobryidae	Control	Mean	2588.9	3932.9	325.4	3211.4	3890.5
		SD	883.4	1437.2	424.1	1548.0	1441.9
		% of control	100.0	100.0	100.0	100.0	100.0
	Acetamiprid 200 SL (0.15 L/ha)	Mean	2192.8	4399.7	396.1	2900.2	5814.5
		SD	852.9	2760.1	362.8	1907.4	4173.9
		% of control	84.7	111.9	121.7	90.3	149.5
	Acetamiprid 200 SL (0.25 L/ha)	Mean	1542.0	2263.5	254.6	3041.6	3650.0
		SD	884.4	2254.5	221.3	1531.1	674.8
		% of control	59.6	57.6	78.3	94.7	93.8
	Acetamiprid 200 SL (0.40 L/ha)	Mean	1471.3	4357.3	679.1	3282.1	4357.3
		SD	499.8	3039.5	602.6	1433.2	1272.3
		% of control	56.8	110.8	208.7	102.2	112.0
	Reference item	Mean	2405.0	367.8	42.4	3282.1	5899.3
		SD	1311.3	244.1	71.0	2228.2	2248.8

	Treatment group		Abundance (ind./m ²)				
			Sampling (days after 1 st application)				
			Pre	1 st	2 nd	3 rd	5 th
			(-4)	(28)	(56)	(182)	(365)
		% of control	92.9	9.4	13.0	102.2	151.6
Entomobryinae	Control	Mean	2532.3	3805.6	254.6	2291.8	3069.9
		SD	871.3	1377.5	347.9	1481.0	1136.8
		% of control	100.0	100.0	100.0	100.0	100.0
	Acetamiprid 200 SL (0.15 L/ha)	Mean	2107.9	4116.8	339.5	1980.6	4739.3
		SD	856.3	2592.9	268.4	1620.1	3459.4
		% of control	83.2	108.2	133.3	86.4	154.4
	Acetamiprid 200 SL (0.25 L/ha)	Mean	1471.3	2206.9	226.4	2348.4	2928.5
		SD	810.0	2246.4	244.1	1360.9	891.5
		% of control	58.1	58.0	88.9	102.5	95.4
	Acetamiprid 200 SL (0.40 L/ha)	Mean	1443.0	4230.0	636.6	2659.7	3395.3
		SD	480.2	2932.3	594.8	1185.5	878.9
		% of control	57.0	111.2	250.0	116.0	110.6
	Reference item	Mean	2376.7	339.5	42.4	2772.8	4711.0
		SD	1291.8	178.1	71.0	1937.6	1414.0
		% of control	93.9	8.9	16.7	121.0	153.5
Lepidocyrtus	Control	Mean	1117.6	1556.2	84.9	1782.5	2051.3
		SD	586.7	1037.7	142.0	1459.4	744.7
		% of control	100.0	100.0	100.0	100.0	100.0
	Acetamiprid 200 SL (0.15 L/ha)	Mean	1259.1	1400.6	141.5	1386.4	3607.5
		SD	561.6	924.8	244.1	1244.8	2475.8
		% of control	112.7	90.0	166.7	77.8	175.9
	Acetamiprid 200 SL (0.25 L/ha)	Mean	933.7	693.2	28.3	1867.4	2504.0
		SD	550.1	925.8	69.3	1184.7	810.2
		% of control	83.5	44.5	33.3	104.8	122.1
	Acetamiprid 200 SL (0.40 L/ha)	Mean	735.6	1485.4	113.2	1952.3	2886.0
		SD	249.9	1499.1	183.4	942.2	911.1
		% of control	65.8	95.5	133.3	109.5	140.7
	Reference item	Mean	1259.1	28.3	14.1	1881.6	3664.1
		SD	877.9	43.8	34.7	1513.1	615.4
		% of control	112.7	1.8	16.7	105.6	178.6
<i>Lepidocyrtus cyaneus</i> / <i>violaceus</i>	Control	Mean	1117.6	1428.9	84.9	1725.9	2023.0
		SD	586.7	945.8	142.0	1351.4	778.7
		% of control	100.0	100.0	100.0	100.0	100.0
	Acetamiprid 200 SL (0.15 L/ha)	Mean	1259.1	1160.1	141.5	1273.2	3607.5
		SD	561.6	801.1	244.1	1256.7	2475.8
		% of control	112.7	81.2	166.7	73.8	178.3
	Acetamiprid 200 SL (0.25 L/ha)	Mean	933.7	509.3	28.3	1683.5	2461.6
		SD	550.1	778.0	69.3	1158.2	747.7
		% of control	83.5	35.6	33.3	97.5	121.7
	Acetamiprid 200 SL (0.40 L/ha)	Mean	735.6	1216.7	113.2	1909.9	2857.7
		SD	249.9	1194.0	183.4	910.7	888.1
		% of control	65.8	85.1	133.3	110.7	141.3
	Reference	Mean	1230.8	28.3	14.1	1796.7	3635.8

	Treatment group		Abundance (ind./m ²)				
			Sampling (days after 1 st application)				
			Pre	1 st	2 nd	3 rd	5 th
			(-4)	(28)	(56)	(182)	(365)
item		SD	899.5	43.8	34.7	1408.6	603.6
		% of control	110.1	2.0	16.7	104.1	179.7
Pseudosinella	Control	Mean	1400.6	2221.1	155.6	282.9	664.9
		SD	806.6	939.7	224.0	219.2	798.8
		% of control	100.0	100.0	100.0	100.0	100.0
	Acetamiprid 200 SL (0.15 L/ha)	Mean	848.8	2673.8	183.9	523.4	679.1
		SD	387.1	1926.5	124.9	417.3	523.3
		% of control	60.6	120.4	118.2	185.0	102.1
	Acetamiprid 200 SL (0.25 L/ha)	Mean	537.6	1443.0	169.8	424.4	367.8
		SD	535.9	1430.5	161.1	405.3	292.4
		% of control	38.4	65.0	109.1	150.0	55.3
	Acetamiprid 200 SL (0.40 L/ha)	Mean	693.2	2659.7	509.3	452.7	438.6
		SD	388.7	1873.3	539.5	404.1	210.8
		% of control	49.5	119.7	327.3	160.0	66.0
	Reference item	Mean	1117.6	311.2	14.1	749.8	891.3
		SD	731.0	191.1	34.7	682.1	1055.8
		% of control	79.8	14.0	9.1	265.0	134.0
Pseudosinella alba	Control	Mean	1386.4	2093.8	127.3	198.1	594.2
		SD	792.0	881.6	158.8	127.8	704.1
		% of control	100.0	100.0	100.0	100.0	100.0
	Acetamiprid 200 SL (0.15 L/ha)	Mean	820.5	2023.0	155.6	311.2	679.1
		SD	428.4	1501.6	99.2	138.6	523.3
		% of control	59.2	96.6	122.2	157.1	114.3
	Acetamiprid 200 SL (0.25 L/ha)	Mean	523.4	1202.5	155.6	311.2	367.8
		SD	518.9	1357.5	146.2	370.6	292.4
		% of control	37.8	57.4	122.2	157.1	61.9
	Acetamiprid 200 SL (0.40 L/ha)	Mean	664.9	2306.0	452.7	282.9	410.3
		SD	388.7	1758.9	502.7	249.9	210.8
		% of control	48.0	110.1	355.6	142.9	69.0
	Reference item	Mean	1103.5	311.2	0.0	509.3	877.1
		SD	740.0	191.1	0.0	500.7	1065.2
		% of control	79.6	14.9	0.0	257.1	147.6
Isotomidae	Control	Mean	5446.6	6691.6	113.2	4753.4	11020.6
		SD	1924.7	4101.0	69.3	2412.8	4599.2
		% of control	100.0	100.0	100.0	100.0	100.0
	Acetamiprid 200 SL (0.15 L/ha)	Mean	4739.3	5446.6	42.4	3084.1	10836.7
		SD	1750.7	2782.5	71.0	1850.9	6198.3
		% of control	87.0	81.4	37.5	64.9	98.3
	Acetamiprid 200 SL (0.25 L/ha)	Mean	3565.1	1966.4	84.9	2772.8	9676.6
		SD	1765.9	980.3	107.4	1697.4	3091.4
		% of control	65.5	29.4	75.0	58.3	87.8
	Acetamiprid 200 SL (0.40 L/ha)	Mean	3508.5	7158.4	70.7	3607.5	9917.1
		SD	985.0	5014.2	83.5	923.2	1375.4
		% of control	64.4	107.0	62.5	75.9	90.0

	Treatment group		Abundance (ind./m ²)				
			Sampling (days after 1 st application)				
			Pre	1 st	2 nd	3 rd	5 th
			(-4)	(28)	(56)	(182)	(365)
Isotoma	Reference item	Mean	5729.6	198.1	0.0	1301.5	11190.4
		SD	3343.4	219.2	0.0	620.7	6736.4
		% of control	105.2	3.0	0.0	27.4	101.5
	Control	Mean	70.7	721.5	0.0	664.9	311.2
		SD	112.8	461.0	0.0	499.1	175.3
		% of control	100.0	100.0	100.0	100.0	100.0
	Acetamiprid 200 SL (0.15 L/ha)	Mean	155.6	99.0	0.0	226.4	410.3
		SD	124.9	136.0	0.0	212.5	270.7
		% of control	220.0	13.7	-	34.0	131.8
Isotoma	Acetamiprid 200 SL (0.25 L/ha)	Mean	155.6	14.1	0.0	353.7	466.9
		SD	236.6	34.7	0.0	236.6	244.5
		% of control	220.0	2.0	-	53.2	150.0
	Acetamiprid 200 SL (0.40 L/ha)	Mean	28.3	198.1	0.0	523.4	212.2
		SD	69.3	191.1	0.0	427.5	158.8
		% of control	40.0	27.5	-	78.7	68.2
	Reference item	Mean	226.4	0.0	0.0	240.5	410.3
		SD	261.2	0.0	0.0	217.5	146.2
		% of control	320.0	0.0	-	36.2	131.8
<i>Isotoma anglicana / viridis</i>	Control	Mean	70.7	721.5	0.0	664.9	297.1
		SD	112.8	461.0	0.0	499.1	158.8
		% of control	100.0	100.0	100.0	100.0	100.0
	Acetamiprid 200 SL (0.15 L/ha)	Mean	141.5	99.0	0.0	226.4	410.3
		SD	116.0	136.0	0.0	212.5	270.7
		% of control	200.0	13.7	-	34.0	138.1
	Acetamiprid 200 SL (0.25 L/ha)	Mean	155.6	14.1	0.0	353.7	452.7
		SD	236.6	34.7	0.0	236.6	225.6
		% of control	220.0	2.0	-	53.2	152.4
Isotomurus	Acetamiprid 200 SL (0.40 L/ha)	Mean	28.3	198.1	0.0	523.4	212.2
		SD	69.3	191.1	0.0	427.5	158.8
		% of control	40.0	27.5	-	78.7	71.4
	Reference item	Mean	226.4	0.0	0.0	240.5	410.3
		SD	261.2	0.0	0.0	217.5	146.2
		% of control	320.0	0.0	-	36.2	138.1
	Control	Mean	452.7	2518.2	14.1	1315.7	5701.3
		SD	277.2	1061.1	34.7	729.7	2036.8
		% of control	100.0	100.0	100.0	100.0	100.0
Isotomurus	Acetamiprid 200 SL (0.15 L/ha)	Mean	198.1	877.1	14.1	693.2	5149.5
		SD	69.3	582.3	34.7	196.6	3014.8
		% of control	43.8	34.8	100.0	52.7	90.3
	Acetamiprid 200 SL (0.25 L/ha)	Mean	169.8	382.0	0.0	594.2	3550.9
		SD	193.6	272.4	0.0	474.1	1735.8
		% of control	37.5	15.2	0.0	45.2	62.3
	Acetamiprid 200 SL (0.40 L/ha)	Mean	141.5	1527.9	0.0	367.8	5022.2
		SD	225.6	1111.9	0.0	358.8	999.2

	Treatment group		Abundance (ind./m ²)				
			Sampling (days after 1 st application)				
			Pre	1 st	2 nd	3 rd	5 th
			(-4)	(28)	(56)	(182)	(365)
		% of control	31.3	60.7	0.0	28.0	88.1
		Mean	396.1	0.0	0.0	99.0	3310.4
		SD	457.6	0.0	0.0	136.0	2396.6
	Reference item	% of control	87.5	0.0	0.0	7.5	58.1
		Mean	3749.0	1853.3	42.4	551.7	3437.7
		SD	1753.9	1456.8	46.5	355.1	4927.5
		% of control	100.0	100.0	100.0	100.0	100.0
Parisotoma	Control	Mean	3749.0	1853.3	42.4	551.7	3437.7
		SD	1753.9	1456.8	46.5	355.1	4927.5
		% of control	100.0	100.0	100.0	100.0	100.0
	Acetamiprid 200 SL (0.15 L/ha)	Mean	3296.3	2504.0	0.0	565.9	3225.5
		SD	1588.4	1669.2	0.0	701.3	3780.9
		% of control	87.9	135.1	0.0	102.6	93.8
	Acetamiprid 200 SL (0.25 L/ha)	Mean	2461.6	749.8	28.3	763.9	4116.8
		SD	1472.2	490.3	69.3	486.1	2837.0
		% of control	65.7	40.5	66.7	138.5	119.8
	Acetamiprid 200 SL (0.40 L/ha)	Mean	2617.2	3635.8	14.1	1301.5	2857.7
		SD	948.9	3596.2	34.7	1078.6	1662.2
		% of control	69.8	196.2	33.3	235.9	83.1
	Reference item	Mean	3975.3	141.5	0.0	438.6	4965.6
		SD	2163.0	175.3	0.0	499.1	5128.1
		% of control	106.0	7.6	0.0	79.5	144.4
Parisotoma notabilis	Control	Mean	3282.1	1796.7	42.4	551.7	3395.3
		SD	1754.1	1426.9	46.5	355.1	4824.4
		% of control	100.0	100.0	100.0	100.0	100.0
	Acetamiprid 200 SL (0.15 L/ha)	Mean	3183.1	2419.2	0.0	565.9	3225.5
		SD	1611.2	1549.2	0.0	701.3	3780.9
		% of control	97.0	134.6	0.0	102.6	95.0
	Acetamiprid 200 SL (0.25 L/ha)	Mean	2164.5	721.5	28.3	763.9	4116.8
		SD	1370.5	425.3	69.3	486.1	2837.0
		% of control	65.9	40.2	66.7	138.5	121.3
	Acetamiprid 200 SL (0.40 L/ha)	Mean	2433.3	3550.9	14.1	1301.5	2857.7
		SD	1001.0	3632.9	34.7	1078.6	1662.2
		% of control	74.1	197.6	33.3	235.9	84.2
	Reference item	Mean	3593.4	141.5	0.0	424.4	4965.6
		SD	2200.2	175.3	0.0	471.1	5128.1
		% of control	109.5	7.9	0.0	76.9	146.3
Poduromorpha	Control	Mean	3055.8	848.8	56.6	282.9	183.9
		SD	2542.5	891.9	138.6	219.2	155.7
		% of control	100.0	100.0	100.0	100.0	100.0
	Acetamiprid 200 SL (0.15 L/ha)	Mean	2758.7	438.6	28.3	113.2	141.5
		SD	1478.8	388.7	43.8	127.8	116.0
		% of control	90.3	51.7	50.0	40.0	76.9
	Acetamiprid 200 SL (0.25 L/ha)	Mean	1867.4	410.3	0.0	282.9	382.0
		SD	1117.1	314.9	0.0	277.2	176.0
		% of control	61.1	48.3	0.0	100.0	207.7
	Acetamiprid 200 SL	Mean	1924.0	325.4	70.7	198.1	169.8

	Treatment group		Abundance (ind./m ²)				
			Sampling (days after 1 st application)				
			Pre	1 st	2 nd	3 rd	5 th
			(-4)	(28)	(56)	(182)	(365)
	(0.40 L/ha)	SD	2508.1	328.4	63.9	198.5	151.8
		% of control	63.0	38.3	125.0	70.0	92.3
		Mean	2829.4	622.5	0.0	212.2	495.1
	Reference item	SD	2274.9	219.2	0.0	256.1	669.3
		% of control	92.6	73.3	0.0	75.0	269.2
		Mean	2956.7	735.6	0.0	254.6	183.9
Onychiuridae	Control	SD	2459.2	896.2	0.0	178.1	155.7
		% of control	100.0	100.0	100.0	100.0	100.0
		Mean	2744.5	382.0	0.0	113.2	127.3
	Acetamiprid 200 SL (0.15 L/ha)	SD	1490.3	261.6	0.0	127.8	89.0
		% of control	92.8	51.9	-	44.4	69.2
		Mean	1810.8	282.9	0.0	141.5	297.1
	Acetamiprid 200 SL (0.25 L/ha)	SD	1133.3	198.5	0.0	175.3	117.0
		% of control	61.2	38.5	-	55.6	161.5
		Mean	1895.7	311.2	14.1	155.6	169.8
	Acetamiprid 200 SL (0.40 L/ha)	SD	2515.5	333.8	34.7	99.2	151.8
		% of control	64.1	42.3	-	61.1	92.3
		Mean	2688.0	580.0	0.0	183.9	481.0
	Reference item	SD	2298.2	265.3	0.0	236.6	636.7
		% of control	90.9	78.8	-	72.2	261.5
		Mean	2857.7	707.4	0.0	212.2	169.8
Tullbergiinae	Control	SD	2491.9	901.0	0.0	219.7	169.8
		% of control	100.0	100.0	100.0	100.0	100.0
		Mean	2673.8	367.8	0.0	99.0	127.3
	Acetamiprid 200 SL (0.15 L/ha)	SD	1530.5	277.2	0.0	136.0	89.0
		% of control	93.6	52.0	-	46.7	75.0
		Mean	1796.7	268.8	0.0	127.3	282.9
	Acetamiprid 200 SL (0.25 L/ha)	SD	1138.1	196.6	0.0	149.5	102.8
		% of control	62.9	38.0	-	60.0	166.7
		Mean	1839.1	311.2	14.1	127.3	169.8
	Acetamiprid 200 SL (0.40 L/ha)	SD	2508.1	333.8	34.7	71.0	151.8
		% of control	64.4	44.0	-	60.0	100.0
		Mean	2673.8	565.9	0.0	183.9	481.0
	Reference item	SD	2265.4	287.4	0.0	236.6	636.7
		% of control	93.6	80.0	-	86.7	283.3
		Mean	2843.6	650.8	0.0	212.2	169.8
Mesaphorura	Control	SD	2504.1	901.0	0.0	219.7	169.8
		% of control	100.0	100.0	100.0	100.0	100.0
		Mean	2673.8	311.2	0.0	99.0	127.3
	Acetamiprid 200 SL (0.15 L/ha)	SD	1530.5	249.9	0.0	136.0	89.0
		% of control	94.0	47.8	-	46.7	75.0
		Mean	1740.1	226.4	0.0	127.3	282.9
	Acetamiprid 200 SL (0.25 L/ha)	SD	1089.3	183.4	0.0	149.5	102.8
		% of control	61.2	34.8	-	60.0	166.7
		Mean	2843.6	650.8	0.0	212.2	169.8
	Reference item	SD	2265.4	287.4	0.0	236.6	636.7

	Treatment group		Abundance (ind./m ²)				
			Sampling (days after 1 st application)				
			Pre	1 st	2 nd	3 rd	5 th
			(-4)	(28)	(56)	(182)	(365)
	Acetamiprid 200 SL (0.40 L/ha)	Mean	1839.1	297.1	14.1	127.3	169.8
		SD	2508.1	302.5	34.7	71.0	151.8
		% of control	64.7	45.7	-	60.0	100.0
	Reference item	Mean	2673.8	537.6	0.0	183.9	438.6
		SD	2265.4	311.5	0.0	236.6	601.2
		% of control	94.0	82.6	-	86.7	258.3
Symphypleona	Control	Mean	1655.2	2999.2	28.3	481.0	438.6
		SD	359.1	1559.3	69.3	266.6	434.2
		% of control	100.0	100.0	100.0	100.0	100.0
	Acetamiprid 200 SL (0.15 L/ha)	Mean	1485.4	2306.0	14.1	551.7	481.0
		SD	1050.3	767.6	34.7	256.1	362.8
		% of control	89.7	76.9	50.0	114.7	109.7
	Acetamiprid 200 SL (0.25 L/ha)	Mean	1103.5	1287.4	56.6	622.5	580.0
		SD	607.4	806.0	87.7	457.6	224.0
		% of control	66.7	42.9	200.0	129.4	132.3
	Acetamiprid 200 SL (0.40 L/ha)	Mean	1216.7	2758.7	99.0	495.1	481.0
		SD	451.3	2007.8	124.9	328.4	358.8
		% of control	73.5	92.0	350.0	102.9	109.7
	Reference item	Mean	1994.7	42.4	14.1	537.6	622.5
		SD	663.5	71.0	34.7	473.1	333.8
		% of control	120.5	1.4	50.0	111.8	141.9
Katiannidae	Control	Mean	1244.9	2900.2	14.1	254.6	396.1
		SD	249.9	1577.5	34.7	107.4	400.5
		% of control	100.0	100.0	100.0	100.0	100.0
	Acetamiprid 200 SL (0.15 L/ha)	Mean	1202.5	2277.7	0.0	339.5	382.0
		SD	956.5	782.4	0.0	227.8	338.5
		% of control	96.6	78.5	0.0	133.3	96.4
	Acetamiprid 200 SL (0.25 L/ha)	Mean	735.6	1259.1	56.6	410.3	367.8
		SD	441.6	809.6	87.7	181.4	225.6
		% of control	59.1	43.4	400.0	161.1	92.9
	Acetamiprid 200 SL (0.40 L/ha)	Mean	820.5	2730.4	84.9	339.5	339.5
		SD	378.3	2009.7	107.4	294.0	93.0
		% of control	65.9	94.1	600.0	133.3	85.7
	Reference item	Mean	1046.9	42.4	0.0	339.5	311.2
		SD	659.0	71.0	0.0	401.7	219.2
		% of control	84.1	1.5	0.0	133.3	78.6

Mean [ind./m²], SD = standard deviation [ind./m²]

Statistics: comparisons of test item treatments vs. control and reference item vs. control (pre-treatment sampling: ANOVA, post-treatment samplings: one-sided t-test): **bold values** indicate statistically significant differences compared to control ($\alpha = 0.05$).

The test item caused no statistically significant reduction in abundance of the majority of the Collembola taxa of the soil surface of all test item treatment groups throughout the test period. Only a few taxa showed transient statistically significant reductions in the low, middle and high test item treatment groups shortly after application (day 28 after 1st application, 1st sampling) and only one taxon showed statistically significant reductions in the middle and high test item treatment group (2 x 0.25 and 2 x 0.40 L test item/ha) on day 182 after 1st application.

Clear recovery took place during the test so that no statistically significant reductions could be observed for all dominant Collembola taxa at the end of the test on day 365 after 1st application (5th sampling).

Table A 205: Mean numbers of the Collembola population of the soil surface (pitfall traps)

	Treatment group		Numbers (ind./pitfall trap)				
			Sampling (days after 1 st application)				
			pre	1 st	2 nd	3 rd	5 th
			(-4)	(28)	(56)	(182)	(365)
Total Collembola	Control	Mean	201.2	347.6	99.8	196.8	3234.1
		SD	78.5	22.0	32.2	55.2	922.7
		% of control	100.0	100.0	100.0	100.0	100.0
	Acetamiprid 200 SL (0.15 L/ha)	Mean	199.7	316.9	132.8	200.4	3205.3
		SD	74.8	105.0	39.3	88.1	905.2
		% of control	99.3	91.2	133.0	101.9	99.1
	Acetamiprid 200 SL (0.25 L/ha)	Mean	180.1	268.9	133.2	179.6	3692.6
		SD	32.3	88.8	46.5	44.7	900.3
		% of control	89.5	77.4	133.4	91.3	114.2
	Acetamiprid 200 SL (0.40 L/ha)	Mean	179.3	238.5	112.7	168.0	3218.6
		SD	38.3	74.9	37.6	53.4	1010.6
		% of control	89.1	68.6	112.9	85.4	99.5
	Reference item	Mean	150.9	35.6	46.8	168.5	3901.5
		SD	39.4	12.1	15.2	24.8	740.9
		% of control	75.0	10.2	46.8	85.6	120.6
Arthropleona	Control	Mean	186.3	234.8	85.7	189.1	3228.3
		SD	76.7	43.8	31.2	56.4	923.0
		% of control	100.0	100.0	100.0	100.0	100.0
	Acetamiprid 200 SL (0.15 L/ha)	Mean	184.5	233.5	122.9	191.1	3201.9
		SD	69.1	84.5	35.9	87.6	904.1
		% of control	99.0	99.5	143.4	101.1	99.2
	Acetamiprid 200 SL (0.25 L/ha)	Mean	159.4	224.8	121.3	167.6	3686.9
		SD	31.9	59.7	49.8	40.6	901.5
		% of control	85.6	95.8	141.5	88.6	114.2
	Acetamiprid 200 SL (0.40 L/ha)	Mean	162.7	177.8	100.9	158.7	3214.0
		SD	42.1	63.0	33.9	51.3	1010.7
		% of control	87.3	75.8	117.7	83.9	99.6
	Reference item	Mean	137.0	35.1	45.2	163.4	3897.7
		SD	39.0	11.8	15.4	26.0	741.4
		% of control	73.6	15.0	52.7	86.4	120.7
Entomobryomorpha	Control	Mean	186.1	234.5	85.7	189.1	3228.1
		SD	76.7	43.8	31.2	56.4	922.9
		% of control	100.0	100.0	100.0	100.0	100.0
	Acetamiprid 200 SL (0.15 L/ha)	Mean	184.4	232.8	122.9	191.1	3201.8
		SD	69.1	84.0	35.9	87.6	904.0

		% of control	99.1	99.3	143.4	101.1	99.2
		Mean	159.1	215.5	121.3	167.6	3686.7
		SD	31.9	65.0	49.8	40.6	901.5
	Acetamiprid 200 SL (0.25 L/ha)	% of control	85.5	91.9	141.5	88.6	114.2
		Mean	162.4	177.3	100.9	158.6	3213.9
		SD	42.1	63.2	33.9	51.4	1010.6
	Acetamiprid 200 SL (0.40 L/ha)	% of control	87.3	75.6	117.7	83.9	99.6
		Mean	136.8	34.3	45.2	163.4	3897.7
		SD	39.1	10.8	15.3	26.0	741.4
	Reference item	% of control	73.5	14.6	52.7	86.4	120.7
Entomobryidae	Control	Mean	172.4	183.4	81.6	130.5	3120.5
		SD	71.7	36.0	29.1	57.3	918.0
		% of control	100.0	100.0	100.0	100.0	100.0
	Acetamiprid 200 SL (0.15 L/ha)	Mean	171.5	189.0	116.0	130.3	3088.0
		SD	64.4	65.3	33.2	76.7	906.8
		% of control	99.5	103.0	142.2	99.9	99.0
	Acetamiprid 200 SL (0.25 L/ha)	Mean	148.7	185.3	115.5	121.4	3572.5
		SD	28.7	49.4	46.8	28.8	917.9
		% of control	86.3	101.0	141.6	93.1	114.5
	Acetamiprid 200 SL (0.40 L/ha)	Mean	150.3	156.5	96.1	112.0	3127.3
		SD	36.5	51.9	31.1	39.8	986.7
		% of control	87.2	85.3	117.8	85.9	100.2
	Reference item	Mean	129.3	31.7	44.8	130.6	3775.9
		SD	36.5	9.7	15.3	27.2	749.0
		% of control	75.0	17.3	54.9	100.1	121.0
Entomobryinae	Control	Mean	170.5	168.0	71.0	68.8	2982.6
		SD	71.3	41.1	28.3	55.8	903.0
		% of control	100.0	100.0	100.0	100.0	100.0
	Acetamiprid 200 SL (0.15 L/ha)	Mean	169.6	166.3	103.0	70.8	2963.3
		SD	64.2	62.3	31.8	69.3	875.9
		% of control	99.5	99.0	145.2	102.8	99.4
	Acetamiprid 200 SL (0.25 L/ha)	Mean	146.4	152.1	104.3	59.5	3427.6
		SD	28.9	43.4	42.5	22.1	908.6
		% of control	85.8	90.5	147.0	86.6	114.9
	Acetamiprid 200 SL (0.40 L/ha)	Mean	148.3	135.8	84.8	48.0	2995.7
		SD	36.7	51.6	27.1	23.6	954.1
		% of control	86.9	80.9	119.4	69.8	100.4
	Reference item	Mean	127.4	29.8	36.5	50.3	3616.7
		SD	35.8	9.2	13.5	17.9	736.4
		% of control	74.7	17.8	51.5	73.0	121.3
Entomobrya	Control	Mean	0.3	7.8	1.0	0.6	1.9
		SD	0.2	5.1	0.8	0.6	1.5
		% of control	100.0	100.0	100.0	100.0	100.0
	Acetamiprid 200 SL (0.15 L/ha)	Mean	0.5	3.2	0.5	0.4	0.7
		SD	0.2	3.5	0.5	0.7	0.4
		% of control	137.5	41.4	52.2	64.3	34.6
	Acetamiprid 200 SL (0.25 L/ha)	Mean	0.4	0.6	0.7	0.5	1.5
		SD	0.3	0.6	0.5	0.6	1.5

		% of control	125.0	8.1	69.6	92.9	77.9
		Mean	0.4	1.4	0.7	0.4	1.5
		SD	0.3	1.7	0.8	0.3	0.6
	Acetamiprid 200 SL (0.40 L/ha)	% of control	125.0	17.7	73.9	64.3	79.4
		Mean	0.3	0.0	0.3	0.3	1.5
		SD	0.3	0.0	0.3	0.3	0.9
	Reference item	% of control	75.0	0.0	30.4	50.0	77.2
<i>Entomobrya multifasciata</i>	Control	Mean	0.3	1.0	0.3	0.5	1.8
		SD	0.2	1.0	0.2	0.5	1.4
		% of control	100.0	100.0	100.0	100.0	100.0
	Acetamiprid 200 SL (0.15 L/ha)	Mean	0.5	0.4	0.1	0.3	0.6
		SD	0.2	0.4	0.1	0.5	0.3
		% of control	137.5	40.0	33.3	54.5	31.5
	Acetamiprid 200 SL (0.25 L/ha)	Mean	0.4	0.5	0.3	0.4	1.3
		SD	0.3	0.6	0.2	0.3	1.4
		% of control	112.5	44.0	116.7	81.8	73.8
	Acetamiprid 200 SL (0.40 L/ha)	Mean	0.4	0.4	0.2	0.2	1.3
		SD	0.3	0.6	0.2	0.2	0.6
		% of control	125.0	36.0	83.3	45.5	73.8
	Reference item	Mean	0.3	0.0	0.0	0.2	1.4
		SD	0.3	0.0	0.1	0.2	0.9
		% of control	75.0	0.0	16.7	45.5	76.2
Lepidocyrtus	Control	Mean	169.5	151.8	67.6	67.6	2979.6
		SD	70.3	34.4	27.3	55.0	901.8
		% of control	100.0	100.0	100.0	100.0	100.0
	Acetamiprid 200 SL (0.15 L/ha)	Mean	168.4	157.4	99.0	70.0	2962.2
		SD	63.6	59.0	30.4	69.2	875.5
		% of control	99.4	103.6	146.4	103.6	99.4
	Acetamiprid 200 SL (0.25 L/ha)	Mean	145.7	146.0	100.0	58.5	3425.3
		SD	29.0	44.5	39.5	21.9	907.9
		% of control	86.0	96.2	147.9	86.6	115.0
	Acetamiprid 200 SL (0.40 L/ha)	Mean	147.8	130.7	79.8	47.1	2992.6
		SD	36.5	51.2	26.4	23.2	953.7
		% of control	87.2	86.1	118.1	69.7	100.4
	Reference item	Mean	126.9	28.6	35.6	49.5	3614.6
		SD	35.8	9.3	13.3	17.6	736.0
		% of control	74.9	18.8	52.7	73.3	121.3
<i>Lepidocyrtus cyaneus / violaceus</i>	Control	Mean	169.4	139.7	64.8	55.3	2965.1
		SD	70.4	36.9	26.2	46.3	894.6
		% of control	100.0	100.0	100.0	100.0	100.0
	Acetamiprid 200 SL (0.15 L/ha)	Mean	168.3	131.4	94.0	61.0	2956.3
		SD	63.7	46.7	29.3	61.8	873.0
		% of control	99.3	94.1	145.1	110.2	99.7
	Acetamiprid 200 SL (0.25 L/ha)	Mean	145.6	119.3	96.3	48.3	3417.9
		SD	29.1	35.3	37.4	16.0	906.6
		% of control	86.0	85.4	148.6	87.3	115.3
	Acetamiprid 200 SL (0.40 L/ha)	Mean	147.7	112.0	74.2	43.1	2985.5
		SD	36.5	49.8	23.6	22.4	949.3

	Reference item	% of control	87.2	80.2	114.4	77.9	100.7
		Mean	126.8	28.3	34.9	44.6	3610.6
		SD	35.9	9.3	13.0	18.3	734.6
		% of control	74.9	20.3	53.8	80.6	121.8
Pseudosinella	Control	Mean	0.7	6.1	2.4	0.3	0.2
		SD	0.8	7.2	1.2	0.5	0.2
		% of control	100.0	100.0	100.0	100.0	100.0
	Acetamiprid 200 SL (0.15 L/ha)	Mean	0.7	4.8	3.5	0.0	0.3
		SD	0.8	3.1	3.1	0.1	0.5
		% of control	100.0	78.9	146.6	14.3	140.0
	Acetamiprid 200 SL (0.25 L/ha)	Mean	0.3	2.9	3.6	0.2	0.1
		SD	0.2	3.1	3.3	0.4	0.2
		% of control	35.3	46.9	148.3	71.4	60.0
	Acetamiprid 200 SL (0.40 L/ha)	Mean	0.1	3.0	3.8	0.3	0.5
		SD	0.1	1.8	2.4	0.3	0.5
		% of control	11.8	49.7	156.9	114.3	260.0
	Reference item	Mean	0.2	1.3	0.6	0.2	0.4
		SD	0.1	1.3	0.5	0.2	0.4
		% of control	29.4	20.4	25.9	71.4	186.7
<i>Pseudosinella alba</i>	Control	Mean	0.7	6.1	2.1	0.3	0.2
		SD	0.8	7.1	1.1	0.4	0.2
		% of control	100.0	100.0	100.0	100.0	100.0
	Acetamiprid 200 SL (0.15 L/ha)	Mean	0.7	4.7	3.1	0.0	0.3
		SD	0.8	3.1	3.1	0.1	0.5
		% of control	100.0	77.4	147.1	16.7	120.0
	Acetamiprid 200 SL (0.25 L/ha)	Mean	0.3	2.9	2.2	0.2	0.1
		SD	0.2	3.1	1.9	0.3	0.2
		% of control	35.3	47.3	103.9	66.7	60.0
	Acetamiprid 200 SL (0.40 L/ha)	Mean	0.1	3.0	3.5	0.3	0.5
		SD	0.1	1.8	2.1	0.3	0.4
		% of control	11.8	50.0	162.7	133.3	240.0
	Reference item	Mean	0.2	1.3	0.5	0.1	0.3
		SD	0.1	1.3	0.5	0.1	0.4
		% of control	29.4	20.5	23.5	33.3	140.0
Orchesellinae	Control	Mean	1.8	8.9	10.3	61.0	133.6
		SD	0.7	1.8	2.0	8.1	22.7
		% of control	100.0	100.0	100.0	100.0	100.0
	Acetamiprid 200 SL (0.15 L/ha)	Mean	1.8	10.6	12.9	58.7	120.9
		SD	0.7	2.9	3.4	20.9	44.0
		% of control	102.4	119.7	126.0	96.2	90.5
	Acetamiprid 200 SL (0.25 L/ha)	Mean	2.2	14.2	11.0	60.9	141.1
		SD	0.5	2.8	5.7	17.1	24.1
		% of control	126.2	159.6	107.3	99.8	105.6
	Acetamiprid 200 SL (0.40 L/ha)	Mean	2.0	12.1	11.3	62.5	126.0
		SD	0.5	1.5	4.3	21.8	37.9
		% of control	114.3	136.2	109.8	102.4	94.3
	Reference item	Mean	1.8	1.8	8.0	79.7	155.3
		SD	1.0	1.0	1.9	19.6	25.2

		% of control	102.4	20.7	78.5	130.6	116.2
Heteromurus / <i>Heteromurus nitidus</i>	Control	Mean	0.0	0.8	1.3	0.7	0.3
		SD	0.0	0.9	1.7	0.5	0.2
		% of control	100.0	100.0	100.0	100.0	100.0
	Acetamiprid 200 SL (0.15 L/ha)	Mean	0.0	0.5	2.5	0.5	0.7
		SD	0.0	0.6	1.7	0.5	0.8
		% of control	-	68.4	190.6	76.5	204.2
	Acetamiprid 200 SL (0.25 L/ha)	Mean	0.0	1.3	1.5	0.8	0.6
		SD	0.1	1.0	1.1	0.4	0.5
		% of control	-	163.2	115.6	111.8	179.2
	Acetamiprid 200 SL (0.40 L/ha)	Mean	0.0	0.8	1.5	0.7	0.6
		SD	0.1	0.8	1.2	0.6	0.4
		% of control	-	94.7	115.6	94.1	187.5
	Reference item	Mean	0.0	0.0	0.1	0.4	0.3
		SD	0.0	0.1	0.2	0.3	0.5
		% of control	-	5.3	9.4	52.9	91.7
Orchesella	Control	Mean	1.8	8.1	8.9	60.3	133.3
		SD	0.7	2.2	2.1	7.9	22.8
		% of control	100.0	100.0	100.0	100.0	100.0
	Acetamiprid 200 SL (0.15 L/ha)	Mean	1.8	10.1	10.4	58.1	120.2
		SD	0.7	2.9	3.2	20.8	44.2
		% of control	102.4	124.7	116.4	96.4	90.2
	Acetamiprid 200 SL (0.25 L/ha)	Mean	2.2	12.9	9.5	60.1	140.5
		SD	0.5	2.9	5.3	16.7	23.8
		% of control	123.8	159.3	106.1	99.7	105.5
	Acetamiprid 200 SL (0.40 L/ha)	Mean	2.0	11.3	9.7	61.8	125.4
		SD	0.5	1.4	3.7	21.8	38.0
		% of control	111.9	140.2	108.9	102.5	94.1
	Reference item	Mean	1.8	1.8	7.9	79.3	155.0
		SD	1.0	1.0	1.9	19.6	25.5
		% of control	102.4	22.2	88.8	131.5	116.3
<i>Orchesella villosa</i>	Control	Mean	1.8	6.1	8.5	44.8	130.3
		SD	0.7	1.8	2.1	10.0	21.8
		% of control	100.0	100.0	100.0	100.0	100.0
	Acetamiprid 200 SL (0.15 L/ha)	Mean	1.8	6.6	9.8	43.9	115.5
		SD	0.7	1.6	2.5	16.7	45.3
		% of control	102.4	107.5	114.6	98.0	88.6
	Acetamiprid 200 SL (0.25 L/ha)	Mean	2.1	8.8	9.4	46.0	137.6
		SD	0.5	2.9	5.3	12.1	23.0
		% of control	121.4	144.2	109.8	102.7	105.6
	Acetamiprid 200 SL (0.40 L/ha)	Mean	2.0	7.5	9.5	49.7	122.1
		SD	0.5	0.7	3.6	20.5	36.9
		% of control	111.9	123.1	111.2	110.9	93.7
	Reference item	Mean	1.8	1.8	7.7	59.6	150.3
		SD	1.0	0.9	2.0	19.8	25.2
		% of control	102.4	28.6	90.2	133.0	115.3
Isotomidae	Control	Mean	13.7	51.0	4.1	58.7	107.7
		SD	6.1	13.7	2.5	13.6	53.4

		% of control	100.0	100.0	100.0	100.0	100.0
		Mean	12.9	43.8	6.9	60.8	113.8
		SD	5.8	22.1	4.1	20.2	36.3
	Acetamiprid 200 SL (0.15 L/ha)	% of control	94.2	85.9	166.7	103.6	105.7
		Mean	10.4	30.1	5.8	46.2	114.3
		SD	5.0	19.9	3.2	23.3	40.8
	Acetamiprid 200 SL (0.25 L/ha)	% of control	76.0	59.0	139.4	78.7	106.1
		Mean	12.2	20.8	4.8	46.6	86.5
		SD	7.1	17.9	3.9	23.9	34.2
	Acetamiprid 200 SL (0.40 L/ha)	% of control	88.8	40.7	116.2	79.4	80.4
		Mean	7.5	2.6	0.4	32.8	121.7
		SD	2.9	1.7	0.3	11.4	35.9
	Reference item	% of control	55.0	5.1	10.1	55.9	113.1
Isotoma	Control	Mean	2.8	5.5	2.3	21.3	19.4
		SD	3.7	3.1	1.6	6.7	8.6
		% of control	100.0	100.0	100.0	100.0	100.0
	Acetamiprid 200 SL (0.15 L/ha)	Mean	1.9	7.0	5.2	30.0	21.3
		SD	0.5	4.3	3.7	14.6	10.3
		% of control	68.2	126.5	225.5	140.9	110.3
	Acetamiprid 200 SL (0.25 L/ha)	Mean	2.1	3.3	4.1	25.0	21.1
		SD	1.4	0.7	2.9	11.1	13.5
		% of control	75.8	59.1	180.0	117.2	108.8
	Acetamiprid 200 SL (0.40 L/ha)	Mean	2.1	3.1	3.2	25.7	22.5
		SD	1.4	3.2	3.5	11.9	11.2
		% of control	75.8	56.1	138.2	120.5	116.0
	Reference item	Mean	1.5	0.5	0.4	19.1	24.0
		SD	0.7	0.4	0.3	8.3	10.1
		% of control	53.0	8.3	18.2	89.6	124.0
<i>Isotoma anglicana / viridis</i>	Control	Mean	2.5	5.3	2.3	21.3	19.4
		SD	3.6	2.9	1.6	6.7	8.6
		% of control	100.0	100.0	100.0	100.0	100.0
	Acetamiprid 200 SL (0.15 L/ha)	Mean	1.3	6.8	5.2	29.9	21.3
		SD	0.6	4.5	3.7	14.6	10.3
		% of control	52.5	128.6	229.6	140.3	110.3
	Acetamiprid 200 SL (0.25 L/ha)	Mean	1.8	2.8	4.1	24.4	21.1
		SD	1.1	0.7	2.9	10.9	13.5
		% of control	68.9	52.4	183.3	114.7	108.8
	Acetamiprid 200 SL (0.40 L/ha)	Mean	1.9	2.6	3.1	25.7	22.5
		SD	1.2	2.8	3.4	11.9	11.2
		% of control	73.8	50.0	138.9	120.5	116.0
	Reference item	Mean	1.2	0.5	0.4	19.0	23.8
		SD	0.6	0.4	0.3	8.3	10.3
		% of control	47.5	8.7	18.5	89.4	123.0
Isotomurus	Control	Mean	5.5	36.4	0.3	31.0	80.0
		SD	2.8	11.0	0.4	8.7	42.7
		% of control	100.0	100.0	100.0	100.0	100.0
	Acetamiprid 200 SL (0.15 L/ha)	Mean	6.7	22.0	0.4	24.2	86.7
		SD	4.3	14.4	0.3	10.3	33.4

		% of control	121.2	60.3	142.9	78.1	108.4
		Mean	5.1	16.7	0.3	15.5	86.4
		SD	3.5	17.7	0.4	10.8	43.0
	Acetamiprid 200 SL (0.25 L/ha)	% of control	92.4	45.8	100.0	50.2	108.0
		Mean	5.6	10.3	0.3	16.0	59.6
		SD	4.4	13.0	0.4	11.6	25.9
	Acetamiprid 200 SL (0.40 L/ha)	% of control	101.5	28.3	114.3	51.8	74.5
		Mean	2.8	1.9	0.0	11.0	94.1
		SD	1.0	1.2	0.0	7.0	38.5
	Reference item	% of control	50.8	5.3	0.0	35.5	117.6
Symphyleona	Control	Mean	14.9	112.8	14.0	7.6	5.7
		SD	5.0	36.9	7.1	2.0	4.9
		% of control	100.0	100.0	100.0	100.0	100.0
	Acetamiprid 200 SL (0.15 L/ha)	Mean	15.1	83.3	9.9	9.3	3.3
		SD	6.6	52.2	5.7	5.2	2.1
		% of control	101.7	73.9	70.5	122.5	58.3
	Acetamiprid 200 SL (0.25 L/ha)	Mean	20.7	44.0	11.9	12.0	5.6
		SD	25.7	33.9	5.4	9.7	3.1
		% of control	139.2	39.1	85.1	157.7	98.3
	Acetamiprid 200 SL (0.40 L/ha)	Mean	16.5	60.6	11.8	9.2	4.6
		SD	8.8	34.3	7.6	2.4	2.2
		% of control	111.2	53.7	84.2	121.4	81.2
	Reference item	Mean	13.8	0.5	1.5	5.1	3.6
		SD	8.2	0.6	0.8	2.6	1.3
		% of control	93.0	0.4	11.0	67.0	62.7
Bourletiellidae	Control	Mean	4.8	6.3	0.7	0.0	1.5
		SD	2.8	2.8	0.5	0.1	1.3
		% of control	100.0	100.0	100.0	100.0	100.0
	Acetamiprid 200 SL (0.15 L/ha)	Mean	5.8	2.7	0.7	0.0	0.7
		SD	2.8	2.5	0.6	0.1	0.6
		% of control	119.8	42.1	100.0	100.0	42.7
	Acetamiprid 200 SL (0.25 L/ha)	Mean	11.8	1.0	1.0	0.0	0.9
		SD	20.7	1.7	0.7	0.1	0.4
		% of control	244.8	15.8	147.1	100.0	59.1
	Acetamiprid 200 SL (0.40 L/ha)	Mean	7.0	0.5	0.8	0.0	1.2
		SD	3.2	0.3	0.6	0.1	0.9
		% of control	145.7	7.9	117.6	100.0	76.4
	Reference item	Mean	7.0	0.1	0.5	0.0	0.4
		SD	6.1	0.2	0.2	0.1	0.4
		% of control	145.7	2.0	64.7	100.0	28.2
Katiannidae	Control	Mean	7.3	105.6	13.1	6.3	3.3
		SD	2.7	35.2	6.7	2.4	3.6
		% of control	100.0	100.0	100.0	100.0	100.0
	Acetamiprid 200 SL (0.15 L/ha)	Mean	6.9	79.0	9.1	7.8	2.1
		SD	4.8	49.6	5.3	5.4	1.7
		% of control	95.4	74.8	69.7	124.7	62.5
	Acetamiprid 200 SL (0.25 L/ha)	Mean	6.5	41.4	10.6	6.4	3.5
		SD	4.3	31.7	5.7	2.8	1.3

		% of control	90.2	39.2	81.2	102.0	105.8
		Mean	7.3	58.1	10.7	7.0	2.9
		SD	5.3	33.5	7.4	3.4	1.3
	Acetamiprid 200 SL (0.40 L/ha)	% of control	100.6	55.0	81.8	112.7	87.5
		Mean	5.0	0.4	1.1	3.7	2.6
		SD	2.4	0.5	0.8	1.9	1.4
Sminthurididae	Reference item	% of control	68.4	0.4	8.3	58.7	76.7
		Mean	0.7	0.5	0.1	1.3	0.8
		SD	0.4	0.6	0.1	2.0	1.6
	Control	% of control	100.0	100.0	100.0	100.0	100.0
		Mean	1.0	1.1	0.0	1.2	0.5
		SD	0.3	1.7	0.0	1.8	0.8
	Acetamiprid 200 SL (0.15 L/ha)	% of control	135.3	216.7	0.0	90.3	57.9
		Mean	0.7	1.2	0.2	5.1	1.1
		SD	0.3	1.7	0.3	8.5	1.9
	Acetamiprid 200 SL (0.25 L/ha)	% of control	100.0	241.7	200.0	396.8	142.1
		Mean	0.9	1.2	0.0	1.9	0.4
		SD	0.6	1.9	0.0	2.5	0.4
	Acetamiprid 200 SL (0.40 L/ha)	% of control	123.5	233.3	0.0	145.2	52.6
		Mean	0.7	0.0	0.0	1.2	0.4
		SD	0.5	0.0	0.0	1.2	0.5
	Reference item	% of control	100.0	0.0	0.0	93.5	56.1
		Mean	0.1	0.1	0.0	1.0	0.7
		SD	0.2	0.1	0.1	2.0	1.4
Sphaeridia	Control	% of control	100.0	100.0	100.0	100.0	100.0
		Mean	0.0	0.5	0.0	0.8	0.4
		SD	0.1	1.2	0.0	1.6	0.7
	Acetamiprid 200 SL (0.15 L/ha)	% of control	33.3	650.0	0.0	87.0	58.8
		Mean	0.2	0.3	0.0	4.8	1.0
		SD	0.1	0.4	0.0	8.5	1.6
	Acetamiprid 200 SL (0.25 L/ha)	% of control	133.3	350.0	0.0	495.7	135.3
		Mean	0.0	0.2	0.0	1.5	0.3
		SD	0.1	0.5	0.0	2.1	0.5
	Acetamiprid 200 SL (0.40 L/ha)	% of control	33.3	250.0	0.0	152.2	47.1
		Mean	0.1	0.0	0.0	1.0	0.4
		SD	0.2	0.0	0.0	1.2	0.5
	Reference item	% of control	100.0	0.0	0.0	100.0	62.7

Mean [ind./pitfall trap], SD = standard deviation [ind./pitfall trap]

The calculations were done with unrounded values.

Statistics: comparisons of test item treatments vs. control and reference item vs. control (pre-treatment sampling: ANOVA, post-treatment sampling: one-sided t-test): **Bold values** indicate statistically significant differences compared to control ($\alpha = 0.05$).

The PRCs indicate short-term effects of the test item on the Collembola community of the upper 5 cm of the soil and the soil surface at all tested application rates shortly after application at 1st sampling (day 28 after 1st application) but clear recovery and no effects at all following samplings (56, 182, and 365 days after 1st application).

Validity criteria

Table A 206: Validity criteria

Validity criteria	Observed in study
Average residue levels of the active substance in the soil samples taken immediately after application should be within the recommended range of 50% to 150% of the nominal values.	Fulfilled
The treatment of the reference item should show a statistically significant reduction of more than 50% in the abundance of the total Collembola	Most of the dominant Collembola taxa showed reductions of > 50% in the course of the test with statistically significant reductions compared to the control determined for numerous taxa.

Conclusion

It can be concluded that the application of Acetamiprid 200 SL tested at application rates of 2 x 0.15 L/ha (corresponding to 2 x 30 g a.s./ha), 2 x 0.25 L/ha (corresponding to 2 x 50 g a.s./ha) and 2 x 0.40 L/ha (corresponding to 2 x 80 g a.s./ha) had no adverse effects on single Collembola taxa and total Collembola as well as on the community structure of Collembola of the upper 5 cm of the soil and the soil surface one year after application.

A 2.5 KCP 10.5 Effects on soil nitrogen transformation

Comments of zRMS:	<p>The study has been already evaluated and accepted by the zRMS in the course of the first zonal evaluation of CA3573 (formerly MCW-2222) finalised in April 2018. As the test guideline has not changed since that time, re-evaluation of the study following renewal of acetamiprid was not necessary and presented below conclusions were taken from the Core Assessment, Part B, Section 6 of April 2018.</p> <p>The study was performed in line with OECD 216 and met all validity criteria.</p> <p>It may be concluded that the effects of the test item on soil nitrogen formation rates were <25% at the end of the study period (28 days) up to 22.74 mg product/kg dws (corresponding to 4.01 mg a.s./kg dws).</p>
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Reference:	KCP 10.5/01
Report	MCW-2222 - Effects on the activity of soil microflora (Nitrogen transformation test) Schulz, L. 2014, R-33843, 14 10 48 018 N
Guideline(s):	OECD 216 (2000)
Deviations:	No
GLP:	Yes, certified laboratory
Acceptability:	Yes
Duplication (if vertebrate study)	Not applicable

Executive Summary

A laboratory study was performed to evaluate the effects of MCW-2222 applied to soil on nitrogen transformation (mineralisation) over a period of 28 days. MCW-2222 was tested with a test item concentration of 2.27 mg test item/kg dry soil and 22.74 mg test item/kg dry soil. Nitrogen transformation was tested by means of soil enriched with lucerne meal as organic nitrogen. To determine nitrogen transformation, 10 g soils portions from treated and untreated replicates were sampled on days 0 (3 hours), 7, 14 and 28 for analysis of NO₃-nitrogen content. MCW-2222 (tested at 2.27 mg/kg dry soil and 22.74 mg test item/kg dry soil) caused no adverse effects (deviation from control <25 %, OECD 216) on soil nitrogen transformation (measured as NO₃-N-production) at the end of the 28-day incubation period.

Materials and methods

Materials

Test item	MCW-2222
Batch #	611-280413-01

Content of active substance	Acetamidiprid 200 g/L (nominal); 202.7 g/L (analysed)
Description	Yellowish liquid
Control	Deionised water
Toxic reference	Dinoterb was tested in a separate study (R 14 10 48 001 N) at concentrations of 6.80, 16.00 and 27.00 mg/kg.
Test organism	
Species	Microflora from an agricultural soil
Source	Wassergut Canitz, Canitz, Sachsen, Germany
Food	Lucerne meal (concentration in soil 0.5 %).
Study design and methods	
Test duration and exposure	28 days. The test item was mixed into the soil.
Experimental dates	13 May – 10 June 2014
Test rates	2.27 mg/kg dw, 22.74 mg/kg dw, equivalent to 1.5 or 15 L test item/ha, respectively
Test units	Wide-mouth glass flasks (500 mL) per concentration, each filled with 200 g soil dry weight
Group size/replicates	3 replicates per treatment group
Soil	Loamy sand (DIN 4220) / sandy loam (USDA), pH 6.6, 1.47 % C _{org} , WHC: 35.72 g/100 g dry soil. No pesticide use since 1990, no fertiliser since 2003 Prior to application, the soil was adapted to test conditions
Environmental conditions	
Temperature	19.8 - 21.4 °C
Photoperiod	None, conducted in darkness
pH	6.2 – 6.3
Water content	15.46 - 15.97 g/100 g dw (equal to approx. 45% of WHC)

Nitrogen measurements

Soil samples (10 g) were taken at 3 hours, 7, 14, and 28 days after application and analysed for NH₄-N, NO₃-N and NO₂-N. Quantitative determination was performed by an extraction with 1M KCl solution followed by a quantitative determination using an Autoanalyzer (Bran + Luebbe).

Results and discussion

Results are given in the following table.

Table A 207: Effects on nitrogen transformation in soil after treatment with the test item

Days after application	Control	2.27 mg/kg dry soil (equivalent to 1.5 L test item/ha)		22.74 mg/kg dry soil (equivalent to 15 L test item/ha)	
	NO ₃ -N [mg/kg dry soil]	NO ₃ -N [mg/kg dry soil]	Deviation from control [%] ¹⁾	NO ₃ -N [mg/kg dry soil]	Deviation from control [%] ¹⁾
0	16.43	16.53	+0.6	16.10	-2.0
7	46.40	44.93	-3.2	45.63	-1.7
14	57.00	56.83	-0.3	56.13	-1.5
28	69.43	69.40	0.0	68.13	-1.9

¹⁾ Based on NO₃-nitrogen production; - = inhibition, + = stimulation

Table A 208: Effects on nitrogen transformation in soil after treatment with the test item based on temporal intervals

Days after application	Control	2.27 mg/kg dry soil (equivalent to 1.5 L test item/ha)		22.74 mg/kg dry soil (equivalent to 15 L test item/ha)	
	NO ₃ -N [mg/kg dry soil]	NO ₃ -N [mg/kg dry soil]	Deviation from control [%] ¹⁾	NO ₃ -N [mg/kg dry soil]	Deviation from control [%] ¹⁾
0 – 7	29.97	28.40	-5.2	29.53	-1.4
0 – 14	40.57	40.30	-0.7	40.03	-1.3
0 – 28	53.00	52.87	-0.3	52.03	-1.8

¹⁾ Based on NO₃-nitrogen production; - = inhibition, + = stimulation

Validity criteria

As shown in the following table, all validity criteria were met.

Table A 209: Validity criteria

Validity criteria according to OECD 216	Observed in study
Variation between replicate control samples $\leq 15\%$	$\leq 4.6\%$

Conclusion

MCW-2222 (tested at 2.27 mg/kg dry soil and 22.74 mg test item/kg dry soil) caused no adverse effects (deviation from control $<25\%$, OECD 216) on soil nitrogen transformation (measured as $\text{NO}_3\text{-N}$ -production) at the end of the 28-day incubation period.

A 2.6 KCP 10.6 Effects on terrestrial non-target higher plants

A 2.6.1 KCP 10.6.1 Summary of screening data

Comments of zRMS:	<p>The study has been already evaluated and accepted by the zRMS in the course of the first zonal evaluation of CA3573 (formerly MCW-2222) finalised in April 2018. As the test guideline has not changed since that time, re-evaluation of the study following renewal of acetamiprid was not necessary and presented below conclusions were taken from the Core Assessment, Part B, Section 6 of April 2018.</p> <p>The study was performed in line with OECD 227 and met all validity criteria.</p> <p>All plants survived after treatment with no phytotoxic effects observed. Effects on shoot fresh weight were $<10\%$ on all tested species.</p> <p>Based on results of the study the NOER was determined to be ≥ 510 g a.s./ha.</p>
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Reference:	KCP 10.6.1/01
Report	Terrestrial plant test with MCW-2222: Vegetative vigour test, Friedrich, S., 2014, 14 10 48 002 P
Guideline(s):	OECD 227 (2006)
Deviations:	No
GLP:	Yes, certified laboratory
Acceptability:	Yes
Duplication (if vertebrate study)	Not applicable

Executive Summary

In a 21-day vegetative vigour test, the phytotoxicity of MCW-2222 to 6 plant species was tested. In the experiments MCW-2222 was applied onto the foliage of plants in the 2 - 4 leaf stage at a nominal application rate of 510 g a.s./ha. Test plants were two monocotyledonous (oats and ryegrass) and four dicotyledonous (turnip, tomato, cucumber and soybean). The toxic effects of the test item were determined on day 21 by assessment of shoot height and shoot fresh weight. The NOER for survival and shoot fresh weight was determined to be > 510 g a.s./ha.

Materials and methods

Materials

Test item	MCW-2222
Batch #	611-280413-01
Content of active substance	Acetamiprid 200 g/L (nominal); 202.7 g/L (analysed)
Description	Yellowish liquid
Control	Deionised water
Test organism	
Species	Monocotyledones: oat (<i>Avena sativa</i>), perennial ryegrass (<i>Lolium</i>

	<i>perenne</i>)
	Dicotyledons: turnip (<i>Brassica rapa</i>), tomato (<i>Lycopersicon esculentum</i>), cucumber (<i>Cucumis sativus</i>), soybean (<i>Glycine max</i>)
Age	2-4 leaf stage BBCH 12-14
Source	Commercial suppliers
Study design and methods	
Test duration and exposure	21 days, spray application at test start
Experimental dates	03 to 24 April 2014
Test rates	510 g a.s./ha in 400 L/ha of water
Test units	Non-porous plastic flower pot (Ø 15 cm), capacity/pot: 1.6 kg fresh soil; actual used amount of soil/pot: 1.4 kg
Group size/replicates	30 - 32 plants per treatment; 2 - 4 plants per replicates in 8 - 15 replicates per treatment
Test soil	Agricultural soil (sandy loam) from site Gerichshain (batch G 01/2014) and stored for at least 1 year before used in the test
Irrigation	Daily bottom watering in pot saucers with tap water
Environmental conditions	
Temperature	14 - 31°C
Photoperiod	16 h light / 8 hours darkness
	310 – 393 µE/m ² /s
Relative humidity	17 – 72 %

Analytical measurements

Analytical verification of spray solution conducted using an HPLC-method with UV-detection.

Biological observations

During the observation period, i.e. up to 21 days after application, the plants were observed weekly for survival/mortality and visual phytotoxicity. Endpoints observed on day 21 after application were survival (mortality), visual phytotoxicity and biomass (shoot fresh weight).

Results and discussion

Analytical measurements

The measured concentration of acetamiprid in the analysed test solution was determined to be 102 % of the nominal value.

Biological results

Biological results are given in the table below.

Table A 210: No Observed Effect Level (NOER) values for non-target terrestrial plants at test termination

	<i>Avena sativa</i>	<i>Lolium perenne</i>	<i>Brassica rapa</i>	<i>Lycopersicon esculentum</i>	<i>Cucumis sativus</i>	<i>Glycine max</i>	<i>Avena sativa</i>
Survival (on day 21 after application)							
NOER	≥ 510	≥ 510	≥ 510	≥ 510	≥ 510	≥ 510	≥ 510
Growth (shoot fresh weight on day 21 after application)							
NOER	≥ 510	≥ 510	≥ 510	≥ 510	≥ 510	≥ 510	≥ 510

Validity criteria

As shown in the following table, all validity criteria were met.

Table A 211: Validity criteria

Validity criteria according to OECD 227	Observed in study
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Seedling emergence should be > 70%	90 – 99%
Controls:	
Mean plant survival during study >90%	100%
No phytotoxic effects should be visible	None observed
Environmental conditions for particular species should be identical and growing media should contain equal amount of soil matrix, support media, or substrate from the same source	Achieved

Conclusion

The foliar application of MCW-2222 at a rate of 510 g a.s./ha to six terrestrial plant species at the 2 to 4 leaf stage did not produce adverse effects on survival and shoot fresh weight. The NOER for survival and shoot fresh weight was determined to be > 510 g a.s./ha.

A 2.7 KCP 10.7 Effects on other terrestrial organisms (flora and fauna)

A 2.8 KCP 10.8 Monitoring data

Appendix 3 Additional information - Exposure overlay profiles

A 3.1 Introduction

Following the EFSA Aquatic Guidance Document (EFSA 2013: AGD), one of the approaches for refining the risk to aquatic organisms is conducting a higher-tier mesocosm study including realistic to worst case pulsed (peak) exposure conditions

In order to demonstrate whether the exposure from a higher-tier mesocosm study (Hommen, 2022) covers the predicted exposure profiles (FOCUS scenarios), a comparison analysis of the exposure profile in the higher-tier study to the predicted exposure profile for acetamiprid was done using the Exposure Overlay Profiles (EOP) analysis. EOP is a visual comparison of predicted environmental exposure (surface water predicted environmental concentration PEC_{SW}) and exposure tested in an ecotoxicologically higher-tier study with an aquatic model ecosystem.

The well-established Exposure Pattern Analysis Tool (EPAT - European Crop Protection Association (ECPA) & Rifcon GmbH) provides statistical and graphical analysis of predicted exposure profiles. In close proximity to EPAT, the newly developed R package (overlapPeaks-package) plots the predicted exposure peaks and the regulatory acceptable profile (RAP - i.e. exposure applied in a toxicity experiment divided by the respective safety-factor) in a diagram.

Accordingly, all relevant concentration curves over time calculated for acetamiprid with the FOCUS surface water models at Step 3 and 4 are presented. The calculated concentration curves in surface water were derived from the maximum Predicted Environmental Concentrations in Surface Water (PEC_{SW}), as presented in the draft Registration Report (CEU - dRR, Section B8) for ADM.00150.1.2.A. No new PEC_{SW} were calculated.

The calculated exposure patterns were compared with the concentration curve of acetamiprid at the most relevant concentration level in a mesocosm study (Hommen, 2021) conducted with the formulated product ADM.00150.1.2.A (a.s. content: 200 g acetamiprid/L).

The model ecosystem in the mesocosm study focused on aquatic insects, benthic macroinvertebrates and zooplankton. However, algae and plants were also monitored to detect indirect effects.

Two applications with nominal concentrations at test start and on day 7 were conducted in this study. In addition to the biological aspects, the correct dosing, fate and distribution of the active substance was monitored in the water body and the sediment.

All figures shown below include the PEC_{SW} timeseries derived from the FOCUS models in blue, the tier 1

RAC (0.0235 µg/L; *C. riparius*; EFSA, 2016) in light gray and the higher tier RAC derived from the mesocosm study in dark gray. The mean initial concentration of 1.12 µg a.s./L in the mesocosm study was used to derive a RAC of 0.56 µg a.s./L based on an assessment factor of 2. Additionally, the figures for the exposure profile analysis also include the Regulatory Acceptable Profile (RAP) represented as green line. This profile was derived from actual measured concentrations of the mesocosm study. Due to its relevance the mean measured no effect concentration curve (nominal concentration: 0.87 µg a.s./L; double application: 7-day intervall) divided by an assessment factor of 2 is shown.

Usually, one graph per FOCUS scenario is shown presenting an overlay of the exposure in the mesocosm and the peak calculated for surface water PECs in the respective FOCUS scenario. For this purpose, usually the maximum calculated peak was aligned with the peak of the mesocosm exposure curve. All relevant peaks from a FOCUS profile were covered by the RAP.

To show the most relevant data in this dRR the amount of data required a significant reduction and in consequence, not all mitigation methods provided in the dRR B8 chapter could be included here. Based on the aquatic risk assessment outcome from dRR B9 chapter, a selection was done and cases where the standard risk assessment ($ETR=PEC/RAC$) would not indicate an acceptable risk but the Exposure Overlay Profiles (EoP) clearly shows that the maximum exposure profile peak/s is/are below the RAP (including the AF of 2) and a safe use could be demonstrated, were reported in the tables below. When all peaks of all FOCUS scenarios of a certain application pattern were below the Tier 2 RAC of 0.56 µg a.s./L (safe use already demonstrated in the dRR B9 chapter), or some of the peaks were breaching the RAP and no safe use could be demonstrated (even with the EoP approach), no EoP results were reported.

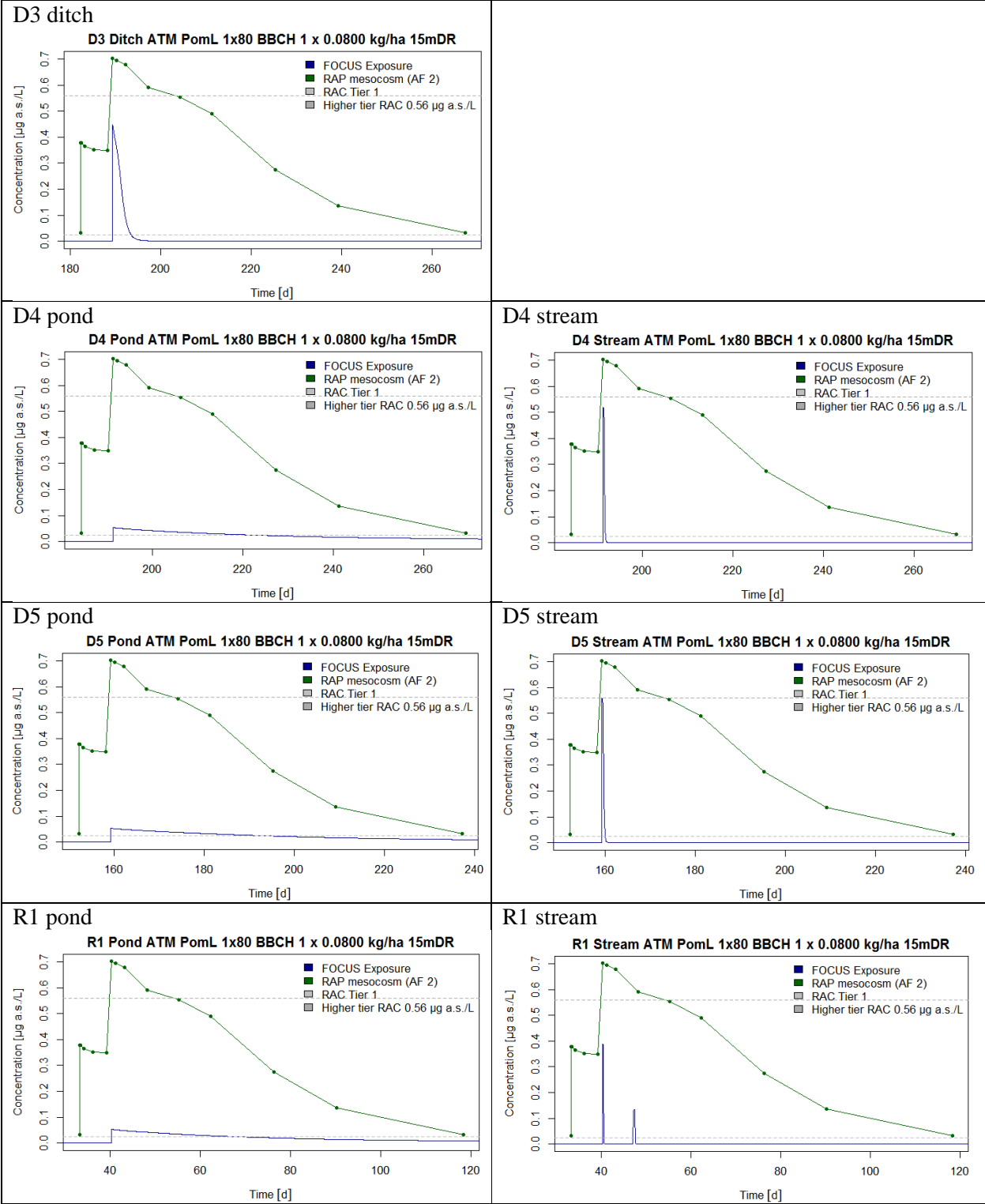
All calculated profiles are available in a compressed datapackage elsewhere.

Please note the different scaling of the X- and Y-axis in the graphs. Due to different scaling, the exposure pattern of the mesocosm study looks different in each scenario though concentrations are always the same. The time given on the x-axis usually starts shortly before the timing of the peak in the FOCUS calculation. The scaling of the x-axis is determined either by the length of the peak in the FOCUS scenario or by the exposure length in the mesocosm study – depending on which of the peaks is longer.

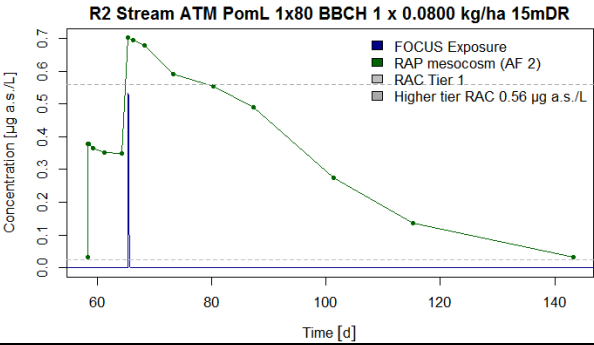
A 3.2 Exposure profile analysis

Use in pome/stone fruit, late applications, 1x80 g a.s./ha of ADM.00150.I.2.A (umbrella use IIa; Jun-Aug / 71-PHI)

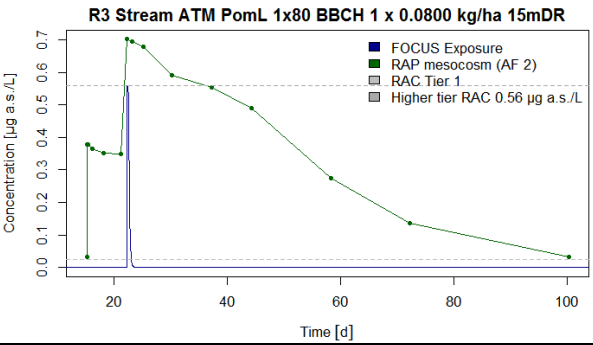
Step 4, 15 m drift buffer zone + 10 m vegetated filter strip, EoP results



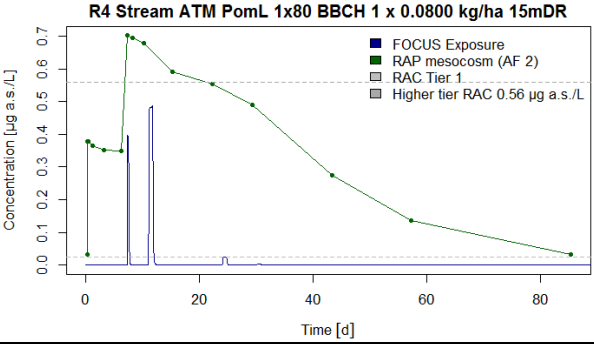
R2 stream



R3 stream

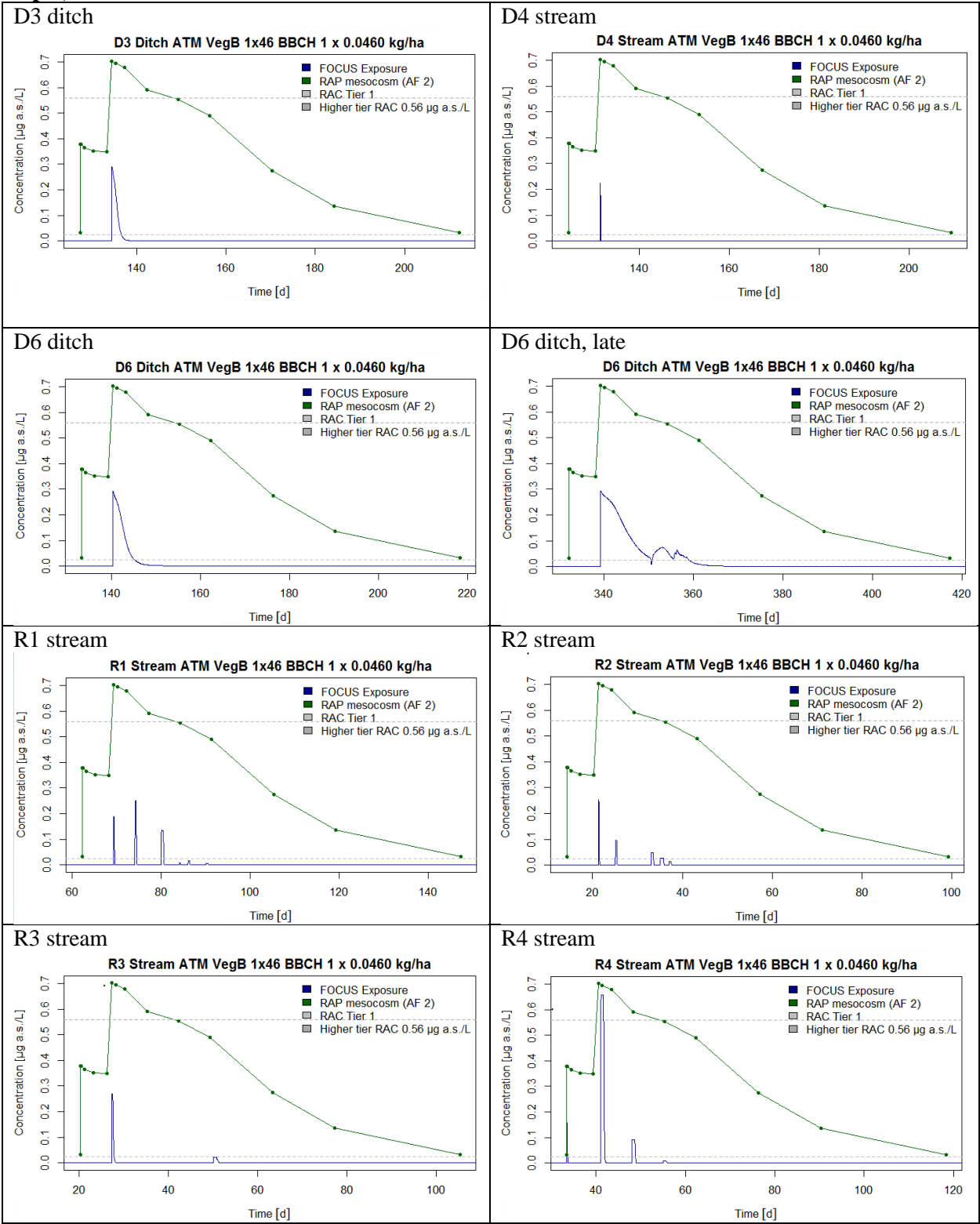


R4 stream



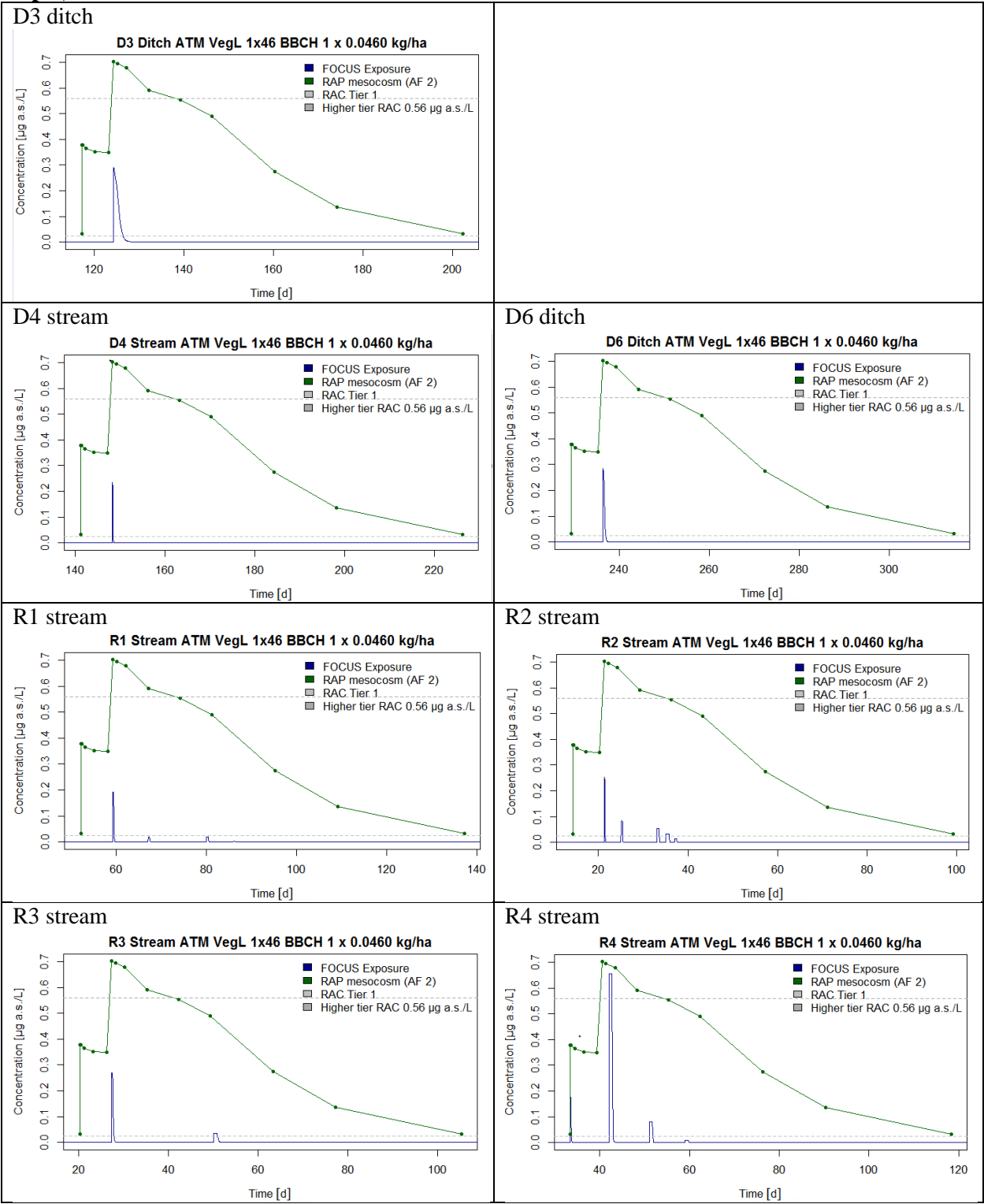
Use in ornamentals (flower bulbs), 1x46 g a.s./ha of ADM.00150.I.2.A (umbrella use IXa; Mar-Jul/BBCH 12 - 91)

Step 3, EoP results



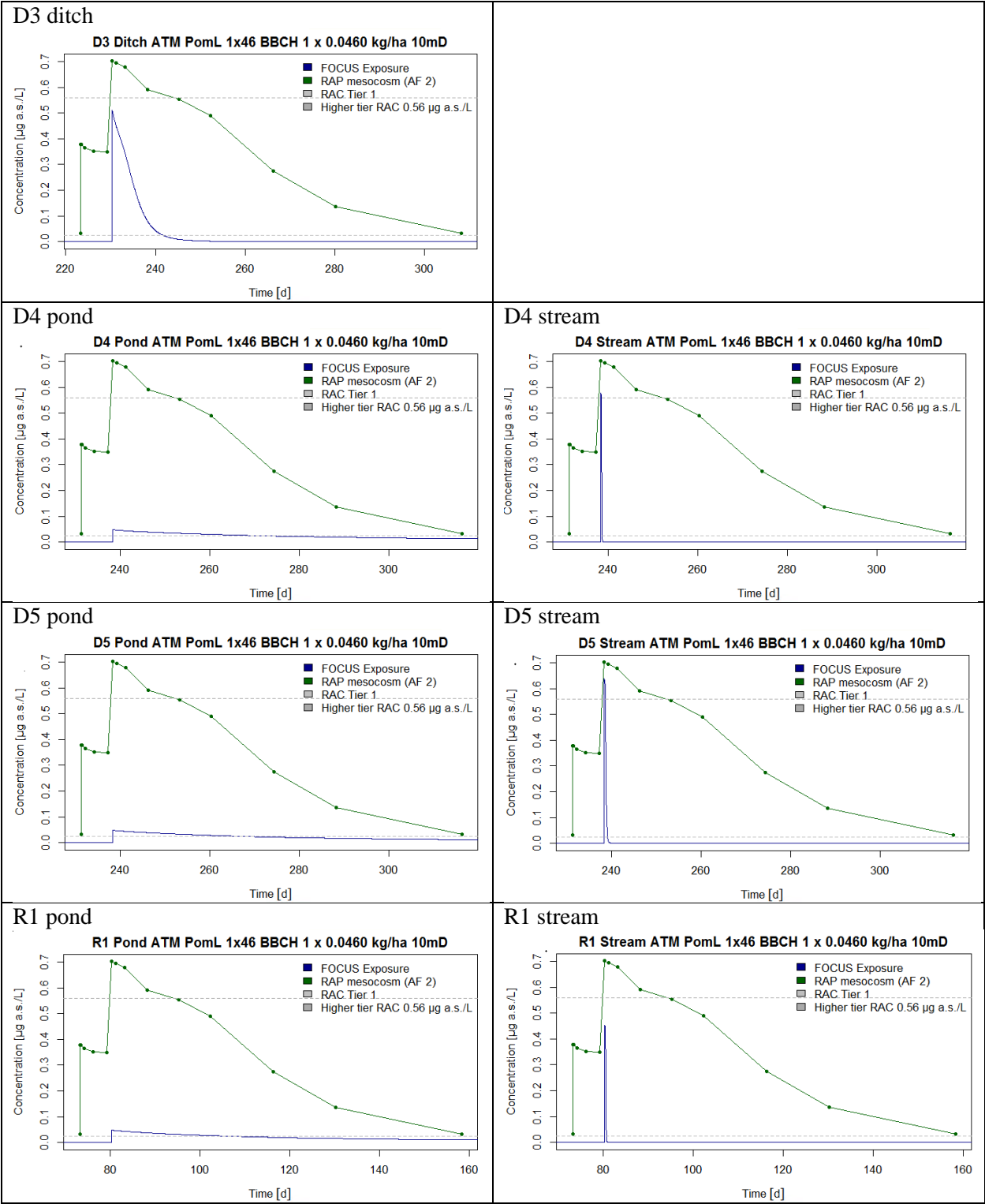
Use in ornamentals (VegL), 1x46 g a.s./ha of ADM.00150.I.2.A (umbrella use Xa; Mar-Aug / BBCH 12)

Step 3, EoP results

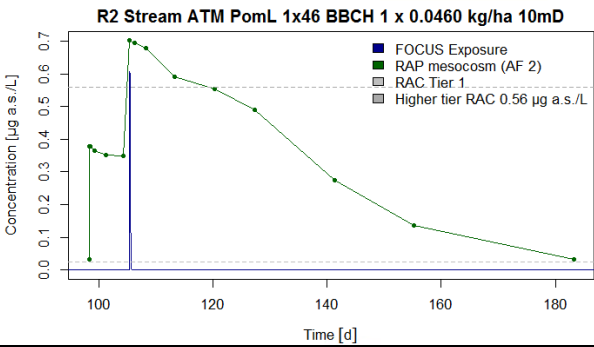


Use in ornamentals (pome/stone fruits, late), 1x46 g a.s./ha of ADM.00150.I.2.A (umbrella use Xa; Mar-Aug / BBCH 91)

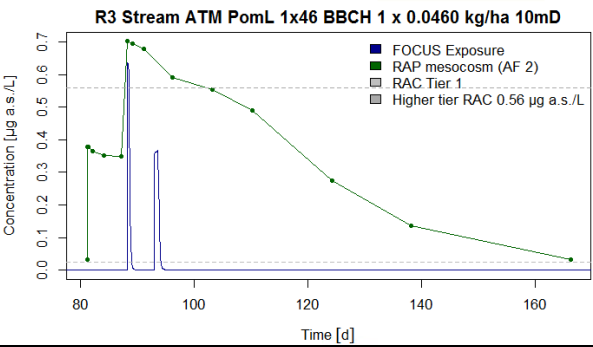
Step 4, 10 m DBZ, EoP results



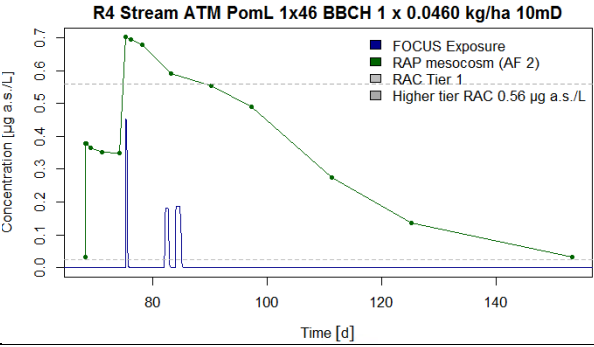
R2 stream



R3 stream

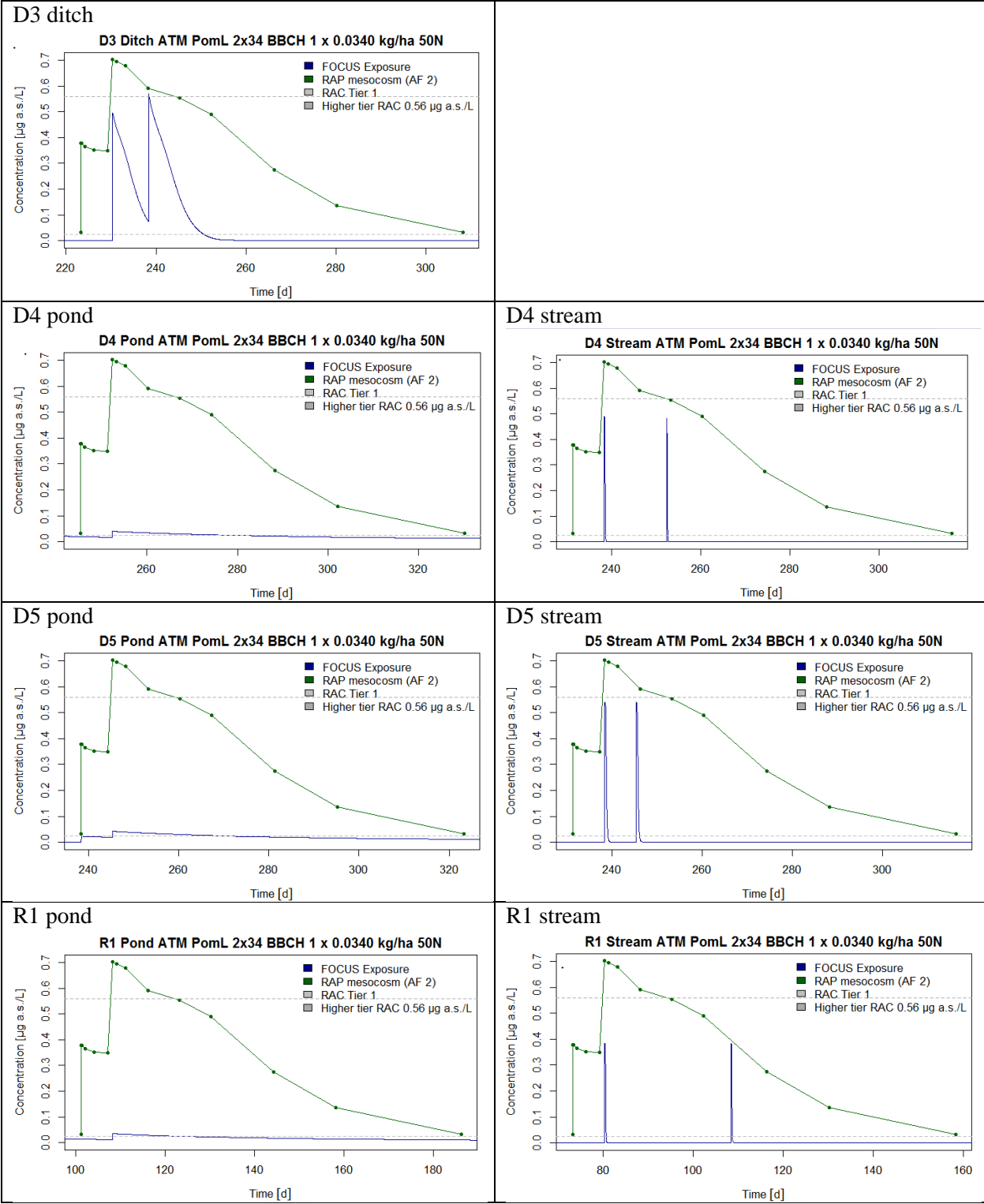


R4 stream

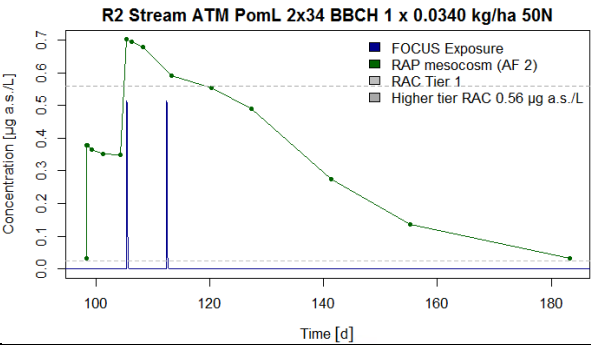


Use in ornamentals (pome/stone fruits, late), 2x34 g a.s./ha of ADM.00150.I.2.A (umbrella use Xb; Mar-Aug / BBCH 91)

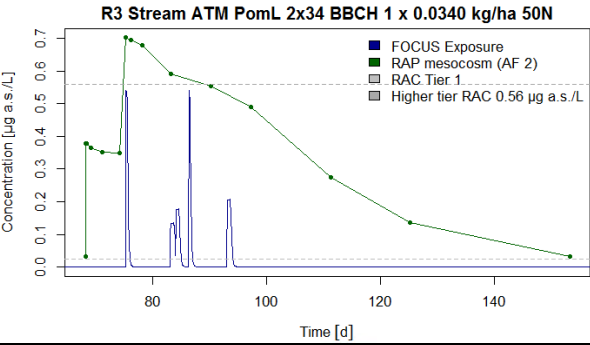
Step 4, 50% DRN, EoP results



R2 stream



R3 stream



R4 stream

