

# REGISTRATION REPORT

## **Part B**

### **Section 9**

#### **Ecotoxicology**

Detailed summary of the risk assessment

Product code: CA3573

Product name(s): Carnadine / Kestrel

Chemical active substance:

Acetamiprid, 200 g/L

Central Zone

Zonal Rapporteur Member State: Poland

#### **CORE ASSESSMENT**

(Re-authorisation acc. to Art. 43)

Applicant: Nufarm Europe GmbH

Submission date: July 2020, August 2021 updated, November  
2021 updated

MS Finalisation date: 31/05/2021 (initial Core Assessment)

November 2021, January 2022 (final Core Assessment)

### Version history

When	What
July 2020	Version 1.0 (application)
May 2021	Initial evaluation by the zRMS
November 2021	Core Assessment updated following the commenting period with consideration of additional information provided by the Applicant in August 2021 and November 2021.  Additional information/assessments included by the zRMS in the report in response to comments recieved from the cMS and the Applicant are highlighted <b>in yellow</b> . Information no longer relevant <del>is struck through and shaded</del> .
January 2022	Final report (Core Assessment after additional round of the commenting period)  GAP table amended after additional round of the commenting period (risk assessment for mammals from use No 2&12 requires confirmation at the cMS level and not from use No 1&11, as mistakenly indicated in the previous version of the Core Assessment). Changes are highlighted <b>in blue</b> . No other amendments or corrections were necessary.

Usually, in dRRs for product renewal, all those paragraphs, endpoints etc. should be highlighted in yellow which were modified in comparison to the dossier submitted for the previous authorisation. This was not done in this B9 document since it would have meant highlighting more or less the entire document. The endpoints summarised in B9.2 to B9.10 were completely reassessed during the last EU renewal and new studies were added. Additionally, the risk assessment in respective chapters was completely redone due to changes in endpoints and guidelines.

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#### **zRMS comments:**

Formulation CA3573 was a subject of the zonal evaluation in April 2018, but under different code name (MCW-2222). Evaluation presented in this report was performed in line with Article 43 of Regulation (EC) No 1107/2009 due to renewal of acetamiprid at the EU level in 2018 (Commission Implementing Regulation (EU) 2018/113) and the new List of Endpoints (LoEP) issued in EFSA Journal 2016;14(11):4610.

Although the code name has been changed from MCW-2222 to CA3573, composition of the product remains the same and results of studies performed during first zonal authorisation with MCW-2222 are relevant for CA3573.

Nufarm GmbH & Co.KG was not the Applicant for the EU renewal of acetamiprid and the data matching process has been carried out by the RMS for acetamiprid (The Netherlands) with final conclusions issued in December 2020. According to the RMS conclusion, Nufarm dossier was acceptable for matching and data matching has been shown sufficiently with all argumentation and submitted alternative studies acceptable. Taking this into account, the list of endpoints reported in EFSA Journal 2016;14(11):4610 may be used for evaluation of formulation CA3573.

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 is struck through and shaded for transparency.

Following the commenting period in August 2021 the Applicant provided the additional information requested by the zRMS (i.e. summaries of the literature data provided to support refinement of the risk to the common vole and further information on EU agreed residue decline data). However, the data protection status of the indicated residue decline data could not be confirmed by the RMS (NL) despite PL requests, so they could not be used for purposes of the risk refinement as being potentially protected. Since the remaining lines of evidence were not sufficient to resolve the risk to common vole from uses in oilseed rape at 60 g a.s./ha, in November 2021 the Applicant proposed to reduce the rate to 50 g a.s./ha. With this rate acceptable risk could be concluded already at Tier 1 and no further refinement for OSR was deemed necessary. The mammalian Tier 1 risk assessment was thus amended accordingly and refinement of the risk for common vole from 60 g a.s./ha in OSR was struck through and shaded in this final version of the zonal report as being no longer necessary. For remaining groups of species the risk assessment performed for application rate of 60 g a.s./ha in OSR was not revised since this evaluation covers lower application rate of 50 g a.s./ha.

All additional information and evaluations inserted to the report after the commenting period are highlighted in yellow. Information and evaluation not relevant anymore has been struck through and shaded.

## **9 Ecotoxicology (KCP 10)**

This application is for CA3573 with the trade name Carnadine (Acetamiprid 200 SL) by Nufarm GmbH & Co.KG. The product was formerly owned by Adama ADAMA Makhteshim Ltd. under the product code MCW-2222. The two products are identical. Therefore all studies conducted with MCW-2222 can be used for CA3573, without any restrictions. Further details are given in Part C.

## 9.1 Critical GAP and overall conclusions

**Table 9.1-1: Critical use pattern of the formulated product**

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 safener/ synergist 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)	L product/ha a) max. rate per appl. b) max. total rate per crop/seaso n	g or kg as/ha a) max. rate per appl. b) max. total rate per crop/seaso n	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)																				
1, 11	Central Zone	Apple (MABSD)	F	<i>Aphis</i> sp. (APHISP)	Foliar spraying overall	May-Oct/ BBCH 70- PHI BBCH 62- PHI	a) 1 b) 1	--	a) 0.125 b) 0.125	a) 25 b) 25	500- 1000	14	Do not apply during flowering (application from BBCH 70)	A	A	R	R From BBC H 70	R 5m NBZ or 75% DR	A	A
2, 12	Central Zone	Apple (MABSD)	F	<i>Cydia pomonella</i> (CARPPO)	Foliar spraying overall	May-Oct/ BBCH 70- PHI BBCH 62- PHI	a) 1 b) 1	--	a) 0.25 b) 0.25	a) 50 b) 50	500- 1000	14	Do not apply during flowering (application from BBCH 70)	A	C	R	R From BBC H 70	R 10m NBZ or 5m NBZ + 50% DR or 75% DR	A	A
3, 13	Central Zone	Potato (SOLTU)	F	<i>Leptinotarsa decemlineata</i> (LPTNDE)	foliar spraying, overall	Jun-Sep/ BBCH 20- 79 BBCH 12- 79	a) 1 b) 1	--	a) 0.18 b) 0.18	a) 36 b) 36	200-400	7	0.12 – 0.18 L/ha Restriction of the application period due to unacceptable risk to soil organisms at BBCH 12-19	A	A	A	A	A	R From BBCH 20	A
4, 5, 6, 7, 14, 15, 16	Central Zone	Winter oilseed rape (BRSNN)	F	<i>Various pests</i>	foliar spraying, overall	May-Jun/ BBCH 31- 71	a) 1 b) 1	--	a) 0.25 0.3 b) 0.25 0.3	a) 50 60 b) 50 60	200-400	28	0.15 – 0.25 0.3 L/ha Application in the evening, after the bee flight	A	A	A	R After bee flight	A	A	A
8, 9, 10, 17, 18	Central Zone	Spring oilseed rape (BRSNN)	F	<i>Various pests</i>	foliar spraying, overall	Mar-Jun/ BBCH 31- 71	a) 1 b) 1	-	a) 0.25 0.3 b) 0.25 0.3	a) 50 60 b) 50 60	200-400	28	In label: 0.15- 0.25 0.3 L/ha Application in the evening, after the bee flight	A	A	A	R After bee flight	A	A	A

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
19, 20	Central Zone	Corn	F	Various pests	foliar spraying, overall	Apr-Aug/ BBCH 51-75	a) 1 b) 1	-	a) 0.28 0.3 b) 0.28 0.3	a) 56 60 b) 56 60	300-500	56	In label: 0.2-0.28 0.3 L/ha Reduction of rate due to unacceptable risk to small herbivorous mammals at 0.3 L/ha	A	R Reduction of appl. rate	A	A	A	A	A

\* F: professional field use, G: 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:

Originally the GAP table presented by the Applicant listed all intended uses of CA3573 in particular countries. However, zonal evaluation in area of ecotoxicology has to cover all countries in the zone and is performed with consideration of the crop, its BBCH stage, number of applications, interval and application rate, while the pests against which the product is applied are not important. Taking this into account the original GAP table has been modified by the zRMS in order to construct the risk envelope GAP, which covers particular uses in each cMS. The detailed GAP for particular countries may be found in the Core Assessment, Part B, Section 0.

GAP table above has been amended accordingly with consideration of additional information provided by the Applicant after the commenting period (in November 2021).

## 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 CA3573 (a.s. acetamiprid) to birds ~~and 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 long term risk to small herbivorous mammals was addressed in a higher tier risk assessment, including data on diet (PD).~~ Risk of secondary poisoning and risk to birds ~~and mammals~~ from exposure via drinking water is **considered to be low** ~~not relevant~~.

The TER values, calculated for recommended scenarios, all exceed the trigger values of 10 for acute risk and 5 for long-term risk, indicating that the risk to birds ~~and mammals~~ is acceptable following use of CA3573 according to the proposed use pattern.

#### Mammals

The acute and long-term risks of CA3573 (a.s. 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 long-term risk to small herbivorous mammals was addressed in a higher tier risk assessment, including data on diet (PD). Risk of secondary poisoning and risk to mammals from exposure via drinking water is considered to be low.

The TER values, calculated for recommended scenarios, all exceed the trigger values of 10 for acute risk, indicating that the acute risk to mammals is acceptable following use of CA3573 according to the proposed use pattern.

Based on the Tier 1 evaluation acceptable long-term risk could be concluded for intended application in orchards at 25 g a.s./ha, potatoes at 36 g a.s./ha **and oilseed rape at 50 g a.s./ha**. For applications in orchards at 50 g a.s./ha **and** ~~oilseed rape at 60 g a.s./ha and~~ maize at 60 g a.s./ha potentially unacceptable long-term risk was concluded for small herbivorous mammals and frugivorous mammals (orchards only). Refinement of the risk to small herbivores has been performed with consideration of the data on the diet composition of the common vole. Based on the performed evaluation acceptable risk could be concluded for application to orchards at 50 g a.s./ha. For maize reduction of the maximum application rate to 56 g a.s./ha (corresponding with 0.28 L product/ha) was necessary to address the long-term risk to small herbivores. ~~The risk to small herbivorous mammals from intended uses in oilseed rape remained unresolved.~~

~~In order to address the risk in oilseed rape and to remove restriction regarding the maximum application rate in maize the Applicant has to clarify the data protection status of the residue decline studies used at the EU level to refine the FTWA value in dicotyledonous plants.~~

The risk to frugivorous mammals from application of acetamiprid in orchards at 50 g a.s./ha has been refined with consideration of the RUD value in large fruits. Acceptable risk could be concluded, but the concerned Member State may wish to reconsider this refinement option at the product authorisation.

Overall it is concluded that acetamiprid will not pose unacceptable risk to mammals following intended application to potatoes, **oilseed rape** and orchards, while for maize the application rate has to be reduced to 0.28 L product/ha. ~~Further data are necessary to address the long term risk in oilseed rape.~~

### 9.1.1.2 Effects on aquatic organisms (KCP 10.2)

The risks to aquatic organisms from the intended uses of CA3573 were evaluated on the basis of the available ecotoxicity studies on the active substance, its metabolites and the formulation. The risks from the metabolites are low and were acceptable on FOCUS Step-1 level. Regarding the formulation and the active substance, the risks to aquatic invertebrates had to be refined by using a mesocosm study. Following this, acceptable risks were demonstrated on FOCUS Step-3 level for the intended uses in spring oil seed rape (1 x 60 g a.s./ha), potatoes (1 x 36 g a.s./ha) and corn (1 x 60 g a.s./ha). Regarding the other intended uses, the following mitigating measures need to be considered (FOCUS Step 4):

Intended use	Mitigating measures	Comment
Apples, 1 x 25 g a.s./ha, <u>early</u> application at BBCH ≥ 62	<ul style="list-style-type: none"> <li>Standard DBZ (3 m) plus 75% DRN, <i>or</i></li> <li>10 m DBZ plus 50% DRN, <i>or</i></li> <li>15 m DBZ</li> </ul>	Covering BBCH ≥ 69
Apples, 1 x 25 g a.s./ha, <u>late</u> application at BBCH ≥ 62	<ul style="list-style-type: none"> <li>Standard DBZ (3 m) plus 50% DRN, <i>or</i></li> <li>5 m DBZ</li> </ul>	Covering BBCH ≥ 69
Apples, 1 x 50 g a.s./ha, <u>early</u> application at BBCH ≥ 62	<ul style="list-style-type: none"> <li>Standard DBZ (3 m) plus 90% DRN, <i>or</i></li> <li>10 m DBZ plus 75% DRN, <i>or</i></li> <li>15 m DBZ plus 50% DRN, <i>or</i></li> <li>20 m DBZ</li> </ul>	Covering BBCH ≥ 69
Apples, 1 x 50 g a.s./ha, <u>late</u> application at BBCH ≥ 62	<ul style="list-style-type: none"> <li>Standard DBZ (3 m) plus 75% DRN, <i>or</i></li> <li>5 m DBZ plus 50% DRN, <i>or</i></li> <li>10 m DBZ</li> </ul>	Covering BBCH ≥ 69
Winter oil seed rape, 1 x 60 g a.s./ha, <u>late</u> application	<ul style="list-style-type: none"> <li>Standard DBZ (1 m)</li> <li>10 m DBZ plus 10 m VFS <sup>1</sup></li> </ul>	Covering <u>early</u> application

<sup>1</sup> for scenario R1 stream only

DBZ: drift buffer zone; DRN: drift reducing nozzles; VFS: vegetated filter strip

#### zRMS comments:

Conclusions presented by the Applicant above are agreed by the zRMS. However, 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. Please note that mitigation measures for early and late application to pome fruits were combined in order to cover the worst case situation.

Application pattern	FOCUS scenarios with respective mitigation measures									
	D1	D2	D3	D4	D5	D6	R1	R2	R3	R4
Apples early and late BBCH ≥ 62 1 x 25 g a.s./ha			15 m BZ  <u>or</u> 5 m BZ + 50% DRN  <u>or</u> 75% DRN	15 m BZ  <u>or</u> 10 m BZ + 50% DRN  <u>or</u> 75% DRN	15 m BZ  <u>or</u> 10 m BZ + 50% DRN  <u>or</u> 75% DRN		15 m BZ  <u>or</u> 50% DRN  <u>or</u> 75% DRN	15 m BZ  <u>or</u> 10 m BZ + 50% DRN  <u>or</u> 75% DRN	15 m BZ  <u>or</u> 10 m BZ + 50% DRN  <u>or</u> 75% DRN	15 m BZ  <u>or</u> 50% DRN  <u>or</u> 75% DRN

Apples early and late BBCH ≥ 62 1 x 50 g a.s./ha			20 m BZ  <u>or</u> 15 m BZ + 50% DRN  <u>or</u> 5 m BZ + 50% DRN  <u>or</u> 90% DRN	20 m BZ  <u>or</u> 15 m BZ + 50% DRN  <u>or</u> 10 m BZ + 75% DRN  <u>or</u> 90% DRN	20 m BZ  <u>or</u> 15 m BZ + 50% DRN  <u>or</u> 10 m BZ + 75% DRN  <u>or</u> 90% DRN		15 m BZ  <u>or</u> 15 m BZ + 50% DRN  <u>or</u> 75% DRN	20 m BZ  <u>or</u> 15 m BZ + 50% DRN  <u>or</u> 10 m BZ + 75% DRN  <u>or</u> 90% DRN	20 m BZ  <u>or</u> 15 m BZ + 50% DRN  <u>or</u> 10 m BZ + 75% DRN  <u>or</u> 90% DRN	15 m BZ  <u>or</u> 15 m BZ + 50% DRN  <u>or</u> 75% DRN
Winter OSR early and late BBCH 31-71 1 x 60 g a.s./ha		None	None	None	None		10 m VFS		None	
Spring OSR BBCH 31-71 1 x 60 g a.s./ha	None		None	None	None		None			
Potatoes early and late BBCH 12-79 1 x 36 g a.s./ha			None	None		None	None	None	None	
Maize BBCH 51-75 1 x 60 g a.s./ha			None	None	None	None	None	None	None	None

**BZ:** unsprayed buffer zone; **VFS:** vegetated filter strip; **DRN:** drift reducing nozzles

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 CA3573, 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.”*

### 9.1.1.3 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 CA3573 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 CA3573 in potatoes and maize.

For apples acceptable acute and chronic risk could be concluded for applications performed after flowering (from BBCH 70 onwards) for all routes of exposure, while for application carried out at BBCH 62-69 unacceptable chronic risk was concluded for adult bees and larvae exposed in the treated crop scenario. For oilseed rape acceptable risk could be concluded for weeds, field margin, adjacent crop and next crop scenarios, but unacceptable risk was concluded for chronic risk was concluded for adult bees and larvae exposed in the treated crop scenario.

Refinement of the risk based on sugar content in nectar of apples and oilseed rape confirmed unacceptable risk following application to apples and acceptable risk following application to oilseed rape. However, these calculations were considered by the zRMS to be not fully reliable and were thus concluded to be illustrative only.

Available higher tier studies (tunnel, semi-field and field trials) were sufficient to demonstrate acceptable risk to bees from application of CA3573 to flowering oilseed rape, provided that application is carried out in the evening, after the bee flight.

Field studies were not sufficient to address the risk to bees following application of CA3573 to flowering apples and for this reason the intended uses in this crop are restricted to the post-flowering period (BBCH 70-PHI).

~~Based on the tunnel, semi field and field studies the risk following application to flowering oilseed rape at 60 g a.s./ha was concluded to be acceptable, provided that application is carried out in the evening, after the bee flight. Almost all HQ/ETR values calculated for the acute risk for bumble bees, the acute and chronic risk for adult honeybees as well as for honeybee larvae, being directly exposed to CA3573 in apple, potatoes, oil seed rape and corn via overspray or via residues in pollen, nectar and water, were below the relevant trigger values at the screening step, 1<sup>st</sup> tier assessment or 2<sup>nd</sup> tier assessment. Exceptions were observed for the chronic exposure of adult honeybees and honeybee larvae via 'treated crops' or 'weeds' with ETRs above the trigger when exposed to an application rate of 50 g a.s./ha in apple orchards. But higher tier risk refinement based on seven semi field and three field studies indicated acceptable risk for bees following the use of CA3573 according to the proposed use pattern.~~

#### 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 CA3573 is applied according to good agricultural practice, except for the intended use in pome fruit at an application rates of 1 x 50 g a.s./ha and 1 x 25 g a.s./ha.

The risk to off-field non-target arthropods is acceptable following use of CA3573 in pome fruit (1 x 25 ~~50~~ 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 CA3573 in pome fruit (1 x 50 g a.s./ha), provided the following risk mitigation measures are applied:

- 75% drift reduction or
- 5 m buffer combined with 50% drift reduction or



- 10 m buffer

In conclusion, no unacceptable risks for non-target arthropods are expected when CA3573 is applied according to good agricultural practice and considering risk mitigation measures as specified above for the uses in pome fruit ( 1 x 50 g a.s./ha and 1 x 25 g a.s./ha).

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

The risk of CA3573 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 CA3573, acetamiprid, its relevant soil metabolites, and the maximum PEC<sub>soil</sub>.

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 CA3573 following intended early uses in potatoes, resulting with the highest exposure. Therefore additional risk assessment has been performed for *Folsomia candida* following each intended use as well as later uses in potatoes at BBCH 20-79. Acceptable risk could be concluded and CA3573 may be thus authorised for intended uses in apples, oilseed rape (spring and winter), maize and potatoes at BBCH 20-79. No authorisation for application to potatoes at BBCH 12-19 may be granted until additional data enabling refinement of the risk to *Folsomia candida* are provided.

Risk from metabolites IM-1-2, IM-1-4 and IC-0 is considered to be covered by evaluation performed for the parent compound.

~~The TER<sub>LT</sub> values for CA3573, acetamiprid and its relevant soil metabolites, are all greater than the recommended trigger value of 5, indicating that the risk to soil meso- and macrofauna is acceptable following use of CA3573 according to the proposed use pattern.~~

#### 9.1.1.6 Effects on soil microbial activity (KCP 10.5)

The risk of CA3573 to soil microorganisms was evaluated by comparison of the maximum concentrations with effects <25% derived from laboratory tests, with maximum PEC<sub>soil</sub>. For metabolite IM-1-5 the evaluation was performed with consideration of the maximum agreed accumulated PEC<sub>soil</sub> and assumption that metabolite is 10 times more toxic for the parent.

No effects > 25% occurred at tested rates exceeding the relevant PEC<sub>soil</sub> values, indicating that the risk to soil microorganisms is acceptable following the use of CA3573 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 compound.

Effects on non-target terrestrial plants (KCP 10.6)

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

The application of CA3573 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) 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 CA3573 grouped according to relevant criteria**

Grouping according to crop group or crop group and application pattern			
Group	Intended uses	Relevant use parameters for grouping	Worst case application pattern
Effects on birds (9.2)			
Orchards	Apple, BBCH 62 – PHI, 1 × 25 g a.s./ha Apple, BBCH 62 – PHI, 1 x 50 g a.s./ha	Crop group according to EFSA/2009/1438 screening assessment, Tier I and application pattern	Apple (BBCH 62 – PHI) 1 × 50 g a.s./ha
Potatoes	Potato, BBCH 12 – 79, 1 x 36 g a.s./ha		Potato (BBCH 12 – 79) 1 × 36 g a.s./ha
Oilseed rape	Winter oilseed rape, BBCH 50 – 60, 61 – 71, 31 – 39, 31 – 59, 31 – 69, 31 – 71, 1 x 60 g a.s./ha Spring oilseed rape, BBCH 50 – 60, 61 – 71, 31 – 59, 31 – 71, 1 x 60 g a.s./ha		Oilseed rape (BBCH 31 – 71) 1 × 60 g a.s./ha
Maize	Corn, BBCH 51 – 75, 1 x 60 g a.s./ha		Maize (BBCH 51 – 75) 1 × 60 g a.s./ha
Effects on mammals (9.3)			
Orchards	Apple, BBCH 62 – PHI, 1 × 25 g a.s./ha Apple, BBCH 62 – PHI, 1 x 50 g a.s./ha	Crop group according to EFSA/2009/1438 screening assessment, Tier I and application pattern	Apple (BBCH 62 – PHI) 1 × 50 g a.s./ha
Potatoes	Potato, BBCH 12 – 79, 36 g a.s./ha		Potato (BBCH 12 – 79) 1 × 36 g a.s./ha
Oilseed rape	Winter oilseed rape, BBCH 50 – 60, 61 – 71, 31 – 39, 31 – 59, 31 – 69, 31 – 71, 1 x 50 60 g a.s./ha Spring oilseed rape, BBCH 50 – 60, 61 – 71, 31 – 59, 31 – 71, 1 x 50 60 g a.s./ha		Oilseed rape (BBCH 31 – 71) 1 × 50 60 g a.s./ha
Maize	Corn, BBCH 51 – 75, 60 g a.s./ha		Maize (BBCH 51 – 75) 1 × 60 g a.s./ha
Effects on aquatic organisms (9.5)			
Apples	Apples, BBCH 62 – PHI, Apples, BBCH 69 – PHI 1 x 25 g a.s./ha	Worst-case PEC values	Apple (BBCH 62/69 – PHI) 1 × 25 g a.s./ha
	Apples, BBCH 62 – PHI, Apples, BBCH 69 – PHI 1 x 50 g a.s./ha	Worst-case PEC values	Apple (BBCH 62/69 – PHI) 1 × 50 g a.s./ha
Oilseed rape	Winter oilseed rape, 1 x 60 g a.s./ha, early and late applications	Worst-case PEC values	Winter oilseed rape, 1 x 60 g a.s./ha, late
	Spring oilseed rape, 1 x 60 g a.s./ha	No grouping	
Potatoes	Potatoes, 1 x 36 g a.s./ha, early and late applications	No grouping	

Grouping according to crop group or crop group and application pattern			
Group	Intended uses	Relevant use parameters for grouping	Worst case application pattern
Maize	Corn, 1 x 60 g a.s./ha	No grouping	
Effects on bees (9.6)			
<u>EPPO approach</u> All proposed uses	All crops	Maximum single application rate	OSR (BBCH 31-71) 1 × 60 g a.s./ha
<u>EFSA approach</u> Orchards	Apples, BBCH 62 – PHI, 25 - 50g a.s./ha	Crop group according to EFSA Bee GD (2013) screening assessment, 1 <sup>st</sup> Tier and 2 <sup>nd</sup> Tier, maximum single application rate	Apples (BBCH 62 – PHI) 1 × 50 g a.s./ha
Potatoes	Potatoes, BBCH 12 – 79, 36 g a.s./ha		Potatoes (BBCH 12 – 79) 1 × 36 g a.s./ha
Oilseed rape	Winter oil seed rape, BBCH 50 – 60, 61 – 71, 31 – 39, 31 – 59, 31 – 69, 31 – 71, 60 g a.s./ha Spring oil seed rape, BBCH 31 – 71, 60 g a.s./ha		OSR (BBCH 31 – 71) 1 × 60 g a.s./ha
Maize	Corn, BBCH 51-75, 1 x 60 g a.s./ha		Corn, BBCH 51-75, 1 x 60 g a.s./ha
Effects on arthropods other than bees (9.7)			
No grouping	-	-	-
Effects on non-target soil meso- and macrofauna (9.8)			
Uses were grouped according to section B8 Environmental Fate	All crops	Worst-case PEC values for acetamiprid, IM-1-2, IM-1-4, IC-0 and IM-1-5	Potatoes (BBCH 12 – 79) 1 × 36 g a.s./ha
Effects on soil microbial activity (9.9)			
Uses were grouped according to section B8 Environmental Fate	All crops	Worst-case PEC values for acetamiprid, IM-1-2, IM-1-4, IC-0 and IM-1-5	Potatoes (BBCH 12 – 79) 1 × 36 g a.s./ha
Effects on non-target terrestrial plants (9.10)			
All proposed uses	All crops	In-field assessment: Maximum annual application rate	OSR (BBCH 31 – 71) 1 × 60 g a.s./ha

**zRMS comments:**

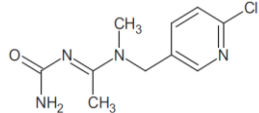
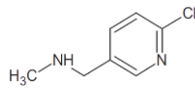
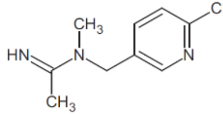
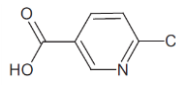
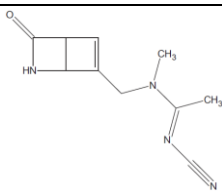
The crop grouping presented in table above is in general agreed by the zRMS. However, in case no acceptable risk could be concluded with the risk envelope approach, the separate evaluation has been performed for lower application rates.

Table above has been amended due to lower application rate in oilseed rape (50 g a.s./ha) proposed by the Applicant following the commenting period in order to address the risk to small herbivorous mammals. The risk assessment for remaining species was performed with consideration of application of CA3573 to oilseed rape at 60 g a.s./ha, covering lower rate, and for this reason Table 9.1-2 was amended only in area of the mammalian risk assessment. Since acceptable risk for remaining species could be concluded for application to OSR at 60 g a.s.

### 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 CA3573 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 on 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 CA3573 were not evaluated as part of the EU assessment of acetamiprid. New data submitted with this application are listed in 0 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	Reference
<i>Anas platyrhynchos</i> (mallard duck)	acetamiprid	Acute	LD <sub>50</sub> = 98 mg/kg bw	EFSA (European Food Safety Authority), 2016. 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
<i>Colinus virginianus</i> (bobwhite quail)	acetamiprid	Acute	LD <sub>50</sub> > 100 mg/kg bw	
<i>Poephila guttata</i> (zebra finch)	acetamiprid	Acute	LD <sub>50</sub> = 5.7 mg/kg bw	
Geometric mean	acetamiprid	Acute	<b>LD<sub>50</sub> = 38.2 mg/kg bw</b>	
	acetamiprid	Long-term	<b>LD<sub>50/10</sub> = 3.8 mg/kg bw</b>	
<i>Anas platyrhynchos</i> (mallard duck)	acetamiprid	Long-term	NOAEL = 9.5 mg/kg bw/d	

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

#### **zRMS comments:**

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

#### 9.2.1.1 Justification for new endpoints

Not relevant.

### 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).

#### 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 CA3573 in apples (BBCH 62-PHI, use no. 1 +2 , 11+12)**

Intended use		Orchards (apple, BBCH 62-PHI)				
Active substance/product		acetamiprid				
Application rate (g/ha)		1 × 50				
Acute toxicity (mg/kg bw)		38.2				
TER criterion		10				
Crop scenario Growth stage	Indicator/generic focal species	SV <sub>90</sub>	MAF <sub>90</sub>	DDD <sub>90</sub> (mg/kg bw/d)	TER <sub>a</sub>	
Spring Summer,	Small insectivorous bird “tit”	46.8	1	2.34	16.3	
BBCH ≥ 40	Small insectivorous/ worm feeding species “thrush”	2.2	1	0.11	347	
BBCH ≥ 40	Small granivorous bird “finch“	8.2	1	0.41	93.2	
Reprod. toxicity (mg/kg bw/d)		3.8				
TER criterion		5				
Crop scenario Growth stage	Indicator/generic focal species	SV <sub>m</sub>	MAF <sub>m</sub> × TWA	DDD <sub>m</sub> (mg/kg bw/d)	TER <sub>tt</sub>	
Spring Summer,	Small insectivorous bird “tit”	18.2	1 × 0.53	0.48	7.92	
BBCH ≥ 40	Small insectivorous/ worm feeding species “thrush”	0.8	1 × 0.53	0.02	190	
BBCH ≥ 40	Small granivorous bird “finch“	3.8	1 × 0.53	0.10	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 9.2-3: First-tier assessment of the acute and long-term/reproductive risk for birds due to the use of CA3573 in potatoes (BBCH 12-79, use no. 3 + 13)**

Intended use		Potatoes (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 Growth stage	Indicator/generic focal species	SV <sub>90</sub>	MAF <sub>90</sub>	DDD <sub>90</sub> (mg/kg bw/d)	TER <sub>a</sub>	
BBCH 10-39	Small omnivorous bird “lark”	24.0	1	0.86	44.4	
BBCH ≥ 40	Small omnivorous bird “lark”	7.2	1	0.26	147	
BBCH 10-19	Small insectivorous bird “wagtail”	26.8	1	0.96	39.8	
BBCH ≥ 20	Small insectivorous bird “wagtail”	25.2	1	0.91	42.0	
Reprod. toxicity (mg/kg bw/d)		3.8				
TER criterion		5				
Crop scenario Growth stage	Indicator/generic focal species	SV <sub>m</sub>	MAF <sub>m</sub> × TWA	DDD <sub>m</sub> (mg/kg bw/d)	TER <sub>t</sub>	
BBCH 10-39	Small omnivorous bird “lark”	10.9	1 × 0.53	0.21	18.1	
BBCH ≥ 40	Small omnivorous bird “lark”	3.3	1 × 0.53	0.06	63.3	
BBCH 10-19	Small insectivorous bird “wagtail”	11.3	1 × 0.53	0.22	17.3	
BBCH ≥ 20	Small insectivorous bird “wagtail”	9.7	1 × 0.53	0.19	20.0	

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 9.2-4: First-tier assessment of the acute and long-term/reproductive risk for birds due to the use of CA3573 in spring and winter oilseed rape (BBCH 31-71, use no. 4-10 + 14-18)**

Intended use		Oilseed rape (BBCH 31-71)				
Active substance/product		acetamiprid				
Application rate (g/ha)		1 × 60				
Acute toxicity (mg/kg bw)		38.2				
TER criterion		10				
Crop scenario	Indicator/generic focal species	SV <sub>90</sub>	MAF <sub>90</sub>	DDD <sub>90</sub> (mg/kg bw/d)	TER <sub>a</sub>	
Growth stage						
late (with seeds) (BBCH 30-99)	Small insectivorous bird “dunnock“	7.4	1	0.44	86.8	
BBCH 30-39	Small omnivorous bird “lark”	7.2	1	0.43	88.8	
BBCH ≥ 40	Small omnivorous bird “lark”	6.0		0.36	106	
BBCH 30-39	Medium herbivorous/ granivorous bird “pigeon“	2.4	1	0.14	273	
BBCH ≥ 40	Medium herbivorous/ granivorous bird “pigeon“	2.0	1	0.12	318	
Reprod. toxicity (mg/kg bw/d)		3.8				
TER criterion		5				
Crop scenario	Indicator/generic focal species	SV <sub>m</sub>	MAF <sub>m</sub> × TWA	DDD <sub>m</sub> (mg/kg bw/d)	TER <sub>t</sub>	
Growth stage						
late (with seeds) (BBCH 30-99)	Small insectivorous bird “dunnock“	2.7	1 × 0.53	0.09	42.2 43.3	
BBCH 30-39	Small omnivorous bird “lark”	3.3	1 × 0.53	0.10	38.0	
BBCH ≥ 40	Small omnivorous bird “lark”	2.7	1 × 0.53	0.09	42.2 43.3	
BBCH 30-39	Medium herbivorous/ granivorous bird “pigeon“	1.1	1 × 0.53	0.03	127 126	
BBCH ≥ 40	Medium herbivorous/ granivorous bird “pigeon“	0.9	1 × 0.53	0.03	127 126	

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 CA3573 in maize (BBCH 51-75, use no. 19 + 20)**

Intended use		Maize (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	Indicator/generic focal species	SV <sub>90</sub>	MAF <sub>90</sub>	DDD <sub>90</sub> (mg/kg bw/d)	TER <sub>a</sub>	
Growth stage						
BBCH ≥ 40	Medium granivorous bird “gamebird”	1.6	1	0.10	382	
BBCH ≥ 40	Small omnivorous bird “lark”	6.0	1	0.36	106	
BBCH ≥ 40	Medium herbivorous/granivorous bird “pigeon”	13.9	1	0.83	46.0	
BBCH ≥ 20	Small insectivorous bird “wagtail”	12.6	1	0.76	50.3	
Reprod. toxicity (mg/kg bw/d)		3.8				
TER criterion		5				
Crop scenario	Indicator/generic focal species	SV <sub>m</sub>	MAF <sub>m</sub> × TWA	DDD <sub>m</sub> (mg/kg bw/d)	TER <sub>it</sub>	
Growth stage						
BBCH ≥ 40	Medium granivorous bird “gamebird”	0.8	1 × 0.53	0.03	127 <del>126</del>	
BBCH ≥ 40	Small omnivorous bird “lark”	2.7	1 × 0.53	0.09	42.2	
BBCH ≥ 40	Medium herbivorous/granivorous bird “pigeon”	5.7	1 × 0.53	0.18	21.1	
BBCH ≥ 20	Small insectivorous bird “wagtail”	4.8	1 × 0.53	0.15	25.3	

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.



The acute and reproductive tier 1 TER values exceed the relevant trigger values, indicating no unacceptable risk following applications of CA3573 (a.s. acetamiprid) in apples, potatoes, oilseed rape and corn according to the intended use pattern.

**zRMS comments:**

The risk assessment for birds provided in Tables 9.2-2 to 9.2-5 above is in general agreed by the zRMS with some minor corrections regarding the derived TER values.

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 CA3573.

Overall, acceptable acute and long-term dietary risk to birds may be concluded from all intended zonal uses of CA3573.

### 9.2.2.2 Higher-tier risk assessment

No higher tier risk assessment required since  $TER_a$  and  $TER_{lt}$  exceed the trigger values of 10 and 5 for acute and reproductive risk assessments, respectively, at tier 1.

### 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 CA3573 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 106.5, 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 oilseed rape~~ **an application rate at 60 g a.s./ha** also covers the risk for birds from all other intended uses. ~~in orchards and potatoes.~~

Effective application rate (g/ha)	60	Ratio effective application rate to relevant endpoint (trigger: < 50)
Acute toxicity (mg/kg bw)	38.2	1.6
Reprod. toxicity (mg/kg bw/d)	3.8	15.8

**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 CA3573 listed in GAP table.

As CA3573 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 CA3573.

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<sub>foc</sub> <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<sub>ow</sub> of acetamiprid amounts to 0.8 and thus does not exceed the trigger value of 3. A risk assessment for effects due to secondary poisoning is not required.

##### **zRMS comments:**

Log P<sub>ow</sub> 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 CA3573 (a.s. 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. Risk of secondary poisoning and risk to birds from exposure via drinking water is **considered to be low** ~~not relevant~~.

The TER values, calculated for recommended scenarios, all exceed the trigger values of 10 for acute risk and 5 for long-term risk, indicating that the risk to birds is acceptable following use of CA3573 according to the proposed use pattern.

## 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) of this report.

Effects on mammals of CA3573 were not evaluated as part of the EU assessment of acetamiprid. New data submitted with this application are listed in 0 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	<b>LD<sub>50</sub> = 146 mg/kg bw</b>	EFSA (European Food Safety Authority), 2016. 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
Rat	Preparation EXP 60707B	Acute	LD <sub>50</sub> = 1065 mg/kg bw ♀ 1000-2000 mg/kg bw ♂	
Rat	acetamiprid	Long-term 90-d study	NOAEL = 12.4 mg/kg bw/d	
Rat	acetamiprid	Long-term Developmental neurotoxicity study	<b>NOAEL = 2.5 mg/kg bw</b>	

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

#### zRMS comments:

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

After the commenting period the RMS for acetamiprid (NL) informed Polish authorities that although in the data matching table for acetamiprid of June 2021 (and also of December 2020) it was concluded that the data matching was shown sufficiently by Nufarm GmbH & Co. KG, there was a mistake made by the RMS and the conclusion has to be amended since Nufarm needs to show the access to the study on oral developmental toxicity by Nemec (2008), which was used to derive the toxicological reference values and for this reason should have been considered necessary for the active substance renewal. It should be noted that the NOAEL of 2.5 mg a.s./kg bw/d used in the mammalian risk assessment also originates from this study.

According to indications of SANTE/2016/11449 (rev 1.5 of October 2021), submission of evidence on ongoing negotiations and steps taken to get access to the vertebrate study are sufficient to conclude matching of the vertebrate data. In support of the zonal evaluation of CA3573, Nufarm submitted copies of the correspondence with the acetamiprid authorisation holder showing that negotiations on the access to the study by Nemec (2008) are ongoing. In addition to that it has to be noted that in line with Article 62 of Regulation (EC) No 1107/2009, the MS authority may use the vertebrate study in evaluation of the application of the prospective Applicant (here: Nufarm) also in case when no agreement with the authorisation holder is reached. Taking this into account, the endpoint from the study may be conditionally used in the mammalian risk assessment, even before the agreement between the two companies is reached.

#### 9.3.1.1 Justification for new endpoints

Not relevant.

## 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 Birds and Mammals on request from EFSA (EFSA Journal 2009; 7(12): 1438; hereafter referred to as EFSA/2009/1438).

### 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 CA3573 in apples (BBCH 62-PHI, use no. 1 + 2, 11+12)**

use of CA375 in apples (BBCH 62-Phi, use no. 1 + 2, 11+12)

Intended use		Orchards (apple, BBCH 62-PHI)				
Active substance/product		acetamiprid				
Application rate (g/ha)		1 × 50				
Acute toxicity (mg/kg bw)		146				
TER criterion		10				
Crop scenario	Indicator/generic focal species	SV <sub>90</sub>	MAF <sub>90</sub>	DDD <sub>90</sub> (mg/kg bw/d)	TER <sub>a</sub>	
Growth stage						
BBCH ≥ 40	Small herbivorous mammal “vole“	40.9	1	2.05	71.2	
BBCH ≥ 71-79	Frugivorous mammal “dormouse”	47.9	1	2.40	60.8	
BBCH ≥ 40	Large herbivorous mammal “lagomorph”	10.5	1	0.53	275	
BBCH ≥ 40	Small omnivorous mammal “mouse”	5.2	1	0.26	561	
Reprod. toxicity (mg/kg bw/d)		2.5				
TER criterion		5				
Crop scenario	Indicator/generic focal species	SV <sub>m</sub>	MAF <sub>m</sub> × TWA	DDD <sub>m</sub> (mg/kg bw/d)	TER <sub>tt</sub>	
Growth stage						
BBCH ≥ 40	Small herbivorous mammal “vole“	21.7	1 × 0.53	0.58	<b>4.31</b>	
BBCH ≥ 71-79	Frugivorous mammal “dormouse”	22.7	1 × 0.53	0.60	<b>4.17</b>	
BBCH ≥ 40	Large herbivorous mammal “lagomorph”	4.3	1 × 0.53	0.11	22.7	
BBCH ≥ 40	Small omnivorous mammal “mouse”	2.3	1 × 0.53	0.06	41.7	

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 9.3-3: First-tier assessment of the acute and long-term/reproductive risk for mammals due to the use of CA3573 in potatoes (BBCH 12-79, use no. 3 + 13)**

Intended use		Potatoes (BBCH 12-79)				
Active substance/product		acetamiprid				
Application rate (g/ha)		1 × 36				
Acute toxicity (mg/kg bw)		146				
TER criterion		10				
Crop scenario Growth stage	Indicator/generic focal species	SV <sub>90</sub>	MAF <sub>90</sub>	DDD <sub>90</sub> (mg/kg bw/d)	TER <sub>a</sub>	
BBCH 10-19	Small insectivorous mammal “shrew”	7.6	1	0.27	541	
BBCH ≥ 20	Small insectivorous mammal “shrew”	5.4	1	0.19	768	
BBCH ≥ 40	Small herbivorous mammal “vole”	40.9	1	1.47	99.3	
BBCH 10-40	Large herbivorous mammal “lagomorph”	35.1	1	1.26	116	
BBCH ≥ 40	Large herbivorous mammal “lagomorph”	10.5	1	0.38	384	
BBCH 10-39	Small omnivorous mammal “mouse”	17.2	1	0.62	235	
BBCH ≥ 40	Small omnivorous mammal “mouse”	5.2	1	0.19	768	
Reprod. toxicity (mg/kg bw/d)		2.5				
TER criterion		5				
Crop scenario Growth stage	Indicator/generic focal species	SV <sub>m</sub>	MAF <sub>m</sub> × TWA	DDD <sub>m</sub> (mg/kg bw/d)	TER <sub>t</sub>	
BBCH 10-19	Small insectivorous mammal “shrew”	4.2	1 × 0.53	0.08	31.3	
BBCH ≥ 20	Small insectivorous mammal “shrew”	1.9	1 × 0.53	0.04	62.5	
BBCH ≥ 40	Small herbivorous mammal “vole”	21.7	1 × 0.53	0.41	6.10	
BBCH 10-40	Large herbivorous mammal “lagomorph”	14.3	1 × 0.53	0.27	9.26	
BBCH ≥ 40	Large herbivorous mammal “lagomorph”	4.3	1 × 0.53	0.08	31.3	
BBCH 10-39	Small omnivorous mammal “mouse”	7.8	1 × 0.53	0.15	16.7	
BBCH ≥ 40	Small omnivorous mammal “mouse”	2.3	1 × 0.53	0.04	62.5	

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 9.3-4:** First-tier assessment of the acute and long-term/reproductive risk for mammals due to the use of CA3573 in spring and winter oilseed rape (BBCH 31-71, use no. 4-10 + 14-18)

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Intended use		Oilseed rape (BBCH 31-71)				
Active substance/product		acetamiprid				
Application rate (g/ha)		1 × 50 <del>60</del>				
Acute toxicity (mg/kg bw)		146				
TER criterion		10				
Crop scenario	Indicator/generic focal species	SV <sub>90</sub>	MAF <sub>90</sub>	DDD <sub>90</sub> (mg/kg bw/d)	TER <sub>a</sub>	
Growth stage						
BBCH ≥ 20	Small insectivorous mammal “shrew”	5.4	1	0.27 <del>0.32</del>	541	456
BBCH ≥ 40	Small herbivorous mammal “vole”	34.1	1	1.71 <del>2.05</del>	85.6	71.2
All season	Large herbivorous mammal “lagomorph”	35.1	1	1.76 <del>2.11</del>	83.2	69.2
BBCH 30-39	Small omnivorous mammal “mouse”	5.2	1	0.26 <del>0.31</del>	562	471
BBCH ≥ 40	Small omnivorous mammal “mouse”	4.3	1	0.22 <del>0.26</del>	679	562
Reprod. toxicity (mg/kg bw/d)		2.5				
TER criterion		5				
Crop scenario	Indicator/generic focal species	SV <sub>m</sub>	MAF <sub>m</sub> × TWA	DDD <sub>m</sub> (mg/kg bw/d)	TER <sub>tt</sub>	
Growth stage						
BBCH ≥ 20	Small insectivorous mammal “shrew”	1.9	1 × 0.53	0.05 <del>0.06</del>	49.7	41.7
BBCH ≥ 40	Small herbivorous mammal “vole”	18.1	1 × 0.53	0.48 <del>0.58</del>	5.21	4.31
All season	Large herbivorous mammal “lagomorph”	14.3	1 × 0.53	0.38 <del>0.45</del>	6.60	5.56
BBCH 30-39	Small omnivorous mammal “mouse”	2.3	1 × 0.53	0.06 <del>0.07</del>	41.0	35.7
BBCH > 40	Small omnivorous mammal “mouse”	1.9	1 × 0.53	0.05 <del>0.06</del>	49.7	41.7

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 9.3-5:** First-tier assessment of the acute and long-term/reproductive risk for mammals due to the use of CA3573 in maize (BBCH 51-75, use no. 19 + 20)

Intended use		Maize (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 <sub>90</sub>	MAF <sub>90</sub>	DDD <sub>90</sub> (mg/kg bw/d)	TER <sub>a</sub>	
Growth stage						
BBCH ≥ 20	Small insectivorous mammal “shrew”	5.4	1	0.32	456	
BBCH ≥ 40	Small herbivorous mammal "vole"	34.1	1	2.05	71.2	
BBCH ≥ 40	Small omnivorous mammal “mouse”	4.3	1	0.26	562	
Reprod. toxicity (mg/kg bw/d)		2.5				
TER criterion		5				
Crop scenario	Indicator/generic focal species	SV <sub>m</sub>	MAF <sub>m</sub> × TWA	DDD <sub>m</sub> (mg/kg bw/d)	TER <sub>tt</sub>	
Growth stage						
BBCH ≥ 20	Small insectivorous mammal “shrew”	1.9	1 × 0.53	0.06	41.7	
BBCH ≥ 40	Small herbivorous mammal “vole”	18.1 21.7	1 × 0.53	0.58 0.69	4.3 3.62	
BBCH ≥ 40	Small omnivorous mammal “mouse”	1.9	1 × 0.53	0.06	41.7	
All season	Large herbivorous mammal “lagomorph”					

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 for small insectivorous, large herbivorous, small herbivorous and small omnivorous mammals exceed the relevant trigger value, indicating no unacceptable acute risk following

applications of CA3573 (a.s. acetamiprid) in apples, potatoes, corn and oilseed rape according to the intended use pattern. However, the  $TER_{it}$  for the frugivorous mammal “dormouse” and small herbivorous mammal “vole” are below the relevant trigger of 5 for uses in orchards, ~~oilseed rape~~ and corn, requiring refinement in a higher tier risk assessment.

#### zRMS comments:

The risk assessment for mammals provided in Tables 9.3-2 to 9.3-5 above is in general agreed by the zRMS with some minor corrections having no impact on the derived conclusions.

It is noted that in case of application to apples two rates are proposed: 50 and 25 g a.s./ha. The TER values were calculated only for the higher rate, forming a risk envelope and covering also lower rate. However, as unacceptable long-term risk was concluded for small herbivorous mammals, the TER values for the lower rate were calculated by the zRMS below in order to check if acceptable risk may be concluded.

#### First-tier assessment of the long-term/reproductive risk for mammals due to the use of CA3573 in apples (BBCH 62-PHI, use no. 2 and 12)

Intended use		Orchards (apple, BBCH 62-PHI)				
Active substance/product		acetamiprid				
Application rate (g/ha)		1 × 25				
Reprod. toxicity (mg/kg bw/d)		2.5				
TER criterion		5				
Crop scenario Growth stage	Indicator/generic focal species	SV <sub>m</sub>	MAF <sub>m</sub> × TWA	DDD <sub>m</sub> (mg/kg bw/d)	TER <sub>it</sub>	
BBCH ≥ 40	Small herbivorous mammal “vole“	21.7	1 × 0.53	0.29	8.6	
BBCH ≥ 71-79	Frugivorous mammal “dormouse”	22.7	1 × 0.53	0.30	8.3	
BBCH ≥ 40	Large herbivorous mammal “lagomorph”	4.3	1 × 0.53	0.06	41.7	
BBCH ≥ 40	Small omnivorous mammal “mouse”	2.3	1 × 0.53	0.03	83.3	

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.

For lower rate of CA3573 in apples acceptable long-term risk may be concluded for all generic focal species. Acute risk is covered from higher rate for all species considered.

Overall, based on the performed calculations acceptable acute dietary risk may be concluded for mammals from all the intended zonal uses. With regard to the long-term risk following conclusions may be taken:

- Apples at 50 g a.s./ha:
  - acceptable risk to large herbivores and small omnivores,
  - unacceptable risk to small herbivores and frugivores.
- Apples at 25 g a.s./ha: acceptable risk for all generic focal species.
- Potatoes at 36 g a.s./ha: acceptable risk for all generic focal species.
- Oilseed rape at ~~50~~ 60 g a.s./ha: **acceptable risk for all generic focal species.**
  - ~~acceptable risk to small insectivores, large herbivores and small omnivores and small herbivores.~~
  - ~~unacceptable risk to small herbivores.~~
- Maize at 60 g a.s./ha:
  - acceptable risk to small insectivores and small omnivores,
  - unacceptable risk to small herbivores.

Refinement of the long-term risk to frugivorous and herbivorous mammals is presented in point 9.3.2.2 below.

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 CA3573.

### 9.3.2.2 Higher-tier risk assessment

The reproductive tier 1  $TER_{It}$  for the small herbivorous mammal “vole” is below the relevant trigger of 5 in orchards, ~~oilseed rape (OSR)~~ and corn, requiring refinement in a higher tier risk assessment. The higher tier risk assessment is conducted according to recommendations of EFSA/2009/1438 as detailed below.

#### **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 orchards, ~~OSR~~ and maize. By definition, the common vole is as such representative and protective for all other small herbivorous mammals potentially exposed to CA3573 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) (Jacob et al. 2013, KCP 10.1.2.2/01, **see study summary Appendix 2, A.2.1.1.2**) 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. 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 preferred dicotyledons (Rinke 1991, KCP 10.1.2.2/02). The portions of monocots and dictos in the diet of common voles vary by season, with 24-25% monocots and 75-76% dicots in spring and summer, opposed to 48% monocots and 52% dicots in autumn.

**Table 9.3-6: 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 portions of diet were found by Leutert (1983, KCP 10.1.2.2/03) in fertilized or unfertilized meadows on 20 study sites located in Northern Switzerland, where voles consumed on average 43% monocots and 57% dicots in spring and summer.

~~On monoculture arable fields, such as OSR, options to choice food are limited. The importance of winter OSR for small rodents has been studied comprehensively by Heroldová and colleagues (Heroldová et al., 2004, KCP 10.1.2.2/04). The study was conducted in winter OSR fields in South Moravia over the course of the year to take a representative sample of the population dynamic of the rodents in relation to the crop phenology. The study provides exhaustive information about the vole species living in OSR fields, their diet, their food intake and their body weight. Therefore it is considered that this study is the most appropriate to refine the risk assessment of voles in rape fields.~~

~~Observations indicate that winter OSR stands are important habitat for the common vole. In autumn, the common vole is dominant in this habitat and partly still reproducing. This species also dominates the small mammal community of winter OSR in early spring when reproduction begins. Data on the diet of common voles was collected during different growth stages of OSR fields: from autumn after germination when leaves rosette is developed to spring on the beginning of prolongation growth (BBCH 20 to 49),~~



flowering starts (BBCH 50 to 69), and before harvest (BBCH 70 to 89). Data is also available for the period after harvest (BBCH >90), when stubble was not yet ploughed and shed seeds had begun to grow and weed infestation started.

The diet of the common vole was examined by microscopic analysis of stomach contents, with the percentage volume (v%) of individual items estimated. Analyses of the spring and autumn diet of common vole in winter OSR have shown that green leaves of this species form the dominant component of its diet. During the period when the rape crop is ripening, the population abundance of the common vole decreases as green food at ground level decreases.

**Table 9.3.7: Diet of common voles in winter rape fields in relation to its main growing phases (Heroldová et al. 2004)**

BBCH growth stage	Rape leaves (% volume)	Rape seeds (% volume)	Weeds leaves (% volume)	Weed seeds (% volume)
20-49	94.4	–	5.1	1.3
50-69	77.2	–	23.2	–
70-89	50.0	10.3	35.4	5.2
≥90	59.9	–	40.3	–

Voles feed on the sappy parts of the leaf blades and avoided the stems. When rape starts to flower it also begins to lignify, with leaves close to soil drying and the biomass of green parts of the plants decreasing. At this time voles also consume the stems and lignified parts of the plant. During ripening, rape was eaten in smaller amounts by the common vole and the consumption of weed increased.

CA3573 is intended to be applied in OSR between March and June, i.e. exclusively during spring and summer, from BBCH 31-71. Thus, for the risk assessment a PD of 100% rape leaves is considered for BBCH 31-71. This value reflects a conservative assumption regarding the diet of common voles during the spring/summer period because also dicotyledonous weeds were consumed which either grow below the crop (i.e. greater interception) or off crop (i.e. no direct overspray and reduced residues) while crop leaves are oversprayed directly and show highest contamination.

Alternatively, a more conservative exposure is calculated for orchards and maize, which is based on a mixed diet (data for spring/summer according to Rinke 1991) including 25% monocot plants (i.e. higher RUD) and average body weight from a large variety of habitats resulting in a higher FIR/bw.

#### **zRMS comments:**

In general, the publication by Rinke (1991) has been already used for refinement of the common vole diet at the EU, zonal and national level. It was also considered in the course of the renewal process of acetamiprid and on the basis of its results the diet consisting of 50% monocots and 50% of dicots has been agreed for voles exposed in orchards during the peer-review.

It should be noted that this conclusion is not fully in line with indications of the Ctgb Evaluation Manual (2017)<sup>1</sup>, where it is stated that based on studies by Rinke (1991) and Lüthi et al. (2010), in monocot dominated underground (as in case of orchards) the proportion of the voles diet in the chronic risk assessment should be 25% of dicotyledons and 75% of monocotyledons (see table below). Nevertheless, for purposes of the risk assessment for voles exposed to CA3573 in orchards the diet as agreed at the EU level will be used for consistency reasons.

#### **PD values for common voles as recommended by Ctgb**

Crop structure	Risk assessment	PD	
		RUD unit: non-grass herbs	RUD unit: grass and cereals
Dicot dominated fields (agricultural crops etc.)	Chronic	50%	50%
Monocot dominated underground (grasslands, orchards etc.)	Chronic	25%	75%

With regard to the oilseed rape, the publication of Heroldová et al. (2004) has been evaluated by the zRMS and considered relevant for determination of the common vole diet. However, in the study proportions of particular food

<sup>1</sup> CTGB Evaluation Manual for the Authorisation of plant protection products according to Regulation (EC) No 1107/2009, Chapter 7 Ecotoxicology: terrestrial; birds and mammals. version 2.2; April 2017

items are given as volume percentage, while for the risk assessment they should be based on the weight proportions and for this reason the study is not fully relevant for quantitative refinement of PD value. Nevertheless, results of the study clearly indicate that oilseed rape represents significant proportion of the voles diet during the whole season and that proportion of dicotyledonous plants in the voles diet will be clearly >50%. Taking this into account, several scenarios will be considered in the risk assessment.

Assumption of oilseed rape representing 100% of the voles diet is relevant only for BBCH stages 20-49, while at later BBCH stages 50-69 the proportion of oilseed rape declines to 77% and remaining part of the diet consists of weed leaves. As from the publication it is not fully clear if grasses were also included in “weed leaves” and voles are known to feed on grasses, as a worst case it should be assumed that the remaining portion of the diet consists of grasses. In order to cover situation where voles are feeding on higher proportion of grass, also the scenario for dicot dominated fields should be considered in line with indications of Ctgb manual.

The summary of the study by Heroldová et al. (2004) has been not provided by the Applicant and the zRMS would like to kindly remind that all publications considered in the risk assessment must be summarised in sufficient detail. The Applicant is thus requested to provide respective summaries during the commenting period.

As application to maize was not considered in the course of the EU renewal, the diet of voles will be refined on the basis of indications of Ctgb manual, as was done for several zonal and national authorisations of other formulations. Since maize is monocotyledonous and according to EFSA (2009) common voles may also feed on maize shoots, the proportion of diet relevant for monocot dominated fields will be used in the refinement, i.e. 75% of monocots and 25% of dicots.

In summary, following PD values will be considered in refinement of the risk for common voles:

- Orchards: 0.5 and 0.5 for dicots and monocots, respectively, in line with EU agreements.
- Oilseed rape: 1 for OSR shoots; 0.75 for OSR shoots and 0.25 for monocots; 0.5 for monocots and 0.5 for dicots.
- Maize: 0.75 for monocots and 0.25 for dicots.

#### Food intake rate of the common vole (100% OSR)

In the study by Heroldová et al. (2004, KCP 10.1.2.2/04), adult and sub-adult, non-breeding common voles were used in experimental feeding trials to determine the amount of winter OSR consumed per day. Feeding experiments were conducted on common voles taken from the autumn population of OSR fields. The weight of individual voles was 18-23 g (20 g in average, SD = 1.91). The average consumption of green biomass consumed was 21.5 g (18-25 g, SD = 2.46) per day. Using these values, a food intake rate per kg body weight for green plant matter (FIR/b.w.) of 1.08 is evident (21.5 g food/20.0 g b.w. = 1.08). Thus, for the risk assessment a FIR of 1.08 was used for rape leaves.

#### zRMS comments:

In the study by Heroldová et al. (2004) the consumption of the green biomass of oilseed rape by voles has been determined. The average consumption was 21.5 g (18 to 25 g), while the average bodyweight of the tested individuals was 20 g (18 to 23 g). The zRMS agrees that based on these data the average FIR/bw would be 1.08, however from the publication it is not possible to conclude if consumption by individuals was proportional to their weight or individuals of lower weight consumed more. In the worst case the FIR/bw of 1.39 could be calculated (25 g consumed/18 g bodyweight), which is not covered by FIR/bw proposed by the Applicant.

It is also noted that only 3 pairs of common voles were used in the experiment, which mean that the food consumption was investigated on only 6 individuals and cannot be thus considered to be sufficiently reliable to derive the FIR/bw relevant for the whole populations of common voles in the Central Zone.

For this reason the food intake rate based on results of the study by Heroldová et al. (2004) is not agreed by the zRMS and the FIR/bw is calculated below using approach described in Appendix G of EFSA (2009).

BW vole (g)	DEE (kJ)	RUD-unit	PD	FE (kJ/g dry)	Moisture Fraction	Assimilation efficiency fraction	FE <sub>total-fresh</sub> (kJ/g fresh weight)	FIR <sub>total-fresh</sub> (g fresh weight/d)	FIR/BW
25	65.09	Non-grass herbs	+	17.8	0.881	0.76	1.867	40.43	1.617

Based on above calculations, the FIR/bw of 1.617 will be considered for voles feeding exclusively on oilseed rape shoots.

The information provided by the Applicant has been struck through as being not agreed upon.

### Food intake rate of the common vole (mixed diet)

Assuming a mixed diet of 25% monocots and 75% dicots (orchards, maize), 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.

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<sub>i</sub>) 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 9.3-8: Calculation of food energy of total mixed diet for common vole**

Parameter	Unit	Grass cereal shoots	Non-grass herbs
Fraction of food item in mixed diet	PD <sub>i</sub> fresh (%)	25.0	75.0
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 <sub>item,fresh</sub> (kJ/g fr. weight)	0.49	1.21
Food energy of total mixed diet	FE <sub>total,fresh</sub>	1.70	

**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 9.3-9: Calculation of food intake rate per body weight for common voles**

Parameter	Unit	Value
Daily energy expenditure	DEE (kJ/day)	65.1
Food energy of total mixed diet	FE <sub>total,fresh</sub> (kJ/g fresh weight)	1.70
Food intake rate of total mixed diet	FIR <sub>total, fresh</sub> (g fresh weight/d)	38.39
	FIR/bw (g fresh weight/d)	1.54

### zRMS comments:

Calculation of FIR/bw for mixed diet consisting of 25% monocots and 75% dicots was performed by the Applicant in line with Appendix G of EFSA (2009) and is agreed by the zRMS (the exact FIR/bw would be 1.536).

However, various mixed diets will be considered depending on the crop and in table below additional calculations for diets consisting of 50% of monocots and 50% of dicots as well as of 75% of monocots and 25% of dicots is calculated by the zRMS.

BW vole (g)	DEE (kJ)	RUD unit	PD	FE (kJ/g dry)	Moisture Fraction	Assimilation efficiency fraction	FE <sub>total fresh</sub> (kJ/g fresh weight)	FIR <sub>total fresh</sub> (g fresh weight/d)	FIR/BW
25	65.09	Grass + cereals	0.5	17.6	0.764	0.47	1.781	36.55	1.462
		Non-grass herbs	0.5	17.8	0.881	0.76			
25	65.09	Grass + cereals	0.75	17.6	0.764	0.47	1.867	34.87	1.395
		Non-grass herbs	0.25	17.8	0.881	0.76			

Overall, following FIR/bw will be used, depending on the mixed diet composition:

- diet consisting of 25% monocots and 75% dicots: 1.536,
- diet consisting of 50% monocots and 50% dicots: 1.462,
- diet consisting of 25% 75% monocots and 75% 25% dicots: 1.395.

### Higher tier calculation for the common vole in orchards

The higher tier TER<sub>it</sub> is calculated based on the refinement options detailed above, considering a conservative mixed diet (25% monocots, 75% dicots) approach.

**Table 9.3-10: Higher tier assessment of the long term/reproductive risk for small herbivorous mammals due to the use of CA3573 in apple orchards – refined parameters (\*) are further described and justified in the text**

Intended use		Orchards (apple, BBCH 62-PHI)						
Active substance/product		Acetamiprid						
Application rate (g/ha)		1 × 50						
Reprod. toxicity (mg/kg bw/d)		2.5						
TER criterion		5						
Focal species	Food category, % in diet <sup>§</sup>	FIR/bw <sup>§</sup>	RUD <sub>m</sub> × DF (mg/kg food)	MAF <sub>m</sub> × TWA	PT	DDD <sub>m</sub> (mg/kg bw/d)	TER <sub>it</sub>	
Common vole ( <i>Microtus arvalis</i> ) Mixed diet	Monocot plants, 25 %	1.54	52.3 × 0.3	1 × 0.53	1	0.16		
	Dicot plants, 75 %	1.54	28.7 × 0.3	1 × 0.53	1	0.26		
	whole diet					0.42	5.95	

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. TER values shown in bold fall below the relevant trigger.

### zRMS comments:

The risk assessment presented in Table 9.3-10 above is not agreed as it is based on assumption of the diet of common voles consisting of 75% of dicotyledonous plants, while in line with EU agreed diet for voles in orchard diet consisting of 50% monocots and 50% dicots should have been assumed. Furthermore, RUD of 52.3 has been considered for monocots, while in line with Appendix F of EFSA (2009) it should be 54.2.

For this reason the risk assessment has been recalculated by the zRMS using the EU agreed diet and respective FIR/bw of 1.462, as calculated above. Deposition factor of 0.3 has been used, in line with EFSA (2009).

Intended use		Orchards (apple, BBCH 62-PHI)						
Active substance/product		Acetamiprid						
Application rate (g/ha)		1 × 50						
Reprod. toxicity (mg/kg bw/d)		2.5						
TER criterion		5						
Focal species	Food category, % in diet	PD	FIR/bw	RUD <sub>m</sub> × DF (mg/kg food)	MAF <sub>m</sub> × TWA	PT	DDD <sub>m</sub> (mg/kg bw/d)	TER <sub>it</sub>
Common vole ( <i>Microtus arvalis</i> ) Mixed diet	Monocot plants	0.5	1.462	54.2 × 0.3	1 × 0.53	1	0.315	
	Dicot plants	0.5	1.462	28.7 × 0.3	1 × 0.53	1	0.167	
	whole diet						0.482	

Based on refinements agreed at the EU level, acceptable risk for small herbivorous mammals exposed following the application of CA3573 to orchards at 50 g a./ha may be concluded.

The Applicants' calculations were struck through as being based on not agreed assumptions.

### Higher tier calculation for the common vole in OSR

The higher tier  $TER_{it}$  is calculated based on the refinement options detailed above, considering a conservative OSR specific approach.

**Table 9.3-11: Higher tier assessment of the long-term/reproductive risk for small herbivorous mammals due to the use of CA3573 in spring and winter oilseed rape refined parameters (\*) are further described and justified in the text**

<b>Intended use</b>		Oilseed rape (BBCH 31-71)					
<b>Active substance/product</b>		Acetamiprid					
<b>Application rate (g/ha)</b>		1 × 60					
<b>Reprod. toxicity (mg/kg bw/d)</b>		2.5					
<b>TER criterion</b>		5					
<b>Focal species</b>	<b>Food category, % in diet*</b>	<b>FIR/bw*</b>	<b>RUD<sub>m</sub> × DF (mg/kg food)</b>	<b>MAF<sub>m</sub> × TWA</b>	<b>PT</b>	<b>DDD<sub>m</sub> (mg/kg bw/d)</b>	<b>TER<sub>it</sub></b>
Common vole ( <i>Microtus arvalis</i> ) OSR-specific approach	Oilseed rape leaves, 100 %	1.08	28.7 × 0.25	1 × 0.53	1	0.25	10.0

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. TER values shown in bold fall below the relevant trigger.

### zRMS comments:

As already discussed above, the common vole diet consisting of 100% oilseed rape shoots has been agreed as one of the diet scenarios possible in OSR fields.

It is, however, noted that in calculations presented in Table 9.3-11 above the Applicant considered DF of 0.25, while deposition factor is relevant for weeds and grasses growing beneath the crop and not for the crop itself. As the spray is targeted on the crop, the deposition factor of 1 should have been considered for voles feeding on the oilseed rape shoots. Furthermore, the FIR/be considered in Applicants' calculations has been not agreed by the zRMS. Taking all this into account, the Applicants' risk assessment is not agreed by the zRMS and is struck through in Table 9.3-11. Respective calculations for each "diet scenario" were performed by the zRMS and are presented below. For non-crop food items the deposition factor of 0.25 has been used in line with EFSA (2009). For oilseed rape DF of 1 has been assumed. Food intake rates as calculated above by the zRMS were considered. Remaining parameters are in line with EFSA (2009).

<b>Intended use</b>		Oilseed rape (BBCH 31-71)						
<b>Active substance/product</b>		Acetamiprid						
<b>Application rate (g/ha)</b>		1 × 60						
<b>Reprod. toxicity (mg/kg bw/d)</b>		2.5						
<b>TER criterion</b>		5						
<b>Focal species</b>	<b>Food category, % in diet</b>	<b>PD</b>	<b>FIR/bw</b>	<b>RUD<sub>m</sub> × DF (mg/kg food)</b>	<b>MAF<sub>m</sub> × TWA</b>	<b>PT</b>	<b>DDD<sub>m</sub> (mg/kg bw/d)</b>	<b>TER<sub>it</sub></b>
Common vole ( <i>Microtus arvalis</i> ) Single diet	100% OSR shoots	1	1.617	28.7 × 1.0	1 × 0.53	1	1.476	1.69
Common vole ( <i>Microtus arvalis</i> ) Mixed diet	Monocot plants	0.25	1.536	54.2 × 0.25	1 × 0.53	1	0.165	2.06
	OSR shoots	0.75	1.536	28.7 × 1.0	1 × 0.53	1	1.051	
	whole diet						1.216	
Common vole ( <i>Microtus arvalis</i> ) Mixed diet	Monocot plants	0.5	1.462	54.2 × 0.25	1 × 0.53	1	0.315	2.55
	OSR shoots	0.5	1.462	28.7 × 1.0	1 × 0.53	1	0.667	
	whole diet						0.982	

For none of the considered “diet scenarios” acceptable risk could be demonstrated and the risk to small herbivorous mammals from intended application of CA3573 to oilseed rape at the maximum rate (60 g a.s./ha) remains unresolved.

It is noted that range of application rates is indicated for oilseed rape in the Central Zone GAP (30-60 g a.s./ha). For this reason additional risk assessment has been performed for the lowest intended rate of 30 g a.s./ha in order to check if acceptable risk could be demonstrated from application of CA3573 at the lower rate.

Intended use		Oilseed rape (BBCH 31-71)						
Active substance/product		Acetamiprid						
Application rate (g/ha)		1 × 30						
Reprod. toxicity (mg/kg bw/d)		2-5						
TER criterion		5						
Focal species	Food category, % in diet	PD	FIR/ bw	RUD <sub>m</sub> × DF (mg/kg food)	MAF <sub>m</sub> × TWA	PT	DDD <sub>m</sub> (mg/kg bw/d)	TER <sub>it</sub>
Common vole ( <i>Microtus arvalis</i> ) Single diet	100% OSR shoots	1	1.617	28.7 × 1.0	1 × 0.53	1	0.738	3.39
Common vole ( <i>Microtus arvalis</i> ) Mixed diet	Monocot plants	0.25	1.536	54.2 × 0.25	1 × 0.53	1	0.331	4.94
	OSR shoots	0.75	1.536	28.7 × 1.0	1 × 0.53	1	0.175	
	whole diet						0.506	
Common vole ( <i>Microtus arvalis</i> ) Mixed diet	Monocot plants	0.5	1.462	54.2 × 0.25	1 × 0.53	1	0.157	5.09
	OSR shoots	0.5	1.462	28.7 × 1.0	1 × 0.53	1	0.334	
	whole diet						0.491	

TER values calculated for lower application rate of 30 g a.s./ha are above the trigger of 5 only for diet consisting of 50% of monocots and 50% of dicots. For remaining two “diet scenarios” the TER values are below 5 indicating potentially unacceptable risk also from application of CA3573 to OSR at lower rate. Further refinement is thus necessary.

It is noted that at the EU level the DT<sub>50</sub> in dicotyledonous plants has been refined with consideration of 17 residue decline trials performed in lettuce and alfalfa. On the basis of their results the geometric mean DT<sub>50</sub> of 2.3 days has been calculated by the RMS and used to refine the fTWA in dicotyledonous plants forming a part of the voles diet. However, none of these trials has been evaluated during first EU review of acetamiprid and their status in terms of the data protection is unclear, as most probably by mistake these residue trials were not included by the RMS into the list of studies relied upon and in the Vol. 2 of the RAR.

Therefore the Applicant is requested to clarify the data protection status of these residue trials before the derived DT<sub>50</sub> is incorporated in the risk refinement for CA3573 applied in oilseed rape.

### Higher tier calculation for the common vole in maize

The higher tier TER<sub>it</sub> is calculated based on the refinement options detailed above, considering a conservative mixed diet (25% monocots, 75% dicots) approach.

**Table 9.3-19: Higher-tier assessment of the long-term/reproductive risk for small herbivorous mammals due to the use of CA3573 in corn – refined parameters (\*) are further described and justified in the text**

<b>Intended use</b>		Maize (corn, BBCH 51-75)					
<b>Active substance/product</b>		Acetamiprid					
<b>Application rate (g/ha)</b>		1 × 60					
<b>Reprod. toxicity (mg/kg bw/d)</b>		2.5					
<b>TER criterion</b>		5					
<b>Focal species</b>	<b>Food category, % in diet*</b>	<b>FIR/bw*</b>	<b>RUD<sub>m</sub> × DF (mg/kg food)</b>	<b>MAF<sub>m</sub> × TWA</b>	<b>PT</b>	<b>DDD<sub>m</sub> (mg/kg bw/d)</b>	<b>TER<sub>It</sub></b>
Common vole ( <i>Microtus arvalis</i> ) Mixed diet	Monocot plants, 25 %	1.54	52.3 × 0.25	1 × 0.53	1	0.16	<b>5.95</b>
	Dicot plants, 75 %	1.54	28.7 × 0.25	1 × 0.53	1	0.26	
	whole diet					0.42	

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. TER values shown in bold fall below the relevant trigger.

#### zRMS comments:

As already discussed above, for monocot dominated fields the voles diet consisting of 75% monocots and 25% dicots, based on studies by Rinke (1991) and Lüthi et al. (2010) and indicated in Ctgb evaluation manual is considered relevant. Taking this into account the Applicants' calculations presented in Table 9.3-12 are not agreed and are struck through for clarity. Respective calculations based on parameters discussed above and agreed by the zRMS are presented in the table below. Deposition factor of 0.25 has been used, in line with EFSA (2009).

<b>Intended use</b>		Maize (BBCH 51-75)						
<b>Active substance/product</b>		Acetamiprid						
<b>Application rate (g/ha)</b>		1 × 60						
<b>Reprod. toxicity (mg/kg bw/d)</b>		2.5						
<b>TER criterion</b>		5						
<b>Focal species</b>	<b>Food category, % in diet</b>	<b>PD</b>	<b>FIR/bw</b>	<b>RUD<sub>m</sub> × DF (mg/kg food)</b>	<b>MAF<sub>m</sub> × TWA</b>	<b>PT</b>	<b>DDD<sub>m</sub> (mg/kg bw/d)</b>	<b>TER<sub>It</sub></b>
Common vole ( <i>Microtus arvalis</i> ) Mixed diet	Monocot plants	0.75	1.395	54.2 × 0.25	1 × 0.53	1	0.451	<b>4.71</b>
	Dicot plants	0.25	1.395	28.7 × 0.25	1 × 0.53	1	0.080	
	whole diet						0.531	

Calculations performed above resulted with TER below the trigger of 5 indicating potentially unacceptable risk to small herbivorous mammals exposed following intended application of CA3573 to maize.

Although the calculated TER is relatively close to the trigger and it is highly unlikely that common voles would spent 100% of their time feeding in maize fields, there is no actual data to confirm this supposition and to reduce the PT. Taking this into account, the risk to small herbivores from uses of CA3573 in maize remains unresolved.

It is noted that range of application rates for maize is indicated in the Central Zone GAP (40-60 g a.s./ha). For this reason additional risk assessment has been performed for the lowest intended rate of 30 g a.s./ha in order to check if acceptable risk could be demonstrated from application of CA3573 at the lower rate.

<b>Intended use</b>		Maize (BBCH 51-75)						
<b>Active substance/product</b>		Acetamiprid						
<b>Application rate (g/ha)</b>		1 × 40						
<b>Reprod. toxicity (mg/kg bw/d)</b>		2.5						
<b>TER criterion</b>		5						
<b>Focal species</b>	<b>Food category, % in diet</b>	<b>PD</b>	<b>FIR/bw</b>	<b>RUD<sub>m</sub> × DF (mg/kg food)</b>	<b>MAF<sub>m</sub> × TWA</b>	<b>PT</b>	<b>DDD<sub>m</sub> (mg/kg bw/d)</b>	<b>TER<sub>It</sub></b>
Common vole ( <i>Microtus arvalis</i> ) Mixed diet	Monocot plants	0.75	1.395	54.2 × 0.25	1 × 0.53	1	0.301	7.06
	Dicot plants	0.25	1.395	28.7 × 0.25	1 × 0.53	1	0.053	
	whole diet						0.354	

TER value calculated for lower application rate of 40 g a.s./ha in maize is above the trigger of 5 indicating acceptable risk to small herbivorous mammals from application of CA3573 to maize at lower rate.

As acceptable risk could be concluded from the lowest intended application rate in maize, additional calculations were performed by the zRMS in order to identify the maximum rate from intended range of 0.2-0.3 L/ha, which would not pose unacceptable risk to small herbivores. Calculations are presented in table below.

<b>Intended use</b>		Maize (BBCH 51-75)							
<b>Active substance/product</b>		Acetamiprid							
<b>Application rate (g/ha)</b>		1 × 58 (corresponding with 0.29 L/ha); 1 x 56 g a.s./ha (corresponding with 0.28 L/ha)							
<b>Reprod. toxicity (mg/kg bw/d)</b>		2.5							
<b>TER criterion</b>		5							
<b>Focal species</b>	<b>Application rate [g a.s./ha]</b>	<b>Food category, % in diet</b>	<b>PD</b>	<b>FIR/ bw</b>	<b>RUD<sub>m</sub> × DF (mg/kg food)</b>	<b>MAF<sub>m</sub> × TWA</b>	<b>PT</b>	<b>DDD<sub>m</sub> (mg/kg bw/d)</b>	<b>TER<sub>it</sub></b>
Common vole ( <i>Microtus arvalis</i> ) Mixed diet	58	Monocot plants	0.75	1.395	54.2 × 0.25	1 × 0.53	1	0.436	
		Dicot plants	0.25	1.395	28.7 × 0.25	1 × 0.53	1	0.077	
		whole diet						0.513	
Common vole ( <i>Microtus arvalis</i> ) Mixed diet	56	Monocot plants	0.75	1.395	54.2 × 0.25	1 × 0.53	1	0.421	
		Dicot plants	0.25	1.395	28.7 × 0.25	1 × 0.53	1	0.074	
		whole diet						0.495	

Performed calculations demonstrated that in order to exclude unacceptable risk to small herbivores, CA3573 in maize may be applied at the maximum rate of 56 g a.s./ha, corresponding with 0.28 L/ha.

It is noted that at the EU level the DT<sub>50</sub> in dicotyledonous plants has been refined with consideration of 17 residue decline trials performed in lettuce and alfalfa. On the basis of their results the geometric mean DT<sub>50</sub> of 2.3 days has been calculated by the RMS and used to refine the fTWA in dicotyledonous plants forming a part of the voles diet. However, none of these trials has been evaluated during first EU review of acetamiprid and their status in terms of the data protection is unclear, as most probably by mistake these residue trials were not included by the RMS into the list of studies relied upon and in the Vol. 2 of the RAR.

In order to finalise the risk assessment for small herbivorous mammals from application of the highest application rate in maize (60 g a.s./ha) the Applicant is requested to clarify the data protection status of these residue trials before the derived DT<sub>50</sub> is incorporated in the risk refinement.

The data protection status of the residue decline studies mentioned above could not be clarified by the RMS for acetamiprid (NL) despite PL requests. Taking this into account, the studies cannot be considered for the risk refinement purposes as potentially they might be protected with no access granted by the authorisation holder to the Applicant for CA3573.

### Weight-of evidence for common vole

Prime habitat for voles comprises of large open, dry, uniform grassy areas such as meadows, heath lands, and fallow land (Jacob et al. 2013, KCP 10.1.2.2/01, see study summary Appendix 2, A.2.1.1.2). Based on results of further studies (Delattre et al. 1992, KCP 10.1.2.2/05; Butet & Leroux 2001, KCP 10.1.2.2/06) some dense perennial crop cultivations, e.g. alfalfa and clover should be included as prime habitats.

In contrast, fields, orchards and vineyards are intensively managed crops, in particular during the reproductive season of voles in spring and summer. Although orchards or vineyards may provide strips of permanent grass, they do not offer the elements which are characteristic for vole habitats or only to limited extent. Field crops may be colonised at late growth stages but mainly during population peaks when numbers increase in primary habitats and populations in field crops are not permanent due to husbandry activities at and after harvest, the latest. In orchards and vineyards, at most, a strip of grass is found between the rows of crop plants. The part of bare soil makes up at least 50% for orchards and vineyards. Insufficient vegetation cover and disturbance lead to reduced numbers of voles on intensive care crops in general. Farming practices causing heavy disturbance, such as ploughing reduce survival



dramatically (Jacob et al. 2013, KCP 10.1.2.2/01), but also harvesting and harrowing appeared to reduce survival too (Jacob and Halle 2001, KCP 10.1.2.2/07; Jacob 2003, KCP 10.1.2.2/08).

Besides the use of pesticides particularly mechanical husbandry activities such as mowing, mulching and pruning take place. Despite the fact that common voles are capable of enormous population increases and thus are able to rapidly colonize new habitats, populations of this species are more sensitive to disturbances (Adamczewska-Andrzejewska 1981, KCP 10.1.2.2/09) compared to other small mammal species, not least due to their small home ranges (Jacob & Hempel 2003, KCP 10.1.2.2/10) and ultradian rhythm with short-term polyphasic activity patterns (i.e. diurnal and nocturnal activity; Halle, 2000, KCP 10.1.2.2/11). Mowing as typical agricultural practice in commercial orchards – if grass strips are available at all – is known to reduce the attractiveness of orchard habitats for voles substantially (Jaworska 1996, KCP 10.1.2.2/12; Sullivan and Hogue, 1987, KCP 10.1.2.2/13). Regular disturbances and lower/lack of vegetation cover (also by herbicidal weeding) lead to vole population decline predominantly through increased exposure to predation through both diurnal and nocturnal predators. In conventional silage grassland, frequent mowing was even followed by ‘crashes’ in common vole numbers (Jacob and Halle 2001, KCP 10.1.2.2/07) which was largely due to an increased predation risk through birds of prey, owls and mammalian predators. Also predator avoidance behaviour is shown in mown grass (Jacob and Brown 2000 KCP 10.1.2.2/14), which confirms that sparse vegetation is unattractive. Likewise, Edge et al., (1995, KCP 10.1.2.2/15) found populations of grey-tailed voles (*Microtus canicaudus*) reduced by 50% after mowing. Hence, the ground vegetation height seems to be a central point for spatial common vole population dynamics and is considered to be a main factor determining the habitat quality. Therefore, intensively managed orchards by mowing, mulching and herbicidal weeding pose adverse habitat conditions for the common vole and are therefore considered only as secondary habitats for this species (Lauenstein 1979, KCP 10.1.2.2/16; Braun and Dieterlen 2005, KCP 10.1.2.2/17).

In summary, it can be concluded that with respect to ecological requirements and preferences as well as behavioural characteristics, orchards and vineyards represent not a preferred habitat for common voles.

Besides the colonization behaviour of primary and secondary habitat of common voles, hints for a possible source-sink model (Pulliam 1988, KCP 10.1.2.2/18; Dias 1996, KCP 10.1.2.2/19; Tattersall et al. 2004, KCP 10.1.2.2/20) were found in a study conducted on voles in old field and orchards habitats in Canada. According to this model animals from “source” populations, which produce surplus individuals (birth rates are higher than mortality rates), migrate to “sink” populations, which cannot sustain themselves alone (birth rate are lower than mortality rates). On the long term “sink” populations cannot survive without the regularly introduction of animals from “source” populations. In the study of Sullivan et al. (2003, KCP 10.1.2.2/21), orchard populations might represent “sink” populations, which are supplied by animals from primary habitats. A four year study on the montane vole (*Microtus montanus*) was conducted in two orchard habitats and ‘old fields’. The orchards were mowed 5-6 times in each summer. The ‘old field’ habitats were abandoned ( $\geq 25$  years) hay fields. The study showed that population dynamics in orchards followed the population dynamic of voles in ‘old fields’, but at a significant lower level. Mean body mass of voles was consistently higher in old fields than orchard sites. The mean survival of voles tended to decline through time in orchard sites. Therefore, the orchards seemed to be linked to source area dynamics of populations in old fields. This effect is even more pronounced in field crops where vegetation cover disappears completely on a regular basis, e.g. after ploughing.

The traditional risk assessment is based on the TER approach, where an estimated theoretical exposure is compared to the endpoint derived in toxicity studies of the active substance in question (EFSA/2009/1438). In order to account for potential uncertainty of the parameters used in the risk assessment due to extrapolation on various levels (e.g. toxicity data from laboratory animals to focal species), uncertainty factors of 10 (acute) and 5 (reproductive) were added to the trigger values. For several reasons presented below, the risk assessment including the common vole as generic focal species is considered to represent a real worst-case (Jacob et al. 2013, KCP 10.1.2.2/01):

- 1) The mammalian toxicity endpoints are usually derived from studies with laboratory Norway rats

- (*Rattus norvegicus*) or house mice (*Mus musculus*) which have a close phylogenetic relationship to field rodent species, thereby reducing the interspecies uncertainty associated with extrapolating laboratory endpoints to wild mammals.
- 2) The preferred primary habitat of common voles is steppe, which comprises grassland, pasture and meadow with mixed grassland, herbs and weeds that provide appropriate cover to avoid predation (Jacob et al. 2013, KCP 10.1.2.2/01; Niethammer and Krapp 1982, KCP 10.1.2.2/22 ; Mitchell-Jones et al. 1999, KCP 10.1.2.2/23). For common voles, cropped areas are considered to be secondary habitats, and significant invasion into them occurs when there is a population outbreak (Jacob et al. 2013, KCP 10.1.2.2/01; Stein 1958, KCP 10.1.2.2/24). In contrast to primary habitats, these secondary habitats cannot maintain common vole populations sustainably for long periods owing to the seasonal nature of farming, where populations are regularly disrupted by agricultural practice, including mowing and mulching of grass strips in orchards/vineyards, if available (Jacob 2003, KCP 10.1.2.2/08; Jacob and Halle 2001, KCP 10.1.2.2/07). Although the common vole is indicated as the representative generic focal species in screening and tier 1 risk assessments under EFSA/2009/1438, population dynamics and habitat preferences indicate that in the period between population outbreaks the likelihood of significant numbers of common voles being found in secondary habitats is low (Jacob et al. 2013, KCP 10.1.2.2/01).
  - 3) The population densities vary seasonally as well as annually. The common vole is well known to show characteristic population cycles with years of mass occurrences (gradation), in which densities may reach up to more than 3000 individuals per hectare (e.g. Truszkowski 1982, KCP 10.1.2.2/25). In Central Europe mass occurrences of common voles take place every 2-4 years and are generally followed by a population break-down, the so-called latency phase (e.g. Heise and Stubbe 1987, KCP 10.1.2.2/26, Niethammer and Krapp 1982, KCP 10.1.2.2/22). During vole population outbreaks, the density of voles in primary habitats is high, which is likely to provide a considerable buffer for potential adverse effects of plant protection products on common vole populations in secondary habitats such as cropped areas. Inclusion of different levels of comparative risk in primary and secondary habitats for a pest such as the common vole is considered to be appropriate to ensure a sufficient population density is maintained in the primary habitat. This contributes to maintaining the protection goal to avoid long-term detrimental effects on common vole populations (Jacob et al. 2013, KCP 10.1.2.2/01). The status of the common vole as pest species is shown by common vole control advices for field crops given by regional departments of agriculture in the European member states.
  - 4) Risk assessment parameters for focal species as defined by EFSA/2009/1438 do not always appear to concur with results of scientific observations in field and laboratory studies (Jacob et al. 2013, KCP 10.1.2.2/01). For example, EFSA/2009/1438 use of energy balance models indicates that a 25 g vole must consume 1.33 times its own body weight to satisfy the theoretical daily energy expenditure (DEE). However, in laboratory studies, common voles have been found to consume only about a third of their body weight per day (reviewed in Jacob et al. 2013, KCP 10.1.2.2/01), and values as low as 10% based on the uptake of dry matter have been reported (Rörig and Knoche 1916 cited in Jacob et al. 2013, KCP 10.1.2.2/01).
  - 5) In Europe, few rodenticide compounds are used regularly for direct control of common vole populations to reduce crop damage. However, even with extensive direct action during outbreaks, *Microtus* populations are seen to recover quickly, although no data are available for common voles. These findings, along with the exceptional reproductive potential of common voles, indicate that common voles are anticipated to overcome potential adverse effects of in-crop application of plant protection products at the landscape level (Jacob et al. 2013, KCP 10.1.2.2/01).

The common vole is a focal species that exists in cropped areas, and, given body weight and food intake rates, represents a worst-case exposure model. Many member states consider the risk of small herbivorous mammal to be covered by the risk assessments for other mammalian species (i.e. lagomorphs, mice), e.g. North Zone, Italy, Greece, in parts UK). If the vole scenario is considered relevant, it seems reasonable to consider an adjustment to the Commission Regulation (EU) No. 546/2011 (trigger value) to account for the reduced uncertainty associated with the evaluation of derived TER values from acute and reproduction dietary risk assessments as the common vole represents a real worst-case focal species which is closely related to laboratory test organisms. This reduction could, for example, follow the model

used in Germany, where lower TER trigger values ( $\geq 5$  in the acute and  $\geq 2$  in the reproductive risk assessment) are applied for common voles and wood mice. German regulators consider these species to be the worst-case exposure models and not simply representatives of the worst-case exposure model (Jacob et al. 2013, KCP 10.1.2.2/01; Nolting, 2010, KCP 10.1.2.2/27). In combination with the ecological reasons detailed above, adjusting the acute and reproductive TER trigger points seems justified given the conservative nature of the vole risk assessment.

In conclusion, the refined  $TER_{it}$  values for common vole exceed the adapted trigger value of 2 in all cases, indicating an acceptable risk. It has to be emphasized, that most intensively used field crops, orchards and vineyards offer no habitat for common voles at all (i.e. grass strips). Based on pest status, population dynamics, habitat preference, resilience, the reproductive potential of the common vole and the very conservative nature of the risk assessment presented above, it has to be concluded that the long-term risk, particularly on population level, is acceptable following application of CA3573 in apple orchards, oilseed rape and corn.

**zRMS comments:**

First of all it should be pointed out that the species identified to be of concern on the basis of the Tier 1 risk assessment may be excluded as the relevant focal species only on the basis of the monitoring field studies performed in line with the current standards in the crop in question. Literature review based on general indications regarding impact of the crop management on attractiveness of the fields to certain mammalian species is not sufficient to support exclusion of representative of the given feeding guild from the evaluation.

~~Furthermore, one of the literature studies submitted by the Applicant (Heroldová et al., 2004) clearly shows that despite intensive cultivation, oilseed rape fields play a significant role as a feeding habitat of the common vole. This is obviously in contradiction to Applicants' conclusions drawn on the basis of the literature review presented above. As no information regarding maize is available, for precautionary reasons it should be assumed that voles will feed in the maize fields, at least until respective evidence is provided by the Applicant enabling exclusion of the common vole as the relevant focal species in this crop.~~

With regard to orchards, the study by Stirkė et al. (2021)<sup>2</sup> found during the zRMS literature search shows that common voles are present in orchards at sufficient quantities to be considered as the focal species. The study was performed in Lithuania, but in opinion of the zRMS it is also representative for conditions in orchards in at least several Central Zone countries, e.g. in Poland. Furthermore, it is expected that the habitat preference is rather species and not country dependent. It should be also noted that the species cannot be excluded only because a given crop is considered to be the secondary habitat. The main criterion is the fact that the species of concern is frequently visiting and foraging in the given crop, while the type of habitat (primary or secondary) is of lesser importance.

Although due to the high reproductive capacity of the common voles and the population outbreaks application of acetamiprid potentially might not lead to population relevant effects, it should be kept in mind that common vole is considered as the best recognised species representative for the whole herbivorous feeding guild. For this reason argument that vole is considered to be the pest of agricultural crops is not acceptable to exclude this species from the risk assessment, since evaluation performed for this species is protective for other herbivorous mammals. As no alternative small herbivorous species is currently indicated in the guidance documents, the risk to common vole has to be addressed and the only way to reject vole as the focal species is submission of the monitoring studies performed in the crop in question. This is in line with conclusions of the Central Zone harmonisation meetings, which state that risk to the common vole has to be addressed in case this species is identified in EFSA (2009) to be relevant for the given crop scenario and no monitoring data are available.

Based on the above discussion the Applicant proposed reduction of the trigger value for common vole from 5 to 2. However, change of the trigger value for the mammalian species is not foreseen by EFSA (2009) and is also not accepted at the Central Zone level, so respective evaluation based on this approach should be presented in National Addenda prepared for countries accepting reduction of the trigger value. In the zonal report the trigger of 5 is applicable for all mammalian species.

<sup>2</sup> Stirkė V., Balčiauskas L., Balčiauskienė L., 2021: Common vole as a focal small mammal species in orchards of Northern Zone. Diversity 2021, 13, 134

## 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. However, the default residues per unit dose/1 kg active substance (RUD) values for fruits were taken from literature and comprise only a few trials of unclear relevance for regulatory purpose. These data based in many cases on studies conducted neither according to nowadays EU agriculture standards, nor according to recommendations given in EFSA/2009/1438 or EC (2017)<sup>3</sup> about how to conduct residue studies.

Therefore, respective field study data of fruit residue levels from applications of pesticides in fruiting crops from five companies (ADAMA, BASF, Bayer, Corteva, FMC, Syngenta), conducted during the last 20 years, were evaluated by Hahne et al. (2019, KCP 10.1.2.2/28). In the final database, 942 residue values in different fruit species such as grapes, berries (currants, raspberries and gooseberries), fruits from orchards (apple, peach, pear, lemon, mandarin, orange, apricot, cherry, plum), gourds (pumpkins, cucumbers, squash and melons) and strawberries. This comprehensive data set provides a solid basis for reviewing registration relevant RUD values for fruits as diet items for birds and mammals in environmental wildlife risk assessments.

The study by Hahne et al. (2019, KCP 10.1.2.2/28) includes 291 field studies (conducted between 1991 and 2017) were available measuring residue levels on fruits in different numbers of separated field trials (1-8) after pesticide application (34% insecticides and 66% fungicides). All study protocols followed regulatory relevant study guidance documents. Samples were collected at the day of application and the following days. All studies were conducted for registration purposes, and only studies fulfilling the following criteria were included in the database and considered for analysis:

- Samples for residue analysis taken at appropriate fruit ripening stages (see below) on the day of application and shortly thereafter.
- Study conducted under GLP and evaluated at EU member state level as appropriate.
- Percentage of recovery during analysis not below 80%. This ensures that the residues found do not significantly underestimate the actual value.
- For 'grapes' and 'large fruits from orchards' only trials with 1 application were considered due to the large number of available trials covering these crop groups.
- For 'berries', 'gourds', 'small fruits from orchards' and 'strawberries', trials with 1 up to 8 applications were used. As a conservative approach, the first residue measurement directly after the last application was evaluated in these cases.

From each study report relevant information regarding the used pesticide (active substance), application method, application rate, concentration of a.s., fruit type, BBCH growth stage, country, time of sampling, and the residue concentrations was extracted. Residue levels after last treatment (DALT0) (i.e. at the day of application or shortly thereafter if maximum value was not measured at DALT0) were taken for further analysis.

The final database consisted of 942 residue values from the following fruits: grapes, currants, raspberries, gooseberries, apples, peaches, pears, lemons, mandarins, oranges, apricots, cherries, plum, pumpkins, cucumbers, squash, melons, and strawberries. The available residue data was grouped into the existing guidance relevant crop groups defining the different food items needed for calculating the exposure in the risk assessment. In general, for the frugivorous scenarios according to EFSA/2009/1438, residue data from growth-stages of development of fruits (BBCH 71-79) and maturity of fruits (BBCH 81-89) are considered.

The proposed new RUD values for different groups of fruits are summarised and compared to current

<sup>3</sup> 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](https://ec.europa.eu/food/sites/food/files/plant/docs/pesticides_mrl_guidelines_app-d.pdf) (accessed 2020 January 10).

EFSA/2009/1438 default values in the following table. Since the data reviewed by Hahne et al. (2019, KCP 10.1.2.2/28) is, according to sample size and methodology, more appropriate for regulatory purposes and wildlife risk assessments (compared to rather obscure public data based on low sample sizes), the proposed new RUD values are considered in the higher tier risk assessment for frugivorous mammals in apple orchards. The following RUD value is considered for relevant crops:

- Apple: **RUD<sub>m</sub>=0.9 mg a.s./kg** (large fruits from orchards, incl. apple and pear residues)

**Table 9.3-12: Proposed new default RUD values for frugivorous scenarios (Hahne et al. 2019) compared to current default values according to EFSA/2009/1438**

Fruit type analysed	Proposed new RUD defaults for frugivorous scenarios [mg/kg]			Current default RUD values of EFSA/2009/1438 [mg/kg]		
	Mean ± s.d.	90 <sup>th</sup> percentile	n (residue values)	Mean ± s.d.	90 <sup>th</sup> percentile	n (residue values)
Grapes	1.6 ± 1.2	3.3	100	8.3 ± 7.2 <sup>5</sup>	16.7 <sup>5</sup>	9 <sup>5</sup>
Berries <sup>1</sup>	5.0 ± 3.6	9.2	180			
Large fruits from orchards <sup>2</sup>	0.9 ± 0.6	1.5	126	19.5 ± 16.8	41.1	33
Small fruits from orchards <sup>3</sup>	2.6 ± 1.4	4.3	126	3.3 ± 2.6	6.5	33
Gourds <sup>4</sup>	0.7 ± 0.6	1.3	267	34.3 ± 54.7	61.5	19
Strawberries	1.3 ± 1.4	2.3	143	Not given, substituted by values of berries		

<sup>1</sup> Currants, raspberries and gooseberries

<sup>2</sup> Apple, peach, pear, lemon, mandarin and orange

<sup>3</sup> Cherry (C-EU, covering apricot and plum (C-EU), and cherry apricot, and plum (S-EU (total of 192 trials)

<sup>4</sup> Pumpkins, cucumbers, squash and melons from studies conducted in S-EU (covering 58 additional RUD values from C-EU)

<sup>5</sup> Grapes and berries merged in EFSA/2009/1438

#### **zRMS comments:**

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.

However, in line with EFSA (2009) the risk assessment should be preferably performed with consideration of the generic default RUD values and refined initial RUD's may be used only in exceptional and well justified cases.

Taking this into account the zRMS consulted the data behind the RUD in EFSA (2009) in order to check if refinement of the initial RUD values proposed by the Applicant could be considered to be justified.

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)<sup>4</sup>. 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.

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.

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).

<sup>4</sup> 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

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.

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.

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 2019<sup>5</sup>, 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)<sup>6</sup> 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 <sup>1)</sup>	Rate [kg/ha]	Sampling day	Matrix	Residue level [mg/kg]	RUD [mg/kg] <sup>1)</sup>
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

<sup>5</sup> 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

<sup>6</sup> 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 DE02 Rheinland-Pfalz 67551 Worms Pfeddersheim Germany  N-EU 2014	Braebum	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
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

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

<sup>2)</sup> 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.

### Higher tier calculation for the dormouse in orchards

The higher tier risk assessment for frugivorous mammal “dormouse” is recalculated with new RUD values as presented in the table below.

**Table 9.3-14: Higher-tier assessment of the long-term/reproductive risk for frugivorous mammals due to the use of CA3573 in apple orchards – refined parameters (\*) are further described and justified in the text**

<b>Intended use</b>		Orchards (apple, BBCH 62-PHI)						
<b>Active substance/product</b>		acetamiprid						
<b>Application rate (g/ha)</b>		1 × 50						
<b>Reprod. toxicity (mg/kg bw/d)</b>		2.5						
<b>TER criterion</b>		5						
<b>Focal species</b>	<b>Food category, % in diet*</b>	<b>FIR/bw*</b>	<b>RUD<sub>max</sub> (mg/kg food)</b>	<b>MAF<sub>m</sub> × TWA</b>	<b>PT</b>	<b>DDD<sub>m</sub> (mg/kg bw/d)</b>	<b>TER<sub>it</sub></b>	
Dormouse ( <i>Microtus arvalis</i> )	Orchard fruits, 100%	1.16	4.8 <del>0.9</del>	1 × 0.53	1	0.15 <del>0.03</del>	16.7 <del>83.3</del>	

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. TER values shown in bold fall below the relevant trigger.

## Conclusion

The TER<sub>it</sub> values for the frugivorous mammal scenario exceed the trigger of 5 for the reproductive risk assessment, indicating an acceptable risk following application of CA3573 (a.s. acetamiprid) in orchards according to the intended GAP.

### zRMS comments:

The risk refinement for frugivorous mammals has been corrected by the zRMS with consideration of the agreed refined RUD value. Acceptable long-term risk could be concluded.

The concerned Member States may wish to reconsider zRMS proposal for refinement of RUD value at the product authorisation in their countries.

### 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 omnivorous 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 (Koc < 500 L/kg) or 3000 in the case of more sorptive substances (Koc ≥ 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 of an application with 60 g a.s./ha in oilseed rape also covers the risk for birds from all other intended uses in orchards and potatoes.

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

### 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 CA3573 listed in GAP table.

As CA3573 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 CA3573.

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 Kfoc <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. 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.

### 9.3.2.5 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 CA3573 (a.s. 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 long-term risk to small herbivorous mammals was addressed in a higher tier risk assessment, including data on diet (PD). Risk of secondary poisoning and risk to mammals from exposure via drinking water is considered to be low ~~not relevant~~.

The TER values, calculated for recommended scenarios, all exceed the trigger values of 10 for acute risk ~~and 5 for long-term risk~~, indicating that the acute risk to mammals is acceptable following use of CA3573 according to the proposed use pattern.

Based on the Tier 1 evaluation acceptable long-term risk could be concluded for intended application in orchards at 25 g a.s./ha, oilseed rape at 50 g a.s./ha and potatoes at 36 g a.s./ha. For applications in orchards at 50 g a.s./ha ~~oilseed rape at 60 g a.s./ha~~ and maize at 60 g a.s./ha potentially unacceptable long-term risk was concluded for small herbivorous mammals and frugivorous mammals (orchards only). Refinement of the risk to small herbivores has been performed with consideration of the data on the diet composition of the common vole. Based on the performed evaluation acceptable risk could be concluded for application to orchards at 50 g a.s./ha. For maize reduction of the maximum application rate to 56 g a.s./ha (corresponding with 0.28 L product/ha) was necessary to address the long-term risk to small herbivores. ~~The risk to small herbivorous mammals from intended uses in oilseed rape remained unresolved.~~

~~In order to address the risk in oilseed rape and to remove restriction regarding the maximum application rate in maize the Applicant has to clarify the data protection status of the residue decline studies used at the EU level to refine the FTWA value in dicotyledonous plants.~~

The risk to frugivorous mammals from application of acetamiprid in orchards at 50 g a.s./ha has been refined with consideration of the RUD value in large fruits. Acceptable risk could be concluded, but the concerned Member State may wish to reconsider this refinement option at the product authorisation.

Overall it is concluded that acetamiprid will not pose unacceptable risk to mammals following intended application to potatoes, oilseed rape and orchards, while for maize the application rate has to be reduced to 0.28 L product/ha. ~~Further data are necessary to address the long-term risk in oilseed rape.~~

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

No relevant data on amphibians and reptiles are available for acetamiprid, consequently no further assessment of potential effects on reptiles and amphibians will be presented in this document.

### **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 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 aquatic organisms of CA3573 were not evaluated as part of the EU assessment of acetamiprid. New data submitted with this application are listed in 0 and summarised in Appendix 2.

The selection of studies and endpoints for the risk assessment is in line with the results of the EU review process. However, for some endpoints it deviates. Justifications are provided below.

**Table 9.5-1: Endpoints and effect values relevant for the risk assessment for aquatic organisms – acetamiprid and relevant metabolites**

Species	Substance	Exposure System	Results	Reference
<b>Fish - acute</b>				
<i>Oncorhynchus mykiss</i>	a.s.	96 h, s	LC <sub>50</sub> > 100 mg a.s./L <sub>nom</sub>	EFSA, 2016
<i>Lepomis macrochirus</i>	a.s.	96 h, f	LC <sub>50</sub> > 119.3 mg a.s./L <sub>mm</sub>	EFSA, 2016
<i>Cyprinodon variegatus</i>	a.s.	96 h, f	LC <sub>50</sub> = 100 mg a.s./L <sub>nom</sub>	EFSA, 2016
<i>Oncorhynchus mykiss</i>	Metabolite IM-1-4	96 h,ss	LC <sub>50</sub> > <b>98.1 mg a.s./L</b>	EFSA, 2016
<b>Fish - chronic</b>				
<i>Pimephales promelas</i>	a.s.	35 d, f	<b>NOEC = 9.4 mg a.s./L<sub>mm</sub></b> <b>EC<sub>10</sub> &gt; 150 mg a.s./L<sub>mm</sub></b>	EFSA, 2016
<b>Amphibians</b>				
<i>Xenopus laevis</i> *	a.s.	21 d, f	NOEC <sub>growth</sub> 2.6 mg a.s./L <sub>mm</sub>	EFSA, 2016
<b>Aquatic invertebrates - acute</b>				
<i>Daphnia magna</i>	a.s.	48 h, s	EC <sub>50</sub> = 49.8 mg a.s./L <sub>mm</sub>	EFSA, 2016
<i>Chironomus riparius</i>	a.s.	48 h, s	EC <sub>50</sub> = 0.0207 mg a.s./L <sub>mm</sub>	EFSA, 2016
<i>Gammarus fasciatus</i>	a.s.	96 h, s	EC <sub>50</sub> = 0.10 mg a.s./L <sub>mm</sub>	EFSA, 2016
<i>Mysidopsis bahia</i>	a.s.	96 h, f	EC <sub>50</sub> = 0.066 mg a.s./L <sub>mm</sub>	EFSA, 2016
<i>Gammarus pulex</i>	a.s.	96 h, s	EC <sub>50</sub> = 0.050 mg a.s./L <sub>mm</sub>	EFSA, 2016
<i>Simulium latigonium</i>	a.s.	96 h, s	EC <sub>50</sub> = 0.0037 mg a.s./L <sub>mm</sub>	EFSA, 2016

Species	Substance	Exposure System	Results	Reference
Geometric mean aquatic insects	a.s.	EC <sub>50</sub>	<b>0.0085 mg a.s./L<sub>mm</sub></b>	EFSA, 2016
<i>Daphnia magna</i>	Metabolite IM-1-2	48 h,ss	<b>EC<sub>50</sub> &gt; 99.8 mg/L</b>	EFSA, 2016
<i>Chironomus riparius</i>	Metabolite IM-1-2	48 h,s	<b>EC<sub>50</sub> = 15.0 mg/L</b>	EFSA, 2016
<i>Daphnia magna</i>	Metabolite IM-1-4	48 h, ss	<b>EC<sub>50</sub> = 43.9 mg/L</b>	EFSA, 2016
<i>Mysidopsis bahia</i>	Metabolite IM-1-4	48 h, s	<b>EC<sub>50</sub> = 19 mg/L</b>	EFSA, 2016
<i>Chironomus riparius</i>	Metabolite IM-1-4	48 h, s	<b>EC<sub>50</sub> = 76.0 mg/L</b>	EFSA, 2016
<i>Daphnia magna</i>	Metabolite IM-1-5	48 h, s	<b>EC<sub>50</sub> = 25 mg/L</b>	EFSA, 2016
<i>Chironomus riparius</i>	Metabolite IM-1-5	48 h,s	<b>EC<sub>50</sub> = 68 mg/L</b>	EFSA, 2016
<i>Daphnia magna</i>	Metabolite IC-0	48 h, ss	<b>EC<sub>50</sub> &gt; 95.1 mg/L</b>	EFSA, 2016
<i>Chironomus riparius</i>	Metabolite IC-0	48 h,s	<b>EC<sub>50</sub> &gt; 100 mg/L</b>	EFSA, 2016
<i>Daphnia magna</i>	Metabolite IB-1-1	48 h,ss	<b>EC<sub>50</sub> &gt; 100.8 mg/L</b>	EFSA, 2016
<i>Chironomus riparius</i>	Metabolite IB-1-1	48 h,s	<b>EC<sub>50</sub> &gt; 100 mg/L</b>	EFSA, 2016
<b>Aquatic invertebrates – chronic</b>				
<i>Daphnia magna</i>	a.s.	21 d, ss	NOEC = 5 mg a.s./L <sub>mm</sub> <b>EC<sub>10</sub> = 2.96 mg a.s./L<sub>mm</sub></b>	EFSA, 2016
<i>Daphnia magna</i>	Metabolite IM-1-5	21 d, ss	<b>NOEC<sub>rep</sub> = 26 mg/L</b>	EFSA, 2016
<b>Sediment dwelling organisms</b>				
<i>Chironomus riparius</i>	a.s.	28 d, s	NOEC <sub>emerg</sub> = 0.00096 mg a.s./L (mm); <b>EC<sub>10,emerg</sub> = 0.000235 mg a.s./L (mm)</b>	EFSA, 2016
<b>Algae</b>				
<i>Scenedesmus subspicatus</i>	a.s.	72 h, s	E <sub>b</sub> C <sub>50</sub> / E <sub>r</sub> C <sub>50</sub> > 98.3 mg a.s./L (mm)	EFSA, 2016
<i>Anabaena flos-aquae</i>	a.s.	120 h, s	<b>EC<sub>50</sub> &gt; 1.3 mg a.s./L (mm)</b>	EFSA, 2016
<b>Higher plant</b>				
<i>Lemna gibba</i>	a.s.	14 d, s	Fronds number, <b>EC<sub>50</sub> &gt; 1.0 mg a.s./L (mm)</b>	EFSA, 2016
<b>Higher-tier studies (micro- or mesocosm studies)<sup>1</sup></b>				
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. 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.				

<sup>1</sup> EFSA, 2016: Peer review of the pesticide risk assessment of the active substance acetamiprid, EFSA Journal 2016;14(11):4610

\* Presented as additional data, no data requirement and not relied upon

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

s: static; ss: semi-static; f: flow-through; nom: based on nominal concentrations; mm: based on mean measured concentrations; im: based on initial measured concentrations;

#### **zRMS comments:**

Aquatic toxicity data for acetamiprid and its metabolites provided in Table 9.5-1 above are in line with EU agreed endpoints reported in EFSA Journal 2016;14(11):4610.

**Table 9.5-2: Endpoints and effect values relevant for the risk assessment for aquatic organisms – CA3573**

Species	Substance	Exposure System	Results	Reference
<b>Fish</b>				
<i>Oncorhynchus mykiss</i>	MCW-2222	96 h, s	<b>LC<sub>50</sub> = 85.8 mg test item/L<sub>nom</sub></b> <b>LC<sub>50</sub> = 15.3 mg a.s./L<sub>nom</sub></b>	xxx, xxx., 2014a R-33831 KCP 10.2.1/01
<b>Aquatic invertebrates</b>				
<i>Daphnia magna</i>	MCW-2222	48 h, s	<b>EC<sub>50</sub> = 100.2 mg test item/L<sub>nom</sub></b> EC <sub>50</sub> = 22.8 mg a.s./L <sub>nom</sub>	Juckeland, D., 2014b R-33832 KCP 10.2.1/02
<i>Chironomus riparius</i>	MCW-2222	48 h, s	<b>EC<sub>50</sub> = 0.0521 mg test item/L<sub>nom</sub></b> EC <sub>50</sub> = 0.00929 mg a.s./L <sub>nom</sub>	Juckeland, D., 2015a R-34873 KCP 10.2.1/03
<b>Algae</b>				
<i>Desmodesmus subspicatus</i>	MCW-2222	72 h, s	<b>ErC<sub>50</sub> = 3110.8 mg test item/L<sub>nom</sub></b> EyC <sub>50</sub> = 204.9 mg test item./L <sub>nom</sub> ErC <sub>50</sub> = 554.5 mg a.s./L <sub>nom</sub> EyC <sub>50</sub> = 204.9 mg a.s./L <sub>nom</sub>	Juckeland, D., 2014c R-33833 KCP 10.2.1/04
<b>Higher-tier studies (micro- or mesocosm studies)</b>				
<p>Outdoor mesocosm study (Hommen, Hennecke, Christmann , 2020; KCP 10.2.3/01 – for full summary see Appendix 2.2.3): Effect assessment on macroinvertebrates, zooplankton, phytoplankton, periphyton and macrophytes in outdoor mesocosms. Test substance: CA3573 (Carnadine); 2 applications with a 7-day interval; three replicates per treatment, 5 replicates for the control; study duration: 84 days; nominal treatment rates: 0.30, 0.51, 0.87, 1.5, 2.5 µg a.s./L corresponding to max. measured (“peak”) concentrations (day 7) of 0.53, 0.89, 1.6, 2.6 and 4.6 µg a.s./L.</p> <p>The following effects were observed at the different test concentrations:</p> <ul style="list-style-type: none"> <li>Maximum measured 0.53 – 1.6 µg a.s./L, nominal up to 0.87 µg a.s./L: No treatment effects were found on any macroinvertebrate or zooplankton taxa. Single statistical findings with NOECs &lt; 1.6 µg a.s./L were found to be not ecotoxicologically relevant due to very low numbers, missing concentration-response, and / or not plausible explanation of delayed or indirect effects.</li> <li>Maximum measured 2.6 µg a.s./L, nominal 1.5 µg a.s./L: This concentration had pronounced effects on larvae and – in consequence – emergence of the mayfly <i>Cloeon dipterum</i> with recovery of emergence demonstrated at the end of the study. A few taxa were slightly affected (e.g. <i>Chaoborus</i>, <i>Gammarus</i>, Tanypodinae, Naididae). Potential effects on the cladoceran <i>Chydorus sphaericus</i> were found late in the study and thus, its duration could not be assessed. Periphyton might have been slightly indirectly promoted. The effects on <i>Cloeon</i> resulted also in significant changes of the macroinvertebrate and insect community.</li> <li>Maximum measured 4.6 µg a.s./L, nominal 2.5 µg a.s./L: Compared to 2.6 µg a.s./L, some effects became more pronounced and for additional species slight direct or indirect effects were found. The mayfly <i>Cloeon</i> could not recover within the study. Emergence of damselflies (Coenagrionidae) was temporarily reduced and since there is only one generation per year, recovery was not possible. Also <i>Gammarus</i> and Naididae showed pronounced effects at the highest test concentration..</li> </ul> <p><i>Proposal for RAC derivation:</i></p> <p>In conclusion, the maximum measured (“peak”) concentration of <u>1.6 µg a.s./L</u> (9.4 µg test item/L; nominal: 0.87 µg a.s./L and 5.1 µg test item/L) is the overall Class 1 concentration which can be used to derive an ETO-RAC. Uncertainty related to this concentration is considered small since clearly no effects on potentially sensitive taxa were found and the results are in line with the findings of a previous mesocosm study with acetamiprid (EFSA 2016).</p> <p>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 (2.6 µg a.s./L maximum measured) effects on the emergence of mayflies lasted longer than eight weeks.</p>				

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

s: static; ss: semi-static; f: flow-through; nom: based on nominal concentrations; mm: based on mean measured concentrations

#### 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 CA3573 (formerly MCW-2222) 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, although not used in the risk assessment. As results of this study were not 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 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, A 2.2.1.5.

#### Toxicity of CA3573 (formerly MCW-2222) to additional aquatic invertebrate species

Organism <sup>1)</sup>	Test substance	Endpoint	Value <sup>1)</sup>	Reference
<i>Daphnia magna</i>	MCW-2222	48h EC <sub>50</sub>	22800 µg a.s./L	See Table 9.5-2
<i>Chironomus riparius</i>	MCW-2222	48h EC <sub>50</sub>	<b>9.29 µg a.s./L</b>	See Table 9.5-2
<i>Aeshna</i> sp.	MCW-2222	48h EC <sub>50</sub>	<b>&gt;2130 µg a.s./L</b>	Appendix 2, A 2.2.1.5 IIIA 10.2.2.2/03, Taylor, S. & Joyce, F., D., 2015 (additional study)
<i>Asellus aquaticus</i>			39.4 µg a.s./L	
<i>Cloeon dipterum</i>			<b>1998 µg a.s./L</b>	
<i>Chaoborus crystallinus</i>			<b>14.4 µg a.s./L</b>	
<i>Ichnura elegans</i>			<b>16.6 µg a.s./L</b>	
<i>Corixidae</i>			<b>30.7 µg a.s./L</b>	
<i>Crangonyx pseudogracilis</i>			115 µg a.s./L	
<i>Gammarus pulex</i>			1351 µg a.s./L	
<i>Phryganea bipunctata</i>			<b>14.8 µg a.s./L</b>	
<i>Notonecta marmorea viridis</i>			<b>1314 µg a.s./L</b>	
		Geometric mean EC <sub>50</sub> (all species)	174.3 µg a.s./L	
		Geometric mean EC <sub>50</sub> (all insect species)	93.1 µg a.s./L	
		Geometric mean EC <sub>50</sub> (insect species, less sensitive species excluded)	15.9 µg a.s./L	

<sup>1)</sup> Insets and insect endpoints are highlighted in **bold**

The geometric mean EC<sub>50</sub> 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 EC<sub>50</sub> of 0.0159 is calculated. All these geometric mean values are higher comparing to EU agreed geometric mean EC<sub>50</sub> 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 EC<sub>50</sub> of 0.0085 mg a.s./L is relevant for the risk assessment purposes as representing worst case.

It is noted that according to EFSA (2013) with data for 12 aquatic invertebrate species it would be more relevant to use SSD approach in calculation of the endpoint relevant for the acute risk assessment. However, at the EU level no HC<sub>5</sub> value was available for aquatic insects and for this reason the geometric mean had to be calculated in order to check if formulation is more toxic than the active compound.

The submitted mesocosm study by Homment et al. (2020) has been evaluated by the zRMS and considered reliable and acceptable with NOEC of 1.6 µg a.s./L based on effects class 1.

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 represented worst case comparing to the intended uses of CA3573 with only single applications indicated for each crop.

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. The NOEC value of

of 1.6 µg a.s./L for Naididae family could be determined based on findings of Hommen et al. (2020). 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, A 2.2.3.1, KCP 10.2.3/01.

#### **9.5.1.1 Justification for new endpoints**

Product studies including a mesocosm study are available for CA3573. The endpoints are summarised in

Table 9.5-2 and study summaries are presented in Appendix 2. The following new endpoints will be used in the risk assessment.

- a) Fish acute toxicity: Based on a.s. content, the LC<sub>50</sub> from the study with CA3573 is lower than from the study with the technical active substance. The value used in the risk assessment is: **96-h LC<sub>50</sub> = 15.3 mg a.s./L.**
- ~~b) Algae: Based on a.s. content, the E<sub>r</sub>C<sub>50</sub> from the study with CA3573 is lower than from the study with the technical active substance. The value used in the risk assessment is: 72-h E<sub>r</sub>C<sub>50</sub> = 554.5 mg a.s./L.~~
- c) Mesocosm study: Due to the uncertainties produced by the first mesocosm study (see Table 9.5-1 and EFSA, 2016), a new mesocosm study was performed. It was conducted in a similar way, but especially addressing the critical points of the first one. The main difference is that in the new mesocosm study, the interval between the two applications was 7 days instead of 14 days. Since acetamiprid dissipates slowly from the water phase, the exposure situation was even worse compared to the first study. 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. In total 11 potentially sensitive taxa fulfil the MDD criterion proposed by Brock et al. (2015). The study is robust and valid. In conclusion, the maximum measured (“peak”) concentration of 1.6 µg a.s./L (9.4 µg test item/L) is considered the overall Class 1 concentration (NOEC), which can be used to derive an ETO-RAC. Uncertainty related to this concentration is considered small since clearly no effects on potentially sensitive taxa were found and the results are in line with the findings of the first mesocosm study with acetamiprid. Therefore and according to the EFSA AGD (2013), an assessment factor of 2 is justified. Following this, a **RAC of 0.8 µg a.s./L (4.7 µg test item/L)** will be used in the risk assessment.

#### **zRMS comments:**

As endpoints from studies on acute toxicity of CA3573 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 EC<sub>50</sub> value of 0.0159 mg a.s./L derived from studies performed with CA3573 (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 1.6 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, A 2.2.3.1, KCP 10.2.3/01.

## **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 in the context of Regulation (EC) No 1107/2009”, as provided by the Commission Services (SANTE-2015-00080, 15 January 2015). The relevant global maximum FOCUS Step 1, 2 and 3 PEC<sub>SW</sub> for risk assessments covering the proposed use pattern and the resulting PEC/RAC ratios are presented in the tables below. To achieve a concise risk assessment, the risk envelope approach is applied. Here, the assessment for the use group apples at BBCH 62 also covers the risk for aquatic organisms for the use group apples at BBCH 69 (see 0).

In the following table, the ratios between predicted environmental concentrations in surface water bodies



( $PEC_{SW}$ ,  $PEC_{SED}$ ) and regulatory acceptable concentrations (RAC) for aquatic organisms are given per intended use for each FOCUS scenario and each organism group.

## Parent compound acetamiprid – FOCUS Steps 1-3

**Table 9.5-3:** Aquatic organisms: acceptability of risk (PEC/RAC < 1) for acetamiprid for each organism group based on FOCUS Steps 1, 2 and 3 calculations for the use of CA3573 in apples 1\*25 g a.s./ha - BBCH ≥ 62 (covering BBCH ≥ 69), early application

Group		Fish acute	Fish prolonged	Invertebrates acute	Invertebrates prolonged	Sed. dwellers prolonged	Algae	Higher plant	Higher-tier information
Test species		<i>Oncorhynchus mykiss</i>	<i>Pimephales promelas</i>	Geomean aquatic insects	<i>Daphnia magna</i>	<i>Chironomus riparius</i>	<i>Anabaena flos-aquae</i>	<i>Lemna gibba</i>	Mesocosm (aquatic invertebrates)
Endpoint (µg/L)		LC <sub>50</sub> 15300	NOEC 9400	EC <sub>50</sub> 8.5	EC <sub>10</sub> 2960	EC <sub>10</sub> 0.235	ErC <sub>50</sub> > 1300	EC <sub>50</sub> > 1000	NOEC 1.6
AF		100	10	100	10	10	10	10	2
RAC (µg/L)		153	940	0.085	296	0.0235	> 130	> 100	0.8
Scenario	PEC <sub>gl-max</sub> (µg/L)								
<b>Step 1</b>									
	9.73	0.06	0.01	<b>114</b>	0.03	<b>414</b>	< 0.07	< 0.10	<b>12</b>
<b>Step 2</b>									
N-Europe	2.43	0.02	0.00	<b>29</b>	0.01	<b>103</b>	< 0.02	< 0.02	<b>3.0</b>
S-Europe	2.43	0.02	0.00	<b>29</b>	0.01	<b>103</b>	< 0.02	< 0.02	<b>3.0</b>
<b>Step 3</b>									
D3/Ditch	1.95	0.01	0.00	<b>23</b>	0.01	<b>83</b>	< 0.02	< 0.02	<b>2.4</b>
D4/Pond	0.118	0.00	0.00	<b>1.4</b>	0.00	<b>5.0</b>	< 0.001	< 0.001	0.15
D4/Stream	2.07	0.01	0.00	<b>24</b>	0.01	<b>88</b>	< 0.02	< 0.02	<b>2.6</b>
D5/Pond	0.118	0.00	0.00	<b>1.4</b>	0.00	<b>5.0</b>	< 0.001	< 0.001	0.15
D5/Stream	2.23	0.01	0.00	<b>26</b>	0.01	<b>95</b>	< 0.02	< 0.02	<b>2.8</b>
R1/Pond	0.118	0.00	0.00	<b>1.4</b>	0.00	<b>5.0</b>	< 0.001	< 0.001	0.15
R1/Stream	1.58	0.01	0.00	<b>19</b>	0.01	<b>67</b>	< 0.01	< 0.02	<b>2.0</b>
R2/Stream	2.12	0.01	0.00	<b>25</b>	0.01	<b>90</b>	< 0.02	< 0.02	<b>2.7</b>
R3/Stream	2.23	0.01	0.00	<b>26</b>	0.01	<b>95</b>	< 0.02	< 0.02	<b>2.8</b>
R4/Stream	1.55	0.01	0.00	<b>18</b>	0.01	<b>66</b>	< 0.01	< 0.02	<b>1.9</b>

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

The PEC/RAC ratios for fish (acute and prolonged) as well as for primary producers (algae and macrophytes) are below the trigger of 1, indicating acceptable risks. For invertebrates the risk assessment needs to be taken to FOCUS Step-4 level.

**Table 9.5-4: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for acetamiprid for each organism group based on FOCUS Steps 1, 2 and 3 calculations for the use of CA3573 in apples 1\*25 g a.s./ha - BBCH ≥ 62 (covering BBCH ≥ 69), late application**

Group		Fish acute	Fish prolonged	Invertebrates acute	Invertebrates prolonged	Sed. dwellers prolonged	Algae	Higher plant	Higher-tier information
Test species		<i>Oncorhynchus mykiss</i>	<i>Pimephales promelas</i>	Geomean aquatic insects	<i>Daphnia magna</i>	<i>Chironomus riparius</i>	<i>Anabaena flos-aquae</i>	<i>Lemna gibba</i>	Mesocosm (aquatic invertebrates)
Endpoint (µg/L)		LC <sub>50</sub> 15300	NOEC 9400	EC <sub>50</sub> 8.5	EC <sub>10</sub> 2960	EC <sub>10</sub> 0.235	ErC <sub>50</sub> > 1300	EC <sub>50</sub> > 1000	NOEC 1.6
AF		100	10	100	10	10	10	10	2
RAC (µg/L)		153	940	0.085	296	0.0235	> 130	> 100	0.8
Scenario	PEC <sub>gl-max</sub> (µg/L)								
<b>Step 1</b>									
	9.73	0.06	0.01	<b>114</b>	0.03	<b>414</b>	< 0.07	< 0.10	<b>12</b>
<b>Step 2</b>									
N-Europe	2.43	0.02	0.00	<b>29</b>	0.01	<b>103</b>	< 0.02	< 0.02	<b>3.0</b>
S-Europe	2.43	0.02	0.00	<b>29</b>	0.01	<b>103</b>	< 0.02	< 0.02	<b>3.0</b>
<b>Step 3</b>									
D3/Ditch	0.919	0.01	0.00	<b>11</b>	0.00	<b>39</b>	< 0.01	< 0.01	<b>1.1</b>
D4/Pond	0.041	0.00	0.00	0.48	0.00	<b>1.7</b>	< 0.0003	< 0.0004	0.05
D4/Stream	0.901	0.01	0.00	<b>11</b>	0.00	<b>38</b>	< 0.01	< 0.01	<b>1.1</b>
D5/Pond	0.041	0.00	0.00	0.48	0.00	<b>1.7</b>	< 0.0003	< 0.0004	0.05
D5/Stream	0.995	0.01	0.00	<b>12</b>	0.00	<b>42</b>	< 0.01	< 0.01	<b>1.2</b>
R1/Pond	0.041	0.00	0.00	0.48	0.00	<b>1.7</b>	< 0.0003	< 0.0004	0.05
R1/Stream	0.706	0.00	0.00	<b>8.3</b>	0.00	<b>30</b>	< 0.01	< 0.01	0.88
R2/Stream	0.946	0.01	0.00	<b>11</b>	0.00	<b>40</b>	< 0.01	< 0.01	<b>1.2</b>
R3/Stream	0.994	0.01	0.00	<b>12</b>	0.00	<b>42</b>	< 0.01	< 0.01	<b>1.2</b>
R4/Stream	0.705	0.00	0.00	<b>8.3</b>	0.00	<b>30</b>	< 0.01	< 0.01	0.88

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

The PEC/RAC ratios for fish (acute and prolonged) as well as for primary producers (algae and macrophytes) are below the trigger of 1, indicating acceptable risks. For invertebrates the risk assessment needs to be taken to FOCUS Step-4 level.

**Table 9.5-5: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for acetamiprid for each organism group based on FOCUS Steps 1, 2 and 3 calculations for the use of CA3573 in apples 1\*50 g a.s./ha - BBCH ≥ 62 (covering BBCH ≥ 69), early application**

Group		Fish acute	Fish prolonged	Invertebrates acute	Invertebrates prolonged	Sed. dwellers prolonged	Algae	Higher plant	Higher-tier information
Test species		<i>Oncorhynchus mykiss</i>	<i>Pimephales promelas</i>	Geomean aquatic insects	<i>Daphnia magna</i>	<i>Chironomus riparius</i>	<i>Anabaena flos-aquae</i>	<i>Lemna gibba</i>	Mesocosm (aquatic invertebrates)
Endpoint (µg/L)		LC <sub>50</sub> 15300	NOEC 9400	EC <sub>50</sub> 8.5	EC <sub>10</sub> 2960	EC <sub>10</sub> 0.235	ErC <sub>50</sub> > 1300	EC <sub>50</sub> > 1000	NOEC 1.6
AF		100	10	100	10	10	10	10	2
RAC (µg/L)		153	940	0.085	296	0.0235	> 130	> 100	0.8
Scenario	PEC <sub>gl-max</sub> (µg/L)								
<b>Step 1</b>									
	19.5	0.13	0.02	<b>229</b>	0.07	<b>830</b>	< 0.15	< 0.20	<b>24</b>
<b>Step 2</b>									
N-Europe	4.87	0.03	0.01	<b>57</b>	0.02	<b>207</b>	< 0.04	< 0.05	<b>6.1</b>
S-Europe	4.87	0.03	0.01	<b>57</b>	0.02	<b>207</b>	< 0.04	< 0.05	<b>6.1</b>
<b>Step 3</b>									
D3/Ditch	3.90	0.03	0.00	<b>46</b>	0.01	<b>166</b>	< 0.03	< 0.04	<b>4.9</b>
D4/Pond	0.236	0.00	0.00	<b>2.8</b>	0.00	<b>10</b>	< 0.002	< 0.002	0.30
D4/Stream	4.13	0.03	0.00	<b>49</b>	0.01	<b>176</b>	< 0.03	< 0.04	<b>5.2</b>
D5/Pond	0.236	0.00	0.00	<b>2.8</b>	0.00	<b>10</b>	< 0.002	< 0.002	0.30
D5/Stream	4.46	0.03	0.00	<b>52</b>	0.02	<b>190</b>	< 0.03	< 0.04	<b>5.6</b>
R1/Pond	0.236	0.00	0.00	<b>2.8</b>	0.00	<b>10</b>	< 0.002	< 0.002	0.30
R1/Stream	3.16	0.02	0.00	<b>37</b>	0.01	<b>134</b>	< 0.02	< 0.03	<b>4.0</b>
R2/Stream	4.24	0.03	0.00	<b>50</b>	0.01	<b>180</b>	< 0.03	< 0.04	<b>5.3</b>
R3/Stream	4.46	0.03	0.00	<b>52</b>	0.02	<b>190</b>	< 0.03	< 0.04	<b>5.6</b>
R4/Stream	3.09	0.02	0.00	<b>36</b>	0.01	<b>131</b>	< 0.02	< 0.03	<b>3.9</b>

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

The PEC/RAC ratios for fish (acute and prolonged) as well as for primary producers (algae and macrophytes) are below the trigger of 1, indicating acceptable risks. For invertebrates the risk assessment needs to be taken to FOCUS Step-4 level.

**Table 9.5-6: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for acetamiprid for each organism group based on FOCUS Steps 1, 2 and 3 calculations for the use of CA3573 in apples 1\*50 g a.s./ha - BBCH ≥ 62 (covering BBCH ≥ 69), late application**

Group		Fish acute	Fish prolonged	Invertebrates acute	Invertebrates prolonged	Sed. dwellers prolonged	Algae	Higher plant	Higher-tier information (aquatic invertebrates)
Test species		<i>Oncorhynchus mykiss</i>	<i>Pimephales promelas</i>	Geomean aquatic insects	<i>Daphnia magna</i>	<i>Chironomus riparius</i>	<i>Anabaena flos-aquae</i>	<i>Lemna gibba</i>	Mesocosm
Endpoint (µg/L)		LC <sub>50</sub> 15300	NOEC 9400	EC <sub>50</sub> 8.5	EC <sub>10</sub> 2960	EC <sub>10</sub> 0.235	ErC <sub>50</sub> > 1300	EC <sub>50</sub> > 1000	NOEC 1.6
AF		100	10	100	10	10	10	10	2
RAC (µg/L)		153	940	0.085	296	0.0235	>130	>100	0.8
Scenario	PEC <sub>gl-max</sub> (µg/L)								
<b>Step 1</b>									
	19.5	0.13	0.02	<b>229</b>	0.07	<b>830</b>	< 0.15	< 0.20	<b>24</b>
<b>Step 2</b>									
N-Europe	4.87	0.03	0.01	<b>57</b>	0.02	<b>207</b>	< 0.04	< 0.05	<b>6.1</b>
S-Europe	4.87	0.03	0.01	<b>57</b>	0.02	<b>207</b>	< 0.04	< 0.05	<b>6.1</b>
<b>Step 3</b>									
D3/Ditch	1.84	0.01	0.00	<b>22</b>	0.01	<b>78</b>	< 0.01	< 0.02	<b>2.3</b>
D4/Pond	0.082	0.00	0.00	0.96	0.00	<b>3.5</b>	< 0.0006	< 0.0008	0.10
D4/Stream	1.80	0.01	0.00	<b>21</b>	0.01	<b>77</b>	< 0.01	< 0.02	<b>2.3</b>
D5/Pond	0.082	0.00	0.00	0.96	0.00	<b>3.5</b>	< 0.0006	< 0.0008	0.10
D5/Stream	1.99	0.01	0.00	<b>23</b>	0.01	<b>85</b>	< 0.02	< 0.02	<b>2.5</b>
R1/Pond	0.082	0.00	0.00	0.96	0.00	<b>3.5</b>	< 0.0006	< 0.0008	0.10
R1/Stream	1.41	0.01	0.00	<b>17</b>	0.00	<b>60</b>	< 0.01	< 0.01	<b>1.8</b>
R2/Stream	1.89	0.01	0.00	<b>22</b>	0.01	<b>80</b>	< 0.01	< 0.02	<b>2.4</b>
R3/Stream	1.99	0.01	0.00	<b>23</b>	0.01	<b>85</b>	< 0.02	< 0.02	<b>2.5</b>
R4/Stream	1.41	0.01	0.00	<b>17</b>	0.00	<b>60</b>	< 0.01	< 0.01	<b>1.8</b>

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

The PEC/RAC ratios for fish (acute and prolonged) as well as for primary producers (algae and macrophytes) are below the trigger of 1, indicating acceptable risks. For invertebrates the risk assessment needs to be taken to FOCUS Step-4 level.

**Table 9.5-7: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for acetamiprid for each organism group based on FOCUS Steps 1, 2 and 3 calculations for the use of CA3573 in winter oilseed rape 1\*60 g a.s./ha late (covering early) application**

Group		Fish acute	Fish prolonged	Invertebrates acute	Invertebrates prolonged	Sed. dwellers prolonged	Algae	Higher plant	Higher-tier information (aquatic invertebrates)
Test species		<i>Oncorhynchus mykiss</i>	<i>Pimephales promelas</i>	Geomean aquatic insects	<i>Daphnia magna</i>	<i>Chironomus riparius</i>	<i>Anabaena flos-aquae</i>	<i>Lemna gibba</i>	Mesocosm
Endpoint (µg/L)		LC <sub>50</sub> 15300	NOEC 9400	EC <sub>50</sub> 8.5	EC <sub>10</sub> 2960	EC <sub>10</sub> 0.235	ErC <sub>50</sub> > 1300	EC <sub>50</sub> > 1000	NOEC 1.6
AF		100	10	100	10	10	10	10	2
RAC (µg/L)		153	940	0.085	296	0.0235	> 130	> 100	0.8
Scenario	PEC <sub>gl-max</sub> (µg/L)								
<b>Step 1</b>									
	18.1	0.12	0.02	<b>213</b>	0.06	<b>770</b>	< 0.14	< 0.18	<b>23</b>
<b>Step 2</b>									
N-Europe <sup>1</sup>	0.843	0.01	0.00	<b>9.9</b>	0.00	<b>36</b>	< 0.01	< 0.01	<b>1.1</b>
S-Europe <sup>1</sup>	0.765	0.01	0.00	<b>9.0</b>	0.00	<b>33</b>	< 0.01	< 0.01	0.96
<b>Step 3</b>									
D2/Ditch	0.385	0.00	0.00	<b>4.5</b>	0.00	<b>16</b>	< 0.003	< 0.004	0.48
D2/Stream	0.343	0.00	0.00	4.04	0.00	<b>15</b>	< 0.003	< 0.003	0.43
D3/Ditch	0.381	0.00	0.00	<b>4.5</b>	0.00	<b>16</b>	< 0.003	< 0.004	0.48
D4/Pond	0.013	0.00	0.00	0.15	0.00	0.55	< 0.0001	< 0.0001	0.02
D4/Stream	0.320	0.00	0.00	<b>3.8</b>	0.00	<b>14</b>	< 0.002	< 0.003	0.40
D5/Pond	0.013	0.00	0.00	0.15	0.00	0.55	< 0.0001	< 0.0001	0.02
D5/Stream	0.355	0.00	0.00	<b>4.2</b>	0.00	<b>15</b>	< 0.003	< 0.004	0.44
R1/Pond	0.058	0.00	0.00	0.68	0.00	<b>2.5</b>	< 0.0004	< 0.001	0.07
R1/Stream	0.930	0.01	0.00	<b>11</b>	0.00	<b>40</b>	< 0.01	< 0.01	<b>1.2</b>
R3/Stream	0.715	0.00	0.00	<b>8.4</b>	0.00	<b>30</b>	< 0.01	< 0.01	0.89

<sup>1</sup> worst-case value

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

The PEC/RAC ratios for fish (acute and prolonged) as well as for primary producers (algae and macrophytes) are below the trigger of 1, indicating acceptable risks. Considering the higher-tier information, the PEC/RAC ratios on FOCUS Step-3 level are also acceptable for invertebrates as all values are below 1 except for R1 stream, where the trigger is slightly not met (PEC/RAC = 1.2).

**Table 9.5-8: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for acetamiprid for each organism group based on FOCUS Steps 1, 2 and 3 calculations for the use of CA3573 in spring oilseed rape 1\*60 g a.s./ha**

Group		Fish acute	Fish prolonged	Invertebrates acute	Invertebrates prolonged	Sed. dwellers prolonged	Algae	Higher plant	Higher-tier information (aquatic invertebrates)
Test species		<i>Oncorhynchus mykiss</i>	<i>Pimephales promelas</i>	Geomean aquatic insects	<i>Daphnia magna</i>	<i>Chironomus riparius</i>	<i>Anabaena flos-aquae</i>	<i>Lemna gibba</i>	Mesocosm
Endpoint (µg/L)		LC <sub>50</sub> 15300	NOEC 9400	EC <sub>50</sub> 8.5	EC <sub>10</sub> 2960	EC <sub>10</sub> 0.235	ErC <sub>50</sub> > 1300	EC <sub>50</sub> > 1000	NOEC 1.6
AF		100	10	100	10	10	10	10	2
RAC (µg/L)		153	940	0.085	296	0.0235	> 130	> 100	0.8
Scenario	PEC <sub>gl-max</sub> (µg/L)								
<b>Step 1</b>									
	18.1	0.12	0.02	<b>213</b>	0.06	<b>770</b>	< 0.14	0.18	<b>23</b>
<b>Step 2</b>									
N-Europe <sup>1</sup>	0.843	0.01	0.00	<b>9.9</b>	0.00	<b>36</b>	< 0.01	0.01	<b>1.1</b>
S-Europe <sup>1</sup>	0.765	0.01	0.00	<b>9.0</b>	0.00	<b>33</b>	< 0.01	0.01	0.96
<b>Step 3</b>									
D1/Ditch	0.388	0.00	0.00	<b>4.6</b>	0.00	<b>17</b>	< 0.003	< 0.004	0.49
D1/Stream	0.337	0.00	0.00	<b>4.0</b>	0.00	<b>14</b>	< 0.003	< 0.003	0.42
D3/Ditch	0.381	0.00	0.00	<b>4.5</b>	0.00	<b>16</b>	< 0.003	< 0.004	0.48
D4/Pond	0.013	0.00	0.00	0.15	0.00	0.55	< 0.0001	< 0.0001	0.02
D4/Stream	0.312	0.00	0.00	<b>3.7</b>	0.00	<b>13</b>	< 0.002	< 0.003	0.39
D5/Pond	0.013	0.00	0.00	0.15	0.00	0.55	< 0.0001	< 0.0001	0.02
D5/Stream	0.331	0.00	0.00	<b>3.9</b>	0.00	<b>14</b>	< 0.003	< 0.003	0.41
R1/Pond	0.043	0.00	0.00	0.51	0.00	<b>1.8</b>	< 0.0003	< 0.0004	0.05
R1/Stream	0.765	0.01	0.00	<b>9.0</b>	0.00	<b>33</b>	< 0.006	< 0.008	0.96

<sup>1</sup> worst-case value

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

The PEC/RAC ratios for fish (acute and prolonged) as well as for primary producers (algae and macrophytes) are below the trigger of 1, indicating acceptable risks. Considering the higher-tier information, the PEC/RAC ratios on FOCUS Step-3 level are also acceptable for invertebrates as all values are below 1.

**Table 9.5-9: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for acetamiprid for each organism group based on FOCUS Steps 1, 2 and 3 calculations for the use of CA3573 in potato 1\*36 g/ha – early application**

Group		Fish acute	Fish prolonged	Invertebrates acute	Invertebrates prolonged	Sed. dwellers prolonged	Algae	Higher plant	Higher-tier information
Test species		<i>Oncorhynchus mykiss</i>	<i>Pimephales promelas</i>	Geomean aquatic insects	<i>Daphnia magna</i>	<i>Chironomus riparius</i>	<i>Anabaena flos-aquae</i>	<i>Lemna gibba</i>	Mesocosm (aquatic invertebrates)
Endpoint (µg/L)		LC <sub>50</sub> 15300	NOEC 9400	EC <sub>50</sub> 8.5	EC <sub>10</sub> 2960	EC <sub>10</sub> 0.235	ErC <sub>50</sub> > 1300	EC <sub>50</sub> > 1000	NOEC 1.6
AF		100	10	100	10	10	10	10	2
RAC (µg/L)		153	940	0.085	296	0.0235	> 130	> 100	0.8
Scenario	PEC <sub>gl-max</sub> (µg/L)								
<b>Step 1</b>									
	10.8	0.07	0.01	<b>127</b>	0.04	<b>460</b>	< 0.08	< 0.11	<b>14</b>
<b>Step 2</b>									
N-Europe <sup>1</sup>	0.933	0.01	0.00	<b>11</b>	0.00	<b>40</b>	< 0.01	< 0.01	<b>1.2</b>
S-Europe <sup>1</sup>	0.801	0.01	0.00	<b>9.4</b>	0.00	<b>34</b>	< 0.01	< 0.01	<b>1.0</b>
<b>Step 3</b>									
D3/Ditch	0.189	0.00	0.00	<b>2.2</b>	0.00	<b>8.0</b>	< 0.001	< 0.002	0.24
D4/Pond	0.008	0.00	0.00	0.09	0.00	0.34	< 0.0001	< 0.0001	0.01
D4/Stream	0.161	0.00	0.00	<b>1.9</b>	0.00	<b>6.9</b>	< 0.001	< 0.002	0.20
D6/Ditch 1 <sup>st</sup>	0.186	0.00	0.00	<b>2.2</b>	0.00	<b>7.9</b>	< 0.001	< 0.002	0.23
D6/Ditch 2 <sup>nd</sup>	0.185	0.00	0.00	<b>2.2</b>	0.00	<b>7.9</b>	< 0.001	< 0.002	0.23
R1/Pond	0.010	0.00	0.00	0.12	0.00	0.43	< 0.0001	< 0.0001	0.01
R1/Stream	0.165	0.00	0.00	<b>1.9</b>	0.00	<b>7.0</b>	< 0.001	< 0.002	0.21
R2/Stream	0.173	0.00	0.00	<b>2.0</b>	0.00	<b>7.4</b>	< 0.001	< 0.002	0.22
R3/Stream	0.209	0.00	0.00	<b>2.5</b>	0.00	<b>8.9</b>	< 0.002	< 0.002	0.26

<sup>1</sup> worst-case value

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

The PEC/RAC ratios for fish (acute and prolonged) as well as for primary producers (algae and macrophytes) are below the trigger of 1, indicating acceptable risks. Considering the higher-tier information, the PEC/RAC ratios on FOCUS Step-3 level are also acceptable for invertebrates as all values are below 1.



**Table 9.5-10: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for acetamiprid for each organism group based on FOCUS Steps 1, 2 and 3 calculations for the use of CA3573 in potato 1\*36 g/ha – late application**

Group		Fish acute	Fish prolonged	Invertebrates acute	Invertebrates prolonged	Sed. dwellers prolonged	Algae	Higher plant	Higher-tier information
Test species		<i>Oncorhynchus mykiss</i>	<i>Pimephales promelas</i>	Geomean aquatic insects	<i>Daphnia magna</i>	<i>Chironomus riparius</i>	<i>Anabaena flos-aquae</i>	<i>Lemna gibba</i>	Mesocosm (aquatic invertebrates)
Endpoint (µg/L)		LC <sub>50</sub> 15300	NOEC 9400	EC <sub>50</sub> 8.5	EC <sub>10</sub> 2960	EC <sub>10</sub> 0.235	ErC <sub>50</sub> > 1300	EC <sub>50</sub> > 1000	NOEC 1.6
AF		100	10	100	10	10	10	10	2
RAC (µg/L)		153	940	0.085	296	0.0235	> 130	> 100	0.8
Scenario	PEC <sub>gl-max</sub> (µg/L)								
<b>Step 1</b>									
	10.8	0.07	0.01	<b>127</b>	0.04	<b>460</b>	< 0.08	< 0.11	<b>14</b>
<b>Step 2</b>									
N-Europe <sup>1</sup>	0.933	0.01	0.00	<b>11</b>	0.00	<b>40</b>	< 0.01	< 0.01	<b>1.2</b>
S-Europe <sup>1</sup>	0.801	0.01	0.00	<b>9.4</b>	0.00	<b>34</b>	< 0.01	< 0.01	<b>1.0</b>
<b>Step 3</b>									
D3/Ditch	0.189	0.00	0.00	<b>2.2</b>	0.00	<b>8.0</b>	< 0.001	< 0.002	0.24
D4/Pond	0.008	0.00	0.00	0.09	0.00	0.34	< 0.0001	< 0.0001	0.01
D4/Stream	0.142	0.00	0.00	<b>1.7</b>	0.00	<b>6.0</b>	< 0.001	< 0.001	0.18
D6/Ditch 1 <sup>st</sup>	0.188	0.00	0.00	<b>2.2</b>	0.00	<b>8.0</b>	< 0.001	< 0.002	0.24
D6/Ditch 2 <sup>nd</sup>	0.189	0.00	0.00	<b>2.2</b>	0.00	<b>8.0</b>	< 0.001	< 0.002	0.24
R1/Pond	0.024	0.00	0.00	0.28	0.00	<b>1.0</b>	< 0.0002	< 0.0002	0.03
R1/Stream	0.408	0.00	0.00	<b>4.8</b>	0.00	<b>17</b>	< 0.003	< 0.004	0.51
R2/Stream	0.176	0.00	0.00	<b>2.1</b>	0.00	<b>7.5</b>	< 0.001	< 0.002	0.22
R3/Stream	0.185	0.00	0.00	<b>2.2</b>	0.00	<b>7.9</b>	< 0.001	< 0.002	0.23

<sup>1</sup> worst-case value

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

The PEC/RAC ratios for fish (acute and prolonged) as well as for primary producers (algae and macrophytes) are below the trigger of 1, indicating acceptable risks. Considering the higher-tier information, the PEC/RAC ratios on FOCUS Step-3 level are also acceptable for invertebrates as all values are below 1.

**Table 9.5-11: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for acetamiprid for each organism group based on FOCUS Steps 1, 2 and 3 calculations for the use of CA3573 in corn 1\*60 g/ha**

Group		Fish acute	Fish prolonged	Invertebrates acute	Invertebrates prolonged	Sed. dwellers prolonged	Algae	Higher plant	Higher-tier information
Test species		<i>Oncorhynchus mykiss</i>	<i>Pimephales promelas</i>	Geomean aquatic insects	<i>Daphnia magna</i>	<i>Chironomus riparius</i>	<i>Anabaena flos-aquae</i>	<i>Lemna gibba</i>	Mesocosm (aquatic invertebrates)
Endpoint (µg/L)		LC <sub>50</sub> 15300	NOEC 9400	EC <sub>50</sub> 8.5	EC <sub>10</sub> 2960	EC <sub>10</sub> 0.235	ErC <sub>50</sub> > 1300	EC <sub>50</sub> > 1000	NOEC 1.6
AF		100	10	100	10	10	10	10	2
RAC (µg/L)		153	940	0.085	296	0.0235	> 130	> 100	0.8
Scenario	PEC <sub>gl-max</sub> (µg/L)								
<b>Step 1</b>									
	18.1	0.12	0.02	<b>213</b>	0.06	<b>770</b>	< 0.14	< 0.18	<b>23</b>
<b>Step 2</b>									
N-Europe <sup>1</sup>	0.778	0.01	0.00	<b>9.2</b>	0.00	<b>33</b>	< 0.01	< 0.01	0.97
S-Europe <sup>1</sup>	0.714	0.00	0.00	<b>8.4</b>	0.00	<b>30</b>	< 0.01	< 0.01	0.89
<b>Step 3</b>									
D3/Ditch	0.315	0.00	0.00	<b>3.7</b>	0.00	<b>13</b>	< 0.002	< 0.003	0.39
D4/Pond	0.013	0.00	0.00	0.15	0.00	0.55	< 0.0001	< 0.0001	0.02
D4/Stream	0.282	0.00	0.00	<b>3.3</b>	0.00	<b>12</b>	< 0.002	< 0.003	0.35
D5/Pond	0.013	0.00	0.00	0.15	0.00	0.55	< 0.0001	< 0.0001	0.02
D5/Stream	0.308	0.00	0.00	<b>3.6</b>	0.00	<b>13</b>	< 0.002	< 0.003	0.39
D6/Ditch	0.310	0.00	0.00	<b>3.6</b>	0.00	<b>13</b>	< 0.002	< 0.003	0.39
R1/Pond	0.033	0.00	0.00	0.39	0.00	<b>1.4</b>	< 0.0003	< 0.0003	0.04
R1/Stream	0.535	0.00	0.00	<b>6.3</b>	0.00	<b>23</b>	< 0.004	< 0.005	0.67
R2/Stream	0.293	0.00	0.00	<b>3.4</b>	0.00	<b>12</b>	< 0.002	< 0.003	0.37
R3/Stream	0.308	0.00	0.00	<b>3.6</b>	0.00	<b>13</b>	< 0.002	< 0.003	0.39
R4/Stream	0.213	0.00	0.00	<b>2.5</b>	0.00	<b>9.1</b>	< 0.002	< 0.002	0.27

<sup>1</sup> worst-case value

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

The PEC/RAC ratios for fish (acute and prolonged) as well as for primary producers (algae and macrophytes) are below the trigger of 1, indicating acceptable risks. Considering the higher-tier information, the PEC/RAC ratios on FOCUS Step-3 level are also acceptable for invertebrates as all values are below 1.

## Relevant metabolites of acetamiprid (FOCUS Step 1 and 2)

**Table 9.5-12:** Aquatic organisms: acceptability of risk (PEC/RAC < 1) for the metabolites IM-1-2 and IM-1-4 for each organism group based on FOCUS Steps 1 and 2 calculations for the use of CA3573 in apples 1\*50 g a.s./ha - BBCH ≥ 62 or ≥ 69 (covering 1\*25 g a.s./ha)

Metabolite	IM-1-2			IM-1-4				
Group		Invertebrates acute	Sed. dwellers acute		Fish acute	Invertebrates acute	Invertebrates acute	Sed. dwellers acute
Test species		<i>Daphnia magna</i>	<i>Chironomus riparius</i>		<i>Oncorhynchus mykiss</i>	<i>Daphnia magna</i>	<i>Mysidopsis bahia</i>	<i>Chironomus riparius</i>
Endpoint (µg/L)		EC <sub>50</sub> > 99800	EC <sub>50</sub> 15000		LC <sub>50</sub> > 98100	EC <sub>50</sub> 43900	EC <sub>50</sub> 19000	EC <sub>50</sub> 76000
AF		100	100		100	100	100	100
RAC (µg/L)		> 998	150		> 981	439	190	760
Scenario	PEC <sub>gl-max</sub> (µg/L)			PEC <sub>gl-max</sub> (µg/L)				
<b>Step 1</b>								
	12.2	< 0.01	0.08	17.4	< 0.02	0.04	0.09	0.02
<b>Step 2</b>								
N-Europe <sup>1</sup>	1.04	< 0.001	0.01	3.61	< 0.004	0.01	0.02	0.00
S-Europe <sup>1</sup>	0.969	< 0.001	0.01	3.37	< 0.003	0.01	0.02	0.00

<sup>1</sup> worst-case value

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

**Table 9.5-13: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for the metabolites IM-1-5, IC-0 and IB-1-1 for each organism group based on FOCUS Steps 1 and 2 calculations for the use of CA3573 in apples 1\*50 g a.s./ha - BBCH ≥ 62 or ≥ 69 (covering 1\*25 g a.s./ha)**

Metabolite	IM-1-5				IC-0			IB-1-1		
Group		Invertebrates acute	Sed. dwellers acute	Sed. dwellers prolonged		Invertebrates acute	Sed. dwellers acute		Invertebrates acute	Sed. dwellers acute
Test species		<i>Daphnia magna</i>	<i>Chironomus riparius</i>	<i>Chironomus riparius</i>		<i>Daphnia magna</i>	<i>Chironomus riparius</i>		<i>Daphnia magna</i>	<i>Chironomus riparius</i>
Endpoint (µg/L)		EC <sub>50</sub> 25000	EC <sub>50</sub> 68000	EC <sub>10</sub> 26000		EC <sub>50</sub> > 95100	EC <sub>50</sub> > 100000		EC <sub>50</sub> > 100800	EC <sub>50</sub> > 100000
AF		100	100	10		100	100		100	100
RAC (µg/L)		250	680	2600		> 951	> 1000		> 1008	> 1000
Scenario	PEC <sub>gl-max</sub> (µg/L)				PEC <sub>gl-max</sub> (µg/L)			PEC <sub>gl-max</sub> (µg/L)		
<b>Step 1</b>										
	2.06	0.01	0.00	0.00	5.15	< 0.005	< 0.005	6.90	< 0.007	< 0.007
<b>Step 2</b>										
N-Europe <sup>1</sup>	0.359	0.00	0.00	0.00	1.06	< 0.001	< 0.001	1.69	< 0.002	< 0.002
S-Europe <sup>1</sup>	0.287	0.00	0.00	0.00	1.03	< 0.001	< 0.001	1.67	< 0.002	< 0.002

<sup>1</sup> worst-case value

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

**Table 9.5-14: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for the metabolites IM-1-2 and IM-1-4 for each organism group based on FOCUS Steps 1 and 2 calculations for the use of CA3573 in winter (covering spring) oilseed rape 1\*60 g a.s./ha**

Metabolite	IM-1-2			IM-1-4				
Group		Invertebrates acute	Sed. dwellers acute		Fish acute	Invertebrates acute	Invertebrates acute	Sed. dwellers acute
Test species		<i>Daphnia magna</i>	<i>Chironomus riparius</i>		<i>Oncorhynchus mykiss</i>	<i>Daphnia magna</i>	<i>Mysidopsis bahia</i>	<i>Chironomus riparius</i>
Endpoint (µg/L)		EC <sub>50</sub> > 99800	EC <sub>50</sub> 15000		LC <sub>50</sub> > 98100	EC <sub>50</sub> 43900	EC <sub>50</sub> 19000	EC <sub>50</sub> 76000
AF		100	100		100	100	100	100
RAC (µg/L)		> 998	150		> 981	439	190	760
Scenario	PEC <sub>gl-max</sub> (µg/L)			PEC <sub>gl-max</sub> (µg/L)				
<b>Step 1</b>								
	13.9	< 0.01	0.09	17.9	< 0.02	0.04	0.09	0.02
<b>Step 2</b>								
N-Europe <sup>1</sup>	0.461	< 0.0005	0.00	1.50	< 0.002	0.00	0.01	0.00
S-Europe <sup>1</sup>	0.384	< 0.0004	0.00	1.26	< 0.001	0.00	0.01	0.00

<sup>1</sup> worst-case value

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

**Table 9.5-15: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for the metabolites IM-1-5, IC-0 and IB-1-1 for each organism group based on FOCUS Steps 1 and 2 calculations for the use of CA3573 in winter (covering spring) oilseed rape 1\*60 g a.s./ha**

Metabolite	IM-1-5				IC-0			IB-1-1		
Group		Invertebrates acute	Sed. dwellers acute	Sed. dwellers prolonged		Invertebrates acute	Sed. dwellers acute		Invertebrates acute	Sed. dwellers acute
Test species		<i>Daphnia magna</i>	<i>Chironomus riparius</i>	<i>Chironomus riparius</i>		<i>Daphnia magna</i>	<i>Chironomus riparius</i>		<i>Daphnia magna</i>	<i>Chironomus riparius</i>
Endpoint (µg/L)		EC <sub>50</sub> 25000	EC <sub>50</sub> 68000	EC <sub>10</sub> 26000		EC <sub>50</sub> > 95100	EC <sub>50</sub> > 100000		EC <sub>50</sub> > 100800	EC <sub>50</sub> > 100000
AF		100	100	10		100	100		100	100
RAC (µg/L)		250	680	2600		> 951	> 1000		> 1008	> 1000
Scenario	PEC <sub>gl-max</sub> (µg/L)				PEC <sub>gl-max</sub> (µg/L)			PEC <sub>gl-max</sub> (µg/L)		
<b>Step 1</b>										
	2.47	0.01	0.00	0.00	5.07	< 0.005	< 0.005	6.59	< 0.007	< 0.007
<b>Step 2</b>										
N-Europe <sup>1</sup>	0.369	0.00	0.00	0.00	0.257	< 0.0003	< 0.0003	0.319	< 0.0003	< 0.0003
S-Europe <sup>1</sup>	0.295	0.00	0.00	0.00	0.226	< 0.0002	< 0.0002	0.290	< 0.0003	< 0.0003

<sup>1</sup> worst-case value

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

**Table 9.5-16: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for the metabolites IM-1-2 and IM-1-4 for each organism group based on FOCUS Steps 1 and 2 calculations for the use of CA3573 in potato 1\*36 g a.s./ha**

Metabolite	IM-1-2			IM-1-4				
Group		Invertebrates acute	Sed. dwellers acute		Fish acute	Invertebrates acute	Invertebrates acute	Sed. dwellers acute
Test species		<i>Daphnia magna</i>	<i>Chironomus riparius</i>		<i>Oncorhynchus mykiss</i>	<i>Daphnia magna</i>	<i>Mysidopsis bahia</i>	<i>Chironomus riparius</i>
Endpoint (µg/L)		EC <sub>50</sub> > 99800	EC <sub>50</sub> 15000		LC <sub>50</sub> > 98100	EC <sub>50</sub> 43900	EC <sub>50</sub> 19000	EC <sub>50</sub> 76000
AF		100	100		100	100	100	100
RAC (µg/L)		> 998	150		> 981	439	190	760
Scenario	PEC <sub>gl-max</sub> (µg/L)			PEC <sub>gl-max</sub> (µg/L)				
<b>Step 1</b>								
	8.31	< 0.01	0.06	10.7	< 0.01	0.02	0.06	0.01
<b>Step 2</b>								
N-Europe <sup>1</sup>	0.700	< 0.001	0.00	2.25	< 0.002	0.01	0.01	0.00
S-Europe <sup>1</sup>	0.569	< 0.001	0.00	1.83	< 0.002	0.00	0.01	0.00

<sup>1</sup> worst-case value

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

**Table 9.5-17: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for the metabolites IM-1-5, IC-0 and IB-1-1 for each organism group based on FOCUS Steps 1 and 2 calculations for the use of CA3573 in potato 1\*36 g a.s./ha**

Metabolite	IM-1-5				IC-0			IB-1-1		
Group		Invertebrates acute	Sed. dwellers acute	Sed. dwellers prolonged		Invertebrates acute	Sed. dwellers acute		Invertebrates acute	Sed. dwellers acute
Test species		<i>Daphnia magna</i>	<i>Chironomus riparius</i>	<i>Chironomus riparius</i>		<i>Daphnia magna</i>	<i>Chironomus riparius</i>		<i>Daphnia magna</i>	<i>Chironomus riparius</i>
Endpoint (µg/L)		EC <sub>50</sub> 25000	EC <sub>50</sub> 68000	EC <sub>10</sub> 26000		EC <sub>50</sub> > 95100	EC <sub>50</sub> > 100000		EC <sub>50</sub> > 100800	EC <sub>50</sub> > 100000
AF		100	100	10		100	100		100	100
RAC (µg/L)		250	680	2600		> 951	> 1000		> 1008	> 1000
Scenario	PEC <sub>gl-max</sub> (µg/L)				PEC <sub>gl-max</sub> (µg/L)			PEC <sub>gl-max</sub> (µg/L)		
<b>Step 1</b>										
	1.48	0.01	0.00	0.00	3.04	0.003	0.003	3.95	0.004	0.004
<b>Step 2</b>										
N-Europe <sup>1</sup>	<b>0.627</b> <del>0.308</del>	0.00	0.00	0.00	0.323	< 0.0003	< 0.0003	0.347	< 0.0003	< 0.0003
S-Europe <sup>1</sup>	<b>0.502</b> <del>0.246</del>	0.00	0.00	0.00	0.271	< 0.0003	< 0.0003	0.299	< 0.0003	< 0.0003

<sup>1</sup> worst-case value

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



**Table 9.5-18: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for the metabolites IM-1-2 and IM-1-4 for each organism group based on FOCUS Steps 1 and 2 calculations for the use of CA3573 in corn 1\*60 g a.s./ha**

Metabolite	IM-1-2			IM-1-4				
Group		Invertebrates acute	Sed. dwellers acute		Fish acute	Invertebrates acute	Invertebrates acute	Sed. dwellers acute
Test species		<i>Daphnia magna</i>	<i>Chironomus riparius</i>		<i>Oncorhynchus mykiss</i>	<i>Daphnia magna</i>	<i>Mysidopsis bahia</i>	<i>Chironomus riparius</i>
Endpoint (µg/L)		EC <sub>50</sub> > 99800	EC <sub>50</sub> 15000		LC <sub>50</sub> > 98100	EC <sub>50</sub> 43900	EC <sub>50</sub> 19000	EC <sub>50</sub> 76000
AF		100	100		100	100	100	100
RAC (µg/L)		> 998	150		> 981	439	190	760
Scenario	PEC <sub>gl-max</sub> (µg/L)			PEC <sub>gl-max</sub> (µg/L)				
<b>Step 1</b>								
	13.9	< 0.01	0.09	17.9	< 0.02	0.04	0.09	0.02
<b>Step 2</b>								
N-Europe <sup>1</sup>	0.397	< 0.0004	0.00	1.30	< 0.001	0.00	0.01	0.00
S-Europe <sup>1</sup>	0.333	< 0.0003	0.00	1.09	< 0.001	0.00	0.01	0.00

<sup>1</sup> worst-case value

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

**Table 9.5-19: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for the metabolites IM-1-5, IC-0 and IB-1-1 for each organism group based on FOCUS Steps 1 and 2 calculations for the use of CA3573 in corn 1\*60 g a.s./ha**

Metabolite	IM-1-5				IC-0			IB-1-1		
Group		Invertebrates acute	Sed. dwellers acute	Sed. dwellers prolonged		Invertebrates acute	Sed. dwellers acute		Invertebrates acute	Sed. dwellers acute
Test species		<i>Daphnia magna</i>	<i>Chironomus riparius</i>	<i>Chironomus riparius</i>		<i>Daphnia magna</i>	<i>Chironomus riparius</i>		<i>Daphnia magna</i>	<i>Chironomus riparius</i>
Endpoint (µg/L)		EC <sub>50</sub> 25000	EC <sub>50</sub> 68000	EC <sub>10</sub> 26000		EC <sub>50</sub> > 95100	EC <sub>50</sub> > 100000		EC <sub>50</sub> > 100800	EC <sub>50</sub> > 100000
AF		100	100	10		100	100		100	100
RAC (µg/L)		250	680	2600		> 951	> 1000		> 1008	> 1000
Scenario	PEC <sub>gl-max</sub> (µg/L)				PEC <sub>gl-max</sub> (µg/L)			PEC <sub>gl-max</sub> (µg/L)		
<b>Step 1</b>										
	2.47	0.01	0.00	0.00	5.07	0.005	0.005	6.59	0.007	0.007
<b>Step 2</b>										
N-Europe <sup>1</sup>	0.308	0.00	0.00	0.00	0.231	< 0.0002	< 0.0002	<b>0.295</b> 0.224	<b>&lt;0.0003</b> <0.0002	<b>&lt;0.0003</b> <0.0002
S-Europe <sup>1</sup>	0.246	0.00	0.00	0.00	0.206	< 0.0002	< 0.0002	0.271	< 0.0003	< 0.0003

<sup>1</sup> worst-case value

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

The PEC/RAC ratios for all relevant aquatic metabolites are below the trigger of 1 for all intended uses already on FOCUS Step-1 level. Therefore, no unacceptable risks from the metabolites are expected when CA3573 is applied according to the GAP.

### Overall summary (FOCUS Step 1-3)

In summary, the risks to aquatic organisms from acetamiprid and its metabolites due to use in CA3573 for application in winter oil seed rape (apart from scenario R1 stream), spring oil seed rape, potatoes (early and late) and corn are acceptable on FOCUS Step 1-3 level. However, due to unacceptable risks to acetamiprid, the scenario R1 stream from the use in winter oil seed rape as well as the uses in apples (early and late; each at 1 x 25 and 1 x 50 g a.s./ha) will need to be refined by using mitigating measures. Therefore, further PEC/RAC ratios were calculated based on FOCUS Step 4 PEC<sub>sw</sub> considering reduced exposure of surface water bodies.

**zRMS comments:**

Aquatic risk assessment based on FOCUS Step 1-3 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. Some minor corrections were introduced in tables for metabolites with no impact on the outcome of the performed evaluation.

The risk to aquatic invertebrates from acetamiprid was refined using endpoint derived from mesocosm study performed with CA3573 and assessment factor of 2, which was agreed by the zRMS.

Based on performed evaluation acceptable acute and chronic risk from acetamiprid in CA3573 could be concluded for application in spring oilseed rape, potatoes and maize with no need for risk mitigation measures.

For remaining crops acceptable acute and chronic risk could be concluded for fish and primary producers. W, while for aquatic invertebrates following conclusions were derived:

1. **Apples at 25 and 50 g a.s./ha:** refinement required in all scenarios (D3, D4, D5, R1, R2,R3, R4).
2. **Winter oilseed rape at 60 g a.s./ha:** refinement required in scenario R1, while acceptable risk with no need for risk mitigation measures demonstrated in scenarios D2, D3, D4, D5 and R3.

Further assessment for acetamiprid based on Step 4 exposure estimates is presented below.

Acceptable risk with no need for risk mitigation measures could be concluded for all acetamiprid metabolites from all intended uses.

## Parent compound acetamiprid – FOCUS Step 4

**Table 9.5-20:** Aquatic organisms: PEC calculation and acceptability of risk (PEC/RAC < 1) for acetamiprid based on FOCUS Step 4 calculations and toxicity data for invertebrates with mitigation of spray drift and run-off for the use of CA3573 in apples 1\*25 g a.s./ha - BBCH ≥ 62 (covering BBCH ≥ 69), early application

<b>Intended use</b>		Apples, early application at BBCH ≥ 62 (covering BBCH ≥ 69) <sup>1</sup>					
<b>Active substance</b>		Acetamiprid					
<b>Application rate (g/ha)</b>		1 × 25 g a.s./ha					
<b>Nozzle reduction</b>	<b>No-spray buffer (m)</b>	FOCUS default buffer 3	5	10	10	15	20
	<b>Vegetated filter strip (m)</b>	-	-	-	10	-	-
<b>No nozzle reduction</b>							
None	D3/ditch	1.95	1.53	0.940	0.940	0.423	-
	D4/stream	2.07	1.77	1.09	1.09	0.490	-
	D5/stream	2.23	1.92	1.18	1.18	0.529 0.530	-
	R1/stream	1.58	1.36	0.835	0.835	0.376	-
	R2/stream	2.12	1.82	1.12	1.12	0.503	-
	R3/stream	2.23	1.92	1.18	1.18	0.529	-
	R4/stream	1.55	1.33	0.816	0.816	0.367	-
<b>RAC (µg/L):</b>		<b>PEC/RAC ratio</b>					
<b>0.8</b>							
None	D3/ditch	2.4	1.9	1.2	1.2	0.53	-
	D4/stream	2.6	2.2	1.4	1.4	0.61	-
	D5/stream	2.8	2.4	1.5	1.5	0.66	-
	R1/stream	2.0	1.7	1.0	1.0	0.47	-
	R2/stream	2.7	2.3	1.4	1.4	0.63	-
	R3/stream	2.8	2.4	1.5	1.5	0.66	-
	R4/stream	1.9	1.7	1.0	1.0	0.46	-
<b>50% nozzle reduction</b>							
50%	D3/ditch	0.974	0.765	0.470	-	-	-
	D4/stream	1.03	0.887	0.545	-	-	-
	D5/stream	1.12	0.958	0.588 0.589	-	-	-
	R1/stream	0.791	0.680	0.417	-	-	-
	R2/stream	1.06	0.911	0.559	-	-	-
	R3/stream	1.12	0.958	0.588	-	-	-
	R4/stream	0.773	0.664	0.408	-	-	-
<b>RAC (µg/L)</b>		<b>PEC/RAC ratio</b>					
<b>0.8</b>							
50%	D3/ditch	1.2	0.96	-	-	-	-
	D4/stream	1.3	1.1	0.68	-	-	-
	D5/stream	1.4	1.2	0.74	-	-	-
	R1/stream	0.99	-	-	-	-	-
	R2/stream	1.3	1.1	0.70	-	-	-
	R3/stream	1.4	1.2	0.74	-	-	-
	R4/stream	0.97	-	-	-	-	-
<b>75% nozzle reduction</b>							
75%	D3/ditch	0.487	-	-	-	-	-
	D4/stream	0.516	-	-	-	-	-
	D5/stream	0.558	-	-	-	-	-
	R1/stream	0.396	-	-	-	-	-
	R2/stream	0.530	-	-	-	-	-
	R3/stream	0.558	-	-	-	-	-
	R4/stream	0.387	-	-	-	-	-
<b>RAC (µg/L)</b>							

<b>Intended use</b>		Apples, early application at BBCH $\geq 62$ (covering BBCH $\geq 69$ ) <sup>1</sup>					
<b>Active substance</b>		Acetamiprid					
<b>Application rate (g/ha)</b>		1 x 25 g a.s./ha					
<b>Nozzle reduction</b>	<b>No-spray buffer (m)</b>	FOCUS default buffer 3	5	10	10	15	20
	<b>Vegetated filter strip (m)</b>	-	-	-	10	-	-
<b>0.8</b>		<b>PEC/RAC ratio</b>					
75%	D3/ditch	0.61	-	-	-	-	-
	D4/stream	0.65	-	-	-	-	-
	D5/stream	0.70	-	-	-	-	-
	R1/stream	0.50	-	-	-	-	-
	R2/stream	0.66	-	-	-	-	-
	R3/stream	0.70	-	-	-	-	-
	R4/stream	0.48	-	-	-	-	-

<sup>1</sup> worst-case value used

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

For the early application in apples (BBCH  $\geq 62$  or  $\geq 69$ ) with an intended use rate of 1 x 25 g a.s./ha, acceptable risk for aquatic organisms is expected when the following mitigating measures are considered depending on scenario:

- a standard drift buffer zone (3 m) with 75% drift reducing nozzles, *or*
- a 10 m drift buffer zone with 50% drift reducing nozzles, *or*
- a drift buffer zone of 15 m (without vegetated filter strip).

**Table 9.5-21: Aquatic organisms: PEC calculation and acceptability of risk (PEC/RAC < 1) for acetamiprid based on FOCUS Step 4 calculations and toxicity data for invertebrates with mitigation of spray drift and run-off for the use of CA3573 in apples 1\*25 g a.s./ha - BBCH ≥ 62 (covering BBCH ≥ 69), late application**

<b>Intended use</b>		Apples, late application at BBCH ≥ 62 (covering BBCH ≥ 69)					
<b>Active substance</b>		Acetamiprid					
<b>Application rate (g/ha)</b>		1 × 25 g a.s./ha					
<b>Nozzle reduction</b>	<b>No-spray buffer (m)</b>	FOCUS default buffer 3	5	10	10	20	20
	<b>Vegetated filter strip (m)</b>	-	-	-	10	-	20
<b>No nozzle reduction</b>							
None	D3/ditch	0.919	0.620	-	-	-	-
	D4/stream	0.901	0.704	-	-	-	-
	D5/stream	0.995	0.777	-	-	-	-
	R2/stream	0.946	0.738	-	-	-	-
	R3/stream	0.994	0.776	-	-	-	-
<b>RAC (µg/L)</b>		<b>PEC/RAC ratio</b>					
<b>0.8</b>							
None	D3/ditch	<b>1.1</b>	0.78	-	-	-	-
	D4/stream	<b>1.1</b>	0.88	-	-	-	-
	D5/stream	<b>1.2</b>	0.97	-	-	-	-
	R2/stream	<b>1.2</b>	0.92	-	-	-	-
	R3/stream	<b>1.2</b>	0.97	-	-	-	-
<b>50% nozzle reduction</b>							
50%	D3/ditch	0.460	-	-	-	-	-
	D4/stream	0.451	-	-	-	-	-
	D5/stream	0.498	-	-	-	-	-
	R2/stream	0.473	-	-	-	-	-
	R3/stream	0.497	-	-	-	-	-
<b>RAC (µg/L)</b>		<b>PEC/RAC ratio</b>					
<b>0.8</b>							
50%	D3/ditch	0.58	-	-	-	-	-
	D4/stream	0.56	-	-	-	-	-
	D5/stream	0.62	-	-	-	-	-
	R2/stream	0.59	-	-	-	-	-
	R3/stream	0.62	-	-	-	-	-

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

For the late application in apples (BBCH ≥ 62 or ≥ 69) with an intended use rate of 1 x 25 g a.s./ha, acceptable risk for aquatic organisms is expected when the following mitigating measures are considered depending on scenario:

- a standard drift buffer zone (3 m) with 50% drift reducing nozzles, *or*
- a drift buffer zone of 5 m.

**Table 9.5-22:** Aquatic organisms: PEC calculation and acceptability of risk (PEC/RAC < 1) for acetamiprid based on FOCUS Step 4 calculations and toxicity data for invertebrates with mitigation of spray drift and run-off for the use of CA3573 in apples 1\*50 g a.s./ha - BBCH ≥ 62 (covering BBCH ≥ 69), early application

<b>Intended use</b>		Apples, early application at BBCH ≥ 62 (covering BBCH ≥ 69)					
<b>Active substance</b>		Acetamiprid					
<b>Application rate (g/ha)</b>		1 × 50 g a.s./ha					
<b>Nozzle reduction</b>	<b>No-spray buffer (m)</b>	FOCUS default buffer 3	5	10	10	15	20
	<b>Vegetated filter strip (m)</b>	-	-	-	10	-	-
<b>No nozzle reduction</b>							
None	D3/ditch	3.90	3.06	1.88	1.88	0.845	0.430
	D4/stream	4.13	3.55	2.18	2.18	0.980	0.498
	D5/stream	4.46	3.83	2.35	2.35	1.06	0.538
	R1/stream	3.16	2.72	1.67	1.67	0.751	0.382
	R2/stream	4.24	3.65	2.24	2.24	1.01	0.512
	R3/stream	4.46	3.83	2.35	2.35	1.06	0.538
	R4/stream	3.09	2.66	1.63	1.63	0.734	0.373
<b>RAC (µg/L)</b>		<b>PEC/RAC ratio</b>					
<b>0.8</b>							
None	D3/ditch	<b>4.9</b>	<b>3.8</b>	<b>2.4</b>	<b>2.4</b>	<b>1.1</b>	0.54
	D4/stream	<b>5.2</b>	<b>4.4</b>	<b>2.7</b>	<b>2.7</b>	<b>1.2</b>	0.62
	D5/stream	<b>5.6</b>	<b>4.8</b>	<b>2.9</b>	<b>2.9</b>	<b>1.3</b>	0.67
	R1/stream	<b>4.0</b>	<b>3.4</b>	<b>2.1</b>	<b>2.1</b>	0.94	-
	R2/stream	<b>5.3</b>	<b>4.6</b>	<b>2.8</b>	<b>2.8</b>	<b>1.3</b>	0.64
	R3/stream	<b>5.6</b>	<b>4.8</b>	<b>2.9</b>	<b>2.9</b>	<b>1.3</b>	0.67
	R4/stream	<b>3.9</b>	<b>3.3</b>	<b>2.0</b>	<b>2.0</b>	0.92	-
<b>50% nozzle reduction</b>							
50%	D3/ditch	1.95	1.53	0.940	0.940	0.423	-
	D4/stream	2.07	1.77	1.09	1.09	0.490	-
	D5/stream	2.23	1.92	1.18	1.18	0.529	-
	R1/stream	1.58	1.36	0.835	0.835	0.376	-
	R2/stream	2.12	1.82	1.12	1.12	0.503	-
	R3/stream	2.23	1.92	1.18	1.18	0.529	-
	R4/stream	1.55	1.33	0.816	0.816	0.367	-
<b>RAC (µg/L)</b>		<b>PEC/RAC ratio</b>					
<b>0.8</b>							
50%	D3/ditch	<b>2.4</b>	<b>1.9</b>	<b>1.2</b>	<b>1.2</b>	0.53	-
	D4/stream	<b>2.6</b>	<b>2.2</b>	<b>1.4</b>	<b>1.4</b>	0.61	-
	D5/stream	<b>2.8</b>	<b>2.4</b>	<b>1.5</b>	<b>1.5</b>	0.66	-
	R1/stream	<b>2.0</b>	<b>1.7</b>	<b>1.0</b>	<b>1.0</b>	0.47	-
	R2/stream	<b>2.7</b>	<b>2.3</b>	<b>1.4</b>	<b>1.4</b>	0.63	-
	R3/stream	<b>2.8</b>	<b>2.4</b>	<b>1.5</b>	<b>1.5</b>	0.66	-
	R4/stream	<b>1.9</b>	<b>1.7</b>	<b>1.0</b>	<b>1.0</b>	0.46	-
<b>75% nozzle reduction</b>							
75%	D3/ditch	0.974	0.765	0.470	-	-	-
	D4/stream	1.03	0.887	0.545	-	-	-
	D5/stream	1.12	0.958	0.588	-	-	-
	R1/stream	0.791	0.680	0.417	-	-	-
	R2/stream	1.06	0.911	0.559	-	-	-
	R3/stream	1.12	0.958	0.588	-	-	-
	R4/stream	0.773	0.664	0.408	-	-	-
<b>RAC (µg/L)</b>		<b>PEC/RAC ratio</b>					
<b>0.8</b>							
75%	D3/ditch	<b>1.2</b>	0.96	-	-	-	-

<b>Intended use</b>		Apples, early application at BBCH $\geq 62$ (covering BBCH $\geq 69$ )					
<b>Active substance</b>		Acetamiprid					
<b>Application rate (g/ha)</b>		1 $\times$ 50 g a.s./ha					
<b>Nozzle reduction</b>	<b>No-spray buffer (m)</b>	FOCUS default buffer 3	5	10	10	15	20
	<b>Vegetated filter strip (m)</b>	-	-	-	10	-	-
	D4/stream	<b>1.3</b>	<b>1.1</b>	0.68	-	-	-
	D5/stream	<b>1.4</b>	<b>1.2</b>	0.74	-	-	-
	R1/stream	0.99	-	-	-	-	-
	R2/stream	<b>1.3</b>	<b>1.1</b>	0.70	-	-	-
	R3/stream	<b>1.4</b>	<b>1.2</b>	0.74	-	-	-
	R4/stream	0.97	-	-	-	-	-
<b>90% nozzle reduction</b>							
90%	D3/ditch	0.390	-	-	-	-	-
	D4/stream	0.413	-	-	-	-	-
	D5/stream	0.446	-	-	-	-	-
	R1/stream	0.316	-	-	-	-	-
	R2/stream	0.424	-	-	-	-	-
	R3/stream	0.446	-	-	-	-	-
	R4/stream	0.309	-	-	-	-	-
<b>RAC (<math>\mu\text{g/L}</math>)</b>							
<b>0.8</b>		<b>PEC/RAC ratio</b>					
90%	D3/ditch	0.49	-	-	-	-	-
	D4/stream	0.52	-	-	-	-	-
	D5/stream	0.56	-	-	-	-	-
	R1/stream	0.40	-	-	-	-	-
	R2/stream	0.53	-	-	-	-	-
	R3/stream	0.56	-	-	-	-	-
	R4/stream	0.39	-	-	-	-	-

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

For the early application in apples (BBCH  $\geq 62$  or  $\geq 69$ ) with an intended use rate of 1 x 50 g a.s./ha, acceptable risk for aquatic organisms is expected when the following mitigating measures are considered, depending on scenario:

- a standard drift buffer zone (3 m) with 90% drift reducing nozzles, *or*
- a 10 m drift buffer zone with 75% drift reducing nozzles, *or*
- a 15 drift drift buffer zone with 50% drift reducing nozzles, *or*
- a 20 m drift buffer zone (without vegetated filter strip).



**Table 9.5-23:** Aquatic organisms: PEC calculation and acceptability of risk (PEC/RAC < 1) for acetamiprid based on FOCUS Step 4 calculations and toxicity data for invertebrates with mitigation of spray drift and run-off for the use of CA3573 in apples 1\*50 g a.s./ha - BBCH ≥ 62 (covering BBCH ≥ 69), late application

<b>Intended use</b>		Apples, late application at BBCH ≥ 62 (covering BBCH ≥ 69)					
<b>Active substance</b>		Acetamiprid					
<b>Application rate (g/ha)</b>		1 × 50 g a.s./ha					
<b>Nozzle reduction</b>	<b>No-spray buffer (m)</b>	FOCUS default buffer 3	5	10	10	20	20
	<b>Vegetated filter strip (m)</b>	-	-	-	10	-	20
<b>No nozzle reduction</b>							
None	D3/ditch	1.84	1.24	0.554	0.554	0.171	0.171
	D4/stream	1.80	1.41	0.629	0.629	0.194	0.194
	D5/stream	1.99	1.55	0.694	0.694	0.214	0.214
	R1/stream	1.41	1.10	0.492	0.492	0.152	0.152
	R2/stream	1.89	1.48	0.660	0.660	0.204	0.204
	R3/stream	1.99	1.55	0.694	0.694	0.214	0.214
	R4/stream	1.41	1.10	0.492	0.492	0.152	0.152
<b>RAC (µg/L)</b>		<b>PEC/RAC ratio</b>					
None	D3/ditch	2.3	1.6	0.69	-	-	-
	D4/stream	2.3	1.8	0.79	-	-	-
	D5/stream	2.5	1.9	0.87	-	-	-
	R1/stream	1.8	1.4	0.62	-	-	-
	R2/stream	2.4	1.9	0.83	-	-	-
	R3/stream	2.5	1.9	0.87	-	-	-
	R4/stream	1.8	1.4	0.62	-	-	-
<b>50% nozzle reduction</b>							
None	D3/ditch	0.919	0.620	-	-	-	-
	D4/stream	0.902	0.704	-	-	-	-
	D5/stream	0.995	0.777	-	-	-	-
	R1/stream	0.706	0.551	-	-	-	-
	R2/stream	0.946	0.738	-	-	-	-
	R3/stream	0.995	0.776	-	-	-	-
	R4/stream	0.706	0.551	-	-	-	-
<b>RAC (µg/L)</b>		<b>PEC/RAC ratio</b>					
None	D3/ditch	1.1	0.78	-	-	-	-
	D4/stream	1.1	0.88	-	-	-	-
	D5/stream	1.2	0.97	-	-	-	-
	R1/stream	0.88	-	-	-	-	-
	R2/stream	1.2	0.92	-	-	-	-
	R3/stream	1.2	0.97	-	-	-	-
	R4/stream	0.88	-	-	-	-	-
<b>75% nozzle reduction</b>							
None	D3/ditch	0.460	-	-	-	-	-
	D4/stream	0.451	-	-	-	-	-
	D5/stream	0.498	-	-	-	-	-
	R1/stream	0.353	-	-	-	-	-
	R2/stream	0.473	-	-	-	-	-
	R3/stream	0.497	-	-	-	-	-
	R4/stream	0.353	-	-	-	-	-
<b>RAC (µg/L)</b>		<b>PEC/RAC ratio</b>					
None	D3/ditch	0.58	-	-	-	-	-

<b>Intended use</b>		Apples, late application at BBCH $\geq 62$ (covering BBCH $\geq 69$ )					
<b>Active substance</b>		Acetamiprid					
<b>Application rate (g/ha)</b>		1 x 50 g a.s./ha					
<b>Nozzle reduction</b>	<b>No-spray buffer (m)</b>	FOCUS default buffer	5	10	10	20	20
	<b>Vegetated filter strip (m)</b>	-	-	-	10	-	20
	D4/stream	0.56	-	-	-	-	-
	D5/stream	0.62	-	-	-	-	-
	R1/stream	0.44	-	-	-	-	-
	R2/stream	0.59	-	-	-	-	-
	R3/stream	0.62	-	-	-	-	-
	R4/stream	0.44	-	-	-	-	-

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

For the late application in apples (BBCH  $\geq 62$  or  $\geq 69$ ) with an intended use rate of 1 x 50 g a.s./ha, acceptable risk for aquatic organisms is expected when the following mitigating measures are considered, depending on scenario:

- a standard drift buffer zone (3 m) with 75% drift reducing nozzles, *or*
- a 5 m drift buffer zone with 50% drift reducing nozzles, *or*
- a 10 m drift buffer zone (without vegetated filter strip).

**Table 9.5-24:** Aquatic organisms: PEC calculation and acceptability of risk (PEC/RAC < 1) for acetamiprid based on FOCUS Step 4 calculations and toxicity data for invertebrates with mitigation of spray drift and run-off for the use of CA3573 in winter oil seed rape 1\*60 g a.s./ha late (covering early) application

Intended use		Winter oil seed rape, late application (covering early application)					
Active substance		Acetamiprid					
Application rate (g/ha)		1 × 60 g a.s./ha					
Nozzle reduction	No-spray buffer (m)	FOCUS default buffer 3	5	10	10	20	20
	Vegetated filter strip (m)	-	-	-	10	-	20
No nozzle reduction							
None	R1/stream	0.930	0.930	0.930	0.422	-	-
RAC (µg/L)		PEC/RAC ratio					
0.8							
None	R1/stream	1.2	1.2	1.2	0.53	-	-

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

For the late application in winter oil seed rape (covering early application) with an intended use rate of 1 x 60 g a.s./ha, acceptable risk for aquatic organisms is expected when the following mitigating measures are considered:

- a 10 m drift buffer zone with a 10 m vegetated filter strip.

## CA3573

**Table 9.5-25:** Acceptability of risk (PEC/RAC < 1) for CA3573 for each organism group following the application in apples 1\*142 g/ha (1\*25 g a.s./ha)

Group				Fish-acute	Inverteb. acute	Inverteb. acute	Algae	Inverteb. higher-tier
Test species				<i>Oncorhynchus mykiss</i>	<i>Daphnia magna</i>	<i>Chironomus riparius</i>	<i>Desmodesmus subspicatus</i>	Mesocosm
Endpoint (µg/L)				LC <sub>50</sub> 85800	EC <sub>50</sub> 100200	EC <sub>50</sub> 52.1	EC <sub>50</sub> 3110800	NOEC 9.4
AF				100	100	100	10	2
RAC (µg/L)				858	1002	0.521	311080	4.7
Application rate	Spray drift buffer (m)	Drift reducing nozzles (%)	PEC (µg/L)					
Apples, 142 g product/ha <sup>a</sup> )	3	-	12.4	0.01	0.01	24	0.00	2.6
		75	3.1	0.00	0.00	6.0	0.00	0.66
	5	-	8.78	0.01	0.01	17	0.00	1.9
		50%	4.39	0.01	0.00	8.4	0.00	0.93
	10	-	5.39	0.01	0.01	10	0.00	1.1
	15	-	2.42	0.00	0.00	4.6	0.00	0.51

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

<sup>a</sup>Based on a formulation density of 1.136 g/mL

The PEC/RAC ratios for CA3573 are below the relevant trigger of 1 indicating acceptable risk following the use of the product in apples with 1 x 142 g product/ha when the following mitigating measures are considered:

- a standard drift buffer zone (3 m) with 75% drift-reducing nozzles, or
- a 5 m drift buffer zone with 50% drift-reducing nozzles, or
- a 15 m drift buffer zone.

**Table 9.5-26: Acceptability of risk (PEC/RAC < 1) for CA3573 for each organism group following the application in apples 1\*284 g/ha (1\*50 g a.s./ha)**

Group				Fish acute	Inverteb. acute	Inverteb. acute	Algae	Inverteb. higher-tier
Test species				<i>Oncorhynchus mykiss</i>	<i>Daphnia magna</i>	<i>Chironomus riparius</i>	<i>Desmodesmus subspicatus</i>	Mesocosm
Endpoint (µg/L)				LC <sub>50</sub> 85800	EC <sub>50</sub> 100200	EC <sub>50</sub> 52.1	EC <sub>50</sub> 3110800	NOEC 9.4
AF				100	100	100	10	2
RAC (µg/L)				858	1002	0.521	311080	4.7
Application rate	Spray drift buffer (m)	Drift reducing nozzles (%)	PEC (µg/L)					
Apples, 284 g product/ha <sup>a</sup> )	3	-	24.7	0.03	0.02	<b>47</b>	0.00	<b>5.3</b>
		90	2.47	0.00	0.00	<b>4.7</b>	0.00	0.53
	5	-	17.6	0.02	0.02	<b>34</b>	0.00	<b>3.7</b>
		75	4.4	0.01	0.00	<b>8.5</b>	0.00	0.94
	10	-	10.8	0.01	0.01	<b>21</b>	0.00	<b>2.3</b>
		50	5.4	0.01	0.01	<b>10</b>	0.00	<b>1.2</b>
	15	-	4.85	0.01	0.00	<b>9.3</b>	0.00	<b>1.0</b>
		50	2.42	0.00	0.00	<b>4.6</b>	0.00	0.51
	20	-	2.47	0.00	0.00	<b>4.7</b>	0.00	0.53

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

<sup>a</sup>Based on a formulation density of 1.136 g/mL

The PEC/RAC ratios for CA3573 are below the relevant trigger of 1 indicating acceptable risk following the use of the product in apples with 1 x 284 g product/ha when the following mitigating measures are considered:

- a standard drift buffer zone (3 m) with 90% drift reducing nozzles, or
- a 5 m drift buffer zone with 75% drift reducing nozzles, or
- a 15 m drift buffer zone with 50% drift reducing nozzles, or
- a 20 m drift buffer zone.

**Table 9.5-27: Acceptability of risk (PEC/RAC < 1) for CA3573 for each organism group following the application in oil seed rape 1\*341 g/ha (1\*60 g a.s./ha)**

Group				Fish acute	Inverteb. acute	Inverteb. acute	Algae	Inverteb. higher-tier
Test species				<i>Oncorhynchus mykiss</i>	<i>Daphnia magna</i>	<i>Chironomus riparius</i>	<i>Desmodesmus subspicatus</i>	Mesocosm
Endpoint (µg/L)				LC <sub>50</sub> 85800	EC <sub>50</sub> 100200	EC <sub>50</sub> 52.1	EC <sub>50</sub> 3110800	NOEC 9.4
AF				100	100	100	10	2
RAC (µg/L)				858	1002	0.521	311080	4.7
Application rate	Spray drift buffer (m)	Drift reducing nozzles (%)	PEC (µg/L)					
OSR, 341 g product/ha <sup>a</sup> )	1	-	2.19	0.00	0.00	<b>4.2</b>	0.00	0.47
	5	-	0.593	0.00	0.00	<b>1.1</b>	0.00	0.13
	10	-	0.315	0.00	0.00	0.60	0.00	0.07

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

<sup>a</sup>Based on a formulation density of 1.136 g/mL

With the standard buffer zone of 1 m, the PEC/RAC ratios for CA3573 are below the relevant trigger of 1 indicating acceptable risk following the use of the product in oil seed rape with 1 x 341 g product/ha.

**Table 9.5-28: Acceptability of risk (PEC/RAC < 1) for CA3573 for each organism group following the application in potato 1\*204 g/ha (1\*36 g a.s./ha)**

Group				Fish-acute	Inverteb. acute	Inverteb. acute	Algae	Inverteb. higher-tier
Test species				<i>Oncorhynchus mykiss</i>	<i>Daphnia magna</i>	<i>Chironomus riparius</i>	<i>Desmodesmus subspicatus</i>	Mesocosm
Endpoint (µg/L)				LC <sub>50</sub> 85800	EC <sub>50</sub> 100200	EC <sub>50</sub> 52.1	ErC <sub>50</sub> 3110800	NOEC 9.4
AF				100	100	100	10	2
RAC (µg/L)				858	1002	0.521	311080	4.7
Application rate	Spray drift buffer (m)	Drift reducing nozzles (%)	PEC (µg/L)					
Potato, 204 g product/ha <sup>a</sup> )	1	-	1.31	0.00	0.00	<b>2.5</b>	0.00	0.28
	5	-	0.356	0.00	0.00	0.68	0.00	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**

<sup>a</sup> Based on a formulation density of 1.136 g/mL

With the standard buffer zone of 1 m, the PEC/RAC ratios for CA3573 are below the relevant trigger of 1 indicating acceptable risk following the use of the product in potato with 1 x 204 g product/ha.

**Table 9.5-29: Acceptability of risk (PEC/RAC < 1) for CA3573 for each organism group following the application in corn 1\*341 g/ha (1\*60 g a.s./ha)**

Group				Fish-acute	Inverteb. acute	Inverteb. acute	Algae	Inverteb. higher-tier
Test species				<i>Oncorhynchus mykiss</i>	<i>Daphnia magna</i>	<i>Chironomus riparius</i>	<i>Desmodesmus subspicatus</i>	Mesocosm
Endpoint (µg/L)				LC <sub>50</sub> 85800	EC <sub>50</sub> 100200	EC <sub>50</sub> 52.1	ErC <sub>50</sub> 3110800	NOEC 9.4
AF				100	100	100	10	2
RAC (µg/L)				858	1002	0.521	311080	4.7
Application rate	Spray drift buffer (m)	Drift reducing nozzles (%)	PEC (µg/L)					
Corn, 341 g product/ha <sup>a</sup> )	1	-	2.19	0.00	0.00	<b>4.2</b>	0.00	0.47
	5	-	0.593	0.00	0.00	<b>1.1</b>	0.00	0.13
	10	-	0.315	0.00	0.00	0.60	0.00	0.07

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

<sup>a</sup> Based on a formulation density of 1.136 g/mL

With the standard buffer zone of 1 m, the PEC/RAC ratios for CA3573 are below the relevant trigger of 1 indicating acceptable risk following the use of the product in corn with 1 x 341 g product/ha.

#### zRMS comments:

The refined risk assessment for aquatic invertebrates performed for acetamiprid with consideration of the risk mitigation measures has been validated by the zRMS and agreed. Some minor correction of the PEC<sub>sw</sub> values has been introduced to tables above, but they had no impact on calculated PEC/RAC values and derived conclusions.

Based on the above calculations it was possible to identify risk mitigation measures necessary to demonstrate acceptable risk. Summary of risk mitigation measures is provided in point 9.5.3, separately for each scenario.

The risk assessment for the formulated product was not validated by the zRMS since the exposure estimates include only one way of migration to surface water bodies (spray drift) with run-off or drainage not taken into account. Furthermore, formulation endpoints has been already considered in the risk assessment performed for acetamiprid, if they were lower than the active substance data. Taking this into account, risk assessment performed for the active compound covers also risk from the formulation.

### 9.5.3 Overall conclusions

The risks to aquatic organisms from the intended uses of CA3573 were evaluated on the basis of the available ecotoxicity studies on the active substance, its metabolites and the formulation. The risks from the metabolites are low and were acceptable on FOCUS Step-1 level. Regarding the formulation and the active substance, the risks to aquatic invertebrates had to be refined by using a mesocosm study. Following this, acceptable risks were demonstrated on FOCUS Step-3 level for the intended uses in spring oil seed rape (1 x 60 g a.s./ha), potatoes (1 x 36 g a.s./ha) and corn (1 x 60 g a.s./ha). Regarding the other intended uses, the following mitigating measures need to be considered (FOCUS Step 4):

Intended use	Mitigating measures	Comment
Apples, 1 x 25 g a.s./ha, <u>early</u> application at BBCH ≥ 62	<ul style="list-style-type: none"> <li>Standard DBZ (3 m) plus 75% DRN, <i>or</i></li> <li>10 m DBZ plus 50% DRN, <i>or</i></li> <li>15 m DBZ</li> </ul>	Covering BBCH ≥ 69
Apples, 1 x 25 g a.s./ha, <u>late</u> application at BBCH ≥ 62	<ul style="list-style-type: none"> <li>Standard DBZ (3 m) plus 50% DRN, <i>or</i></li> <li>5 m DBZ</li> </ul>	Covering BBCH ≥ 69
Apples, 1 x 50 g a.s./ha, <u>early</u> application at BBCH ≥ 62	<ul style="list-style-type: none"> <li>Standard DBZ (3 m) plus 90% DRN, <i>or</i></li> <li>10 m DBZ plus 75% DRN, <i>or</i></li> <li>15 m DBZ plus 50% DRN, <i>or</i></li> <li>20 m DBZ</li> </ul>	Covering BBCH ≥ 69
Apples, 1 x 50 g a.s./ha, <u>late</u> application at BBCH ≥ 62	<ul style="list-style-type: none"> <li>Standard DBZ (3 m) plus 75% DRN, <i>or</i></li> <li>5 m DBZ plus 50% DRN, <i>or</i></li> <li>10 m DBZ</li> </ul>	Covering BBCH ≥ 69
Winter oil seed rape, 1 x 60 g a.s./ha, <u>late</u> application	<ul style="list-style-type: none"> <li>Standard DBZ (1 m)</li> <li>10 m DBZ plus 10 m VFS <sup>1</sup></li> </ul>	Covering <u>early</u> application

<sup>1</sup> for scenario R1 stream only

DBZ: drift buffer zone; DRN: drift reducing nozzles; VFS: vegetated filter strip

#### zRMS comments:

Conclusions presented by the Applicant above are agreed by the zRMS. However, 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. Please note that mitigation measures for early and late application to pome fruits were combined in order to cover the worst case situation.

Application pattern	FOCUS scenarios with respective mitigation measures									
	D1	D2	D3	D4	D5	D6	R1	R2	R3	R4
Apples early and late BBCH ≥ 62 1 x 25 g a.s./ha			15 m BZ  <u>or</u> 5 m BZ + 50% DRN  <u>or</u> 75% DRN	15 m BZ  <u>or</u> 10 m BZ + 50% DRN  <u>or</u> 75% DRN	15 m BZ  <u>or</u> 10 m BZ + 50% DRN  <u>or</u> 75% DRN		15 m BZ  <u>or</u> 50% DRN  <u>or</u> 75% DRN	15 m BZ  <u>or</u> 10 m BZ + 50% DRN  <u>or</u> 75% DRN	15 m BZ  <u>or</u> 10 m BZ + 50% DRN  <u>or</u> 75% DRN	15 m BZ  <u>or</u> 50% DRN  <u>or</u> 75% DRN

Apples early and late BBCH $\geq 62$ 1 x 50 g a.s./ha			20 m BZ  <u>or</u> 15 m BZ + 50% DRN  <u>or</u> 5 m BZ + 50% DRN  <u>or</u> 90% DRN	20 m BZ  <u>or</u> 15 m BZ + 50% DRN  <u>or</u> 10 m BZ + 75% DRN  <u>or</u> 90% DRN	20 m BZ  <u>or</u> 15 m BZ + 50% DRN  <u>or</u> 10 m BZ + 75% DRN  <u>or</u> 90% DRN		15 m BZ  <u>or</u> 15 m BZ + 50% DRN  <u>or</u> 75% DRN	20 m BZ  <u>or</u> 15 m BZ + 50% DRN  <u>or</u> 10 m BZ + 75% DRN  <u>or</u> 90% DRN	20 m BZ  <u>or</u> 15 m BZ + 50% DRN  <u>or</u> 10 m BZ + 75% DRN  <u>or</u> 90% DRN	15 m BZ  <u>or</u> 15 m BZ + 50% DRN  <u>or</u> 75% DRN
Winter OSR early and late BBCH 31-71 1 x 60 g a.s./ha		None	None	None	None		10 m VFS		None	
Spring OSR BBCH 31-71 1 x 60 g a.s./ha	None		None	None	None		None			
Potatoes early and late BBCH 12-79 1 x 36 g a.s./ha			None	None		None	None	None	None	
Maize BBCH 51-75 1 x 60 g a.s./ha			None	None	None	None	None	None	None	None

**BZ:** unsprayed buffer zone; **VFS:** vegetated filter strip; **DRN:** drift reducing nozzles

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 CA3573, 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 and related documents.

Effects on bees of the formulation CA3573 were not evaluated as part of the EU assessment of acetamiprid. New data submitted with this application are listed in **Błąd! Nie można odnaleźć źródła o** **dwolania.** and Appendix 1 and are summarised in Appendix 2.

New studies on the 10-day chronic toxicity of CA3573 to adult honey bees and on the toxicity of repeated oral administration of CA3573 to honey bee larvae were conducted to fill deficiencies in the respective older ones.

In total, 10 higher tier studies (*i.e.* 7 semi-field and 3 field studies) on effects of acetamiprid on bees are available for CA3573 / 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>	Exp 60707A	Acute, oral	<b>LD<sub>50</sub> = 8.85 µg a.s./bee</b>	EFSA, 2016
		Acute, contact	<b>LD<sub>50</sub> = 9.26 µg a.s./bee</b>	EFSA, 2016
<i>Bombus terrestris</i>	EXP 60707A	Acute, contact	LD <sub>50</sub> > 100 µg a.s./bee	EFSA, 2016
<i>Apis mellifera</i>	a.s.	Chronic, oral, 10 days	LDD <sub>50</sub> = 11.7 µg a.s./bee/day	EFSA, 2016
<i>Apis mellifera</i>	a.s.	Chronic larvae, Oral feeding for 6 days	EC <sub>10</sub> = 1.3 µg/larvae/developmental period	EFSA, 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 (CA3573)**

Species	Substance	Exposure System	Results	Reference
<i>Apis mellifera</i>	MCW-2222	Acute, Oral	<b>LD<sub>50</sub> = 9.1 µg a.s./bee</b>	Franke, M., 2014 R-33834 KCP 10.3.1.1.1/01
		Acute, Contact	<b>LD<sub>50</sub> = 3.8 µg a.s./bee</b>	Franke, M., 2014 R-33834 KCP 10.3.1.1.2/01
<i>Bombus terrestris</i>	MCW-2222	Acute, Oral	LD <sub>50</sub> = 24.3 µg a.s./bumble bee	Röhlig, U., 2014 R-33837 KCP 10.3.1.2.1/01
		Acute, Contact	LD <sub>50</sub> > 200 µg a.s./bumble bee	Röhlig, U., 2014 R-33837 KCP 10.3.1.2.2/01
<i>Apis mellifera</i>	CA3573 Acetamiprid 200 SL (Carnadine)	Chronic, Oral	LDD <sub>50</sub> = 3.71 µg a.s./bee/day <b>NOEDD = 1.54 µg a.s./bee/day</b>	Dressler, K., 2019 19 48 BAC 0028 KCP 10.3.1.2/01
<i>Apis mellifera</i>	MCW-2222	Chronic, Oral	LDD <sub>50</sub> = 3.994 µg a.s./bee/day <b>NOEDD = 0.546 µg a.s./bee/day</b>	Kleebaum, K., 2014a R-33835 KCP 10.3.1.2/02
<i>Apis mellifera</i>	CA3573 Acetamiprid 200 SL (Carnadine)	Chronic, Oral, larvae	<b>22d NOEDD ≥ 0.486 µg a.s./larvae</b>	Scheller, K., 2020 KCP 10.3.1.3/01
<i>Apis mellifera</i>	MCW-2222	Chronic, Oral, larvae	8d NOEDD = 3.8 µg a.s./larvae	Kleebaum, K., 2014b R-33836 KCP 10.3.1.3/02

Values shown in **bold** are used for the risk assessment acc. to EPPO; values shown in *italics* used for the risk assessment acc. to the EFSA Bee GD; values shown in **bold** and *italics* used for both risk assessment approaches



**Table 9.6-3: Summary and results of semi-field and field studies supporting the evaluation risk with MCW-2222 (CA3573)**

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"> <li>Temporary, significant effects on daily adult mortality until D+2 in T1 and T2,</li> <li>No significant differences on cumulative adult mortality in T1 and T2,</li> <li>Temporary effects on foraging activity until D+1 and behaviour (few bees with signs of intoxication) until D+2 in T1 and T2,</li> <li>No impact on colony strength and colony development in T1 and T2.</li> </ul>	Mamet, O. & Molitor, C., 2015, 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"> <li>Statistically significant effect on daily mortality from D+1 to D+3,</li> <li>No significant difference on cumulative adult mortality,</li> <li>Effects on foraging activity were observed until D+3,</li> <li>Bees hesitated to forage and few bees displayed signs of intoxication until D+1,</li> <li>No impact on colony strength and colony development.</li> </ul> <p>T2:</p> <ul style="list-style-type: none"> <li>Statically significant effect on daily mortality at D+2 and D+3,</li> <li>No significant difference on cumulative adult mortality,</li> <li>Effects on foraging activity were observed until D+3,</li> <li>Bees hesitated to forage until D+2 and few bees displayed signs of intoxication until D+1,</li> <li>No impact on colony strength and colony development.</li> </ul>	Mamet, O., 2015, 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"> <li>Statistically significant effect on daily mortality on D+1,</li> <li>No significant difference on cumulative adult mortality,</li> <li>Effects on foraging activity were observed until D+1 and on behaviour on the day of application,</li> <li>No signs of intoxication,</li> <li>No impact on colony strength and colony development.</li> </ul> <p>T2:</p> <ul style="list-style-type: none"> <li>No significant effects on daily mortality,</li> <li>No significant difference on cumulative adult mortality,</li> <li>Effects on foraging activity were observed until D+3 and effects on behaviour until D+2,</li> <li>No signs of intoxication,</li> <li>No impact on colony strength and colony development.</li> </ul>	Mamet, O., 2015, 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"> <li>Slight but significant effect on daily adult mortality on D+1 in T1, no effect on adult mortality in T2.</li> <li>No significant differences on cumulative adult mortality in T1 and T2.</li> <li>No effects on foraging activity and behaviour in T1 and T2.</li> <li>No impact on colony strength and colony development in T1 and T2.</li> <li>Observations up to 8 DAA.</li> <li>No residue analysis, but treated crop was the only food source during the study.</li> </ul>	Mamet, O. & Molitor, C., 2015, 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 (T1) and after (T2) bee flight to flowering <i>Phacelia</i>	<p>T1 and T2</p> <ul style="list-style-type: none"> <li>No significant effects on daily adult mortality.</li> <li>No significant differences on cumulative adult mortality.</li> <li>No effects on foraging activity and behaviour.</li> <li>No impact on colony strength and colony development.</li> <li>Observations up to 8 DAA.</li> </ul>	Mamet, O. & Molitor, C., 2015, R-34876 KCP 10.3.1.5/05

Higher-tier studies – semi-field studies (tunnels)			
Species	Substance	Endpoint used for risk assessment	Reference
		<ul style="list-style-type: none"> <li>No residue analysis, but treated crop was the only food source during the study.</li> </ul>	
<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>	T1 and T2 <ul style="list-style-type: none"> <li>No significant effects on daily adult mortality.</li> <li>No significant differences on cumulative adult mortality.</li> <li>No effects on foraging activity and behaviour.</li> <li>No impact on colony strength and colony development.</li> <li>Observations up to 8 DAA.</li> <li>No residue analysis, but treated crop was the only food source during the study.</li> </ul>	Molitor, C., 2015, 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 <sup>st</sup> application just before the flowering period and 2 <sup>nd</sup> application during the flowering period in the evening after the flight	<ul style="list-style-type: none"> <li>No impact on adult and pupal bee mortality.</li> <li>No impact on foraging activity and behaviour.</li> <li>No impact on colony strength and colony development.</li> <li>No impact on the detailed brood development based on the assessed indices, i.e. brood termination rate, brood index, brood compensation index.</li> <li>Residue analysis prove clearly that bees and colonies were exposed to MCW-2222.</li> <li>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.</li> <li>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).</li> </ul> <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 <i>Phacelia tanacetifolia</i> oil-seed rape 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"> <li>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).</li> <li>No impact on foraging activity and behaviour.</li> <li>No impact on colony strength and colony development.</li> <li>No impact on the detailed brood development based on the assessed indices, i.e. brood termination rate, brood index, brood compensation index.</li> <li>Residue analysis prove clearly that colonies were exposed to MCW-2222.</li> <li>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.</li> <li>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).</li> <li>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 pronounced in test item groups; in some hives the number of nursery bees too low to assure correct development of brood</li> </ul>	Molitor, C., 2015, R-34877 KCP 10.3.1.6/01

Higher-tier studies – semi-field studies (tunnels)			
Species	Substance	Endpoint used for risk assessment	Reference
		<p>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"> <li>Overwintering success not investigated.</li> </ul> <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>	MCW 2222, applied twice at 0.5 L/ha (100 g/ha acetamiprid) to <u>oil seed rape</u> in the field; 1 <sup>st</sup> application just before the flowering period and 2 <sup>nd</sup> application during the flowering period in the evening after the flight	<ul style="list-style-type: none"> <li>No impact on adult and pupal bee mortality.</li> <li>No impact on foraging activity and behaviour.</li> <li>No impact on colony strength and colony development.</li> <li>No impact on the detailed brood development based on the assessed indices, i.e. brood termination rate, brood index, brood compensation index.</li> <li>Residue analysis prove clearly that colonies were exposed to MCW-2222.</li> <li>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).</li> <li>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.</li> <li>Overwintering success not investigated.</li> </ul> <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>	Molitor, C., 2015, R-35844 KCP 10.3.1.6/02
<i>Apis mellifera</i>	MCW 2222, applied twice at 0.5 L/ha (100 g/ha acetamiprid) to <u>apple</u> trees in an orchard; 1 <sup>st</sup> application just before the flowering period and 2 <sup>nd</sup> application during the flowering period in the evening after the flight	<ul style="list-style-type: none"> <li>No impact on adult, larval and pupal bee mortality.</li> <li>No impact on <del>foraging and</del> flight activity.</li> <li>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.</li> <li>No impact on colony strength and brood amount.</li> <li>No statistically significant 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).</li> <li>Compensation indices were not calculated.</li> <li>Rain at 0.8, 7.0 and 10.6 mm was observed on 1 DALA, 2 DALA and 3 DALA respectively. Precipitation on 2 and 3</li> </ul>	Aucejo, C., 2015, R-35961 KCP 10.3.1.6/03

Higher-tier studies – semi-field studies (tunnels)			
Species	Substance	Endpoint used for risk assessment	Reference
		<p>DALA was high enough to significantly reduce the exposure.</p> <ul style="list-style-type: none"> <li>Residue analysis of nectar and pollen prove clearly that colonies were exposed to MCW-2222, but could be reduced due to rain on 1 DALA, 2 DALA nad 3 DALA. Flowers were not analysed for acetamiprid residues.</li> </ul> <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>	

#### 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 CA3573 (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, A 2.3.1.1.

In support of evaluation of CA3573 due to acetamiprid renewal also chronic and larvae toxicity studies performed with the product were provided. Studies were evaluated and accepted by the zRMS. For study summaries and zRMS conclusions, please refer to Appendix 2, A 2.3.1.2 and A 2.3.1.4, respectively.

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). No longer relevant endpoints are struck through in Table 9.6-2 above.

For the quantitative risk assessment endpoints from studies performed with CA3573 (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 for purposes of the current assessment, because higher tier data were not required to support the risk assessment in 2018 and initial evaluation was rather brief. Summaries of studies together with zRMS evaluation and conclusions are presented in Appendix 2, A 2.3.1.7 and A 2.3.1.8. 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 were struck through in Table 9.6-3 as being not relevant for the intended use pattern of CA3574 and thus not considered in the risk assessment.

#### 9.6.1.1 Justification for new endpoints

Additional laboratory studies were performed with CA3573 on 10-day chronic toxicity to adult honey bees and toxicity of repeated oral exposure to honey bee larvae due to deficiencies in respective older one. In more specific terms, this means that the chronic oral study on adult honey bees of Kleebaum (2014a) did not contain an analytical verification of the diets, which was state of the art at this time since no official guideline was available. The larval testing of Kleebaum (2014b) contained a single exposure until day 8 and the absence of analytical verification as no official guideline was in force. To fill these gaps, new studies on the adult and larval toxicity after chronic or repeated exposure have been conducted to current guidelines, i.e. Dressler (2019) and Scheller (2020), respectively.

**zRMS comments:**

The studies on chronic and larval bee toxicity by Dressler (2019) and Scheller (2020) were evaluated by the zRMS and considered acceptable. Results of these studies supersede not fully reliable endpoints derived in older tests by Kleebaum (2014a and 2014b), which were not performed fully in line with the current guidelines.

Details of the studies evaluation together with zRMS conclusions are given in Appendix 2, A 2.3.1.2 and A 2.3.1.4.

## 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) following two approaches.

~~In the first, the acute risk assessment was conducted based on the revised EPPO scheme (2010), 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, also the EPPO scheme (2010) was used. For the chronic oral exposure of adult honey bees and larvae, the EPPO scheme suggests to calculate the toxicity exposure ratio (TER) between the NOEL and the exposure. Originally, this approach has been developed for seed treatments, but can be directly applied for foliar uses as well. The risk assessments according to the EPPO approach are presented in chapter 9.6.2.1 and were conducted with the highest single application rate of 0.3 L CA3573/ha ( $\triangleq$  0.060 kg acetamiprid/ha) in OSR (uses 4 to 10, 14 to 18) and corn (uses 19 and 20) as a worst case scenarios, covering all other uses.~~

Due to different requirements within Europe, a second approach was followed which is based on the principles of the EFSA Bee Guidance Document (EFSA 2013b) in order to consider also the evaluation of the chronic risk for adult honey bees and honey bee larvae, as well as the acute risk for adult bumble bees. As test methods for acute solitary bee testing as well as chronic oral and larval testing of bumble bees and solitary bees are not yet developed and are regarded as long-term research projects (EC, 2014), the current risk assessment is carried out using the required endpoints according to the draft roadmap of the European Commission (EC, 2014), dated on 16<sup>th</sup> May, 2014:

- Honey bee: acute oral and contact (adult), chronic adult, larvae
- Bumble bee: acute oral and contact (adult)

The acute risk assessment for adults and bumble bees as well as the chronic risk assessment for adult honey bees and honey bee larvae according to the EFSA Bee GD is presented in chapter 9.6.2.2 and covers the GAPs in Poland and Slovakia in orchards (apples at BBCH 62-PHI for Poland and BBCH 69-PHI for Slovakia, 1 x 0.050 kg a.s./ha), potatoes (BBCH 12-79, 1 x 0.036 kg a.s./ha) and oil seed rape (winter and spring oil seed rape, overall BBCH 31-71, 1 x 0.060 kg a.s./ha) and corn (BBCH 51-75, 1 x 0.060 kg a.s./ha, only in Slovakia).

Potential effects on the acute and chronic exposure of adult bees as well on the bee brood development were evaluated based on a total of 7 semi-field and 3 field studies.

According to the EFSA Bee Guidance Document (2013) also the risk of bees being exposed to contaminated water via guttation water, surface water and puddle water has to be assessed. Based on the ‘Pesticides Peer Review Meeting 145’(7–9 June 2016) regarding the exposure of bees to contaminated water which was summarized within the Belgium ‘Data requirements and risk assessment for bees’, version 2.3 from 04.04.2019, the authority concluded that ‘it is not required to perform a risk assessment for exposure through guttation water for product authorisation for the time being. For the risk from exposure through the consumption of surface water and puddle water, experience from the assessment of active substance with a high toxicity to bees shows that the exposure and risk for these scenarios can also be considered of relatively low relevance. Therefore, a risk assessment for these exposure scenarios does not need to be performed for the authorisation of plant protection products for the time being.’ Consequently, no risk assessment on contaminated water was conducted here.

#### zRMS comments:

In general, according to conclusions of the Central Zone Steering Committee (CZSC) at the Central Zone level the risk assessment for bees should be performed in line with recommendations of SANCO/10329/2002 rev 2 final, while recommendations of EFSA (2013) should not be considered until the guidance is noted at the EU level.

However, as acetamiprid is an insecticide with a specific mode of action, the risk assessment based solely on acute toxicity endpoints may be not sufficiently protective for bees and the chronic risk to adult bees as well as risk to bee larvae should be sufficiently addressed before authorisation is granted.

As SANCO/10329/2002 rev 2 final does not provide any indications on how to perform the chronic and larvae risk assessment, consideration of indications of EFSA (2013) to perform dietary oral risk assessment seems to be reasonable approach, even if the guidance itself is not noted yet.

Taking this into account the risk assessment performed using both guidance documents (SANCO and EFSA) has been validated by the zRMS in points below.

With regard to the risk via contaminated water the Applicant refers to indications of Belgium guidance “*Data requirements and risk assessment for bees*”, which states that the risk via contaminated water is considered to be of low relevance and is currently not required. It should be, however, noted that the document mentioned is the national guidance document and its indications may not be relevant for the zonal level. Nevertheless, the zRMS agrees with information provided in the Belgium guidance that assumptions in the EFSA (2013) scheme for risk assessment via contaminated water are extremely conservative with no or only little options for refinement in case the risk assessment fails. For example, the same potential exposure via guttation fluid is assumed regardless of the crop, although it is known that guttation does not occur in all crops and its extent may also depend on the growth stage (also in crops known for intensive guttation during early BBCH stages like cereals and maize). However, very little data is available to support extent of guttation in particular crops and actually the only refinement option for exposure via guttation fluid is to perform the residue study instead of first check if exposure via guttation water in the considered crop is possible at all.

Taking this into account the risk via contaminated water was not performed for CA3573 and exposure via treated crops is considered to be most relevant and protective also for exposure via contaminated water.

The evaluation of the chronic and larvae risk based on indications of EPPO (2010) has not been validated by the zRMS, as the scheme for chronic and larvae risk assessment has been developed for seed treatments and although in the past due to absence of any other guidance it was sometimes used for spray applications, it may be not fully relevant for this route of exposure. Furthermore, the chronic and larvae risk assessment is considered to be sufficiently addressed on the basis of evaluation based on EFSA (2013), so additional evaluation performed in line with EPPO (2010) is not considered necessary.

### 9.6.2.1 Hazard quotients for bees (based on SANCO/10329/2002 and EPPO 2010)

#### Acute Risk Assessment for adult honey bees

**Table 9.6-4: First-tier assessment of the acute risk for adult bees due to the use of CA3573 in winter and spring oil seed rape and corn (worst case)**

<b>Intended use</b>	OSR (winter/spring); corn		
<b>Active substance</b>	acetamiprid		
<b>Application rate (g/ha)</b>	1 × 60		
<b>Test design</b>	<b>LD<sub>50</sub> (lab.) (µg a.s./bee)</b>	<b>Single application rate (g a.s./ha)</b>	<b>Q<sub>HO</sub>, Q<sub>HC</sub> criterion: Q<sub>H</sub> ≤ 50</b>
Oral toxicity	8.85	60*	6.8
Contact toxicity	9.26		6.5
<b>Product</b>	CA3573		
<b>Application rate (g/ha)</b>	1 × 60		
<b>Test design</b>	<b>LD<sub>50</sub> (lab.) (µg a.s./bee)</b>	<b>Single application rate (g a.s./ha)</b>	<b>Q<sub>HO</sub>, Q<sub>HC</sub> criterion: Q<sub>H</sub> ≤ 50</b>
Oral toxicity	9.1	60*	6.6
Contact toxicity	3.8		15.8

Q<sub>HO</sub>, Q<sub>HC</sub>: Hazard quotients for oral and contact exposure. Q<sub>H</sub> values shown in bold breach the relevant trigger.

\* worst-case application rate, covering all other intended uses

The hazard quotients for acetamiprid and CA3573 are less than 50, indicating that the acute risk to adult honey bees is acceptable following use according to the proposed use pattern.

### Chronic Risk Assessment of adult honey bees

For adult honey bees, the exposure is assessed by the amount of residues that may be ingested by a bee in one day (= daily residue intake expressed as µg a.s./bee/day). The risk assessment for the chronic exposure of adult bees is performed calculating the toxicity exposure ratio (TER) between the NOEL (= NOEDD in µg a.s./bee/day) and the predicted exposure (daily residue intake):

$$TER = \frac{NOEDD}{\text{daily residue intake}}$$

According to EPPO (2010) the obtained TER is compared to a trigger of 1.

To calculate the expected daily intake, EPPO 2010 refers to the review of Rortais et al. (2005, KCP 10.3.1/01). For adult honey bees, only nectar consumption is relevant whereas no pollen is consumed. Based on the authors the maximum daily sugar amount an adult bee consumes is 128 mg/bee/day. Based on a nectar concentration of 30% this corresponds to a total consumption of 426.7 mg nectar/bee/day. The sugar concentration in nectar was taken from Rortais et al. (2005) which gave a range of 5 to 80%, but specifically mentioning 40% as representative for bee attractive crops. This is also confirmed by Kim et al. (2011, KCP 10.3.1/02, cited by Pamminger et al. 2019, KCP 10.3.1/03) who determined the bee optimal range of 35 to 65%. Thus, a 30% sugar concentration can be considered as conservative for crop plants.

To calculate the daily residue intake of acetamiprid by adult honey bees the consumed amount of nectar (426.7 mg nectar/bee day) is multiplied with the maximum residue concentration in nectar on day 3 (0.16 mg a.s./kg = 0.16 µg a.s./g = 0.16 ng a.s./mg nectar), deriving from the field bee brood study on oil seed rape with an application rate of 100 g a.s./ha (Molitor 2015, R 35844):

$$426.7 \text{ mg nectar/bee/day} \times 0.16 \text{ ng a.s./mg nectar} = 68.3 \text{ ng a.i./bee/day} = \mathbf{0.0682 \text{ µg a.i./bee/day}}$$

For the risk assessment, the endpoint of the study of Dressler (2019) was used, which was conducted according to the current guideline. As the study by Kleebaum (2014a) results in a lower NOEDD, the risk assessment was also conducted using this endpoint. For this study it has to be noted, that the endpoint is regarded as not fully reliable, as the study was not conducted according to current guideline and missed the analytical verification of the test item in the diets. The resulting TERs are presented in Table 9.6 5.

**Table 9.6 5: First-tier assessment of the chronic risk for adult honey bees due to the use of CA3573 in winter and spring oil seed rape and corn (worst case)**

Product	CA3573						
Application rate (g/ha)	1 × 60						
Test design	NOEDD (lab.) (µg a.s./bee/day)	Sugar consumption of a bee (mg/day)	Concentration <sup>‡</sup> (%)	Nectar consumption (mg nectar/bee/day)	Residue concentration <sup>‡</sup> (µg a.s./g = ng a.s./mg)	Daily intake of acetamiprid (µg a.s./bee/day)	TER Trigger: ≥1
Chronic oral toxicity	1.54	128	30	426.7	0.16	0.0682	22.58
Chronic oral toxicity	0.546 <sup>††</sup>	128	30	426.7	0.16	0.0682	8.01

<sup>‡</sup> Sugar concentration in nectar;

<sup>‡‡</sup> Maximum mean residue concentration of acetamiprid in in-hive nectar on day 3;

<sup>††</sup> Endpoint regarded as not fully reliable, as study was not conducted according to current guideline without analytical verification of the test item in the diets

TER: Toxicity Exposure Ratio for chronic oral exposure of adult honey bees. TER value shown in non bold is above the relevant trigger (safe use).

The calculated TERs for CA3573 and acetamiprid display to be 8.01 (using the endpoint of Kleebaum, 2014a) and 22.58 (using the endpoint of Dressler, 2019) and thus being higher than the trigger of 1, indicating that the chronic oral risk to bees is acceptable following the use of CA3573 according to the proposed use pattern.

### Chronic Risk Assessment of honey bee larvae

For honey bee larvae, the exposure is assessed by the amount of residues that may be ingested by a bee larva during its development period (expressed as µg a.s./larva). For honey bee larvae it has to be considered that they are not exposed to the test substance during the entire study duration of 22 days, but rather during the feeding period of the larvae which lasts 5 days. As the needed and supplied amount of food increases with the growing of the larvae the level of exposure for larvae equals the total amount of residues ingested by the larvae during the complete larval stage of five days.

The risk assessment for the chronic oral exposure of honey bee larvae is performed calculating the toxicity exposure ratio (TER) between the NOEL (= NOEDD in µg a.s./larva/development period) and the predicted exposure (total residue intake over the 5-day feeding period in µg a.i./larva):

$$\text{TER} = \frac{\text{NOED}}{\text{total residue intake over the 5-day feeding period}}$$

According to EPPO (2010a) the obtained TER is compared to a trigger of 1.

To calculate the expected food consumption, EPPO (2010b) refers to the review of Rortais et al. (2005). For honey bee larvae, both nectar and pollen consumption is relevant. Based on the authors, the maximum total amount of sugar consumed by a larva during its development period is 59.4 mg/5 days which corresponds to a total consumption of 198 mg nectar/5 days, taking a 30% sugar concentration into account (for justification: see above). In addition to the nectar requirements honey bee larvae consume up to 2 mg pollen/5 days. This consumption value is taken from the original publication of Babendreier et al. (2004, KCP 10.3.1/04) as their results were erroneously cited as being 5.4 mg in Rortais et al. (2005).

To calculate the overall residue intake of acetamiprid by honey bee larvae, the consumed amount of nectar (198 mg nectar/5 days) and pollen (2 mg pollen/5 days) is multiplied with the maximum residue concentration on day 3 in nectar (0.16 mg a.s./kg = 0.16 µg a.s./g = 0.16 ng a.s./mg nectar) (deriving from the field bee brood study on oil seed rape with an application rate of 100 g a.s./ha, Molitor 2015, R-35844) and pollen (8.2 mg a.s./kg = 8.2 µg a.s./g = 8.2 ng a.s./mg pollen), deriving from the semi field bee brood study on Phacelia with an application rate of 80 g a.s./ha (Hecht Rost & Mayer 2018, R-37336).

For residues in nectar this is:

$$198 \text{ mg nectar/5 days} * 0.16 \text{ ng a.s./mg nectar} = 31.68 \text{ ng a.s./5 days} = \mathbf{0.032 \text{ } \mu\text{g a.s./5 days in nectar}}$$

For residues in pollen this is:

$$2.0 \text{ mg pollen/5 days} * 8.2 \text{ ng a.s./mg pollen} = 16.4 \text{ ng a.s./5 days} = \mathbf{0.016 \text{ } \mu\text{g a.i./5 days in pollen}}$$

This sums up to a total amount of acetamiprid residues of **0.048 µg a.s./5 days**.

The resulting TER is presented in Table 9.6-6.



**Table 9.6-6: First-tier assessment of the chronic risk for honey bee larvae due to the use of CA3573 in winter and spring oil seed rape and corn (worst case)**

<b>Product</b>	CA3573							
<b>Application rate (g/ha)</b>	1 × 60							
<b>Test design</b>	<b>NOED (lab.) (µg a.s./develop. period)</b>	<b>Food consumption (mg/5-days)</b>	<b>Concentration* (%)</b>	<b>Food consumption (mg/5-days)</b>	<b>Residue concentration** (µg a.s./g = ng a.i./mg)</b>	<b>Total intake of acetamiprid (µg a.s./5-days)</b>		<b>TER Trigger: ≥1</b>
Chronic-bee larvae toxicity	≥0.486	Sugar: 59.4 Pollen: 2.0	Nectar: 30 Pollen: —	Nectar: 198.0 Pollen: 2.0	Nectar: 0.16 Pollen: 8.2	Nectar: 0.032 Pollen: 0.016	0.048	≥10.13

\* Sugar concentration in nectar

\*\* Maximum residue concentration of acetamiprid in nectar / pollen on day 3

TER: Toxicity Exposure Ratio for chronic oral exposure of honey bee larvae. TER value shown in non bold is above the relevant trigger (safe use).

The calculated TER for CA3573 and acetamiprid is ≥ 10.13 and thus above the trigger of 1, indicating that the chronic risk to bee larvae is acceptable following the use of CA3573 according to the proposed use pattern.

#### **zRMS comments:**

The acute risk assessment performed in compliance with SANCO/10329/2002 rev 2 final is agreed by the zRMS.

Calculations provided in Table 9.6-4 above were performed with consideration of the maximum intended application rate of acetamiprid in CA3573 and on their basis acceptable acute risk to bees may be concluded from all intended uses of CA3573.

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 and respective calculations are provided in point 9.6.2.2 below, while calculations based on EPPO indications are struck through above.

For detailed justification of the zRMS approach, please refer to zRMS comments in point 9.6.2 above.

### **9.6.2.2 Risk assessment according to the ‘EFSA Bee GD’ (EFSA, 2013)**

All steps for the risk assessment, i.e. the screening step, 1<sup>st</sup> and 2<sup>nd</sup> 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 CA3573 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 CA3573 for crops with a maximum single application rate of 0.060 kg a.s./ha (worst case)**

Application rate of 0.000 kg a.s./ha (worst case)					
Test	Endpoint	Calculation factor <sup>a)</sup>	HQ or ETR <sup>a)</sup>	Trigger <sup>a)</sup>	Risk acceptable?
Contact route of exposure					
Honey bee	3.8 µg a.s./bee	1	15.8	42 / 85	Yes
Bumble bee	> 200 µg a.s./bee		< 0.3	7 / 14	Yes
Oral route of exposure					
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	<b>0.123 / 0.171</b>	0.03	<b>No</b>
Honey bee, larvae	≥ 0.486 µg a.s./larva	4.4 / 6.1	<b>≤ 0.54 / ≤ 0.75</b>	0.2	<b>No</b>
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

<sup>a)</sup> Application scenario used for calculations: downward spraying / up- and sideward spraying

Considering the proposed uses of CA3573 at a maximum application rate of 0.06 kg a.s./ha, no unacceptable effects are expected for honey bees and bumble 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, 1<sup>st</sup> tier oral risk assessments were carried out (see Table 9.6-8).

### 1<sup>st</sup> tier, oral risk assessment

In the screening step, potential risk was indicated for adult honey bees following the chronic exposure as well as for honey bee larvae. In the following, a crop and life stage-specific (adult/larvae) risk assessment is carried out, which is a first step of refinement. On the one hand, this takes into account crop dependent exposure factors (Ef), and on the other hand it considers SV values, which depend on default values for pollen and nectar consumption, sugar content in nectar, residues (RUDs) in pollen and nectar as well as crop attractiveness (see table below).

**Table 9.6-8: 1<sup>st</sup> tier oral risk assessment for honey bees (chronic and larvae) of CA3573**

Crop (Crop group according to EFSA tool)	Endpoint	ETR (oral exposure scenario) <sup>a)</sup>					Trigger
		Treated crop	Weeds	Field margin	Adjacent crop	Next crop	
Maximum single application rate: 0.050 kg a.s./ha, BBCH 62-69 PHI							
Apple (orchards 1)	adult, chronic	0.080 <del>0.040</del>	0.008	0.003	0.004	0.005	0.03
	larvae	0.53	0.06	0.02	0.03	0.03	0.2
Maximum single application rate: 0.050 kg a.s./ha BBCH 70–PHI							
Apple (orchards 1)	adult, chronic	0.000	0.008	0.003	0.004	0.005	0.03
	larvae	0.00	0.06	0.02	0.03	0.03	0.2
Maximum single application rate: 0.025 kg a.s./ha, BBCH 62-69							
Apple (orchards 1) BBH 62-69	adult, chronic	0.040	0.004	0.001	0.002	0.003	0.03
	larvae	0.27	0.03	0.01	0.01	0.02	0.2
Maximum single application rate: 0.025 kg a.s./ha, BBCH 70–PHI							
Apple (orchards 1) BBCH 70-PHI	adult, chronic	0.000	0.004	0.001	0.002	0.003	0.03
	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	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
Maximum single application rate: 0.06 kg a.s./ha, BBCH 51-75							
Corn (maize)	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

ETR values in **bold** are above the trigger value

<sup>a)</sup> All BBCH scenarios were used according to the proposed application timing. In the table only the worst-case (highest) values are presented

Based on the 1<sup>st</sup> tier oral risk assessment, no unacceptable effects are expected for the chronic oral exposure of adult honey bees and honey bee larvae regarding the exposure scenarios ‘weeds’, ‘field margin’, ‘adjacent crop’ and ‘next crop’ following the proposed uses of CA3573. For the use in potatoes no unacceptable effects are expected considering all oral routes of exposure.

However, for the use in apple and spring and winter oil seed rape, a chronic risk for adult honey bees and honey bee larvae cannot be ruled out based on the oral exposure in the ‘treated field’ scenario. Therefore a 2<sup>nd</sup> 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 CA3573.

#### **zRMS comments:**

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 by the Applicant above were validated by the zRMS using EFSA Bee-Tool v.3 and are confirmed to be correct with exception of the ETR value for apples at 50 g a.s./ha (treated crop scenario), which is higher than this reported in Table 9.6-8 above.

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 1<sup>st</sup> 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 and maize (all considered scenarios).

For application to apples and oilseed rape acceptable chronic and larvae risk could be concluded for “weeds”, “field margin”, “adjacent crop” and “next crop” scenarios, but unacceptable risk was indicated for “treated crop” scenario.

It is noted that 1<sup>st</sup> 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 CA3573 will be applied to apples. Additional calculations demonstrated acceptable risk for the “treated crop” scenario when the product is applied from BBCH 70 onward (i.e. after the flowering period).

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.

Furthermore, additional assessment was performed by the zRMS for lower application rate in apples (25 g a.s./ha). The same conclusions as for higher application rate were taken: unacceptable risk in “treated crop” scenario (with exception of application carried out at BBCH  $\geq 70$ ) and acceptable risk in remaining scenarios.

All additional calculations has been included by the zRMS in Table 9.6-8 above.

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.

## 2<sup>nd</sup> tier oral risk assessment

Based on the 1<sup>st</sup> tier oral risk assessment, a potential risk could not be excluded regarding chronically exposed honey bee adults as well as for honey bee larvae, although crop and life stage-specific (adult/larvae) assumptions have been considered. This is primarily driven by very conservative default shortcut values (SV) for the oral exposure that are based on sugar content, food consumption and default Residue per Unit Dose (RUD) values which are proposed by the ‘EFSA Bee Guidance Document’ (EFSA 2013).

As one option in the 2<sup>nd</sup> step of refinement, EFSA (2013) proposes to refine the nectar sugar content. The current SV used for the 1<sup>st</sup> tier oral risk assessment based on a sugar concentration of 15% for the treated crop, which was considered by the EFSA Working Group as the worst-case value (i.e. nectar with the lowest sugar content from the ranges which maybe foraged by the honey bees). The sugar concentration in nectar was taken from Rortais et al. (2005) which gave a range of 5% to 80%, but specifically mentioning 40% as representative for bee attractive crops. A comprehensive literature data analysis on the sugar content in nectar was done by Pamminger et al. (2018, KCP 10.3.1/03, see study summary Appendix 2, A.2.3.1) to compile a comprehensive geographically explicit dataset on nectar quality (i.e. total sugar concentration), offered to bees both within fields (crop and weed species) as well as outside fields (wild species) around the globe. For apples (*Malus domestica*, total<sub>n</sub> = 10) the authors found a range of the sugar content in nectar between 32.9% and 55%, median 42% (see supplied available data of Pamminger et al. 2019). For different species of *Brassica* (*B. napus*, *B. oleracea* and *B. rapa*, total<sub>n</sub> = 33), to which oil seed rape belongs (*B. napus*), the range was 36% to 62%, median 41.5%. These ranges well support the literature for flowers that provide nectar suitable for their pollinators, which suggests optimal values ranging from 35% to 65% (e.g. Kim et al. 2011). Based on EFSA’s procedure to choose the lowest sugar content from the ranges, a content of **32.9% for apples** and **36% for oil seed rape** was chosen to refine the shortcut values.

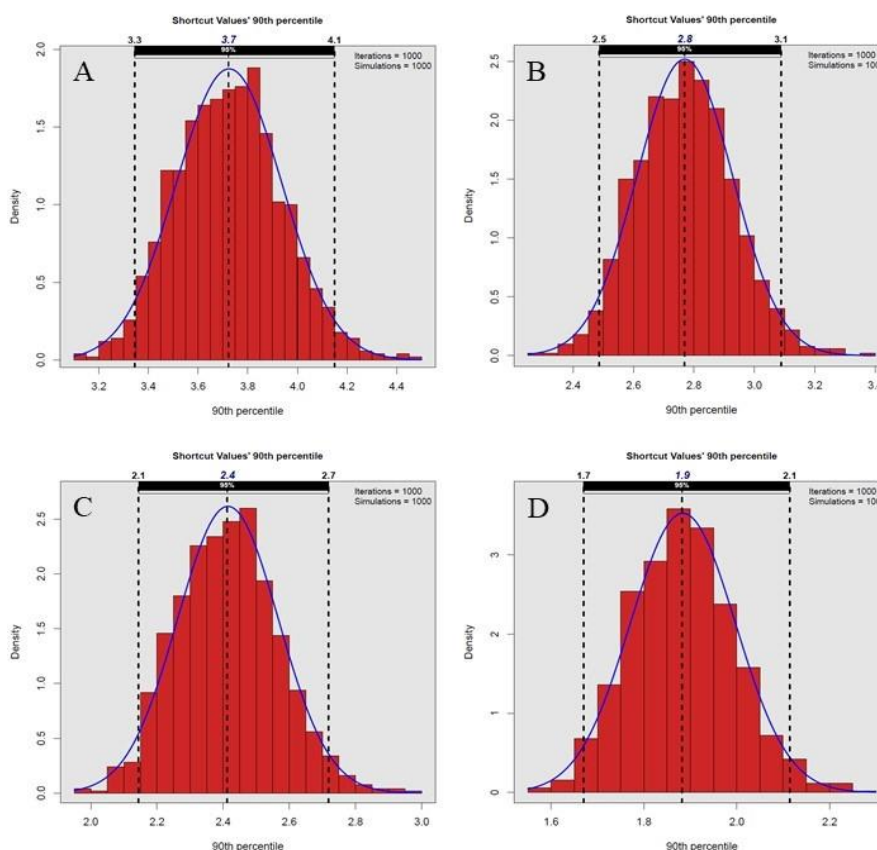
Calculations were performed using EFSA's 'SHVAL tool, version 1.1' (EFSA 2014). Input parameters (RUDS, consumption rates) for downward (DW) and side/upward foliar spray applications (SUW) were taken from Table J4 and Table J5 of the EFSA Bee GD, respectively. An overview of the input parameters and the results of the SV refinement are presented below:

**Table 9.6-9: Refinement of shortcut values (SV) for the 'treated crop' scenario based on refined sugar concentration in apple and oil seed rape using the 'SHVAL tool'**

Crop / application direction	Exposed life stage	Input parameters					SV value	
		Pollen consumption [mg/bee/day] or [mg/larva] <sup>a)</sup>	Range or value for the sugar consumption [mg/bee/day] or [mg/larva]	Sugar content [%]	Median ± SD of RUDs in pollen [mg a.s./kg]	Median ± SD of RUDs in nectar [mg a.s./kg]	Standard <sup>a)</sup>	Refined
Apple / SUW	Honey bee adult, chronic	0	32-128	32.9	1.180 ± 1.127	4.018 ± 1.044	8.2	3.7
	Honey bee larvae	2	59.4				6.1	2.8
Oil seed rape /DW	Honey bee adult, chronic	0	32-128	36	13.02 ± 1.386	2.478 ± 1.153	5.8	2.4
	Honey bee larvae	2	59.4				4.4	1.9

<sup>a)</sup> According to tables J4-J5 in EFSA (2013). For the 'treated crop' scenario values differ for up- and sideward and downward application

The figure below shows an overview of the refined SV values of the 2 exposure scenarios (i.e. apple, oil seed rape) for adult honey bees and honey bee larvae:



**Figure 9.6-1: Refinement of shortcut values (SV) based on refined sugar concentration values using 'SHVAL tool' for the 'apple' (A: 'adult chronic honey bees', B: 'honey bee larvae') and the 'oil seed rape' scenario (C: 'adult chronic honey bees', D: 'honey bee larvae')**

The refined SV values were accordingly used to refine the risk assessment, which is presented below.

**Table 9.6-10: 2<sup>nd</sup> tier oral risk assessment for honey bees (chronic, adults and larvae) in the ‘treated crop’ scenario**

Crop (Crop group according to EFSA tool) <sup>a)</sup>	Endpoint	ETR (1 <sup>st</sup> tier, default SV value)	ETR (2 <sup>nd</sup> tier, refined SV value)	Trigger
<b>Maximum single application rate: 0.050 kg a.s./ha, BBCH 62–69 PHH (risk acceptable for application at BBCH ≥70)</b>				
Apple (orchards 1)	adult, chronic	<b>0.080</b>	<b>0.036</b>	0.03
	larvae	<b>0.53</b>	<b>0.245</b>	0.2
<b>Maximum single application rate: 0.06 kg a.s./ha, BBCH 31-71</b>				
Spring and winter oil seed rape (oil seed rape)	adult, chronic	<b>0.068</b>	0.028	0.03
	larvae	<b>0.46</b>	0.199	0.2

ETR values in **bold** are above the trigger value

For the chronic oral exposure of adult honey bee and honey bee larvae via ‘treated crop’, no unacceptable risks are expected following the proposed use of CA3573 in spring and winter oil seed rape, as the refined ETR values are below the trigger values of 0.03 or 0.2, respectively.

However, for the use in apple, a chronic risk for adult honey bees and honey bee larvae still cannot be ruled out in the ‘treated field’ scenario, as both ETRs were slightly above the trigger values.

Therefore, a higher tier risk assessment is presented in the following chapter.

#### **zRMS comments:**

Calculation of the SV values based on the sugar content is an acceptable refinement option indicated in EFSA (2013). Tool SHVAL has been developed by EFSA to aid respective calculations.

However, in order to refine the sugar content in nectar, the respective data must be available and rules for their derivation are described in Appendix S of EFSA (2013). It is pointed out that the sugar content should be determined in several varieties most frequently grown in the area of the intended use. Furthermore, studies should cover various field conditions, as sugar content in nectar depends not only on crop and its variety, but also on soil properties and climatic conditions (especially air humidity). Therefore EFSA (2013) indicates that sugar content in nectar should be determined in at least 5 varieties (lower number may be accepted in case it represents significant area from the area of the intended use). Samples for each variety should be taken from at least 20 individual plants from 5 different fields in order to obtain at least 25 average data for 5 varieties investigated.

No such targeted study has been provided by the Applicant. Instead, publication by Pamminger et al. (2018) has been submitted, presenting sugar content in various plants (crops and weeds) obtained during extensive literature search. The summary of the publication has been not included in Appendix 2 and the Applicant is kindly reminded that detailed summaries of all studies used in evaluation, also from public literature, must be presented in the dRR.

The original publication has been reviewed by the zRMS and it is confirmed that sugar content in nectar of apples (n=10) and *Brassica* species (n=38) is also included in the dataset. Apples varieties are available for 5 studies (Booskoop, Jonathan, Yellow Transp., Cox Orange and Golden Delicious), but no such information is available for remaining 5 studies. Among 38 *Brassica* species, only 3 results are relevant specifically for oilseed rape and varieties are given for only two studies (Candal and Regent). No analysis of representativeness of tested varieties for particular cMS has been provided by the Applicant. Furthermore, no details regarding the sampling procedure is available in the publication (e.g. number of fields, number of samples, etc.). Taking this into account, available data are not fully reliable for purposes of the refinement of the sugar content in nectar of apples and oilseed rape. However, given the large dataset it may be expected that various climatic and soil conditions have been covered in the studies.

Although based on the available information it is not possible to decide on suitability of the available sugar content for conditions of intended uses of CA3573 in particular cMS, obtained results clearly indicate that the sugar content of 15% considered in calculation of default SV values in EFSA (2013) represent unrealistic worst case, as in all the studies the sugar content in apples and *Brassica* sp. was greater than 30% and in majority of studies exceeded 40%. As already mentioned above, only 3 studies were performed with oilseed rape and most of the results were relevant

for *Brassica rapa* (var. Toria, n=25). Remaining data points were obtained for *Raphanus raphanistrum*, *Brassica oleracea*, *Sinapis alba* and *Sinapis arvensis*. Nevertheless, due the similarity of the plants from Brassica family the zRMS is of the opinion that results obtained for other Brassica species are also representative for oilseed rape.

Overall, due to deficiencies indicated above, the results presented in publication by Pamminger et al. (2018) are considered to be not fully reliable to take the final conclusions regarding acceptability of the risk to bees in apples and oilseed rape. However, they are considered as information supporting much higher sugar content in nectar of apples and oilseed rape taken into account in calculation of default SV values in EFSA (2013).

In the above calculations the lowest value of available ranges for particular crops has been taken into account and obtained results are confirmed to be correct. Based on obtained results potentially acceptable risk to bees is indicated following intended applications in oilseed rape. However, as the considered sugar content in nectar is not fully reliable, calculations presented in Table 9.6-10 above are considered to be only indicative and are further discussed in point 9.6.2.3 together with results of semi-field and field studies.

The final discussion will be taken based on all available lines of evidence.

The Applicant is requested to provide detailed summary of Pamminger et al. (2018) during the commenting phase (the summary has been provided and is presented in Appendix 2 under KCP 10.3.1/03). Study by Kim et al. (2011) also mentioned above does not provide any useful information in order to support the sugar content in nectar of particular crops, so presentation of the abstract is deemed sufficient.

### 9.6.2.3 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 of the seven tunnel tests were carried out according to the CEB 230 methodology and followed also the EPPO 170/4 standard for semi-field tests. Three of them were conducted on *Phacelia* during the flowering period, whereas the other ones, were performed on winter wheat. 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 as 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)

At all these studies MCW 2222 was applied once at a rate of 0.5 L/ha (= 100 g a.s./ha acetamiprid).

The application of the test item out of bee presence is requested in order to be in line with the risk mitigation measure fixed in several countries like France. This risk mitigation measure requests farmers to apply insecticide or acaricide out of bee foraging, for example in the evening, during the flowering period or in case of honeydew production on crops by sucking pests. In France this risk mitigation measure was reported in the decree of September 12<sup>th</sup>, 2006. This decree obliges farmers to apply out of bee presence insecticide or acaricide having the french bee label (Mention abeilles).

Additionally, the seventh study was a bee brood study which 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 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 a.s./ha acetamiprid), the 1<sup>st</sup> before the flowering period and the

~~2<sup>nd</sup> during the flowering period but after the daily bee flight activity which is a risk mitigation measure applied during this period.~~

~~Finally, three honey bee studies under field conditions were carried out with MCW 2222, one in *Phacelia*, one in oil seed rape and one in an apple orchard 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 first one, MCW 2222 was applied once at 0.5 L/ha (= 100 g a.s./ha acetamiprid) during the flowering period of *Phacelia* whereas in the second and third one it was applied twice at 0.5 L/ha, just before and during the flowering period. In all the studies, the application during the flowering period was carried outside the foraging activity (bee flight activity). The application outside the foraging activity is regarded as a risk mitigation measure applied during this period.~~

~~Overall, the results of the CEB 230 studies indicate, that if MSC 2222 was applied to sugar syrup treated wheat, temporary effects on daily adult bee mortality occurred in two of the three studies, which lasted up to three days (D+3) after the application, independent from its timing, i.e. when applied during (T1) or after bee flight (T2). In one study, effects on the daily adult bee mortality was recorded when MCW 2222 was applied during bee flight but not if applied after. Nevertheless, no significant differences were observed compared to the control regarding the cumulative mortality in both treatment groups and all three studies at the end of the study.~~

~~Effects on the foraging activity and the behaviour (e.g. intoxication symptoms) were observed in all three studies lasting up D+3, regardless the application timing.~~

~~Despite these observations, no impact on the colony strength and the colony development was observed at any of the three studies.~~

~~In the three other CEB 230 studies, MSC 2222 was applied to flowering *Phacelia*. In contrast to the CEB 230 ‘wheat studies’ no effects on the daily adult bee mortality was observed in all three studies if MCW 2222 was applied after bee flight and in two of the three studies, if the application took place during bee flight. In the latter case, the observed effects were just slight but nevertheless significant and occurred just up to the day after application (D+1). Overall, no significant differences were observed compared to the control regarding the cumulative mortality in both treatment groups and all three studies at the end of the study. Moreover, no effects on the foraging activity, behaviour, colony strength and the colony development were observed at any of the three studies.~~

~~The results of the semi field bee rood study according to OECD GD 75 showed, that in the course of the study no effects on the daily and overall adult and pupal mortality (covering acute and chronic exposure of adult bees and larvae) as well as on the foraging activity and behaviour occurred, after having MSC 2222 been applied in the evening after bee flight. Moreover, the regular assessments of the colony strength and the colony development as well 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 than the control.~~

~~The results of the semi field bee brood study were confirmed by the field studies. 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 noticed. Moreover, the regular assessments of the colony strength and the colony development as well 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 rates, brood indices and brood compensation indices for cells filled with eggs, young and old larvae were on the control level. Especially when CA3573 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

There may be some concerns because of some rain in the 'Phacelia' field study between 2DAA and 4DAA. But precipitation on 2DAA and 4DAA was rather low (3 mm and 1 mm, respectively). Moreover, foraging activity was very high on 1DAA (10.4 bees/m<sup>2</sup>, meaning 208,000 bees foraging on the 2 ha field). And even on 2DAA (3.5 bees/m<sup>2</sup>, meaning 70,000 bees foraging) and 3DAA (0.9 bees/m<sup>2</sup>, meaning 18,000 bees foraging) a sufficient number of foraging of bees was observed, indicating a sufficient exposure. In the 'oil seed rape' study, concerns might attribute to short distances between the study fields and other fields with oil seed rape not treated with acetamiprid. Although it cannot be fully excluded that bees did not forage on the treated field, the study field size of 3 ha offers a huge foraging area for seven honey bee colonies only, which is thus more attractive than rape fields in a distance of at least 1 km. This is indicated by the data on the foraging activity, which can be regarded as quite high for that time of the year. Moreover, study fields were also surrounded by non attractive cereals fields and woods which can be regarded as natural barriers for bees and reduce their foraging range. Finally, residue data of the 'phacelia' and 'oil seed rape' field study obtained from in hive nectar on DAA 3/DALA 3 indicate similar or even higher levels compared to the study in the apple orchard, where no limitations of the study were observed. Overall, exposure in the three field studies can be considered to be sufficient and thus observed results can be regarded reliable.

Overall, based on the presented data it can be concluded, that MCW-2222 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 crops up to a rate of 100 g a.s./ha acetamiprid after daily bee flight. Moreover, application in the evening after bee flight activity is regarded as a suitable risk mitigation measure to avoid any risk for honey bees foraging on bee attractive crops.

#### **zRMS comments:**

The performed risk assessment demonstrated potentially unacceptable chronic and larvae risk following application of CA3573 to apples and oilseed rape. The Applicant presented refinement based on the measured sugar content in apples and OSR nectar, derived from the literature data and demonstrating acceptable risk following application of the product in oilseed rape. However, evaluation of the publication by Pamminer et al. (2018) by the zRMS demonstrated that not sufficient information regarding the considered crop varieties and collection of nectar samples is available, therefore refinement of the risk based on data from Pamminer et al. (2018) could be considered as indicative, but not reliable enough to be the basis for definite conclusions on acceptability of the risk.

As acceptable risk was indicated by the Applicant based on Pamminer et al. (2018) data, refinement of the risk with consideration of semi-field and field studies was carried out only for apples, while oilseed rape was not taken into account. However, as indicated above, no firm conclusions could be taken also for oilseed rape and further refinement of the risk in this crop is also needed.

In addition to that, evaluation of the semi-field and field studies by the zRMS revealed some deficiencies, which were not taken into account in the Applicants' evaluation above. For this reason the zRMS performed its own refinement of the risk based on higher-tier data, while Applicants' evaluation above has been struck through.

The summaries of higher-tier studies together with detailed zRMS evaluation may be found in in Appendix 2, A 2.3.1.7 and A 2.3.1.8. Obtained results and observed deficiencies are also shortly summarised in Table 9.6-3 above.

#### 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 is 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, 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 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.

Unacceptable chronic risk to adult bees and larvae was also indicated based on calculations performed with consideration of the refined sugar content in apples nectar for application rate of 50 g a.s./ha. The ETR values could be lower for application rate of 25 g a.s./ha, however the data on nectar sugar content collected by Pamminer et al. (2018) was considered to be not fully reliable by the zRMS, so performed calculations cannot be used to exclude the risk to bees following application to apples at lower rate.

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.

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 evaluation are presented in Appendix 2, while the summary of obtained results is presented in Table 9.6-3 in point 9.6.1 above.

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). The summary of obtained results is provided in Table 9.6-3 in point 9.6.1 above.

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 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. This is further confirmed by additional refinement of the risk assessment performed in line with EFSA (2013) with acceptable ETR values calculated with consideration of the lowest nectar sugar content in *Brassica* family flowers indicated in publication by Pamminger et al. (2018). As already mentioned by the zRMS, calculation based on data collected by Pamminger et al. (2018) was itself not sufficient to conclude on acceptable risk to bees but it may be considered as additional support to the higher tier data.

### 9.6.3 Effects on bumble bees

See chapter 9.6.1, **Błąd! Nie można odnaleźć źródła odwołania..**

### 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

#### **zRMS comments:**

The risk assessment performed in line with SANCO/1039/2002 demonstrated acceptable risk to bees following application of CA3573 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 CA3573 in potatoes and maize.

For apples acceptable acute and chronic risk could be concluded for applications performed after flowering (from BBCH 70 onwards) for all routes of exposure, while for application carried out at BBCH 62-69 unacceptable chronic risk was concluded for adult bees and larvae exposed in the treated crop scenario. For oilseed rape acceptable risk could be concluded for weeds, field margin, adjacent crop and next crop scenarios, but unacceptable risk was concluded for chronic risk was concluded for adult bees and larvae exposed in the treated crop scenario.

Refinement of the risk based on sugar content in nectar of apples and oilseed rape confirmed unacceptable risk following application to apples and acceptable risk following application to oilseed rape. However, these calculations were considered by the zRMS to be not fully reliable and were thus concluded to be illustrative only.

Available higher tier studies were not sufficient to address the risk to bees following application of CA3573 to flowering apples and for this reason the intended uses in this crop are restricted to the post-flowering period (BBCH 70-PHI).

Based on the tunnel, semi-field and field studies the risk following application to flowering oilseed rape at 60 g a.s./ha was concluded to be acceptable, provided that application is carried out in the evening, after the bee flight.

~~Almost all HQ/ETR values calculated for the acute risk for bumble bees, the acute and chronic risk for adult honey bees as well as for honey bee larvae, being directly exposed to CA3573 in apple, potatoes, oil seed rape and corn via overspray or via residues in pollen, nectar and water, were below the relevant trigger values at the screening step, 1<sup>st</sup> tier assessment or 2<sup>nd</sup> tier assessment. Exceptions were observed for the chronic exposure of adult honeybees and honeybee larvae via ‘treated crops’ or ‘weeds’ with ETRs above the trigger when exposed to an application rate of 50 g a.s./ha in apple orchards. But higher tier risk refinement based on seven semi field and three field studies indicated acceptable risk for bees following the use of CA3573 according to the proposed use pattern.~~

## 9.7 Effects on arthropods other than bees (KCP 10.3.2)

### 9.7.1 Toxicity data

Effects on non-target arthropods of CA3573 were not evaluated as part of the EU assessment of acetamiprid. New data submitted with this application are listed in 0 and summarised in Appendix 2.

**Table 9.7-1: Endpoints and effect values relevant for the risk assessment for non-target arthropods - CA3573**

Species	Substance	Exposure System	Results	Reference
<b>Laboratory studies</b>				
<i>Typhlodromus pyri</i> (protonymphs)	MCW-2222	Laboratory test glass plates (2D)	<b>LR<sub>50</sub> = 9.13 g a.s./ha</b> ER <sub>50</sub> = > 6.17 g a.s./ha	Röhlig, U., 2014 R-33838 KCP 10.3.2.1/01
<i>Aphidius rhopalosiphi</i> (adults)	MCW-2222	Laboratory test glass plates (2D)	<b>LR<sub>50</sub> = 0.0243 g a.s./ha</b> ER <sub>50</sub> -	Röhlig, U., 2014 R-33839 KCP 10.3.2.1/02
<b>Extended laboratory studies</b>				
<i>Typhlodromus pyri</i> (protonymphs)	MCW-2222	Extended laboratory test, bean leafs (2D)	<b>LR<sub>50</sub> = 31.9 g a.s./ha</b> <b>ER<sub>50</sub> = &gt; 12.5 g a.s./ha</b>	Röhlig, U., 2014 R-34780 KCP 10.3.2.2/01
<i>Aphidius rhopalosiphi</i> (adults)	MCW-2222	Extended laboratory bean leafs (2D)	<b>LR<sub>50</sub> = 0.111 g a.s./ha</b> ER <sub>50</sub> = 0.1 g a.s./ha	Stevens, J., 2015 R-35026 KCP 10.3.2.2/02
<i>Aphidius rhopalosiphi</i> (adults)	MCW-2222	Extended laboratory test, barley plants (3D)	<b>LR<sub>50</sub> = 3.56 g/ha</b> ER <sub>50</sub> ≤ 0.64 g/ha <sup>a</sup>	Röhlig, U., 2014 R-33839A KCP 10.3.2.2/03
<i>Chrysoperla carnea</i>	MCW-2222	Extended laboratory test, bean leafs (2D)	<b>LR<sub>50</sub> = 106 g a.s./ha</b> ER <sub>50</sub> > 116 g a.s./ha	Röhlig, U., 2014 R-34781 KCP 10.3.2.2/04
<i>Coccinella septempunctata</i>	MCW-2222	Extended laboratory test, bean leaf (2D)	<b>LR<sub>50</sub> = 22.1 g a.s./ha</b> ER <sub>50</sub> = 20.7 g a.s./ha	Röhling, U. 2014 R-34782 KCP 10.3.2.2/05
<i>Aleochara bilineata</i>	Metabolite IM-1-5	Extended laboratory test, sand, 2D	ER <sub>50</sub> = 62.5 mg/kg <sup>b</sup>	Schmitzer, S., 2003 RD-03101, 2 <sup>nd</sup> Addendum 2 of the DAR (2003)

Species	Substance	Exposure System	Results	Reference
<b>Aged residue studies</b>				
<i>Typhlodromus pyri</i>	MCW-2222	Aged Residue Test (leaves of potted apples plants, 2D <del>3D</del> )	<p><b><u>102 g a.s./ha</u></b></p> <p>Mortality: 1.06% at 0 DAT -4.30% at 35 DAT 2.13% at 42 DAT</p> <p>Red. of reproduction: 7.41% at 0 DAT -16.61% at 35 DAT -5.66% at 42 DAT</p> <p><b><u>170 g a.s./ha</u></b></p> <p>Mortality: 42.55% at 0 DAT 0% at 35 DAT 3.19% at 42 DAT</p> <p>Red. of reproduction 27.65% at 0 DAT -3.37% at 35 DAT 9.24% at 42 DAT</p>	Luna, F., 2017b R-37335 KCP 10.3.2.3/05
<i>Aphidius rhopalosiphi</i>	MCW-2222	Aged Residue Test (leaves of potted bean plants, 2D <del>3D</del> )	<p><b><u>45 g a.s./ha</u></b></p> <p>Mortality: 100% at 0 DAT 10% at 28 DAT 5% at 36 DAT</p> <p>Red. of reproduction: Not determined for 0 DAT -35.0% at 28 DAT 12.0% at 36 DAT</p>	Luna, F., 2016a R-36938A / TRC15-242BA KCP 10.3.2.3/01
<i>Aphidius rhopalosiphi</i>	MCW-2222	Aged Residue Test (leaves of potted bean plants, 2D <del>3D</del> )	<p><b><u>70 g a.s./ha</u></b></p> <p>Mortality: 100% at 0 DAT 27.5% at 28 DAT 20% at 36 DAT</p> <p>Red. of reproduction: Not determined for 0 DAT -6.56% at 28 DAT 15.92 % at 36 DAT</p>	Luna, F., 2016b TRC15-243BA KCP 10.3.2.3/02
<i>Aphidius rhopalosiphi</i>	MCW-2222	Aged Residue Test (leaves of potted bean plants, 2D <del>3D</del> )	<p><b><u>102 g a.s./ha</u></b></p> <p>Mortality: 100% at 0 DAT 75% at 28 DAT 42.5% at 36 DAT 23.5% at 42 DAT</p> <p>Red. of reproduction: Not determined for 0 DAT, 28 DAT and 36 DAT 11.7% at 42 DAT</p>	Luna, F., 2016c TRC15-244BA KCP 10.3.2.3/03

Species	Substance	Exposure System	Results	Reference
<i>Aphidius rhopalosiphi</i>	MCW-2222	Aged Residue Test ( <del>branches</del> leaves of potted apple plants, 3D)	<b>170 g a.s./ha</b>  Mortality: 100% at 0 DAT 28.57% at 42 DAT 14.29% at 49 DAT  Red. of reproduction N/A at 0 DAT 41.97% at 42 DAT -8.41% at 49 DAT	Luna, F., 2017a R-37333 / TRC16-073BA KCP 10.3.2.3/04
<i>Coccinella septempunctata</i>	MCW-2222	Aged Residue Test ( <del>branches</del> leaves of potted apple plants, 3D)	<b>102 g a.s./ha</b>  Mortality: 48.72% at 0 DAT 5.26% at 35 DAT 2.83% at 42 DAT  No of fertile eggs/female/day: 25.77 at 0 DAT 10.43 at 35 DAT 24.20 at 42 DAT  Egg viability: 96.62% at 0 DAT 89.43% at 35 DAT 97.24% at 42 DAT  <b>170 g a.s./ha</b>  Mortality: 61.54% at 0 DAT 5.13% at 35 DAT 3.05% at 42 DAT  No of fertile eggs/female/day: not assayed at 0 DAT 11.98 at 35 DAT 19.94 at 42 DAT  Egg viability: not assayed at 0 DAT 93.93% at 35 DAT 95.71% at 42 DAT	Luna, F., 2017c R-37334 / TRC16-075BA KCP 10.3.2.3/06
<b>Higher-tier studies</b>				
Species	Substance	Endpoint used for risk assessment		Reference
Non-target arthropod fauna	MCW - 2222	Lowest Observed Ecological Adverse Effect Rate (LOEAER) for population = 7.2 g a.s./ha. No Observed Ecological Adverse Effect Rate (NOEAER) = <del>3.4 g a.s./ha</del> <del>3.6 g a.s./ha</del> No Observed Ecological Effect rate (NOER) = 1.4 g a.s./ha No Observed Ecological Effect rate (NOER) = <del>7.2 g a.s./ha</del>		Appeltaufer, A 2016, R-35848, KCP 10.3.2.4/01

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

<sup>a</sup> no ER<sub>50</sub> could be determined in this study

<sup>b</sup> presented as additional data, no data requirement and not relied upon

#### **zRMS comments:**

All the laboratory and extended laboratory studies on effects of CA3573 (formerly 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, A 2.3.2.1 and A 2.3.2.2.

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 conclusions on acceptability are provided in Appendix 2, A 2.3.2.4.

Aged residue studies were submitted in support of the re-evaluation process. Summaries of the studies together with their evaluation by the zRMS may be found in Appendix 2, A 2.3.2.3.

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.

#### **9.7.1.1 Justification for new endpoints**

CA3573/MCW-2222 was not the representative formulation for the renewal of the active substance acetamiprid. New studies on effects of the formulation CA3573 on non-target arthropods were carried out as required by Regulation (EU) 284/2013. The studies with CA3573 were conducted in accordance with the most recent guidelines.

#### **9.7.2 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.

### 9.7.2.1 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 CA3573 in apple**

<b>Intended use</b>	Apple		
<b>Product</b>	CA3573		
<b>Application rate (g/ha)</b>	1 x 50		
<b>MAF</b>	1		
<b>3D crop correction factor</b>	0.5		
<b>Test species</b>	<b>LR<sub>50</sub> (lab.)</b>	<b>PER<sub>in-field</sub></b>	<b>HQ<sub>in-field</sub></b>
<b>Tier I</b>	<b>(g/ha)</b>	<b>(g/ha)</b>	<b>criterion: HQ ≤ 2</b>
<i>Typhlodromus pyri</i>	LR <sub>50</sub> = 9.13 g a.s./ha	25	<b>2.74</b>
<i>Aphidius rhopalosiphi</i>	LR <sub>50</sub> = 0.0243 g a.s./h		<b>1029</b>
<b>Extended laboratory studies</b>	<b>Rate with ≤ 50 % effect*</b>	<b>PER<sub>in-field</sub></b>	<b>PER<sub>in-field</sub> below rate with</b>
	<b>(g/ha)</b>	<b>(g/ha)</b>	<b>≤ 50 % effect?</b>
<i>Typhlodromus pyri</i>	LR <sub>50</sub> = 31.9 g a.s./ha ER <sub>50</sub> = > 12.5 g a.s./ha	25	<b>no</b>
<i>Aphidius rhopalosiphi</i>	LR <sub>50</sub> = 0.111 g a.s./ha ER <sub>50</sub> = 0.100 g a.s./ha		<b>no</b>
<i>Aphidius rhopalosiphi</i>	LR <sub>50</sub> = 3.56 g a.s./ha ER <sub>50</sub> < 0.64 g a.s./ha		<b>no</b>
<i>Chrysoperla carnea</i>	LR <sub>50</sub> = 106 g a.s./ha ER <sub>50</sub> = 116 g a.s./ha		yes
<i>Coccinella septempunctata</i>	LR <sub>50</sub> = 22.1 g a.s./ha ER <sub>50</sub> = 20.7 g a.s./ha		<b>no</b>
<b>Aged residue studies</b>	<b>Rate with ≤ 50 % effect</b>	<b>PER<sub>in-field</sub></b>	<b>PER<sub>in-field</sub> below rate with</b>
	<b>(g/ha) at xxx DAT</b>	<b>(g/ha)</b>	<b>≤ 50 % effect?</b>
<i>Typhlodromus pyri</i>	102 g a.s./ha at 0 DAT	25	yes
<i>Aphidius rhopalosiphi</i>	45 g a.s./ha at 28 DAT		yes
<i>Aphidius rhopalosiphi</i>	70 g a.s./ha at 28 DAT		yes
<i>Aphidius rhopalosiphi</i>	102 g a.s./ha at 42 <del>36</del> DAT		yes
<i>Coccinella septempunctata</i>	102 g a.s./ha at 0 DAT		yes

MAF: Multiple application factor; PER: Predicted environmental rate; HQ: Hazard quotient; DAT: Days after last treatment.

Criteria values shown in bold breach the relevant trigger.

\* If an LR<sub>50</sub> or ER<sub>50</sub> 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 CA3573 in apple**

<b>Intended use</b>	Apple		
<b>Product</b>	CA3573		
<b>Application rate (g/ha)</b>	1 × 25		
<b>MAF</b>	1		
<b>3D crop correction factor</b>	0.5		
<b>Test species</b>	<b>LR<sub>50</sub> (lab.)</b>	<b>PER<sub>in-field</sub></b>	<b>HQ<sub>in-field</sub></b>
<b>Tier I</b>	<b>(g/ha)</b>	<b>(g/ha)</b>	<b>criterion: HQ ≤ 2</b>
<i>Typhlodromus pyri</i>	LR <sub>50</sub> = 9.13 g a.s./ha	12.5	1.37
<i>Aphidius rhopalosiphi</i>	LR <sub>50</sub> = 0.0243 g a.s./h		<b>514</b>
<b>Extended laboratory studies</b>	<b>Rate with ≤ 50 % effect*</b>	<b>PER<sub>in-field</sub></b>	<b>PER<sub>in-field</sub> below rate with</b>
	<b>(g/ha)</b>	<b>(g/ha)</b>	<b>≤ 50 % effect?</b>
<i>Typhlodromus pyri</i>	LR <sub>50</sub> = 31.9 g a.s./ha ER <sub>50</sub> = > 12.5 g a.s./ha	12.5	yes
<i>Aphidius rhopalosiphi</i>	LR <sub>50</sub> = 0.111 g a.s./ha ER <sub>50</sub> = 0.100 g a.s./ha		<b>no</b>
<i>Chrysoperla carnea</i>	LR <sub>50</sub> = 3.56 g a.s./ha ER <sub>50</sub> < 0.64 g a.s./ha		yes
<i>Coccinella septempunctata</i>	LR <sub>50</sub> = 106 g a.s./ha ER <sub>50</sub> = 116 g a.s./ha		yes
<b>Aged residue studies</b>	<b>Rate with ≤ 50 % effect</b>	<b>PER<sub>in-field</sub></b>	<b>PER<sub>in-field</sub> below rate with</b>
	<b>(g/ha) at x DAT</b>	<b>(g/ha)</b>	<b>≤ 50 % effect?</b>
<i>Typhlodromus pyri</i>	102 g a.s./ha at 0 DAT	12.5	yes
<i>Aphidius rhopalosiphi</i>	45 g a.s./ha at 28 DAT		yes
<i>Aphidius rhopalosiphi</i>	70 g a.s./ha at 28 DAT		yes
<i>Aphidius rhopalosiphi</i>	102 g a.s./ha at <b>42</b> <del>36</del> DAT		yes
<i>Coccinella septempunctata</i>	102 g a.s./ha at 0 DAT		yes

MAF: Multiple application factor; PER: Predicted environmental rate; HQ: Hazard quotient; DAT: Days after last treatment.  
Criteria values shown in bold breach the relevant trigger.

\* If an LR<sub>50</sub> or ER<sub>50</sub> 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 CA3573 in potato**

<b>Intended use</b>	Potato		
<b>Product</b>	CA3573		
<b>Application rate (g/ha)</b>	1 × 36		
<b>MAF</b>	1		
<b>Test species</b>	<b>LR<sub>50</sub> (lab.)</b>	<b>PER<sub>in-field</sub></b>	<b>HQ<sub>in-field</sub></b>
<b>Tier I</b>	<b>(g/ha)</b>	<b>(g/ha)</b>	<b>criterion: HQ ≤ 2</b>
<i>Typhlodromus pyri</i>	LR <sub>50</sub> = 9.13 g a.s./ha	36	<b>3.94</b>
<i>Aphidius rhopalosiphi</i>	LR <sub>50</sub> = 0.0243 g a.s./h		<b>1481</b>
<b>Extended laboratory studies</b>	<b>Rate with ≤ 50 % effect*</b>	<b>PER<sub>in-field</sub></b>	<b>PER<sub>in-field</sub> below rate with</b>
	<b>(g/ha)</b>	<b>(g/ha)</b>	<b>≤ 50 % effect?</b>
<i>Typhlodromus pyri</i>	LR <sub>50</sub> = 31.9 g a.s./ha ER <sub>50</sub> = > 12.5 g a.s./ha	36	<b>no</b>
<i>Aphidius rhopalosiphi</i>	LR <sub>50</sub> = 0.111 g a.s./ha ER <sub>50</sub> = 0.100 g a.s./ha		<b>no</b>
<i>Aphidius rhopalosiphi</i>	LR <sub>50</sub> = 3.56 g a.s./ha ER <sub>50</sub> < 0.64 g a.s./ha		<b>no</b>
<i>Chrysoperla carnea</i>	LR <sub>50</sub> = 106 g a.s./ha ER <sub>50</sub> = 116 g a.s./ha		yes
<i>Coccinella septempunctata</i>	LR <sub>50</sub> = 22.1 g a.s./ha ER <sub>50</sub> = 20.7 g a.s./ha		<b>no</b>
<b>Aged residue studies</b>	<b>Rate with ≤ 50 % effect</b>	<b>PER<sub>in-field</sub></b>	<b>PER<sub>in-field</sub> below rate with</b>
	<b>(g/ha) at x DAT</b>	<b>(g/ha)</b>	<b>≤ 50 % effect?</b>
<i>Typhlodromus pyri</i>	102 g a.s./ha at 0 DAT	36	yes
<i>Aphidius rhopalosiphi</i>	45 g a.s./ha at 28 DAT		yes
<i>Aphidius rhopalosiphi</i>	70 g a.s./ha at 28 DAT		yes
<i>Aphidius rhopalosiphi</i>	102 g a.s./ha at 42 <del>36</del> DAT		yes
<i>Coccinella septempunctata</i>	102 g a.s./ha at 0 DAT		yes

MAF: Multiple application factor; PER: Predicted environmental rate; HQ: Hazard quotient; DAT: Days after last treatment.

Criteria values shown in bold breach the relevant trigger.

\* If an LR<sub>50</sub> or ER<sub>50</sub> 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 CA3573 in oilseed rape and corn**

<b>Intended use</b>	OSR (winter/spring); corn		
<b>Product</b>	CA3573		
<b>Application rate (g/ha)</b>	1 × 60		
<b>MAF</b>	1		
<b>Test species</b>	<b>LR<sub>50</sub> (lab.)</b>	<b>PER<sub>in-field</sub></b>	<b>HQ<sub>in-field</sub></b>
<b>Tier I</b>	<b>(g/ha)</b>	<b>(g/ha)</b>	<b>criterion: HQ ≤ 2</b>
<i>Typhlodromus pyri</i>	LR <sub>50</sub> = 9.13 g a.s./ha	60	<b>6.57</b>
<i>Aphidius rhopalosiphi</i>	LR <sub>50</sub> = 0.0243 g a.s./h		<b>2469</b>
<b>Extended laboratory studies</b>	<b>Rate with ≤ 50 % effect*</b>	<b>PER<sub>in-field</sub></b>	<b>PER<sub>in-field</sub> below rate with</b>
	<b>(g/ha)</b>	<b>(g/ha)</b>	<b>≤ 50 % effect?</b>
<i>Typhlodromus pyri</i>	LR <sub>50</sub> = 31.9 g a.s./ha ER <sub>50</sub> = > 12.5 g a.s./ha	60	<b>no</b>
<i>Aphidius rhopalosiphi</i>	LR <sub>50</sub> = 0.111 g a.s./ha ER <sub>50</sub> = 0.100 g a.s./ha		<b>no</b>
<i>Aphidius rhopalosiphi</i>	LR <sub>50</sub> = 3.56 g a.s./ha ER <sub>50</sub> < 0.64 g a.s./ha		<b>no</b>
<i>Chrysoperla carnea</i>	LR <sub>50</sub> = 106 g a.s./ha ER <sub>50</sub> = 116 g a.s./ha		yes
<i>Coccinella septempunctata</i>	LR <sub>50</sub> = 22.1 g a.s./ha ER <sub>50</sub> = 20.7 g a.s./ha		<b>no</b>
<b>Aged residue studies</b>	<b>Rate with ≤ 50 % effect</b>	<b>PER<sub>in-field</sub></b>	<b>PER<sub>in-field</sub> below rate with</b>
	<b>(g/ha) at xxx DAT</b>	<b>(g/ha)</b>	<b>≤ 50 % effect?</b>
<i>Typhlodromus pyri</i>	102 g a.s./ha at 0 DAT	60	yes
<i>Aphidius rhopalosiphi</i>	45 g a.s./ha at 28 DAT		<b>no</b>
<i>Aphidius rhopalosiphi</i>	70 g a.s./ha at 28 DAT		yes
<i>Aphidius rhopalosiphi</i>	102 g a.s./ha at 42 <del>36</del> DAT		yes
<i>Coccinella septempunctata</i>	102 g a.s./ha at 0 DAT		yes

MAF: Multiple application factor; PER: Predicted environmental rate; HQ: Hazard quotient; DAT: Days after last treatment.  
Criteria values shown in bold breach the relevant trigger.

\* If an LR<sub>50</sub> or ER<sub>50</sub> 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 only exception was application to apples at 25 g a.s./ha (12.5 g a.s./ha after correction for 3D crop, in line with ESCORT 2), for which acceptable risk at Tier II could be concluded for majority of species with exception of *A. rhopalosiphi*.

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 CA3573 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 CA3573.

## 9.7.2.2 Risk assessment for off-field exposure

The results of the first and higher tier risk assessments are summarised in the following tables.

**Table 9.7.6: First and higher tier assessment of the off-field risk for non-target arthropods due to the use of CA3573 in apple**

<b>Intended use</b>	Apple				
<b>Product</b>	CA3573				
<b>Application rate (g/ha)</b>	1 x 50				
<b>MAF</b>	1				
<b>vdf</b>	10 (2D) / not applicable (3D)				
<b>Test species</b>	<b>LR<sub>50</sub> (lab.)</b>	<b>Drift rate</b>	<b>PER<sub>off-field</sub></b>	<b>CF</b>	<b>HQ<sub>off-field</sub></b>
<b>Tier I</b>	<b>(g/ha)</b>		<b>(g/ha)</b>		<b>criterion: HQ ≤ 2</b>
<i>Typhlodromus pyri</i>	LR <sub>50</sub> = 9.13 g a.s./ha	0.1573	0.7865	10	0.73
<i>Aphidius rhopalosiphii</i>	LR <sub>50</sub> = 0.0243 g a.s./h				27.6
<i>Typhlodromus pyri</i>	LR <sub>50</sub> = 9.13 g a.s./ha	0.2920		10	
<i>Aphidius rhopalosiphii</i>	LR <sub>50</sub> = 0.0243 g a.s./h				
<b>Extended laboratory studies</b>	<b>Rate with ≤ 50 % effect*</b>	<b>Drift rate</b>	<b>PER<sub>off-field</sub></b>	<b>CF</b>	<b>corr. PER<sub>off-field</sub> below rate with ≤ 50 % effect?</b>
	<b>(g/ha)</b>		<b>(g/ha)</b>		
<i>Typhlodromus pyri</i>	ER <sub>50</sub> = > 12.5 g a.s./ha	0.1573	0.7865	5	yes
<i>Aphidius rhopalosiphii</i>	LR <sub>50</sub> = 0.111 g a.s./ha		0.7865		no
<i>Aphidius rhopalosiphii</i>	LR <sub>50</sub> = 3.56 g a.s./ha		7.865		no
<i>Chrysoperla carnea</i>	LR <sub>50</sub> = 106 g a.s./ha		0.7865		yes
<i>Coccinella septempunctata</i>	ER <sub>50</sub> = 20.7 g a.s./ha		0.7865		yes
<b>Aged-residue studies</b>	<b>Rate with ≤ 50 % effect</b>	<b>Drift rate</b>	<b>PER<sub>off-field</sub></b>	<b>CF</b>	<b>PER<sub>in-field</sub> below rate with ≤ 50 % effect?</b>
	<b>(g/ha) at x DAT</b>		<b>(g/ha)</b>		
<i>Typhlodromus pyri</i>	102 g a.s./ha at 0 DAT	0.1573	7.865	5	yes
<i>Aphidius rhopalosiphii</i>	45 g a.s./ha at 28 DAT				yes
<i>Coccinella septempunctata</i>	102 g a.s./ha at 0 DAT				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 LR<sub>50</sub> or ER<sub>50</sub> 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 off-field risk for non-target arthropods due to the use of CA3573 in apple**

<b>Intended use</b>	Apple				
<b>Product</b>	CA3573				
<b>Application rate (g/ha)</b>	1 x 25				
<b>MAF</b>	Not applicable				
<b>vdf</b>	10 (2D) / not applicable (3D)				
<b>Test species</b>	<b>LR<sub>50</sub> (lab.)</b>	<b>Drift rate</b>	<b>PER<sub>off-field</sub></b>	<b>CF</b>	<b>HQ<sub>off-field</sub></b>
<b>Tier I</b>	<b>(g/ha)</b>		<b>(g/ha)</b>		<b>criterion: HQ ≤ 2</b>
<i>Typhlodromus pyri</i>	LR <sub>50</sub> = 9.13 g a.s./ha	0.1573	0.39	10	0.43
<i>Aphidius rhopalosiphii</i>	LR <sub>50</sub> = 0.0243 g a.s./h				160
<b>Extended laboratory studies</b>	<b>Rate with ≤ 50 % effect*</b>	<b>Drift rate</b>	<b>PER<sub>off-field</sub></b>	<b>CF</b>	<b>corr. PER<sub>off-field</sub> below rate with ≤ 50 % effect?</b>
	<b>(g/ha)</b>		<b>(g/ha)</b>		
<i>Typhlodromus pyri</i>	ER <sub>50</sub> = > 12.5 g a.s./ha	0.1573	0.39	5	yes
<i>Aphidius rhopalosiphii</i>	LR <sub>50</sub> = 0.111 g a.s./ha		0.39		no
<i>Aphidius rhopalosiphii</i>	LR <sub>50</sub> = 3.56 g a.s./ha		3.9		no
<i>Chrysoperla carnea</i>	LR <sub>50</sub> = 106 g a.s./ha		0.39		yes
<i>Coccinella septempunctata</i>	ER <sub>50</sub> = 20.7 g a.s./ha		0.67		yes

Aged-residue-studies	Rate with $\leq 50\%$ effect (g/ha) at xx DAT	Drift-rate	PER <sub>off-field</sub> (g/ha)	CF	PER <sub>in-field</sub> -below-rate with $\leq 50\%$ effect?
<i>Typhlodromus pyri</i>	102 g a.s./ha at 0 DAT	0.1573	3.9	5	yes
<i>Aphidius rhopalosiphi</i>	45 g a.s./ha at 28 DAT				yes
<i>Coccinella septempunctata</i>	102 g a.s./ha at 0 DAT				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 LR<sub>50</sub> or ER<sub>50</sub> from a relevant extended laboratory test is available, it should be considered in place of the rate with  $\leq 50\%$  effect.

**Table 9.7-8: First- and higher tier assessment of the off-field risk for non-target arthropods due to the use of CA3573 in potato**

Intended use	OSR (winter/spring); corn				
Product	CA3573				
Application rate (g/ha)	1 x 36				
MAF	1				
vdf	10 (2D) / not applicable (3D)				
Test species	LR <sub>50</sub> (lab.) (g/ha)	Drift-rate	PER <sub>off-field</sub> (g/ha)	CF	HQ <sub>off-field</sub> criterion: HQ $\leq 2$
Tier I					
<i>Typhlodromus pyri</i>	LR <sub>50</sub> = 9.13 g a.s./ha	0.0277	0.0997	10	0.11
<i>Aphidius rhopalosiphi</i>	LR <sub>50</sub> = 0.0243 g a.s./h				41
Extended laboratory studies	Rate with $\leq 50\%$ effect* (g/ha)	Drift-rate	PER <sub>off-field</sub> (g/ha)	CF	corr. PER <sub>off-field</sub> -below-rate with $\leq 50\%$ effect?
<i>Typhlodromus pyri</i>	ER <sub>50</sub> = > 12.5 g a.s./ha	0.0277	0.0997	5	yes
<i>Aphidius rhopalosiphi</i>	LR <sub>50</sub> = 0.111 g a.s./ha		0.0997		no
<i>Aphidius rhopalosiphi</i>	LR <sub>50</sub> = 3.56 g a.s./ha		0.997		no
<i>Chrysoperla carnea</i>	LR <sub>50</sub> = 106 g a.s./ha		0.0997		yes
<i>Coccinella septempunctata</i>	ER <sub>50</sub> = 20.7 g a.s./ha		0.0997		yes
Aged-residue-studies	Rate with $\leq 50\%$ effect (g/ha) at x DAT	Drift-rate	PER <sub>off-field</sub> (g/ha)	CF	PER <sub>in-field</sub> -below-rate with $\leq 50\%$ effect?
<i>Typhlodromus pyri</i>	102 g a.s./ha at 0 DAT	0.0277	0.997	5	yes
<i>Aphidius rhopalosiphi</i>	45 g a.s./ha at 28 DAT				yes
<i>Coccinella septempunctata</i>	102 g a.s./ha at 0 DAT				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 LR<sub>50</sub> or ER<sub>50</sub> from a relevant extended laboratory test is available, it should be considered in place of the rate with  $\leq 50\%$  effect.

**Table 9.7-9: First- and higher-tier assessment of the off-field risk for non-target arthropods due to the use of CA3573 in oilseed rape and corn**

<b>Intended use</b>	OSR (winter/spring); corn				
<b>Product</b>	CA3573				
<b>Application rate (g/ha)</b>	1 x 60				
<b>MAF</b>	1				
<b>vdf</b>	10 (2D) / not applicable (3D)				
<b>Test species</b>	<b>LR<sub>50</sub> (lab.)</b>	<b>Drift rate</b>	<b>PER<sub>off-field</sub></b>	<b>CF</b>	<b>HQ<sub>off-field</sub></b>
<b>Tier I</b>	<b>(g/ha)</b>		<b>(g/ha)</b>		<b>criterion: HQ ≤ 2</b>
<i>Typhlodromus pyri</i>	LR <sub>50</sub> = 9.13 g a.s./ha	0.0277	0.1662	10	0.18
<i>Aphidius rhopalosiphi</i>	LR <sub>50</sub> = 0.0243 g a.s./ha				68
<b>Extended laboratory studies</b>	<b>Rate with ≤ 50 % effect*</b>	<b>Drift rate</b>	<b>PER<sub>off-field</sub></b>	<b>CF</b>	<b>corr. PER<sub>off-field</sub> below rate with ≤ 50 % effect?</b>
	<b>(g/ha)</b>		<b>(g/ha)</b>		
<i>Typhlodromus pyri</i>	ER <sub>50</sub> = > 12.5 g a.s./ha	0.0277	0.1662	5	yes
<i>Aphidius rhopalosiphi</i>	LR <sub>50</sub> = 0.111 g a.s./ha		0.1662		no
<i>Aphidius rhopalosiphi</i>	LR <sub>50</sub> = 3.56 g a.s./ha		1.662		no
<i>Chrysoperla carnea</i>	LR <sub>50</sub> = 106 g a.s./ha		0.1662		yes
<i>Coccinella septempunctata</i>	ER <sub>50</sub> = 20.7 g a.s./ha		0.1662		yes
<b>Aged residue studies</b>	<b>Rate with ≤ 50 % effect</b>	<b>Drift rate</b>	<b>PER<sub>off-field</sub></b>	<b>CF</b>	<b>PER<sub>in-field</sub> below rate with ≤ 50 % effect?</b>
	<b>(g/ha) at x DAT</b>		<b>(g/ha)</b>		
<i>Typhlodromus pyri</i>	102 g a.s./ha at 0 DAT	0.0277	1.662	5	yes
<i>Aphidius rhopalosiphi</i>	45 g a.s./ha at 28 DAT				yes
<i>Coccinella septempunctata</i>	102 g a.s./ha at 0 DAT				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 LR<sub>50</sub> or ER<sub>50</sub> from a relevant extended laboratory test is available, it should be considered in place of the rate with ≤ 50 % effect.

#### **zRMS comments:**

Although most of Tier I and Tier II calculations presented above is correct, in opinion of the zRMS the risk assessment is presented in not fully transparent way, as presentation of not corrected PER<sub>off-field</sub> makes comparison with the endpoints more difficult and the reviewer has to perform additional calculation in order to see whether corrected PER<sub>off-field</sub> is actually lower than the toxicity value. In order to avoid multiple correction of the table above and to present all the necessary information, the zRMS decided to struck through the Applicants' tables and present respective evaluation including all necessary information enabling rapid review.

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 CA3573 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 eaves will be fully developed at that time.

Detailed risk assessment is presented in zRMS modified tables below. Please note that in Tier II calculations the lower of LR<sub>50</sub> and ER<sub>50</sub> was considered, since both parameters have to be covered.

### Risk assessment for NTAs following application to apples at 1x50 g a.s./ha

<b>Intended use</b>	Apple					
<b>Product</b>	CA3573					
<b>Application rate (g/ha)</b>	1 x 50					
<b>MAF</b>	1					
<b>Test species Tier I</b>	<b>LR<sub>50</sub> (lab.) (g/ha)</b>	<b>Drift rate</b>	<b>VDF</b>	<b>CF</b>	<b>corrected PER<sub>off-field</sub> (g/ha)</b>	<b>HQ<sub>off-field</sub> criterion: HQ ≤ 2</b>
<i>Typhlodromus pyri</i>	LR <sub>50</sub> = 9.13 g a.s./ha	0.1573	10 (2D study)	10	7.87	0.86
<i>Aphidius rhopalosiphi</i>	LR <sub>50</sub> = 0.0243 g a.s./h					323.9
<b>Extended laboratory studies</b>	<b>Rate with ≤ 50 % effect* (g/ha)</b>	<b>Drift rate</b>	<b>VDF</b>	<b>CF</b>	<b>corrected PER<sub>off-field</sub> (g/ha)</b>	<b>corr. PER<sub>off-field</sub> below rate with ≤ 50 % effect?</b>
<i>Typhlodromus pyri</i>	ER <sub>50</sub> > 12.5 g a.s./ha	0.1573	10 (2D study)	5	3.93	yes
<i>Aphidius rhopalosiphi</i>	ER <sub>50</sub> = 0.10 g a.s./ha		10 (2D study)		3.93	no
<i>Aphidius rhopalosiphi</i>	LR <sub>50</sub> = 3.56 g a.s./ha ER <sub>50</sub> < 0.64 g a.s./ha		none (3D study)		39.33	no
<i>Chrysoperla carnea</i>	LR <sub>50</sub> = 106 g a.s./ha		10 (2D study)		3.93	yes
<i>Coccinella septempunctata</i>	ER <sub>50</sub> = 20.7 g a.s./ha		10 (2D study)		3.93	yes

### Risk assessment for NTAs following application to apples at 25 g a.s./ha

<b>Intended use</b>	Apple					
<b>Product</b>	CA3573					
<b>Application rate (g/ha)</b>	1 x 25					
<b>MAF</b>	1					
<b>Test species Tier I</b>	<b>LR<sub>50</sub> (lab.) (g/ha)</b>	<b>Drift rate</b>	<b>VDF</b>	<b>CF</b>	<b>corrected PER<sub>off-field</sub> (g/ha)</b>	<b>HQ<sub>off-field</sub> criterion: HQ ≤ 2</b>
<i>Typhlodromus pyri</i>	LR <sub>50</sub> = 9.13 g a.s./ha	0.1573	10 (2D study)	10	3.93	0.43
<i>Aphidius rhopalosiphi</i>	LR <sub>50</sub> = 0.0243 g a.s./h					161.7
<b>Extended laboratory studies</b>	<b>Rate with ≤ 50 % effect* (g/ha)</b>	<b>Drift rate</b>	<b>VDF</b>	<b>CF</b>	<b>corrected PER<sub>off-field</sub> (g/ha)</b>	<b>corr. PER<sub>off-field</sub> below rate with ≤ 50 % effect?</b>
<i>Typhlodromus pyri</i>	ER <sub>50</sub> > 12.5 g a.s./ha	0.1573	10 (2D study)	5	1.97	yes
<i>Aphidius rhopalosiphi</i>	ER <sub>50</sub> = 0.10 g a.s./ha		10 (2D study)		1.97	no
<i>Aphidius rhopalosiphi</i>	LR <sub>50</sub> = 3.56 g a.s./ha ER <sub>50</sub> < 0.64 g a.s./ha		none (3D study)		19.7	no
<i>Chrysoperla carnea</i>	LR <sub>50</sub> = 106 g a.s./ha		10 (2D study)		1.97	yes
<i>Coccinella septempunctata</i>	ER <sub>50</sub> = 20.7 g a.s./ha		10 (2D study)		1.97	yes

### Risk assessment for NTAs following application to potatoes at 1x36 g a.s./ha

<b>Intended use</b>	Potatoes					
<b>Product</b>	CA3573					
<b>Application rate (g/ha)</b>	1 x 36					
<b>MAF</b>	1					
<b>Test species Tier I</b>	<b>LR<sub>50</sub> (lab.) (g/ha)</b>	<b>Drift rate</b>	<b>VDF</b>	<b>CF</b>	<b>corrected PER<sub>off-field</sub> (g/ha)</b>	<b>HQ<sub>off-field</sub> criterion: HQ ≤ 2</b>
<i>Typhlodromus pyri</i>	LR <sub>50</sub> = 9.13 g a.s./ha	0.0277	10 (2D study)	10	1.0	0.11
<i>Aphidius rhopalosiphi</i>	LR <sub>50</sub> = 0.0243 g a.s./h					41.2

Extended laboratory studies	Rate with $\leq 50\%$ effect* (g/ha)	Drift rate	VDF	CF	corrected PER <sub>off-field</sub> (g/ha)	corr. PER <sub>off-field</sub> below rate with $\leq 50\%$ effect?
<i>Typhlodromus pyri</i>	ER <sub>50</sub> > 12.5 g a.s./ha	0.0277	10 (2D study)	5	0.50	yes
<i>Aphidius rhopalosiphi</i>	ER <sub>50</sub> = 0.10 g a.s./ha		10 (2D study)		0.50	no
<i>Aphidius rhopalosiphi</i>	LR <sub>50</sub> = 3.56 g a.s./ha ER <sub>50</sub> < 0.64 g a.s./ha		none (3D study)		5.0	no
<i>Chrysoperla carnea</i>	LR <sub>50</sub> = 106 g a.s./ha		10 (2D study)		0.50	yes
<i>Coccinella septempunctata</i>	ER <sub>50</sub> = 20.7 g a.s./ha		10 (2D study)		0.50	yes

#### Risk assessment for NTAs following application to OSR and maize at 1x60 g a.s./ha

Intended use	OSR (winter, spring), maize					
Product	CA3573					
Application rate (g/ha)	1 x 60					
MAF	1					
Test species Tier I	LR <sub>50</sub> (lab.) (g/ha)	Drift rate	VDF	CF	corrected PER <sub>off-field</sub> (g/ha)	HQ <sub>off-field</sub> criterion: HQ $\leq 2$
<i>Typhlodromus pyri</i>	LR <sub>50</sub> = 9.13 g a.s./ha	0.0277	10 (2D study)	10	1.66	0.18
<i>Aphidius rhopalosiphi</i>	LR <sub>50</sub> = 0.0243 g a.s./h					68.3
Extended laboratory studies	Rate with $\leq 50\%$ effect* (g/ha)	Drift rate	VDF	CF	corrected PER <sub>off-field</sub> (g/ha)	corr. PER <sub>off-field</sub> below rate with $\leq 50\%$ effect?
<i>Typhlodromus pyri</i>	ER <sub>50</sub> > 12.5 g a.s./ha	0.0277	10 (2D study)	5	0.83	yes
<i>Aphidius rhopalosiphi</i>	ER <sub>50</sub> = 0.10 g a.s./ha		10 (2D study)		0.83	no
<i>Aphidius rhopalosiphi</i>	LR <sub>50</sub> = 3.56 g a.s./ha ER <sub>50</sub> < 0.64 g a.s./ha		none (3D study)		8.31	no
<i>Chrysoperla carnea</i>	LR <sub>50</sub> = 106 g a.s./ha		10 (2D study)		0.83	yes
<i>Coccinella septempunctata</i>	ER <sub>50</sub> = 20.7 g a.s./ha		10 (2D study)		0.83	yes

Based on presented above calculations, acceptable risk may be concluded for most of the non-target arthropods from all intended uses of CA3573. With regard to *Aphidius rhopalosiphi*, no acceptable risk could be concluded based on Tier I toxicity data and the PER<sub>off-field</sub> was higher than Tier II LR<sub>50</sub>. 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

It is noted that in order to refine the risk the Applicant performed evaluation based on results of aged residue studies. However, consideration of aged residue studies to address the off-field risk is unacceptable, as design of the studies is relevant to investigate potential for re-colonisation, while in case of the off-field risk the potential for recovery within an ecologically relevant time (i.e. max 1 year) must be demonstrated. This cannot be deduced from the aged residue studies. Taking this into account, the results of aged residue studies were not taken into account in the zRMS tables above.

The risk was further refined on the basis of the field study by Appeltauer (2016). For details, see point 9.7.2.3 below.

### 9.7.2.3 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 a field study is presentend in the following. In the available field study assessing the effects of acetamiprid, applied at drift rates, on the non target arthropod fauna in a meadow in Germany it was concluded the arthropod community 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).

In laboratory studies, *Aphidius rhopalosiphii* (Braconidae) was the most sensitive arthropod species tested. Braconid wasps were also recorded in the field study and no adverse effects on this family of hymenopterans occurred at any of the tested rates.

As the community NOER as well as the NOER for the most sensitive arthropod family in laboratory and extended laboratory studies is higher than the predicted environmental rate emanating from the intended uses of acetamiprid it can be concluded that no adverse effects on non-target arthropods in off field areas are to be expected. Only for the intended use in pomefruit at an application rate of 1 x 50 g a.s./ha, a risk cannot be excluded at this stage and risk mitigation needs to be taken into account.

#### zRMS comments:

The field study by Appeltauer (2016) on effects of CA3573 on off-field population of non-target arthropods has been evaluated and agreed by the zRMS (for details, please, refer to Appendix 2, A 2.3.2.4).

The study was performed on a meadow in Germany and covered two applications of CA3573 (formerly MCW-2222) with 6 days interval, which represents worst case comparing to the current GAP, including single applications only. 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 50 g a.s./ha.

Although effects on all caught species were evaluated by the zRMS, special attention was paid to the *Braconidae* family (parasitic wasps), as in the laboratory studies *Aphidius rhopalosiphii* turned out to be particularly sensitive to acetamiprid in CA3573. During the field study the *Braconidae* family was present on the study plots but 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 of the study was sufficient to detect effects on these insects.

Overall, application of CA3573 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 and in line with indications of ESCORT 2, this endpoint is relevant for purposes of refinement of the risk.

Taking into account the agreed endpoints, the evaluation provided by the Applicant above is not agreed by the zRMS, as it is based on not correct assumptions – in the discussion the Applicant considered the community NOER of 7.2 g a.s./ha, ignoring statistically significant and treatment related reduction of juvenile *Thysanoptera* abundance with no recovery observed during the study period (effect class 8). The Applicants' text above has been thus struck through.

In order to address the risk to off-field population of NTAs, the NOEAER from the study was compared directly with the drift rates expected after application of CA3573 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.

Crop	Application rate [g a.s./ha]	Drift rate	PER <sub>off-field</sub> [g a.s./ha]	NOEAER [g a.s./ha]	PER <sub>off-field</sub> < NOEAER?
Apples	50	15.73%	7.87	3.4	No
Apples	25	15.73%	3.93		No
Potatoes	36	2.77%	1.0		Yes
Oilseed rape	60	2.77%	1.66		Yes
Maize	60	2.77%	1.66		Yes

As may be seen in table above, the expected off-field exposure after application of CA3573 to potatoes, oilseed rape and maize is lower than the NOEAER from the field study. On this basis acceptable risk to non-target arthropods may be concluded for these uses with no need for risk mitigation measures.

The expected off-field exposure following both intended applications of CA3573 to apples is higher than the NOEAER indicating potentially unacceptable risk. 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 9.7.2.4 below.

## 9.7.2.4 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 5m; drift-reducing nozzles with reduction by 50%) are summarised in the following table:

**Table 9.7-10: Risk assessment for non-target arthropods due to the use of 1 x 50 g/ha of CA3573 in apple considering risk mitigation measures (spray buffer zones, and drift-reducing nozzles)**

Intended use Active substance/product Application rate (g/ha) MAF Drift rate (%) vdf	Apple CA3573 1 x 50 1 15.73% (3m) 8.41% (5 m) 10 (2D) / not applicable (3D)			
Higher-tier field study NOER (g a.s./ha)	Buffer Zone	Drift-reducing nozzles	PER <sub>off-field</sub> (g a.s./ha)	Acceptable risk
7.2 g a.s./ha	—	—	7.865	no
	—	50%	3.933	yes
	5	—	4.205	yes

MAF: Multiple application factor; vdf: Vegetation distribution factor; (corr.) PER Predicted environmental rate; CF: Correction factor; HQ: Hazard quotient. Criteria values shown in bold breach the relevant trigger.

\* If an LR<sub>50</sub> or ER<sub>50</sub> from a relevant extended laboratory test is available, it should be considered in place of the rate with ≤ 50 % effect.

### zRMS comments:

The risk assessment performed with consideration of risk mitigation measures presented by the Applicant above has been based on not agreed endpoint. Furthermore, application to apples at 25 g a.s./ha was not considered in Applicants' calculations. Taking this into account, table above has been struck through and respective calculations were performed by the zRMS in tables below. As in evaluation presented in point 9.7.2.3, the PER<sub>off-field</sub> was not corrected as NOEAER was derived from field study with high abundance of various non-target arthropods species. Off-field exposure greater than NOEAER has been highlighted in bold indicating unacceptable risk.

Intended use Active substance/product Application rate [g/ha] MAF vdf Endpoint from field study	Apples Acetamiprid in CA3573 1 x 50 1 Not relevant for higher tier assessment based on field data NOEAER = 3.4 g a.s./ha				
Buffer strip [m]	Drift rate	PER <sub>off-field</sub> [g/ha]	PER <sub>off-field</sub> 50 % drift red. [g/ha]	PER <sub>off-field</sub> 75 % drift red. [g/ha]	PER <sub>off-field</sub> 90 % drift red. [g/ha]
3	15.73%	<b>7.87</b>	<b>3.94</b>	1.97	-
5	8.41%	<b>4.21</b>	2.11	1.05	-
10	3.60%	1.80	0.90	0.45	-

PER<sub>off-field</sub> highlighted in **bold** exceeds NOEAER and indicates unacceptable risk

Based on above calculations acceptable risk following application of CA3573 to apples at 50 g a.s./ha may be concluded provided that:

- 10 m unsprayed buffer zone to non-agricultural land is respected, or
- 5 m unsprayed buffer zone to non-agricultural land is respected in combination with 50% drift reduction, or
- the spray drift is reduced by 75% using relevant drift reducing techniques.

Intended use		Apples			
Active substance/product		Acetamiprid in CA3573			
Application rate [g/ha]		1 x 25			
MAF		1			
vdf		Not relevant for higher tier assessment based on field data			
Endpoint from field study		NOEAER = 3.4 g a.s./ha			
Buffer strip [m]	Drift rate	PER <sub>off-field</sub> [g/ha]	PER <sub>off-field</sub> 50 % drift red. [g/ha]	PER <sub>off-field</sub> 75 % drift red. [g/ha]	PER <sub>off-field</sub> 90 % drift red. [g/ha]
3	15.73%	3.93	1.97	-	-
5	8.41%	2.10	1.05	-	-
10	3.60%	0.90	0.45	-	-

Based on above calculations acceptable risk following application of CA3573 to apples at 25 g a.s./ha may be concluded provided that:

- 5 m unsprayed buffer zone to non-agricultural land is respected, or
- the spray drift is reduced by 75% using relevant drift reducing techniques.

Concerned Member States should check applicability of indicated risk mitigation measures in their countries.

### 9.7.3 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 CA3573 is applied according to good agricultural practice, except for the intended use in pome fruit at an application rates of 1 x 50 g a.s./ha and 1 x 25 g a.s./ha.

The risk to off-field non-target arthropods is acceptable following use of CA3573 in pome fruit (1 x 25 ~~50~~ 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 CA3573 in pome fruit (1 x 50 g a.s./ha), provided the following risk mitigation measures are applied:

- 75% drift reduction or
- 5 m buffer combined with 50% drift reduction or
- 10 m buffer

In conclusion, no unacceptable risks for non-target arthropods are expected when CA3573 is applied according to good agricultural practice and considering risk mitigation measures as specified above for the uses in pome fruit ( 1 x 50 g a.s./ha and 1 x 25 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.

Effects on earthworms and other non-target soil organisms (meso- and macrofauna) of CA3573 were not evaluated as part of the EU assessment of acetamiprid. New data submitted with this application are listed in 0 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) - acetamiprid**

Species	Substance	Exposure System	Results	Reference
<i>Eisenia fetida</i>	IM-1-5	Homogenous mixing, chronic	Growth, reproduction, behaviour <b>NOEC = 62.5 mg/kg d.w. soil</b>	EFSA, 2016 KCP 10.4.1.1/02
<i>Folsomia candida</i>	IM-1-5	Homogenous mixing, chronic	NOEC <sub>mortality</sub> = 62.7 mg/kg soil d.w. No EC values could be calculated as there were no effects below the highest tested value. <b>NOEC<sub>reproduction</sub> = 12.5 mg/kg soil d.w.</b> No EC values were calculated as the data were not appropriate for modelling.	EFSA, 2016 KCP 10.4.2.1/02

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

\* Corrected value derived by dividing the endpoint by a factor of 2 in accordance with the EPPO earthworm scheme 2002.

<sup>a</sup> Based on standard assumptions of soil bulk density 1.5 g/cm<sup>3</sup> and incorporation depth 5 cm.

**Table 9.8-2: Endpoints and effect values relevant for the risk assessment for earthworms and other non-target soil organisms (meso- and macrofauna) – CA3573**

Species	Substance	Exposure System	Results	Reference
<i>Eisenia fetida</i>	MCW-2222	Mixed into substrate, 56 d, chronic, 10 % peat content	<b>NOEC = 0.85 mg a.s./kg dw</b> EC <sub>10</sub> = 0.90 mg a.s./kg dw	Friedrich, S. 2014a, R-33840 KCP 10.4.1.1/01
<i>Folsomia candida</i>	MCW-2222	Mixed into substrate, 28 d, chronic, 5 % peat content	<b>NOEC = 0.18 mg a.s./kg dw</b> EC <sub>10</sub> = 0.41 mg a.s./kg dw <sup>***</sup>	Friedrich, S. 2014b, R-33841 KCP 10.4.2.1/01
<i>Hypoaspis aculeifer</i>	MCW-2222	Mixed into substrate, 14 d, chronic, 5 % peat content	<b>NOEC = 100 mg a.s./kg dw</b> <del>NOEC = 200 mg a.s./kg dw</del> <del>EC<sub>10</sub> &gt; 200 mg a.s./kg dw</del>	Schulz, L., 2014, R-33842 KCP 10.4.2.1/02
<b>Field studies</b>				
Not required				

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

\* Corrected value derived by dividing the endpoint by a factor of 2 in accordance with the EPPO earthworm scheme 2002.

<sup>a</sup> Based on standard assumptions of soil bulk density 1.5 g/cm<sup>3</sup> and incorporation depth 5 cm.

<sup>\*\*\*</sup> As no mortality and no decreased reproduction was noted at the NOEC, the EC<sub>10</sub> is considered to be the more relevant endpoint

#### 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 CA3573 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 CA3573 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 EC<sub>10</sub> 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, A 2.4.1 and A 2.4.2.

It is noted that for purposes of the risk assessment for *Folsomia candida* the Applicant proposed to use EC<sub>10</sub> of 0.41 mg a.s./kg dws derived from study by Friedrich (2014b) as no mortality and no effects on reproduction were observed at the NOEC. It should be, however, pointed out that according to EFSA Supporting publication 2015: EN-924:

*Regarding the use of EC<sub>x</sub> in the risk assessment, the experts agreed that where a reliable median EC<sub>10</sub> could be calculated, then the lower between this value and the NOEC should be used (unless a recent guidance document explicitly indicates a preference - i.e. currently only EFSA PPR Panel (2013b)).*

As no preference is given in the current guidance document SANCO/10329/2002 rev 2 final, the risk assessment for soil macro- and meso-fauna is performed with consideration of the lower of EC<sub>10</sub> and NOEC values. In case of study by Friedrich (2014b), NOEC is lower than EC<sub>10</sub> and is thus relevant for the risk assessment purposes.

The zRMS is aware that there is an extensive discussion regarding reliability of the NOEC values, which are strongly design dependent, do not consider the dose-response relationship and may be not representative of real lack of effects. Furthermore, lack of clear indication regarding consideration of EC<sub>10</sub> value in the soil risk assessment is due to the outdated guidance from 2002 (not changed since that time) and not due to higher reliability of the NOEC over EC<sub>10</sub>. Nevertheless, the risk assessment has to be performed in line with current guidance documents and conclusions of the EFSA meetings on general recurring issues in ecotoxicology. All these documents clearly indicate that the soil risk assessment must be performed with consideration of the NOEC values.

### 9.8.1.1 Justification for new endpoints

CA3573/MCW-2222 was not the representative formulation for the renewal of the active substance acetamiprid. New studies on effects of the formulation CA3573 on non-target arthropods were carried out as required by Regulation (EU) 284/2013. The studies with CA3573 were conducted in accordance with the most recent guidelines.

### 9.8.2 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.

#### 9.8.2.1 First-tier risk assessment

The relevant PEC<sub>soil</sub> for risk assessments covering the proposed use pattern are taken from Section 8 (Environmental Fate), Chapter 8.7.2, Table 8.7-3. According to the assessment of environmental-fate data, multi-annual accumulation in soil does not need to be considered for acetamiprid and its metabolites IM-1-2 and IC-0. For the metabolites IM-1-4 and 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.

**Table 9.8-3: First-tier assessment of the acute and chronic risk for earthworms and other non-target soil organisms (meso- and macrofauna) due to the intended uses of acetamiprid in potato (based on max PEC<sub>soil</sub> values)**

Intended use	Potato 1 × 36 g a.s/ha		
Chronic effects on earthworms			
Product/active substance	NOEC (mg/kg dw)	PEC <sub>soil</sub> (mg/kg dw)	TER <sub>it</sub> (criterion TER ≥ 5)
Acetamiprid	0.85	0.041	20.7
MCW-2222	4.77	0.230	20.7
IM-1-2	62.5 <sup>b</sup>	0.024	2604
IM-1-4	62.5 <sup>b</sup>	0.022 <sup>a</sup>	2841
IM-1-5	62.5	0.021 <sup>a</sup> 0.014 <sup>a</sup>	2976 4464
IC-0	62.5 <sup>b</sup>	0.003	20833
Chronic effects on other soil macro- and mesofauna - Folsomia			
Product/active substance	NOEC/EC <sub>10</sub> (mg/kg dw)	PEC <sub>soil</sub> (mg/kg dw)	TER <sub>it</sub> (criterion TER ≥ 5)
Acetamiprid	0.18 0.41 <sup>c</sup>	0.041	4.4 10
MCW-2222	2.30 <sup>c</sup>	0.230	10
IM-1-2	12.5 <sup>b</sup>	0.024	521
IM-1-4	12.5 <sup>b</sup>	0.022 <sup>a</sup>	568
IM-1-5	12.5	0.021 <sup>a</sup> 0.014 <sup>a</sup>	595 893
IC-0	12.5 <sup>b</sup>	0.003	4167
Chronic effects on other soil macro- and mesofauna - Hypoaspis			
Product/active substance	NOEC (mg/kg dw)	PEC <sub>soil</sub> (mg/kg dw)	TER <sub>it</sub> (criterion TER ≥ 5)
Acetamiprid	100 200	0.041	2439 4878
MCW-2222	1122	0.330	4878
IM-1-2	20 <sup>d</sup>	0.024	833
IM-1-4	20 <sup>d</sup>	0.022 <sup>a</sup>	909
IM-1-5	20 <sup>d</sup>	0.021 <sup>a</sup> 0.014 <sup>a</sup>	952 1429
IC-0	20 <sup>d</sup>	0.003	6666

TER values shown in **bold** fall below the relevant trigger.

<sup>a</sup> Maximum PEC<sub>soil</sub>, accumulation

<sup>b</sup> IM-1-5 values are also used for RA of other metabolites since none of the metabolites is considered to be more toxic or persistent

<sup>c</sup> An acceptable risk (TER ≥ 5) is also shown if the lower CI of the EC<sub>10</sub> is used

<sup>d</sup> Chronic toxicity of the metabolite is assumed to be 10-fold higher than the parent

All TER values for soil meso- and macrofauna are above the trigger of 5, demonstrating acceptable risk to soil meso- and macrofauna when MCW-2222 is applied according to good agricultural practice.

#### **zRMS comments:**

The risk assessment for earthworms from acetamiprid (formulated as CA3573) is agreed by the zRMS.

For *Hypoaspis aculeifer* lower endpoint has been agreed from study with CA3573 and the risk assessment in Table 9.8-3 was amended accordingly. The TER value based on lower NOEC was far above the trigger of 5 and acceptable risk could be thus concluded.

With regard to the risk assessment for *Folsomia candida* from acetamiprid in CA3573, the TER was based on EC<sub>10</sub> value from study performed with the formulated product, although the NOEC value was lower. In line with current requirements, lower of EC<sub>10</sub> and NOEC value must be used in the risk assessment for soil organisms and the risk assessment in Table 9.8-3 recalculated by the zRMS with consideration of the NOEC of 0.18 mg a.s./kg dws

resulted with TER below the threshold of 5, indicating potentially unacceptable risk. It is, however, noted that calculations presented in Table 9.8-3 were performed with consideration of the worst case PEC<sub>soil</sub> value derived for early uses in potatoes (BBCH 12-19), while intended uses in potatoes include wide range of BBCH (12-79) and considerably lower PEC<sub>soil</sub> could be calculated for applications at BBCH 20-79 due to higher crop interception. Therefore additional risk assessment was performed by the zRMS for these later uses in potatoes. However, as for these uses PEC<sub>soil</sub> is not protective for all intended uses, separate risk assessment was performed for each crop intended in the Central Zone GAP and is presented in table below.

<b>Risk assessment for <i>Folsomia candida</i></b>					
<b>Compound</b>	<b>Crop</b>	<b>Application rate [g/ha]</b>	<b>NOEC [mg/kg dw]</b>	<b>PEC<sub>soil</sub> [mg/kg dw]<sup>2)</sup></b>	<b>TER<sub>it</sub> [criterion TER ≥ 5]</b>
Acetamiprid	Potatoes (BBCH 12-19)	1 x 36	0.18	0.041	<b>4.4</b>
	Potatoes (BBCH 20-79)	1 x 36		0.019	9.5
	Orchards (BBCH >62)	1 x 50 <sup>1)</sup>		0.027	6.7
	Oilseed rape (BBCH 31-71)	1 x 60		0.016	11.3
	Maize (BBCH 51-75)	1 x 60		0.020	9.0

<sup>1)</sup> Covering lower application rate of 1 x 25 g a.s./ha

<sup>2)</sup> PEC<sub>soil</sub> as agreed in Core Assessment, Part B, Section 8

Based on provided above calculations acceptable risk may be concluded for potatoes at BBCH 20-79 and all other intended uses of CA3574. Taking this into account, the use pattern in potatoes has to be restricted to BBCH stages >20, while application at BBCH 12-19 cannot be authorised until additional data enabling refinement of the risk to *Folsomia candida* is available.

Endpoints considered in the risk assessment to earthworms and *Folsomia candida* from metabolite IM-1-5 are in line with values reported in EFSA Journal 2016;14(11):4610. As no endpoint for metabolite IM-1-5 was available from the EU review for *H. aculeifer*, 10 times toxicity of the parent has been assumed as a worst case. It was noted that maximum accumulation PEC<sub>soil</sub> agreed in area of Section 9 was 0.021 mg pm/kg dws for application at 50 g a.s./ha for apples, while PEC<sub>soil</sub> of 0.014 mg pm/kg dws was taken into account in Applicants' calculations. For this reason calculations in Table 9.8-3 were amended accordingly, but this had no impact on the on the outcome of the evaluation and acceptable risk from metabolite may be concluded for all intended uses of CA3573.

In Table 9.8-3 the Applicant presented also separate TER calculations for the formulated product, however the formulation endpoint (expressed in terms of the active substance) has been used in calculations for acetamiprid and already accounted for potential toxicity of the co-formulants. As consideration of endpoint and PEC<sub>soil</sub> value for the formulation does not provide any additional information as exactly the same TER values are calculated, evaluation for the formulation has been struck through as being already covered by the risk assessment for acetamiprid.

It is also noted that the risk assessment for metabolites IM-1-2, IM-1-4 and IC-0 was based on the toxicity endpoint derived for metabolite IM-1-5. In general, in absence of toxicity data for metabolites, 10 times toxicity of the parent is assumed in the risk assessment and neither of metabolites mentioned is formed directly from IM-1-5. Therefore consideration of endpoints for this metabolite to address the risk from other metabolites is not justified. Furthermore, assumption of the endpoint for the parent or another metabolite would require detailed justification, including comparison of the structure and analysis of presence or absence of the toxophore. As this was not done by the Applicant, the risk assessment for metabolites IM-1-2, IM-1-4 and IC-0 is not agreed by the zRMS and is thus struck through in Table 9.8-3.

Nevertheless, during the EU review it was concluded that metabolites IM-1-2, IM-1-4 and IC-0 are not expected to be more toxic than the parent and no risk assessment was performed for these compounds. A data gap for respective toxicity studies with metabolites was also not 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 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 and 2, respectively. Maximum occurrence of metabolite IM-1-4 was observed on day 14, so it was formed in study with *Eisenia foetida* and *Folsomia candida*, lasting for 56 and 28 days, but it could not be formed at its maximum in study with *Hypoaspis aculeifer*, which is carried out for 14 days. However, based on the toxicity data for CA3574 and the representative formulation, the most sensitive species is obviously *Folsomia candida* while *Hypoaspis aculeifer* is not particularly sensitive to acetamiprid. Thus, based on the available information it may be concluded that the risk to soil macro- and meso-fauna from metabolites IM-1-2, IM-1-4 and IC-0 is sufficiently covered by evaluation performed for acetamiprid in formulation CA3574.

### 9.8.2.2 Higher-tier risk assessment

Not relevant.

### 9.8.3 Overall conclusions

The risk of CA3573 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 CA3573 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 CA3573 following intended early uses in potatoes, resulting with the highest exposure. Therefore additional risk assessment has been performed for *Folsomia candida* following each intended use as well as later uses in potatoes at BBCH 20-79. Acceptable risk could be concluded and CA3573 may be thus authorised for intended uses in apples, oilseed rape (spring and winter), maize and potatoes at BBCH 20-79. No authorisation for application to potatoes at BBCH 12-19 may be granted until additional data enabling refinement of the risk to *Folsomia candida* are provided.

~~The risk of CA3573 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 CA3573, acetamiprid, its relevant soil metabolites, and the maximum  $PEC_{soil}$ . The  $TER_{LT}$  values CA3573, from acetamiprid and its relevant soil metabolites, are all greater than the recommended trigger value of 5, indicating that the risk to soil meso and macrofauna is acceptable following use of CA3573 according to the proposed use pattern.~~



## 9.9 Effects on soil microbial activity (KCP 10.5)

### 9.9.1 Toxicity data

Studies on effects on soil microorganisms have been carried out with acetamiprid. Full details of these studies are provided in the respective EU DAR and related documents.

Effects on soil microorganisms of CA3573 were not evaluated as part of the EU assessment of acetamiprid. New data submitted with this application are listed in 0 and summarised in Appendix 2.

**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 <sup>a)</sup>	28 d, aerobic	No significant effects > 25% at 0.2 kg a.s./ha	EFSA, 2016
N-mineralisation	MCW-2222	28 d, aerobic loamy sand	<b>NOEC = 22.74 mg test item/kg dw corresponding to 4.01 mg a.s./kg dw <sup>b)</sup></b>	Schulz, L., 2014 R-33843 KCP 10.5/01

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

<sup>a)</sup> Representative formulation, Acetamiprid 20 SG

<sup>b)</sup> 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 CA3573.

Study on effects of CA3573 on soil nitrogen transformation listed in Table 9.9-1 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, A 2.5.

#### 9.9.1.1 Justification for new endpoints

New studies are available for CA3573 which are required to fulfil the data requirements for plant protection products in accordance with Regulation (EC) No 1107/2009. The endpoints are summarised in

#### **zRMS comments:**

The risk assessment for soil micro-organisms from acetamiprid in CA3573 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 CA3573 may be concluded.

In Table 9.9-2 the Applicant presented also separate evaluation for the formulated product, however the formulation endpoint (expressed in terms of the active substance) has been used in calculations for acetamiprid and already accounted for potential toxicity of the co-formulants. As consideration of endpoint and PEC<sub>soil</sub> value for the formulation does not provide any additional information, evaluation for the formulation has been struck through as being already covered by the risk assessment for acetamiprid.

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 CA3574.

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 by the zRMS using the maximum accumulated PEC<sub>soil</sub> 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 CA3573.

and a study summary is presented in Appendix 2.

## 9.9.2 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<sub>soil</sub> for risk assessments covering the proposed use pattern are taken from Section 8 (Environmental Fate), Chapter 8.7.2, Table 8.7-3 and were already used in the risk assessment for earthworms and other non-target soil organisms (meso- and macrofauna) (see 0).

To achieve a concise risk assessment, the risk envelope approach is applied. Here, the assessment for the use in potatoe also covers the risk for soil microorganisms from all other intended uses (see 0).

**Table 9.9-2: Assessment of the risk for effects on soil micro-organisms due to the use of CA3573 in crop potato**

Intended use		Potato 1 × 36 g a.s./ha	
N-mineralisation			
Product/active substance	Max. conc. with effects ≤ 25 % (mg/kg dw)	PEC <sub>soil</sub> (mg/kg dw)	Risk acceptable?
Acetamiprid	> 4.01 (at 28 d)	0.041	yes
IM-1-5	>0.401 (at 28 d) <sup>a</sup>	0.021 <sup>b</sup>	yes
formulation	> 22.74 (at 28 d)	0.330	yes

<sup>a</sup> 10 times toxicity of the parent assumed as a worst case

<sup>b</sup> Maximum PEC<sub>soil</sub>, accumulation

### zRMS comments:

The risk assessment for soil micro-organisms from acetamiprid in CA3573 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 CA3573 may be concluded.

In Table 9.9-2 the Applicant presented also separate evaluation for the formulated product, however the formulation endpoint (expressed in terms of the active substance) has been used in calculations for acetamiprid and already accounted for potential toxicity of the co-formulants. As consideration of endpoint and PEC<sub>soil</sub> value for the formulation does not provide any additional information, evaluation for the formulation has been struck through as being already covered by the risk assessment for acetamiprid.

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 CA3574.

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 by the zRMS 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 CA3573.

### 9.9.3 Overall conclusions

The risk of CA3573 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 CA3573 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

Effects on non-target terrestrial plants of CA3573 were not evaluated as part of the EU assessment of acetamiprid. New data submitted with this application are listed in 0 summarised in Appendix 2.

The selection of studies and endpoints for the risk assessment deviates from the results of the EU review process. Justifications are provided below.

**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	MCW-2222	21 d Vegetative vigour	<b>ER<sub>50</sub> plant weight &gt; 510 g a.s./ha</b>	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 CA3573 on non-target terrestrial plants listed in Table 9.9-1 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, A 2.6.3.

#### 9.10.1.1 Justification for new endpoints

CA3573/MCW-2222 was not the representative formulation for the renewal of the active substance acetamiprid. New studies on effects of the formulation CA3573 on non-target terrestrial plants were carried out as required by Regulation (EU) 284/2013.

### 9.10.2 Risk assessment

#### 9.10.2.1 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 0).

Limit tests at rates up to 510 g a.s./ha were conducted with CA3573 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 equal/exceed the highest field application rate in oil seed rape and corn and consequently it is concluded that the risk for non-target terrestrial plants is acceptable following the use of CA3573 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 CA3573 (60 g a.s./ha in oilseed rape and maize)) is more than 8 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 CA3573 is concluded to be acceptable and calculation of TER values is deemed not necessary.

**9.10.2.2 Tier-2 risk assessment (based on dose-response data)**

Not relevant.

**9.10.2.3 Higher-tier risk assessment**

Not relevant.

**9.10.2.4 Risk mitigation measures**

No risk mitigation needed.

**9.10.3 Overall conclusions**

The application of CA3573 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)**

Tests on other non-target species are not required.

**9.12 Monitoring data (KCP 10.8)**

There are no other relevant data for the active substance or product on organisms in the environment generated from monitoring schemes.

## 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 CA3573/MCW-2222 the following data is available:

Acute data: Fish, *Daphnia*, *Chironomus* 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 CA3573**

Ecotoxicology/Environment data relevant for classification of CAS 575				
Substance tested	Study Type (duration)	Findings	Triggered classification and labelling	Reference
Acute (short-term) aquatic hazard				
MCW-2222	<i>Oncorhynchus mykiss</i> (96 h)	96 h LC <sub>50</sub> = 15.3 mg a.s./L	No aquatic acute hazard	xxxx, xxx., 2014a R-33831 KCP 10.2.1/01
MCW-2222	<i>Daphnia magna</i> (48 h)	48 h EC <sub>50</sub> = 22.8 mg a.s./L	No aquatic acute hazard	Juckeland, D., 2014b R-33832 KCP 10.2.1/02
MCW-2222	<i>Chironomus riparius</i> (48 h)	<b>48 h EC<sub>50</sub> = 0.0929 mg a.s/L nom</b>	<b>Aquatic acute hazard cat. 1 (H400)</b>	Juckeland, D., 2015a R-34873 KCP 10.2.1/03
MCW-2222	<i>Desmodesmus subspicatus</i> (72 h)	72 h E <sub>r</sub> C <sub>50</sub> = 553.5 mg a.s./L	No aquatic acute hazard	Juckeland, D., 2014b R-33833 KCP 10.2.1/04
		72 h E <sub>r</sub> C <sub>10</sub> = 146.6 mg a.s./L	No aquatic chronic hazard	
Long-term aquatic hazard				
Acetamiprid <sup>1)</sup>	<i>P. promelas</i> (35 d)	NOEC = 9.4 mg a.s./L	No aquatic chronic hazard	EFSA, 2016
	<i>D. magna</i> (21 d)	EC <sub>10</sub> = 2.96 mg a.s./L	No aquatic chronic hazard	EFSA, 2016
	<i>C. riparius</i> (28 d)	<b>EC<sub>10</sub> = 0.000235 mg a.s./L</b>	<b>Aquatic chronic hazard cat 1 (H410), M = 100</b>	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

<sup>1)</sup> Nominal contents within the formulated product CA3573: 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 CA3573.

For the chronic classification of the product CA3573 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, CA3573 is classified as Chronic 1 (H410).

**Table 9.13-2: Chronic classification of acetamiprid CA3573 using the summation method according to (EC) No 1272/2008**

Chronic classification of CA3573					
Formulation component					
Name	Chronic Category	M-Factor	Content in CA3573 Acetamiprid 200 SL/ Carnadine [%]	Result (% Content x M-Factor)	
Acetamiprid	1	100	20	200	
1 <sup>st</sup> equation	SUM ( <i>M x Chronic 1</i> )			2000	≥ 25 %
					CA3573: Aquatic Chronic Hazard Category 1

## Conclusion

In conclusion the following classification and labelling is proposed for CA3573: 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 CA3573 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 CA3573 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 CA3573 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

EC, 2014: Draft implementation plan for the EFSA Guidance Document on the Risk Assessment of Plant Protection Products on Bees (*Apis mellifera*, *Bombus* spp. and solitary bees). SANCO/10606/2014, 16<sup>th</sup> May 2014.

EFSA, 2009: Guidance of EFSA, Risk Assessment for Birds and Mammals. EFSA Journal 7(12):1438, 358 pp.

EFSA, 2013: Scientific Opinion. Guidance on tiered risk assessment for plant protection products for aquatic organisms in edge-of-field surface waters. EFSA Journal 11(7): 3290, 268 pp.

EFSA, 2013b: EFSA Guidance Document on the risk assessment of plant protection 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, 268 pp.

EFSA, 2014: User Manual - A small application developed in R for the estimation of the residue intake rate for certain bee species under given conditions: the SHVAL tool. EFSA supporting publication: EN-623: 15 pp.

EFSA, 2016b. Conclusion on the peer review of the pesticide risk assessment of the active substance acetamiprid. EFSA Journal 14(11): 4610,

EPPO, 2010: EPPO Standards PP 3 / 10 (3). Environmental risk assessment for plant protection products. Chapter 10: honeybees. Bulletin OEPP/EPPO Bulletin 40, 323–331.

OECD, 2007: Guidance document on the honeybee (*Apis mellifera* L.) brood test under semi-field conditions. OECD Environment, Health and Safety Publications, Series on Testing and Assessment No. 75, ENV/JM/MONO (2007) 22, 27 pp, Lists of data considered in support of the evaluation

## 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	Previously used  Y/N  If yes, for which data point
KCP 10.1.2.2/01	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	Not relevant  Public literature data, not a subject of data protection
KCP 10.1.2.2/02	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	Not relevant  Public literature data, not a subject of data protection
KCP 10.1.2.2/03	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	Not relevant  Public literature data, not a subject of data protection
KCP 10.1.2.2/04	Heroldová, M., Zejda, J., Zapletal, M., Obdržálová, D., Jánová, E., Bryja, J., Tkadlec, E.	2004	Importance of winter rape for small rodents. Plant soil and environment, 50(4), 175-181. GLP: no Published: yes	N	Public	Not relevant  Public literature data, not a subject of data protection

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	Previously used  Y/N  If yes, for which data point
KCP 10.1.2.2/05	Delattre, P., Giraudoux, P. et al.	1992	Effects of agriculture development on vole dynamics and conservation of Montagu`s harrier in western French wetlands. Biological Conservation 100: 289-295. GLP: no Published: yes	N	Public	Not relevant  Public literature data, not a subject of data protection
KCP 10.1.2.2/06	Butet, A., Leroux, A.B.A.	2001	Effects of agriculture development on vole dynamics and conservation of Montagu`s harrier in western French wetlands. Biological Conservation 100: 289-295. GLP: no Published: yes	N	Public	Not relevant  Public literature data, not a subject of data protection
KCP 10.1.2.2/07	Jacob, J., Halle, S.	2001	The importance of land management for population parameters and spatial behaviour in common voles ( <i>Microtus arvalis</i> ). Advances in Vertebrate Pest Management. H.-J. Pelz and C. J. Feare. Fürth, Filander-Verlag. 2: 319-330. GLP: no Published: yes	N	Public	Not relevant  Public literature data, not a subject of data protection
KCP 10.1.2.2/08	Jacob, J.	2003	Short-term effects of farming practices on populations of common voles. Agriculture Ecosystems and Environment 95: 321-325. GLP: no Published: yes	N	Public	Not relevant  Public literature data, not a subject of data protection
KCP 10.1.2.2/09	Adamczewska- Andrzejewska, K.A.	1981	Populations structure of <i>Microtus arvalis</i> (Pall.) against the background of a community of rodents in crop fields. Polish ecological studies 7(2): 193-211. GLP: no Published: yes	N	Public	Not relevant  Public literature data, not a subject of data protection

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	Previously used  Y/N  If yes, for which data point
KCP 10.1.2.2/10	Jacob, J., Hempel, N	2003	Effects of farming practices on spatial behaviour of common voles. Journal of Ethology 21: 45-50. GLP: no Published: yes	N	Public	Not relevant  Public literature data, not a subject of data protection
KCP 10.1.2.2/11	Halle, S.	2000	Voles - small graminivores with polyphasic patterns. Activity patterns in small mammals. S. Halle and N. C. Stenseth. Berlin, Heidelberg, New York, Barcelona, Hong Kong, London, Milan, Paris, Singapore, Tokyo, Springer-Verlag: 191-215. GLP: no Published: yes	N	Public	Not relevant  Public literature data, not a subject of data protection
KCP 10.1.2.2/12	Jaworska, K	1996	The cover of herbaceous plants in an IPM apple orchard and its influence on the occurrence of rodents. Acta Horticulturae 422: 431-432. GLP: no Published: yes	N	Public	Not relevant  Public literature data, not a subject of data protection
KCP 10.1.2.2/13	Sullivan, T.P., van Hogue, E.J.	2003	Influence of Orchard Floor Management on Vole and Pocket Gopher Populations and Damage in Apple Orchards. Journal of the American Society for Horticultural Science 112: 972-977. GLP: no Published: yes	N	Public	Not relevant  Public literature data, not a subject of data protection
KCP 10.1.2.2/14	Jacob, J., Brown, J.S	2000	Microhabitat use, giving-up densities and temporal activity as short- and long-term anti-predator behaviors in common voles. Oikos 91: 131-138. GLP: no Published: yes	N	Public	Not relevant  Public literature data, not a subject of data protection

<b>Data point</b>	<b>Author(s)</b>	<b>Year</b>	<b>Title</b> <b>Company Report No.:</b> <b>Source (where different from company)</b> <b>GLP or GEP status</b> <b>Published or not</b>	<b>Vertebrate study</b> <b>Y/N</b>	<b>Owner</b>	<b>Previously used</b>  <b>Y/N</b>  <b>If yes, for which data point</b>
KCP 10.1.2.2/15	Edge, W., Wolff, J. et al.	1995	Density-dependent responses of gray-tailed voles to mowing. Journal of Wildlife Management 59: 245-251. GLP: no Published: yes	N	Public	Not relevant  Public literature data, not a subject of data protection
KCP 10.1.2.2/16	Lauenstein, G.	1979	Zur Problematik der Bekämpfung von Feldmäusen. Zeitschrift für angewandte Zoologie 66: 35-59. GLP: no Published: yes	N	Public	Not relevant  Public literature data, not a subject of data protection
KCP 10.1.2.2/17	Braun, M., Dieterlen,	2005	Die Säugetiere Baden-Württembergs Band 2. Stuttgart, Verlag Eugen Ulmer. Pp. 297-311. GLP: no Published: yes	N	Public	Not relevant  Public literature data, not a subject of data protection
KCP 10.1.2.2/18	Pulliam, H.R.	1988	Sources, Sinks, and Population Regulation. The American Naturalist 132(5): 652-661. GLP: no Published: yes	N	Public	Not relevant  Public literature data, not a subject of data protection
KCP 10.1.2.2/19	Dias, P.C.	1996	Sources and sinks in population biology. Tree 11: 326-330. GLP: no Published: yes	N	Public	Not relevant  Public literature data, not a subject of data protection

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	Previously used  Y/N  If yes, for which data point
KCP 10.1.2.2/20	Tattersall, F.H., MacDonald, D.W. et al.	2004	2004: Balanced dispersal or source-sink - do both models describe wood mice in farmed landscapes? Oikos 106: 536-550. GLP: no Published: yes	N	Public	Not relevant  Public literature data, not a subject of data protection
KCP 10.1.2.2/21	Sullivan, T.P., Sullivan, D.S., van Hogue, E.J.	2003	Demography of montane voles in old field and orchard habitats in Southern British Columbia. Northwest Science 77: 228-236. GLP: no Published: yes	N	Public	Not relevant  Public literature data, not a subject of data protection
KCP 10.1.2.2/22	Niethammer, J., and F. Krapp	1982	<i>Microtus arvalis</i> (Pallas, 1779) - Feldmaus. Handbuch der Säugetiere Europas. J. Niethammer and F. Krapp. Wiesbaden, Aula-Verlag. 2/I: 285-318. GLP: no Published: yes	N	Public	Not relevant  Public literature data, not a subject of data protection
KCP 10.1.2.2/23	Mitchell-Jones, A., G. Amori, et al.	1999	The Atlas of European Mammals. London, Academic Press. Pp. 220-257. GLP: no Published: yes	N	Public	Not relevant  Public literature data, not a subject of data protection
KCP 10.1.2.2/24	Stein, G.H.W	1958	Die Feldmaus: <i>Microtus arvalis</i> Pallas. Wittenberg, Lutherstadt, A. Ziemsen Verlag. GLP: no Published: yes	N	Public	Not relevant  Public literature data, not a subject of data protection

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	Previously used  Y/N  If yes, for which data point
KCP 10.1.2.2/25	Truszkowski, J.	1982	The impact of the common vole on the vegetation of agroecosystems. Acta Theriologica 27: 305-345. GLP: no Published: yes	N	Public	Not relevant  Public literature data, not a subject of data protection
KCP 10.1.2.2/26	Heise, S., Stubbe, M.	1987	Populationsökologische Untersuchungen zum Massenwechsel der Feldmaus <i>Microtus arvalis</i> (Pallas, 1779). Säugetierkundliche Informationen 2: 403-414 GLP: no Published: yes	N	Public	Not relevant  Public literature data, not a subject of data protection
KCP 10.1.2.2/27	Nolting, H.-G	2010	Bekanntmachung über die Umsetzung des EFSA-Guidance Document zur Risikobewertung für Vögel und Säuger (BVL 10/02/14). Bundesamt für Verbraucherschutz und Lebensmittelsicherheit, Bundesanzeiger. 62: 2228-2229. GLP: no Published: yes	N	Public	Not relevant  Public literature data, not a subject of data protection
KCP 10.1.2.2/28	Hahne, J., Schabacker, J., Foudoulakis, M., Ludwigs, J.-D., Murfitt, R., Ristau, K.	2019	New proposed Residues on Fruits (RUDs) for frugivorous scenarios in EFSA Bird and Mammal Risk Assessment. Poster-Presentation SETAC Helsinki, June 2019. GLP: no Published: yes	N	Public	Not relevant  Public literature data, not a subject of data protection
KCP 10.1.2.2/29	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. <a href="https://doi.org/10.1002/ieam.4345">https://doi.org/10.1002/ieam.4345</a> ; <a href="https://setac.onlinelibrary.wiley.com/doi/epdf/10.1002/ieam.4345">https://setac.onlinelibrary.wiley.com/doi/epdf/10.1002/ieam.4345</a> GLP: no Published: yes	N	Public	Not relevant  Public literature data, not a subject of data protection

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KCP 10.2.1/01	xxx, xxxx.	2014a	Acute toxicity of MCW-2222 to the rainbow trout <i>Oncorhynchus mykiss</i> in a 96-hour static test Report No.: R-33831 xxxx. GLP: yes Published: no	Y	Adama	Y  RR KIIIA1 10.2.2.1/01
KCP 10.2.1/02	Juckeland, D.	2014b	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	Y  RR KIIIA1 10.2.2.2/01
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	Y  RR KIIIA1 10.2.2.2/02
KCP 10.2.1/04	Juckeland, D.	2014	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	Y  RR KIIIA1 10.2.2.3/01
KCP 10.2.1/02	Taylor, S. & Joyce, F., D.	2015	Acetamiprid 200 SL – Acute Toxicity to Aquatic Organisms Report no. R-35057 Cambridge Environmental Assessments, Battlegate Road, Boxworth, Cambridgeshire, CB23 4NN, UK GLP: yes Published: no	N	Adama	Y  RR KIIIA1 10.2.2.2/03
KCP 10.2.3/01	Hommen U., Hennecke S., Christmann R	2020	Carnadine – Outdoor mesocosm study Report No.: NFM-001/7-52 Fraunhofer Institute for Molecular Biology and Applied Ecology (IME), Auf dem Aberg 1, 57392 Schmallenberg, Germany GLP: Yes Published: no	N	Nufarm	N



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	Previously used  Y/N  If yes, for which data point
KCP 10.3.1/01	Rortais A., Arnold G., Halm M. P., Touffet-Briens F.	2005	Modes of honey bee exposure to systemic insecticides: estimated amounts of contaminated pollen and nectar consumed by different categories of bees. Apidologie, 36(1), 71-83. GLP: no Published: yes	N	Public	Not relevant  Public literature data, not a subject of data protection
KCP 10.3.1/02	Kim W., Gilet T., Bush J.W.	2011	Optimal concentrations in nectar feeding. Proceedings of the National Academy of Sciences of the United States of America 108(40): 16618-16621. GLP: no Published: yes	N	Public	Not relevant  Public literature data, not a subject of data protection
KCP 10.3.1/03	Pamminger T., Becker R., Himmelreich S., Schneider C. W., Bergtold M.	2019	The nectar report: quantitative review of nectar sugar concentrations offered by bee visited flowers in agricultural and non-agricultural landscapes. PeerJ 7, e6329, 15 pp. GLP: no Published: yes	N	Public	Not relevant  Public literature data, not a subject of data protection
KCP 10.3.1/04	Babendreier D., Kalberer N., Romeis J., Fluri P., Bigler, F.	2004	Pollen consumption in honey bee larvae: a step forward in the risk assessment of transgenic plants. Apidologie 35(3), 293-300. GLP: no Published: yes	N	Public	Not relevant  Public literature data, not a subject of data protection
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 Report No.: R-33834 BioChem agrar Labor für biologische und chemische Analytik GmbH. Kupferstraße 6. 04827 Gerichshain, Germany. GLP: yes Published: no	N	Adama	Y  RR KIIIA1 10.4.2.1/01

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KCP 10.3.1.2/01	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	N
KCP 10.3.1.3/01	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	N
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 Report No.: R-33837 BioChem agrar Labor für biologische und chemische Analytik GmbH. Kupferstraße 6. 04827 Gerichshain, Germany. GLP: yes Published: no	N	Adama	Y  RR KIIIA1 10.4.2.2/01
KCP 10.3.1.6/03	Aucejo, S.	2015	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. Report No.: R-35961 TESTAPI, Sarré, 49350 Gennes, France. GLP: yes Published: no	N	Adama	Y  RR KIIIA1 10.4.5/03
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.) Report No.: R-37336 RIFCON GmbH Goldbeckstr. 13 D-69493 Hirschberg, Germany. GLP: no Published: no	N	Adama	N

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	Previously used  Y/N  If yes, for which data point
KCP 10.3.1.5/04	Mamet, O. & Molitor, C.	2015	Assessment of toxicity on honeybees ( <i>Apis mellifera</i> ) of the product MCW-2222 (acetamiprid200 g/L) applied under insect proof tunnels on a phacelia crop in Northern France. Report No.: R-34875 TESTAPI, Sarré, 49350 Gennes, France. GLP: yes Published: no	N	Adama	Y  RR KIIIA1 10.4.7/04
KCP 10.3.1.5/05	Mamet, O. & Molitor, C.	2015	Assessment of toxicity on honey bees ( <i>Apis mellifera</i> ) of the product MCW-2222 (acetamiprid200 g/L) applied under insect proof tunnels on a phacelia crop during summer in France Report No.: R-34876 TESTAPI, Sarré, 49350 Gennes, France. GLP: yes Published: no	N	Adama	Y  RR KIIIA1 10.4.7/05
KCP 10.3.1.5/06	Mamet, O. & Molitor, C.	2015	Assessment of toxicity on honey bees ( <i>Apis mellifera</i> ) of the product MCW-2222 (acetamiprid200 g/L) applied under insect proof tunnels on a phacelia crop during summer in France. Report No.: R-35847 TESTAPI, Sarré, 49350 Gennes, France. GLP: yes Published: no	N	Adama	Y  RR KIIIA1 10.4.7/06
KCP 10.3.1.6/01	Molitor, C.	2015	Field Study to Evaluate Potential Side Effects of the product MCW-2222 (acetamiprid200 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> Report No.: R-34877 TESTAPI, Sarré, 49350 Gennes, France. GLP: yes Published: no	N	Adama	Y  RR KIIIA1 10.4.5/01
KCP 10.3.1.6/02	Molitor, C.	2015	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 Study no R-35844 TESTAPI, Sarré, 49350 Gennes, France. GLP: yes Published: no	N	Adama	Y  RR KIIIA1 10.4.5/02

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KCP 10.3.2.4/01	Appeltauer, A.	2018	A Field Study Assessing the Impact of Drift Rates of Acetamiprid on the Non-Target Arthropod Fauna on a Meadow in Germany Report No.: R-35848 Eurofins Agrosience GmbH, Eutinger Str. 24 D-75223 Niefern-Öschelbronn, Germany GLP: yes Published: no	N	Adama	Y  RR KIIIA1 10.5.4/01
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 rhopalosiphi</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	N
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 rhopalosiphi</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	N
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 rhopalosiphi</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	N
KCP 10.3.2.3/04	Luna, F.	2017a	Aged residue test with the formulation “MCW-2222” at 170 g a.s. /ha on the parasitic wasp <i>Aphidius rhopalosiphi</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	N

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KCP 10.3.2.3/05	Luna, F.	2017b	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	N	Adama	N
KCP 10.3.2.3/06	Luna, F.	2017c	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	N	Adama	N
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 (LR <sub>50</sub> ) - Report No.: R-33838 BioChem agrar Labor für biologische und chemische Analytik GmbH. Kupferstraße 6. 04827 Gerichshain, Germany. GLP: yes Published: no	N	Adama	Y  RR KIIIA1 10.5.1/02
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 (LR <sub>50</sub> ) - Report No.: R-33839 BioChem agrar Labor für biologische und chemische Analytik GmbH. Kupferstraße 6. 04827 Gerichshain, Germany. GLP: yes Published: no	N	Adama	Y  RR KIIIA1 10.5.1/01
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 (LR <sub>50</sub> ) - Report No.: R-34780 BioChem agrar Labor für biologische und chemische Analytik GmbH. Kupferstraße 6. 04827 Gerichshain, Germany. GLP: yes Published: no	N	Adama	Y  RR KIIIA1 10.5.2/01

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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 (LR <sub>50</sub> ) - Report No.: R-34781 BioChem agrar Labor für biologische und chemische Analytik GmbH. Kupferstraße 6. 04827 Gerichshain, Germany. GLP: yes Published: no	N	Adama	Y  RR KIIIA1 10.5.2/02
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 (LR <sub>50</sub> ) - Report No.:R-33839A BioChem agrar Labor für biologische und chemische Analytik GmbH. Kupferstraße 6. 04827 Gerichshain, Germany. GLP: yes Published: no	N	Adama	Y  RR KIIIA1 10.5.2/03
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 (LR <sub>50</sub> ) - Report No.: R-34782 BioChem agrar Labor für biologische und chemische Analytik GmbH. Kupferstraße 6. 04827 Gerichshain, Germany. GLP: yes Published: no	N	Adama	Y  RR KIIIA1 10.5.2/04
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	Y  RR KIIIA1 10.5.2/05
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	Y  RR KIIIA1 10.6.3/01

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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	Y  RR KIIIA1 10.6.6/01
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	Y  RR KIIIA1 10.6.6/02
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	Y  RR KIIIA1 10.7.1/01
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	Y  RR KIIIA1 10.8.1.2/01

**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
KCP 10.4.1.1/02	Lühns, U	2003	Effects of IM-1-5 on reproduction and growth of earthworms Eisenia foetida in artificial Soil RD-03058 IBACON GLP: Yes Published: No	N	Nippon Soda (no data protection)
KCP 10.4.2.1/02	Klein, S. & Rosenkraus, B.	2003	Effects of IM -1-5 on Reproduction of the Collembola Folsomia Candida in Artificial Soil C029229 / RD-03096 Aventis CropScience GLP: Yes Published: No	N	Nippon Soda (no data protection)

**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	Reason for rejection
KCP 10.1.2.2/04	Heroldová, M., Zejda, J., Zapletal, M., Obdržálová, D., Jánová, E., Bryja, J., Tkadlec, E.	2004	Importance of winter rape for small rodents. Plant soil and environment, 50(4), 175-181. GLP: no Published: yes	N	Public	Not required for the use pattern supported by the Applicant
KCP 10.3.1.2/02	Kleebaum, K.	2014a	Chronic toxicity of MCW-2222 to the honeybee <i>Apis mellifera</i> L. under laboratory conditions Report No.: R-33835 BioChem agrar Labor für biologische und chemische Analytik GmbH. Kupferstraße 6. 04827 Gerichshain, Germany. GLP: yes Published: no	N	Adama	Study no longer valid, superseded by study presented under KCP 10.3.1.2/01



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	Reason for rejection
KCP 10.3.1.3/02	Kleebaum, K.	2014b	Chronic toxicity of MCW-2222 to honeybee larvae ( <i>Apis mellifera</i> L.) under laboratory conditions (in vitro) Report No.: R-33836 BioChem agrar Labor für biologische und chemische Analytik GmbH. Kupferstraße 6. 04827 Gerichshain, Germany. GLP: yes Published: no	N	Adama	Study no longer suitable for the risk assessment purposes, superseded by study presented under KCP 10.3.1.3/01
KCP 10.3.1.5/02	Mamet, O.	2015	Assessment of toxicity on honeybees ( <i>Apis mellifera</i> ) of MCW-2222 on wheat crop in a tunnel trial in France. Report No.: R-35845 TESTAPI, Sarré, 49350 Gennes, France. GLP: yes Published: no	N	Adama	Study performed on crop not included in the Central Zone GAP for CA3573, thus not relevant for the risk assessment
KCP 10.3.1.5/03	Mamet, O.	2015	Assessment of toxicity on honey bees ( <i>Apis mellifera</i> ) of the product MCW-2222 on cereals in a tunnel trial in France. Report No.: R-35846 TESTAPI, Sarré, 49350 Gennes, France. GLP: yes Published: no	N	Adama	Study performed on crop not included in the Central Zone GAP for CA3573, thus not relevant for the risk assessment

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	Reason for rejection
KCP 10.3.1.5/01	Mamet, O. & Molitor, C.	2014	Assessment of toxicity on honey bees ( <i>Apis mellifera</i> ) of the product MCW-2222 (acetamiprid200 g/L) applied under insect proof tunnels on a cereal crop Report No.: R-34874 TESTAPI, Sarré, 49350 Gennes, France. GLP: yes Published: no	N	Adama	Study performed on crop not included in the Central Zone GAP for CA3573, thus not relevant for the risk assessment

**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
None					

## 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

##### 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

Public literature evaluated by zRMS and considered in the risk assessment are summarized below.

Comments of zRMS:	For comments of the zRMS on acceptability and applicability of this literature study for the higher-tier risk assessment, please refer to point 9.3 of this document.
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Reference:	KCP 10.1.2.2/01
Report	Common vole ( <i>Microtus arvalis</i> ) ecology and management: implications for risk assessment of plant protection products. Jacob, J., Manson, P., Barfknecht, R., Fredricks, T. 2013 Pest Management Science 70: 869-878.
Guideline(s):	Not applicable (publication)
Deviations:	Not applicable (publication)
GLP:	No
Acceptability:	Please, refer to point 9.3 for zRMS comments on acceptability and applicability of the study
Duplication (if vertebrate study)	Not applicable

### Executive Summary

Common voles (*Microtus arvalis*) are common small mammals in some European landscapes. They can be a major rodent pest in European agriculture and they are also a representative generic focal small herbivorous mammal species used in risk assessment for plant protection products. In this paper, common vole population dynamics, habitat and food preferences, pest potential and use of the common vole as a model small wild mammal species in the risk assessment process are reviewed. Common voles are a component of agroecosystems in many parts of Europe, inhabiting agricultural areas (secondary habitats) when the carrying capacity of primary grassland habitats is exceeded. Colonisation of secondary habitats occurs during multiannual outbreaks, when population sizes can exceed 1000 individuals ha<sup>-1</sup>. In such cases, in-crop common vole population control management has been practised to avoid significant crop damage. The species' status as a crop pest, high fecundity, resilience to disturbance and intermittent colonisation of crop habitats are important characteristics that should be reflected in risk assessment. Based on the information provided in the scientific literature, it seems justified to modify elements of the current risk assessment scheme for plant protection products, including the use of realistic food intake rates, reduced assessment factors or the use of alternative focal rodent species in particular European regions. Some of these adjustments are already being applied in some EU member states. Therefore, it seems reasonable consistently to apply such pragmatic and realistic approaches in risk assessments for plant protection products across the EU.

## Materials and methods

This article presents a review of common vole population dynamics, biology and behaviour, including habitat preferences and crop damage potential relevant to risk assessment. In the review, refined approaches to the use of common voles in the risk assessment of plant protection products within the EU regulatory framework on the basis of realistic and scientifically based information are discussed.

## Conclusion

Common voles are widely distributed in agroecosystems. The risk of side effects of plant protection products for common voles is limited to individuals present in crops during product application, while (extensive) populations in off-crop primary habitat refuges remain unaffected. For many crops the occurrence of common voles is restricted to population outbreaks and is associated with voles becoming significant agricultural pests. Their pest status, highly fluctuating population dynamics, habitat preferences, resilience and high reproductive potential should reduce potential pesticide impact upon common vole populations, but this is not fully reflected in the current risk assessment scheme. Overall, based on the compelling evidence provided in this document, it is proposed that it would be justified to modify elements of the current risk assessment, for example by refining consumption estimates on the basis of expanded field collected data on common voles, applying reduced TER trigger values universally across all member states and/or advocating alternative focal species where this is considered to be geographically appropriate. This will ensure that a more realistic and pragmatic approach to wild mammal risk assessment is taken in the assessment of plant protection products.

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Comments of zRMS:	For comments of the zRMS on acceptability and applicability of this literature study for the higher-tier risk assessment, please refer to point 9.3 of this document.
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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. <a href="https://doi.org/10.1002/ieam.4345">https://doi.org/10.1002/ieam.4345</a>
Guideline(s):	Not applicable (publication)
Deviations:	Not applicable (publication)
GLP:	No
Acceptability:	Please, refer to point 9.3 for zRMS comments on acceptability and applicability of the study
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 <sup>1</sup>	Large fruits <sup>2</sup>	Small fruits <sup>3</sup>	Gourds <sup>4</sup>	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

<sup>1</sup> Currants, raspberries and gooseberries

<sup>2</sup> Apple, peach, pear, lemon, mandarin and orange

<sup>3</sup> RUD value from Cherries (C-EU), covering apricot and plum (C-EU), and cherry apricot, and plum (S-EU) (192 trials)

<sup>4</sup> 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  $\geq 100$  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 2.1.3                      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 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 203 and met all validity criteria. Following endpoints based on nominal concentrations were agreed:</p> <p>LC<sub>50</sub> = 85.8 mg product/L (corresponding to 15.3 mg a.s./L)</p>
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<b>Reference:</b>	KCP 10.2.1/01
<b>Report</b>	Acute toxicity of MCW-2222 to the rainbow trout <i>Oncorhynchus mykiss</i> in a 96-hour static test, xxx, xxx., 2014, R-33831
<b>Guideline(s):</b>	OECD 203 (1992)
<b>Deviations:</b>	<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) This is not considered to have any impact on the study integrity or outcome</p>
<b>GLP:</b>	Yes, certified laboratory
<b>Acceptability:</b>	Acceptable
<b>Duplication (if vertebrate study)</b>	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 LC<sub>10</sub>, LC<sub>20</sub>, LC<sub>50</sub> 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

<b>Test item</b>	MCW-2222
<b>Batch #</b>	611-280413-01
<b>Purity</b>	Acetamiprid 200 g/L (nominal); 202.7 g/L (analysed)
<b>Description</b>	Yellowish liquid
<b>Control</b>	Test medium
<b>Test organism</b>	

<b>Species</b>	Rainbow trout <i>Oncorhynchus mykiss</i>
<b>Source</b>	mean length: $5.0 \pm 0.2$ cm, mean weight: $1.18 \pm 0.2$ g. Forellenzucht Trostadt GbR” Dorfstraße 7, 98646 Trostadt OT Reurieth, Germany
<b>Study design and methods</b>	
<b>Test duration and exposure</b>	96 hours, static
<b>Experimental dates</b>	03 to 14 February 2014
<b>Test concentrations</b>	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
<b>Test units</b>	One 13 L stainless steel container per concentration, each filled with 10 L of test solution
<b>Group size/replicates</b>	8 organisms per concentration; 1 replicate per concentration
<b>Test medium</b>	Reconstituted water according to ISO 6341 Conductivity of deionised water: $\leq 10$ $\mu$ S/cm (measured 1.9 $\mu$ S/cm)
<b>Adaptation</b>	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.
<b>Aeration</b>	Yes
<b>Environmental conditions</b>	
<b>Temperature</b>	13.4 – 14.5 °C
<b>Photoperiod</b>	16 hours light / 8 hours darkness
<b>pH</b>	7.56 - 8.22
<b>Dissolved oxygen</b>	$\geq 7.46$

### **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<sub>x</sub> 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 1: 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 2: 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,  $p \geq 0.05$ )

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 3: Endpoints after 96 hours**

	Concentration [mg test item/L]	Concentration [mg a.s./L]
LC <sub>10</sub> (95%-CI)	34.5 (12.2 – 54.0)	6.14 (2.17 – 9.62)
LC <sub>20</sub> (95%-CI)	47.1 (21.5 – 70.2)	8.40 (3.83 – 12.5)
LC <sub>50</sub> (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 4: Validity criteria**

Validity criteria according to OECD 203 (2019)	Observed in study
Mortality in the control $\leq 10\%$	0%
O <sub>2</sub> 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 LC<sub>10</sub>, LC<sub>20</sub>, LC<sub>50</sub> 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. 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 202 and met all validity criteria. Following endpoints based on nominal concentrations were agreed:</p> <p>EC<sub>50</sub> = 100.2 mg product/L (corresponding to 17.9 mg a.s./L)</p>
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<b>Reference:</b>	KCP 10.2.1/02
<b>Report</b>	Acute toxicity of MCW-2222 to <i>Daphnia magna</i> in a 48-hour static test Juckeland, D., 2014b, R-33832
<b>Guideline(s):</b>	OECD 202 (2004)
<b>Deviations:</b>	No
<b>GLP:</b>	Yes, certified laboratory
<b>Acceptability:</b>	Acceptable
<b>Duplication (if vertebrate study)</b>	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 EC<sub>10</sub>, EC<sub>20</sub> and EC<sub>50</sub> 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

<b>Test item</b>	MCW-2222
<b>Batch #</b>	611-280413-01
<b>Content of active substance</b>	Acetamiprid 200 g/L (nominal); 202.7 g/L (analysed)
<b>Description</b>	Yellowish liquid
<b>Control</b>	Test medium
<b>Toxic reference</b>	Potassium chloride was tested in a separate study
<b>Test organism</b>	
<b>Species</b>	<i>Daphnia magna</i> ; neonate (less than 24 hours old)
<b>Source</b>	In-house culture, originally obtained from Landesanstalt für Umweltschutz Baden-Württemberg, Griesbachstr. 1, 76185 Karlsruhe, Germany

#### Study design and methods

<b>Test duration and exposure</b>	48 hours, static exposure
<b>Experimental dates</b>	04 to 06 Feb 2014

<b>Test concentrations</b>	19.2, 42.2, 93.1, 204.5, 450.2 mg test item/L corresponding to 3.42, 7.53, 16.6, 36.5, 80.3 mg a.s./L
<b>Test units</b>	150 mL glass beakers, each filled with 10 mL of test solution.
<b>Group size/replicates</b>	20 organisms per concentration; 5 in each of 4 replicates
<b>Test medium</b>	M-4 Medium (OECD 202, 2004)
<b>Environmental conditions</b>	
<b>Temperature</b>	19.7 – 20.7 °C
<b>Photoperiod</b>	16 hours light / 8 hours darkness
<b>pH</b>	7.77 - 8.28
<b>Dissolved oxygen</b>	≥ 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<sub>x</sub> 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 5: 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 6: 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 7: Endpoints after 48 hours**

	Concentration [mg test item/L]	Concentration [mg a.s./L]
EC <sub>10</sub> (95%-CI)	36.0 (24.0 – 54.7)	6.42 (4.28 – 9.75)
EC <sub>20</sub> (95%-CI)	51.4 (36.8 – 71.7)	9.16 (6.56 – 12.8)
EC <sub>50</sub> (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 8: 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 EC<sub>10</sub>, EC<sub>20</sub> and EC<sub>50</sub> 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. 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 235 and met all validity criteria. Following endpoints based on nominal concentrations were agreed:</p> <p>EC<sub>50</sub> = 0.0521 mg product/L (corresponding to 0.00929 mg a.s./L)</p>
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<b>Reference:</b>	KCP 10.2.1/03
<b>Report</b>	Acute toxicity of MCW-2222 to <i>Chironomus riparius</i> in a 48-hour static test, Juckeland, D., 2015, R-34873
<b>Guideline(s):</b>	OECD 235 (2011)
<b>Deviations:</b>	No
<b>GLP:</b>	Yes, certified laboratory
<b>Acceptability:</b>	Acceptable
<b>Duplication (if vertebrate study)</b>	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 EC<sub>10</sub>, EC<sub>20</sub> and EC<sub>50</sub> 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

<b>Test item</b>	MCW-2222
<b>Batch #</b>	611-280413-01
<b>Content of active substance</b>	Acetamiprid 200 g/L (nominal); 202.7 g/L (analysed)
<b>Description</b>	Yellowish liquid
<b>Control</b>	Test medium
<b>Toxic reference</b>	Potassium chloride was tested in a separate study.
<b>Test organism</b>	
<b>Species</b>	<i>Chironomus riparius</i> ; first instar (~48 hours old)
<b>Source</b>	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

<b>Test duration and exposure</b>	48 hours, static exposure
<b>Experimental dates</b>	29 to 31 Jul 2014
<b>Test concentrations</b>	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
<b>Test units</b>	Glass beakers, each filled with 10 mL of test solution
<b>Group size/replicates</b>	20 organisms per concentration; 5 in each of 4 replicates
<b>Test medium</b>	M-4 Medium (OECD 235, 2011)
<b>Environmental conditions</b>	
<b>Temperature</b>	19.8 – 20.4 °C
<b>Photoperiod</b>	16 hours light / 8 hours darkness
<b>pH</b>	7.87 - 8.17
<b>Dissolved oxygen</b>	≥ 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<sub>x</sub> 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 9: 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
	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  $\mu\text{g a.s./L}$

### Biological results

Biological results are given in the table below.

**Table A 10: Percent of immobilised chironomids in a 48-hour acute immobilisation test exposed to MCW-2222**

Nominal concentration [ $\mu\text{g test item/L}$ ]	Control	26.1	36.4	51.0	71.4	100.0
Nominal concentration [ $\mu\text{g a.s./L}$ ]	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,  $p \alpha 0.05$ , one-sided greater)

**Table A 11: Endpoints after 48 hours**

	Concentration [ $\mu\text{g test item/L}$ ]	Concentration [ $\mu\text{g a.s./L}$ ]
EC <sub>10</sub> (95%-CI)	37.9 (32.8 – 43.8)	6.76 (5.85 – 7.81)
EC <sub>20</sub> (95%-CI)	42.3 (37.6 – 47.6)	7.54 (6.70 – 8.49)
EC <sub>50</sub> (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 12: Validity criteria**

Validity criteria according to OECD 235	Observed in study
Number of immobilised larvae must be $\leq 15\%$	0%
Dissolved oxygen concentration at the end of the test must be $\geq 3 \text{ mg/L}$ in control(s) and test solutions.	$\geq 7.91 \text{ mg/L}$
<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 EC<sub>10</sub>, EC<sub>20</sub> and EC<sub>50</sub> 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. 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 201 and met all validity criteria. Following endpoints based on nominal concentrations were agreed:</p> <p>E<sub>r</sub>C<sub>50</sub> = 3110.8 mg product/L (corresponding to 554.5 mg a.s./L)  E<sub>y</sub>C<sub>50</sub> = 1149.5 mg product/L (corresponding to 204.9 mg a.s./L)  NOEC = 218.8 mg product/L (corresponding to 39.0 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:	Acceptable
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 E<sub>r</sub>C<sub>50</sub> of 554.5 and an E<sub>y</sub>C<sub>50</sub> 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

<b>Toxic reference</b>	The reference item potassium dichromate was tested in a separate study to verify the sensitivity of the test system.
<b>Test organism</b>	
<b>Species</b>	<i>Desmodesmus subspicatus</i>
<b>Source</b>	ScienceBridge GmbH, Hans-Adolf-Krebs-Weg 1, 37077 Göttingen, Germany; strain: 86.81 SAG
<b>Study design and methods</b>	
<b>Test duration and exposure</b>	72 hours, static
<b>Experimental dates</b>	04 Feb to 07 Feb 2014
<b>Test concentrations</b>	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 analysed content of a.s.)
<b>Test units</b>	250 mL glass vessels filled with 100 mL test solution.
<b>Group size/replicates</b>	3 replicates for each test concentration and 6 replicates for the control
<b>Test medium</b>	OECD medium
<b>Preculture</b>	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.
<b>Aeration</b>	None
<b>Renewal of test solutions</b>	None
<b>Initial cell density</b>	Approximately $5 \times 10^3$ cells/mL
<b>Environmental conditions</b>	
<b>Temperature</b>	22.7 – 22.8 °C
<b>Lighting</b>	Continuously at $95 \mu\text{E m}^{-2}\text{s}^{-1}$
<b>pH</b>	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  $\text{EC}_x$  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 13: Nominal and measured concentrations of test item**

Table 12.12.1						
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Limit of quantification: 0.344 mg/L

### Biological results

Biological result are given in the following table:

**Table A 14: 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 <sup>4</sup> cells/mL]	% Inhibition (0-72 h)	
		Growth rate	Yield
0 (Control)	23.21	0	0
218.8	23.50	0 (-0.3) <sup>a</sup>	0 (-1.3) <sup>a</sup>
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 15: EC<sub>50</sub>-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 <sub>r</sub> C <sub>50</sub>	3110.8 (2701.4 – 3754.0)
E <sub>y</sub> C <sub>50</sub>	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 16: 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 <sup>-1</sup> .	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<sub>r</sub>C<sub>10</sub>, E<sub>r</sub>C<sub>20</sub> and E<sub>r</sub>C<sub>50</sub> 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<sub>y</sub>C<sub>10</sub>, E<sub>y</sub>C<sub>20</sub> and E<sub>y</sub>C<sub>50</sub> 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 2.2.1.5 KCP 10.2.1/02 Acute toxicity to additional invertebrate species

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 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 adopted OECD 202 and met all validity criteria. Following endpoints based on nominal concentrations were agreed:</p> <table border="1"> <thead> <tr> <th>Species</th><th>EC<sub>50</sub> (mg a.s./L)</th></tr> </thead> <tbody> <tr> <td><i>Aeshna</i> sp</td><td>&gt;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>Corixinae</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> </tbody> </table>	Species	EC <sub>50</sub> (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	Corixinae	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 <sub>50</sub> (mg a.s./L)																						
<i>Aeshna</i> sp	>2.13																						
<i>Asellus aquaticus</i>	0.0394																						
<i>Chaoborus crystallinus</i>	1.998																						
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Corixinae	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																						

<b>Report:</b>	KIIIA1 10.2.2.2/03 S. Taylor & F. Joyce., 2015
<b>Title:</b>	Acetamiprid 200 SL – Acute Toxicity to Aquatic Organisms
<b>Document No:</b>	R-35057
<b>Guidelines:</b>	The study was not conducted according to any specific regulatory guideline, but the following was consulted: OECD Guidelines for Testing of Chemicals, No. 202: “Daphnia sp. Acute Immobilisation Test”, adopted, 2004.
<b>Acceptability:</b>	Acceptable
<b>GLP</b>	Yes

#### Executive Summary

The objective of this study was to determine the effect of acetamiprid 200 SL (MCW-2222) on 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*. The test item was a formulated product containing the active substance acetamiprid (17.51% w/w).

The invertebrates were exposed to a range of concentrations of the test item prepared in filtered (30 µm ) pond water over a 48 hour exposure period in a laboratory under static conditions, in order to provide relevant Effect Concentrations (ECx) and Lethal Concentrations (LCx), based on immobility and mortality observations. The definitive test concentrations, which were selected for testing for each individual taxon are presented below:

Taxon	Nominal Test Conc. (µg a.s./L)
<i>Aeshna</i> sp.	150, 255, 434, 737, 1253, 2130
<i>Asellus aquaticus</i>	1.94, 4.27, 9.39, 20.7, 45.5, 100
<i>Cloeon dipterum</i>	1.94, 4.27, 9.39, 20.7, 45.5, 100
<i>Chaoborus crystallinus</i>	150, 255, 434, 737, 1253, 2130
<i>Ischnura elegans</i>	150, 255, 434, 737, 1253, 2130
Corixinae	1.94, 4.27, 9.39, 20.7, 45.5, 100
<i>Crangonyx pseudogracilis</i>	1.94, 4.27, 9.39, 20.7, 45.5, 100
<i>Gammarus pulex</i>	79, 118, 177, 267, 400, 600
<i>Phryganea bipunctata</i>	1.94, 4.27, 9.39, 20.7, 45.5, 100
<i>Notonecta marmorea viridis</i>	176, 299, 509, 865, 1470, 2500

During the exposure period the invertebrates were inspected at 24 hr intervals for signs of mortality and immobility. Statistical analyses of the available data for immobility after 48 hours revealed that the following EC<sub>10</sub>, EC<sub>20</sub> and EC<sub>50</sub> values were reliably calculated:

Taxon	48-hr (µg a.s./L)		
	EC <sub>10</sub> (95% cl)	EC <sub>20</sub> (95% cl)	EC <sub>50</sub> (95% cl)
<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)

## I. MATERIALS AND METHODS

### A. Materials

- Test material:** Acetamiprid 200 SL (MCW-2222)  
**Description:** Clear yellow liquid  
**Lot/Batch #:** 135410-20-7  
**Purity:** Acetamiprid 200 g/L (nominal)  
**Composition:** 17.51% w/w (199.2 ± 1.3 g/L)  
**Stability of test compound:** Expiry date: 13 March 2016
- Vehicle and/or positive control:** None
- Test animals:**  
**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*)).  
  
**Size:**

<i>Aeshna</i> sp.	Larvae (5 - 20 mm total length)
<i>Asellus aquaticus</i>	Adult (4 - 10 mm total length)
<i>Chaoborus crystallinus</i>	Larvae (5 - 10 mm total length)

<i>Cloeon dipterum</i>	Adult (2 - 8 mm total length)
<i>Corixinae</i>	Adult (5 - 15 mm total length)
<i>Crangonyx pseudogracilis</i>	Adult (3 - 6 mm total length)
<i>Gammarus pulex</i>	Adult (5 - 15 mm total length)
<i>Ischnura elegans</i>	Larvae (5 - 20 mm total length)
<i>Phryganea bipunctata</i>	Adult (10 - 20 mm total length)
<i>Notonecta marmorea viridis</i>	Adult (15 - 20 mm total length)

**Source:**

CEA mesocosm facility; Blades Biological, UK

**Acclimation period:**

The organism cultures were maintained in either a 5 L glass beaker or a 12 L plastic box. The temperature of the culture media was maintained at 17.9 to 20.9°C (measured from spot measurements and from a continuous min-max thermometer in a separate vessel) and a pH of 7.05 to 8.69. Water quality parameters (pH, dissolved oxygen and temperature) were recorded at least twice during each acclimation period.

**Environmental conditions:**

Temperature:

20°C ± 2°C

Photoperiod:

16 hours light/8 hours dark cycle

## B. Study design and methods

### 1. In life dates:

29 October 2014 to 05 March 2015

### 2. Experimental treatments

A 48-hour static exposure regime was employed. The test media were prepared at the start of each definitive test and used on the same day they were made.

The definitive test concentrations, which were selected for testing for each individual taxon are presented below:

Taxon	Nominal Test Conc. (µg a.s/L)
<i>Aeshna</i> sp.	150, 255, 434, 737, 1253, 2130
<i>Asellus aquaticus</i>	1.94, 4.27, 9.39, 20.7, 45.5, 100
<i>Cloeon dipterum</i>	1.94, 4.27, 9.39, 20.7, 45.5, 100
<i>Chaoborus crystallinus</i>	150, 255, 434, 737, 1253, 2130
<i>Ischnura elegans</i>	150, 255, 434, 737, 1253, 2130
<i>Corixinae</i>	1.94, 4.27, 9.39, 20.7, 45.5, 100
<i>Crangonyx pseudogracilis</i>	1.94, 4.27, 9.39, 20.7, 45.5, 100
<i>Gammarus pulex</i>	79, 118, 177, 267, 400, 600
<i>Phryganea bipunctata</i>	1.94, 4.27, 9.39, 20.7, 45.5, 100
<i>Notonecta marmorea viridis</i>	176, 299, 509, 865, 1470, 2500

6 treatments in 4 (group housed) or 10 (individually housed) replicates per group with 5 (group housed) or 1 (individually housed) organism per replicate were conducted plus one diluent control per taxon.

### 3. Observations

The test organisms were observed daily at approximate 24-hour intervals for signs of mortality and immobility. The temperature, pH, and dissolved oxygen of freshly prepared (0 hr) and pooled expired (48 hr) media per concentration or control group were recorded for each test. Air and media temperature in the test area was monitored continuously using a min-max thermometer in a replicate vessel.

## II. RESULTS UND DISCUSSION

### A. Findings

#### Environmental Conditions:

Environmental conditions are presented in table below, separately for each species.

Taxon	Media Temperature (°C)*		Continuous Culture Temperature (°C)		pH		Dissolved Oxygen (% ASV)	
	Min	Max	Min	Max	Min	Max	Min	Max
<i>Aeshna sp.</i>	18.8	18.9	17.9	19.8	7.05	7.32	84.4	89.3
<i>Asellus aquaticus</i>	19.9	19.9	18.1	20.2	7.92	8.29	62.1	92.7
<i>Cloeon dipterum</i>	19.9	20.0	18.1	20.2	8.34	8.55	86.5	93.0
<i>Chaoborus crystallinus</i>	20.5	20.5	17.9	19.8	7.38	7.43	97.7	98.2
<i>Ischnura elegans</i>	18.9	20.0	17.9	19.8	7.33	7.36	94.8	101.4
<i>Corixinae</i>	20.8	20.9	18.8	19.6	8.20	8.37	91.0	91.4
<i>Crangonyx pseudogracilis</i>	19.6	19.7	18.1	20.2	8.30	8.61	78.2	93.2
<i>Gammarus pulex</i>	20.3	20.4	18.8	19.6	8.27	8.41	90.5	90.9
<i>Phryganea bipunctata</i>	18.0	18.0	18.1	20.2	8.13	8.69	87.0	96.7
<i>Notonecta marmorea viridis</i>	20.7	20.7	18.8	20.3	7.95	7.95	83.7	83.7

#### Exposure Concentrations:

Chemical analysis 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), highlighted below, which is not considered to have an impact on the overall results. Therefore, the nominal concentrations were used in the calculation of the results. Overall mean measured concentrations of acetamiprid for each batch of taxa were as follows:

*Aeshna sp.*, *Chaoborus crystallinus* and *Ischnura elegans*

- 128, 243, 390, 645, 1185 and 1812 µg a.s./L (85 to 95% of nominal).

*Asellus aquaticus*, *Cloeon dipterum*, *Crangonyx pseudogracilis* and *Phryganea bipunctata*

- 1.92, 5.17, 9.17, 18.8, 45.6 and 94.4 µg a.s./L (91 to 121% of nominal).

*Notonecta marmorea viridis*

- 166, 281, 474, 813, 1425 and 2480 µg a.s./L (93 to 99% of nominal).

*Gammarus pulex*

- 74.1, 112, 166, 248, 384 and 577 µg a.s./L (93 to 106% of nominal).

*Corixinae*

- 1.76, 3.80, 8.33, 22.0, 43.7, 83.5 µg a.s./L (84 to 106% of nominal).

#### Immobilisation:

No immobility was observed in the control and the test groups with *Aeshna sp.* Data on immobility are provided in the table below.

### Percent of immobilized organisms in a 48-hour acute immobilisation test exposed to MCW-2222

	Immobilisation [%]						
<i>Asellus aquaticus</i>							
Nominal concentration (µg a.s./L)	Control	1.94	4.27	9.39	20.7	45.5	100
48 h	0	0	0	0	30	50	90
<i>Chaoborus crystallinus</i>							
Nominal concentration (µg a.s./L)	Control	150	255	434	737	1253	2130
48 h	0	0	0	0	10	10	60
<i>Cloeon dipterum</i>							
Nominal concentration (µg a.s./L)	Control	1.94	4.27	9.39	20.7	45.5	100
48 h	0	0	5	5	95	100	100
<i>Corixinae</i>							
Nominal concentration (µg a.s./L)	Control	1.94	4.27	9.39	20.7	45.5	100
48 h	12.5	15	15	35	55	85	100
<i>Crangonyx pseudogracilis</i>							
Nominal concentration (µg a.s./L)	Control	1.94	4.27	9.39	20.7	45.5	100
48 h	0	0	0	0	30	70	100
<i>Gammarus pulex</i>							
Nominal concentration (µg a.s./L)	Control	79	118	177	267	400	600
48 h	5	30	30	90	100	100	100
<i>Ischnura elegans</i>							
Nominal concentration (µg a.s./L)	Control	150	255	434	737	1253	2130
48 h	0	0	0	0	20	40	80
<i>Phryganea bipunctata</i>							
Nominal concentration (µg a.s./L)	Control	1.94	4.27	9.39	20.7	45.5	100
48 h	0	0	0	22	70	100	100
<i>Notonecta marmorea viridis</i>							
Nominal concentration (µg a.s./L)	Control	176	299	509	586	1470	2500
48 h	0	0	0	0	0	60	100

### EC values of immobilisation (based on analytically confirmed nominal concentrations)

Taxon	48-hr (µg a.s./L)		
	EC <sub>10</sub> (95% cl)	EC <sub>20</sub> (95% cl)	EC <sub>50</sub> (95% cl)
<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)
<i>Corixinae</i>	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:

This test for each taxon can regarded as valid since:

- In the control, mortality of organisms did not exceed 10% (except *Corixinae*)
- The dissolved oxygen was maintained at >60% ASV for the duration of the test

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.

## III. CONCLUSIONS

The concentrations of acetamiprid were determined in freshly prepared (0 hour) and pooled expired (48 hour) test media for each batch of tests conducted. 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.

Statistical analyses of the available data for immobility after 48 hours revealed that the following EC<sub>10</sub>, EC<sub>20</sub> and EC<sub>50</sub> values were reliably calculated:

Taxon	48-hr (µg a.s./L)		
	EC <sub>10</sub> (95% cl)	EC <sub>20</sub> (95% cl)	EC <sub>50</sub> (95% cl)
<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)

## 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 2.2.3.1 KCP 10.2.3/01 Mesocosm study

Comments of zRMS:	<p>The newly submitted mesocosm study with CA3573 (Hommen et al., 2020) has been validated by the zRMS against criteria given in:</p> <ul style="list-style-type: none"> <li>• EFSA aquatic guidance (2013),</li> <li>• OECD 53 (2006): Guidance document on simulated freshwater lentic field tests (outdoor microcosms and mesocosms),</li> <li>• Giddings et al. (2002): Community Level Aquatic System Studies – Interpretation Criteria (CLASSIC).</li> </ul> <p>In the study formulation CA3573 was applied to established (&gt;1 year) systems twice with 7 days interval. The exposure in test systems represented thus worst case comparing to this expected under field conditions, since the current Central Zone GAP includes only single applications. 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 84 days (12 weeks after dosing in order to allow detection of delayed effects on e.g. emergence of insects).</p> <p>The concentration of acetamiprid in water was verified approximately 3 hours after the both applications (day 0 and 7) and on days 1, 3, 6, 8, 10, 15, 22, 30, 42, 56 and 84, 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 5, 15, 23, 28, 42, 56 and 54, which was sufficient to confirm dissipation of the active substance to this compartment</p>
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	<p>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>1. <u>Is the test system adequate and does the test system represent a realistic freshwater community?: Yes</u></p> <p>The test system is considered to be adequate to investigate acute and long-term effects of the test item on populations and communities of aquatic invertebrates.</p> <p>In order to ensure the development of a representative community of populations, algae from several classes, zooplankton (several species of Cladocerans, Copepods, Rotifiers), midge larvae of <i>Chaoborus</i> sp. and macroinvertebrates (e.g. <i>Asellus aquaticus</i>, <i>Chironomidae</i>, <i>Ephemeroptera</i>, <i>Odonata</i>, <i>Hirudinea</i>, <i>Oligochaeta</i>, <i>Gastropoda</i>, <i>Bivalvia</i>) were introduced with the sediment and water as well as via aerial colonisation. Taxa identified to be most sensitive in Tier 1 or in the first mesocosm study with acetamiprid were present in the test ponds.</p> <p>The test system contained also various macrophytes (e.g. <i>Chara globularis</i>, <i>Myriophyllum spicatum</i>) growing in the sediment. Macrophytes were partially removed before the application in order to achieve coverage of less than 30%. No macrophytes were removed after the application. .</p> <p>The abundance of taxa and populations is considered to be sufficient with 33 taxa or stages of macroinvertebrates, 44 taxa or stages of zooplankton and 24 taxa of emerging insects.</p> <p>Overall, the mesocosms represented realistic natural freshwater community.</p> <p>2. <u>Is the description of the experimental set-up adequate and unambiguous?: Yes</u></p> <p>The description of the experimental set-up are very detailed and unambiguous. All necessary information regarding the experimental ecosystems, measured endpoints, sampling frequency and sampling techniques may be found in the study report and shorter version is presented below, in the study summary.</p> <p>3. <u>Is the exposure regime adequately described?: Yes</u></p> <p>The exposure regime is adequately described with all necessary information regarding preparation of the test solutions, method of application, sampling of water and sediment for verification of the test item concentrations and analytical methods used (including detection limits). Since the test item concentration was measured at sufficient frequency until the study termination, the dissipation dynamics could be sufficiently characterised, including determination of the DT<sub>50</sub> values. Respective graphs illustrated the exposure pattern and aid comparison with FOCUS exposure profiles.</p> <p>4. <u>Are the investigated endpoints sensitive and in accordance with the working mechanisms of the compound, and with the results of the first-tier studies?: Yes</u></p> <p>The endpoints were determined based on changes in abundance of the tested species. Since acetamiprid is an insecticide, the main goal of the study was to investigate its effects on aquatic invertebrates with special attention paid to species most sensitive in Tier 1 studies and first mesocosm (emerging insects, <i>Cloeon dipterum</i> and family Naididae). Nevertheless, statistical analyses were performed on all investigated species. Respective calculations were also performed for algae and macrophytes, which gave indications regarding effects of acetamiprid on productivity of the aquatic communities.</p> <p>5. <u>Is it possible to evaluate the observed effects statistically and ecologically?: Yes</u></p>
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The biological results of the study were statistically analysed by uni- and multivariate statistics required for this type of studies. Williams-test was used to determine the NOEC values for each taxon (species or higher taxonomic level, is appropriate). Furthermore, diversity analysis, ordination analysis, principal component analysis (PCA), redundancy analysis (RDA) and principal response curve analysis (PCR) were performed. The Minimum Detectable Differences (MDD) were calculated for each taxon, in line with requirements of EFSA (2013). Analyses were performed at species, population and community level. All results are presented in both, tabular and graphical form, enabling validation of results and obtained endpoints.

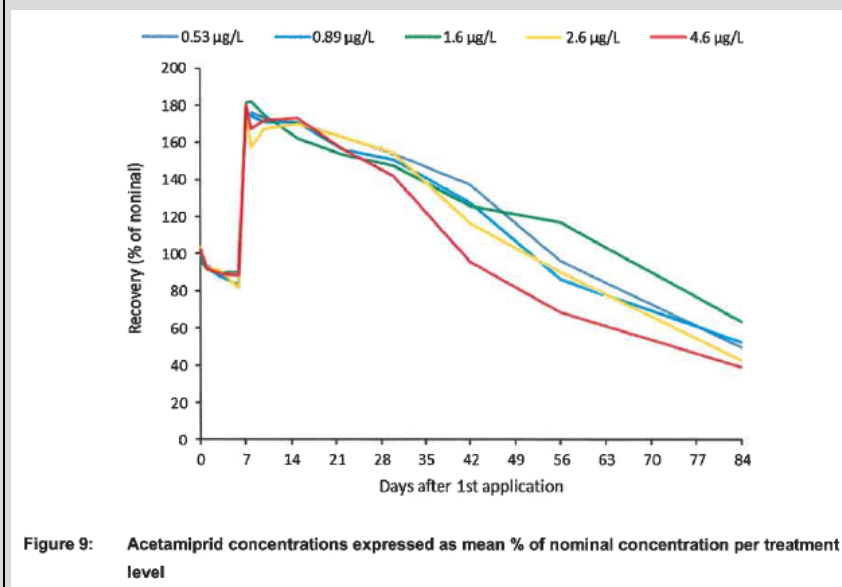
The reliability of the study was further evaluated using checklist provided in Table 32 in point 9.3.3 of EFSA (2013).

Items	Notes	Reliability index 1-3
<b>Methodology and test description</b>		
<b>1. Substance</b>	<b>Properly characterised and reported?</b>	<b>1</b>
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.
<b>2. Test site, duration</b>	<b>Properly characterised and reported?</b>	<b>1</b>
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
<b>3. Application</b>	<b>Properly characterised and reported?</b>	<b>1</b>
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	Necessary to make a link between the	All details given in the study

	scheme	test and the intended use of the PPP	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 with exception of the wind speed. However, this information was not necessary due to application in separating funnels directly to the water column (no possibility for the spray drift).
	<b>4. Test design</b>	<b>Properly designed and reported?</b>	<b>1</b>
	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 (>1 year)
	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 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
	<b>5. Biological system</b>	<b>Representative and properly reported?</b>	<b>1</b>
	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.
<b>Results</b>			
<b>7. Endpoints</b>	<b>Properly reported?</b>		<b>1</b>
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.
<b>8. Elaboration of results</b>	<b>Are conclusions based on measured data? Methodology correct?</b>		<b>1</b>
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 all species and populations
8.3 Population-level responses	Sufficiently reported?		Yes
8.3 Community-level responses	Sufficiently reported?		Yes
<b>9. Control</b>			<b>1</b>
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).
<b>10 Classification of effects</b>	<b>Properly derivable?</b>		Yes
<b>11 Biological meaning of statistically significant differences</b>	<b>Sufficiently explained?</b>		Yes
<p>Explanation to reliability index:</p> <p>1 Reliable</p> <p>2 Less reliable</p> <p>3 Not reliable</p> <p>In test item groups concentration of acetamiprid exceeded 80% of nominal up to day 56 of the study, with exception 2.5 µg a.s./L group, in which the measured concentration on day</p>			

56 was at 75.6% 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 to >170% of nominal and were at ~150% of nominal up to day 30-42. Clear decrease in measured concentrations was observed on day 84 in all test item groups, but it was still at the relatively high level for a mesocosm study (39.3-63.7% of nominal). Since in the Central Zone formulation CA3573 is intended to be applied once during the season and acetamiprid turned out to be stable throughout the study with measured concentrations far above the nominal after the second application, the zRMS agrees with the study authors that the endpoints may be expressed as the peak measured concentrations after the second application. The graph giving more clear overview of exposure to acetamiprid during the study has been copied from the study report and presented below:



Concentration of acetamiprid in control samples was <LOQ at all sampling occasions.

As in case of all studies of this type, sufficiently low MDD values could not be calculated for all the taxa investigated. Nevertheless, the abundance of the most sensitive species was sufficient to obtain MDD values <70%, sufficient to detect medium effects, while for some MDD values were <50%, so also small effects could be determined.

In general, the number of taxa and populations (including the most sensitive ones) with MDD <70% was sufficient to consider the statistical power of the study to be adequate.

Overall, the evaluation the mesocosm study with CA3573 by Hommen et al. (2020) indicates that the study acceptable and sufficiently reliable to be used for purposes of refinement of the risk assessment for aquatic invertebrates.

Based on the effect classes and statistical evaluation it may be concluded that no effects were observed up to the concentration of 1.6 µg a.s./L (peak measured) and this concentration is considered to be the NOEC from the study to derive the ETO-RAC.

NOEAEC to derive ERO-RAC could not be determined due to effects on emergence of mayflies at the next higher concentration (2.6 µg a.s./L) lasting >8 weeks.

Some additional comments on results of the study has been introduced by the zRMS in commenting boxes directly in the summary text.

<b>Reference:</b>	KCP 10.2.3/01
<b>Report</b>	Carnadine – Outdoor mesocosm study – Test item: Carnadine (16–19% acetamiprid). Hommen U., Hennecke S., Christmann R., 2020; NFM-001/7-52
<b>Guideline(s):</b>	OECD Guidance Document “Freshwater Lentic Field Tests” (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)
<b>Deviations:</b>	1. No TOC samples were taken on day -1. Instead, samples from the control enclosures (enclosures A1-A5) were taken on day 6 2. 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. → Both deviations are considered minor with no influence on the integrity or outcome of the study.
<b>GLP:</b>	Yes, certified laboratory
<b>Acceptability:</b>	Acceptable
<b>Duplication (if vertebrate study)</b>	Not applicable

## Executive Summary

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 a few macrophytes and many algae and invertebrate species from a large variety of taxonomic groups. Fungi, protozoa and bacteria were also present but not monitored.

Because dissipation of acetamiprid from the water column was slow and thus, 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 the nominal concentrations.

The study provides effect data for more than eight potentially sensitive populations. 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 to acetamiprid than e.g. the mayflies evaluated in this study. In addition, exposure duration is expected to be much shorter in streams than in the lentic test systems and thus, the same maximum concentration has probably less severe effects in streams than in the test systems used in this study.

The maximum measured 1.6 µg a.s./L (9.4 µg test item/L; nominal: 0.87 µg a.s./L and 5.1 µg test item/L) is the overall Class 1 concentration which can be used to derive an ETO-RAC. Uncertainty related to this concentration is considered small since clearly no effects on potentially sensitive taxa were found and the 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 the mayflies lasted longer than eight weeks.

## Materials and methods

### Materials

<b>Test item</b>	Carnadine
<b>Code No.</b>	CA3573
<b>Synonyms</b>	Kestrel, MCW-2222, Acetamiprid 200 SL
<b>Batch #</b>	981101035
<b>Purity</b>	Acetamiprid 200 g/L (nominal); analysed: 17% (w/w) = 195.5 g/L
<b>Description</b>	Liquid, clear, yellow, brown

### Test organisms

<b>Species</b>	Algae from several classes, zooplankton (several species of cladocerans,
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	<p>copepods, rotifers), midge larvae of <i>Chaoborus</i> sp. and macroinvertebrates (e.g. <i>Asellus aquaticus</i>, Chironomidae, Ephemeroptera, Odonata, Hirudinea, Oligochaeta, Gastropoda, Bivalvia) were introduced with the sediment and water as well as via aerial colonisation.</p> <p>The test systems contained various macrophytes (e.g. <i>Chara globularis</i>, <i>Myriophyllum spicatum</i>) growing in the sediment. Coverage of macrophytes was not more than 30% of the sediment surface at application. In addition, an <i>in-situ</i> bioassay with 20 individuals of <i>Gammarus</i> sp. was introduced into each replicate one day before the 1<sup>st</sup> application. Introduction of amphibians or fish was avoided.</p>
<b>Source</b>	Natural water bodies on the test site of Mesocosm GmbH, Homberg/Ohm, Germany
<b>Study design and methods</b>	
<b>Test duration and exposure</b>	84 days (1 <sup>st</sup> application on 22-May-2019 = day 0; end of in-life phase on 16-August-2019); The duration of the study after dosing was 12 weeks to allow the detection of delayed effects, e.g. on the emergence of insects.
<b>Application no. and dates</b>	1 <sup>st</sup> application on 22-May-2019, 2 <sup>nd</sup> application on 29-May-2019
<b>Application method</b>	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.
<b>Test concentrations</b>	<p>Nominal: 0.0 (control), 1.8, 3.0, 5.1, 8.8, 15 µg test item/L corresponding to 0.0 (control), 0.30, 0.51, 0.87, 1.5, 2.5 µg a.s./L</p> <p>Max. measured: 0.0 (control), 0.53, 0.89, 1.6, 2.6, 4.6 µg a.s./L</p>
<b>Test units</b>	Stainless-steel enclosures each with a diameter of approximately 1.43 m (surface approximately 1.6 m <sup>2</sup> ) and a depth of approximately 1.5 m. With a depth of the water body of about 110 cm ± 15%, the total volume of each enclosure was approximately 1850 L. 29 stainless steel enclosures were pressed into the sediment of “Big Pond A”. Twenty enclosures were selected for the study before the 1 <sup>st</sup> application.
<b>Group size/replicates</b>	1 enclosure = 1 replicate; 3 replicates per test concentration and 5 replicates for the control
<b>Test medium</b>	The water of “Big Pond A” was originally taken from a lake on site in April 2018. The water was mixed with rain water to give the ultimate water body.
<b>Adaptation</b>	The period for equilibration of the enclosures was more than one year (installation of the pond April 2018, start of the study May 2019).
<b>Aeration</b>	Yes. Pumps were switched off at least 15 minutes prior to each sampling and were switched on again after the sampling was completed.
<b>Environmental conditions</b>	
<b>Temperature</b>	The water temperature measured 50 cm below water surface ranged between a minimum temperature of 11.1 °C in the mid of May and a maximum during the study with 24.3 °C in the beginning of July.
<b>Photoperiod</b>	Natural
<b>pH</b>	7.10 – 9.95
<b>Dissolved oxygen</b>	4.88 – 15.23 mg/L
<b>Conductivity</b>	159 – 257 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 litre
- Macroinvertebrates: Abundance of taxa per sample using two types of substrate samplers and netting. Macroinvertebrates were enumerated alive and identified using taxonomic keys on live organisms to the species level when possible and then returned to the appropriate enclosure. The data of the samplers and the netting were combined and expressed as sum of individuals sampled per enclosure and date.
- Oligochaetes and Chironomidae in sediment: Since a previous mesocosm study with the same active substance indicated a potential risk for Naididae, 12 baskets filled with sediment were introduced on the ground of each enclosure at the start of the test especially to sample sediment organisms. The data were combined with the other macroinvertebrate data.
- In-situ *Gammarus* assay: 20 individuals of *Gammarus* sp. were introduced one day before the 1<sup>st</sup> 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, bluegreen, 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 samples at three water depths on the days of application) and sediment samples

**Table A 17: Time schedule for sampling and measurements**

Date	Day	Emerging insects	Macroinvertebrates*	<i>Gammarus</i> sp.. Bioassay	Zooplankton	Phytoplankton	Periphyton	Macrophytes	Chemical parameters	TOC/DOC	Functional parameters	Residue analysis water	Residue analysis sediment	Water level
15/04/2019	-37	Introduction of Enclosures												
02/05/2019	-20		intro				intro							
13/05/2019	-9		x											
14/05/2019	-8				x						x			
15/05/2019	-7	intro				x								x
20/05/2019	-2		x								x			
21/05/2019	-1			intro	x	x	x	x	x					x
22/05/2019	0	x										x <sup>3</sup>		X
23/05/2019	1											x		x
24/05/2019	2				x									
25/05/2019	3											x		x
27/05/2019	5				x								x	
28/05/2019	6		x	x		x				x <sup>1</sup>		x		x
29/05/2019	7	x									x	x <sup>2</sup> , x <sup>3</sup>		x
30/05/2019	8											x		x
31/05/2019	9				x									
01/06/2019	10											x		x
03/06/2019	12				x		x				x			
04/06/2019	13		x	x										
05/06/2019	14	x				x						x		x
06/06/2019	15							x					x	
11/06/2019	20				x						x			
12/06/2019	21		x	x										
13/06/2019	22	x				x						x		x

Date	Day	Emerging insects	Macroinvertebrates*	<i>Gammarus</i> sp. Bioassay	Zooplankton	Phytoplankton	Periphyton	Macrophytes	Chemical parameters	TOC/DOC	Functional parameters	Residue analysis water	Residue analysis sediment	Water level
14/06/2019	23												x	
17/06/2019	26				x		x				x			
18/06/2019	27		x	x										
19/06/2019	28												x	
21/06/2019	30	x				x		x	x	x		x		x
24/06/2019	33										x			
26/06/2019	35	(x)												
01/07/2019	40				x		x				x			
02/07/2019	41		x	x										
03/07/2019	42	x				x						x	x	x
08/07/2019	47										x			
10/07/2019	49	(x)												
15/07/2019	54				x						x			
16/07/2019	55		x	x										
17/07/2019	56	x				x			x	x		x	x	x
19/07/2019	58						x	x						
22/07/2019	61				(x)						x			
25/07/2019	64	(x)												
29/07/2019	68				x						x			
30/07/2019	69		x	x										
31/07/2019	70	x				x								
02/08/2019	72						x							
05/08/2019	75				(x)						x			
07/08/2019	77	(x)												
12/08/2019	82				x						x			
13/08/2019	83		x											
14/08/2019	84	x				x			x	x		x	x	x
16/08/2019	86						x	x						

Note:

The date of application shaded grey.

intro: introduction of the respective trap

(x): samples were taken, but not analysed

x: samples were taken and analysed and/or evaluated

\*: Macroinvertebrates: indicates sampling with all sampling techniques: traps, netting and sediment baskets

1: No TOC samples were taken on day -1. Instead, samples from the control enclosures (enclosures A1-A5) were taken on day 6

2: before application

3: 3 to 3.5 hours after application

### **Analytical measurements**

Acetamiprid in water and sediment samples taken was 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 NFM-002/6-22). The limit of quantification (LOQ) was set during the method development: LOQ = 10 ng a.s./L and LOQ = 50.0 ng a.s./kg dw for sediment.

### **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 the NOEC (No Observed Effect Concentration).

Effects on the community levels were analysed by diversity indices and Principal Response Curves (PRC). At the community level, all taxa including the rare ones were used. If identification of the species



was not possible, the abundance of the genus or next higher taxonomic level was used.

#### *Williams-test, NOEC calculation, MDDs*

For each taxon (species or higher taxonomic level if appropriate) and sampling date, the multiple t-test by Williams 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 to the EFSA aquatic guidance document (EFSA PPR panel, 2013) and Brock et al. (2015<sup>7</sup>). The abundance data of the organisms were log-transformed ( $y' = \ln(a \cdot y + 1)$ ) before the analysis to approximate normality and homoscedasticity (homogeneity of variances) requirements (van den Brink et al., 2000<sup>8</sup>). 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 detected by the statistical test.

**Table A 18: 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 might have been 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

<sup>7</sup> Brock TCM, Hammers-Wirtz M, Hommen U, Preuss TG, Ratte HT, Roessink I, Strauss T, Van den Brink PJ (2015): The minimum detectable difference (MDD) and the interpretation of treatment-related effects of pesticides in experimental ecosystems. *Environ Sci Pollut Res.* 22:1160–1174.

<sup>8</sup> Van den Brink PJ, Hattink J, Bransen F, Van Donk E, Brock TCM (2000): Impact of the fungicide carbendazim in freshwater microcosms. II. Zooplankton, primary producers and final conclusions. *Aquatic Toxicology* 48, 251–264.

treatment effect. Taxa falling into MDD category 3 (high MDDs and no significant difference from control) were not further considered.

### *Diversity analysis*

The diversity of a community was described using three different measures. Firstly, the number of present species (taxa richness) per treatment was plotted against time. Secondly, the Shannon-Index, a frequently used diversity measure, was calculated. Thirdly, the evenness was calculated by dividing the Shannon-Index by the maximum possible value (Shannon-Index, if all species are equally abundant). In this way the influence of the number of species is neglected. The maximum evenness is 1 while dominant species result in low evenness values.

### *Ordination analysis (PRC, RDA)*

Principal Response Curves (PRCs, van den Brink & Ter Braak, 1998<sup>9</sup>, 1999<sup>10</sup>) 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 abundance 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<sup>11</sup>). 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).

### *Biological observations*

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. 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 categorisation of taxa according to

<sup>9</sup> Van den Brink, PJ, Ter Braak, CJF (1998): Multivariate analysis of stress in experimental ecosystems by Principal Response Curves and similarity analysis. *Aquatic Ecology* 32, 163 – 178.

<sup>10</sup> Van den Brink, PJ, Ter Braak, CJF (1999): Principal Response Curves: analysis of timedependent multivariate responses of a biological community to stress. *Env. Toxicol.Chem.* 18: 138-148.

<sup>11</sup> Hommen U, Veith D, Dülmer U (1994): A computer program to evaluate plankton data of freshwater field tests. In I.R. Hill, F. Heimbach, P. Leeuwangh, P. Matthiessen (eds.): *Freshwater Field Tests for Hazard Assessment of Chemicals*. Lewis Publ., Boca Raton, FL, 503-513.

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.

Note 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 to achieve a monotonous concentration response before the testing. 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?

**Table A 19: 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.

Effect class	Description	Criteria
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 and discussion

### *Analytical measurements*

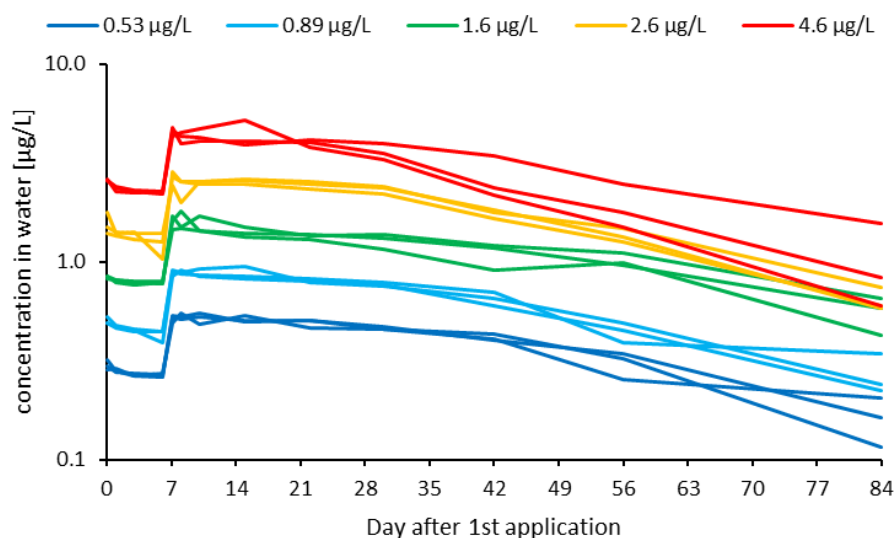
#### *Water*

From the measured concentrations in the application solutions, the volume of the application solutions and the water volume of the enclosures, the expected (theoretical) initial concentrations in the enclosures were calculated. These theoretical initial concentrations were on average 94% of the refined nominal concentrations for the first and 90% for the second application. The mean recovery rate over all application solutions was 92%.

The first water samples were taken 3 hours after application with a mean recovery of 100% after the first and 177% after the second application if related to the refined nominal concentrations. Variability was relatively large, probably caused by not complete homogeneous distribution in the water. If for each enclosure the measured concentration on day 6 is subtracted from the concentrations measured three hours after the second application, on average 90 % of the refined nominal concentration was found after the second application.

The course of acetamiprid concentrations in the water is shown in the figure below for the measured concentrations per enclosure. The variability between the replicates per test concentration was very low and the general pattern over time was very similar over the different test concentrations. At the end of the study, eleven weeks after the second application, on average 50% of the nominal concentrations were still found in the water. The DT<sub>50</sub> based on the data after the second application varied between 26 and 64 days with an average of 44 days. The DT<sub>50</sub> of the single enclosures showed no concentration related trend.

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

### Sediment

In the treated enclosures, all sediment samples revealed concentrations above the LOQ of 0.05 µg/kg. Concentrations increased slowly over time and reached e.g. in the highest treatment level a mean maximum of 7.5 µg/kg dw eight weeks after the first application. No concentrations above the LOQ were found in the control samples.

A summary of the analytical results in the water and sediment phases is given in the table below.

**Table A 20:** Nominal and measured concentrations of acetamiprid

	Treatment level				
	1	2	3	4	5
Nominal concentration [µg a.s./L]	0.30	0.51	0.87	1.5	2.5
<b>Maximum measured means in water [µg a.s./L]</b>	<b>0.53</b>	<b>0.89</b>	<b>1.6</b>	<b>2.6</b>	<b>4.6</b>
Maximum measured means in sediment [µg a.s./kg dw]	1.43	1.02	1.94	2.46	7.48

Nominal concentrations are used in the following to indicate the different treatment levels in figures and tables and to express NOEC and effect concentrations. However, since the dissipation of acetamiprid from the water column was slow and thus, the second application resulted in measured concentrations clearly above the nominal concentrations, the maximum measured concentrations are probably better suited for derivation of a regulatory acceptable concentration (RAC) from this mesocosm study.

## Biological results

### Macroinvertebrates

#### Population level analysis

Thirty-three taxa or stages were differentiated in the 200 combined samples of the macroinvertebrate data set. Half of the individuals counted were the mayfly *Cloeon dipterum* and the phantom midge *Chaoborus* sp. Also abundant were the leech *Helobdella stagnalis*, worms of the family of Naididae, the water louse *Asellus aquaticus*, Tanypodinae, damselflies (Zygoptera), and the snails *Lymnea stagnalis* and *Planorbis planorbis*. For the evaluation, also the sum of Odonata, the sum of Diptera, the sum of *Chaoborus* sp. (larvae and pupae) and the sum of Chironomidae (larvae and pupae) were calculated.

Ten macroinvertebrate taxa (excluding pooled taxa) fulfil the MDD criterion by Brock et al. (2015). For all of them the MDD was at least once below 70% during the eight weeks after the first application and thus, belonged to the preferred MDD class III or IV. Due to the slow dissipation of acetamiprid, five of

them, i.e. the mayfly *Cloeon dipterum*, damselflies (Zygoptera), the midges *Chaoborus* sp. and Chironomidae, and the water louse *Asellus aquaticus*, are insects or crustaceans and thus, are considered potentially sensitive to an insecticide as acetamiprid. In addition, worms of the family of Naididae were found in a previous mesocosm study with acetamiprid as potentially sensitive and showed also sufficiently low MDDs. Thus, direct effects could be analysed on six potentially sensitive taxa in the macroinvertebrate data set according to the MDDs.

Also the leech *Helobdella stagnalis* and some snails fulfilled the MDD criterion by Brock et al. (2015), but they were not considered potentially sensitive. For some other taxa, the MDDs were higher but, nevertheless, a significant difference to the controls was found at least once after application. These MDD category 2 taxa were analysed for treatment effects. The remaining taxa with high MDDs and without any significant findings after applications are not further considered.

Mayflies are known to be especially sensitive to neonicotinoids and also in a previous mesocosm study with acetamiprid (EFSA 2016), *Cloeon dipterum* was the most sensitive species. This was confirmed in the present study where *Cloeon dipterum* was the only species in the macroinvertebrate data set with pronounced effects at 2.6 and 4.6 µg a.s./L. The effect at 2.6 µg a.s./L was significant over less than eight weeks and thus, considered class 3A. Larvae numbers at 4.6 µg a.s./L did not reach the level of the controls within the study. Thus, effect class 5 B was used for 4.6 µg a.s./L. Up to 1.6 µg a.s./L, clearly no treatment effect was given (see summary table on NOECs and MDDs below).

In a previous mesocosm study (EFSA 2016, effects of acetamiprid on Naididae (Oligochaeta) were uncertain due to generally low numbers 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 samplers were used in this study to increase sampling success. The calculated MDDs were sufficiently low to detect effects below or equal to 70% on days 6 and 21. However, after day 21 the mean abundance in the control was below 5 per sample and variability between replicates and over time was partly large. For example, in one enclosure treated with 4.6 µg a.s./L 24 Naididae were found on day 69, while no other were found in this enclosure on the samplings before or afterwards, as well as in the other replicates of 4.6 µg a.s./L taken on day 69. The data indicate clearly no effects up to 1.6 µg a.s./L. On day 6, a NOEC of 1.6 µg a.s./L was calculated. However, it is unlikely a treatment effect since mean abundance at 2.6 µg a.s./L was relatively stable until day 21. Though, on days 27 and 41 mean abundance was about 70%, but not significantly below the mean of controls. Thus, effects at 2.6 µg a.s./L were considered Class 2. On days 21 and 27 significantly lower abundances were found at 4.6 µg a.s./L. Since in one replicate 24 worms were found on day 69, the effect was considered temporary (class 3A).

**Table A 21: Macroinvertebrate NOECs [ $\mu\text{g a.s./L}$ , nominal] and %MDD (in brackets)**

Macroinvertebrates	Day after application										Mean	Min	MDD
	-9	-2	6	13	21	27	41	55	69	83	MDD	MDD	Cat
<i>Cloeon sp.</i> (Ephemeroptera)	≥4.6 (61)	≥4.6 (50)	≥4.6 (46)	2.6- (51)	2.6- (55)	1.6- (54)	1.6- (61)	1.6- (79)	2.6- (69)	2.6- (84)	58	46	1
Sum Odonata	≥4.6 (80)	≥4.6 (41)	≥4.6 (73)	2.6- (36)	2.6- (49)	2.6- (63)	≥4.6 (99)	≥4.6 (117)	≥4.6 (104)	≥4.6 (100)	73	36	1
<b>Zygoptera</b>	≥4.6 (81)	≥4.6 (41)	≥4.6 (73)	2.6- (35)	2.6- (49)	2.6- (63)	≥4.6 (99)	≥4.6 (117)	≥4.6 (105)	≥4.6 (99)	73	35	1
Anisoptera	≥4.6 (204)	≥4.6 (301)	≥4.6 (226)	≥4.6 (n.c.)		≥4.6 (154)		2.6+ (n.c.)	≥4.6 (144)	≥4.6 (198)	190	154	2
Sum Diptera	≥4.6 (52)	≥4.6 (57)	≥4.6 (61)	≥4.6 (69)	≥4.6 (64)	≥4.6 (62)	≥4.6 (58)	≥4.6 (69)	≥4.6 (67)	2.6- (61)	64	58	1
<b>Chaoborus sp.</b>	≥4.6 (54)	≥4.6 (59)	≥4.6 (62)	≥4.6 (71)	≥4.6 (70)	≥4.6 (71)	1.6- (79)	≥4.6 (92)	≥4.6 (91)	2.6- (84)	74	62	1
<i>Chaoborus sp. (larvae)</i>	≥4.6 (56)	≥4.6 (60)	≥4.6 (69)	≥4.6 (77)	≥4.6 (62)	≥4.6 (66)	≥4.6 (85)	≥4.6 (92)	≥4.6 (92)	2.6- (82)	75	62	1
<b>Chironomidae</b>	≥4.6 (143)	≥4.6 (106)	≥4.6 (248)	≥4.6 (156)	≥4.6 (136)	≥4.6 (86)	<0.53- (49)	≥4.6 (67)	≥4.6 (65)	≥4.6 (68)	124	49	1
Chironomidae indet. (larvae)	≥4.6 (301)	≥4.6 (198)	≥4.6 (234)	≥4.6 (156)	≥4.6 (155)	≥4.6 (91)	2.6- (70)	≥4.6 (82)	≥4.6 (74)	≥4.6 (94)	131	70	1
Tanypodinae (larvae)	≥4.6 (143)	≥4.6 (127)	≥4.6 (n.c.)		≥4.6 (208)	2.6+ (266)	≥4.6 (85)	≥4.6 (79)	≥4.6 (82)	≥4.6 (95)	160	79	2
Ceratopogonidae	≥4.6 (154)		≥4.6 (252)	≥4.6 (n.c.)			≥4.6 (n.c.)	≥4.6 (n.c.)	2.6+ (n.c.)	≥4.6 (n.c.)	252	252	2
Coleoptera	≥4.6 (n.c.)		2.6+ (n.c.)			2.6+ (n.c.)	≥4.6 (n.c.)	≥4.6 (n.c.)					2
Coleoptera (adult)	≥4.6 (n.c.)		2.6+ (n.c.)			2.6+ (n.c.)	≥4.6 (n.c.)						2
Corixidae	≥4.6 (168)	≥4.6 (114)	≥4.6 (n.c.)	≥4.6 (154)	2.6- (92)	≥4.6 (139)	≥4.6 (n.c.)	≥4.6 (293)	≥4.6 (n.c.)	≥4.6 (133)	170	92	2
<i>Notonecta glauca</i>	≥4.6 (98)	≥4.6 (196)	≥4.6 (198)	≥4.6 (115)	≥4.6 (129)	≥4.6 (120)	≥4.6 (97)	1.6- (93)	≥4.6 (92)	≥4.6 (115)	125	93	2
<b>Asellus aquaticus</b>	<0.53- (95)	≥4.6 (87)	≥4.6 (66)	≥4.6 (79)	≥4.6 (92)	≥4.6 (86)	≥4.6 (85)	≥4.6 (81)	≥4.6 (63)	≥4.6 (79)	82	66	1
Acari	≥4.6 (124)	≥4.6 (120)	≥4.6 (100)	≥4.6 (105)	≥4.6 (n.c.)	≥4.6 (129)	≥4.6 (104)	2.6- (93)	≥4.6 (160)	0.53- (84)	106	93	2
Sum Oligochaeta	≥4.6 (80)	≥4.6 (83)	1.6- (70)	≥4.6 (75)	2.6- (68)	≥4.6 (81)	≥4.6 (86)	≥4.6 (92)	≥4.6 (102)	2.6- (88)	79	68	1
<b>Naididae</b>	≥4.6 (80)	≥4.6 (83)	1.6- (65)	≥4.6 (85)	2.6- (70)	2.6- (83)	≥4.6 (86)	≥4.6 (91)	≥4.6 (128)	2.6- (84)	80	65	1
<i>Helobdella stagnalis</i>	≥4.6 (77)	≥4.6 (79)	≥4.6 (86)	≥4.6 (58)	≥4.6 (88)	≥4.6 (76)	≥4.6 (81)	≥4.6 (130)	≥4.6 (75)	≥4.6 (105)	87	58	1
Sum Lymnaidae	≥4.6 (81)	≥4.6 (60)	≥4.6 (46)	2.6- (32)	≥4.6 (52)	≥4.6 (64)	≥4.6 (41)	1.6+ (33)	≥4.6 (36)	≥4.6 (42)	45	32	1
<i>Lymnaea stagnalis</i>	≥4.6 (112)	≥4.6 (72)	≥4.6 (62)	≥4.6 (51)	≥4.6 (66)	≥4.6 (61)	≥4.6 (72)	≥4.6 (43)	≥4.6 (40)	≥4.6 (53)	59	43	1
Lymnaeidae (smaller 0.5 cm)					≥4.6 (138)	≥4.6 (124)	≥4.6 (80)	0.53+ (57)	≥4.6 (78)	≥4.6 (86)	100	57	1
<i>Radix sp.</i>	≥4.6 (108)	≥4.6 (84)	0.89- (51)	2.6- (67)	≥4.6 (68)	≥4.6 (82)	≥4.6 (71)	≥4.6 (86)	2.6+ (50)	≥4.6 (81)	71	51	1
<i>Planorbis planorbis</i>	≥4.6 (80)	≥4.6 (95)	≥4.6 (94)	≥4.6 (96)	≥4.6 (69)	≥4.6 (56)	≥4.6 (58)	≥4.6 (58)	≥4.6 (74)	≥4.6 (95)	72	56	1

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 8 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 (i.e. MDD category 1).

### zRMS comments:

Comments regarding single NOEC values below the overall NOEC of 1.6 µg a.s./L.

### Chironomidae

In general, the total number of Chironomidae increased during the study. A single NOEC <0.53 µg a.s./L was calculated for day 41. However, the mean abundance in the two highest treatment levels were closest to the mean in controls and at 2.6 µg a.s./L the mean was even higher comparing to controls. It is also noted that abundance at the maximum concentration tested (4.6 µg a.s./L) on day 41 was higher comparing to 0.89 and 1.6 µg a.s./L. Based on that, no plausible concentration-response could be found and the significance was caused by the moving average procedure of the Williams test. This may be seen on the graph below.

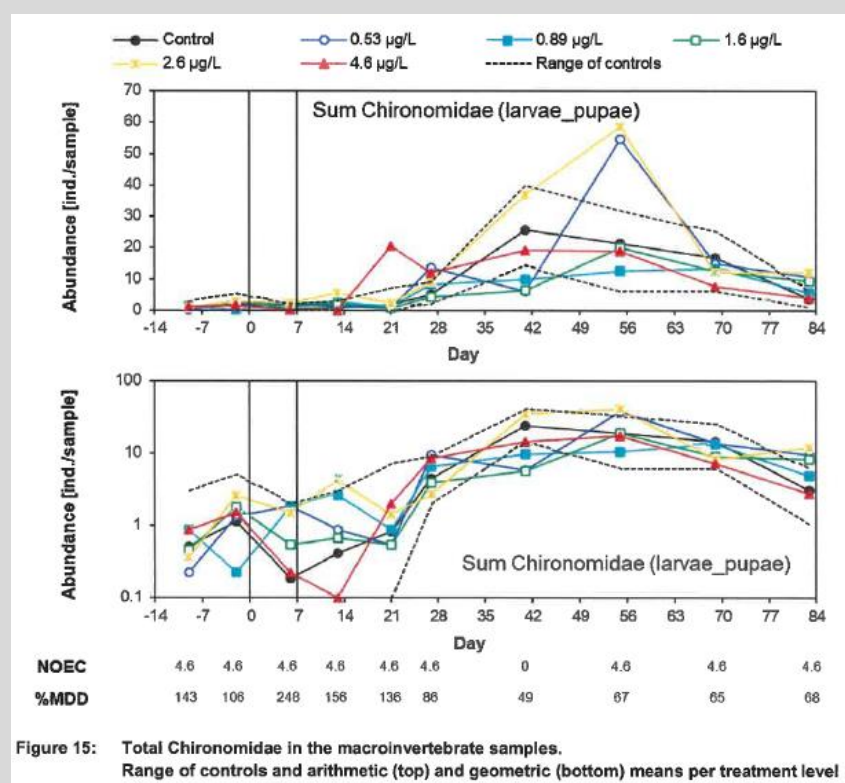


Figure 15: Total Chironomidae in the macroinvertebrate samples.  
Range of controls and arithmetic (top) and geometric (bottom) means per treatment level

### Asellus aquaticus

The NOEC <0.53 µg a.s./L was determined for day -9 (before the application). It is thus not relevant for determination of the NOEC from exposure to CA3573. In general, abundance of *A. aquaticus* at first and second application was low with 0-5 individuals in all test groups. However, in the course of the study the abundance was increasing in all test item groups. The graph below shows that no adverse effects were observed at test item concentrations up to 2.6 µg a.s./L. The drop in abundance at 2.6 µg a.s./L after second application cannot be considered reliable, due to only single individual observed at that time. At 4.6 µg a.s./L the abundance was lower, but due to low number at the test start it is difficult to conclude if this was a result of exposure to acetamiprid.



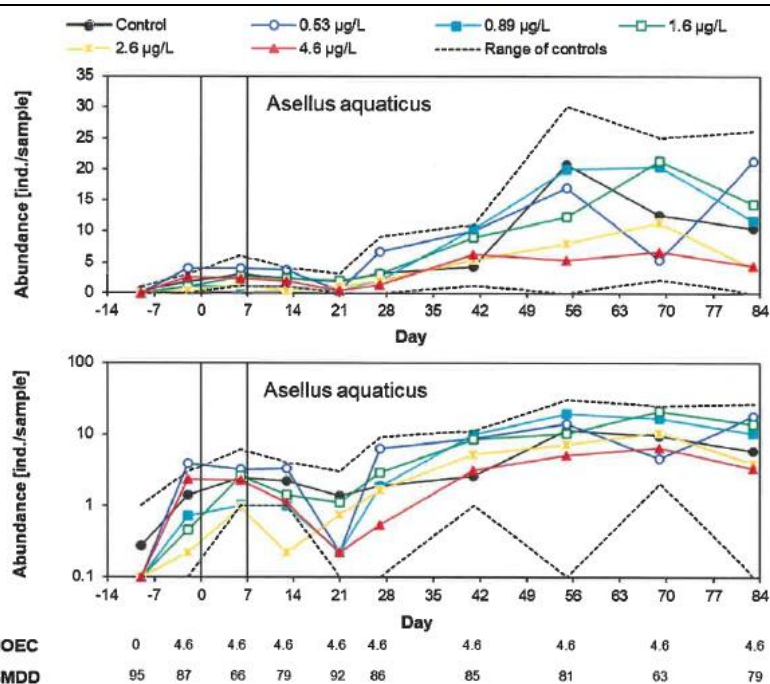


Figure 18: *Asellus aquaticus* (Isopoda) in the macroinvertebrate samples. Range of controls and arithmetic (top) and geometric (bottom) means per treatment level

### Lymnaeidae (smaller, 0.5 cm)

No small Lymnaeidae (<0.5 cm) were found before day 21. The NOEC of 0.53 µg a.s./L was determined for promotion on day 55, but no clear concentration-response could be determined and abundance (see graph below).

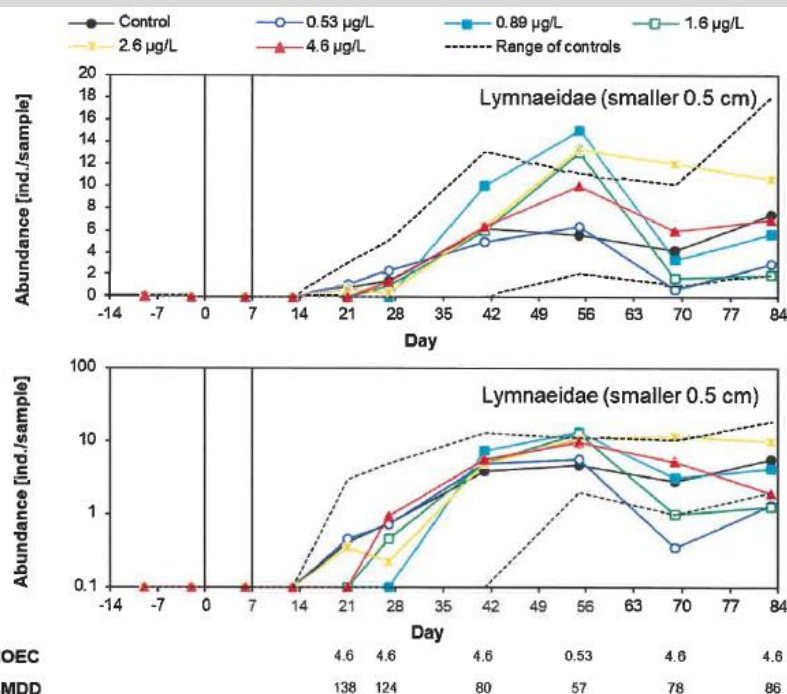
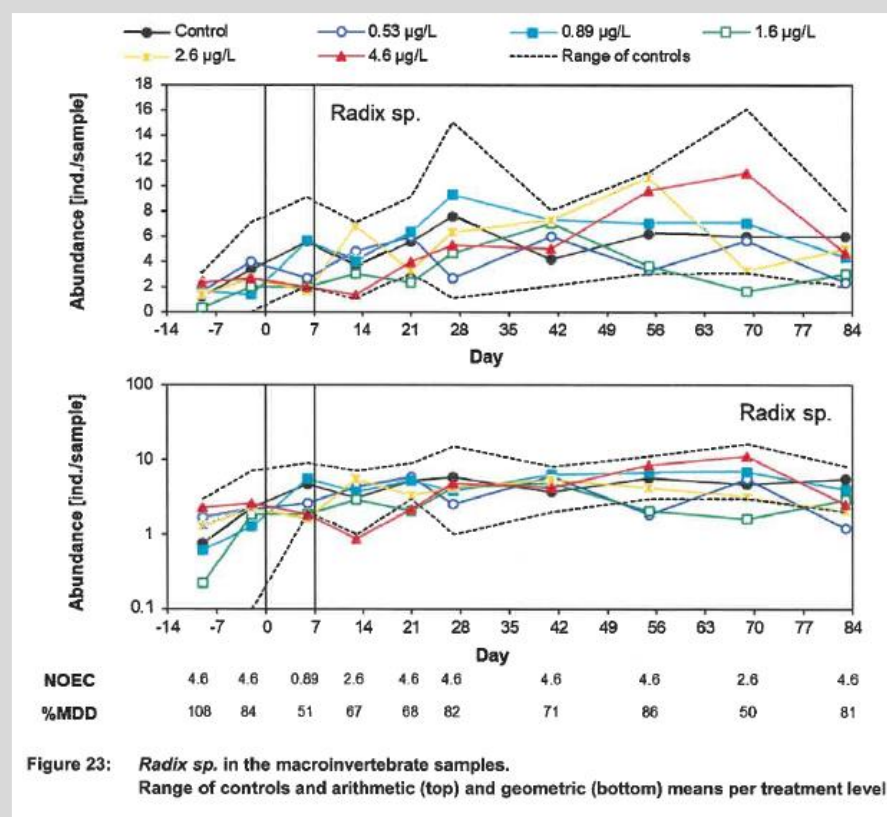


Figure 22: Small Lymnaeidae in the macroinvertebrate samples. Range of controls and arithmetic (top) and geometric (bottom) means per treatment level

### Radix sp.

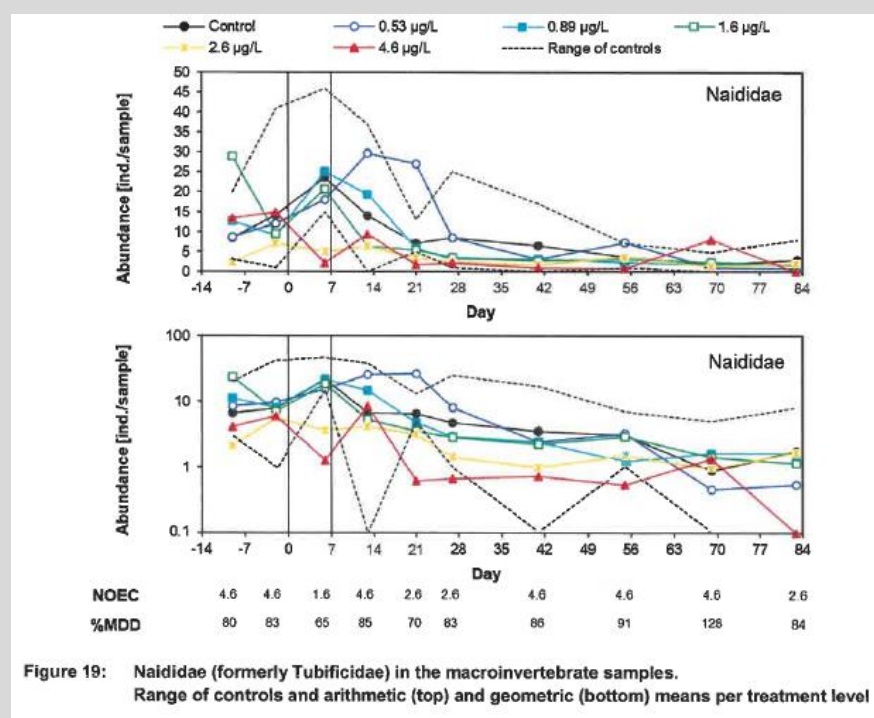
On day 6 a NOEC of 0.89 µg a.s./L was calculated, but the mean abundance at 0.53 µg a.s./L was also below the

controls, while abundance at 2.6  $\mu\text{g a.s./L}$  increased directly after the second application despite higher exposure. Therefore effects seen seems to be not treatment related. Furthermore, the number of individuals in days 0-7 was low in all treatment levels (2-6, see graph below) and in opinion of the zRMS statistical analysis for these days is not sufficiently reliable.



## Naididae

Since at the EU level Naididae were identified as potentially sensitive family, the graph presenting effects on their abundance has been copied from the study report and presented below for reference of the CMS. As may be seen, no treatment related effects were observed up to 1.6  $\mu\text{g a.s./L}$



### **Acari**

Although for Acari the NOEC of 0.53 µg a.s./L was determined for day 83, this group was not considered for effect classification due to low numbers in all enclosures throughout the period of the study. Based on raw data, the number of individuals was usually 0-4 with more individuals (8-12) observed at only single sampling occasions in a single enclosures. Based on that, the derived NOEC is considered to be not reliable.

### *Community level analysis*

The diversity analysis for the macroinvertebrates indicated a reduced number of taxa in the samples of the 4.6 µg a.s./L treatment level. Effects on the Shannon index and evenness were less clear except for day 6. The effects on the macroinvertebrate community are clearer shown by the PRCs which are driven mainly by the effects on the mayflies. The PRCs indicate no effects up to 1.6 µg a.s./L, pronounced effects at 2.6 µg a.s./L, but over less than eight weeks, and long-term effects at 4.6 µg a.s./L. Redundancy analysis per sampling data revealed only for day 21 a significant treatment effect. However, according to the Williams-test applied to the PCA sample scores, the community NOEC is 1.6 µg a.s./L. On the last two samplings, no significance was found. In summary, effects on the macroinvertebrate community are considered Class 1 up to 1.6 µg a.s./L, Class 3A at 2.6 µg a.s./L and Class 5B at 4.6 µg a.s./L.

**Table A 22: Macroinvertebrate NOECs [µg a.s./L, nominal] for diversity indices and results of ordination analysis**

Macroinvertebrate community level	Day after application									
	-9	-2	6	13	21	27	41	55	69	83
n_taxa	≥4.6	≥4.6	≥4.6	≥4.6	2.6-	≥4.6	2.6-	2.6-	≥4.6	2.6-
Shannon	≥4.6	≥4.6	2.6-	≥4.6	2.6-	≥4.6	≥4.6	≥4.6	≥4.6	2.6-
Evenness	≥4.6	≥4.6	1.6-	≥4.6	≥4.6	≥4.6	≥4.6	≥4.6	≥4.6	≥4.6
p-value RDA	0.314	0.306	0.38	0.142	0.04	0.066	0.35	0.458	0.608	0.69
NOEC PCA scores	n.d.	n.d.	n.d.	≥4.6	2.6	1.6	1.6	1.6	≥4.6	≥4.6

### **zRMS comments:**

The PRCs for the macroinvertebrates data set have been copied from the study report and presented below, for reference of the cMS.

As may be seen, the macroinvertebrates community has been not adversely affected up to concentration of 1.6 µg a.s./L.

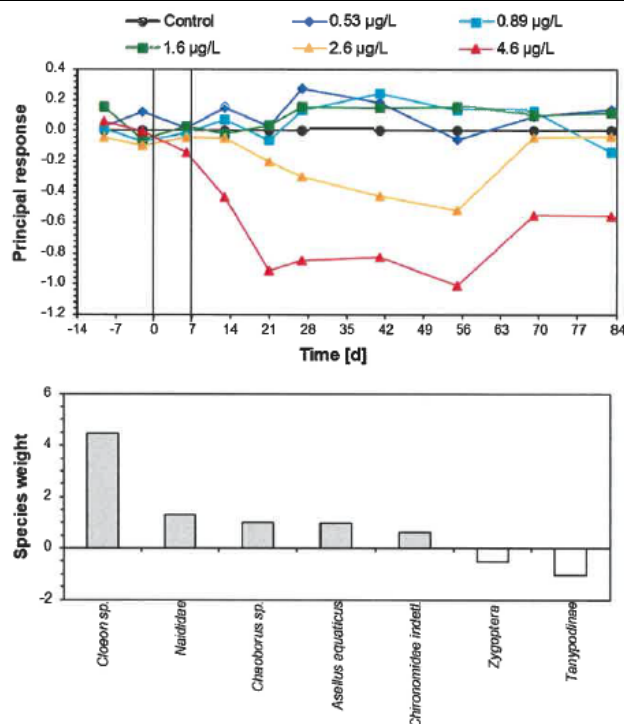


Figure 26: PRCs for the macroinvertebrate data set.  
40% of total variance explained by time, 21% of total variance explained by treatment  
( $p=0.002$ ), 38% of the variance explained by treatment are captured by the PRC ( $p=0.002$ )

### Summary

No effects on macroinvertebrates were found up to the nominal concentration of 1.6 µg a.s./L. Direct effects were found at higher concentrations for four insect populations (*Cloeon dipterum*, Zygoptera, *Chaoborus* and Tanypodinae), two crustaceans (*Asellus aquaticus* and, in an in-situ bioassay, *Gammarus* sp.) and oligochaetes (Naididae). At 2.6 µg a.s./L effects on *Cloeon* were pronounced but recovery was demonstrated within eight weeks, while no recovery within 12 weeks was found at 4.6 µg a.s./L. The other species were less sensitive: *Gammarus* and Naididae showed only slight effects at 2.6 µg a.s./L and pronounced effects at 4.6 µg a.s./L. Chironomidae were slightly affected at 4.6 µg a.s./L. Response of the community level was driven by *Cloeon dipterum*.

**Table A 23: Effect classification for macroinvertebrates**

Nominal conc. [µg a.s./L]		0.30	0.51	0.87	1.5	2.5
Max. measured conc. [µg a.s./L]		0.53	0.89	1.6	2.6	4.6
Macroinvertebrates	<b><i>Cloeon dipterum</i></b>	1	1	1	3A	5B
	<b>Zygoptera</b>	1	1	1	1	3A
	<b><i>Chaoborus</i> sp.</b>	1	1	1	2	2
	Total Chironomidae	1	1	1	1	1
	<b>Tanypodinae</b>	1	1	1	1	2+
	Chironomidae indet.	1	1	1	1	2
	<b><i>Asellus aquaticus</i></b>	1	1	1	1	1
	<b><i>Gammarus</i> sp. (bioassay)</b>	1	1	1	2	3A/4A
	<b>Naididae</b>	1	1	1	2	3A
	<b><i>Helobdella stagnalis</i></b>	1	1	1	1	1
	<b>Lymnaeidae</b>	1	1	1	1	2
	<b><i>Planorbis planorbis</i></b>	1	1	1	1	1
	<b>Community structure</b>	1	1	1	3A	5B

Taxa in bold represent potentially sensitive populations with sufficiently low MDDs to assess direct effects.

### Emerging insects

#### Population level analysis

EFSA (2019) recommends to report emergence per sampling date, but also cumulative emergence of insects since emergence is a measure of production rather than population dynamics. The cumulative emergence has the advantage to be independent of variability over time and due to the increasing numbers over time the sampling error and thus, the MDDs become smaller. Therefore, the focus is on the statistics and figures for the cumulative emergence. On the other hand, it is more difficult to assess the duration of an effect based on cumulative emergence and thus, emergence per week is also shown if useful for evaluation of recovery.

Nine taxa (without pooled taxa) fulfil the MDD criterion by Brock et al. (2015) in the data set of cumulative emergence. For six, at least once the MDD was below 70%. In the data set of emergence per sampling date four taxa (without pooled taxa or overlapping taxa) belong to MDD category 1 and five had at least once an MDD < 70%.

**Table A 24:** NOECs [ $\mu\text{g a.s./L}$ , nominal] and %MDD (in brackets) for the cumulative emergence of insects (only MDD Category 1 and 2 taxa shown)

Cumulative emergence	Day after application									Mean	Min	MDD
	0	7	14	22	30	42	56	70	84	MDD	MDD	Cat
Total emergence	$\geq 4.6$ (38)	$\geq 4.6$ (21)	$\geq 4.6$ (21)	$\geq 4.6$ (25)	$\geq 4.6$ (27)	$\geq 4.6$ (26)	$\geq 4.6$ (24)	2.6- (26)	2.6- (26)	25	21	1
<b><i>Cloeon dipterum</i></b>	2.6- (60)	$\geq 4.6$ (62)	2.6- (58)	2.6- (56)	1.6- (56)	1.6- (53)	1.6- (44)	1.6- (42)	1.6- (40)	51	40	1
Sum Odonata	$\geq 4.6$ (133)	1.6- (44)	2.6- (36)	$\geq 4.6$ (31)	2.6- (28)	2.6- (27)	2.6- (28)	2.6- (28)	2.6- (28)	31	27	1
<b>Coenagrionidae</b>	$\geq 4.6$ (181)	2.6- (44)	2.6- (34)	$\geq 4.6$ (31)	2.6- (28)	2.6- (28)	2.6- (29)	2.6- (29)	2.6- (29)	32	28	1
Zygoptera indet.	$\geq 4.6$ (242)	1.6- (65)	$\geq 4.6$ (61)	$\geq 4.6$ (60)	$\geq 4.6$ (61)	$\geq 4.6$ (63)	$\geq 4.6$ (63)	$\geq 4.6$ (63)	$\geq 4.6$ (63)	62	60	1
Sum Diptera	$\geq 4.6$ (40)	$\geq 4.6$ (27)	$\geq 4.6$ (27)	$\geq 4.6$ (32)	$\geq 4.6$ (35)	$\geq 4.6$ (36)	$\geq 4.6$ (34)	$\geq 4.6$ (34)	$\geq 4.6$ (34)	32	27	1
Sum Chironomidae	$\geq 4.6$ (111)	$\geq 4.6$ (81)	$\geq 4.6$ (72)	$\geq 4.6$ (76)	$\geq 4.6$ (75)	$\geq 4.6$ (74)	$\geq 4.6$ (62)	$\geq 4.6$ (53)	$\geq 4.6$ (51)	68	51	1
<b>Orthocladiinae</b>			$\geq 4.6$ (135)	$\geq 4.6$ (94)	$\geq 4.6$ (95)	$\geq 4.6$ (86)	$\geq 4.6$ (84)	$\geq 4.6$ (72)	$\geq 4.6$ (74)	91	72	1
<b>Tanyptodinae</b>	$\geq 4.6$ (108)	$\geq 4.6$ (65)	$\geq 4.6$ (69)	$\geq 4.6$ (63)	$\geq 4.6$ (62)	$\geq 4.6$ (63)	$\geq 4.6$ (57)	$\geq 4.6$ (61)	$\geq 4.6$ (60)	63	57	1
Chironominae indet.	$\geq 4.6$ (n.c.)	2.6+ (n.c.)	$\geq 4.6$ (148)	$\geq 4.6$ (150)	$\geq 4.6$ (119)	1.6+ (103)	1.6+ (87)	1.6+ (86)	1.6+ (88)	112	86	2
Chironomidae indet.	$\geq 4.6$ (366)	$\geq 4.6$ (95)	$\geq 4.6$ (84)	$\geq 4.6$ (88)	$\geq 4.6$ (87)	$\geq 4.6$ (83)	$\geq 4.6$ (71)	$\geq 4.6$ (62)	$\geq 4.6$ (60)	79	60	1
<b><i>Chaoborus sp.</i></b>	$\geq 4.6$ (44)	$\geq 4.6$ (35)	$\geq 4.6$ (37)	$\geq 4.6$ (44)	$\geq 4.6$ (48)	$\geq 4.6$ (48)	$\geq 4.6$ (49)	$\geq 4.6$ (48)	$\geq 4.6$ (48)	45	35	1
<i>Anopheles sp.</i>			$\geq 4.6$ (154)	$\geq 4.6$ (154)	$\geq 4.6$ (154)	<0.53- (95)	<0.53- (95)	<0.53- (95)	$\geq 4.6$ (119)	124	95	2
Sum Coleoptera	0.89+ (192)	2.6- (71)	$\geq 4.6$ (83)	$\geq 4.6$ (84)	$\geq 4.6$ (68)	$\geq 4.6$ (65)	$\geq 4.6$ (58)	<0.53- (55)	$\geq 4.6$ (58)	68	55	1
Dytiscidae	$\geq 4.6$ (n.c.)	$\geq 4.6$ (138)	$\geq 4.6$ (101)	$\geq 4.6$ (97)	2.6- (76)	2.6- (71)	2.6- (70)	2.6- (70)	2.6- (70)	87	70	1
<i>Helophorus sp.</i>			$\geq 4.6$ (196)	$\geq 4.6$ (226)	$\geq 4.6$ (93)	$\geq 4.6$ (87)	$\geq 4.6$ (84)	$\geq 4.6$ (84)	$\geq 4.6$ (84)	122	84	1
<i>Haliphus sp.</i>	2.6+ (n.c.)	2.6+ (n.c.)	2.6+ (n.c.)	2.6+ (n.c.)	2.6+ (n.c.)	2.6+ (n.c.)	2.6+ (n.c.)	2.6+ (n.c.)	2.6+ (n.c.)			2
Thysanoptera	$\geq 4.6$ (108)	$\geq 4.6$ (80)	$\geq 4.6$ (75)	$\geq 4.6$ (75)	2.6- (57)	2.6- (48)	$\geq 4.6$ (52)	$\geq 4.6$ (46)	$\geq 4.6$ (47)	60	46	1

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 with sufficiently low MDDs (i.e. MDD category 1).



**Table A 25:** NOECs [ $\mu\text{g a.s./L}$ , nominal] and %MDD (in brackets) for the emergence of insects per date (only MDD Category 1 and 2 taxa shown)

Emergence of insects	Day after application									Mean	Min	MDD
	0	7	14	22	30	42	56	70	84	MDD	MDD	Cat
Total emergence	$\geq 4.6$ (38)	$\geq 4.6$ (29)	$\geq 4.6$ (32)	$\geq 4.6$ (48)	$\geq 4.6$ (44)	2.6- (46)	$\geq 4.6$ (42)	0.89- (49)	2.6- (57)	43	29	1
<b><i>Cloeon dipterum</i></b>	2.6- (60)	$\geq 4.6$ (66)	1.6- (63)	1.6- (71)	1.6- (68)	1.6- (58)	2.6- (82)	1.6- (70)	2.6- (63)	68	58	1
Sum Odonata	$\geq 4.6$ (133)	1.6- (45)	$\geq 4.6$ (41)	$\geq 4.6$ (39)	$\geq 4.6$ (38)	$\geq 4.6$ (47)	$\geq 4.6$ (86)	$\geq 4.6$ (133)	$\geq 4.6$ (n.c.)	61	38	1
<b>Coenagrionidae</b>	$\geq 4.6$ (181)	2.6- (44)	$\geq 4.6$ (39)	$\geq 4.6$ (39)	$\geq 4.6$ (38)	$\geq 4.6$ (47)	$\geq 4.6$ (86)	$\geq 4.6$ (133)	$\geq 4.6$ (n.c.)	61	38	1
Sum Diptera	$\geq 4.6$ (40)	$\geq 4.6$ (45)	$\geq 4.6$ (39)	$\geq 4.6$ (56)	$\geq 4.6$ (63)	$\geq 4.6$ (74)	$\geq 4.6$ (42)	0.89- (53)	$\geq 4.6$ (60)	54	39	1
Sum Chironomidae	$\geq 4.6$ (111)	$\geq 4.6$ (83)	$\geq 4.6$ (68)	$\geq 4.6$ (82)	$\geq 4.6$ (77)	$\geq 4.6$ (75)	$\geq 4.6$ (42)	0.89- (53)	$\geq 4.6$ (65)	68	42	1
Chironominae indet.	$\geq 4.6$ (n.c.)	1.6+ (n.c.)	$\geq 4.6$ (130)	$\geq 4.6$ (n.c.)	$\geq 4.6$ (301)	1.6+ (351)	$\geq 4.6$ (141)	$\geq 4.6$ (n.c.)	$\geq 4.6$ (154)	215	130	2
<b>Tanypodinae</b>	$\geq 4.6$ (108)	$\geq 4.6$ (68)	$\geq 4.6$ (82)	$\geq 4.6$ (85)	$\geq 4.6$ (81)	0.53- (71)	0.89- (59)	2.6- (78)	2.6- (72)	75	59	1
Chironomidae indet.	$\geq 4.6$ (366)	$\geq 4.6$ (95)	$\geq 4.6$ (80)	$\geq 4.6$ (93)	$\geq 4.6$ (94)	$\geq 4.6$ (80)	$\geq 4.6$ (48)	2.6- (62)	$\geq 4.6$ (71)	78	48	1
<b><i>Chaoborus sp.</i></b>	$\geq 4.6$ (44)	$\geq 4.6$ (43)	$\geq 4.6$ (44)	$\geq 4.6$ (77)	$\geq 4.6$ (85)	$\geq 4.6$ (181)	2.6- (97)	1.6- (80)	$\geq 4.6$ (99)	88	43	1
Sum Coleoptera	0.89+ (192)	<0.53- (72)	$\geq 4.6$ (112)	2.6+ (241)	<0.53- (64)	$\geq 4.6$ (88)	1.6- (88)	$\geq 4.6$ (105)	$\geq 4.6$ (n.c.)	110	64	2
Curculionidae	$\geq 4.6$ (154)	$\geq 4.6$ (154)	$\geq 4.6$ (196)	2.6+ (n.c.)	$\geq 4.6$ (n.c.)		<0.53- (95)	$\geq 4.6$ (105)	2.6+ (n.c.)	138	95	2
Dytiscidae	$\geq 4.6$ (n.c.)	$\geq 4.6$ (133)	$\geq 4.6$ (130)	$\geq 4.6$ (154)	1.6- (76)	$\geq 4.6$ (196)	<0.53- (83)	$\geq 4.6$ (n.c.)	$\geq 4.6$ (n.c.)	129	76	2
Hydrophilidae	$\geq 4.6$ (n.c.)	$\geq 4.6$ (154)	$\geq 4.6$ (n.c.)	2.6+ (n.c.)		$\geq 4.6$ (n.c.)	$\geq 4.6$ (226)	$\geq 4.6$ (249)	$\geq 4.6$ (n.c.)	210	154	2
Mymaridae		$\geq 4.6$ (n.c.)	$\geq 4.6$ (196)	$\geq 4.6$ (n.c.)	$\geq 4.6$ (196)	$\geq 4.6$ (n.c.)	$\geq 4.6$ (105)	$\geq 4.6$ (119)	2.6- (96)	142	96	2

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 with sufficiently low MDDs (i.e. MDD category 1).

#### zRMS comments:

Comments regarding single NOEC values below the overall NOEC of  $1.6 \mu\text{g a.s./L}$ .

#### Total emergence

Based on the cumulative emergence, the lowest NOEC of  $2.6 \mu\text{g a.s./L}$  was determined for days 70 and 84, while based on emergence per day, the lowest NOEC of  $0.89 \mu\text{g a.s./L}$  was determined on day 70. However, based on the graph for total emergence (see below), in opinion of the zRMS this could be due to higher mean total emergence at  $0.89 \mu\text{g a.s./L}$ , since the mean emergence at 0.53 and  $1.6 \mu\text{g a.s./L}$  was at the level observed in controls and similar trend was observed. The total emergence was lower at 2.6 and  $4.6 \mu\text{g a.s./L}$ , but clear increase was observed throughout the study period comparing to the test initiation. Shortly after each application of the test item no drop in emergence at any of the concentrations has been observed.

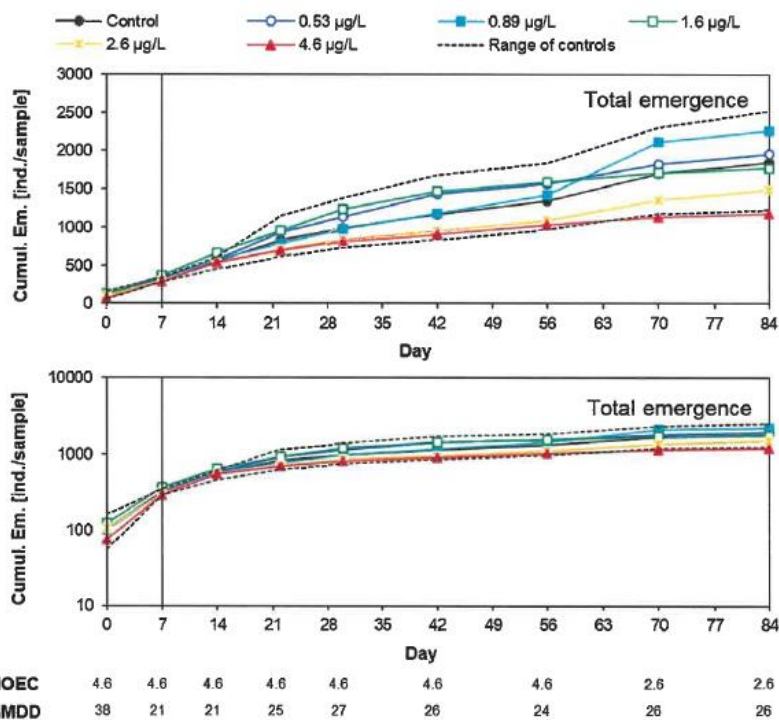


Figure 28: Cumulative total emergence of insects.  
Range of controls and arithmetic (top) and geometric (bottom) means per treatment level

### Sum Diptera

Based on the cumulative emergence, the NOEC of 4.6 µg a.s./L was determined for the whole study period, while based on emergence per day, the lowest NOEC of 0.89 µg a.s./L was determined on day 70. However, based on the graph for total emergence (see below), in opinion of the zRMS this could be due to higher mean total emergence at 0.89 µg a.s./L, since the mean emergence at 0.53, 1.6 and 2.6 µg a.s./L was at the level observed in controls and similar trend was observed. The total emergence was lower at 4.6 µg a.s./L, but clear increase was observed throughout the study period comparing to the test initiation. Shortly after each application of the test item no drop in emergence at any of the concentrations has been observed.

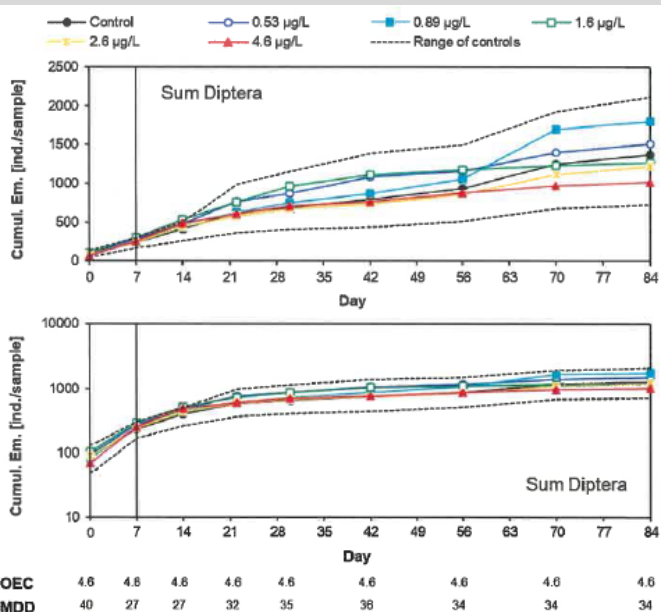
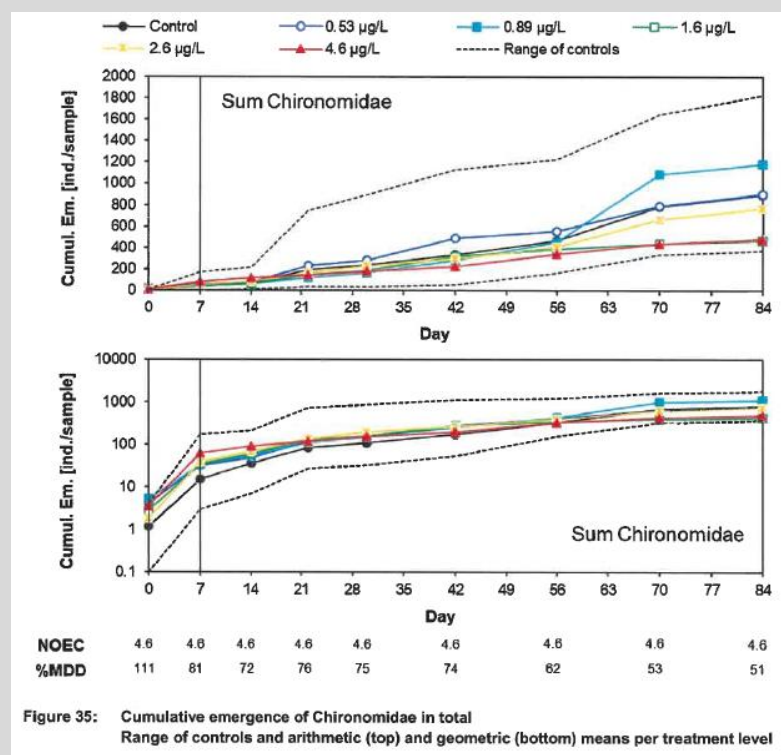


Figure 33: Cumulative emergence of Diptera  
Range of controls and arithmetic (top) and geometric (bottom) means per treatment level



## Sum Chironomidae

Based on the cumulative emergence, the NOEC of 4.6 µg a.s./L was determined for the whole study period, while based on emergence per day, the lowest NOEC of 0.89 µg a.s./L was determined on day 70. However, based on the graph for total emergence (see below), in opinion of the zRMS no clear concentration-response may be found, since the total emergence of Chironomidae at 0.89 µg a.s./L was much higher comparing to controls, at 0.53 and 2.6 µg a.s./L was at the control level, while it was lower at 1.6 and 4.6 µg a.s./L. No effects were observed directly after either application and up to day 63. Based on that, effect observed at 1.6 µg a.s./L on day 70 is considered to be not treatment related.



This is confirmed by results obtained for abundance (see graph below) with abundance at 2.6 and 4.6 µg a.s./L higher than at 1.6 µg a.s./L.

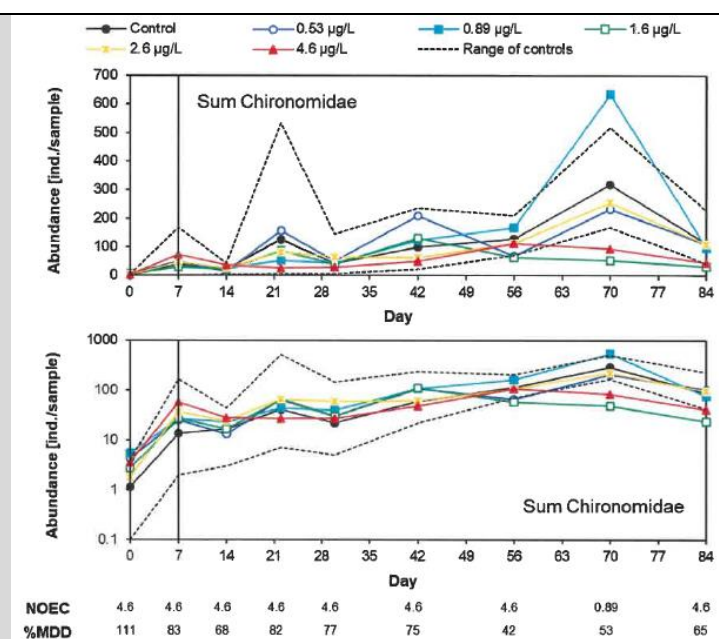


Figure 36: Sum Chironomidae, emergence per week.  
Range of controls and arithmetic (top) and geometric (bottom) means per treatment level

## Tanypodinae

Based on the cumulative emergence, the NOEC of 4.6 µg a.s./L was determined for the whole study period, while based on emergence per day, the lowest NOEC of 0.53 µg a.s./L was determined on day 42 and 0.89 µg a.s./L on day 56. However, based on the graph for total emergence (see below), in opinion of the zRMS no clear concentration-response may be found, since the total emergence of Tanypodinae at 1.6 µg a.s./L on days 42 and 56 was at the level observed in controls and was clearly higher comparing to this observed in 0.53 µg a.s./L, where it was the lowest. Based on that, effect observed at 1.6 µg a.s./L on days 42 and 56 is considered to be not treatment related.

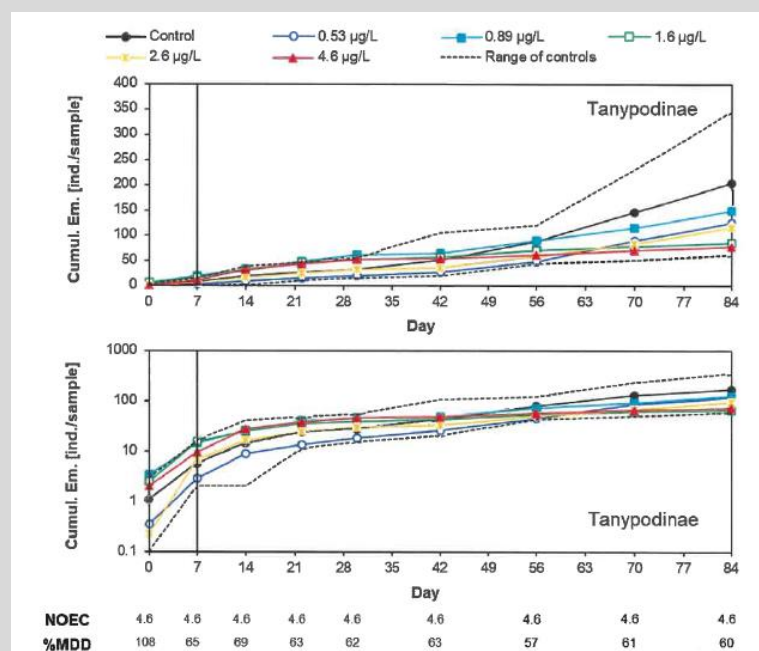


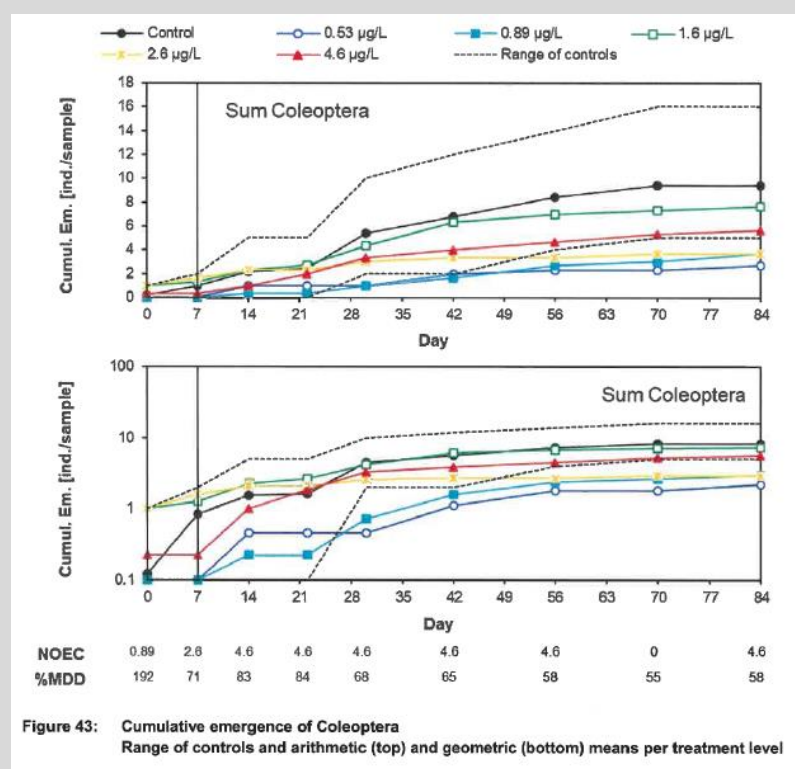
Figure 38: Cumulative emergence of Tanypodinae  
Range of controls and arithmetic (top) and geometric (bottom) means per treatment level

### Anopheles sp.

Although for *Anopheles* sp. the NOEC of 0.53 µg a.s./L was determined for days 42, 56 and 70 based on the cumulative emergence, this species was found very rarely in all enclosures (including controls) throughout the period of the study with 1 individual found at only single sampling occasions in some enclosures. The MDDs were always >90% and on days 14, 22, 30 and 84 they were >100%. For this reason all the statistical analyses for this species are not reliable and the determined NOEC cannot be taken into account for interpretation of the study results.

### Sum Coleoptera

Based on the cumulative emergence, the lowest NOEC of 0.89 µg a.s./L was determined for day 0 (promotion of emergence), while based on emergence per day, the NOEC of 0.53 µg a.s./L was determined for days 7 and 30 due to emergence reduction. However, effects observed on these days were due to presence/absence of single beetle taxa as the number of beetles found in all samples was low with usually 0-3 individuals and max 6 individuals at some sampling occasions in single enclosures (see graph below). Hence, calculated NOEC values are not sufficiently reliable. Furthermore, no clear concentration-response could be found, since emergence in higher test concentrations (2.6 and 4.6 µg a.s./L) was higher comparing to 0.53 and 0.89 µg a.s./L, while at 1.6 µg a.s./L the highest emergence was observed among test item groups.



### Curculionidae

Although for Curculionidae the NOEC of 0.53 µg a.s./L was determined for day 56, this group was not considered for effect classification due to low numbers in all enclosures throughout the period of the study. Based on raw data, the number of individuals ranged from 0 to 1 with 3 individuals observed at only one sampling occasion in a single enclosure. Based on that, the derived NOEC is considered to be not reliable.

### Dytiscidae

Although for Dytiscidae the NOEC of 0.53 µg a.s./L was determined for day 56, this group was not considered for effect classification due to low numbers in all enclosures throughout the period of the study. Based on raw data, the number of individual ranged from 0 to 1 at all sampling occasions in all enclosures. Based on that, the derived NOEC is considered to be not reliable.

### Community level analysis

The community level analysis was only done for the emergence data per week. The diversity analysis revealed a few significant differences to controls on the last three samplings but these were not very pronounced. The NOEC of 0.89 µg a.s./L on day 56 for the number of taxa is not considered reliable because the lowest number was found at 1.6 µg a.s./L, and the generally low number of taxa per sample is largely affected by finding a rare taxon or not.

In contrast to the diversity indices, the PRCs indicate clear effects of 1.9 and 4.6 µg a.s./L but no effects up to 1.6 µg a.s./L on emergence. As for the larvae, the highest weight is calculated for the mayfly *Cloeon* but also *Chaoborus* sp. and Tanyptodinae responded in a similar way. As for the macroinvertebrates, the PRCs for insect emergence indicate no effects up to 1.6 µg a.s./L, pronounced effects at 2.6 µg a.s./L, but with recovery until the end of the study and long-term effects at 4.6 µg a.s./L. Redundancy analysis per sampling data revealed a significant treatment effect on day 22 and 42, but on day 30 the p-value was only slightly higher than 5%. However, according to the Williams-test applied to the PCA sample scores, the community NOEC is 1.6 µg a.s./L from day 30 to 70. On the last sampling, no significance was found.

In summary, effects on the insect community assessed via emergence are Class 1 up to 1.6 µg a.s./L, Class 5A at 2.6 µg a.s./L and Class 5B at 4.6 µg a.s./L.

**Table A 26:** NOECs [µg a.s./L, nominal] for diversity indices of the emerged insects and results of ordination analysis

Emergence Community analysis	Day after application								
	0	7	14	22	30	42	56	70	84
n_taxa	≥4.6	≥4.6	≥4.6	≥4.6	≥4.6	≥4.6	0.89-	2.6-	2.6-
Shannon	≥4.6	≥4.6	≥4.6	≥4.6	≥4.6	≥4.6	2.6-	≥4.6	2.6-
Evenness	≥4.6	≥4.6	≥4.6	≥4.6	≥4.6	≥4.6	2.6-	≥4.6	≥4.6
p-value RDA	0.648	0.112	0.358	0.042	0.088	0.018	0.32	0.08	0.076
NOEC PCA scores	n.d.	2.6	4.6	4.6	1.6	1.6	1.6	1.6	2.6

#### zRMS comments:

The PCR for the emerging insects data set have been copied from the study report and presented below, for reference of the cMS.

As may be seen, the effects on emerging insects community are not fully consistent with more pronounced effects at 0.53 µg a.s./L comparing to 1.6 and 0.89 µg a.s./L. Clear effects were seen at 2.6 and 4.6 µg a.s./L with recovery observed at 2.6 µg a.s./L.

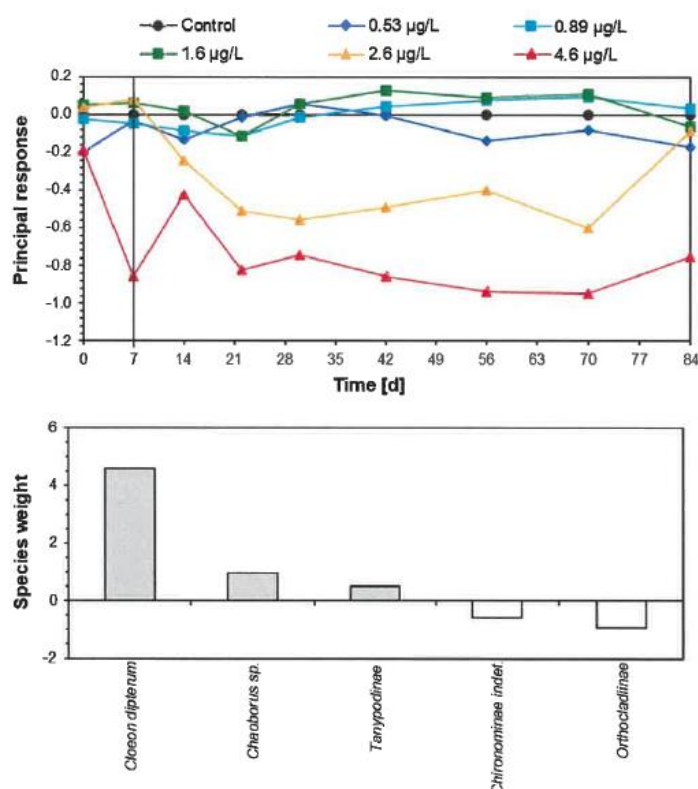


Figure 46: PRCs for the emerged insects  
53% of total variance explained by time, 19% of total variance explained by treatment  
( $p=0.002$ ), 48% of the variance explained by treatment are captured by the PRC ( $p=0.002$ )

### Summary

Insect emergence was not affected up to 1.6 µg a.s./L. At 2.6 µg a.s./L emergence of *Cloeon* was clearly and of Tanypodinae slightly affected. Emergence of mayflies increase again at the end of the study. At 4.6 µg a.s./L emergence of mayflies and damselflies were reduced until the end of the study. Total emergence was affected only at 4.6 µg a.s./L with unclear duration, while the community structure was strongly affected by the response of *Cloeon*.

Table A 27: Effect classification for the emerged insects

Nominal conc. [µg a.s./L]		0.30	0.51	0.87	1.5	2.5
Max. measured conc. [µg a.s./L]		0.53	0.89	1.6	2.6	4.6
Insect emergence	Total emergence	1	1	1	1	3A/4A
	<i>Cloeon dipterum</i>	1	1	1	5A	5B
	Coenagrionidae	1	1	1	1	5B
	Diptera	1	1	1	1	1
	Chironomidae	1	1	1	1	2
	Orthocladinae	1	1	1	1	1
	Tanypodinae	1	1	1	2	3A/4A
	Chironomidae indet.	1	1	1	1	2
	<i>Chaoborus sp.</i>	1	1	1	1	1
	Community structure	1	1	1	5A	5B

### ***Zooplankton***

44 taxa or stages were differentiated in the zooplankton samples. About half of the counted individuals were nauplius larvae of copepods. Based on the numbers of copepodites and adults, most of them were probably Cyclopidae. About 46 %% of the counted animals were rotifers (dominated by *Keratella quadrata*) and only about 2 %% were cladocerans. Ostracods and midges were even rarer.

### ***Population level analysis***

Three taxa (*Chydorus sphaericus*, *Simocephalus sp.* and Cyclopidae (adults and copepodites) fulfil the MDD criterion by Brock et al. (2015) and are considered as potentially sensitive crustaceans. Nauplius larvae are not counted here since most of them belonged to Cyclopidae. For *Chydorus* and Cyclopidae, but also for Chironomidae a MDD < 70% was calculated at least once within the eight weeks after the first application. Other taxa with relatively low MDDs are rotifers and thus, not considered potentially sensitive.

**Table A 28: NOECs [ $\mu\text{g a.s./L}$ , nominal] and %MDD (in brackets) for the zooplankton (only MDD Category 1 and 2 taxa shown)**

Zooplankton	Day after application												Mean	Min	MDD
	-8	-1	2	5	9	12	20	26	40	54	68	82	MDD	MDD	Cat
Cladocera	≥4.6 (84)	≥4.6 (72)	≥4.6 (71)	≥4.6 (73)	≥4.6 (66)	≥4.6 (77)	≥4.6 (60)	≥4.6 (66)	≥4.6 (67)	≥4.6 (77)	≥4.6 (76)	0.89+ (72)	70	60	1
<i>Chydorus sphaericus</i>	≥4.6 (77)	≥4.6 (78)	≥4.6 (68)	≥4.6 (77)	≥4.6 (64)	≥4.6 (65)	≥4.6 (71)	≥4.6 (71)	≥4.6 (75)	1.6+ (79)	1.6+ (69)	0.53+ (81)	71	64	1
<i>Simocephalus sp.</i>	≥4.6 (92)	≥4.6 (78)	≥4.6 (94)	≥4.6 (81)	≥4.6 (84)	≥4.6 (88)	≥4.6 (88)	≥4.6 (92)	≥4.6 (79)	≥4.6 (87)	1.6+ (89)	≥4.6 (112)	87	79	1
<i>Alona sp.</i>									≥4.6 (183)		2.6+ (n.c.)	≥4.6 (148)	183	183	2
<i>Daphnia longispina</i>						2.6+ (n.c.)		≥4.6 (n.c.)	≥4.6 (179)	≥4.6 (131)	≥4.6 (122)	≥4.6 (98)	155	131	2
<i>Graptoleberis sp.</i>		≥4.6 (n.c.)	≥4.6 (149)			≥4.6 (149)	≥4.6 (114)	≥4.6 (295)	≥4.6 (84)	≥4.6 (82)	≥4.6 (82)	0.89+ (96)	146	82	2
Copepoda	≥4.6 (47)	≥4.6 (43)	≥4.6 (39)	≥4.6 (40)	≥4.6 (24)	≥4.6 (40)	≥4.6 (58)	<0.53+ (44)	0.89+ (34)	≥4.6 (69)	≥4.6 (67)	≥4.6 (65)	44	24	1
Nauplius larvae	≥4.6 (47)	≥4.6 (43)	≥4.6 (38)	≥4.6 (41)	≥4.6 (26)	≥4.6 (45)	≥4.6 (64)	<0.53+ (45)	0.89+ (34)	≥4.6 (69)	≥4.6 (72)	≥4.6 (74)	45	26	1
Cyclopidae	≥4.6 (80)	≥4.6 (65)	≥4.6 (64)	≥4.6 (52)	1.6- (46)	≥4.6 (52)	≥4.6 (49)	≥4.6 (62)	≥4.6 (44)	≥4.6 (75)	≥4.6 (75)	≥4.6 (78)	56	44	1
Cyclopidae (adult)	≥4.6 (80)	≥4.6 (73)	≥4.6 (80)	≥4.6 (77)	≥4.6 (50)	≥4.6 (58)	≥4.6 (52)	2.6- (74)	≥4.6 (53)	≥4.6 (71)	2.6+ (68)	≥4.6 (66)	64	50	1
Cyclopidae (Copepodit)	≥4.6 (82)	≥4.6 (65)	≥4.6 (63)	≥4.6 (58)	2.6- (52)	≥4.6 (54)	2.6- (55)	≥4.6 (63)	≥4.6 (73)	≥4.6 (78)	≥4.6 (81)	≥4.6 (81)	62	52	1
Ostracoda		≥4.6 (148)	≥4.6 (223)	≥4.6 (n.c.)	≥4.6 (n.c.)	≥4.6 (149)	≥4.6 (107)	≥4.6 (204)	≥4.6 (195)	≥4.6 (211)	≥4.6 (121)	<0.53+ (153)	182	107	2
Chironomidae	≥4.6 (131)	≥4.6 (138)	≥4.6 (n.c.)	≥4.6 (138)	≥4.6 (105)	0.53- (65)	≥4.6 (92)	≥4.6 (95)	≥4.6 (92)	≥4.6 (85)	2.6+ (63)	≥4.6 (81)	96	65	1
<i>Chaoborus sp. (larvae)</i>	2.6+ (193)	≥4.6 (79)	≥4.6 (128)	≥4.6 (122)	≥4.6 (148)	≥4.6 (205)	≥4.6 (329)	≥4.6 (191)	≥4.6 (101)	≥4.6 (187)	≥4.6 (113)	2.6- (91)	176	101	2
<i>Chaoborus sp. (pupa)</i>	≥4.6 (179)	≥4.6 (234)	≥4.6 (231)		1.6- (82)	≥4.6 (194)	≥4.6 (n.c.)	≥4.6 (n.c.)		≥4.6 (n.c.)		≥4.6 (n.c.)	169	82	2
Rotifera	≥4.6 (58)	≥4.6 (66)	≥4.6 (72)	≥4.6 (81)	≥4.6 (86)	≥4.6 (89)	≥4.6 (84)	≥4.6 (81)	≥4.6 (76)	≥4.6 (81)	≥4.6 (84)	≥4.6 (68)	81	72	1
<i>Keratella quadrata</i>	≥4.6 (91)	≥4.6 (89)	≥4.6 (90)	≥4.6 (89)	≥4.6 (89)	≥4.6 (91)	≥4.6 (88)	≥4.6 (84)	≥4.6 (83)	≥4.6 (85)	≥4.6 (85)	≥4.6 (67)	87	83	1
<i>Testudinella sp.</i>	≥4.6 (76)	≥4.6 (93)	≥4.6 (93)	≥4.6 (90)	≥4.6 (77)	≥4.6 (68)	≥4.6 (79)	≥4.6 (83)	≥4.6 (97)	≥4.6 (95)	≥4.6 (94)	≥4.6 (87)	85	68	1
<i>Habrotrocha sp. cf.</i>	≥4.6 (n.c.)	≥4.6 (211)	≥4.6 (132)	≥4.6 (205)	≥4.6 (n.c.)	≥4.6 (n.c.)	2.6+ (n.c.)	≥4.6 (n.c.)	≥4.6 (n.c.)	1.6+ (n.c.)	≥4.6 (273)	≥4.6 (160)	169	132	2
<i>Lecane sp.</i>	≥4.6 (n.c.)		≥4.6 (n.c.)		1.6+ (n.c.)	≥4.6 (n.c.)	≥4.6 (n.c.)	2.6+ (n.c.)		≥4.6 (172)	2.6+ (n.c.)		172	172	2
<i>Lepadella sp.</i>	≥4.6 (n.c.)	≥4.6 (264)	≥4.6 (n.c.)	2.6+ (136)	≥4.6 (89)	≥4.6 (89)	≥4.6 (134)	≥4.6 (145)	≥4.6 (234)	≥4.6 (251)	≥4.6 (238)	≥4.6 (95)	154	89	2
<i>Mytilina sp.</i>	≥4.6 (147)	≥4.6 (107)	≥4.6 (225)	≥4.6 (104)	≥4.6 (102)	≥4.6 (88)	≥4.6 (79)	≥4.6 (82)	≥4.6 (168)	≥4.6 (148)	2.6+ (n.c.)	≥4.6 (90)	125	79	2
<i>Platylabus quadricornis</i>	≥4.6 (n.c.)	≥4.6 (223)	≥4.6 (184)	≥4.6 (149)	≥4.6 (n.c.)	≥4.6 (103)	≥4.6 (120)	≥4.6 (156)	<0.53- (96)	≥4.6 (127)	≥4.6 (135)	≥4.6 (162)	134	96	2
<i>Polyarthra sp.</i>	≥4.6 (53)	≥4.6 (43)	≥4.6 (64)	≥4.6 (72)	≥4.6 (83)	≥4.6 (96)	≥4.6 (139)	2.6+ (338)	≥4.6 (n.c.)	≥4.6 (158)	≥4.6 (n.c.)	≥4.6 (n.c.)	136	64	2
<i>Rotaria sp.</i>		≥4.6 (n.c.)			2.6+ (n.c.)	≥4.6 (n.c.)		≥4.6 (n.c.)			≥4.6 (n.c.)	≥4.6 (n.c.)			2
<i>Squatinella sp.</i>	≥4.6 (n.c.)									≥4.6 (n.c.)	≥4.6 (n.c.)	2.6+ (n.c.)			2
<i>Synchaeta pectinata</i>	≥4.6 (103)	≥4.6 (97)	≥4.6 (96)	≥4.6 (101)	2.6- (95)	≥4.6 (112)	≥4.6 (n.c.)	≥4.6 (n.c.)	≥4.6 (n.c.)	≥4.6 (n.c.)	≥4.6 (231)	≥4.6 (107)	101	95	2
<i>Synchaeta spp.</i>	≥4.6 (173)		≥4.6 (n.c.)	≥4.6 (n.c.)	≥4.6 (n.c.)	≥4.6 (n.c.)	2.6+ (n.c.)		≥4.6 (183)	≥4.6 (n.c.)	2.6+ (n.c.)	≥4.6 (n.c.)	183	183	2
<i>Trichocerca sp.</i>	≥4.6 (267)	≥4.6 (124)	≥4.6 (104)	≥4.6 (200)	≥4.6 (105)	≥4.6 (101)	2.6+ (101)	2.6+ (92)	≥4.6 (112)	≥4.6 (94)	1.6+ (101)	2.6+ (94)	114	92	2

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 8 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 (i.e. MDD category 1).

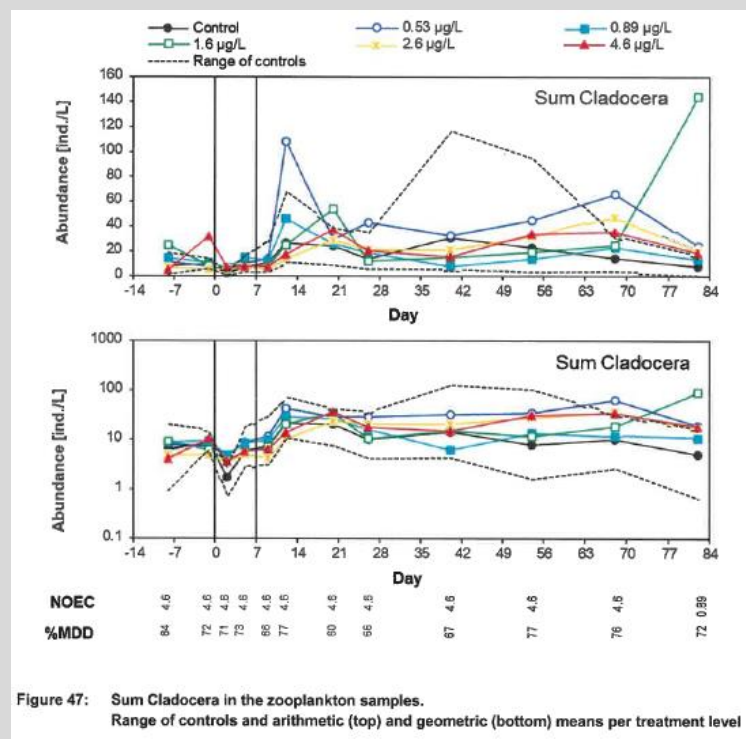


## zRMS comments:

Comments regarding single NOEC values below the overall NOEC of 1.6 µg a.s./L.

### Cladocera

The NOEC of 0.89 µg a.s./L was determined for day 72 due to proportion of Cladocera abundance at 1.6 µg a.s./L. However, from the below graph it seems that this effect was random, as at all remaining test concentrations abundance was not promoted and no concentration-response has been observed. Therefore the effect at 1.6 µg a.s./L is considered to be not treatment related.



### Chydorus sphaericus

Significantly higher abundance has been observed at 2.6 and 4.6 µg a.s./L from day 54 until the end of the study. It is not clear if the higher abundance was caused by the test item, since the mean abundance at the lowest concentration of 0.53 µg a.s./L was also higher comparing to controls at these dates. The overall trend in *C. sphaericus* abundance in all treatment levels was similar into controls. It is also noted that abundance promotion was observed at the time, when the number of individuals in controls was low with single animals found (see graph below). In such a case even one individual more in the treatment level would lead to 100% increase in abundance, which seems to be significant, but is in opinion of the zRMS irrelevant when it is based on absence or presence of single individuals. Based on that the zRMS is of the opinion that the observed effect is not treatment related.



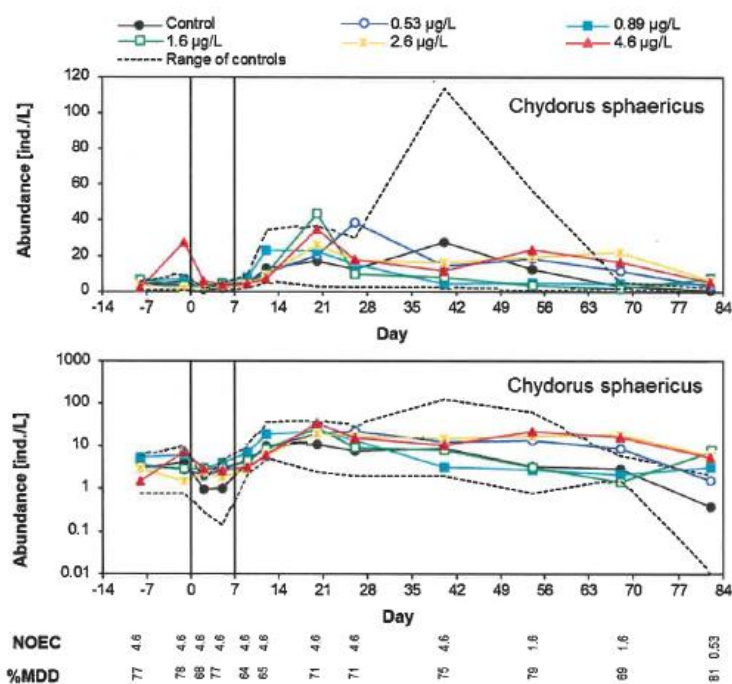


Figure 48: *Chydorus sphaericus* in the zooplankton samples.  
Range of controls and arithmetic (top) and geometric (bottom) means per treatment level

### Graptoleberis sp.

For day 82 the NOEC of 0.89 µg a.s./L has been determined for this group.

It is, however, noted that until day 26 *Graptoleberis* sp. were found at 0-1 individuals in all treatment levels and in all enclosures. From day 26 the numbers started to increase, but were still at the low level of 0 to 4 with slightly higher number of 5-7 found in some enclosures. The abundance clearly increased in all groups from day 68 and then dropped in controls on day 82 to single individuals in 4 replicates (0-0.43) and slightly higher number in 5<sup>th</sup> replicate (3.92). In the treatment groups the number of *Graptoleberis* sp. was more consistent among replicates with 1-4 individuals. The only exception was single replicate at 2.6 µg a.s./ha, where ~20 individuals were found. Overall, no treatment related effects could be found and no concentration-response could be observed. Therefore it is concluded that the NOEC of 0.89 µg a.s./L was rather due to low number of individuals found in 4/5 control replicates and more consistent abundance at level of the single control replicate observed in the test item groups than due to actual abundance promotion by the test item. No graph is available for *Graptoleberis* since this species was found late in the study and long-term observation of potential effects was not possible.

### Copepoda

For days 26 and 40 the NOEC of 0.53 and 0.89 µg a.s./L were determined, respectively, due to increase in abundance. As may be seen on the graph below, no clear concentration-response could be determined with highest promotion seen at 1.6 µg a.s./L and the lowest seen at 2.6 µg a.s./L. No parameters that could cause indirect effects were affected (i.e. reduction of predators or increase in food levels). Based on the raw data it seems that statistical significance of this effects could be due unexpected drop in abundance of Copepoda in controls and high number of Copepoda in single enclosures of the treatment levels. It is noted that increase in abundance at 1.6 µg a.s./L could be also observed after the first application followed by reduction before the second application, but this effect was not significant since it was not consistent among the treatments, where either reduction was followed by promotion or promotion was followed by reduction. Furthermore, the trend in abundance from day 26 was very similar in all test groups (including controls), just the number of individuals were different. Overall, increase in abundance of Copepoda is considered to be not treatment related.

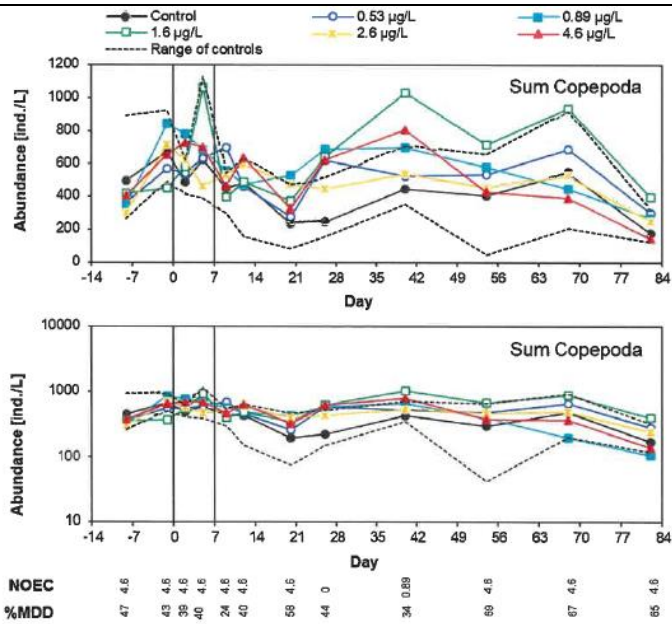


Figure 50: Sum Copepoda in the zooplankton samples.  
Range of controls and arithmetic (top) and geometric (bottom) means per treatment level

### Nauplius larvae

Effect pattern observed for Nauplius larvae was very similar to this observed for Copepoda. This was due to the fact that very large part of Copepoda found in samples were not further determined Nauplius larvae. For this reason graphs and statistical results for nauplii and copepods are almost identical. Hence, the same conclusions as derived for Copepoda apply.

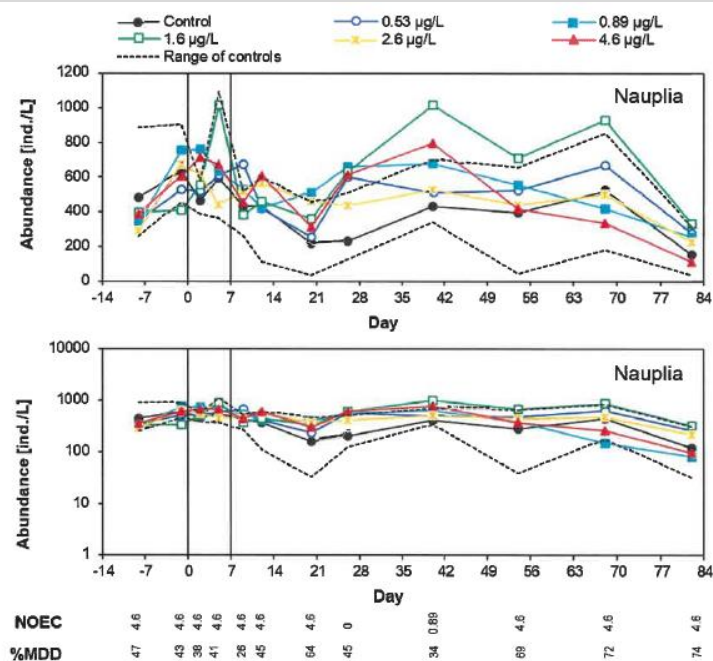


Figure 51: Nauplius larvae in the zooplankton samples.  
Range of controls and arithmetic (top) and geometric (bottom) means per treatment level

### Ostracoda

Due to abundance promotion the NOEC of 0.53 µg a.s./L has been determined for this group at the end of the study (day 82). However, Ostracoda were not considered for effect classification due to low numbers in all enclosures

throughout the period of the study with 0-1 individuals at all sampling points and higher number of individuals (1.78 and 4.0) found in single replicates of the treatment groups at the end of the test. Based on that, the derived NOEC is considered to be not reliable, which is also confirmed by high MDD values (always >100%).

### Chironomidae

Chironomidae do not belong to the zooplankton, but some larvae were found in samples with MDD partly <70%, so they were taken into account in statistical analyses. For day 12 the NOEC of 0.53 µg a.s./L was determined due to abundance reduction. As may be, however, seen on the graph below, reduction could be seen only at 1.6 µg a.s./L, while at remaining treatment levels the numbers were comparable with controls. Furthermore it is noted that Chironomidae were present at very low number in the zooplankton samples during the whole study period (<1 individuals) with slightly higher numbers (up to 8 individuals) observed in single enclosures at some sampling occasions. Overall, effects observed on day 12 is considered to be not treatment related.

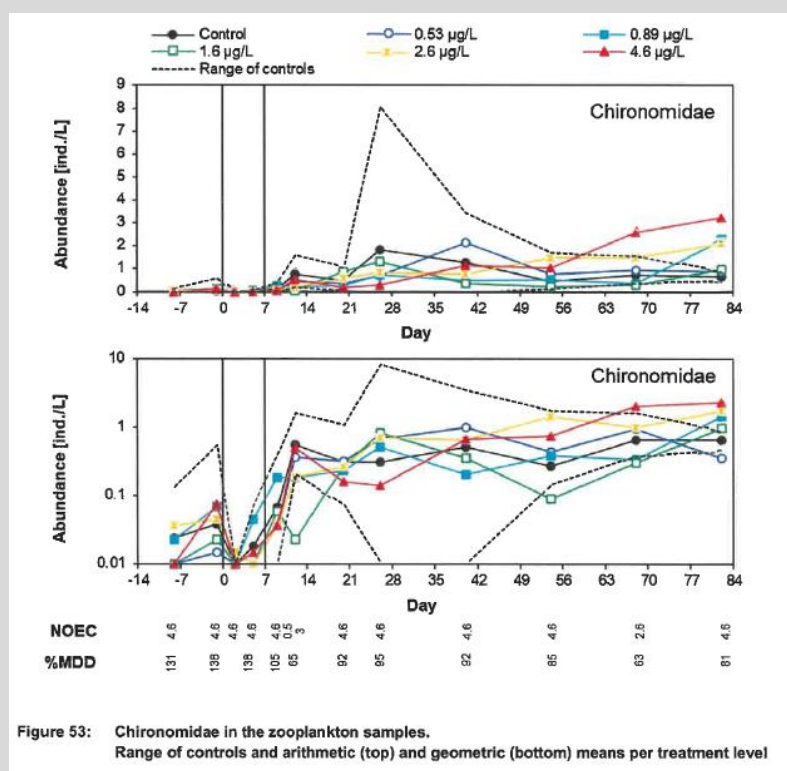


Figure 53: Chironomidae in the zooplankton samples. Range of controls and arithmetic (top) and geometric (bottom) means per treatment level

### Platvias quadricornis

Although for this species the NOEC of 0.53 µg a.s./L was determined for day 40, this species was not considered for effect classification due to low numbers in all enclosures throughout the period of the study. Based on raw data, the number of individuals found in samples was usually 0 with single individuals found at some occasions. Based on that, the derived NOEC is considered to be not reliable which is also confirmed by high MDD values (always >100%).

### Community level analysis

The diversity analysis of the zooplankton revealed temporarily more taxa at the two highest treatment levels and increased Shannon diversity at 1.6 µg a.s./L on the last sampling date. However, the latter was not clearly related to the exposure levels. The PRCs indicate a positive general response of the community at 2.6 and 4.6 µg a.s./L, which increased over the course of the study. The taxa with the highest weights were *Lepadella sp.*, *Graptoleberis sp.*, *Chydorus sphaericus*, Chironomidae, *Simocephalus sp.*, *Testudinella sp.*, and *Trichocerca sp.* *Keratella quadrata*, *Daphnia sp.* and *Daphnia longispina* had negative weights. Thus, for these species lower abundance at the highest test concentration is predicted by the PRC analysis. Based on the PRCs, effects on the zooplankton were considered Class 2 up to 1.6 µg a.s./L and Class 3A/4A for 1.9 and 4.6 µg a.s./L, respectively.

**Table A 29:** NOECs [ $\mu\text{g a.s./L}$ , nominal] for diversity indices of the zooplankton and results of ordination analysis

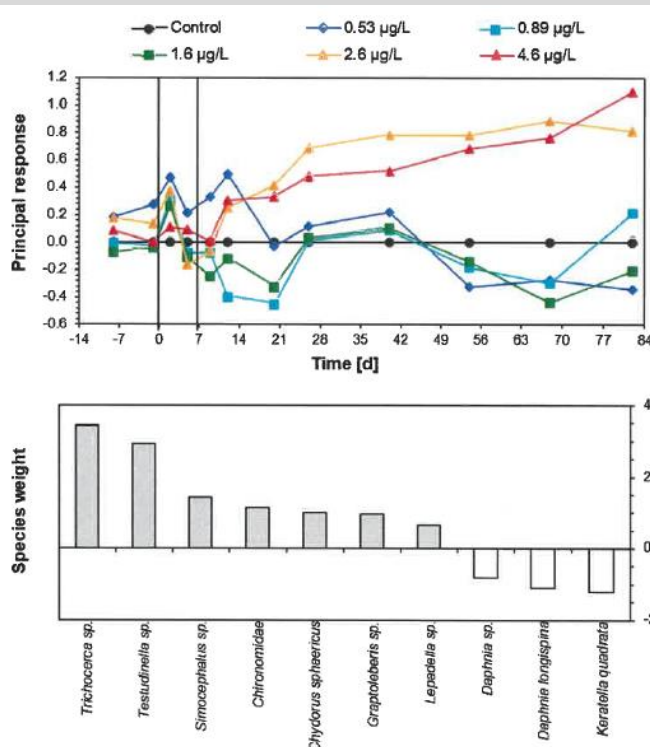
Zooplankton community analysis	Day after application											
	-8	-1	2	5	9	12	20	26	40	54	68	82
n_taxa	4.6	4.6	4.6	4.6	4.6	4.6	4.6	1.6+	4.6	4.6	1.6+	4.6
Shannon	4.6	4.6	4.6	4.6	4.6	4.6	4.6	4.6	4.6	4.6	4.6	0.89+
Evenness	4.6	4.6	4.6	4.6	4.6	4.6	4.6	4.6	4.6	4.6	4.6	4.6
p-value RDA	0.926	0.624	0.586	0.108	0.138	0.16	0.29	0.16	0.534	0.594	0.564	0.044

#### zRMS comments:

The PCRs for zooplankton data set have been copied from the study report and presented below, for reference of the cMS.

As may be seen, the effects on zooplankton community were not consistent with temporarily reduction observed at lower treatment levels and performance better at higher test concentrations.

44% of the total variance in the zooplankton data was attributed to time. Only 16% (not significant) could be explained by the treatment levels and only 26% of that (not significant) was captured by the PRCs. Redundancy analysis per date revealed significant treatment effect only for the last sampling date (promotion) and was thus not analysed further. Effects on zooplankton were considered Class 1 up to  $1.6 \mu\text{g a.s./L}$  and Class 3A/4A for  $2.6$  and  $4.6 \mu\text{g a.s./L}$ .



**Figure 60:** PRCs for the zooplankton.  
44% of total variance explained by time, 16% of total variance explained by treatment ( $p=0.248$ ), 26% of the variance by treatment captured by the PRC ( $p=0.082$ )

#### Summary

There were no treatment effects on the zooplankton up to  $1.6 \mu\text{g a.s./L}$  (nominal). At the two highest test concentrations, there were effects on a few species and the community structure. Since these occurred later in the study, their duration could not be assessed.

**Table A 30: Effect classification for the zooplankton**

Nominal conc. [µg a.s./L]		0.30	0.51	0.87	1.5	2.5
Max. measured conc. [µg a.s./L]		0.53	0.89	1.6	2.6	4.6
Zooplankton	Cladocera	1	1	1	1	1
	<i>Chydorus sphaericus</i>	1	1	1	3A+/4A+	3A+/4A+
	<i>Simocephalus sp.</i>	1	1	1	2+	2+
	Copepoda (mainly nauplii)	1	1	1	1	1
	<b>Cyclopidae (without nauplii)</b>	1	1	1	2	2
	Rotifera	1	1	1	1	1
	<i>Keratella quadrata</i>	1	1	1	1	1
	<i>Testudinella sp.</i>	1	1	1	1	1
	Community structure	1	1	1	3A/4A	3A/4A

Taxa in bold represent potentially sensitive populations with sufficiently low MDDs to assess direct effects.

### Primary producers

Direct effects of acetamiprid on primary producers were not expected and thus, they were monitored more as descriptors of the systems and for indirect effects. Phytoplankton and periphyton were monitored just by chlorophyll measurements which allowed to differentiate four major groups. The macrophytes growing in the sediment were assessed via area coverage.

### Phytoplankton

Some statistically significant effects were found for the phytoplankton until day 22. For total chlorophyll-a, but also blue-greens, diatoms and cryptophytes, a NOEC of < 0.53 µg a.s./L was calculated for a promotion on day 6. However, a promotion of the phytoplankton could only be caused indirectly by a decrease of grazers in the zooplankton but this was not observed. In addition, a direct effect on grazers should be more pronounced after the 2<sup>nd</sup> application and thus, also a promotion of algae should be then found for a longer time period. To the contrary, slightly lower chlorophyll concentrations at 1.6 µg a.s./L and higher concentrations after the second application were determined. Since a direct reduction of algae is very unlikely for the mode of action of a neonicotinoid, such a reduction should be caused by an effect on the zooplankton, e.g. an (indirect) promotion, which was also not observed. All significant deviations of chlorophyll-a concentrations were still very close to the range of the controls. Thus, in summary, no effects on the phytoplankton are concluded.

**Table A 31: NOECs [µg a.s./L, nominal] and %MDD (in brackets) for the phytoplankton chlorophyll-a values**

Phytoplankton chlorophyll-a	Day after application										Mean	Min	MDD
	-7	-1	6	14	22	30	42	56	70	84	MDD	MDD	Cat
Total chlorophyll-a content	<0.53- (38)	≥4.6 (47)	<0.53+ (48)	≥4.6 (53)	0.89- (32)	≥4.6 (56)	≥4.6 (34)	≥4.6 (60)	≥4.6 (57)	≥4.6 (52)	47.7	32	1
Blue Greens	≥4.6 (37)	≥4.6 (40)	<0.53+ (50)	0.89- (49)	0.89- (55)	≥4.6 (92)	≥4.6 (85)	≥4.6 (72)	≥4.6 (68)	≥4.6 (58)	60.6	37	1
Greens	≥4.6 (93)		≥4.6 (154)	≥4.6 (90)	2.6- (47)	≥4.6 (60)	≥4.6 (49)	≥4.6 (75)	≥4.6 (99)	≥4.6 (99)	85.11	47	1
Diatoms	<0.53- (45)	≥4.6 (54)	<0.53+ (40)	≥4.6 (46)	0.89- (40)	≥4.6 (51)	≥4.6 (47)	≥4.6 (60)	≥4.6 (52)	≥4.6 (51)	48.6	40	1
Cryptophytes	<0.53- (36)	≥4.6 (47)	<0.53+ (56)	0.89- (76)	≥4.6 (113)	≥4.6 (117)	≥4.6 (111)	≥4.6 (96)	≥4.6 (86)	≥4.6 (65)	80.3	36	1

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.

Colours indicate the NOECs.



## zRMS comments:

Due to the mode of action of acetamiprid direct effects on primary producers were not expected and they were monitored rather as descriptors of the test systems and for indirect effects.

## Phytoplankton

Some statistically significant effects were found for phytoplankton until day 22. For total chlorophyll-a, but also for blue-greens, diatoms and Cryptophytes the NOEC of  $<0.53 \mu\text{g a.s./L}$  was determined due to promotion on day 6. However, promotion of the phytoplankton could be caused only indirectly by a decrease of grazers in zooplankton, which was not observed. Furthermore, direct effect on grazer should be more pronounced after the second application resulting in promotion of algae for a longer period of time. Nevertheless, after second application slightly lower chlorophyll concentrations were found at  $1.6 \mu\text{g a.s./L}$  and above. Since a direct reduction of algae is unlikely given the MoA of acetamiprid and low sensitivity of this group seen in Tier 1 studies, this reduction could be indirect effect of promotion of zooplankton abundance, which was not observed. It is also noted that trends in abundance of phytoplankton in treatment levels were similar comparing to controls (see graphs below) and all significant deviations of chlorophyll-a concentrations were still very close to range in controls. In summary, no effects on phytoplankton are concluded.

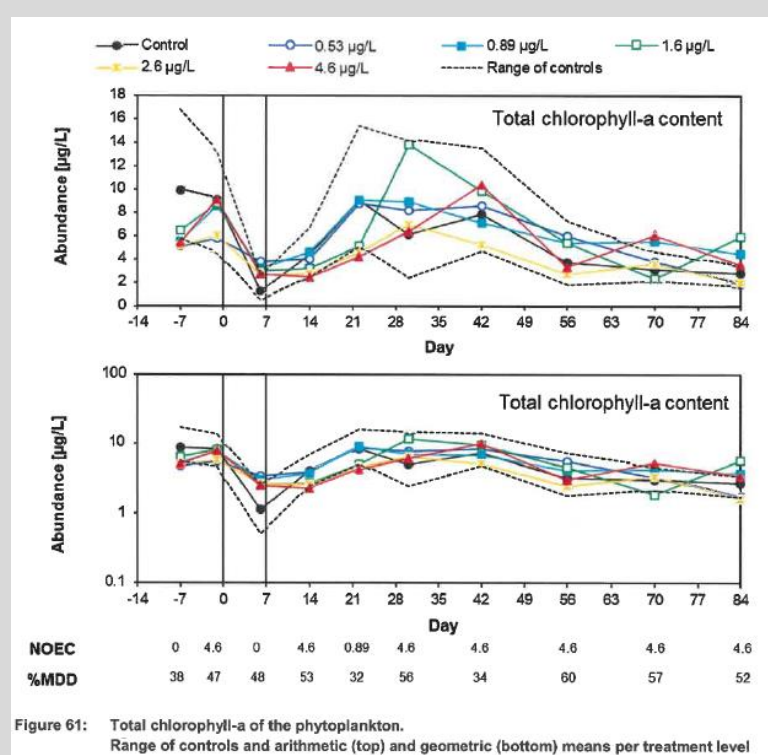


Figure 61: Total chlorophyll-a of the phytoplankton.  
Range of controls and arithmetic (top) and geometric (bottom) means per treatment level

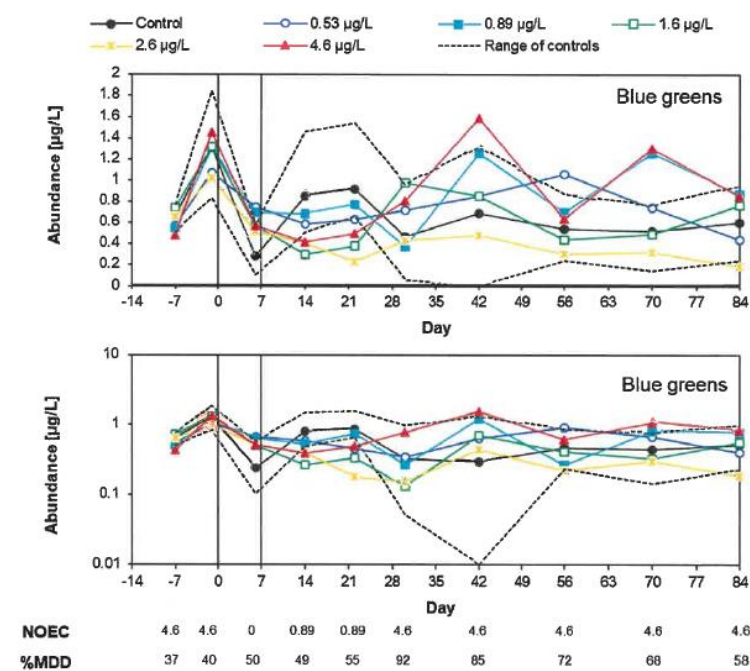


Figure 62: Cyanophyceae (blue-greens) chlorophyll-a of the phytoplankton. Range of controls and arithmetic (top) and geometric (bottom) means per treatment level

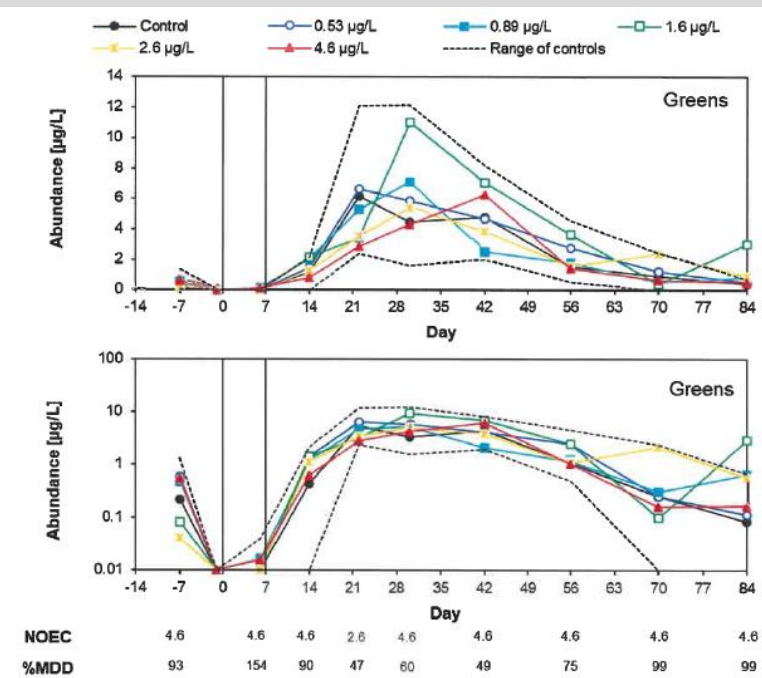
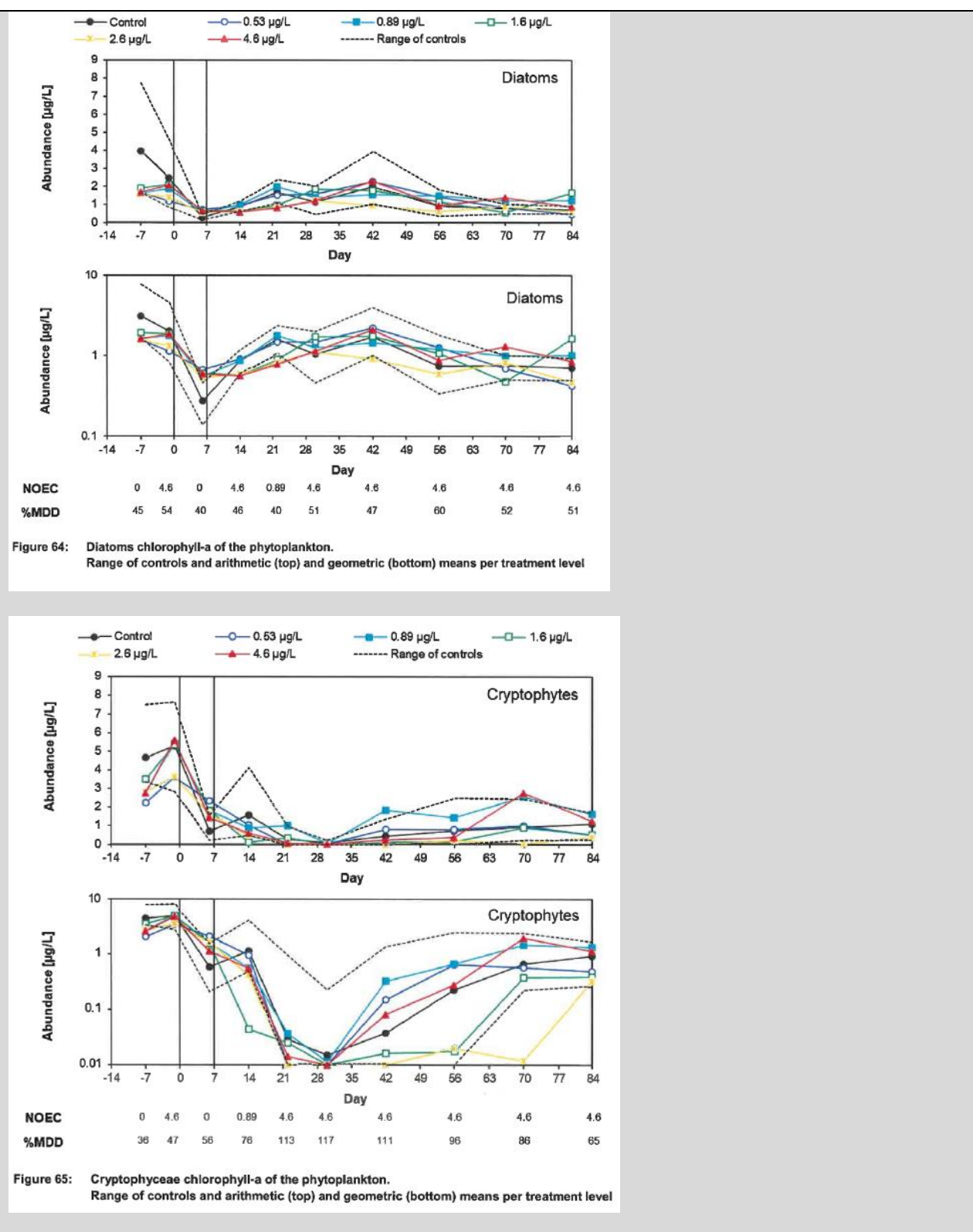


Figure 63: Chlorophyceae and others ('greens') chlorophyll-a of the phytoplankton. Range of controls and arithmetic (top) and geometric (bottom) means per treatment level



### Periphyton

According to the Williams-test, the periphyton was promoted on day 40 and partly also on day 26. However, the mean total chlorophyll-a value at 4.6 µg a.s./L was at its maximum on day 26 less than twice the mean of the control, but still within the range of the controls. Significance was caused by a very low MDD. On day 40 the chlorophyll values were in general much lower and absolute differences were also much smaller.



Considering the figures per group, a slight temporary promotion at the two highest test concentrations might have occurred, which could have been caused by the reduced number of mayflies grazing at the periphyton. Since there were no effects on mayflies at concentrations up to 1.6 µg a.s./L an indirect effect on the periphyton would be hard to explain. Thus, a slight promotion (Class 2+) of the periphyton is assumed for 2.6 and 4.6 µg a.s./L.

**Table A 32:** NOECs [µg a.s./L, nominal] and %MDD (in brackets) for the periphyton chlorophyll-a values

Periphyton chlorophyll-a	Day after application						
	-1	12	26	40	58	72	86
Total chlorophyll-a content	≥4.6 (34)	≥4.6 (73)	2.6+ (26)	0.89+ (65)	≥4.6 (67)	≥4.6 (82)	≥4.6 (58)
Blue Greens	≥4.6 (123)	≥4.6 (73)	≥4.6 (49)	1.6+ (76)	≥4.6 (81)	≥4.6 (77)	≥4.6 (72)
Greens	≥4.6 (38)	≥4.6 (79)	2.6+ (25)	0.53+ (66)	≥4.6 (71)	≥4.6 (88)	≥4.6 (63)
Diatoms	≥4.6 (47)	≥4.6 (71)	≥4.6 (37)	0.89+ (61)	≥4.6 (65)	≥4.6 (83)	≥4.6 (56)
Cryptophytes	≥4.6 (137)	≥4.6 (n.c.)	≥4.6 (n.c.)	1.6+ (90)	≥4.6 (102)	≥4.6 (96)	≥4.6 (96)

Signs +/- indicate the direction of a significant effect and colours indicate the different NOECs.  
Colours indicate the NOECs.

#### **zRMS comments:**

Due to the mode of action of acetamiprid direct effects on primary producers were not expected and they were monitored rather as descriptors of the test systems and for indirect effects.

#### **Periphyton**

According to the Williams-test, the periphyton was promoted on day 40 and partly also on day 26. However, the mean total chlorophyll-a value as 4.6 µg a.s./L was at its maximum on day 26 less than twice the mean in control, but still within the control range. Significance was caused by very low MDD. On day 40 the chlorophyll values were in general much lower and absolute differences were also much smaller.

Considering the below graphs per group, a slight temporary promotion at the two highest concentrations might have occurred, which could be caused by the reduced number of mayflies grazing at periphyton. Since there were no effects on mayflies up to 1.6 µg a.s./L, an indirect on periphyton due to reduced grazing was difficult to explain.

It is also noted that trends in abundance of periphyton in treatment levels were similar comparing to controls (see graphs below) and all significant deviations of chlorophyll-a concentrations were still very close to range in controls.

Overall, slight promotion (class 2) of periphyton is concluded for concentrations of 2.6 and 4.6 µg a.s./L.

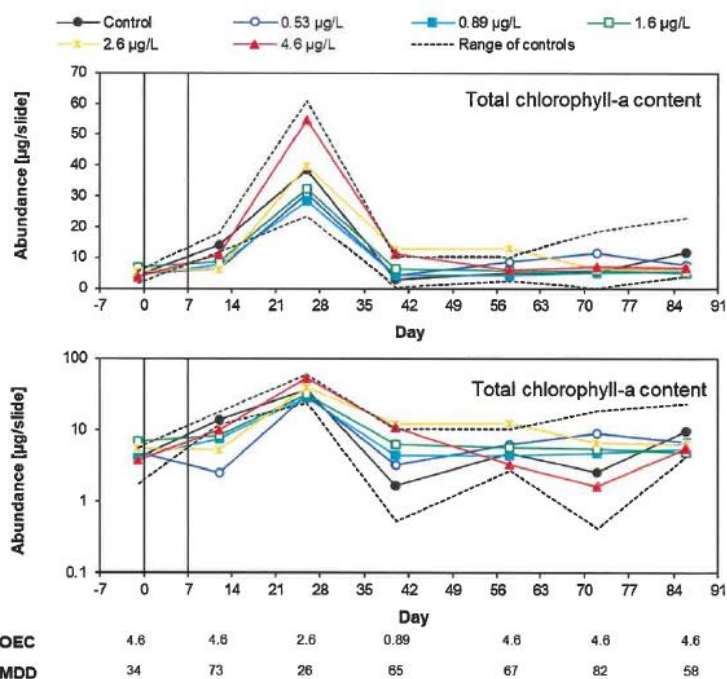


Figure 66: Total chlorophyll-a of the periphyton. Range of controls and arithmetic (top) and geometric (bottom) means per treatment level. Slide area 192.5  $\text{cm}^2$ .

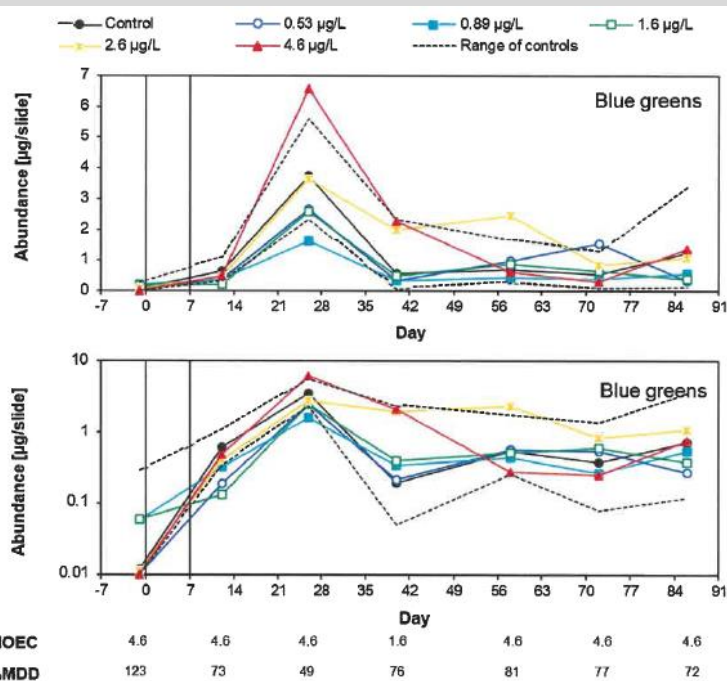


Figure 67: Cyanophyceae (blue-greens) chlorophyll-a of the periphyton. Range of controls and arithmetic (top) and geometric (bottom) means per treatment level. Slide area 192.5  $\text{cm}^2$ .

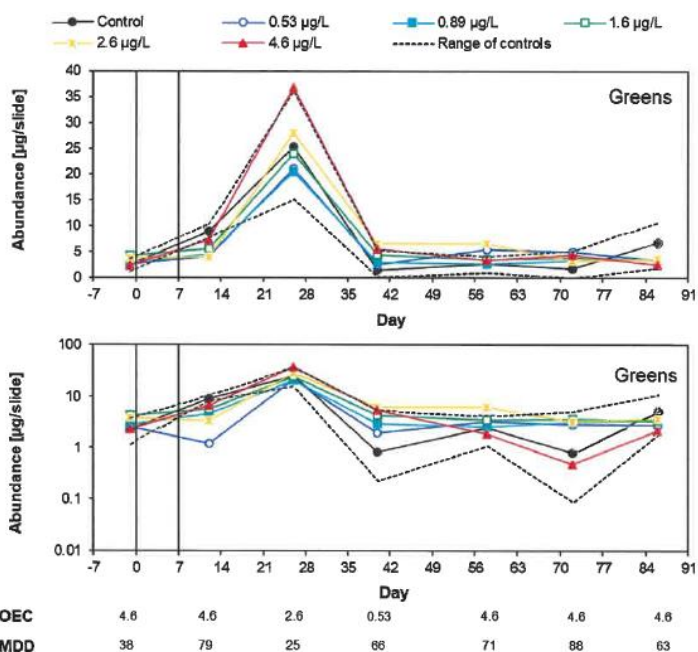


Figure 68: Chlorophyceae and other ('greens') chlorophyll-a of the periphyton. Range of controls and arithmetic (top) and geometric (bottom) means per treatment level. Slide area 192.5 cm<sup>2</sup>.

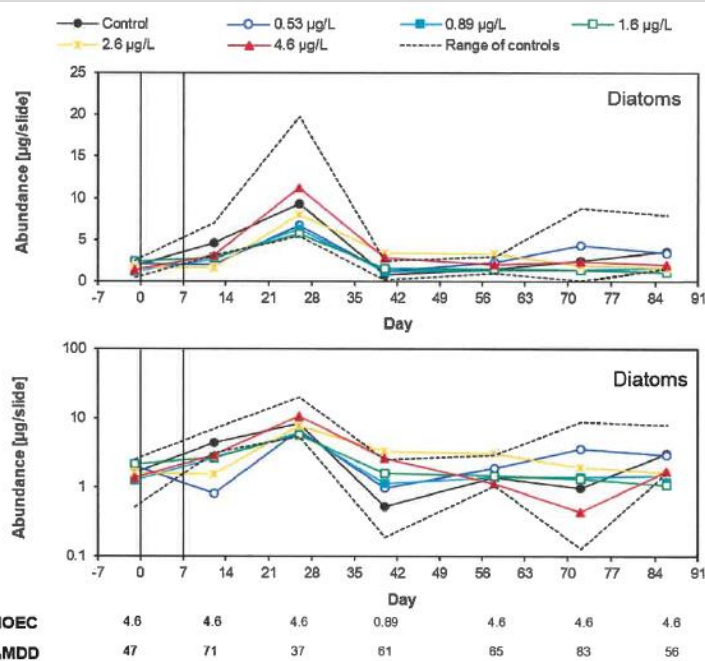
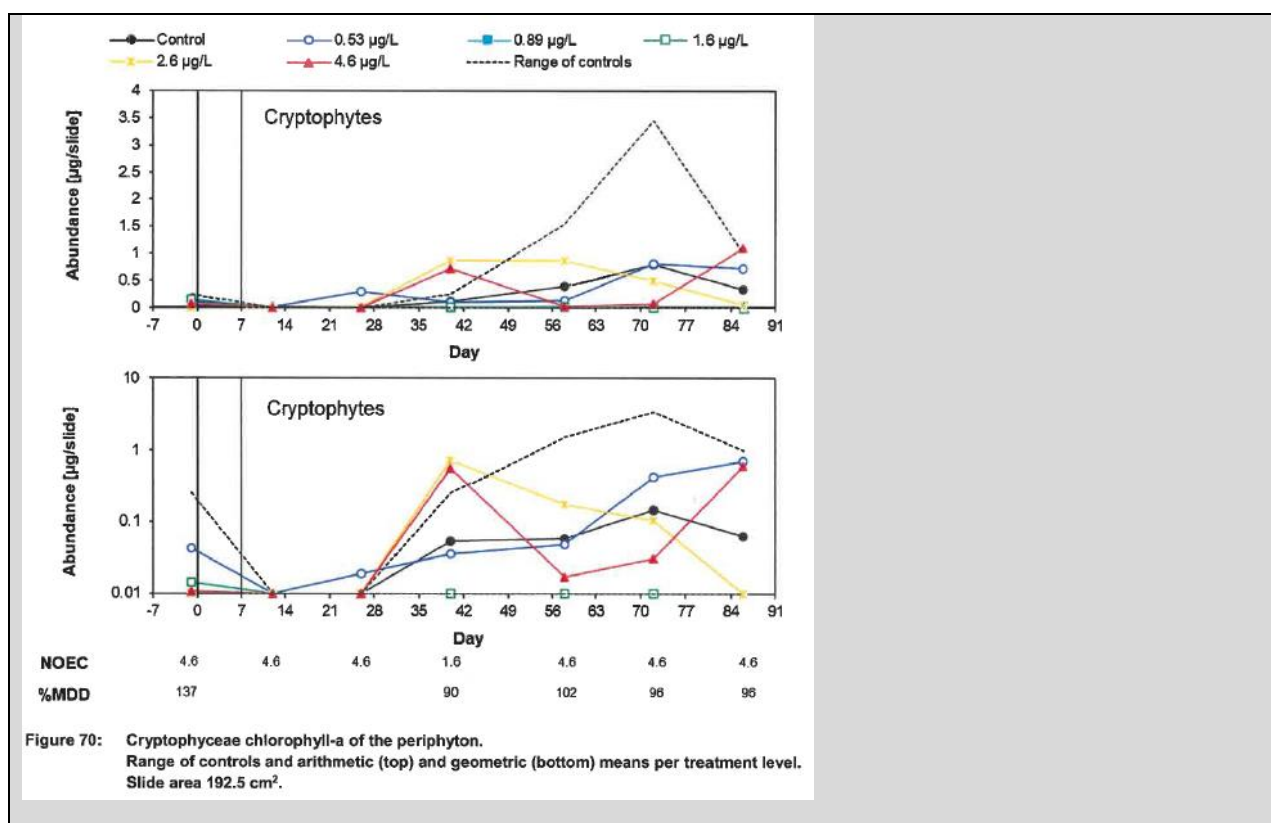


Figure 69: Diatoms chlorophyll-a of the periphyton. Range of controls and arithmetic (top) and geometric (bottom) means per treatment level. Slide area 192.5 cm<sup>2</sup>.



### Macrophytes

Three macrophytes species were present in the enclosures. In addition, filamentous algae were considered for mapping area coverage. MDDs for these measurements were relatively low but except for *Myriophyllum spicatum* on a single day, no significant deviations to controls were found. However, the NOEC of 0.89 µg a.s./L for a promotion of *Myriophyllum* on day 30 is related to slightly higher coverage at 1.6 and 2.6 µg a.s./L, but not 4.6 µg a.s./L. Therefore, this was not considered to be a treatment effect and no effects were assumed for macrophytes and filamentous algae.

**Table A 33:** NOECs [µg a.s./L, nominal] and %MDD (in brackets) for the macrophyte coverage data

Macrophytes	Day after application				
	-1	15	30	58	86
Sum Coverage	≥4.6 (24)	≥4.6 (29)	≥4.6 (15)	≥4.6 (14)	≥4.6 (20)
<i>Chara globularis</i>	≥4.6 (23)	≥4.6 (30)	≥4.6 (18)	≥4.6 (13)	≥4.6 (14)
<i>Myriophyllum spicatum</i>	≥4.6 (43)	≥4.6 (49)	0.89+ (22)	≥4.6 (40)	≥4.6 (35)
<i>Zannichéllia palustris</i>		≥4.6 (n.c.)			
Filamentous algae	≥4.6 (49)	≥4.6 (56)	≥4.6 (43)	≥4.6 (47)	≥4.6 (64)

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.

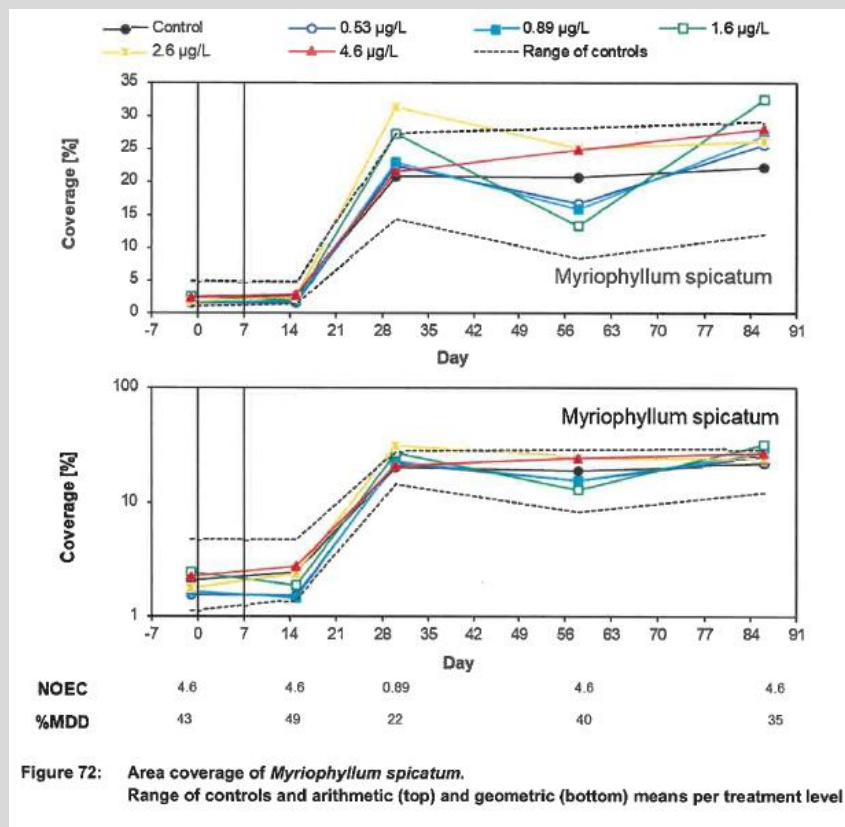
Colours indicate the NOECs

#### zRMS comments:

Due to the mode of action of acetamiprid direct effects on primary producers were not expected and they were monitored rather as descriptors of the test systems and for indirect effects.

#### Macrophytes

The respective explanation regarding observed effects is provided above, in the Applicants' summary. Below the graph for *M. spicatum* is presented in order to demonstrate the trend observed and further justify that promotion of growth on day 40 resulting with the NOEC of 0.89 µg a.s./L was random and not treatment related.



#### Community metabolisms

Dissolved oxygen concentrations, pH and conductivity can be considered as indicators of community metabolisms since they are affected by primary production and partly, respiration. Already before application, dissolved oxygen concentration and pH at the higher treatment levels were above the mean of the controls. Thus, the NOECs indicating a promotion after application until day 20 are not considered reliable. Later the pH at 4.6 µg a.s./L was temporarily higher than the mean pH of the controls but it was always within or close to the upper range of the controls. After day 42, the mean conductivity in the treated enclosures was partly significantly lower than the control mean with the lowest values often found at 1.6 µg a.s./L.

It should be noted that the observed differences were small and had no consequences on the water quality. Significance was often found due to very low MDDs (often less than 10%), e.g. the NOEC of 0.53 µg a.s./L for an increase of pH was related to a mean pH in the controls of 8.3 and 8.6 at 0.89 µg a.s./L. Significance in conductivity on day 47 was related to 203 µS/cm in the controls compared to 190 µS/cm at 0.89 µg a.s./L.

Thus, the statistical differences of these physico-chemical parameters were not considered to indicate relevant effects on the community metabolism, especially photosynthesis.

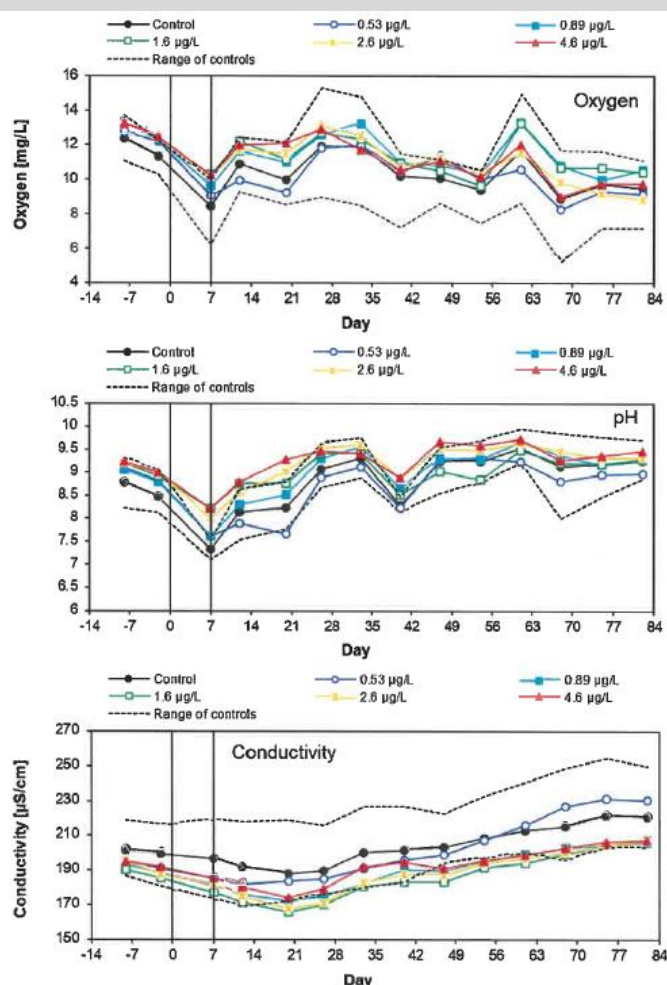


**Table A 34:** NOECs [ $\mu\text{g a.s./L}$ , nominal] for dissolved oxygen concentrations, pH and conductivity as indicators for community metabolism

Community metabolism	Day after application													
	-8	-2	7	12	20	26	33	40	47	54	61	68	75	82
oxygen [mg/L]	≥4.6	0.89+	0.89+	≥4.6	2.6+	≥4.6	≥4.6	≥4.6	≥4.6	≥4.6	≥4.6	≥4.6	≥4.6	≥4.6
pH	2.6+	1.6+	<0.53+	0.89+	0.89+	≥4.6	≥4.6	0.53+	2.6+	≥4.6	≥4.6	≥4.6	≥4.6	≥4.6
conductivity [μS/cm]	≥4.6	≥4.6	≥4.6	≥4.6	≥4.6	≥4.6	≥4.6	≥4.6	0.53-	0.89-	0.89-	≥4.6	≥4.6	≥4.6

**zRMS comments:**

The respective explanation regarding effects observed on oxygen, pH and conductivity is provided above, in the Applicants' summary. Below the graph for these parameters is presented in order to demonstrate the trends observed and illustrate that observed changes were very small with no impact on the water quality.



**Figure 75:** Indicators for community metabolism (dissolved oxygen, pH, conductivity of the water). Range of controls and arithmetic means per treatment level

**Summary**

Despite a potential slight temporary promotion of periphyton at 1.9 and 4.6  $\mu\text{g a.s./L}$ , no effects on primary producers were found.

**Table A 35: Effect classification for primary producers**

Nominal conc. [ $\mu\text{g a.s./L}$ ]		0.30	0.51	0.87	1.5	2.53
Max. measured conc. [ $\mu\text{g a.s./L}$ ]		0.53	0.89	1.6	2.6	4.6
Producers	Phytoplankton chlorophyll-a	1	1	1	1	1
	Periphyton chlorophyll-a	1	1	1	2+	2+
	Macrophytes (coverage)	1	1	1	1	1
	Community metabolism	1	1	1	1	1

## 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 a few macrophytes and many algae and invertebrate species from a large variety of taxonomic groups. Fungi, protozoa and bacteria were also present but not monitored.

### Exposure

The analysis of acetamiprid in application solutions (on average 92%) confirmed the intended loading. Three hours after applications, on average, 100% after the first and 90% 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. Thus, the measurements confirm that the theoretical exposure was achieved. Variability between the replicates of the water samples taken shortly after application was relatively large, probably due to not yet homogenous distribution in the water column.

Dissipation of acetamiprid from the water was relatively slow with a mean  $DT_{50}$  of 44 days. Thus, twelve weeks after the first application still about 50% of the nominal concentrations were measured in the water. Acetamiprid dissipated at least partly into the sediment since it could be measured in all sediment samples (except from control enclosures). In the highest treatment level ( $3.2 \mu\text{g a.s./L}$ ), a mean maximum of  $7.5 \mu\text{g a.s./kg}$  dry weight was measured eight weeks after the first application.

Thus, organisms were exposed over the full study duration to acetamiprid in water and sediment. Because the second application resulted in measured concentrations clearly above the nominal concentrations, the maximum measured concentrations are probably better suited for comparison with maximum PEC values.

### 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, also some Oligochaetes (Naididae) might 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 11 potentially sensitive taxa fulfil the MDD criterion proposed by Brock et al. (2015), including mayflies (*Cloeon dipterum*), midges (*Chaoborus crystallinus*, Tanypodinae, Orthocladiinae), damselflies (Zygoptera, Coenagrionidae). Amphipoda (*Gammarus pulex*), Isopoda (*Asellus aquaticus*), Cladocera (two species), Copepoda (Cyclopidae) 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. However, also if only the first eight weeks are considered, when exposure was larger than 50% of nominal, more than eight taxa revealed at least once MDDs of up to 70%, which is sufficient to detect medium effects following EFSA PPR panel (2013). Thus, a statistical analysis of direct effect 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:

- Maximum measured 0.53 – 1.6 µg a.s./L, nominal up to 0.87 µg a.s./L:  
No treatment effects were found on any macroinvertebrate or zooplankton taxa. Single statistical findings with NOECs < 1.6 µg a.s./L were found to be not ecotoxicologically relevant due to very low numbers, missing concentration-response, and / or not plausible explanation of delayed or indirect effects.
- Maximum measured 2.6 µg a.s./L, nominal 1.5 µg a.s./L:  
This concentration had pronounced effects on larvae and – in consequence – emergence of the mayfly *Cloeon dipterum* with recovery of emergence demonstrated at the end of the study. A few taxa were slightly affected (e.g. *Chaoborus*, *Gammarus*, Tanypodinae, Naididae). Potential effects on the cladoceran *Chydorus sphaericus* were found late in the study and thus, its duration could not be assessed. Periphyton might have been slightly indirectly promoted. The effects on *Cloeon* resulted also in significant changes of the macroinvertebrate and insect community.
- Maximum measured 4.6 µg a.s./L, nominal 2.5 µg a.s./L:  
Compared to 2.6 µg a.s./L, some effects became more pronounced and for additional species slight direct or indirect effects were found. The mayfly *Cloeon* could not recover within the study. Emergence of damselflies (Coenagrionidae) was temporarily reduced and since there is only one generation per year, recovery was not possible. Also *Gammarus* and Naididae showed pronounced effects at the highest test concentration.

#### *Proposal for RAC derivation*

**In conclusion, the maximum measured concentration of 1.6 µg a.s./L (9.4 µg test item/L; nominal: 0.87 µg a.s./L and 5.1 µg test item/L) is the overall Class 1 concentration** which can be used to derive an ETO-RAC. Uncertainty related to this concentration is considered small since clearly no effects on potentially sensitive taxa were found and the 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 (2.6 µg a.s./L maximum measured) effects on the emergence of mayflies lasted longer than eight weeks.



**Table A 36: Effect classification for all data sets of the study**

Nominal conc. [µg a.s./L]		0.30	0.51	0.87	1.5	2.5
Max. measured conc. [µg a.s./L]		0.53	0.89	1.6	2.6	4.6
Macroinvertebrates	<b><i>Cloeon dipterum</i></b>	1	1	1	3A	5B
	<b>Zygoptera</b>	1	1	1	1	3A
	<b><i>Chaoborus sp.</i></b>	1	1	1	2	2
	Total Chironomidae	1	1	1	1	1
	<b>Tanypodinae</b>	1	1	1	1	2+
	Chironomidae indet.	1	1	1	1	2
	<b><i>Asellus aquaticus</i></b>	1	1	1	1	1
	<b><i>Gammarus sp.</i> (bioassay)</b>	1	1	1	2	3A/4A
	<b>Naididae</b>	1	1	1	2	3A
	<i>Helobdella stagnalis</i>	1	1	1	1	1
	Lymnaeidae	1	1	1	1	2
	<i>Planorbis planorbis</i>	1	1	1	1	1
	Community structure	1	1	1	3A	5B
	Total emergence	1	1	1	1	3A/4A
Insect emergence	<b><i>Cloeon dipterum</i></b>	1	1	1	5A	5B
	<b>Coenagrionidae</b>	1	1	1	1	5B
	Diptera	1	1	1	1	1
	Chironomidae	1	1	1	1	2
	<b>Orthocladinae</b>	1	1	1	1	1
	<b>Tanypodinae</b>	1	1	1	2	3A/4A
	Chironomidae indet.	1	1	1	1	2
	<i>Chaoborus sp.</i>	1	1	1	1	1
	Community structure	1	1	1	5A	5B
	Cladocera	1	1	1	1	1
Zooplankton	<b><i>Chydorus sphaericus</i></b>	1	1	1	3A+/4A+	3A+/4A+
	<b><i>Simocephalus sp.</i></b>	1	1	1	2+	2+
	Copepoda (mainly nauplii)	1	1	1	1	1
	<b>Cyclopidae (without nauplii)</b>	1	1	1	2	2
	Rotifera	1	1	1	1	1
	<i>Keratella quadrata</i>	1	1	1	1	1
	<i>Testudinella sp.</i>	1	1	1	1	1
	Community structure	1	1	1	3A/4A	3A/4A
	Phytoplankton chlorophyll-a	1	1	1	1	1
Producers	Periphyton chlorophyll-a	1	1	1	2+	2+
	Macrophytes (coverage)	1	1	1	1	1
	Community metabolism	1	1	1	1	1
	<b>Overall effect classification</b>	<b>1</b>	<b>1</b>	<b>1</b>	<b>5A</b>	<b>5B</b>

Taxa in bold represent potentially sensitive populations with sufficiently low MDDs to assess direct effects.

## A 2.3 KCP 10.3 Effects on arthropods

### A 2.3.1 KCP 10.3.1 Effects on bees

Comments of zRMS:	For comments of the zRMS on acceptability and applicability of this literature study for the higher-tier risk assessment, please refer to point 9.6 of this document.
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Reference:	KCP 10.3.1/03
Report	The nectar report: quantitative review of nectar sugar concentrations offered by bee visited flowers in agricultural and non-agricultural landscapes, PeerJ 7, e6329, 15 pp. Franke, M Pamminger T., Becker R., Himmelreich S., Schneider C. W., Bergtold M., 2019
Guideline(s):	Not applicable (publication)
Deviations:	Not applicable (publication)
GLP:	No
Acceptability:	Please, refer to point 9.6 for zRMS comments on acceptability and applicability of the study
Duplication (if vertebrate study)	Not applicable

### Executive Summary

A comprehensive literature data analysis on the sugar content in nectar was done to compile a comprehensive geographically explicit dataset on nectar quality (i.e. total sugar concentration), offered to bees both within fields (crop and weed species) as well as outside fields (wild species) around the globe. In total, 444 individual measurements of sugar concentration in nectar for bee pollinated flowers were collected, ranging from 6.3 to 85%. With similar sampling sizes for plant species in crop (n = 151) and wild plants (n = 141), but fewer measurements for weeds (n = 30). On a genus level the authors found that the wild community has the highest phylogenetic diversity in terms of number of genera recorded (n = 63) followed by the crop community (n = 29) and lowest diversity in the weed community (n = 18). In general, the recorded data is evenly spread across the geographic regions, however only a limited number of weed species could be identified in Africa (n = 13) and South America (n = 18). With respect to the risk assessment, for apples (*Malus domestica* and *M. sp.*, total<sub>n</sub> = 10) the authors found a range of the sugar content in nectar between 32.9% and 55%, median 42%. For different species of *Brassica* (*B. napus*, *B. oleracea*, *B. rapa*, and *B. sp.* total<sub>n</sub> = 33), to which oil seed rape belongs (*B. napus*), the range was 36% to 62%, median 41.5%. For *B. napus* alone (n = 4) it ranged between 39 to 62% with a median of 43.5%.

### Materials and methods

#### Materials

#### Test item

#### Species

Not relevant

*Malus domestica* (apple), *Brassica* spec. (*B. napus*, *B. oleracea* and *B. rapa*), *Brassica napus* (oil seed rape)

#### Group size/replicates

*Malus domestica* (n = 1), *Brassica* spec. (n = 33), *Brassica napus* (n = 4)

#### Experimental treatments

Sugar content in nectar.

### Biological observations

A comprehensive literature data analysis was made on the sugar content in nectar of crop and weed species as well as for wild species around the globe. With respect to the current risk assessment, the content was analysed for apples (*Malus domestica*), different species of *Brassica* (*B. napus*, *B. oleracea* and *B. rapa*) and for oil seed rape alone (*B. napus*).

## Results and discussion

### Biological results – sugar content

Biological results on the sugar content in nectar are given in the table below.

**Table A 37: Honeybee mortality after contact application of MCW-2222**

Sample no.	Sugar content in nectar [%]							
	<i>Malus domestica</i> (apple)			<i>Brassica</i> sp. ( <i>B. napus</i> , <i>B. oleracea</i> and <i>B. rapa</i> )			<i>Brassica napus</i> (oil seed rape)	
	Species	Content	Variety	Species	Content	Variety	Content	Variety
1	<i>M. sp.</i>	42	NA	<i>B. sp.</i>	39	NA	39	Candal
2	<i>M. sp.</i>	42	NA	<i>B. oleracea</i>	56	NA	42	Regent
3	<i>M. sp.</i>	42	NA	<i>B. rapa</i>	38	Toria	62	NA
4	<i>M. sp.</i>	42	NA	<i>B. rapa</i>	43.8	Toria	45	NA
5	<i>M. domestica</i>	45.3	Booskoop	<i>B. rapa</i>	42	Toria		
6	<i>M. domestica</i>	47.4	Jonathan	<i>B. rapa</i>	41.5	Toria		
7	<i>M. domestica</i>	32.9	Yellow Transp.	<i>B. rapa</i>	40	Toria		
8	<i>M. domestica</i>	36.4	Cox Orange	<i>B. rapa</i>	38.6	Toria		
9	<i>M. domestica</i>	44.9	Golden Delicious	<i>B. rapa</i>	43	Toria		
10	<i>M. domestica</i>	55	NA	<i>B. rapa</i>	40	Toria		
11				<i>B. rapa</i>	41	Toria		
12				<i>B. rapa</i>	38	Toria		
13				<i>B. rapa</i>	42	Toria		
14				<i>B. rapa</i>	41.5	Toria		
15				<i>B. rapa</i>	40	Toria		
16				<i>B. rapa</i>	38	Toria		
17				<i>B. rapa</i>	42	Toria		
18				<i>B. rapa</i>	40	Toria		
19				<i>B. rapa</i>	41.5	Toria		
20				<i>B. rapa</i>	36	Toria		
21				<i>B. rapa</i>	38	Toria		
22				<i>B. rapa</i>	41	Toria		
23				<i>B. rapa</i>	42	Toria		
24				<i>B. rapa</i>	38	Toria		
25				<i>B. rapa</i>	38	Toria		
26				<i>B. rapa</i>	42.5	Toria		
27				<i>B. rapa</i>	49	Sarson		
28				<i>B. rapa</i>	48.5	Toria		
29				<i>B. napus</i>	39	Candal		
30				<i>B. napus</i>	42	Regent		
31				<i>B. rapa</i>	57	NA		
32				<i>B. napus</i>	62	NA		
33				<i>B. napus</i>	45	NA		
Min.		32.5			36		39	
Mean		43			42.5		47	
Median		42			41.5		43.5	
Max.		55			62		62	

NA = not applicable

For apples (*Malus domestica* and *M. sp.*, total<sub>n</sub> = 10) the sugar content in nectar was between 32.9% and 55%, median 42%. For different species of *Brassica* (*B. napus*, *B. oleracea*, *B. rapa*, and *B. sp.* total<sub>n</sub> = 33), to which oil seed rape belongs (*B. napus*), the range was 36% to 62%, median 41.5%. For *B. napus* alone (n = 4) it ranged between 39 to 62% with a median of 43.5%.

## Conclusion

For apples (*Malus domestica* and *M. sp.*, total<sub>n</sub> = 10) the sugar content in nectar was between 32.9% and 55%, median 42%, for different species of *Brassica* (*B. napus*, *B. oleracea*, *B. rapa*, and *B. sp.* total<sub>n</sub> =

33) the range was 36% to 62%, median 41.5% and for *B. napus* alone (n = 4) it ranged between 39 to 62% with a median of 43.5%.

### 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. 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 LD<sub>50</sub> = 51.3 µg product/bee (corresponding to 9.1 µg a.s./bee) 48 h contact LD<sub>50</sub> = 21.2 µg product/bee (corresponding to 3.8 µg a.s./bee)</p>
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<b>Reference:</b>	KCP 10.3.1.1.1/01 & KCP 10.3.1.1.2/01
<b>Report</b>	Acute toxicity of MCW-2222 to the honeybee <i>Apis mellifera</i> L. under laboratory conditions, Franke, M., 2014, R-33834
<b>Guideline(s):</b>	OECD 213/214 (1998)
<b>Deviations:</b>	No
<b>GLP:</b>	Yes, certified laboratory
<b>Acceptability:</b>	Acceptable
<b>Duplication (if vertebrate study)</b>	Not applicable

### 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 LD<sub>50</sub>-values were determined. Based on the effective food consumption the 48 h LD<sub>50</sub> for contact 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 LD<sub>50</sub> 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).

### Materials and methods

#### Materials

<b>Test item</b>	MCW-2222
<b>Batch #</b>	611-280413-01
<b>Content of active substance</b>	Acetamiprid 200 g/L (nominal); 202.7 g/L (analysed)
<b>Description</b>	Yellowish liquid
<b>Control (contact)</b>	Deionised water
<b>Vehicle control (contact)</b>	Deionised water + 1.0 % v/v Tween®80
<b>Control (oral)</b>	50% (w/v) sucrose solution
<b>Toxic reference</b>	Dimethoate EC 400 (BAS 152 11 l)
<b>Test organism</b>	
<b>Species</b>	<i>Apis mellifera iberica</i> , adult worker bees
<b>Source</b>	Joaquin Cordero (Beekeeper), Paseo de Colón No. 19, 41370 Cazalla (Seville), Spain

## Study design and methods

<b>Test duration</b>	48 hours
<b>Experimental dates</b>	31 March to 02 April 2014
<b>Test doses (nominal)</b>	2.6, 5.7, 12.4, 27.3 and 60.0 µg test item/bee for both, contact and oral tests. 2.6, 5.6, 12.4, 27.3 and 55.2 µg test item/bee (oral test)
<b>Test doses (actually consumed)</b>	
<b>Test units</b>	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.
<b>Group size/replicates</b>	30 bees per dose; 10 in each of 3 replicates
<b>Experimental treatments (oral)</b>	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.
<b>Experimental treatments (contact)</b>	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.
<b>Acclimatisation</b>	The bees were transferred immediately after collection at the hive to the laboratory and acclimatised for 1 h.
<b>Environmental conditions</b>	
<b>Temperature</b>	23.4 - 27.0 °C
<b>Photoperiod</b>	Continuous darkness
<b>Relative humidity</b>	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 LD<sub>50</sub> 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 38: 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 [µg a.s./bee]	0.251	0.0	73.3	80.0
	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 39: 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 40: Endpoints for contact and oral toxicity after 48 hours**

Treatment	Reference unit of endpoint	Contact toxicity 48 hours	Oral toxicity <sup>a)</sup> 48 hours
Test item	LD <sub>50</sub> [µg test item/bee] (lower and upper 95 %-CL)	21.2 (16.8 – 26.7)	51.3 (40.6 – 65.0)
	LD <sub>50</sub> [µg a.s./bee] (lower and upper 95 %-CL)	3.8 (3.0 – 4.8)	9.1 (7.2 – 11.6)
Reference item	LD <sub>50</sub> [µg a.s./bee] (lower and upper 95 %-CL)	0.188 (0.169 – 0.210)	0.136 (0.116 – 0.158)

<sup>a)</sup>Values refer to consumed dosages

### Validity criteria

As shown in the following table, all validity criteria were met.

**Table A 41: Validity criteria**

Validity criteria according to OECD 213 oral (1998)	Observed in study
Mortality in control ≤ 10%	0%
The 24 h LD <sub>50</sub> 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 <sub>50</sub> 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 LD<sub>50</sub>-values were

determined.

The 48 h LD<sub>50</sub> 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 LD<sub>50</sub> 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 2.3.1.1.1.2 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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<b>Reference:</b>	KCP 10.3.1.1.1/01 & KCP 10.3.1.1.2/01 Please see A 2.3.1.1.1.1 for full summary
<b>Report</b>	Acute toxicity of MCW-2222 to the honeybee <i>Apis mellifera</i> L. under laboratory conditions, Franke, M., 2014, R-33834
<b>Guideline(s):</b>	OECD 213/214 (1998)
<b>Deviations:</b>	No
<b>GLP:</b>	Yes, certified laboratory
<b>Acceptability:</b>	Acceptable
<b>Duplication (if vertebrate study)</b>	Not applicable

#### A 2.3.1.1.1.3 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"> <li><u>Bumblebees in the contact test were not kept individually (3 replicates with 10 bumblebees were used).</u></li> </ol> <p>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.</p> <ol style="list-style-type: none"> <li><u>Bumblebees were not individually weighed.</u></li> </ol> <p>Lack of individual weighing is also not considered to have impact on the test results. 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> <ol style="list-style-type: none"> <li><u>Acclimatisation time of 3 hours was shorter than 8 hours recommended by the guidelines.</u></li> </ol> <p>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>
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	<p>4. <u>The feeding solutions used in oral test and chemical solutions used in contact test were not analysed during the test.</u></p> <p>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. <u>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).</u></p> <p>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 LD<sub>50</sub> = 136.0 µg product/bee (corresponding to 24.3 µg a.s./bee) 48 h contact LD<sub>50</sub> &gt;1122.0 µg product/bee (corresponding to &gt;200.0 µg a.s./bee)</p>
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<b>Reference:</b>	KCP 10.3.1.2.1/01 & KCP 10.3.1.2.2/01
<b>Report</b>	Acute toxicity of MCW-2222 to the bumblebee <i>Bombus terrestris</i> L. under laboratory conditions, Röhlig, U., 2014a, R-33837
<b>Guideline(s):</b>	OECD 213 (1998), OECD 214 (1998), EFSA (2013); 11(7):3295
<b>Deviations:</b>	<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>
<b>GLP:</b>	Yes, certified laboratory
<b>Acceptability:</b>	Acceptable
<b>Duplication (if vertebrate study)</b>	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 LD<sub>50</sub> values were determined. The LD<sub>50</sub> for contact exposure (48 h) was estimated to be > 200 µg a.s./bumblebee (corresponding to > 1122 µg test item/bumblebee). The LD<sub>50</sub> 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

<b>Test item</b>	MCW-2222
<b>Batch #</b>	611-280413-01



<b>Content of active substance</b>	Acetamiprid 200 g/L (nominal); 202.7 g/L (analysed)
<b>Description</b>	Yellowish liquid
<b>Control (contact)</b>	Deionised water
<b>Vehicle control (contact)</b>	Acetone 1.0 % v/v Tween® 80 solution
<b>Control (oral)</b>	50% (w/v) sucrose solution
<b>Toxic reference</b>	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.
<b>Test organism</b>	
<b>Species</b>	<i>Bombus terrestris</i> L., young adult worker bees
<b>Source</b>	Katz Biotech AG, An der Birkenpfuhlheide 10, 15837 Baruth, Germany
<b>Study design and methods</b>	
<b>Test duration</b>	48 hours
<b>Experimental dates</b>	05 March to 08 March 2014
<b>Test doses (contact; nominal)</b>	70, 140, 280, 561 and 1122 µg test item/bumblebee
<b>Test doses (oral; nominal)</b>	35, 70, 140, 281 and 561 µg test item/bumblebee
<b>Test doses (oral consumed)</b>	33, 68, 136, 267 and 532 µg test item/bumblebee
<b>Test units contact</b>	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.
<b>Test units oral</b>	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.
<b>Group size/replicates contact</b>	30 bees per dose; 10 in each of 3 replicates per dose level
<b>Group size/replicates oral</b>	30 bees per dose; 1 in each of 30 replicates per dose level
<b>Experimental treatments (oral)</b>	Oral treatment was done by administration of the test item dispersed in a 50% (w/v) sucrose solution.
<b>Experimental treatments (contact)</b>	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 <sub>2</sub> .
<b>Acclimatisation</b>	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.
<b>Environmental conditions</b>	
<b>Temperature</b>	24 - 27 °C
<b>Photoperiod</b>	Continuous darkness
<b>Relative humidity</b>	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 LD<sub>50</sub> values were calculated by probit analysis. Statistical significance was determined by Fishers' 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 42: 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
	20.0	0.0	100*	100*
Toxic reference [µg a.s./bee]	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 LD<sub>50</sub> (48 h) was calculated to be 24.3 µg a.s./bumblebee (corresponding to 136 µg test item/bumblebee).

**Table A 43: Cumulative bumblebee 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
	2.4	83.3*	86.7*
Toxic reference [µg a.s./bee]	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 44: Endpoints for contact and oral toxicity after 48 hours**

Treatment	Reference unit of endpoint	Contact toxicity 48 hours	Oral toxicity <sup>a)</sup> 48 hours
Test item	LD <sub>50</sub> [µg test item/bee] (lower and upper 95 %-CL)	> 1122	136 (103-180)
	LD <sub>50</sub> [µg a.s./bee] (lower and upper 95 %-CL)	> 200	24.3 (18.4 - 32.0)
Reference item	LD <sub>50</sub> [µg a.s./bee] (lower and upper 95 %-CL)	5.14 (4.44 – 5.95)	0.54 (0.30 – 0.98)

<sup>a)</sup> Values refer to consumed dosages

### Validity criteria

As shown in the following table, all validity criteria were met.

**Table A 45: Validity criteria**

Validity criteria according to OECD 246 oral (2017)	Observed in study
Mortality in control $\leq 10\%$	0%
Mortality in the toxic reference substance group should be $\geq 50\%$ 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 $\leq 10\%$	0%
Mortality in the toxic reference substance group should be $\geq 50\%$ 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 LD<sub>50</sub>-values were determined. The LD<sub>50</sub> for contact exposure (48 h) was estimated to be  $> 200 \mu\text{g a.s./bumblebee}$  (corresponding to  $> 1122 \mu\text{g test item/bumblebee}$ ). The LD<sub>50</sub> for oral exposure (48 h) was calculated to be  $24.3 \mu\text{g a.s./bumblebee}$  (corresponding to  $136 \mu\text{g test item/bumblebee}$ ).

### A 2.3.1.1.1.4 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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<b>Reference:</b>	KCP 10.3.1.2.1/01 & KCP 10.3.1.2.2/01 Please see A 2.3.1.1.1.3 for full summary
<b>Report</b>	Acute toxicity of MCW-2222 to the bumblebee <i>Bombus terrestris</i> L. under laboratory conditions, Röhlig, U., 2014, R-33837
<b>Guideline(s):</b>	OECD 213 (1998), OECD 214 (1998), EFSA (2013); 11(7):3295
<b>Deviations:</b>	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.
<b>GLP:</b>	Yes, certified laboratory
<b>Acceptability:</b>	Acceptable
<b>Duplication (if vertebrate study)</b>	Not applicable

### A 2.3.1.2 KCP 10.3.1.2/01 Chronic toxicity to bees

Comments of zRMS:	<p>The study on chronic toxicity of CA3573 to bees (Dressler, 2019) has been submitted in support of the re-evaluation of CA3573 due to renewal of acetamiprid and was not evaluated earlier. The validation of the study was thus performed by the zRMS.</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>LDD<sub>50</sub> = <math>21.8 \mu\text{g product/bee/day}</math> (corresponding to <math>3.71 \mu\text{g a.s./bee/day}</math>) NOEDD = <math>9.04 \mu\text{g product/bee/day}</math> (corresponding to <math>1.54 \mu\text{g a.s./bee/day}</math>)</p>
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<b>Reference:</b>	KCP 10.3.1.2/01
<b>Report</b>	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
<b>Guideline(s):</b>	OECD 245 (2017)
<b>Deviations:</b>	None
<b>GLP:</b>	Yes, certified laboratory
<b>Acceptability:</b>	Acceptable

## 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 LDD<sub>50</sub> was determined to be 21.8 µg test item/bee/day (equivalent to 3.71 µg a.s./bee/day) and the LC<sub>50</sub> 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 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

<b>Test item</b>	CA3573 Acetamiprid 200 SL (Carnadine)
<b>Batch #</b>	981101035
<b>Content of active substance</b>	Acetamiprid 200 g/L (nominal); 195.5 g/L (analysed)
<b>Description</b>	Clear yellow-brown liquid
<b>Control</b>	Untreated diet
<b>Toxic reference</b>	Dimethoate technical 400 g/L (nominal) 429 g/L (analysed)
<b>Test organism</b>	
<b>Species</b>	<i>Apis mellifera</i> subspecies Buckfast (max 2 days old)
<b>Source</b>	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

<b>Test duration and exposure</b>	10 days with continuous exposure via food (spiked sucrose solution)
<b>Experimental dates</b>	25 June – 05 July 2019
<b>Test doses test item</b>	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
<b>Test doses reference item</b>	Nominal dosing: 0.0273 µg a.s./bee/day Effective dosing (based on actual daily intake): 0.0154 µg a.s./bee/day
<b>Test units</b>	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

<b>Group size/replicates contact</b>	(one in front and one in the back) for observation of the bees 30 bees per treatment (1 control, 5 test item dosages, 1 reference treatment); 10 bees in each of 3 replicates per treatment
<b>Acclimatisation</b>	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 $33 \pm 2$ °C and relative humidity of $70 \pm 10$ % 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 $33 \pm 2$ °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.
<b>Environmental conditions</b>	
<b>Temperature:</b>	31.6 – 33.6 °C
<b>Photoperiod:</b>	Darkness (except assessments)
<b>Relative humidity</b>	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  $24 \pm 2$  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 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 46: 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 47: 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		
	nominal	effective	nominal	effective	[mg test item/kg food]	[mg a.s./kg food]	Mean mortality [%]		Bees showing behav. abnormalities**
							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
Reference item	[ng ref. item/bee/day]		[ng a.s./ bee/day]		[mg ref item/ kg food]	[mg a.s./ kg food]	-	-	-
	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 48: Endpoints**

Treatment	Endpoints	Day 10
Test item doses*	LDD <sub>50</sub> [µg test item/bee/day] <sup>1</sup>	21.8 (18.5 – 25.8)
	LDD <sub>50</sub> [µg a.s./bee/day] <sup>1</sup>	3.71 (3.15 – 4.40)
	NOEDD [µg test item/bee/day] <sup>2</sup>	9.04
	NOEDD [µg a.s./bee/day] <sup>2</sup>	1.54
Test item concentrations	LC <sub>50</sub> [mg test item/kg food] <sup>1</sup>	700 (601 – 821)
	LC <sub>50</sub> [mg a.s./kg food] <sup>1</sup>	119 (102 – 140)
	NOEC [mg test item/kg food] <sup>2</sup>	303
	NOEC [mg a.s./kg food] <sup>2</sup>	51.4

<sup>1</sup> Median lethal dietary doses/concentrations (95%-CI lower-upper) were calculated using Probit analysis (linear max. likelihood regression)

<sup>2</sup> 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 49: Validity criteria**

Validity criteria according to OECD 245 (2017)	Observed in study
Mortality in control ≤ 15%	3.3%
Mortality in the toxic reference substance group should be ≥ 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 LDD<sub>50</sub> was determined to be 21.8 µg test item/bee/day (equivalent to 3.71 µg a.s./bee/day) and the LC<sub>50</sub> 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 2.3.1.3 KCP 10.3.1.2/02 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"> <li><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).</li> <li><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.</li> <li><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.</li> </ol> <p>Following deviations are considered to have potentially significant impact on the test results:</p> <ol style="list-style-type: none"> <li><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.</li> <li><u>Evaporation of the test solution from feeders was not determined.</u> 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</li> </ol>
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	<p>(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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<b>Reference:</b>	KCP 10.3.1.2/02
<b>Report</b>	Chronic toxicity of MCW-2222 to honeybee ( <i>Apis mellifera</i> L.) under laboratory conditions, Kleebaum, K., 2014a; R-33835
<b>Guideline(s):</b>	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)
<b>Deviations:</b>	<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"> <li>Maximum age of workers bees was 3 days instead of 2 days</li> <li>Replicates contained 20 bees instead of 10 bees</li> <li>Evaporation of test solution from feeders was not determined</li> <li>No analytical verification of the test substance was conducted</li> </ul> <p>Minor deviation:</p> <ul style="list-style-type: none"> <li>Relative humidity during exposure was 46.2 – 60.0 % instead of 50 – 70 %</li> </ul>
<b>GLP:</b>	Yes, certified laboratory
<b>Acceptability:</b>	No longer valid, superseded by study presented under KCP 10.3.1.2/01
<b>Duplication (if vertebrate study)</b>	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 LD<sub>50</sub> was determined to be 3.994 µg consumed a.s./bee/day. This corresponds to a LC<sub>50</sub> 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)



<b>Source</b>	Joaquin Cordero (Beekeeper), Paseo de Colón No. 19, 41370 Cazalla (Seville), Spain
<b>Study design and methods</b>	
<b>Test duration</b>	10 days of exposure
<b>Experimental dates</b>	09 May to 30 June 2014
<b>Test doses</b>	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)
<b>Test units</b>	Aluminum 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.
<b>Group size/replicates contact</b>	60 bees per treatment (1 control, 5 test item dosages, 1 reference item); 20 in each of 3 replicates per treatment
<b>Acclimatisation</b>	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 $33 \pm 2$ °C and relative humidity of $70 \pm 10$ % 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 $33 \pm 2$ °C and $50 \pm 10$ % RH and provided with sugar solution and pollen food. Moribund and dead bees were rejected and replaced by healthy bees before starting the test.
<b>Environmental conditions</b>	
<b>Temperature:</b>	33.3–35.0 °C
<b>Photoperiod:</b>	Darkness (except assessments)
<b>Relative humidity</b>	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<sub>50</sub> 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 50: Mean mortality and behaviour of bees in the chronic toxicity feeding test with MCW-2222 after 10 days**

Treatment group	Dosage of a.s. [ $\mu\text{g}/\text{bee}/\text{day}$ ]		Concentration [g a.s./ kg food]	D10		
				Mean mortality [%] <sup>1</sup>		Mean BA [%]
	nominal	consumed		Absolute	Corrected	
Control	-	-	-	1.7	-	0.0
Test substance [ $\mu\text{g}$ 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 [ $\mu\text{g}$ 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

<sup>1</sup>) 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 51: Endpoints after 96 and 120 hours of exposure**

Treatment	Endpoints	Day 10
Test item doses	LD <sub>50</sub> [ $\mu\text{g}$ consumed a.s./bee/day] <sup>1</sup>	3.994
	NOED [ $\mu\text{g}$ consumed a.s./bee/day] <sup>2</sup>	0.546
Test item concentrations	LC <sub>50</sub> [g a.s./kg food] <sup>2</sup>	0.100
	NOEC [g a.s./kg food] <sup>3</sup>	0.012
Reference item	LD <sub>50</sub> [ $\mu\text{g}$ consumed a.s./bee/day]	12.661
	LC <sub>50</sub> [mg a.s./kg food]	0.423

<sup>1</sup> Median lethal dose was calculated by using Probit analysis (linear max. likelihood regression)

<sup>2</sup> Median lethal concentration was calculated by using Weibull analysis (linear max. likelihood regression)

<sup>3</sup> 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 52: 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 LD<sub>50</sub> was determined to be 3.994  $\mu\text{g}$  consumed a.s./bee/day. This corresponds to a LC<sub>50</sub> of 0.100 g a.s./kg food. The NOED was determined to be 0.546  $\mu\text{g}$  consumed a.s./bee/day, and the NOEC was 0.012 g 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 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 not evaluated earlier. The validation of the study was thus performed by the zRMS.</p> <p>The study was carried out in line with OECD 239 with following deviations:</p> <ol style="list-style-type: none"> <li>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,</li> </ol>
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	<p>slightly higher amount of water).</p> <p>2. On D8 bees at pre-pupal stage were transferred to new culture plates.</p> <p>3. Culture plates were covered with lids throughout development between D8 and D15.</p> <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>ED<sub>10</sub> &gt;2.861 µg product/larvae/developmental period (corresponding to &gt;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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<b>Reference:</b>	KCP 10.3.1.3/01
<b>Report</b>	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
<b>Guideline(s):</b>	Guidance Document on Honey Bee Larval Toxicity Test following Repeated Exposure, Series on Testing and Assessment, No. 239, OECD (2016)
<b>Deviations:</b>	<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"> <li>• diet composition (more water and less royal jelly in diet A),</li> <li>• a pre-pupal transfer step to a new culture plate on D8,</li> <li>• changes to the rearing environment (a lid placed upon the culture plates throughout development between D8 and D15)</li> </ul>
<b>GLP:</b>	Yes, certified laboratory
<b>Acceptability:</b>	Acceptable
<b>Duplication (if vertebrate study)</b>	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  $> 0.486 \mu\text{g a.i./larva}$  ( $> 2.861 \mu\text{g product/larva}$ ) which is equivalent to an  $EC_{50/20/10}$  of  $> 3.075 \text{ mg a.i./kg food}$  ( $> 18.087 \text{ mg product/kg food}$ ). The NOED was determined to be  $\geq 0.485 \mu\text{g a.i./larva}$  ( $\geq 2.861 \mu\text{g product/larva}$ ) which is equivalent to a NOEC of  $\geq 3.075 \text{ mg a.i./kg food}$  ( $\geq 18.087 \text{ mg product/kg food}$ )

## Materials and methods

### Materials

<b>Test item</b>	CA3573 Acetamiprid 200 SL (Carnadine))
<b>Batch #</b>	981101035
<b>Content of active substance</b>	200 g/L acetamiprid (nominal), 195.5 g/L (analysed)
<b>Density</b>	1.15 g/mL
<b>Description</b>	Clear yellow-brown liquid
<b>Control</b>	Untreated diet A, B and C (see below for details on diet)
<b>Toxic reference</b>	Dimethoate technical, $98.8 \pm 0.5 \%$
<b>Test organism</b>	
<b>Species</b>	Honey bee ( <i>Apis mellifera</i> , hybrid line Buckfast), 3-day old worker larvae at test start
<b>Source</b>	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.
<b>Food/feeding</b>	<p>Three different diets, adapted to the needs of the larvae at different stages of development:</p> <ul style="list-style-type: none"> <li>- 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</li> <li>- 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</li> <li>- 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.</li> </ul> <p>The treated diets (prepared daily), were warmed in an incubator before use. Feeding volumes using a sterile pipette:</p> <p>D1 &amp; D3: 20 <math>\mu\text{L}</math>/larva (no diet administered on D2)  D4: 30 <math>\mu\text{L}</math>/larva  D5: 40 <math>\mu\text{L}</math>/larva  D6: 50 <math>\mu\text{L}</math>/larva</p> <p>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.</p>
<b><u>Test design</u></b>	
<b>Test duration and exposure</b>	<p>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</p>
<b>Experimental dates</b>	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 Chi<sup>2</sup> 2x2 Table Test with Bonferroni Correction was used. The accepted significance level was alpha = 0.05 (one-sided greater). Prior to the Chi<sup>2</sup> 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<sub>x</sub>/EC<sub>x</sub> 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 53: 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 54: 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"

OO: Other observations (remaining food, small body size)

\* result significantly different to control (Step-down Cochran-Armitage tests)

Calculations were performed with non-rounded values.

**Table A 55: Endpoints on D22**

Endpoint	[product]	[a.s.]
Dose [µg/larva]		
ED <sub>10</sub>	> 2.861	> 0.486
ED <sub>20</sub>	> 2.861	> 0.486
ED <sub>50</sub>	> 2.861	> 0.486
NOED	≥ 2.861	≥ 0.486
Concentration [mg/kg feeding solution]		
EC <sub>10</sub>	> 18.087	> 3.075
EC <sub>20</sub>	> 18.087	> 3.075
EC <sub>50</sub>	> 18.087	> 3.075
NOEC	≥ 18.087	≥ 3.075

### Validity criteria

As shown in the following table, all validity criteria were met.

**Table A 56: 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 ED<sub>50</sub> was determined to be > 0.486 µg a.s./larva, corresponding to an EC<sub>50</sub> of > 3.075 mg a.s./kg. The NOED was determined to be ≥ 0.486 µg a.s./larva, corresponding to a NOEC of ≥ 3.075 mg a.s./kg. The analytical part proved correct dosing.

### 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 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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<b>Reference:</b>	KCP 10.3.1.3/02
<b>Report</b>	Chronic toxicity of MCW-2222 to honeybee larvae ( <i>Apis mellifera</i> L.) under laboratory conditions ( <i>in vitro</i> ), Kleebaum, K., 2014, R-33836
<b>Guideline(s):</b>	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)
<b>Deviations:</b>	<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"> <li>No data on pupation or emergence were recorded</li> <li>The test duration was not 22 days but only 8 days</li> </ul>
<b>GLP:</b>	Yes, certified laboratory
<b>Acceptability:</b>	No longer suitable for the risk assessment purposes, superseded by study presented under KCP 10.3.1.3/01
<b>Duplication (if vertebrate study)</b>	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 LD<sub>50</sub> (96 h) was determined to be 21.1 µg a.s./larva, which is equivalent to a LC<sub>50</sub> (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 LD<sub>50</sub> (120 h) was determined to be 10.2 µg a.s./larva, which is equivalent to a LC<sub>50</sub> (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



<b>Batch #</b>	611-280413-01
<b>Content of active substance</b>	Acetamiprid 200 g/L (nominal); 202.7 g/L (analysed)
<b>Description</b>	Yellowish liquid
<b>Control</b>	Untreated diet
<b>Toxic reference</b>	Dimethoate technical (99.8%)
<b>Test organism</b>	
<b>Species</b>	<i>Apis mellifera iberica</i> , first instar larvae
<b>Source</b>	Joaquín Cordero (Beekeeper), Paseo de Colón No. 19, 41370 Cazalla (Seville), Spain
<b>Study design and methods</b>	
<b>Test duration</b>	11 days; 120 hours of exposure
<b>Experimental dates</b>	16 to 23 Jun 2014
<b>Test doses</b>	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
<b>Test units</b>	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.
<b>Group size/replicates/contact</b>	36 bees per treatment (control/test item/reference); 12 in each of 3 replicates per treatment
<b>Environmental conditions</b>	
<b>Temperature:</b>	35.2 °C – 35.8 °C
<b>Photoperiod:</b>	Continuous darkness (except assessments)
<b>Relative humidity</b>	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/LD<sub>50</sub> 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 57:** 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 [%] <sup>1</sup>		Mean mortality [%] <sup>1</sup>	
			Absolute	Corrected	Absolute	Corrected
Control	-	-	11.1	0.0	11.1	0.0
Test substance <sup>+</sup> [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 <sup>+</sup> [µg a.s./bee]	6.2	0.039	55.6*	50.0	61.1*	56.3

<sup>1</sup> 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;  $\alpha=0.05$ ; one sided greater)

**Table A 58:** Endpoints after 96 and 120 hours of exposure

Treatment	Endpoints	D7 (96 h after 1 <sup>st</sup> application)	D8 (120 h after 1 <sup>st</sup> application)
Test item doses	LD50 [µg a.s./larva]	21.1	10.2
	(95 % CL / lower-upper)	(12.0—36.9)	(6.0—17.3)
	NOED [µg a.s./larva]	3.8	3.8
Test item concentrations	LC50 [g a.s./kg food]	0.117	0.060
	(95 % CL / lower-upper)	(0.070—0.196)	(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 59:** Validity criteria

Validity criteria according to OECD 237 (2013)	Observed in study
Mortality in control $\leq 15\%$	11%
Mortality in the toxic reference substance group should be $\geq 50\%$ on D7.	55.6%

## Conclusion

In a chronic larval toxicity study with MCW 2222, the LD<sub>50</sub> (96 h) was determined to be 21.1 µg a.s./larva, which is equivalent to a LC<sub>50</sub> (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 LD<sub>50</sub> (120 h) was determined to be 10.2 µg a.s./larva, which is equivalent to a LC<sub>50</sub> (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 2.3.1.6 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 test with honeybees on cereals**

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>However, the relevance of the study for the intended use pattern of CA3573 as well as possibility of the trial to detect long-term effects were specifically considered by the zRMS for purposes of the evaluation of CA3573 following acetamiprid renewal.</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"> <li>– during bee foraging activity: water control, MCW-2222 at 100 g a.s./ha and the reference dimethoate,</li> <li>– out of the presence of forager bees: MCW-2222 at 100 g a.s./ha at night.</li> </ul> <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> <p>It is also noted that the study was performed on winter wheat, while cereals are currently not included in the GAP table for CA3573. Taking this into account, the study is not relevant for purposes of the risk assessment for CA3573 following acetamiprid renewal.</p>
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<b>Reference:</b>	KCP 10.3.1.5/01
<b>Report</b>	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 cereal crop. Mamet, O. & Molitor, C., 2015, R-34874
<b>Guideline(s):</b>	C.E.B methodology n°230, part IV
<b>Deviations:</b>	<p>Yes, minor deviations:</p> <p>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</p> <p>This minor deviation did not have an impact on the reliability and the outcome of the study.</p>
<b>GLP:</b>	Yes, certified laboratory
<b>Acceptability:</b>	Acceptable to detect acute effects; suitability for risk assessment discussed in point 9.6 of this document
<b>Duplication (if vertebrate study)</b>	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 m<sup>2</sup>, 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	

<b>Species</b>	<p>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.</p> <p>All colonies at the beginning of the study</p> <ul style="list-style-type: none"> <li>- with at 2 to 4 frames containing all brood stages</li> <li>- with 0 to 2 storage frames</li> <li>- with 0 to 2 empty frames</li> <li>- were free of clear clinical symptoms of disease; no treatment against <i>Varroa</i> for at least 4 weeks before beginning of the study.</li> </ul>
<b>Source</b>	local beekeeper, GAEC Mélibocage
<b>Food/feeding</b>	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).
<b>Study design and methods</b>	
<b>Test duration</b>	<p>Pre-exposure phase (D -10 to D0) within the tunnels: 10 days</p> <p>Exposure phase (D 0 to D+7) within the tunnels: 7 days</p>
<b>Experimental dates</b>	18 <sup>th</sup> May to 5 <sup>th</sup> June 2014
<b>Test doses</b>	<p><b>Test item</b></p> <p>T1 (during bee flight): 100 g a.s./ha</p> <p>T2 (after bee flight): 100 g a.s./ha</p> <p><b>Toxic reference</b></p> <p>R (during bee flight): 400 g a.s./ha</p>
<b>Test units</b>	<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 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.</p> <p>All actual treatment rates were within <math>\pm 5\%</math> from the target application rate.</p> <p>Tunnels with an area of 140 m<sup>2</sup>, containing 64 m<sup>2</sup> (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.</p>
<b>Endpoints and assessments</b>	<p><i>mortality of bees:</i></p> <p>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</p> <p><i>foraging activity:</i></p> <p>D -7 to D+6, on the entire 4 plots/tunnel (4 x 16 m<sup>2</sup> 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"> <li>- immediately before application</li> <li>- 30 minutes after application, followed by three other assessments</li> </ul> <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 -9) and once at the end (D+8) of the study; assessment of:</p> <ul style="list-style-type: none"> <li>- estimated number of bees (colony strength)</li> <li>- number of cells containing brood (total of cells with eggs, larvae and capped brood)</li> <li>- presence of queens (e.g. presence of eggs)</li> <li>- number of storage frames.</li> </ul>
<b>Group size/replicates contact</b>	Three tunnels per treatment group

## Adaptation of bees

Colonies were set-up in the tunnel on 10 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 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 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.

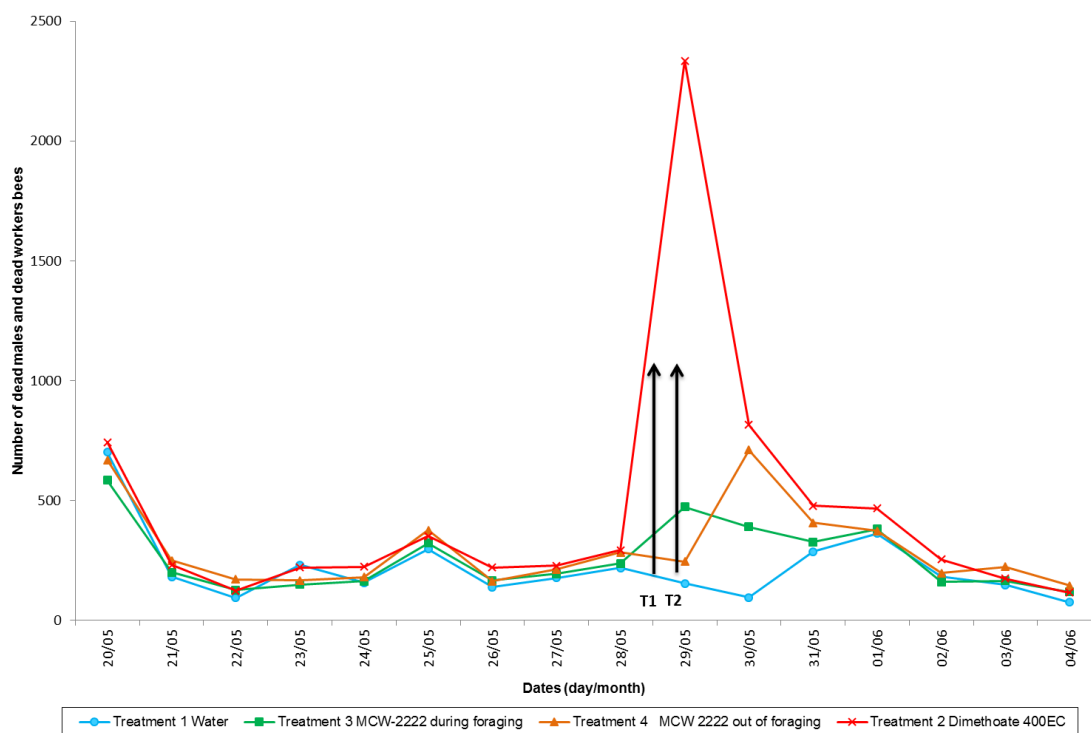
MCW-2222 applied during the bee flight (T1) showed a slight increase of the average mortality at D+1 (from 237 dead bees at D0 to 474 the day after application) and at D+2 (390 dead bees). Nevertheless, the mortality increase observed at D+1 and D+2 was much lower than the one recorded in the toxic reference 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 2: Total daily mortality**



**Table A 60: 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 61: Relative toxicity index**

Treatments	Time after Treatment	I tox Value*	
		I tox <sub>1</sub> (D+1 versus D0) During foraging	I tox <sub>2</sub> (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 60 and Figure A 2). 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/m<sup>2</sup> on 26/05 at 14h15.

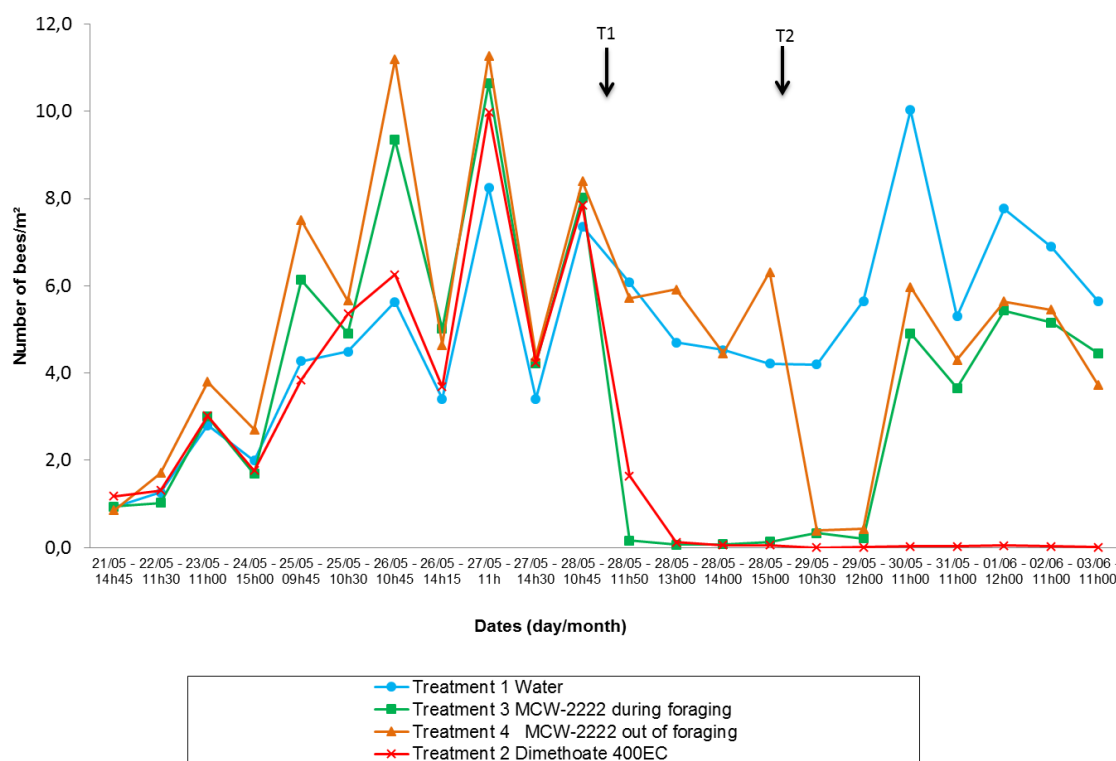
On the day of application, foraging activity was high (from 5 to 11 bees/m<sup>2</sup>) and always superior to the required level (3 bees/m<sup>2</sup>).

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/m<sup>2</sup> and was superior to the required level of 3 bees/m<sup>2</sup>.



**Figure A 3: Foraging activity - Average number of bees/m<sup>2</sup>**

**Table A 62: Foraging activity - Average number of bees/m<sup>2</sup>**

Dates (day/month-hours) x = delay from application day	Average number of bees/m <sup>2</sup>			
	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 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

### *Behaviour*

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 63: Validity criteria**

Validity criteria according to CEB 230 (2003), part IV	Observed in study
<b>Before treatment:</b>	
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 <sup>2</sup> on flowering plants and three bees / m <sup>2</sup> on wheat shortly before application	C: 7.4 bees/m <sup>2</sup> T1: 8.0 bees/m <sup>2</sup> T2: 8.4 bees/m <sup>2</sup> , assessed during bee flight 0 bees/m <sup>2</sup> , assessed after bee flight R: 7.8 bees/m <sup>2</sup>
Foraging activity in different tunnels must be comparable.	Achieved
<b>After treatment:</b>	
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 m<sup>2</sup>, 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/m<sup>2</sup> 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 test with honeybees on wheat

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>However, the relevance of the study for the intended use pattern of CA3573 as well as possibility of the trial to detect long-term effects were specifically considered by the zRMS for purposes of the evaluation of CA3573 following acetamiprid renewal.</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"> <li>– during bee foraging activity: water control, MCW-2222 at 100 g a.s./ha and the reference dimethoate,</li> <li>– out of the presence of forager bees: MCW-2222 at 100 g a.s./ha at night.</li> </ul> <p>In these experimental conditions, MCW-2222 applied in bee presence triggered a statistically significant effect on daily mortality from D+1 to D+3. When applied out of the bee presence, 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 observed in control 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. No signs of behavioral abnormalities were recorded after D+2 in the tunnels treated with MCW-2222. The foraging activity was reduced after the application of MCW-2222 during the foraging activity of honeybees (from D0+ to D+3) with a statistically significant difference from the control. When applied out of the bee presence, the foraging activity was significantly reduced at D+1 and D+2. From D+4 the trend was similar to control with lower values until the end of the trial. 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 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> <p>It is also noted that the study was performed on winter wheat, while cereals are currently not included in the GAP table for CA3573. Taking this into account, the study is not relevant for purposes of the risk assessment for CA3573 following acetamiprid renewal.</p>
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<b>Reference:</b>	KCP 10.3.1.5/02
<b>Report</b>	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., 2015, R-35845
<b>Guideline(s):</b>	C.E.B methodology n°230, part IV
<b>Deviations:</b>	<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</p>

	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
	This minor deviation did not have an impact on the reliability and the outcome of the study.
<b>GLP:</b>	Yes, certified laboratory
<b>Acceptability:</b>	Acceptable to detect acute effects; suitability for risk assessment discussed in point 9.6 of this document
<b>Duplication (if vertebrate study)</b>	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 m<sup>2</sup>, 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

<b>Test item</b>	MCW-2222
<b>Batch #</b>	659-030314-01
<b>Content of active substance</b>	Acetamiprid 200 g/L (nominal); 199.2 ± 1.3 g/L (analysed)
<b>Description</b>	Yellowish liquid
<b>Control</b>	C: Water treated crop, applied during foraging activity
<b>Toxic reference</b>	R: Rogor Plus (400 g a.s./ha dimethoate), applied during the foraging activity
<b>Test organism</b>	
<b>Species</b>	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.
<b>Source</b>	local beekeeper, Apistory
<b>Food/feeding</b>	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

<b>Test duration</b>	Pre-exposure phase (D -6 to D0) within the tunnels: 6 days Exposure phase (D 0 to D+7) within the tunnels: 7 days
<b>Experimental dates</b>	16 <sup>th</sup> May to 29 <sup>th</sup> May 2015
<b>Test doses</b>	<b>Test item</b> T1 (during bee flight): 100 g a.s./ha T2 (after bee flight): 100 g a.s./ha  <b>Toxic reference</b> 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.
<b>Test units</b>	Tunnels with an area of 140 m <sup>2</sup> , containing 64 m <sup>2</sup> (subdivided in 4 plots and separated and surrounded by plastic lanes) of flowering winter wheat (variety: Euclide), each with one colony; tunnels equipped with a water and pollen supply.
<b>Endpoints and assessments</b>	<i>mortality of bees:</i> 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  <i>foraging activity:</i> D -4 to D+7, on the entire 4 plots/tunnel (4 x 16 m <sup>2</sup> 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  
Adaptation of bees**

Three tunnels per treatment group  
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
<b>Temperature:</b>	16 °C	15 °C	2 to 21 °C
<b>Wind speed:</b>	3 km/h	0 km/h	not measure
<b>Rel. humidity:</b>	50%	60%	not reported
<b>Precipitation:</b>	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 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.

$$Itox = (Mt \times Ta) / (Ma \times Tt)$$

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.

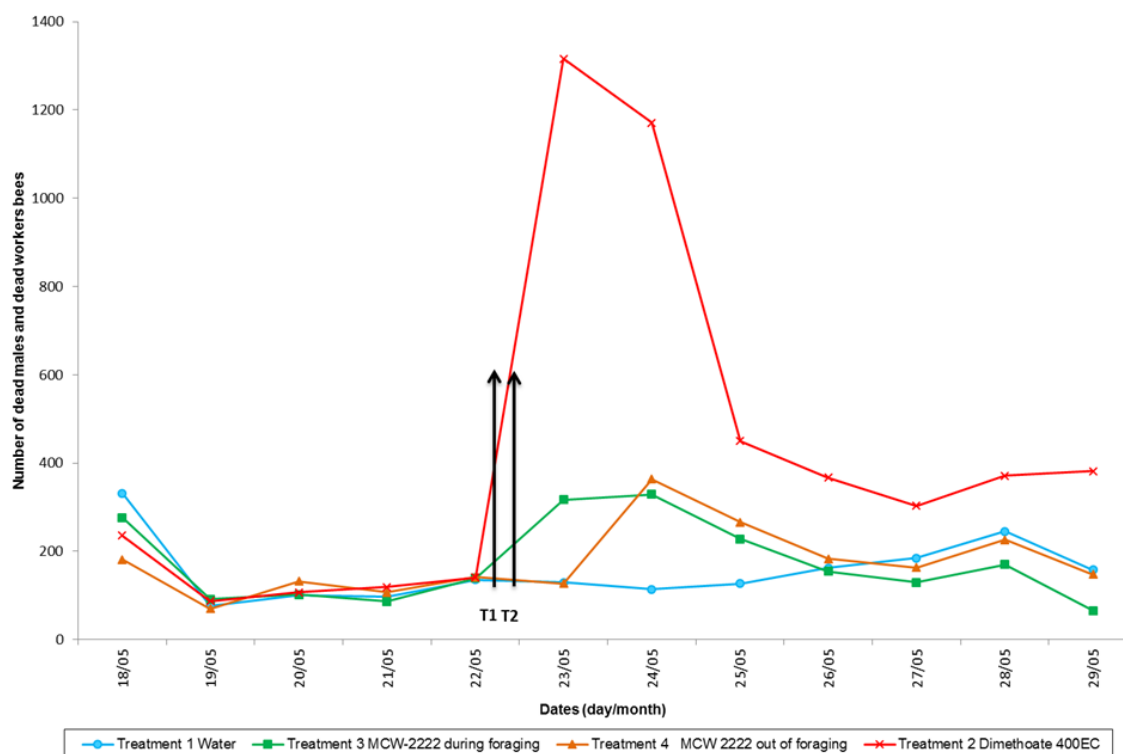
MCW-2222 applied during bee flight (T1) showed a statistically significant higher average mortality from D+1 to D+3 compared to that of the control. Nevertheless, this level of mortality observed during this period was much lower than the one recorded in the toxic reference treatment (317 versus 1316 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 4). 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 4:** Total daily mortality

**Table A 64:** 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 65: Relative toxicity index**

Treatments	Time after Treatment	I tox Value*	
		I tox <sub>1</sub> (D+1 versus D0) During foraging	I tox <sub>2</sub> (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 value = (Mt x Ta) / (Ma x Tt)

### *Foraging activity*

On the day of application, the bee activity was high (from 5.2 to 7.2 bees/m<sup>2</sup>) and always superior to the required level (3 bees/m<sup>2</sup>).

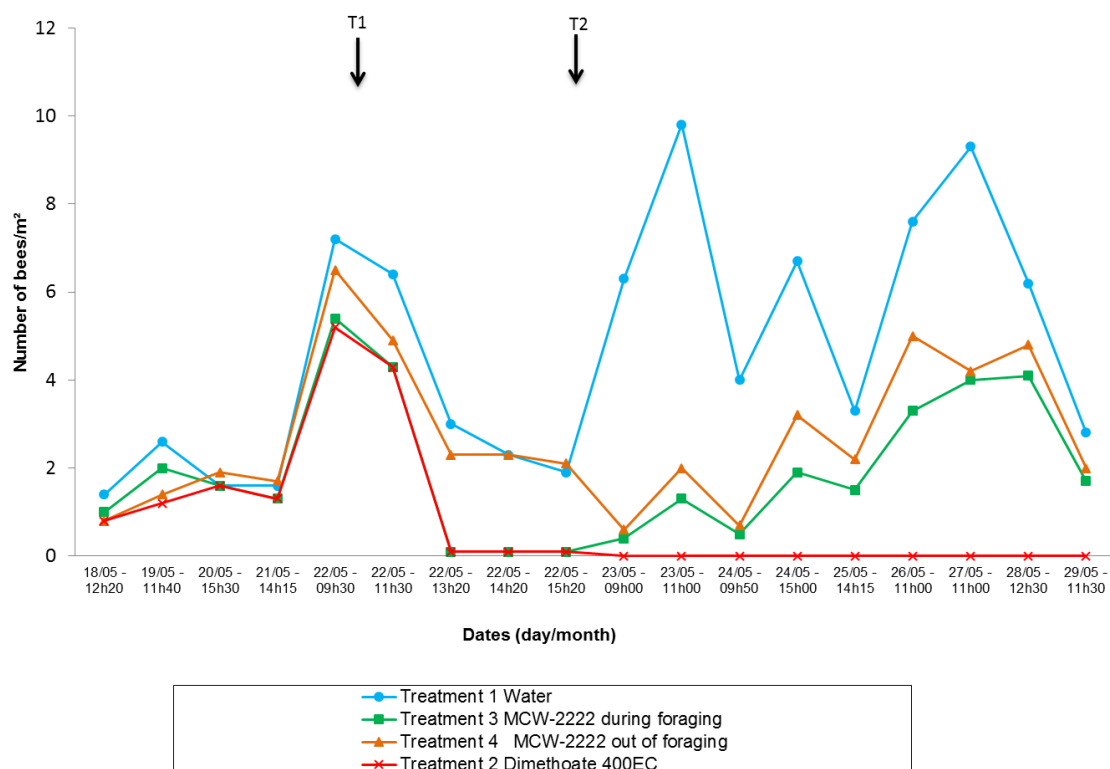
The foraging activity in the control tunnels declined after the application T1 from 6.8 up to 1.9 bees/m<sup>2</sup> in average. This activity was higher the following days and the number of bees/m<sup>2</sup> was above 3 up to 9.8 bees/m<sup>2</sup>.

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/me<sup>2</sup> 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/m<sup>2</sup>). 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 5: Foraging activity - Average number of bees/m²**

**Table A 66: Foraging activity - average number of bees/m²**

Dates	Average number of bees/m²			
(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-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 67: Validity criteria**

Validity criteria according to CEB 230 (2012), part IV	Observed in study
<b>Before treatment:</b>	
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 <sup>2</sup> on flowering plants and three bees / m <sup>2</sup> on wheat shortly before application	C: 6.8 bees/m <sup>2</sup> T1: 4.8 bees/m <sup>2</sup> T2: 5.7 bees/m <sup>2</sup> , assessed during bee flight 0 bees/m <sup>2</sup> , assessed after bee flight R: 4.8 bees/m <sup>2</sup>
Foraging activity in different tunnels must be comparable.	Achieved
<b>After treatment:</b>	
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 m<sup>2</sup>, 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/m<sup>2</sup> 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 test with honeybees on wheat

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"> <li>– during bee foraging activity: water control, MCW-2222 at 100 g a.s./ha and the reference dimethoate,</li> <li>– out of the presence of forager bees: MCW-2222 at 100 g a.s./ha at night.</li> </ul> <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.</p>
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	<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 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> <p>It is also noted that the study was performed on winter wheat, while cereals are currently not included in the GAP table for CA3573. Taking this into account, the study is not relevant for purposes of the risk assessment for CA3573 following acetamiprid renewal.</p>
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<b>Reference:</b>	KCP 10.3.1.5/03
<b>Report</b>	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., 2015, R-35846
<b>Guideline(s):</b>	C.E.B methodology n°230, part IV
<b>Deviations:</b>	None
<b>GLP:</b>	Yes, certified laboratory
<b>Acceptability:</b>	Acceptable to detect acute effects; suitability for risk assessment discussed in point 9.6 of this document
<b>Duplication (if vertebrate study)</b>	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 m<sup>2</sup>, 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

<b>Test item</b>	MCW-2222
<b>Batch #</b>	659-030314-01
<b>Content of active substance</b>	Acetamiprid 200 g/L (nominal); 199.2 ± 1.3 g/L (analysed)
<b>Description</b>	Yellowish liquid
<b>Control</b>	C: Water treated crop, applied during foraging activity
<b>Toxic reference</b>	R: Rogor Plus (400 g a.s./ha dimethoate), applied during the foraging activity
<b>Test organism</b>	
<b>Species</b>	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.
<b>Source</b>	local beekeeper, Apistory
<b>Food/feeding</b>	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

<b>Test duration</b>	Pre-exposure phase (D -8 to D0) within the tunnels: 8 days Exposure phase (D 0 to D+7) within the tunnels: 7 days
<b>Experimental dates</b>	9 <sup>th</sup> June to 25 <sup>th</sup> June 2015
<b>Test doses</b>	<b>Test item</b> 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.

Tunnels with an area of 140 m<sup>2</sup>, containing 64 m<sup>2</sup> (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.

### Test units

### 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+7, on the entire 4 plots/tunnel (4 x 16 m<sup>2</sup> 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+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 Adaptation of bees

Three tunnels per treatment group  
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 16<sup>th</sup> (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 18<sup>th</sup>. 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
<b>Temperature:</b>	21 °C	18 °C	9 to 27 °C
<b>Wind speed:</b>	1 to 2 km/h	0 km/h	not measured
<b>Rel. humidity:</b>	68 %	70 %	not reported
<b>Precipitation:</b>	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  $\text{Log}(x+1)$  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 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.

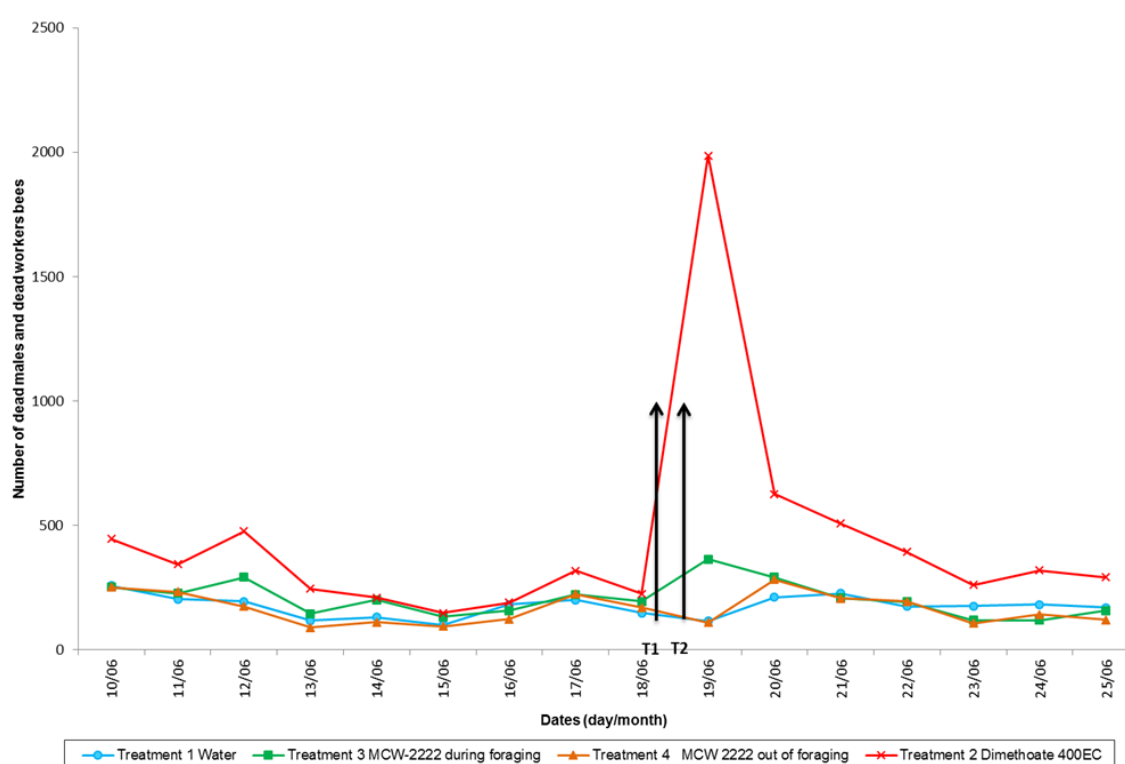
MCW-2222 after bee flight (T2) showed a slight increase of the mortality only at D+2. Compared to the control there was no statically significant difference at all the assessment timings.

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 68)** 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 6:** Total daily mortality

**Table A 68: 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
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 69: Relative toxicity index**

Treatments	Time after Treatment	I tox Value*	
		I tox <sub>1</sub> (D+1 versus D0) During foraging	I tox <sub>2</sub> (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/m<sup>2</sup> in average) and always superior to the required level (3 bees/m<sup>2</sup>).

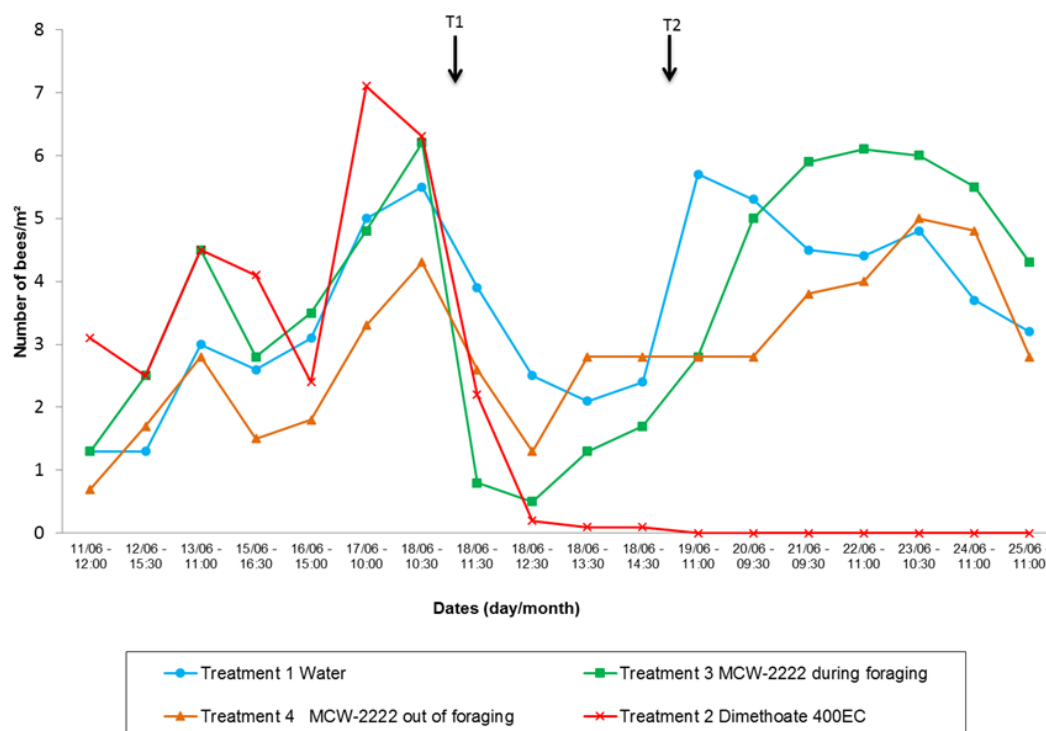
The foraging activity in the control tunnels declined after the application of T1 from 5.5 up to 2.1 bees/m<sup>2</sup>. This activity was higher the following days and the number of bees/m<sup>2</sup> was above 3 up to 5.3 bees/m<sup>2</sup>. This activity in the control and the MCW-2222 tunnels decreased 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 7: Foraging activity - Average number of bees/m<sup>2</sup>**

**Table A 70: Foraging activity - average number of bees/m<sup>2</sup>**

Dates  (day/month-hours) x= delay from application day	Average number of bees/m <sup>2</sup>			
	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 71: Validity criteria**

Validity criteria according to CEB 230 (2012), part IV	Observed in study
<b>Before treatment:</b>	
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 <sup>2</sup> on flowering plants and three bees / m <sup>2</sup> on wheat shortly before application	C: 5.5 bees/m <sup>2</sup> T1: 6.2 bees/m <sup>2</sup> T2: 4.3 bees/m <sup>2</sup> , assessed during bee flight 0 bees/m <sup>2</sup> , assessed after bee flight R: 6.3 bees/m <sup>2</sup>
Foraging activity in different tunnels must be comparable.	Achieved
<b>After treatment:</b>	
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 m<sup>2</sup>, 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 test with honeybees 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 for purposes of the current assessment, because higher tier data were not required to support the risk assessment in 2018 and initial evaluation was rather brief.</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"> <li>– during bee foraging activity: water control, MCW-2222 at 100 g a.s./ha and the reference dimethoate,</li> <li>– out of the presence of forager bees: MCW-2222 at 100 g a.s./ha at night.</li> </ul> <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.</p> <p>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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<b>Reference:</b>	KCP 10.3.1.5/04
<b>Report</b>	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 <i>phacelia</i> crop in Northern France. Mamet, O. & Molitor, C., 2015, R-34875
<b>Guideline(s):</b>	C.E.B methodology n°230, part IV
<b>Deviations:</b>	<p>Yes, minor deviations:</p> <p>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/m<sup>2</sup>) 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,</p> <p>This minor deviation did not have an impact on the reliability and the outcome of the study.</p>
<b>GLP:</b>	Yes, certified laboratory
<b>Acceptability:</b>	Acceptable to detect acute effects; suitability for risk assessment discussed in point 9.6 of this document
<b>Duplication (if vertebrate study)</b>	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 m<sup>2</sup>, 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

<b>Test item</b>	MCW-2222
<b>Batch #</b>	93191024
<b>Content of active substance</b>	Acetamiprid 20% (nominal); 19.8% (analysed)
<b>Description</b>	Yellowish liquid
<b>Control</b>	C: Water treated crop, applied during foraging activity

<b>Toxic reference</b>	R: Rogor Plus (400 g a.s./ha dimethoate), applied during the foraging activity
<b>Test organism</b>	
<b>Species</b>	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 <ul style="list-style-type: none"> <li>- with at 2 to 4 frames containing all brood stages</li> <li>- with 0 to 2 storage frames</li> <li>- with 0 to 2 empty frames</li> <li>- were free of clear clinical symptoms of disease; no treatment against <i>Varroa</i> for at least 4 weeks before beginning of the study.</li> </ul>
<b>Source</b>	local beekeeper, GAEC Mélibocage
<b>Food/feeding</b>	Full flowering <i>Phacelia tanacetifolia</i> served as food supply, no additional feeding throughout the study. Each tunnel was provided with a water supply.
<b>Study design and methods</b>	
<b>Test duration</b>	Pre-exposure phase (D -10 to D0) within the tunnels: 10 days Exposure phase (D 0 to D+7) within the tunnels: 7 days
<b>Experimental dates</b>	23 <sup>rd</sup> June to 9 <sup>th</sup> July 2014
<b>Test doses</b>	<b>Test item</b> T1 (during bee flight): 100 g a.s./ha T2 (after bee flight): 100 g a.s./ha
	<b>Toxic reference</b> 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.
<b>Test units</b>	Tunnels with an area of 140 m <sup>2</sup> , containing 64 m <sup>2</sup> (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.
<b>Endpoints and assessments</b>	<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 <sup>2</sup> 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"> <li>- approx. 1.5 hours before application</li> <li>- immediately before application</li> <li>- 30 minutes after application, followed by two other assessments</li> </ul> <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"> <li>- estimated number of bees (colony strength)</li> <li>- number of cells containing brood (total of cells with eggs, larvae and capped brood)</li> <li>- presence of queens (e.g. presence of eggs)</li> <li>- number of storage frames.</li> </ul>

**Group size/replicates contact  
Adaptation of bees**

Three tunnels per treatment group  
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 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/m<sup>2</sup> 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).

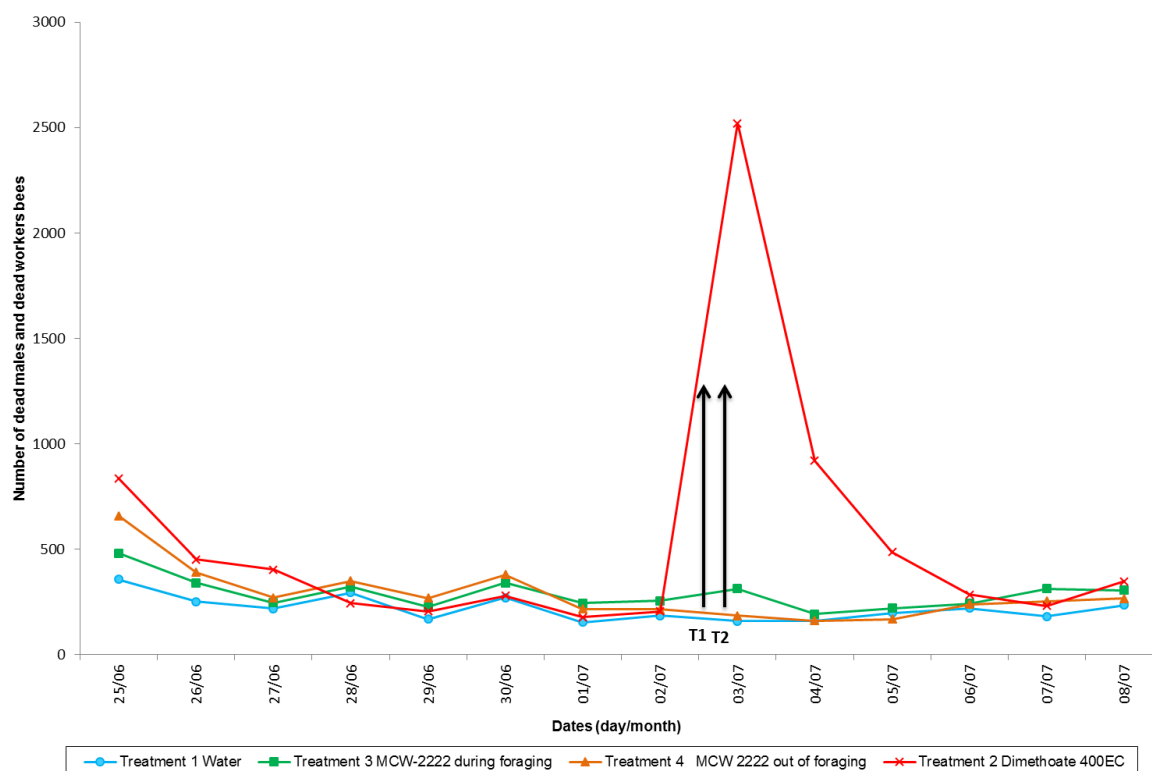
MCW-2222 after bee flight (T2) induced no effect on mortality (from 186 dead bees at D+1 to 162 at D+2 in average). Compared to the control, there was no significant difference during the whole experimental phase.

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 8: Total daily mortality**

**Table A 72: Total daily mortality**

Dates (day/month, D±x) x= delay from application day	Average number of dead bees			
	Treatment 1 Water	Treatment 3 (T 1) MCW-2222 during foraging	Treatment 4 (T 2) MCW-2222 out of foraging	Treatment 2 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 73: Relative toxicity index**

Treatments	Time after Treatment	I tox Value*	
		I tox <sub>1</sub> (D+1 versus D0) During foraging	I tox <sub>2</sub> (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)

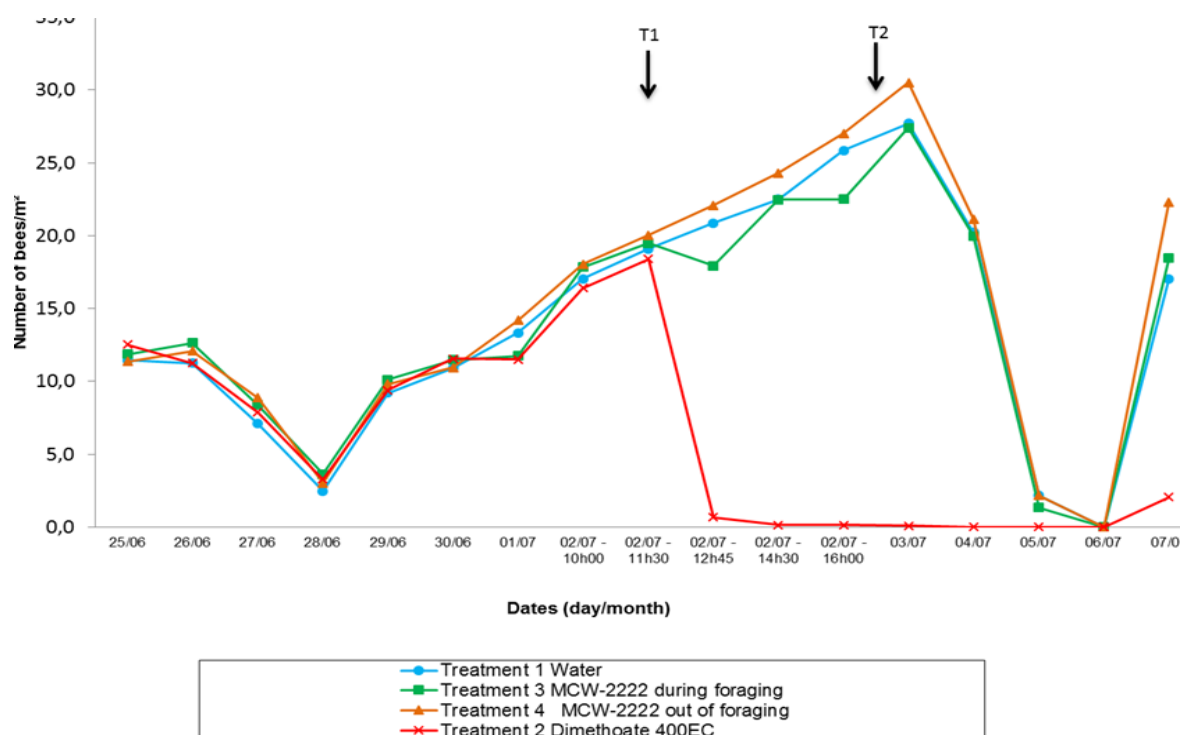
### Foraging activity

The day of the application, the bee activity was high (from 16 to 23 bees/m<sup>2</sup> at D0) and always superior to the required level (5 bees/m<sup>2</sup>).

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 9: Foraging activity - Average number of bees/m<sup>2</sup>**

**Table A 74: Foraging activity - average number of bees/m<sup>2</sup>**

Dates (day/month-hours) x= delay from application day	Average number of bees/m <sup>2</sup>			
	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 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 75: Validity criteria**

Validity criteria according to CEB 230 (2012), part IV	Observed in study
<b>Before treatment:</b>	
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 <sup>2</sup> on flowering plants and three bees / m <sup>2</sup> on wheat shortly before application	C: 18.1 bees/m <sup>2</sup> T1: 18.7 bees/m <sup>2</sup> T2: 19.0 bees/m <sup>2</sup> , assessed during bee flight 0 bees/m <sup>2</sup> , assessed after bee flight R: 17.4 bees/m <sup>2</sup>
Foraging activity in different tunnels must be comparable.	Achieved
<b>After treatment:</b>	
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 m<sup>2</sup>, 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 test with honeybees 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 for purposes of the current assessment, because higher tier data were not required to support the risk assessment in 2018 and initial evaluation was rather brief.</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"> <li>– during bee foraging activity: water control, MCW-2222 at 100 g a.s./ha and the reference dimethoate,</li> <li>– out of the presence of forager bees: MCW-2222 at 100 g a.s./ha at night.</li> </ul> <p>In these experimental conditions, when MCW-2222 was applied at 0.5 L/ha during the</p>
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	<p>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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<b>Reference:</b>	KCP 10.3.1.5/05
<b>Report</b>	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., 2015, R-34876
<b>Guideline(s):</b>	C.E.B methodology n°230, part IV
<b>Deviations:</b>	<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>
<b>GLP:</b>	Yes, certified laboratory
<b>Acceptability:</b>	Acceptable to detect acute effects; suitability for risk assessment discussed in point 9.6 of this document
<b>Duplication (if vertebrate study)</b>	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 m<sup>2</sup>, 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

<b>Test item</b>	MCW-2222
<b>Batch #</b>	93191024
<b>Content of active substance</b>	Acetamiprid 20% (nominal); 19.8% (analysed)
<b>Description</b>	Yellowish liquid
<b>Control</b>	C: Water treated crop, applied during foraging activity
<b>Toxic reference</b>	R: Rogor Plus (400 g a.s./ha dimethoate), applied during the foraging activity
<b>Test organism</b>	
<b>Species</b>	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.
<b>Source</b>	local beekeeper, M. Coueron
<b>Food/feeding</b>	Full flowering <i>Phacelia tanacetifolia</i> served as food supply, no additional feeding throughout the study. Each tunnel was provided with a water supply.
<b>Study design and methods</b>	
<b>Test duration</b>	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<sup>th</sup> August to 4<sup>th</sup> 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

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 *Phacelia*) 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<sup>2</sup>, containing 64 m<sup>2</sup> (subdivided in 4 plots and separated and surrounded by plastic lanes) of flowering *Phacelia tanacetifolia* (variety: Meva), each with one colony; tunnels equipped with a water supply.

## Endpoints and assessments

### mortality of bees:

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

### foraging activity:

D -8 to D+5, on the entire 4 plots/tunnel (4 x 16 m<sup>2</sup> 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

### 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 -10) and once at the almost end (D+5) 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 -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
<b>Temperature:</b>	20 °C	18 °C	9 to 25 °C
<b>Wind speed:</b>	0 km/h	0 km/h	not measured
<b>Rel. humidity:</b>	49 %	62 %	not reported
<b>Precipitation:</b>	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 (i tox) 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.

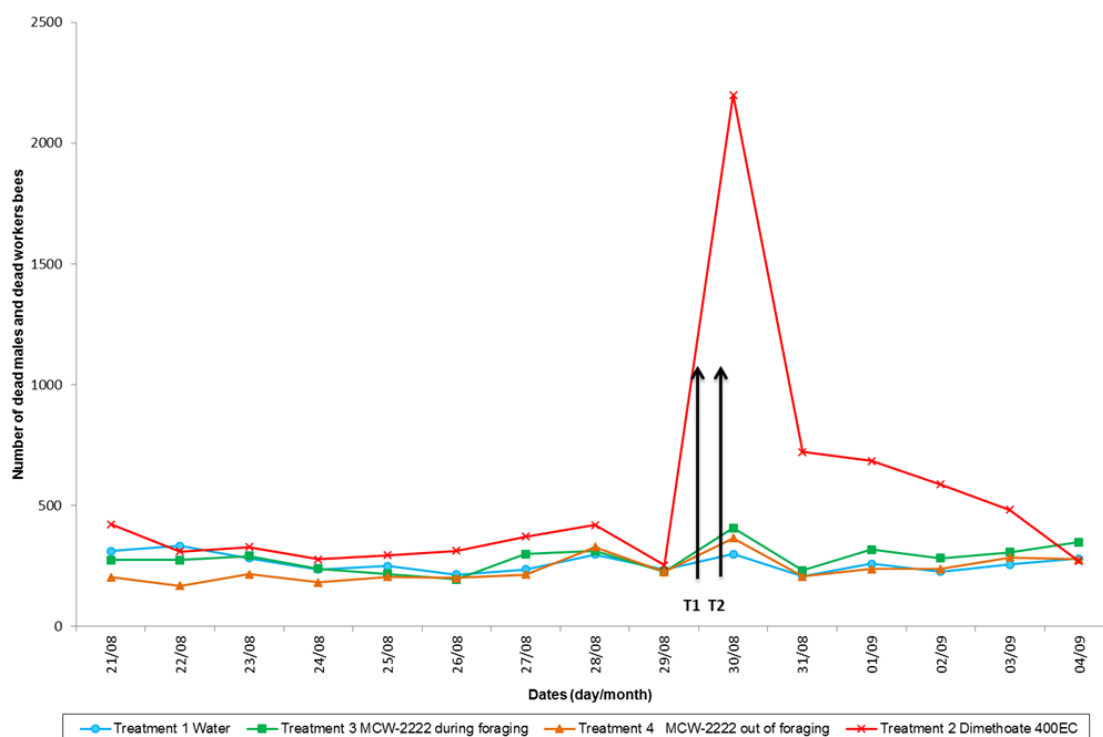


Figure A 10: Total daily mortality

**Table A 76: 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
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

← Application T1 and T2

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 77: Relative toxicity index**

Treatments	Time after Treatment	I tox Value*	
		I tox <sub>1</sub> (D+1 versus D0) During foraging	I tox <sub>2</sub> (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/m<sup>2</sup> at D0) and always superior to the required level (5 bees/m<sup>2</sup>). 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/m<sup>2</sup>. At D+3, the foraging activity increased drastically to more than 16 bees/m<sup>2</sup> (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/m<sup>2</sup>. At D+3, the foraging activity increased drastically to more than 14 bees/m<sup>2</sup> 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.

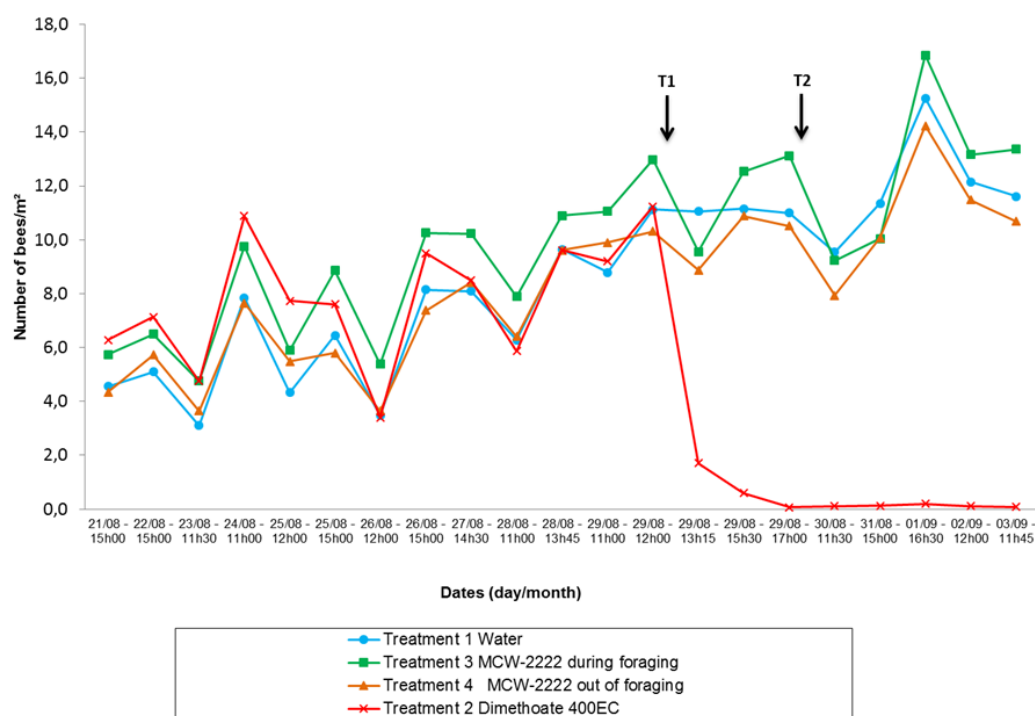


Figure A 11: Foraging activity - Average number of bees/m<sup>2</sup>

**Table A 78: Foraging activity - average number of bees/m<sup>2</sup>**

Dates (day/month-hours) x= delay from application day	Average number of bees/m <sup>2</sup>			
	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-8 21/08 - 15:00	4.6	5.74	4.34	6.3
D-7 22/08 - 15:00	5.1	6.5	5.7	7.1
D-6 23/08 - 11:30	3.1	4.8	3.7	4.8
D-5 24/08 - 11:00	7.9	9.8	7.6	10.9
D-4 25/08 - 12:00	4.3	5.9	5.5	7.7
D-4 25/08 - 15:00	6.5	8.9	5.8	7.6
D-3 26/08 - 12:00	3.5	5.4	3.6	3.4
D-3 26/08 - 15:00	8.1	10.3	7.4	9.5
D-2 27/08 - 14:30	8.1	10.2	8.4	8.5
D-1 28/08 - 11:00	6.3	7.9	6.4	5.9
D-1 28/08 - 13:45	9.6	10.9	9.6	9.6
D0 29/08 - 11:00	8.8	11.1	9.9	9.2
D0 29/08 - 12:00	11.1	13.0	10.3	11.2
D0+ 29/08 - 13:15	11.1	9.6	8.9	1.7
D0+ 29/08 - 15:30	11.1	12.5	10.9	0.6
D0+ 29/08 - 17:00	11.0	13.1	10.5	0.1
D+1 30/08 - 11:30	9.5	9.2	7.9	0.1
D+2 31/08 - 15:00	11.4	10.0	10.1	0.1
D+3 01/09 - 16:30	15.3	16.8	14.2	0.2
D+4 02/09 - 12:00	12.2	13.17	11.47	0.1
D+5 03/09 - 11:45	11.6	13.35	10.69	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 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 79: Validity criteria**

Validity criteria according to CEB 230 (2012), part IV	Observed in study
<b>Before treatment:</b>	
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 <sup>2</sup> on flowering plants and three bees / m <sup>2</sup> on wheat shortly before application	C: 10.0 bees/m <sup>2</sup> T1: 12.0 bees/m <sup>2</sup> T2: 10.1 bees/m <sup>2</sup> , assessed during bee flight 0 bees/m <sup>2</sup> , assessed after bee flight R: 10.2 bees/m <sup>2</sup>
Foraging activity in different tunnels must be comparable.	Achieved
<b>After treatment:</b>	
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 m<sup>2</sup>, 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 10.3.1.5/06 Tunnel test with honeybees 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 for purposes of the current assessment, because higher tier data were not required to support the risk assessment in 2018 and initial evaluation was rather brief.</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"> <li>– during bee foraging activity: water control, MCW-2222 at 100 g a.s./ha and the reference dimethoate,</li> <li>– out of the presence of forager bees: MCW-2222 at 100 g a.s./ha at night.</li> </ul> <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</p>
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	<p>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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<b>Reference:</b>	KCP 10.3.1.5/06
<b>Report</b>	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., 2015, R-35847
<b>Guideline(s):</b>	C.E.B methodology n°230, part IV
<b>Deviations:</b>	<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>
<b>GLP:</b>	Yes, certified laboratory
<b>Acceptability:</b>	Acceptable to detect acute effects; suitability for risk assessment discussed in point 9.6 of this document
<b>Duplication (if vertebrate study)</b>	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

<b>Test item</b>	MCW-2222
<b>Batch #</b>	659-030314-01
<b>Content of active substance</b>	Acetamiprid 200 g/L (nominal); 199.2 ± 1.3 g/L (analysed)
<b>Description</b>	Yellowish liquid
<b>Control</b>	C: Water treated crop, applied during foraging activity
<b>Toxic reference</b>	R: Rogor Plus (400 g a.s./ha dimethoate), applied during the foraging activity
<b>Test organism</b>	
<b>Species</b>	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.
<b>Source</b>	local beekeeper, Apistory
<b>Food/feeding</b>	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

<b>Test duration</b>	Pre-exposure phase (D -3 to D0) within the tunnels: 3 days Exposure phase (D 0 to D+7) within the tunnels: 7 days
<b>Experimental dates</b>	14 <sup>th</sup> July to 24 <sup>th</sup> July 2015
<b>Test doses</b>	<b>Test item</b> 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

### Test units

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 *Phacelia*) 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.

Tunnels with an area of 140 m<sup>2</sup>, containing 64 m<sup>2</sup> (subdivided in 4 plots and separated and surrounded by plastic lanes) of flowering *Phacelia tanacetifolia* (variety: Meva), each with one colony; tunnels equipped with a water supply.

### Endpoints and assessments

#### *mortality of bees:*

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

#### *foraging activity:*

D -2 to D+7, on the entire 4 plots/tunnel (4 x 16 m<sup>2</sup> 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

#### *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 Adaptation of bees

Three tunnels per treatment group

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
<b>Temperature:</b>	22 °C	22 °C	14 to 32 °C
<b>Wind speed:</b>	0 to 5 km/h	0 km/h	not measured
<b>Rel. humidity:</b>	44 %	60 %	not reported
<b>Precipitation:</b>	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 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.

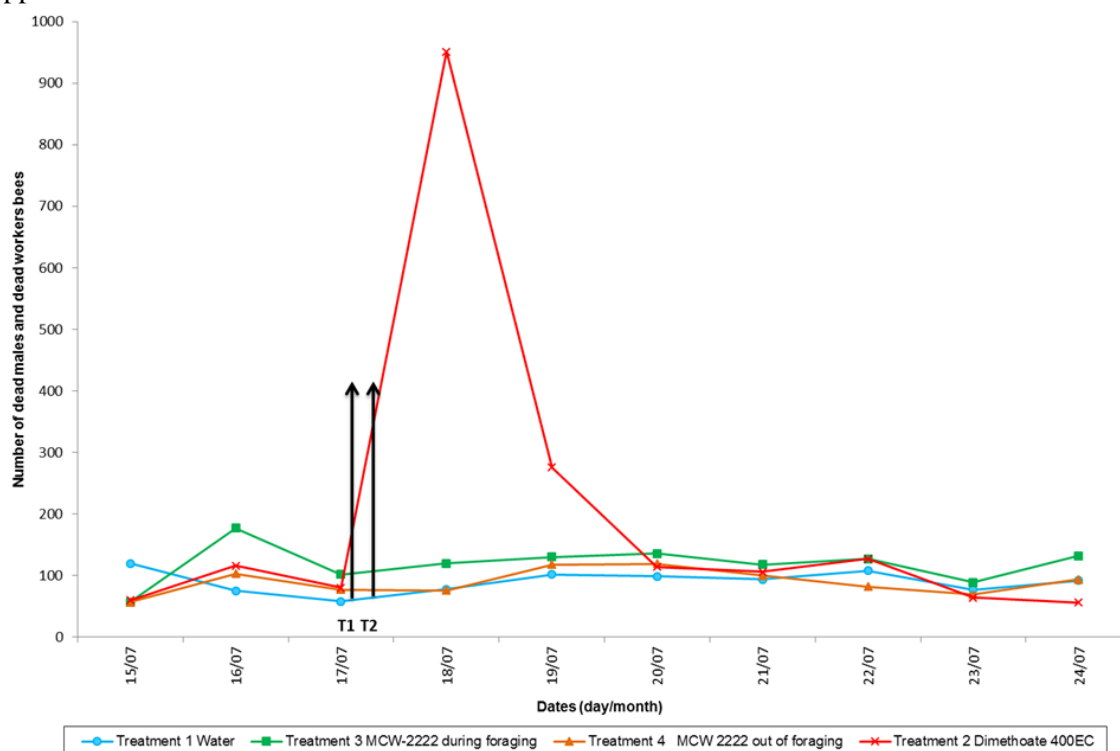
The lack of effect of MCW-2222 on mortality is also confirmed by the low values of the relative toxicity index (itox). Two types of relative toxicity index (i tox) were calculated, one for application during the foraging activity and another one for application outside the foraging activity.

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 12:** Total daily mortality

**Table A 80:** 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

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.

Application T1  
and T2

**Table A 81: Relative toxicity index**

Treatments	Time after Treatment	I tox Value*	
		I tox <sub>1</sub> (D+1 versus D0) During foraging	I tox <sub>2</sub> (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 m<sup>2</sup> instead of 64 m<sup>2</sup>. Phacelia in the other test tunnels were not affected and the assessments were carried out on 64 m<sup>2</sup>. This deviation was taken into account in the calculation of number of bees/m<sup>2</sup>.

The day of the application, the average bee activity was high (from 7.1 to 8.6 bees/m<sup>2</sup> at D0) and always superior to the required level (5 bees/m<sup>2</sup>).

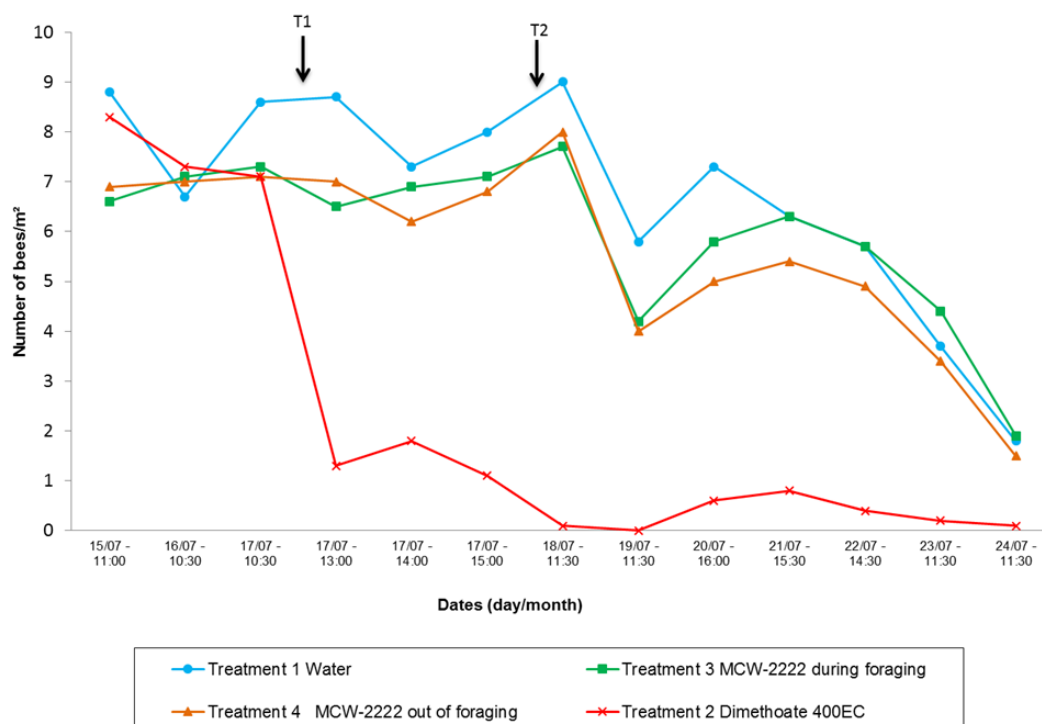
The foraging activity started to decrease in the control tunnel and in the test items tunnels from D+5 (22/07) because the crop became less attractive to bees.

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/m<sup>2</sup>). 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/m<sup>2</sup> 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 13:** Foraging activity - Average number of bees/m²

**Table A 82:** Foraging activity - average number of bees/m²

Dates (day/month-hours) x= delay from application day	Average number of bees/m²			
	Treatment 1 Water	Treatment 3 (T 1) MCW-2222 during foraging	Treatment 4 (T 2) MCW-2222 out of foraging	Treatment 2 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 83: Validity criteria**

Validity criteria according to CEB 230 (2012), part IV	Observed in study
<b>Before treatment:</b>	
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 <sup>2</sup> on flowering plants and three bees / m <sup>2</sup> on wheat shortly before application	C: 8.6 bees/m <sup>2</sup> T1: 7.3 bees/m <sup>2</sup> T2: 7.1 bees/m <sup>2</sup> , assessed during bee flight 0 bees/m <sup>2</sup> , assessed after bee flight R: 7.1 bees/m <sup>2</sup>
Foraging activity in different tunnels must be comparable.	Achieved
<b>After treatment:</b>	
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 m<sup>2</sup>, 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 Semi-field honeybee 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 due to renewal of acetamiprid and was not evaluated earlier. 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-Wurttemberg. During the study the product was applied twice at rate of 80 g a.s./ha - 1<sup>st</sup> 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> <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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<b>Reference:</b>	KCP 10.3.1.5/07
<b>Report</b>	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
<b>Guideline(s):</b>	OECD GD 75 (2007)
<b>Deviations:</b>	None
<b>GLP:</b>	Yes, certified laboratory
<b>Acceptability:</b>	Acceptable; suitability for risk assessment discussed in point 9.6 of this document
<b>Duplication (if vertebrate study)</b>	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 m<sup>2</sup>). 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 2<sup>nd</sup> 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 2<sup>nd</sup> 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 2<sup>nd</sup> T application (DAA -1) shortly before application, and the day after the 2<sup>nd</sup> 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 1m<sup>2</sup> plots per tunnel for 15 seconds; additional assessments were conducted shortly before the 2<sup>nd</sup> 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 2<sup>nd</sup> 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 DAA7 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 2<sup>nd</sup> 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

<b>Test item</b>	MCW-2222
<b>Batch #</b>	811-021115-01
<b>Content of active substance</b>	Acetamiprid 200 g/L (nominal); 205.1 ± 1.1 g/L (analysed)
<b>Description</b>	Liquid / clear yellow to brown
<b>Control</b>	C: Tap water
<b>Toxic reference</b>	R: Insegar 25 WG (250 g fenoxycarb/kg)
<b>Test organism</b>	
<b>Species</b>	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, nosemosis, 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).
<b>Source</b>	Company's own apiary
<b>Food/feeding</b>	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

<b>Test duration</b>	Exposure phase before the 2 <sup>nd</sup> application (DAA -5 to DAA -1): 4 days in the tunnels Exposure phase after the 2 <sup>nd</sup> 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
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<b>Experimental dates</b>	3 <sup>rd</sup> July to 8 <sup>th</sup> August 2016
<b>Test doses</b>	<p><b>Test item (T):</b> 2 x 80 g a.s./ha 1<sup>st</sup> application on 3<sup>rd</sup> July 2016 (DAA -8; BBCH 59-61, single plants with open flowers) without hives present in the tunnels 2<sup>nd</sup> application on 10<sup>th</sup> July 2016 (DAA -1; BBCH 60-65) with hives present in the tunnels but applied after bee flight</p> <p><b>Toxic reference (R):</b> 1 x 300 g a.s./ha, applied on 11<sup>th</sup> July (DAA 0), during bee flight</p> <p>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<sup>nd</sup> 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 <math>\pm 5\%</math> from the target application rate.</p>
<b>Test units</b>	Tunnels with an area of 108 m <sup>2</sup> , containing 84 m <sup>2</sup> of <i>Phacelia tanacetifolia</i> , each with one colony; tunnels equipped with a water supply.
<b>Group size/replicates contact</b>	Four tunnels per treatment group, two additional tunnels for the generation of samples for residue analysis.
<b>Endpoints and assessments</b>	<p><i>mortality of worker bees, drones and puape/larvae:</i> DAA -6 &amp; -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:</p> <ul style="list-style-type: none"><li>- DAA -5 shortly before transport of colonies to the tunnels</li><li>- the day of the 2<sup>nd</sup> T application (DAA -1) shortly before application;</li><li>- the day after the 2<sup>nd</sup> T application (DAA 0) in the morning, 2h after R application and in the evening.</li></ul> <p><i>foraging activity:</i> DAA -4 to DAA 7, counting the number of foraging bees on three 1m<sup>2</sup> plots per tunnel for 15 seconds; assessed once a day during the flight activity of the bees; additional assessments on:</p> <ul style="list-style-type: none"><li>- the day of 2<sup>nd</sup> T application (=DAA -1) shortly before treatment to ensure no bees were actively foraging</li><li>- the day after the 2<sup>nd</sup> T application (DAA 0)<ul style="list-style-type: none"><li>- shortly before application of R to ensure that enough bees were actively foraging (only in the reference item tunnels),</li><li>- 4 times within the first hour after application of R (in all tunnels)</li><li>- 2 hours after application</li><li>- 4 hours after application</li><li>- 6 hours after application</li></ul></li></ul> <p><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</p> <p><i>condition of the colonies:</i> 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</p>

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 Agrosience 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 = 1<sup>st</sup> day on which the bees were

exposed to the water treated control, the 2<sup>nd</sup> 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 2<sup>nd</sup> 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**

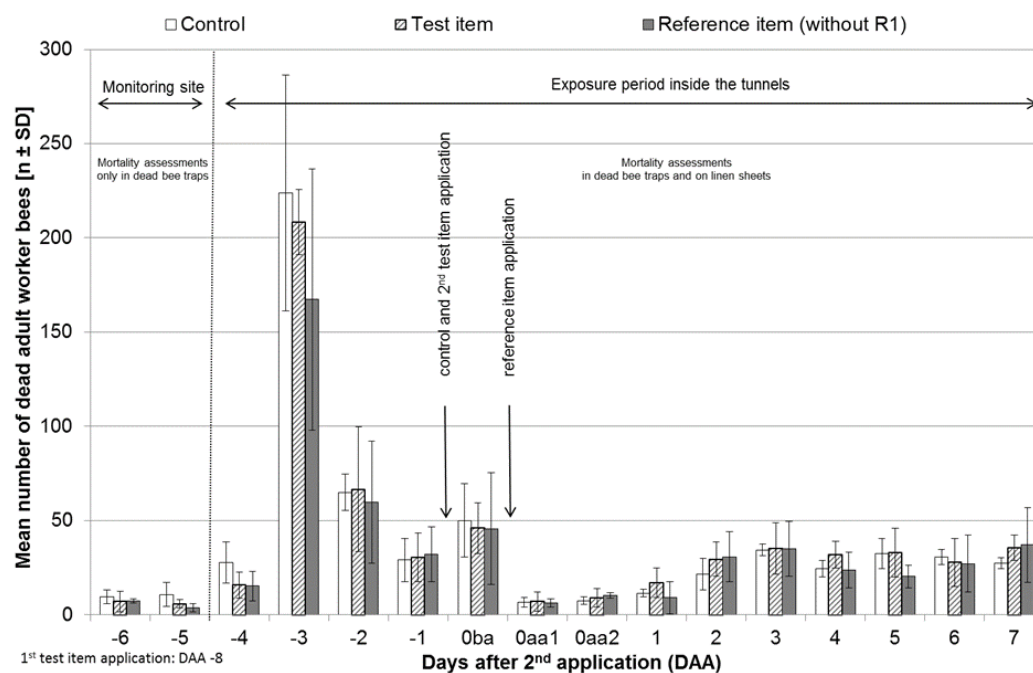
##### *Mortality, adult honeybees*

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.

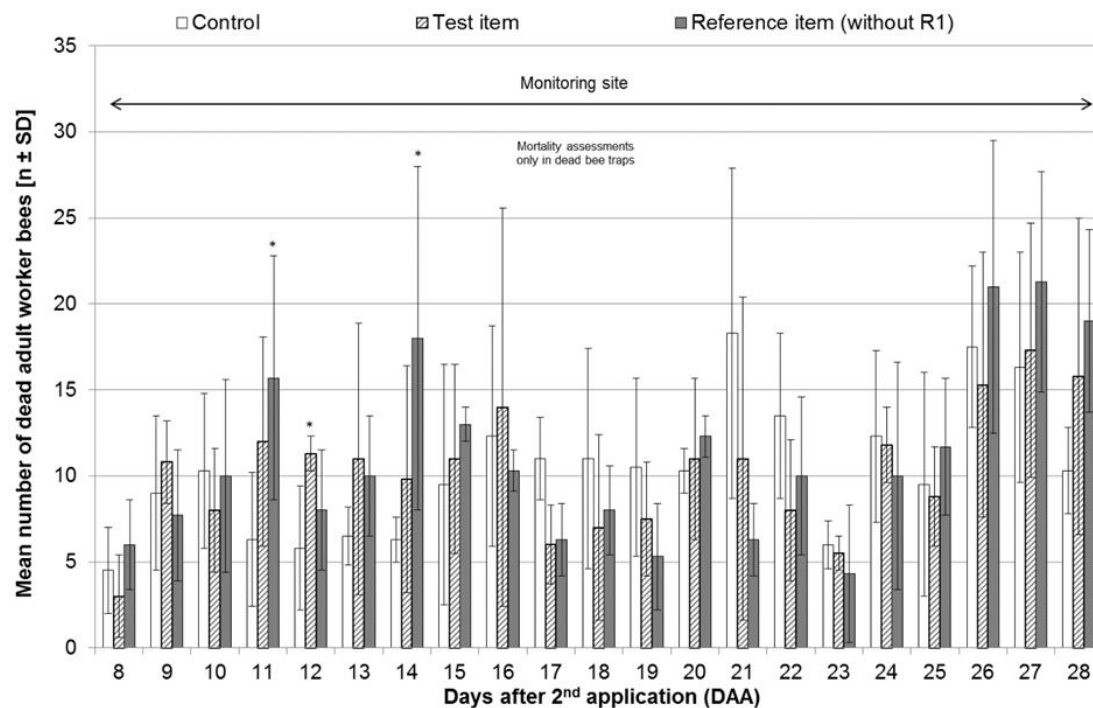
Regarding the post-exposure period at the monitoring site (DAA 8 to 28) and the post-application periods from DAA -4 to DAA 28 and DAA 0aa to 28, the mean mortalities were in general comparable and within the range of the normal expected biological variability in all treatment groups. Thus, a test item related adverse effect on the adult bee mortality can be excluded.



DAA 0 = 11.07.2016 (1<sup>st</sup> day on which the bees were exposed to the water treated control, the 2<sup>nd</sup> 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 14: Adult worker bee mortality during the pre-exposure and exposure periods**





DAA 0 = 11.07.2016 (1<sup>st</sup> day on which the bees were exposed to the water treated control, the 2<sup>nd</sup> test item and the reference item application); SD

**Figure A 15: Adult worker bee mortality during the post-exposure period at the monitoring site**

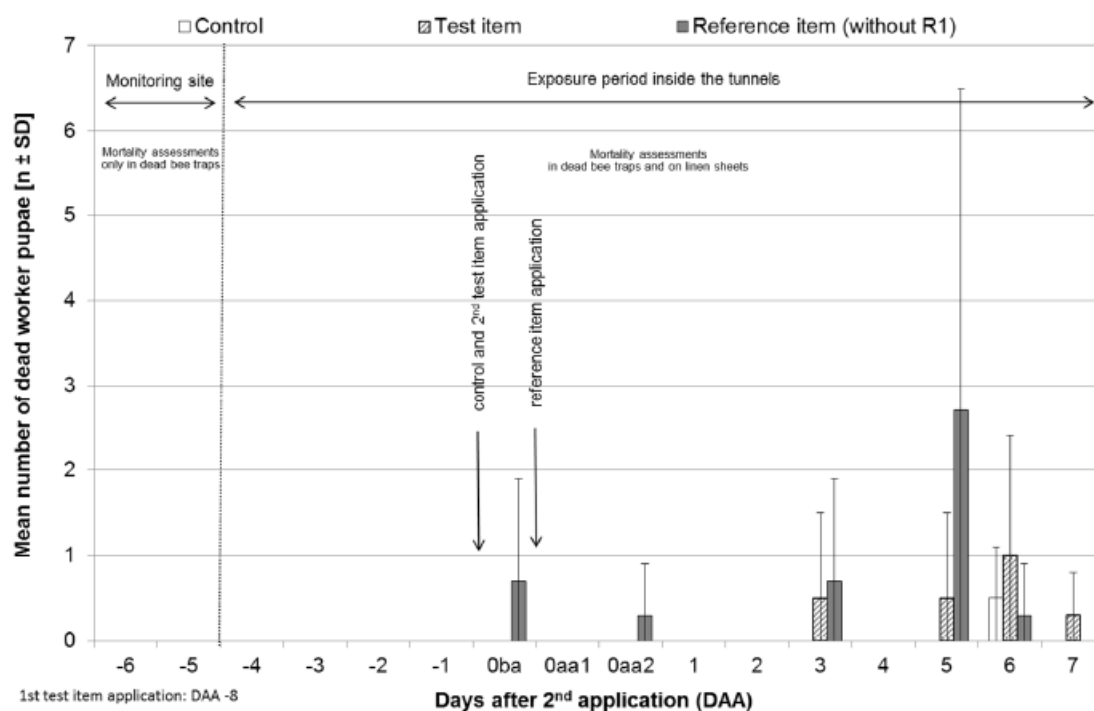
**Table A 84: Adult worker bee mortality during the entire period of the study**

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.
<b>Mean (-6 to -5)<sup>1)</sup></b>		<b>10.1</b>	<b>4.9<sup>3)</sup></b>	<b>6.4</b>	<b>3.9<sup>3)</sup></b>	<b>n.s.</b>	<b>5.5</b>	<b>2.5<sup>3)</sup></b>	<b>n.s.</b>
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.
<b>Mean (-4 to -1)<sup>2)</sup></b>		<b>86.4</b>	<b>88.3<sup>3)</sup></b>	<b>80.3</b>	<b>80.8<sup>3)</sup></b>	<b>n.s.</b>	<b>68.6</b>	<b>70.2<sup>3)</sup></b>	<b>n.s.</b>
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.
<b>Σ 0aa<sup>2)</sup></b>		<b>14.0</b>	<b>3.6<sup>3)</sup></b>	<b>16.0</b>	<b>10.0<sup>3)</sup></b>	<b>n.s.</b>	<b>16.7</b>	<b>3.5<sup>3)</sup></b>	<b>n.s.</b>
12.07.2016	1	11.5	2.1	17.0	7.7	n.s.	9.0	8.7	n.s.
<b>Σ0aa+1<sup>2)</sup></b>		<b>25.5</b>	<b>4.2</b>	<b>33.0</b>	<b>17.1</b>	<b>n.s.</b>	<b>25.7</b>	<b>5.7</b>	<b>n.s.</b>
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.
<b>Mean (Σ0aa+1 to 7)<sup>2)</sup></b>		<b>28.0</b>	<b>6.4<sup>3)</sup></b>	<b>32.3</b>	<b>10.8<sup>3)</sup></b>	<b>n.s.</b>	<b>28.5</b>	<b>12.2<sup>3)</sup></b>	<b>n.s.</b>
<b>Mean (-4 to 7)<sup>2)</sup></b>		<b>49.3</b>	<b>57.2<sup>3)</sup></b>	<b>49.4</b>	<b>51.6<sup>3)</sup></b>	<b>n.s.</b>	<b>43.3</b>	<b>45.1<sup>3)</sup></b>	<b>n.s.</b>
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.
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.
<b>Mean (8 to 28)<sup>1)</sup></b>		<b>10.3</b>	<b>5.7<sup>3)</sup></b>	<b>10.3</b>	<b>6.2<sup>3)</sup></b>	<b>n.s.</b>	<b>11.2</b>	<b>6.4<sup>3)</sup></b>	<b>n.s.</b>
<b>Mean (Σ0aa+1 to 28)<sup>1) &amp; 2)</sup></b>		<b>14.7</b>	<b>9.7<sup>3)</sup></b>	<b>15.8</b>	<b>12.2<sup>3)</sup></b>	<b>n.s.</b>	<b>15.5</b>	<b>11.1<sup>3)</sup></b>	<b>n.s.</b>
<b>Mean (-4 to 28)<sup>1) &amp; 2)</sup></b>		<b>24.5</b>	<b>39.4<sup>3)</sup></b>	<b>24.5</b>	<b>36.6<sup>3)</sup></b>	<b>n.s.</b>	<b>22.8</b>	<b>31.5<sup>3)</sup></b>	<b>n.s.</b>

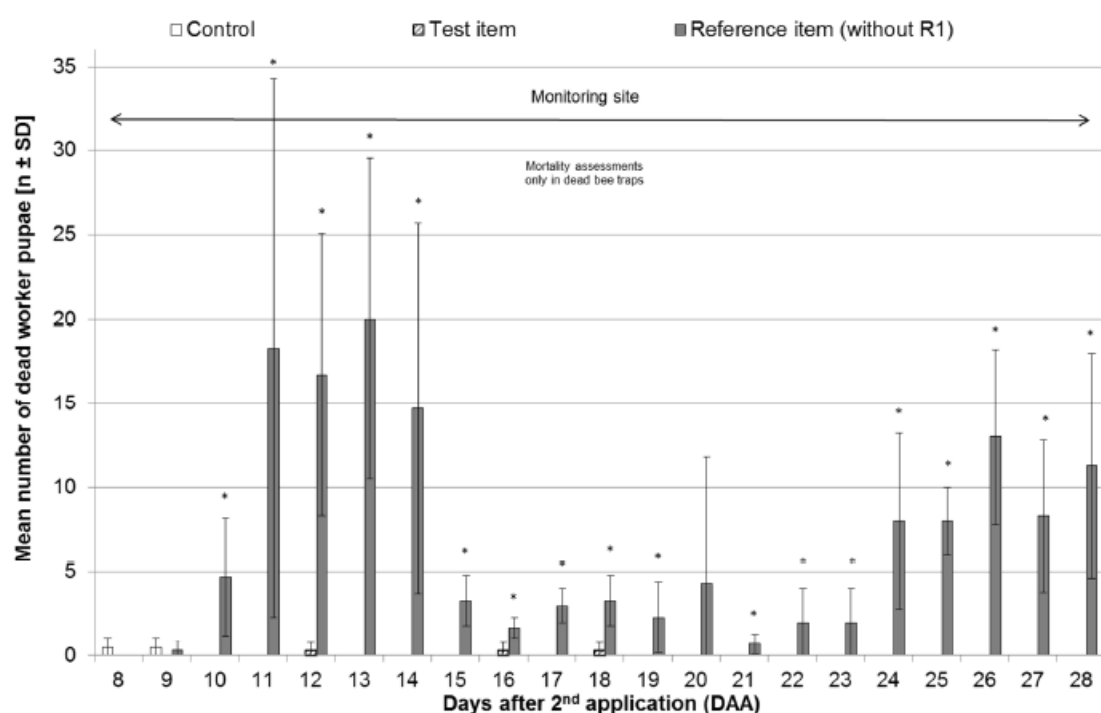
For all calculations (means, SDs) DAA 0ba and DAA Σ 0aa1+2 were considered as daily values although covering less than 24 hours. DAA = days after application (DAA 0 = 11.07.2016 (1<sup>st</sup> day on which the bees were exposed to the water treated control, the 2<sup>nd</sup> test item and the reference 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); <sup>D</sup> Dunnett test; <sup>U</sup> U test; <sup>M</sup> Median test; <sup>1)</sup> mortality in dead bee traps; <sup>2)</sup> mortality in dead bee traps and on sheets; <sup>3)</sup> standard deviation calculated for the individual values of the respective group

### 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.



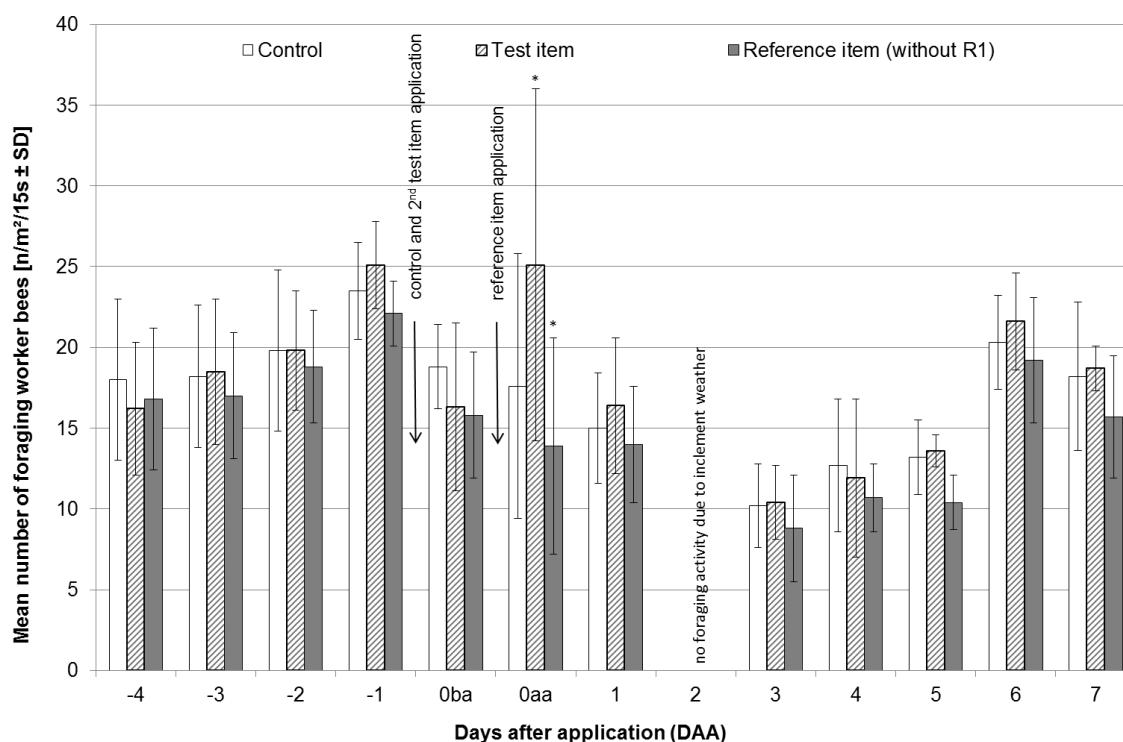
Mean pupal mortality of the different treatment groups during the pre-exposure and exposure periods



## Mean pupal mortality of the different treatment groups during the post- exposure period at the monitoring site

### 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 (1<sup>st</sup> day on which the bees were exposed to the water treated control, the 2<sup>nd</sup> test item and the reference item application); ba = before application; aa = after application; SD = standard deviation

**Figure A 16: Foraging activity - Average number of bees/m²**

**Table A 85: Foraging activity - average number of bees/m<sup>2</sup>**

Date [dd.mm.yyyy]	DAA	Control		Test item			Reference item (without R1)		
		Mean [n/m <sup>2</sup> /15s] <sup>1)</sup>	SD	Mean [n/m <sup>2</sup> /15s] <sup>1)</sup>	SD	Stat.	Mean [n/m <sup>2</sup> /15s] <sup>1)</sup>	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.
<b>Mean (-4 to -1)</b>		<b>19.9</b>	<b>4.0</b>	<b>19.9</b>	<b>4.4</b>	<b>n.s.</b>	<b>18.7</b>	<b>3.0</b>	<b>n. s.</b>
<b>11.07.2016</b>	<b>0ba</b>	<b>18.8</b>	<b>2.6</b>	<b>16.3</b>	<b>5.2</b>	<b>n.s.</b>	<b>15.8</b>	<b>3.9</b>	<b>n.s.</b>
	<b>0aa</b>	<b>17.6</b>	<b>8.2</b>	<b>25.1</b>	<b>10.9</b>	<b>* M</b>	<b>13.9</b>	<b>6.7</b>	<b>* U</b>
12.07.2016	1	15.0	3.4	16.4	4.2	n.s.	14.0	3.6	n.s.
13.07.2016	2 <sup>2)</sup>	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.
<b>Mean (0aa to 7)</b>		<b>13.4</b>	<b>6.6</b>	<b>14.7</b>	<b>7.6</b>	<b>n.s.</b>	<b>11.6</b>	<b>5.9</b>	<b>n.s.</b>
<b>Mean (-4 to 7)</b>		<b>15.8</b>	<b>6.4</b>	<b>16.4</b>	<b>6.9</b>	<b>n.s.</b>	<b>14.1</b>	<b>5.9</b>	<b>n.s.</b>

DAA = days after application (DAA 0 = 11.07.2016 (1<sup>st</sup> day on which the bees were exposed to the water treated control, the 2<sup>nd</sup> 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 ( $p < 0.05$ ); <sup>M</sup> Median test; <sup>U</sup> U test; <sup>1)</sup> mean foraging activity on DAA -4, DAA -3, DAA -2 and DAA 1 (3 assessments) and on DAA 0aa (7 assessments); <sup>2)</sup> foraging activity was low due to bad weather conditions

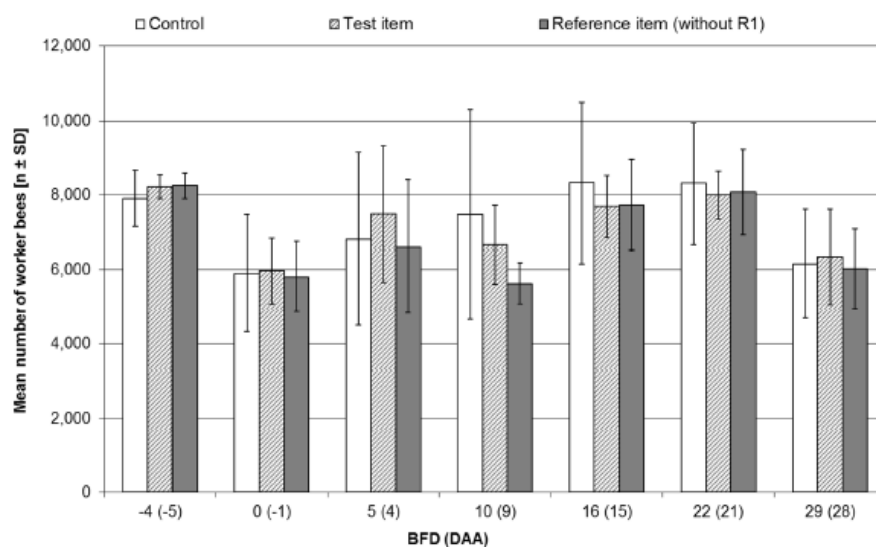
### Behaviour

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.



## Development of the mean colony strength (bees/colony) in particular treatment groups

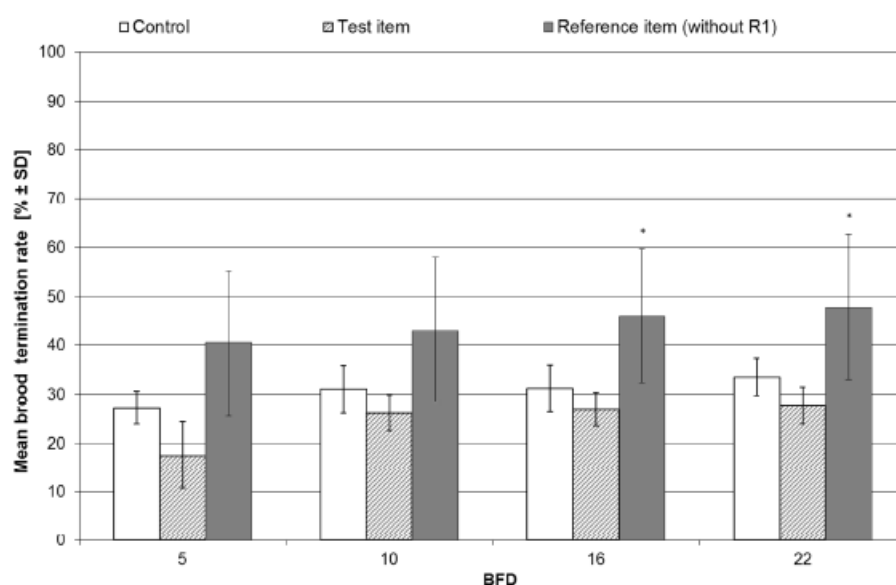
### Detailed brood development

#### 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  $3.6 \pm 0.2$  and therefore slightly higher than that of the control group with  $3.3 \pm 0.2$ . 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  $2.6 \pm 0.7$ .

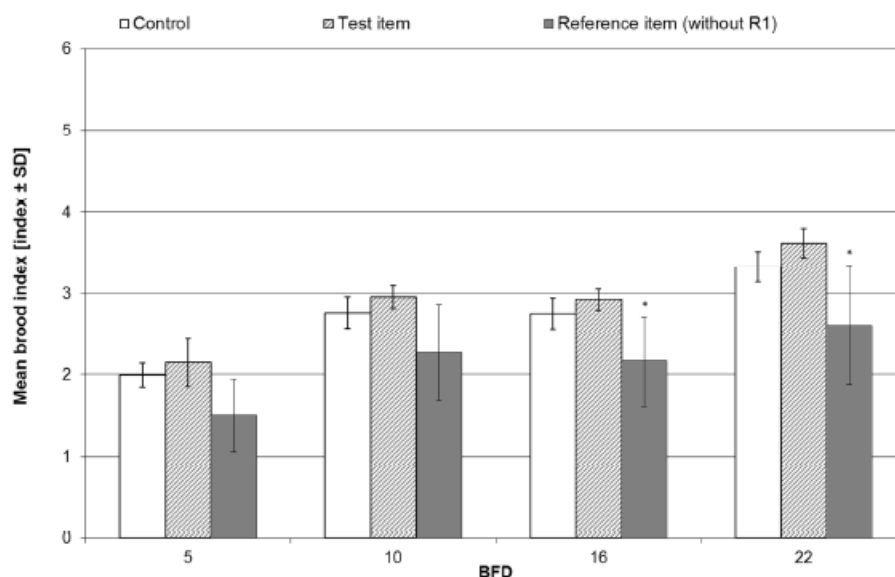


### Mean brood termination rates of the particular treatment groups

#### Brood index

The BI values correlate with the BTRs: the higher the BTRs the lower the BIs and vice versa. Consequently the mean BI of the test item group was consistently slightly higher than that of the control group and ended up with  $3.6 \pm 0.2$  at the final BFD assessment (BFD 22), compared to  $3.3 \pm 0.2$  in the control group. The BI value in the reference item group at the final assessment (BFD 22) was with  $2.6 \pm 0.7$  the lowest.

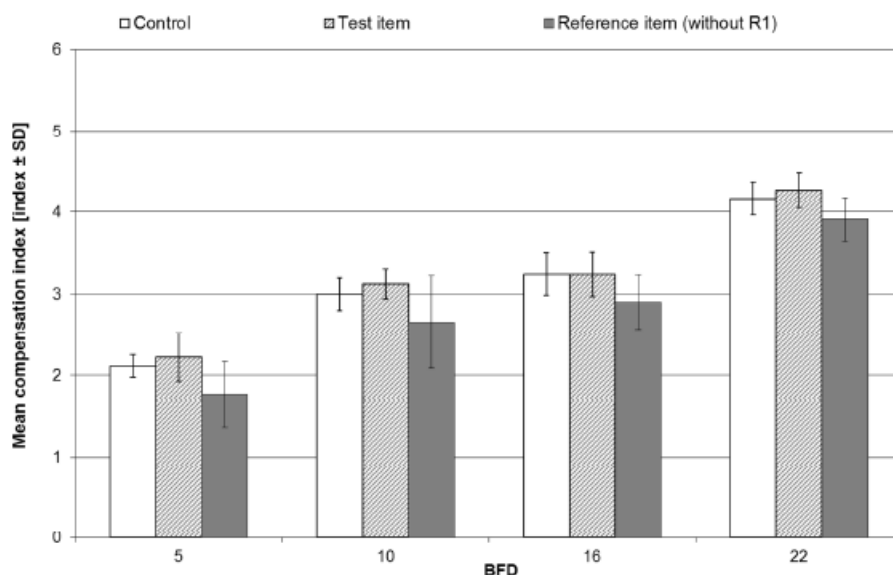
No statistically significant differences were calculated between the control and the test item group, though at BFD 16 and BFD 22 statistically significant differences were found between the control and the reference item group. Thus, on the basis of these data, it is also possible to exclude a test item related adverse effect on the bee brood.



**Mean brood indices of the particular treatment groups**

### 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  $4.2 \pm 0.2$ ,  $4.3 \pm 0.2$  and  $3.9 \pm 0.3$  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.



**Mean compensation indices of the particular treatment groups**

### *Residue analysis*

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 im-pact 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 86: Validity criteria**

No validity criteria are given by OECD GD 75 (2007) but by the study plan	Observed in study
<b>Before treatment:</b>	
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 <sup>2</sup> )	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.  <b>Criterion was achieved</b>
Mean foraging activity shortly before the application of the reference item should be >10 bees/m <sup>2</sup> in the respective reference item tunnels	Foraging activit before the reference item application was: C: 18.8 bees/m <sup>2</sup> T: 16.3 bees/m <sup>2</sup> R: 15.8 bees/m <sup>2</sup>  <b>Criterion was achieved</b>
<b>After treatment:</b>	
A detectable effect of the reference item should 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).  <b>Criterion was achieved</b>

## 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 honeybees

#### A 2.3.1.8.1 KCP 10.3.1.6/01 Field study with honeybees 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 for purposes of the current assessment, because higher tier data were not required to support the risk assessment in 2018 and initial evaluation was rather brief.</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/m<sup>2</sup>). 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>
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	<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 at 2<sup>nd</sup> but also on 3<sup>rd</sup> 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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<b>Reference:</b>	KCP 10.3.1.6/01
<b>Report</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> . Molitor, C., 2015, R-34877
<b>Guideline(s):</b>	EPPO 1/170 (4) (2010), C.E.B methodology n°230, part IV (2003), OECD GD75 (2007)
<b>Deviations:</b>	None
<b>GLP:</b>	Yes, certified laboratory
<b>Acceptability:</b>	Acceptable with some restrictions (see commenting box above)
<b>Duplication (if vertebrate study)</b>	Not applicable

## 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 exposed 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 m<sup>2</sup> 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 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 clinical 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/m<sup>2</sup> in the control and 8 bees/m<sup>2</sup> 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/m<sup>2</sup> 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/m<sup>2</sup> at 3DAA and 4DAA. At 5DAA, the activity again increased drastically in both treatments (around 8 bees/m<sup>2</sup>). 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/m<sup>2</sup> 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 ( $0.018 \pm 0.004$  mg/kg in in-hive nectar at 8DAA,  $0.066 \pm 0.035$  mg/kg in bee bread at 8DAA and  $0.020 \pm 0.005$  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  $0.111 \pm 0.086$  mg/kg at 3DAA in pollen,  $0.033 \pm 0.013$  mg/kg at 8DAA in nectar,  $0.109 \pm 0.048$  mg/kg at 8DAA in bee bread and  $0.031 \pm 0.008$  mg/kg at 20 DAA in honey specimens. Pollen specimens collected at 8DAA showed a much lower level ( $< \text{LOQ}$  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/m<sup>2</sup> (trigger: 3 bees/m<sup>2</sup>) 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

<b>Test item</b>	MCW-2222
<b>Batch #</b>	93191024
<b>Content of active substance</b>	Acetamiprid 200 g/L (nominal); 198 g/L (analysed)
<b>Description</b>	Yellowish liquid
<b>Control</b>	C: Untreated crop
<b>Toxic reference</b>	none
<b>Test organism</b>	

<b>Species</b>	<p>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.</p> <p>All colonies at the beginning of the study</p> <ul style="list-style-type: none"><li>- with at 4 to 9 frames containing all brood stages</li><li>- with 1 to 5 storage frames</li><li>- with 0 to 2 empty frames</li><li>- were free of clear clinical symptoms of disease; no treatment against <i>Varroa</i> for at least 4 weeks before beginning of the study.</li></ul>
<b>Source</b>	local beekeeper, Alban Couëron
<b>Food/feeding</b>	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.
<b>Study design and methods</b>	
<b>Test duration</b>	<p>Pre-exposure phase (6DBA to 0DBA): 7 days at the study fields</p> <p>Exposure phase (0DAA to 37DAA): 37 days at the study fields</p> <p>Post-Exposure phase (38DAA to 40DAA): 3 days at the monitoring site</p>
<b>Experimental dates</b>	1 <sup>st</sup> July to 12 <sup>th</sup> August 2014
<b>Test doses</b>	<p>T: 100 g a.s./ha, applied after bee flight</p> <p>Application was performed after bee flight (from 22:30 to 22:45) at BBCH 64 (full flowering of <i>Phacelia</i>) of the crop with a volume of 200 L water/ha. The actual treatment rate was 98% of the target application rate.</p>
<b>Test units</b>	Study fields with flowering <i>Phacelia tanacetifolia</i> (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.
<b>Group size/replicates</b>	One study field per treatment group, 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.
<b>Endpoints and assessments</b>	<p><i>mortality of adult bees and pupae:</i></p> <p>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</p> <p><i>foraging activity:</i></p> <p>Daily recording of the number of forager bees daily on two areas of 10 m<sup>2</sup> 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.</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 = 1DBA), on BFD 28 and on BFD 42 (end of the study); assessment of:</p> <ul style="list-style-type: none"><li>- estimated number of bees (colony strength) acc. to Liebefeld method</li><li>- number of cells containing brood (total of cells with eggs, larvae and capped brood) to Liebefeld method</li><li>- presence of queens (e.g. presence of eggs)</li></ul>

- 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 a 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 ± 1 after BFD00	Young larvae or old larvae	2 or 3
10 days ± 1 after BFD00	Capped cells	4
16 days ± 2 after BFD	Capped cells shortly before hatch	4
22 days ± 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 ± 1 after BFD00	Old larvae or capped cells	3 or 4
10 days ± 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.

#### **Adaptation of bees**

Colonies were set-up at the fields ten days before application on 7DBA to get familiar with the new conditions.

#### **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 recorded 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***

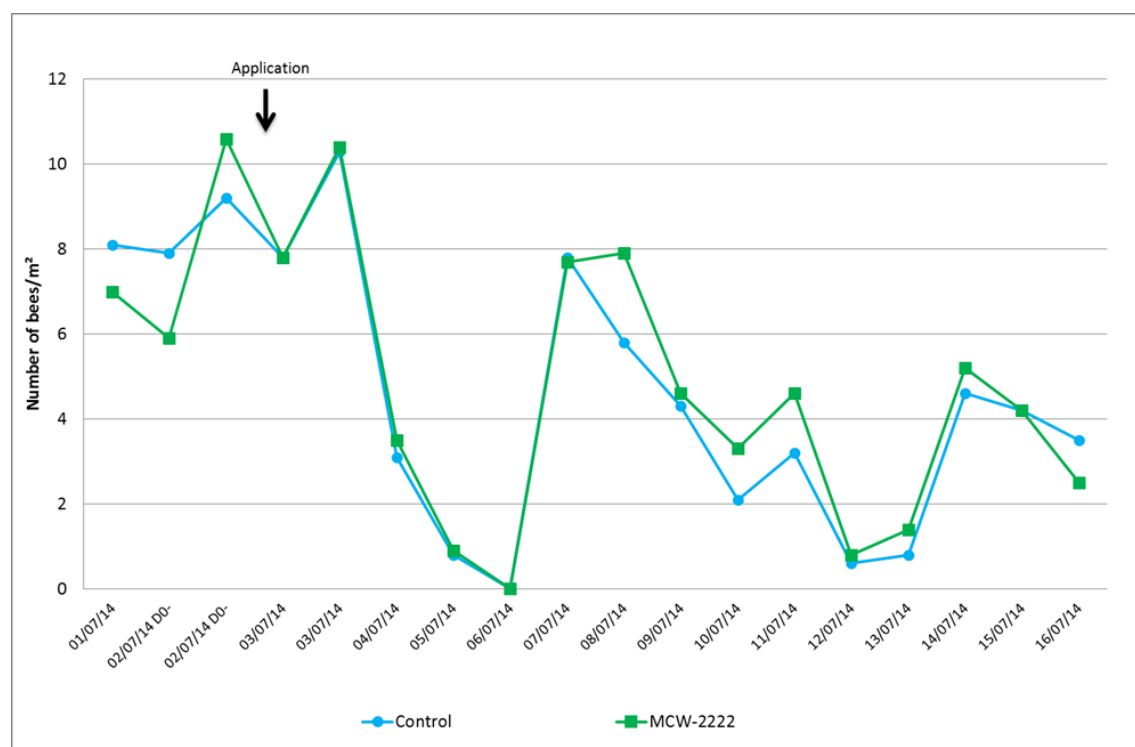
#### ***Foraging activity***

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/m<sup>2</sup> in the control field and 8 bees/m<sup>2</sup> 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/m<sup>2</sup> 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/m<sup>2</sup> at 3DAA and 4DAA. At 5DAA, the activity increased drastically in both treatments (around 8 bees/m<sup>2</sup>). 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/m<sup>2</sup> 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 17:** Daily mean foraging activity  
**Table A 87:** Daily mean foraging activity

Date	Timing	Number of bees/m <sup>2</sup>	
		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

DBA = days before application; DAA = days after application

← Application after bee flight

### 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.

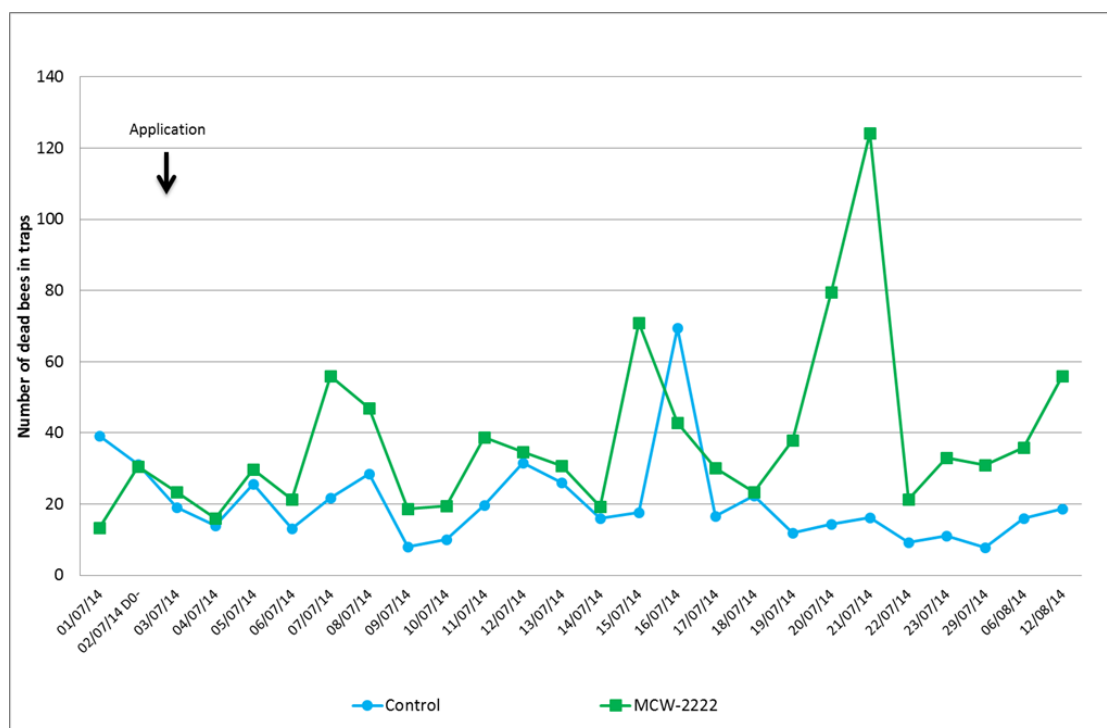
During two days (18 DAA & 19DAA), a higher mortality was recorded for all hives in the test item treatment compared to the control one. A peak of mortality in hive R017 was observed and the dead bodies found in the catch trap were sampled. A multi-residue analysis was performed on the 19DAAs' sample 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.

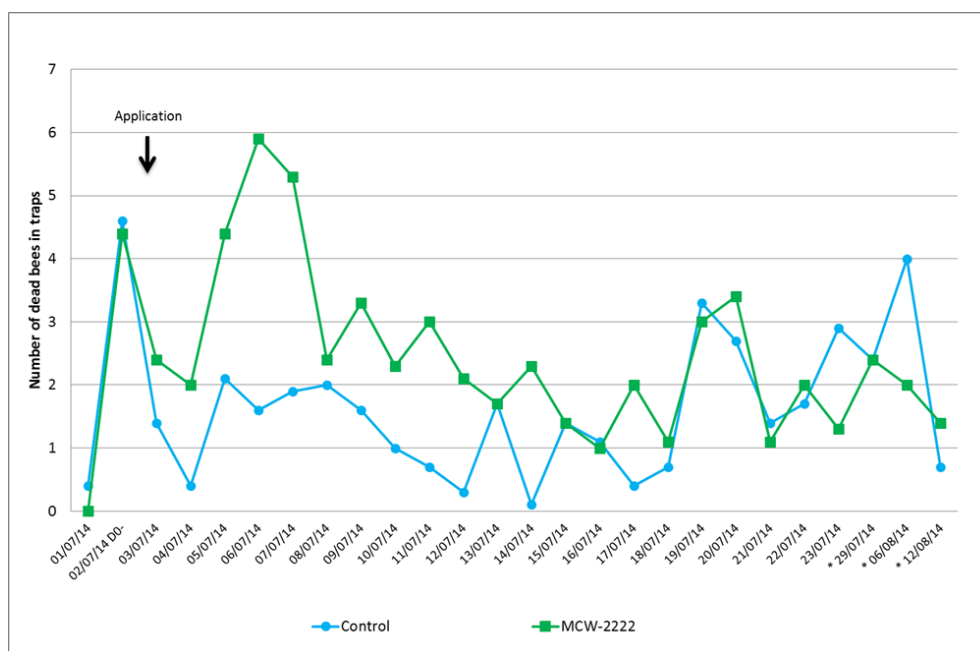


**Figure A 18:** Daily mean mortality of adult bees

**Table A 88: 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

← Application after bee flight



**Figure A 19: Daily mean mortality of bee pupae**

**Table A 89: 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

← Application after bee flight

### *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.

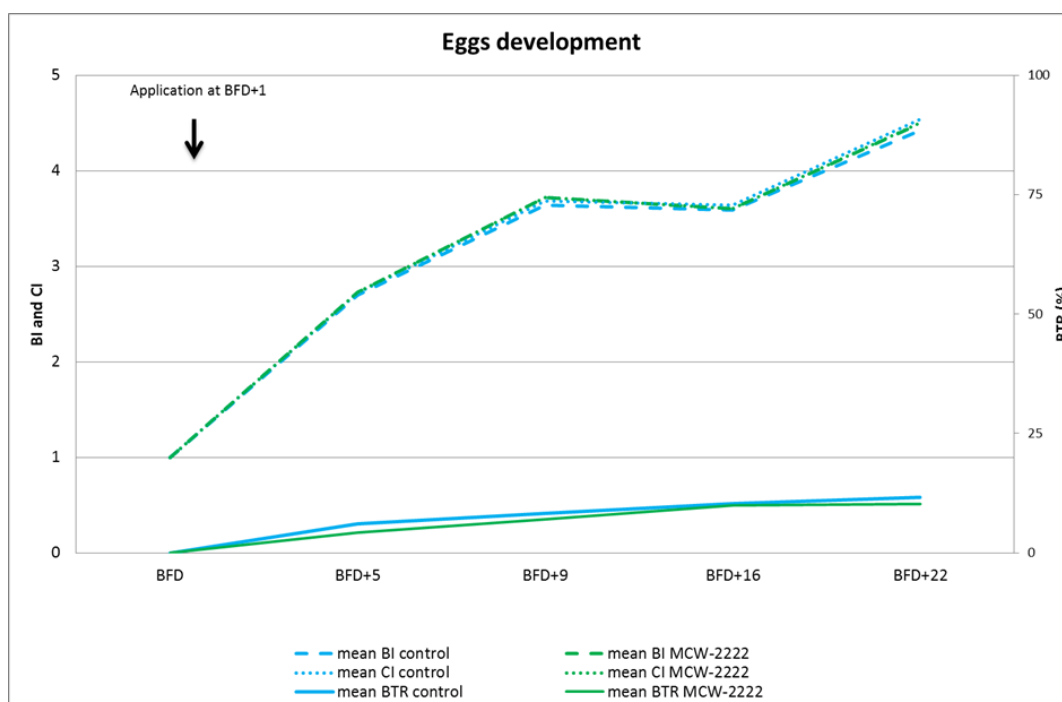


Figure A 20: Development of eggs (BTR, BI, BCI)

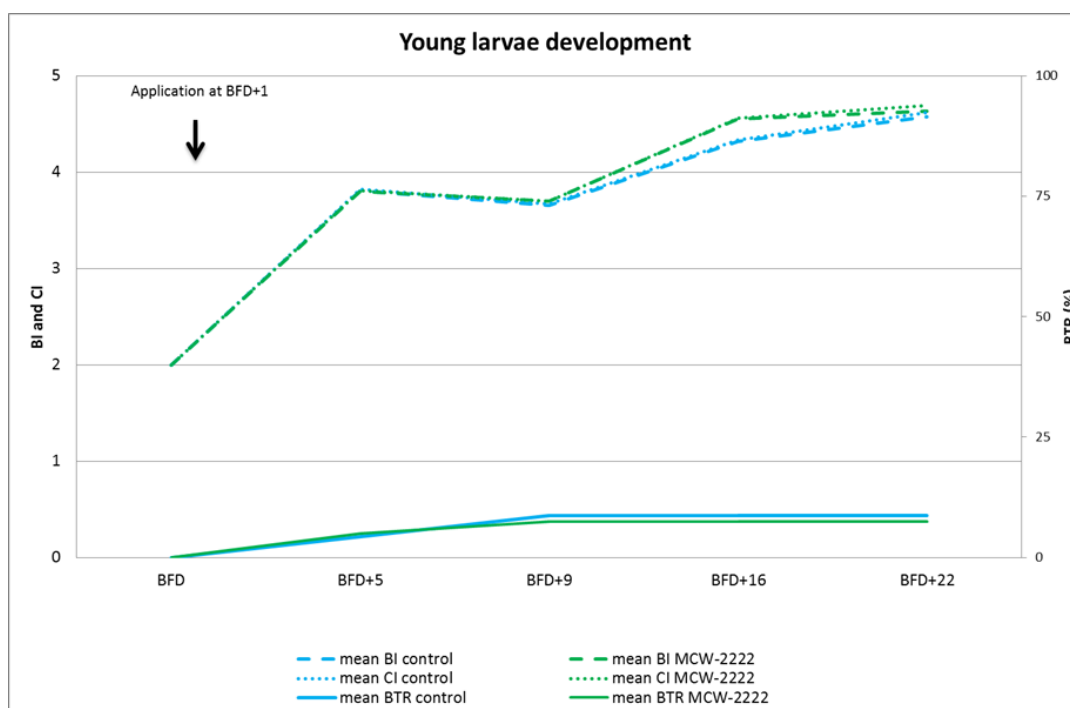
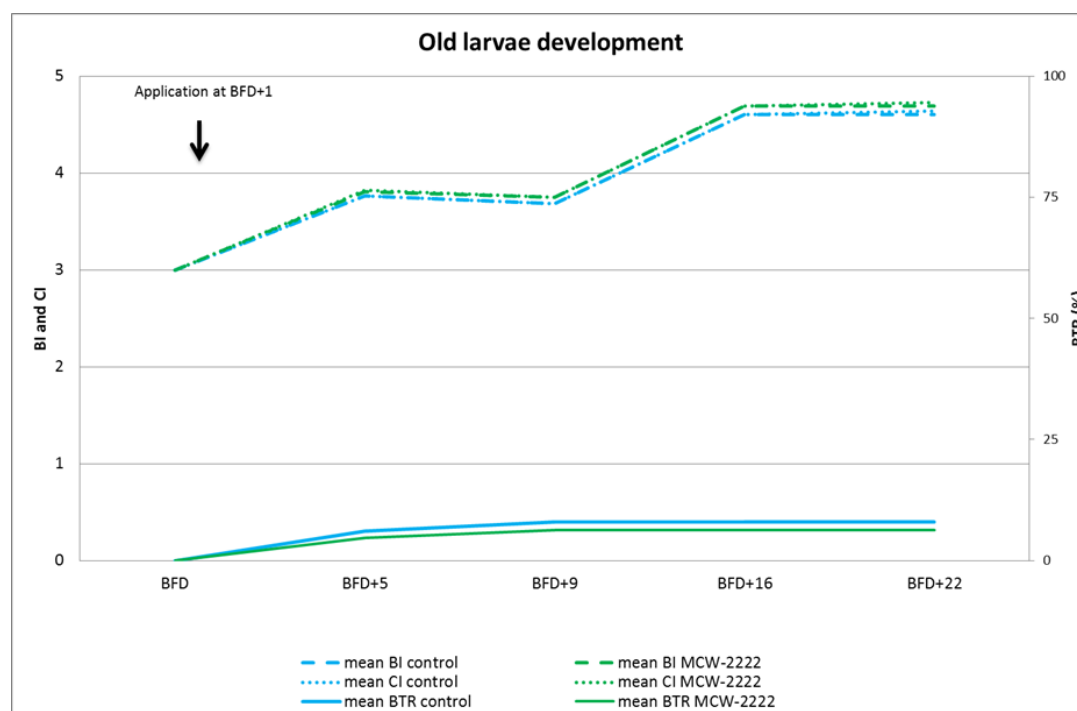


Figure A 21: Development of young larvae (BTR, BI, BCI)



**Figure A 22: Development of old larvae (BTR, BI, BCI)**

**Table A 90: 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 91: 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 92: 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

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<sup>-1</sup> (mean of 0.018 ±0.004 mg.kg<sup>-1</sup>) 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 93: 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
<b>Before treatment:</b>	
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.  <b>Criterion was achieved</b>
<b>After treatment:</b>	
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%  <b>Criterion was achieved</b>
<b>Additional validity criteria according to study plan</b>	<b>Observed in study</b>
<b>Before treatment:</b>	

The foraging activity should be significant in each field (over 3 bee/m <sup>2</sup> ) and comparable between treatments	C: 7.9 to 9.2 bees/m <sup>2</sup> T: 5.9 to 10.6 bees/m <sup>2</sup>  <b>Criterion was achieved</b>
<b>After brood fixing day:</b>	
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  <b>Criterion was achieved</b>
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%  <b>Criterion was achieved</b>
Weather conditions must remain favourable (mean temperature between 15°C and 30°C)	<b>Criterion was achieved at most days</b>
Any other phenomena that have been considered as abnormal in the course of the study will be reported	None observed  <b>Criterion was achieved</b>

## 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 honeybees 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 for purposes of the current assessment, because higher tier data were not required to support the risk assessment in 2018 and initial evaluation was rather brief.</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</p>
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	<p>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> <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</p>
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	<p>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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<b>Reference:</b>	KCP 10.3.1.6/02
<b>Report</b>	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> L.) on Oilseed Rape. Molitor, C., 2015, R-35844
<b>Guideline(s):</b>	EPPO 1/170 (4) (2010), C.E.B methodology n°230, part IV (2003), OECD GD75 (2007)
<b>Deviations:</b>	None
<b>GLP:</b>	Yes, certified laboratory
<b>Acceptability:</b>	In general the test design acceptable, but results must be treated with caution due to other oilseed rape fields present at ~1 km from the test field
<b>Duplication (if vertebrate study)</b>	Not applicable

## 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. 25<sup>th</sup> 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. 9<sup>th</sup> 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 (2<sup>nd</sup>) 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 m<sup>2</sup> 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 Liebefeld 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 (2<sup>nd</sup>) 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 m<sup>2</sup> in the control field and 4.5 bees/10 m<sup>2</sup> 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/10m<sup>2</sup> in the control plot and 6 and 13 bees/10m<sup>2</sup> 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 statically 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 treatments 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

<b>Test item</b>	MCW-2222
<b>Batch #</b>	659-030314-01
<b>Content of active substance</b>	Acetamiprid 200 g/L (nominal); 199.2 ± 1.3 g/L (analysed)
<b>Description</b>	Not given
<b>Control</b>	C: Untreated crop
<b>Toxic reference</b>	none
<b>Test organism</b>	
<b>Species</b>	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 - with 1 to 5 storage frames

	<p>-with 0 to 2 empty frames</p> <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>
<b>Source</b>	local beekeeper, Bernard Bru
<b>Food/feeding</b>	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.
<b>Study design and methods</b>	
<b>Test duration</b>	<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>
<b>Experimental dates</b>	25 <sup>th</sup> March to 20 <sup>th</sup> May 2015
<b>Test doses</b>	<p>2 x 100 g a.s./ha</p> <p>1<sup>st</sup> application on 25<sup>th</sup> March 2015 (15DBLA) without hives present at the field</p> <p>2<sup>nd</sup> application on 9<sup>th</sup> April 2015 (0DBLA), with hives present at the field, applied after bee flight</p> <p>The 2<sup>nd</sup> 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>
<b>Test units</b>	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.
<b>Group size/replicates</b>	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.
<b>Endpoints and assessments</b>	<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 m<sup>2</sup> 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"> <li>- estimated number of bees (colony strength) acc. to Liebefeld method</li> <li>- number of cells containing brood (total of cells with eggs, larvae and capped brood) to Liebefeld method</li> <li>- presence of queens (e.g. presence of eggs)</li> </ul>



- 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), an 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 2<sup>nd</sup> 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 <sup>st</sup> application	2 <sup>nd</sup> 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 m<sup>2</sup> in the control field and 4.5 bees/10 m<sup>2</sup> 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/10m<sup>2</sup> in the control plot and 6 and 13 bees/10m<sup>2</sup> 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.

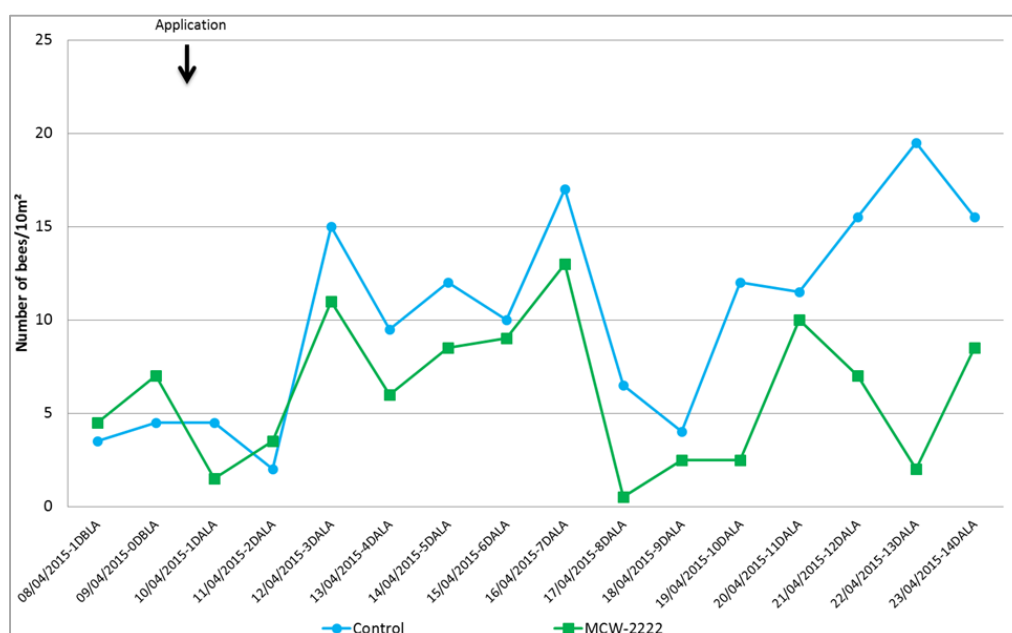


Figure A 23: Daily mean foraging activity

**Table A 94: Daily mean foraging activity**

Date	Timing	Number of bees/m <sup>2</sup>	
		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

← 2<sup>nd</sup> 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.

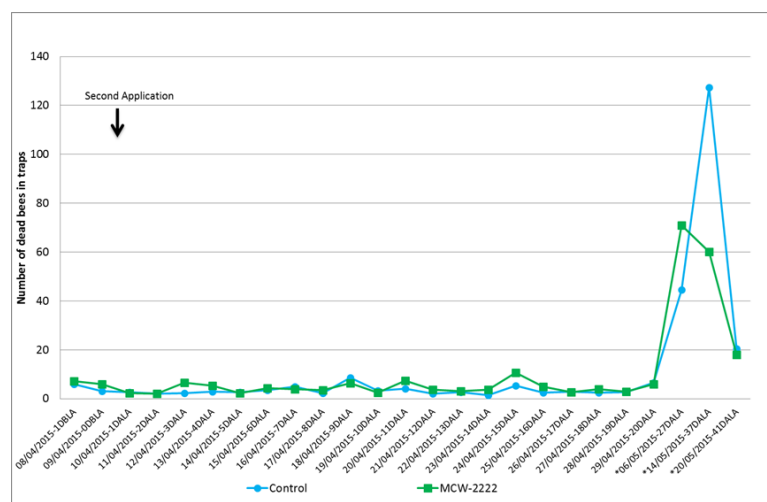
### Mortality

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 24: Daily mean mortality of adult bees**

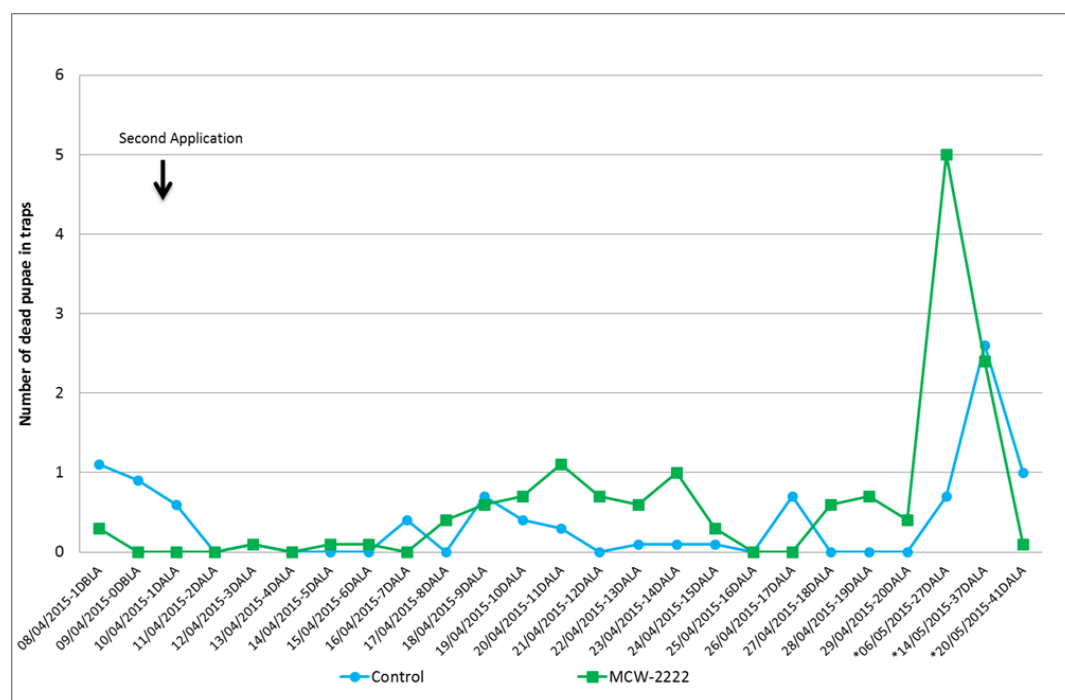
**Table A 95: Daily mean mortality of adult bees**

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

← 2<sup>nd</sup> Application after  
bee flight

DBLA = days before last (second) application; DALA = days after lat (second) application

\* The mortality at 27, 3 and 41DALA is a cumulative mortality from the previous assessment timing



**Figure A 25: Daily mean mortality of bee pupae**

**Table A 96: 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

← 2<sup>nd</sup> Application after  
bee flight

DBLA = days before last (second) application; DALA = days after lat (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 ietm groupo 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.

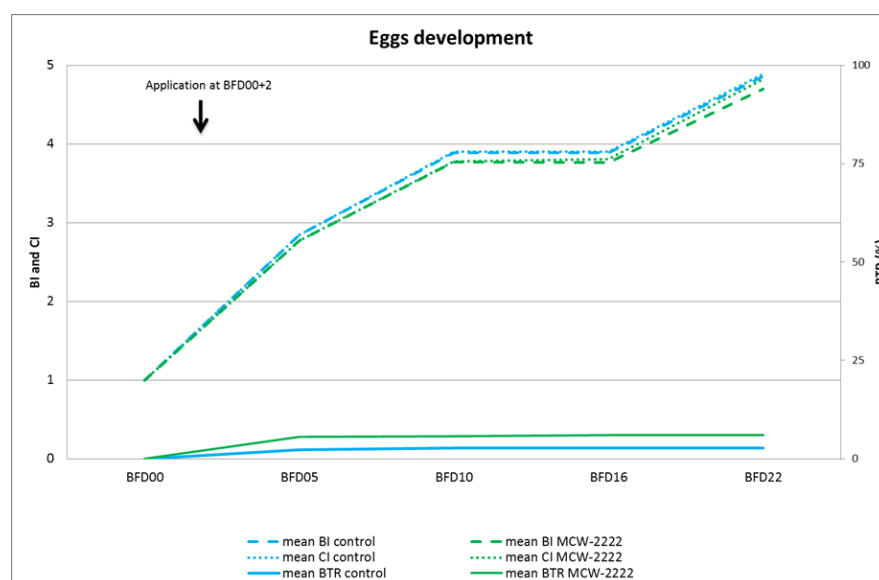
The value of 5 (successful development) was reached at BFD22 in most cells. Mean BIs at the end of the experimental phase were very close to 5 whatever the development stage selected at BFD00:

- 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.

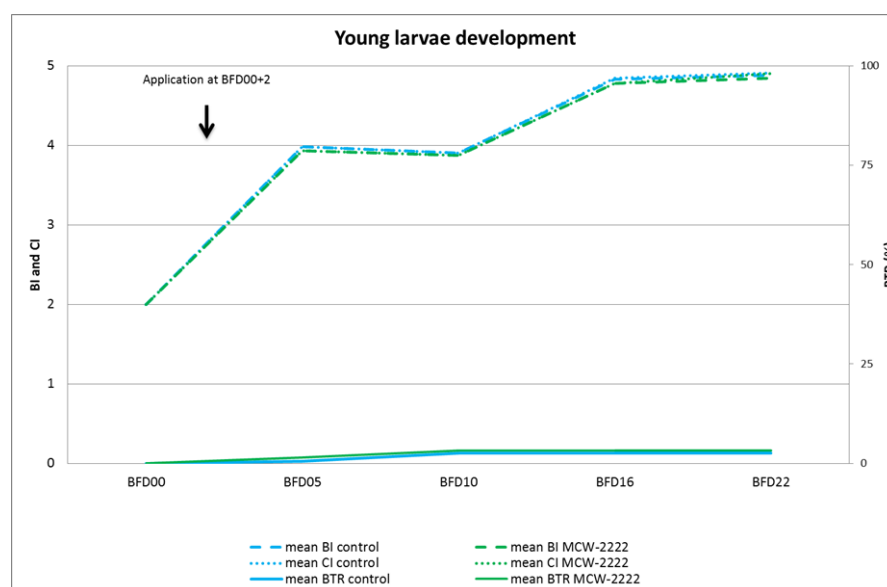
No statistical difference between both treatment and between both treatments over the day was found.

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.

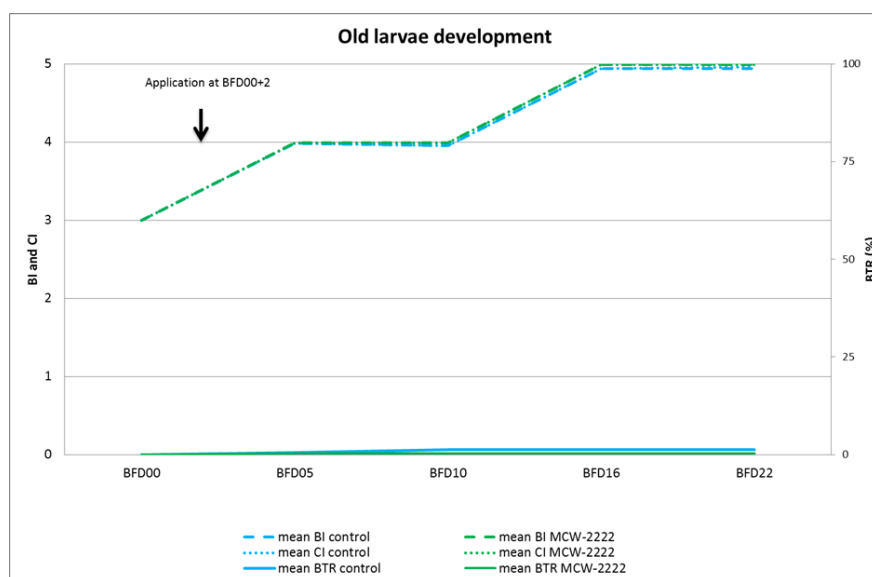


**Figure A 26: Development of eggs (BTR, BI, BCI)**



**Figure A 27: Development of young larvae (BTR, BI, BCI)**





**Figure A 28:** Development of old larvae (BTR, BI, BCI)

**Table A 97:** Brood termination rate (%) per hive and per treatment over the time

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 98: Brood index per hive and per treatment over the time**

Table A 30: 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 99: Brood compensation index per hive and per treatment over the time**

Table A 99: 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	<b>1.00</b>	1.00	1.00	1.00	1.00	<b>1.00</b>
12/04/15	BFD05	3 DALA	2.94	2.72	3.10	2.60	<b>2.84</b>	3.11	2.41	2.40	3.16	<b>2.77</b>
17/04/15	BFD10	8 DALA	4.00	3.88	3.80	3.90	<b>3.90</b>	3.88	3.96	3.39	3.89	<b>3.78</b>
23/04/15	BFD16	14 DALA	4.00	3.88	3.80	3.92	<b>3.90</b>	3.87	3.96	3.45	3.96	<b>3.81</b>
29/04/15	BFD22	20 DALA	5.00	4.85	4.76	4.93	<b>4.89</b>	4.93	4.95	4.48	4.93	<b>4.82</b>
	Young Larvae											
07/04/15	BFD00	2 DBLA	2.00	2.00	2.00	2.00	<b>2.00</b>	2.00	2.00	2.00	2.00	<b>2.00</b>
12/04/15	BFD05	3 DALA	4.00	3.96	4.00	3.96	<b>3.98</b>	3.96	3.95	3.81	4.00	<b>3.93</b>
17/04/15	BFD10	8 DALA	4.00	3.88	3.88	3.84	<b>3.90</b>	3.72	3.96	3.80	4.00	<b>3.87</b>
23/04/15	BFD16	14 DALA	4.88	4.81	4.84	4.84	<b>4.84</b>	4.53	4.92	4.71	4.95	<b>4.78</b>
29/04/15	BFD22	20 DALA	5.00	4.85	4.86	4.91	<b>4.91</b>	4.80	4.97	4.82	5.00	<b>4.90</b>
	Old Larvae											
07/04/15	BFD00	2 DBLA	3.00	3.00	3.00	3.00	<b>3.00</b>	3.00	3.00	3.00	3.00	<b>3.00</b>
12/04/15	BFD05	3 DALA	4.00	3.92	4.00	4.00	<b>3.98</b>	3.96	4.00	4.00	4.00	<b>3.99</b>
17/04/15	BFD10	8 DALA	4.00	3.88	4.00	3.92	<b>3.95</b>	3.96	4.00	4.00	4.00	<b>3.99</b>
23/04/15	BFD16	14 DALA	5.00	4.85	5.00	4.90	<b>4.94</b>	4.95	5.00	5.00	5.00	<b>4.99</b>
29/04/15	BFD22	20 DALA	5.00	4.85	5.00	4.92	<b>4.96</b>	4.95	5.00	5.00	5.00	<b>4.99</b>

### Residue analysis

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 100: 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
<b>Before treatment:</b>	
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.  <b>Criterion was achieved</b>
The foraging activity should be significant in each field and comparable between treatments	C: 4.5 bees/10m <sup>2</sup> T: 7 bees/10m <sup>2</sup>  <b>Criterion was achieved</b>
<b>After treatment:</b>	
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%  <b>Criterion was achieved</b>
<b>Additional validity criteria according to study plan</b>	<b>Observed in study</b>
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  <b>Criterion was achieved</b>
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%  <b>Criterion was achieved</b>
Weather conditions must remain favourable	<b>Criterion was achieved</b>
Any other phenomena that have been considered as abnormal in the course of the study will be reported	None observed  <b>Criterion was achieved</b>

## 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 assesment 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 honeybee 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 for purposes of the current assessment, because higher tier data were not required to support the risk assessment in 2018 and initial evaluation was rather brief.</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 7<sup>th</sup> 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</p>
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	<p>were present just next to the test plots. Their flowering status is not provided.</p> <p>The colonies comprising of approximately 6000 young bees, &gt;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) 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.</p> <p>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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<b>Reference:</b>	KCP 10.3.1.6/03
<b>Report</b>	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
<b>Guideline(s):</b>	OECD GD75 (2007), adapted to field situations
<b>Deviations:</b>	None
<b>GLP:</b>	Yes, certified laboratory
<b>Acceptability:</b>	Results not fully reliable due to potentially reduced exposure. Furthermore, clear effect on BTR was seen, which could be of biological relevance, even if statistically not significant.
<b>Duplication (if vertebrate study)</b>	Not applicable

## 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. 9<sup>th</sup> 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. 16<sup>th</sup> 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 (2<sup>nd</sup>) 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 4.5m<sup>2</sup> 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 (2<sup>nd</sup>) 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

<b>Test item</b>	MCW-2222
<b>Batch #</b>	659-030314-01
<b>Content of active substance</b>	Acetamiprid 200 g/L (nominal); 199.2 L (analysed)
<b>Description</b>	Clear yellowish liquid
<b>Control</b>	Untreated crop
<b>Toxic reference</b>	none
<b>Test organism</b>	
<b>Species</b>	<p>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.</p> <p>Test colonies were housed in 6 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 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>
<b>Source</b>	Commercial farm, in 12100 Cuneo (CN), Italy
<b>Food/feeding</b>	Full flowering apple ( <i>Malus domestica</i> , variety Gala, red group) served as food supply, no additional feeding throughout the study.
<b>Study design and methods</b>	
<b>Test duration</b>	<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>
<b>Experimental dates</b>	9 <sup>th</sup> April to 11 <sup>th</sup> May 2015
<b>Test doses</b>	<p>2 x 100 g a.s./ha</p> <p>1<sup>st</sup> application on 9<sup>th</sup> April 2015 (-7 DAB; BBCH 57) without hives present in</p>



the plots

2<sup>nd</sup> application on 16<sup>th</sup> April 2015 (0 DAB; BBCH 65) with hives present in the plots but applied after bee flight

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.

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.

#### Test units

Three study orchards (plots) with flowering apple (*Malus domestica*) (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.

Each study field with 8 colonies.

#### Group size/replicates

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.

#### Endpoints and assessments

##### *foraging and flight activity:*

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.

##### *mortality of adult bees, larvae and pupae:*

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 m<sup>2</sup> (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.

The number of dead bees was recorded daily from - 3 DAB (= BFD 0) until 21 DAB (Days after Application B) (= BFD 24).

##### *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 cm<sup>2</sup> 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 dm<sup>2</sup> (colony strength) the number of squares was multiplied by a conversion factor of 125 bees/dm<sup>2</sup>, while the estimated area containing brood was multiplied by a conversion factor of 380 cells/dm<sup>2</sup>.

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/2<sup>nd</sup> application)

BFD = brood area fixing day

#### **Adaptation of bees**

Colonies were set-up at the fields seven days before the 2<sup>nd</sup> 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<sup>st</sup> application    2<sup>nd</sup> 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 <sup>st</sup> application	2 <sup>nd</sup> 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 recored 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 trasformations) 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 29: 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 101: 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

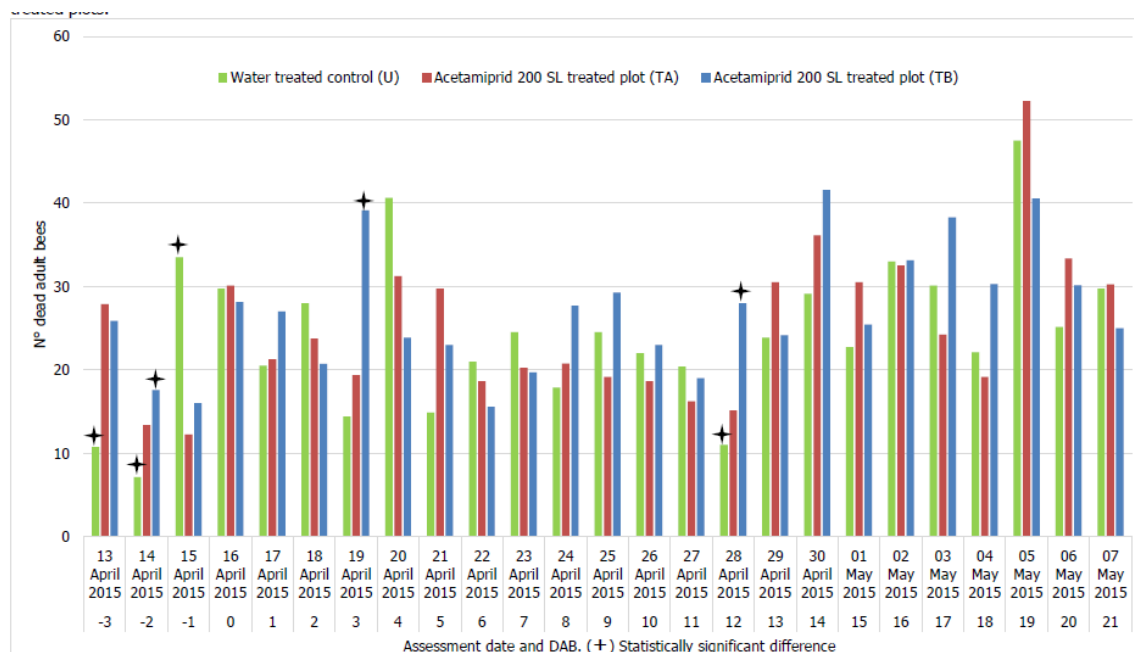
rate of 100 g a.s./ha did not cause any adverse effect on the adult worker bee population.

**Table A 102: Daily mean mortality of adult bees**

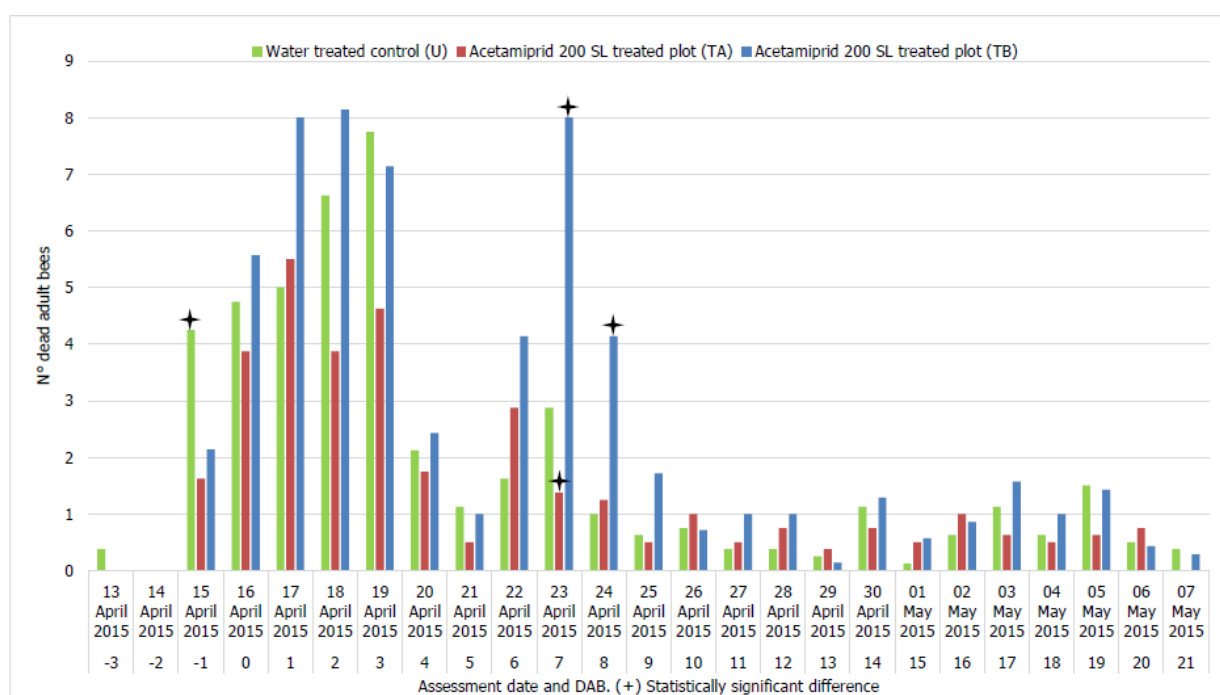
Date	Timing (DAB)	Mean adult bee mortality [n]recorded by					
		bee traps			collecting sheets		
		Control	Acetamiprid 200 SL Plot A	Acetamiprid 200 SL Plot B	Control	Acetamiprid 200 SL Plot A	Acetamiprid 200 SL Plot B
13/04/2015	-3	10.75 b	27.88 a	25.60 a	0.38 a	0.00 a	0.04 a
14/04/2015	-2	7.13 b	13.38 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.



**Mean number of dead adult bees found in dead bee traps**



**Mean number of dead adult bees found on the collecting sheets**

### 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 103: 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 104: 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 105: 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.

Figure 3. Mean values of % Brood Termination Rate at each assessment

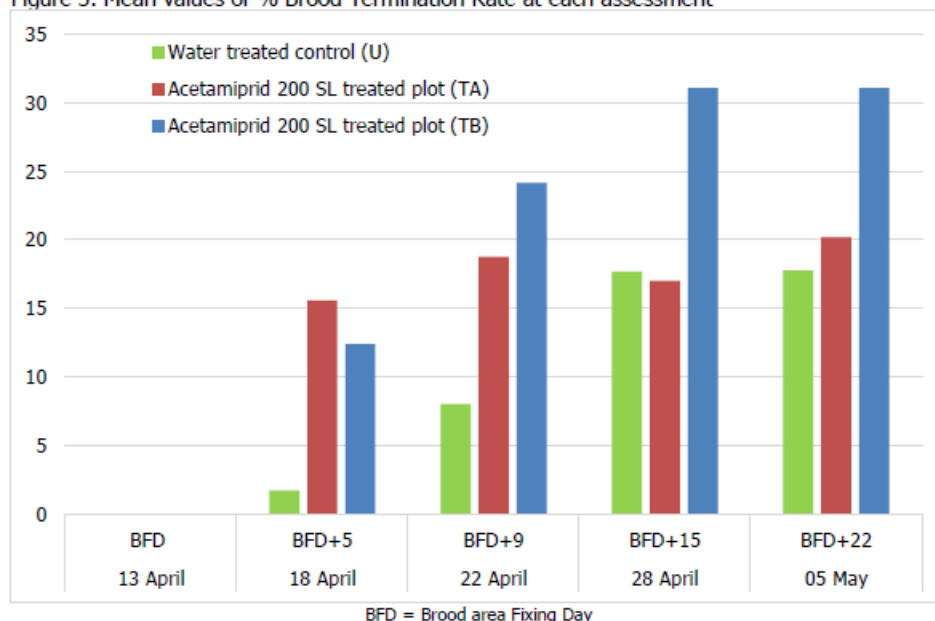
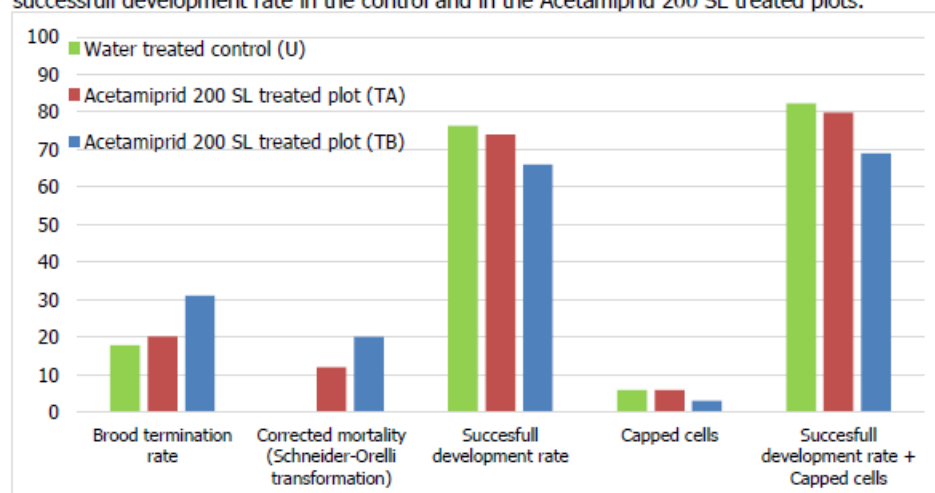


Figure 5. Mean values of % brood termination rate, % capped cells and % total brood successful development rate in the control and in the Acetamiprid 200 SL treated plots.



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 106: Mean Brood Index on each assessment date after BFD 0

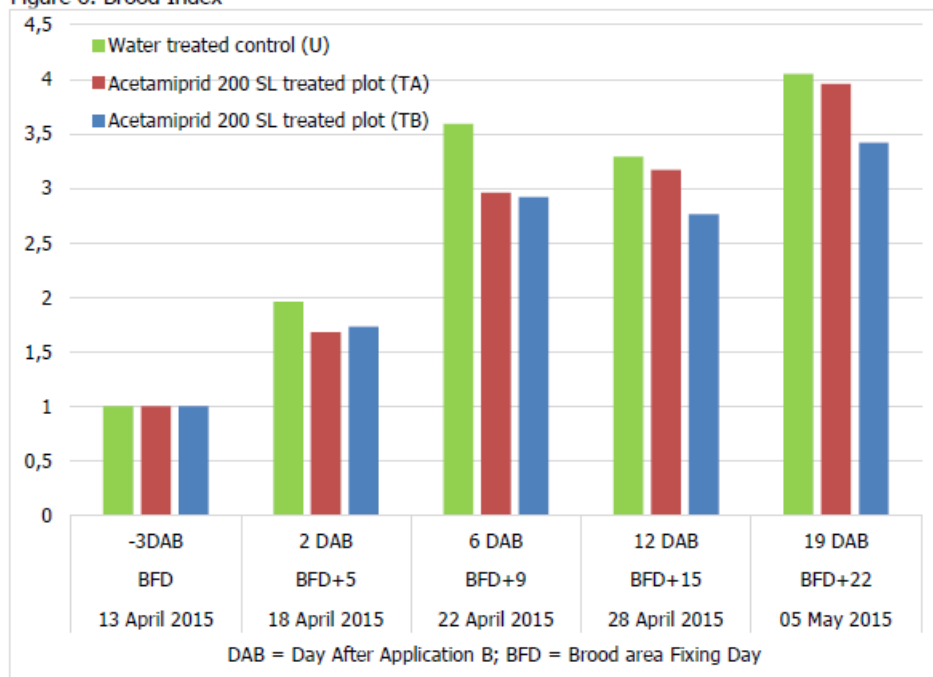
	-3 DAB	2 DAB	6 DAB	12 DAB	19 DAB
Treatment	BFD 0	BFD +5	BFD +9	BFD +15	BFD +22
Control	1.00	1.96 a	3.59 a	3.29 a	4.05 a
Acetamiprid 200 SL Plot A	1.00	1.68 a	2.96 a	3.17 a	3.96 a
Acetamiprid 200 SL Plot B	1.00	1.73 a	2.92 a	2.76 a	3.42 a

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.



Figure 6. Brood Index



### Residue analysis

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 107:** 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

Treatment	-1 DAB BFD+2	1 DAB BFD +4	7 DAB BFD +10	14 DAB 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<sup>-1</sup>

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 108: 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<sup>-1</sup>

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 intervall (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 109: Validity criteria**

No validity criteria are given by OECD GD 75 (2007) but by the study plan	Observed in study
<b>before treatment</b>	
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.  <b>Criterion was achieved</b>
<b>After treatment</b>	
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 the study can be considered valid because: <ul style="list-style-type: none"> <li>the mortality values of adults, pupae and larvae in dead bee traps and in 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,</li> <li>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</li> </ul> <b>Criterion was achieved</b>

## 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

#### A 2.3.2.1.1 KCP 10.3.2.1/01 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>LR<sub>50</sub> = 9.13 g a.s./ha ER<sub>50</sub> &gt;6.17 g a.s./ha</p>
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<b>Reference:</b>	KCP 10.3.2.1/01
<b>Report</b>	Effects of MCW-2222 on the predatory mite <i>Typhlodromus pyri</i> SCHEUTEN in a laboratory test - Rate-Response-Test (LR <sub>50</sub> ) -, Röhlig, U., 2014, R-33838
<b>Guideline(s):</b>	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.
<b>Deviations:</b>	No
<b>GLP:</b>	Yes, certified laboratory
<b>Acceptability:</b>	Acceptable
<b>Duplication (if vertebrate study)</b>	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 LR<sub>50</sub> for MCW-2222 was calculated to be 9.13 g a.s./ha. The ER<sub>50</sub> for MCW-2222 was estimated to be > 6.17 g a.s./ha in 200 L water/ha.

### Materials and methods

#### Materials

<b>Test item</b>	MCW-2222
<b>Batch #</b>	611-280413-01
<b>Content of active substance</b>	Acetamiprid 200 g/L (nominal); 202.7 g/L (analysed)
<b>Description</b>	Yellowish liquid
<b>Control</b>	Deionised water
<b>Toxic reference</b>	Dimethoate (content: 400 g/L)
<b>Test organism</b>	
<b>Species</b>	<i>Typhlodromus pyri</i> , protonymphs (< 24 hours old)
<b>Source</b>	In-house culture, originally obtained from Dr. Peter Katz, PK-Nützlingszuchten, Industriestraße 38, D-73642 Welzheim
<b>Acclimatisation</b>	The test organisms were and kept in the test arenas with conditions similar to the test conditions
<b>Study design and methods</b>	
<b>Test duration and exposure</b>	14 days of exposure on a dried glass plate prior sprayed with respective test rate.
<b>Experimental dates</b>	18 February to 04 March 2014.

<b>Test rates</b>	1.91, 3.43, 6.17, 11.1 and 20 g a.s./ha
<b>Test units</b>	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
<b>Food</b>	Pollen, <i>Pinus nigra</i> and <i>Betula pendula</i> (each assessment day)
<b>Group size/replicates</b>	100 organisms per treatment; 20 in each of 5 replicates per treatment group
<b>Environmental conditions</b>	
<b>Temperature</b>	23 – 27 °C
<b>Photoperiod</b>	16 h light/8 h dark; 1950 lx
<b>Relative humidity</b>	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 LR<sub>50</sub> 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 110: 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 (%) <sup>1</sup>	7.0	8.0	20.0*	60.0*	96.0*	3.0	81.0*
Corrected mortality (%) <sup>2</sup>	4.1	5.2	17.5	58.8	95.9	-	80.4

<sup>1</sup> 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 ( $\alpha = 0.05$ ).

<sup>2</sup> 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 111: Reproduction of female mites during the 7 day egg laying period**

Mites	Control	Test substance [mL test item/ha]				
		1.91	3.43	6.17	11.1	20.0
Reproduction rate (mean no. of eggs/female) <sup>1</sup>	6.41	6.45	5.03*	4.67*	-	-
Effect on reproduction (%) <sup>2</sup>	-	-0.6	21.5	27.1	-	-

<sup>1</sup> Results for reproduction compared by WILLIAMS t-test ( $\alpha > 0.05$ ).

<sup>2</sup> Negative values indicate an increase in reproduction

\*Statistically significantly different compared to the control

**Table A 112: Endpoints**

	Endpoints
<b>LR<sub>50</sub></b> <b>(95% CI)</b>	9.13 g a.s./ha 5.72 - 14.56 g a.s./ha
<b>ER<sub>50</sub></b>	> 6.17 g a.s./ha

### Validity criteria

As shown in the following table, all validity criteria were met.

**Table A 113: 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 LR<sub>50</sub> for MCW-2222 was calculated to be 9.13 g a.s./ha. The ER<sub>50</sub> 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 were agreed:</p> <p>LR<sub>50</sub> = 0.0243 g a.s./ha</p> <p>Effects on reproduction were not investigated.</p>
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<b>Reference:</b>	KCP 10.3.2.1/02
<b>Report</b>	Effects of MCW-2222 on the parasitic wasp <i>Aphidius rhopalosiphi</i> (DESTEFANI-PEREZ) in a laboratory test - Rate-Response-Test (LR <sub>50</sub> ) - Röhlig, U., 2014, R-33839
<b>Guideline(s):</b>	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)
<b>Deviations:</b>	No
<b>GLP:</b>	Yes, certified laboratory
<b>Acceptability:</b>	Acceptable
<b>Duplication (if vertebrate study)</b>	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 LR<sub>50</sub> for MCW-2222 was calculated to 0.02430 g a.s./ha.

## Materials and methods

### Materials

<b>Test item</b>	MCW-2222
<b>Batch #</b>	611-280413-01
<b>Content of active substance</b>	Acetamiprid 200 g/L (nominal); 202.7 g/L (analysed)
<b>Description</b>	Yellowish liquid
<b>Control</b>	Deionised water
<b>Toxic reference</b>	Dimethoate (content: 400 g/L)
<b>Test organism</b>	
<b>Species</b>	<i>Aphidius rhopalosiphi</i> , less than 48 hours old
<b>Source</b>	In-house culture, originally obtained from Katz Biotech AG, an der Birkenpfuhlheide 10, 15837 Baruth, Germany
<b>Rearing</b>	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

<b>Test duration and exposure</b>	48 hours of exposure on a dried glass plate prior sprayed with respective test rate.
<b>Experimental treatments</b>	
<b>Experimental dates</b>	17 - 19 February 2014
<b>Test rates</b>	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
<b>Test units</b>	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)
<b>Group size/replicates</b>	40 organisms (28 females, 12 males) per treatment; 10 in each of 4 replicates per treatment group
<b>Environmental conditions</b>	
<b>Temperature</b>	19-22 °C
<b>Photoperiod</b>	light / dark 16 / 8 h, 1020 lx
<b>Relative humidity</b>	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 EC<sub>50</sub> 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 114: 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 (%) <sup>1</sup>	0	0	15*	47.5*	77.5*	82.5*	97.5*	100

<sup>1</sup> 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 115: Acute toxicity of test item to *Aphidius rhopalosiphi***

	Endpoints
LR <sub>50</sub> 48 h (95% CI)	0.02430 g a.s./ha 0.01966-0.03005 g a.s./ha

#### Validity criteria

As shown in the following table, all validity criteria were met.

**Table A 116: 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 LR<sub>50</sub> 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>LR<sub>50</sub> = 31.9 g a.s./ha ER<sub>50</sub> &gt;12.5 g a.s./ha</p>
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Reference:	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 (LR <sub>50</sub> ) -, Röhlig, U., 2014, R-34780
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:	No
GLP:	Yes, certified laboratory
Acceptability:	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 LR<sub>50</sub> 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 ER<sub>50</sub> 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
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 (TEMMEN 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 LR<sub>50</sub> 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 117: Mortality and effect on the reproductive capacity of *Typhlodromus pyri* following seven days exposure to MCW-2222**

Treatment	Rate <sup>a</sup> [g a.s./ha]	Mortality <sup>b</sup> [%]	Corrected Mortality <sup>c</sup> [%]	Reproduction <sup>d</sup> [eggs/female]	Effect on reproduction <sup>e</sup> [%]
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	-	-

<sup>a</sup> Application rate in 200 L water/ha

<sup>b</sup> 7 day mortality rate (Fisher Exact Binomial Test with BONFERRONI correction ( $\alpha = 0.05$ ))

<sup>c</sup> Corrected mortality according to Abbott (1925)

<sup>d</sup> 14 day reproduction rate (WILLIAMS-t-test ( $\alpha = 0.05$ ))

<sup>e</sup> Calculated on the exact raw data; negative values mean increased reproduction compared to control

\*Statistically significantly different compared to the control

**Table A 118: Endpoints**

	Endpoints
<b>LR<sub>50</sub></b> <b>(95% CI)</b>	31.9 g a.s./ha 16.3 – 62.4 g a.s./ha
<b>ER<sub>50</sub></b>	> 12.5 g a.s./ha

### Validity criteria

As shown in the following table, all validity criteria were met.

**Table A 119: 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 LR<sub>50</sub> 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 ER<sub>50</sub> 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 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>LR<sub>50</sub> = 0.555 mL product/ha (corresponding to 0.111 g a.s./ha) ER<sub>50</sub> &gt;0.502 mL product/ha (corresponding to 0.100 g a.s./ha)</p>
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Reference:	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, R-35026
Guideline(s):	Mead-Briggs <i>et al.</i> 2009
Deviations:	No
GLP:	Yes, certified laboratory
Acceptability:	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 (LR<sub>50</sub>) 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. In terms of effects on the reproductive performance of surviving wasps, the median effect rate (ER<sub>50</sub>) for MCW-2222 was > 0.502 mL test item/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 Birkenpfuhlheide 10, 15837 Baruth, Germany

#### Study design and methods

Test duration and exposure	14 days, exposure phase (48 h) followed by a reproduction phase (10 days). 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> )
Experimental dates	01 October - 17 November 2014

<b>Test rates</b>	2.008, 1.004, 0.502, 0.251 and 0.126 mL test item/ha
<b>Test units</b>	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
<b>Group size/replicates</b>	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
<b>Environmental conditions</b>	
<b>Temperature</b>	21 -22 °C
<b>Photoperiod/Intensity</b>	light / dark 16 / 8 h, 1664 lx
<b>Relative humidity</b>	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 LR<sub>50</sub> 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 120: Mortality of *Aphidius rhopalosiphi* after exposure to MCW-2222**

<b>Treatment</b>	<b>Rate [mL test item/ha]</b>	<b>Mortality <sup>a</sup> [%]</b>	<b>Corrected mortality <sup>b</sup> [%]</b>
Control	0	5.0	-
MCW-2222	0.126	12.5	7.9
MCW-2222	0.251	32.5*	28.9
MCW-2222	0.502	45.0*	42.1
MCW-2222	1.004	72.5*	71.1
MCW-2222	2.008	87.5*	86.8

<sup>a</sup> Individual treatments were compared to the control by Fisher’s Exact Test ( $\alpha = 0.05$ ) and an asterisk (\*) indicates where they differed significantly.

<sup>b</sup> Corrected mortality according to Abbott.

**Table A 121: Reproduction of *Aphidius rhopalosiphi* after exposure to MCW-2222**

Treatment	Rate [mL test item/ha]	Reproduction [mean number of mummies/female] <sup>a</sup>	Effects on reproduction [%] <sup>b</sup>
Control	0	14.1	-
MCW-2222	0.126	17.1	-21.3
MCW-2222	0.251	15.0	-6.6
MCW-2222	0.502	14.3	-1.9
MCW-2222	1.004	n.d.	-
MCW-2222	2.008	n.d.	-

<sup>a</sup> Reproduction: mean number of parasitised aphids (mummies)/surviving female. The results for the test item

<sup>b</sup> 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 122: Acute toxicity of test item to *Aphidius rhopalosiphi***

	Endpoints
<b>LR<sub>50</sub> 48 h (95% CI)</b>	0.555 mL test item/ha 0.408-0.733 mL test item/ha
<b>ER<sub>50</sub></b>	> 0.502 mL test item/ha (effects)
<b>NOER</b>	0.126 mL test item/ha (mortality) 0.502 mL test item/ha (reproduction)

### Validity criteria

As shown in the following table, all validity criteria were met.

**Table A 123: 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 $\geq 5$ 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 (LR<sub>50</sub>) 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. In terms of effects on the reproductive performance of surviving wasps, the median effect rate (ER<sub>50</sub>) for MCW-2222 was > 0.502 mL test item/ha and the NOER was 0.502 mL test item/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>LR<sub>50</sub> = 3.56 g a.s./ha ER<sub>50</sub> could not be determined (effects &gt;50% at 0.64 g a.s./ha, the lowest rate tested)</p>
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Reference:	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 (LR <sub>50</sub> ) -, Röhlig, U., 2014, R-33839A, 14 10 48 037 A
Guideline(s):	IOBC (Mead-Briggs <i>et al.</i> 2009)
Deviations:	No
GLP:	Yes, certified laboratory
Acceptability:	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 LR<sub>50</sub> 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 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	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.
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	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> )
<b>Experimental dates</b>	07 - 21 April 2014
<b>Test rates</b>	0.64, 1.4, 3.1, 6.8 and 15 g a.s./ha
<b>Test units</b>	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.
<b>Group size/replicates</b>	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.
<b>Environmental conditions</b>	
<b>Temperature</b>	19 – 22 °C
<b>Photoperiod/Intensity</b>	light / dark 16 / 8 h, Mortality phase: 1020 lx Paratisation phase: 4210 lx Reproduction phase: 6250 lx
<b>Relative humidity</b>	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 LR<sub>50</sub>, 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 LR<sub>50</sub> 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 124: Mortality of *Aphidius rhopalosiphi* after exposure to MCW-2222**

Treatment	Rate <sup>a</sup> [g a.s./ha]	Mortality <sup>b</sup> [%]	Corrected mortality <sup>c</sup> [%]
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

<sup>a</sup>) Application rate in 400 L water/ha.

<sup>b</sup>) 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 ( $\alpha = 0.05$ ).

<sup>c</sup>) Corrected mortality according to Abbott (1925).

\* statistically significantly different compared to the control.

**Table A 125: Reproduction of *Aphidius rhopalosiphi* after exposure to MCW-2222**

Treatment	Rate <sup>a</sup> [g a.s./ha]	Reproduction [mean number of mummies/female] <sup>b</sup>	Effects on reproduction [%] <sup>c</sup>
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.	-

<sup>a</sup>) Application rate in 400 L water/ha.

<sup>b</sup>) Reproduction: mean number of parasitised aphids (mummies)/surviving female. The results for the test item

<sup>c</sup>) 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 126: Acute toxicity of test item to *Aphidius rhopalosiphi***

	Endpoints
LR <sub>50</sub> 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 127: 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 LR<sub>50</sub> 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>LR<sub>50</sub> = 106.0 g a.s./ha ER<sub>50</sub> &gt;116.0 g a.s./ha</p>
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Reference:	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 (LR <sub>50</sub> ) -, Röhlig, U., 2014, R-34781
Guideline(s):	IOCB (Vogt <i>et al.</i> , 2000)
Deviations:	No
GLP:	Yes, certified laboratory
Acceptability:	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 LR<sub>50</sub> 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 ER<sub>50</sub> 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
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<b>Experimental treatments</b>	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.
<b>Experimental dates</b>	12 June to 18 July 2008
<b>Test rates</b>	11, 24, 53, 116 and 255 g a.s./ha
<b>Test units</b>	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)
<b>Food</b>	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
<b>Group size/replicates</b>	40 organisms per treatment; 1 larvae in each of 40 replicates per treatment group
<b>Environmental conditions</b>	
<b>Temperature</b>	23 – 27 °C
<b>Photoperiod</b>	16 h light, 8 h dark; 1260 lx
<b>Relative humidity</b>	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 LR<sub>50</sub> 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 128: Mortality of *Chrysoperla carnea* after exposure to MCW-2222**

Treatment	Rate <sup>a</sup> [g a.s./ha]	Pre-imaginal mortality <sup>b</sup> [%]	Corrected pre-imaginal mortality <sup>c</sup> [%]
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

<sup>a</sup> Application rate in 200 L water/ha

<sup>b</sup> FISHER`s Exact Binomial test with Bonferroni Correction ( $\alpha = 0.05$ )

<sup>c</sup> Corrected pre-imaginal mortality according to Abott (1925)

\*Statistically significantly different compared to the control

**Table A 129: Reproduction of *Chrysoperla carnea* after exposure to MCW-2222**

Treatment	Rate <sup>a</sup> [g a.s./ha]	Reproduction [eggs/female/day] <sup>b</sup>	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	-	-

<sup>a</sup>Application rate in 200 L water/ha

<sup>b</sup> Based on all eggs laid on the fibrous tissue sheet

**Table A 130: Endpoints**

	Endpoints
LR <sub>50</sub> (95% CI)	106 g a.s./ha 89 - 12516.3 – 62.4 g a.s./ha
ER <sub>50</sub>	> 116 g a.s./ha

### Validity criteria

As shown in the following table, all validity criteria were met.

**Table A 131: 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 $\geq 15$ 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 LR<sub>50</sub> 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 ER<sub>50</sub> 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>LR<sub>50</sub> = 22.1 g a.s./ha ER<sub>50</sub> &gt;20.7 g a.s./ha</p>
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<b>Reference:</b>	KCP 10.3.2.2/05
<b>Report</b>	Effects of MCW-2222 on the ladybird <i>Coccinella septempunctata</i> L. in an extended laboratory test - Rate-Response-Test (LR <sub>50</sub> ) -, Röhlig, U., 2014, R-34782
<b>Guideline(s):</b>	IOBC (Schmuck <i>et al.</i> 2000) modified for the exposure on natural substrate (extended laboratory test)
<b>Deviations:</b>	No
<b>GLP:</b>	Yes, certified laboratory
<b>Acceptability:</b>	Acceptable
<b>Duplication (if vertebrate study)</b>	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 LR<sub>50</sub> 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

<b>Test item</b>	MCW-2222
<b>Batch #</b>	611-280413-01
<b>Content of active substance</b>	Acetamiprid 200 g/L (nominal); 202.7 g/L (analysed)
<b>Description</b>	Yellowish liquid
<b>Control</b>	Deionised water
<b>Toxic reference</b>	Dimethoate (content: 400 g/L)
<b>Test organism</b>	
<b>Species</b>	Larvae, 3-5 days old of seven pointed ladybird <i>Coccinella septempunctata</i> L. (Coleoptera: Coccinellidae)
<b>Source</b>	Katz Biotech AG, an der Birkenpfuhlheide 10, 15837 Baruth, Germany
<b>Food</b>	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:  $\geq 23$  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 LR<sub>50</sub> 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 132: Mortality of *Coccinella septempunctata* after exposure to MCW-2222**

Treatment	Rate <sup>a</sup> [g a.s./ha]	Mortality <sup>b</sup> [%]	Corrected mortality <sup>c</sup> [%]
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

<sup>a</sup> Application rate in 200 L water/ha.

<sup>b</sup> 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 ( $\alpha = 0.05$ )

<sup>c</sup> Corrected mortality according to Abbott (1925) \* statistically significantly different compared to the control.

**Table A 133: Reproduction of *Coccinella septempunctata* after exposure to MCW-2222**

Treatment	Rate <sup>a</sup> [g a.s./ha]	Reproduction [fertile eggs/vemal/day] <sup>b</sup>	Hatching rate [%] <sup>c</sup>
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.	-

<sup>a</sup> 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 134: Acute toxicity of test item to *Coccinella septempunctata***

	Endpoints
LR <sub>50</sub>	22.1
(95% CI)	14.5 – 33.7 g a.s./ha

### Validity criteria

As shown in the following table, all validity criteria were met.

**Table A 135: 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 LR<sub>50</sub> 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 *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 and was not evaluated earlier. 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 (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</p>
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	<p>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 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 &lt;50% after 28 days of aging.</p>
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<b>Reference:</b>	KCP 10.3.2.3/01
<b>Report</b>	Aged residue test with the formulation “MCW-2222” (Acetamiprid 20% w/v SL) at 45 g a.s./ha on the parasitic wasp <i>Aphidius rhopalosiphi</i> (Hymenoptera: Braconidae), Luna, F., 2016a, TRC15-242BA
<b>Guideline(s):</b>	Mead-Briggs <i>et al.</i> 2010, and an unpublished draft guideline by Mead-Briggs and Longley 1997
<b>Deviations:</b>	No
<b>GLP:</b>	Yes, certified laboratory
<b>Acceptability:</b>	Acceptable

## 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

<b>Test item</b>	MCW-2222 (Acetamiprid 20% w/v SL)
<b>Batch #</b>	659-030314-01
<b>Content of active substance</b>	acetamiprid: 199.2 ± 1.3 g/L, (nominal: 200 g/L)
<b>Control</b>	Tap water
<b>Toxic reference</b>	Deltamethrin 2.5% EC at 0.05% = 7.65 g a.s./ha
<b>Test organism</b>	
<b>Species</b>	Parasitic wasp <i>Aphidius rhopalosiphi</i> Adults, < 48 h old
<b>Source</b>	Katz Biotech AG”, An der Birkenpfuhlheide 10, 15837 Baruth, Germany
<b>Study design and methods</b>	
<b>Aging periods</b>	1, 28 and 36 days
<b>Exposure duration</b>	48 hours
<b>Experimental dates</b>	30 Nov 2015 to 18 Jan 2016
<b>Test doses (nominal)</b>	45 g acetamiprid/ha, equivalent to 0.2259 L/ha of formulated test item

<b>Test units</b>	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 <sup>2</sup> (5 m × 1 m) for the treatments and they were arranged in two crop rows (0.5 m to each other).
<b>Group size/replicates</b>	5 females and 5 males per replicate, 4 replicates (40 adults) per treatment
<b>Experimental treatments</b>	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 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.
<b>Environmental conditions</b>	
<b>Temperature</b>	1 DAA: 19.6–20.5 °C 28 DAA: 19.0–21.0 °C 36 DAA: 18.4–20.7 °C
<b>Photoperiod</b>	16 h light (4000–20000 lx): 8 h dark
<b>Relative humidity</b>	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 136: Effects of MCW-2222 (Acetamiprid 20% w/v SL) on mortality of the parasitoid, *Aphidius rhopalosiphi***

	Rate <sup>1)</sup> [g a.s./ha]	Bioassay					
		1 DAA <sup>2)</sup>		28 DAA		36 DAA	
		M (%)	C <sub>m</sub> (%)	M (%)	C <sub>m</sub> (%)	M (%)	C <sub>m</sub> (%)
Control	0	0.0	-	0.0	-	0.0	-
MCW-2222 (Acetamiprid 20% w/v SL)	45	100 <sup>SD</sup>	100	10.0 <sup>NS</sup>	10.0	5.0 <sup>NS</sup>	5.0
Reference Item (Deltamethrin 2.5% EC)	7.65	100	100	70	70	85	85

<sup>1)</sup> Application rate in 600 L water/ha

<sup>2)</sup> DAA = Days after application; M [%] = Mortality [%]; C<sub>m</sub> [%] = 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 137: Effects of MCW-2222 (Acetamiprid 20% w/v SL) on fecundity of the parasitoid, *Aphidius rhopalosiphi***

	Rate <sup>1)</sup> [g a.s./ha]	Bioassay					
		1 DAA <sup>2)</sup>		28 DAA		36 DAA	
		F [m/f]	R [%] <sup>4)</sup>	F [m/f]	R [%]	F [m/f]	R [%]
Control	0	N/S <sup>3)</sup>		24.4	-	42.7	-
MCW-2222 (Acetamiprid 20% w/v SL)	45	N/S <sup>3)</sup>		32.9 <sup>NS</sup>	-35.0	37.6 <sup>NS</sup>	12.0

<sup>1)</sup> Application rate in 600 L water/ha

<sup>2)</sup> DAA = Days after application; ; F [m/f]= Fecundity [mummies per female]; R [%]= Reduction [%].

<sup>3)</sup> N/S = Reproduction was not studied as mortality was > 50% in T.

<sup>4)</sup> 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 138: 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%	≥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 2.3.2.3.2 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) due to renewal of acetamiprid and was not evaluated earlier. 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 (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 &lt;50% after 28 days of aging.</p>
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<b>Reference:</b>	KCP 10.3.2.3/02
<b>Report</b>	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, TRC15-243BA
<b>Guideline(s):</b>	Mead-Briggs <i>et al.</i> 2000, and an unpublished draft guideline by Mead-Briggs and Longley 1997
<b>Deviations:</b>	No
<b>GLP:</b>	Yes, certified laboratory
<b>Acceptability:</b>	Acceptable

### 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

<b>Test item</b>	MCW-2222 (Acetamiprid 20% w/v SL)
<b>Batch #</b>	659-030314-01
<b>Content of active substance</b>	acetamiprid: 199.2 ± 1.3 g/L, (nominal: 200 g/L)
<b>Control</b>	Tap water
<b>Toxic reference</b>	Deltamethrin 2.5% EC at 0.05% = 7.65 g a.s./ha
<b>Test organism</b>	
<b>Species</b>	Parasitic wasp <i>Aphidius rhopalosiphi</i> Adults, < 48 h old
<b>Source</b>	Katz Biotech AG”, An der Birkenpfuhlheide 10, 15837 Baruth, Germany
<b>Study design and methods</b>	
<b>Ageing periods</b>	1, 28 and 36 days
<b>Exposure duration</b>	48 hours
<b>Experimental dates</b>	30 Nov 2015 to 18 Jan 2016
<b>Test doses (nominal)</b>	70 g acetamiprid/ha, equivalent to 0.3514 L/ha of formulated test item
<b>Test units</b>	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 <sup>2</sup> (5 m × 1 m) for the treatments and they were arranged in two crop rows (0.5 m to each other).
<b>Group size/replicates</b>	10 adults (≥ 5 females) per replicate, 4 replicates (40 adults) per treatment
<b>Experimental treatments</b>	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.
<b>Environmental conditions</b>	
<b>Temperature</b>	1DAA: 19.6–20.5 °C 28 DAA: 19.0–21.0 °C 36 DAA: 18.4–20.7 °C
<b>Photoperiod</b>	16 h light (4000–20000 lx) : 8 h dark
<b>Relative humidity</b>	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 139: Effects of MCW-2222 (Acetamiprid 20% w/v SL) on mortality of the parasitoid, *Aphidius rhopalosiphii***

	Rate <sup>1)</sup> [g a.s./ha]	Bioassay					
		1 DAA <sup>2)</sup>		28 DAA		36 DAA	
		M (%)	C <sub>m</sub> (%)	M (%)	C <sub>m</sub> (%)	M (%)	C <sub>m</sub> (%)
Control	0	0.0	-	0.0	-	0.0	-
MCW-2222 (Acetamiprid 20% w/v SL)	70	100 <sup>SD</sup>	100	27.5 <sup>SD 3)</sup>	27.5	20.0 <sup>SD</sup>	20.0
Reference Item (Deltamethrin 2.5% EC)	7.65	100	100	70	70	85	85

<sup>1)</sup> Application rate in 600 L water/ha

<sup>2)</sup> DAA = Days after application; M [%] = Mortality [%]; C<sub>m</sub> [%] = Corrected mortality [%].

<sup>3)</sup> 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 140: Effects of MCW-2222 (Acetamiprid 20% w/v SL) on fecundity of the parasitoid, *Aphidius rhopalosiphi***

	Rate <sup>1)</sup> [g a.s./ha]	Bioassay					
		1 DAA <sup>2)</sup>		28 DAA		36 DAA	
		F [m/f]	R [%] <sup>4)</sup>	F [m/f]	R [%]	F [m/f]	R [%]
Control	0	N/S <sup>3)</sup>		24.4	-	42.7	-
MCW-2222 (Acetamiprid 20% w/v SL)	70	N/S <sup>3)</sup>		26.0 <sup>NS</sup>	-6.6	35.9 <sup>NS</sup>	15.9

<sup>1)</sup> Application rate in 600 L water/ha

<sup>2)</sup> DAA = Days after application; ; F [m/f]= Fecundity [mummies per female]; R [%]= Reduction [%].

<sup>3)</sup> N/S = Reproduction was not studied as mortality was > 50% in T.

<sup>4)</sup> 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 141: 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 $\geq 5$ mummies per female	$\geq 22.4$
Not more than two wasps should produce no mummies	$\leq 1$

### 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 2.3.2.3.3 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) due to renewal of acetamiprid and was not evaluated earlier. 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 (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>
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	<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 &lt;50% after 36 days of aging. Effects on fecundity were &lt;50% after 42 days of aging (no fecundity assessment carried out after 36 days of aging).</p>
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<b>Reference:</b>	KCP 10.3.2.3/03
<b>Report</b>	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, TRC15-244BA
<b>Guideline(s):</b>	Mead-Briggs <i>et al.</i> 2000, and an unpublished draft guideline by Mead-Briggs and Longley 1997
<b>Deviations:</b>	No
<b>GLP:</b>	Yes, certified laboratory
<b>Acceptability:</b>	Acceptable

## 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

<b>Test item</b>	MCW-2222 (Acetamiprid 20% w/v SL)
<b>Batch #</b>	659-030314-01
<b>Content of active substance</b>	acetamiprid: 199.2 ± 1.3 g/L, (nominal: 200 g/L)
<b>Control</b>	Tap water
<b>Toxic reference</b>	Deltamethrin 2.5% EC at 0.05% = 7.65 g a.s./ha
<b>Test organism</b>	
<b>Species</b>	Parasitic wasp <i>Aphidius rhopalosiphi</i> Adults, < 48 h old
<b>Source</b>	Katz Biotech AG”, An der Birkenpfuhlheide 10, 15837 Baruth, Germany
<b>Study design and methods</b>	

<b>Ageing periods</b>	1, 28, 36 and 42 days
<b>Exposure duration</b>	48 hours
<b>Experimental dates</b>	30 Nov 2015 to 25 Jan 2016
<b>Test doses (nominal)</b>	102 g acetamiprid/ha, equivalent to 0.5120 L/ha of formulated test item
<b>Test units</b>	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 <sup>2</sup> (5 m × 1 m) for the treatments and they were arranged in two crop rows (0.5 m to each other).
<b>Group size/replicates</b>	10 adults (≥ 5 females) per replicate, 4 replicates (40 adults) per treatment
<b>Experimental treatments</b>	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.
<b>Environmental conditions</b>	
<b>Temperature</b>	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
<b>Photoperiod</b>	16 h light (4000–20000 lx) : 8 h dark
<b>Relative humidity</b>	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 142:** Effects of MCW-2222 (Acetamiprid 20% w/v SL) on mortality of the parasitoid, *Aphidius rhopalosiphi*

	Rate <sup>1)</sup> [g a.s./ha]	Bioassay							
		1 DAA <sup>2)</sup>		28 DAA		36 DAA		42 DAA	
		M (%)	C <sub>m</sub> (%)	M (%)	C <sub>m</sub> (%)	M (%)	C <sub>m</sub> (%)	M (%) <sup>3)</sup>	C <sub>m</sub> (%)
Control	0	0.0	-	0.0	-	0.0	-	2.5	-
MCW-2222 (Acetamiprid 20% w/v SL)	102	100 <sup>SD</sup>	100	75.0 <sup>SD</sup>	75.0	42.5 <sup>SD</sup>	42.5	25.0 <sup>NS</sup>	23.1
Reference Item (Deltamethrin 2.5% EC)	7.65	100	100	70.0	70.0	85.0	85.0	70.0	69.2

<sup>1)</sup> Application rate in 600 L water/ha

<sup>2)</sup> DAA = Days after application; M [%] = Mortality [%]; C<sub>m</sub> [%] = Corrected mortality [%].

<sup>3)</sup> 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 143:** Effects of MCW-2222 (Acetamiprid 20% w/v SL) on fecundity of the parasitoid, *Aphidius rhopalosiphi*

	Rate <sup>1)</sup> [g a.s./ha]	Bioassay							
		1 DAA <sup>2)</sup>		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 <sup>NS</sup>	11.6

<sup>1)</sup> Application rate in 600 L water/ha

<sup>2)</sup> 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 144: 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 $\geq 5$ mummies per female	$\geq 69.5$
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 2.3.2.3.4 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) due to renewal of acetamiprid and was not evaluated earlier. 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 &lt;50% after 42 days of aging.</p>
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<b>Reference:</b>	KCP 10.3.2.3/04
<b>Report</b>	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, TRC16-073BA, R-37333
<b>Guideline(s):</b>	Mead-Briggs <i>et al.</i> 2010
<b>Deviations:</b>	No
<b>GLP:</b>	Yes, certified laboratory
<b>Acceptability:</b>	Acceptable

## 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

<b>Test item</b>	MCW-2222 (Acetamiprid 20% w/v SL)
<b>Batch #</b>	811-021115-01
<b>Content of active substance</b>	acetamiprid: 205.1 ± 1.1 g/L, (nominal: 200 g/L)
<b>Control</b>	Tap water
<b>Toxic reference</b>	Deltamethrin 2.5% EC at 1.15 L test item/ha = 29.325 g a.s./ha
<b>Test organism</b>	
<b>Species</b>	Parasitic wasp <i>Aphidius rhopalosiphi</i> Adults, < 48 h old
<b>Source</b>	Katz Biotech AG”, An der Birkenpfuhlheide 10, 15837 Baruth, Germany

### Study design and methods

<b>Ageing periods</b>	0, 42 and 49 days
<b>Exposure duration</b>	48 hours
<b>Experimental dates</b>	12 Jul 2016 to 12 Sep 2016
<b>Test doses (nominal)</b>	170 g acetamiprid/ha, equivalent to 0.8289 L/ha of formulated test item
<b>Test units</b>	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 <sup>2</sup> (10 m × 2 m) for the treatments and they were arranged in two rows (0.5 m to each other).
<b>Group size/replicates</b>	5 females per replicate, 6 replicates (30 adults) per treatment
<b>Experimental treatments</b>	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.
<b>Environmental conditions</b>	
<b>Temperature</b>	0DAA: 19.4–21.2 °C 42 DAA: 19.6–21.6 °C 49 DAA: 19.7–21.6 °C
<b>Photoperiod</b>	16 h light (432–699 lx mortality phase, 4582–5591 lx parasitisation phase, 9309–10982 lx reproduction phase) : 8 h dark
<b>Relative humidity</b>	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  $20 \pm 2$  °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 145: Effects of MCW-2222 (Acetamiprid 20% w/v SL) on mortality of the parasitoid, *Aphidius rhopalosiphii***

	Rate [g a.s./ha]	Bioassay					
		0 DAA <sup>1)</sup>		42 DAA		49 DAA	
		M (%) <sup>2)</sup>	C <sub>m</sub> (%)	M (%) <sup>3)</sup>	C <sub>m</sub> (%)	M (%) <sup>2)</sup>	C <sub>m</sub> (%)
Control	0	3.33	-	6.67	-	6.67	-
MCW-2222 (Acetamiprid 20% w/v SL)	170	100 <sup>SD</sup>	100	33.33 <sup>ND</sup>	28.57	20.00 <sup>NS</sup>	14.29
Reference Item (Deltamethrin 2.5% EC)	29.325	100	100	56.67	53.57	20.00	14.29

<sup>1)</sup> DAA = Days after application; M [%] = Mortality [%]; C<sub>m</sub> [%] = Corrected mortality [%].

<sup>2)</sup> SD = statistically significant different compared to the control (Mann-Whitney test, exact sig., 1-tailed greater,  $\alpha=0.05$ ).

<sup>3)</sup> 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 146: Effects of MCW-2222 (Acetamiprid 20% w/v SL) on fecundity of the parasitoid, *Aphidius rhopalosiphi***

	Rate [g a.s./ha]	Bioassay					
		0 DAA <sup>1)</sup>		42 DAA		49 DAA	
		F [m/f]	R [%]	F [m/f] <sup>2)</sup>	R [%]	F [m/f]	R [%] <sup>3)</sup>
Control	0	N/S		16.43	-	29.33	-
MCW-2222 (Acetamiprid 20% w/v SL)	170	N/S		9.53 <sup>SD</sup>	41.97	31.80	-8.41

N/S = Reproduction was not studied as mortality was > 50% in T.

<sup>1)</sup> DAA = Days after application; ; F [m/f]= Fecundity [mummies per female]; R [%]= Reduction [%].

<sup>2)</sup> SD = statistically significant different compared the control (T-Test,  $\alpha=0.05$ ).

<sup>3)</sup> 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 147: 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 $\geq 5$ mummies per female	$\geq 16.43$
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 2.3.2.3.5 KCP 10.3.2.3/05 Aged residue study with *Typhlodromus pyri*

Comments of zRMS:	<p>The aged residue study on effects to <i>T.pyri</i> has been submitted in support of the re-evaluation of CA3573 (formerly MCW-2222) due to renewal of acetamiprid and was not evaluated earlier. 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"> <li>1. In group exposed to fresh residues (0 DAA) the maximum temperature (40.2°C) during mortality phase was clearly above the maximum of 27°C recommended by the guideline.</li> <li>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.</li> <li>3. In group exposed to residues aged for 35 and 42 days the maximum relative humidity (95.6%) was above the maximum of 90% recommended by the guideline.</li> </ol> <p>It is noted that the temperature during the mortality phase was exceeded for three days, while the relative humidity was too low for 2 days.</p> <p>Nevertheless, as all validity criteria were met all mentioned deviations are considered to have no impact on test results.</p> <p>Overall, the study is considered acceptable.</p> <p>Obtained results demonstrated that lethal and sub-lethal effects on <i>T. pyti</i> following application of CA3573 at 102 and 170 g a.s./ha are &lt;50% for all aging periods and after exposure to fresh residues.</p>
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<b>Reference:</b>	KCP 10.3.2.3/05
<b>Report</b>	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
<b>Guideline(s):</b>	Blümel <i>et al.</i> 2000
<b>Deviations:</b>	No
<b>GLP:</b>	Yes, certified laboratory
<b>Acceptability:</b>	Acceptable

#### 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

<b>Test item</b>	MCW-2222 (Acetamiprid 20% w/v SL)
<b>Batch #</b>	811-021115-01
<b>Content of active substance</b>	acetamiprid: 205.1 ± 1.1 g/L, (nominal: 200 g/L)
<b>Control</b>	Tap water
<b>Toxic reference</b>	Deltamethrin 2.5% EC at 1.15 L test item/ha = 29.325 g a.s./ha
<b>Test organism</b>	
<b>Species</b>	Predatory mite <i>Typhlodromus pyri</i> Protonymphs not later than 24 hours from moulting
<b>Source</b>	Katz Biotech AG”, An der Birkenpfuhlheide 10, 15837 Baruth, Germany

### Study design and methods

<b>Ageing periods</b>	0, 32 and 45 days
<b>Exposure duration</b>	7 days
<b>Experimental dates</b>	12 Jul 2016 to 6 Sep 2016
<b>Test doses (nominal)</b>	102 and 170 g acetamiprid/ha, equivalent to 0.4973 and 0.8289 L/ha of formulated test item
<b>Test units</b>	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 <sup>2</sup> (10 m × 2 m) for the treatments and they were arranged in two rows (0.5 m to each other).
<b>Group size/replicates</b>	20 protonymphs per replicate, 5 replicates per treatment
<b>Experimental treatments</b>	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

<b>Temperature</b>	0 DAA: 24.5–40.2 °C 35 DAA: 24.8–25.3 °C 42 DAA: 24.8–25.3 °C
<b>Photoperiod</b>	16 h light (1699–2184 lx mortality phase, 1164–3213 lx fecundity phase) : 8 h dark
<b>Relative humidity</b>	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  $\leq 50\%$  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,  $\alpha=0.05$ ) 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 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 148:** Effects of MCW-2222 (Acetamiprid 20% w/v SL) on mortality of the predatory mite, *Phytodromus pyri*

	Rate [g a.s./ha]	Exposure					
		0 DAA <sup>1)</sup>		35 DAA		42 DAA	
		M (%) <sup>2)</sup>	C <sub>m</sub> (%)	M (%)	C <sub>m</sub> (%) <sup>3)</sup>	M (%)	C <sub>m</sub> (%)
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 <sup>SD</sup>	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

<sup>1)</sup> DAA = Days after application; M [%] = Mortality [%]; C<sub>m</sub> [%] = Corrected mortality [%].

<sup>2)</sup> SD = statistically significant different compared to the control (T-test,  $\alpha=0.05$ ).

<sup>3)</sup> 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 149: 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 <sup>1)</sup>		35 DAA		42 DAA	
		e/f <sup>2)</sup>	R [%]	e/f	R [%] <sup>3)</sup>	e/f	R [%] <sup>3)</sup>
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 <sup>SD</sup>	27.65	9.20	-3.37	8.47	9.24

<sup>1)</sup> DAA = Days after application; ; F [m/f]= Fecundity [mummies per female]; R [%]= Reduction [%].

<sup>2)</sup> SD = statistically significant different compared the control (T-test,  $\alpha=0.05$ ).

<sup>3)</sup> 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 150: 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 <sup>1)</sup>
More than 4 eggs per female should be achieved	$\geq 8.90$

<sup>1)</sup> 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 2.3.2.3.6 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) due to renewal of acetamiprid and was not evaluated earlier. 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"> <li>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.</li> <li>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.</li> <li>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.</li> </ol>
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	<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 &amp; McEwen (1998)<sup>12</sup> 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 &gt;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 &lt;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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<b>Reference:</b>	KCP 10.3.2.3/06
<b>Report</b>	Aged residue test with the formulation “MCW-2222” on <i>Coccinella septempunctata</i> L (Coleoptera:Coccinellidae), Luna, F., 2017c, TRC16-075BA / R-37334
<b>Guideline(s):</b>	Schmuck et al. 2000
<b>Deviations:</b>	No
<b>GLP:</b>	Yes, certified laboratory
<b>Acceptability:</b>	Acceptable

## 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.

<sup>12</sup> Haskell P.T., McEwen P. (1998): Pesticides and beneficial organisms. Springer-Science+Business media B.V.

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

<b>Test item</b>	MCW-2222 (Acetamiprid 20% w/v SL)
<b>Batch #</b>	811-021115-01
<b>Content of active substance</b>	acetamiprid: 205.1 ± 1.1 g/L, (nominal: 200 g/L)
<b>Control</b>	Tap water
<b>Toxic reference</b>	Deltamethrin 2.5% EC at 1.15 L test item/ha = 29.325 g a.s./ha
<b>Test organism</b>	
<b>Species</b>	Aphid predatory <i>Coccinella septempunctata</i> Larvae, 3–4 days old
<b>Source</b>	Katz Biotech AG”, An der Birkenpfehlheide 10, 15837 Baruth, Germany

### Study design and methods

<b>Ageing periods</b>	0, 35 and 42 days
<b>Experimental dates</b>	12 Jul 2016 to 4 Oct 2016
<b>Test doses (nominal)</b>	102 and 170 g acetamiprid/ha, equivalent to 0.4973 and 0.8289 L/ha of formulated test item
<b>Test units</b>	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 <sup>2</sup> (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).
<b>Group size/replicates</b>	40 larvae per treatment
<b>Experimental treatments</b>	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

<b>Temperature</b>	0DAA: 21.5–40.2 °C 35 DAA: 22.5–31.3 °C 42 DAA: 22.5–31.3 °C
<b>Photoperiod</b>	16 h light (1467–3224 lx mortality phase, 1391–4751 lx reproduction phase) : 8 h dark
<b>Relative humidity</b>	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  $25 \pm 2$  °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 151: Effects of MCW-2222 (Acetamiprid 20% w/v SL) on mortality of *Coccinella septempunctata***

	Rate [g a.s./ha]	Exposure					
		0 DAA <sup>1)</sup>		35 DAA		42 DAA	
		M (%) <sup>2)</sup>	C <sub>m</sub> (%)	M (%)	C <sub>m</sub> (%)	M (%)	C <sub>m</sub> (%)
Control	0	0.00	-	0.00	-	5.00	-
MCW-2222 (Acetamiprid 20% w/v SL)	102	48.72 <sup>SD</sup>	48.72	5.26	5.26	7.69	2.83
	170	61.54 <sup>SD</sup>	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

<sup>1)</sup> DAA = Days after application; M [%] = Mortality [%]; C<sub>m</sub> [%] = Corrected mortality [%].

<sup>2)</sup> 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 152: Effects of MCW-2222 (Acetamiprid 20% w/v SL) on fecundity of *Coccinella septempunctata***

	Rate [g a.s./ha]	Exposure		
		0 DAA <sup>1)</sup>	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 <sup>2)</sup>	11.98	19.94

<sup>1)</sup> DAA = Days after application.

<sup>2)</sup> 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 <sup>1)</sup>	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 <sup>2)</sup>	93.93	95.71

<sup>1)</sup> DAA = Days after application.

<sup>2)</sup> 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 153: 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 $\geq 2$	$\geq 10.32$
Mortality in the reference treatment should be $\geq 40\%$	100% at 0 DAA, 42.50% at 35 DAA, 17.5% at 42 DAA <sup>1)</sup>

<sup>1)</sup> 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 50 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 <b>NOER from the study was set to 1.4 g a.s./ha</b>, i.e. highest rate tested with only effects class 1 and 2, relevant for determination of NOER.</p>
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	<p><b>Determination of NOEAER</b></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 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 <b>NOEAER from this study was set to 3.4 g a.s./ha</b>, 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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<b>Reference:</b>	KCP 10.3.2.4/01
<b>Report</b>	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, R-35848
<b>Guideline(s):</b>	Candolfi <i>et al.</i> (2000); De Jong <i>et al.</i> (2010); Alix <i>et al.</i> , 2011
<b>Deviations:</b>	No
<b>GLP:</b>	Yes, certified test facility
<b>Acceptability:</b>	Acceptable
<b>Duplication (if vertebrate study)</b>	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 2<sup>nd</sup> 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

<b>Test item</b>	MCW-2222
<b>Batch #</b>	659-030314-01
<b>Content of active substance</b>	Acetamiprid 200 g/L (nominal); 199.2 g/L (analysed)
<b>Description</b>	Liquid / clear yellow to brown
<b>Control</b>	Water
<b>Toxic reference</b>	Dimethoate (content: 400 g/L)

### Test organism

<b>Species</b>	Naturally occurring populations of arthropods in the meadow at the field site
<b>Source</b>	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<sup>st</sup> application and the final sampling was 27 days after the 2<sup>nd</sup> 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 m<sup>2</sup> each.

### Test duration and exposure

27 days after 2<sup>nd</sup> application (6 days between 1<sup>st</sup> and 2<sup>nd</sup> 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<sup>2</sup>

### 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						
	C	T1	T2	T3	T4	R	Total
<b>Pitfall traps</b>	3462	3760	3410	3353	3527	1869	<b>19381</b>
<b>Photoeclector</b>	2197	2119	1927	2909	2196	1335	<b>12683</b>
<b>Foliage/Litter</b>	7257	5508	6718	7317	8546	5915	<b>41261</b>
<b>Vortis</b>	207420	235228	216956	226004	202440	44137	<b>1132185</b>
<b>Total</b>	<b>220336</b>	<b>246615</b>	<b>229011</b>	<b>239583</b>	<b>216709</b>	<b>53256</b>	<b>1205510</b>

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 Photoelectors 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, Photoelectors, 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 1<sup>st</sup> application and the succeeding samplings 5, 10, 15, 20 and 27 days after the 2<sup>nd</sup> 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 3<sup>rd</sup> sampling (10DAA2). Numbers were already higher at the 2<sup>nd</sup> 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 5<sup>th</sup> 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 2<sup>nd</sup> sampling (5DAA2). No statistically significant differences occurred in the two higher test item rates T1 and T2, though abundances decreased from the 1<sup>st</sup> to the 2<sup>nd</sup> 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 3<sup>rd</sup> 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 5<sup>th</sup> sampling (20DAA2) and in test item treatment T4 at the 2<sup>nd</sup> and 5<sup>th</sup> 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 1<sup>st</sup> 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 2<sup>nd</sup> sampling (5DAA2). From the 3<sup>rd</sup> 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 2<sup>nd</sup> sampling.

The Coleoptera suborder Polyphaga was decreased to a statistically significant extent in test item treatment T2 at the 2<sup>nd</sup> sampling (5DAA2) and in test item treatment T1 at the 2<sup>nd</sup>, 3<sup>rd</sup> and 5<sup>th</sup> 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 2<sup>nd</sup> and 4<sup>th</sup> sampling (5 and 15DAA2) and for the lower test rates T2, T3 and T4 at the 2<sup>nd</sup> 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 3<sup>rd</sup> 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 2<sup>nd</sup> sampling (5DAA2). No specimens were caught in the control and all test item treatments at the 1<sup>st</sup> sampling (8DBA2). Abundances increased at the 2<sup>nd</sup> 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 6<sup>th</sup> 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 1<sup>st</sup> sampling was taken 2 days before the 1<sup>st</sup> application and the succeeding samplings 5, 10, 15, 20 and 27 days after the 2<sup>nd</sup> 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 4<sup>th</sup> 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 4<sup>th</sup> 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 2<sup>nd</sup> 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 2<sup>nd</sup> 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 1<sup>st</sup> 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 3<sup>rd</sup> sampling (10DAA2). Until the 4<sup>th</sup> 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 4<sup>th</sup> and 5<sup>th</sup> sampling (15 and 20DAA2). However, abundances were clearly below those in the control from the 1<sup>st</sup> 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 2<sup>nd</sup> 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 2<sup>nd</sup> 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 1<sup>st</sup> sampling was taken 3 days before the 1<sup>st</sup> application and the succeeding samplings 3, 8, 14, 20 and 27 days after the 2<sup>nd</sup> 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 5<sup>th</sup> 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 6<sup>th</sup> 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 2<sup>nd</sup> sampling (3DAA2). However, from the 3<sup>rd</sup> 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 2<sup>nd</sup> 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 3<sup>rd</sup> sampling (8DAA2). At the 4<sup>th</sup> 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 4<sup>th</sup> 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 1<sup>st</sup> to the 3<sup>rd</sup> sampling (9DBA2 to 8DAA2), statistically significantly lower numbers were observed in test item treatment T2 at the 3<sup>rd</sup> sampling (8DAA2); in test item treatment T1 abundances were not statistically significantly lower at the 3<sup>rd</sup> 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 6<sup>th</sup> sampling (27DAA2). This was most likely due to chance or normal population dynamics as specimens of this species were only caught from the 4<sup>th</sup> 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 1<sup>st</sup> to the 3<sup>rd</sup> sampling (9DBA2 to 8DAA2) resulting in statistically significantly lower numbers compared to the control in test item treatment T1 at the 3<sup>rd</sup> 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 1<sup>st</sup> sampling (9DBA2) on for the species *Sminthurinus aureus*, though not on a statistically significant level. Moreover, no statistically significant effects occurred for total Symphyleona 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 4<sup>th</sup> sampling (14DAA2).

For the family Staphylinidae abundances in test item treatment T1 were statistically significantly lower at the 4<sup>th</sup> 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 3<sup>rd</sup> 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 5<sup>th</sup> sampling (20DAA2) after lower numbers were observed at the 2<sup>nd</sup> and 3<sup>rd</sup> 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 Sciarioidea (fungus gnats) in the control. Abundances of superfamily Sciarioidea were statistically significantly lower compared to the control in test item treatment T2 at the 2<sup>nd</sup> sampling (3DAA2). In test item treatment T1 both taxa showed statistically significantly lower abundances compared to the control at the 3<sup>rd</sup> sampling (8DAA2). Further at the 3<sup>rd</sup> sampling in T2 abundances of the superfamily Sciarioidea 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 midgets) were present at statistically significantly higher numbers in test item treatment T3 at the 4<sup>th</sup> 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 2<sup>nd</sup> to the 3<sup>rd</sup> sampling (3 to 8DAA2), resulting in statistically significantly lower numbers at the 3<sup>rd</sup> 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 2<sup>nd</sup> sampling (3DAA2) and were still lower at the 5<sup>th</sup> and 6<sup>th</sup> 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 5<sup>th</sup> 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 4<sup>th</sup> 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 3<sup>rd</sup> 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 3<sup>rd</sup> 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 1<sup>st</sup> sampling (9DBA2) on. A steep increase from the 3<sup>rd</sup> to the 4<sup>th</sup> sampling (8 to 14DAA2) resulted in statistically significantly higher numbers compared to the control at 14DAA2. At the 5<sup>th</sup> 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 5<sup>th</sup> and 6<sup>th</sup> sampling (20 and 27DAA2). Lower numbers were already observed directly after the 2<sup>nd</sup> application (3 and 8 DAA2), though not on a statistically significant level, and at the 4<sup>th</sup> 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 1<sup>st</sup> application and the succeeding samplings 4, 14 and 27 days after the 2<sup>nd</sup> 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 2<sup>nd</sup> 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 2<sup>nd</sup> 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 154: 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 155: 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 LOEAER	Population NOEAER		

\* = 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 2<sup>nd</sup> 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<sub>mortality</sub> = 4.10 mg a.s./kg dws  NOEC<sub>biomass</sub> = 1.44 mg a.s./kg dws  NOEC<sub>reproduction</sub> = 0.85 mg a.s./kg dws  EC<sub>10</sub> = 0.90 mg a.s./kg dws</p> <p>According the conclusions presented in EFSA Supporting publication 2019:EN-1673, reliability of the derived EC<sub>10</sub> 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"> <li>NW (normalised width) of 0.16 was calculated, which results with rating “excellent” in line with Table E9,</li> <li>median EC<sub>10</sub> is lower than EC<sub>20,low</sub>,</li> <li>the dose-response curve is medium with steepness of 0.60 (i.e. in range of 0.33-0.66).</li> </ul> <p>Based on above indications, the calculated EC<sub>10</sub> 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:	Acceptable
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) mixed with 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 EC<sub>10</sub>, EC<sub>20</sub> and EC<sub>50</sub> 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

<b>Test item</b>	MCW-2222
<b>Batch #</b>	611-280413-01
<b>Purity</b>	Acetamiprid 200 g/L (nominal); 202.7 g/L (analysed)
<b>Description</b>	Yellowish liquid
<b>Control</b>	Deionised water
<b>Toxic reference</b>	Nutdazim 50 FLOW (Carbendazim, SC 500), tested in a separate study (BioChem project No. R 13 10 48 005 S, (November 2013).

### Test organism

<b>Species</b>	<i>Eisenia fetida</i> (Earthworm), about 3 month old (with clitellum), weight: 280 – 469 mg/worm
<b>Source</b>	In-house culture, originally obtained from W. Neudorff GmbH KG”, An der Mühle 3, 31860 Emmerthal, Germany
<b>Food</b>	5 g of dried horse manure per replicate and week

### Study design and methods

<b>Test duration and exposure</b>	8 weeks (overall) 4 weeks mortality and sublethal observations 4 weeks for reproductive success The test item was mixed with the artificial soil containing 10% peat
<b>Experimental dates</b>	18 February - 15 April 2015
<b>Test rates</b>	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
<b>Test units</b>	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
<b>Group size/replicates</b>	Test rates: 40 organisms per concentration; 10 in each of 4 replicates Control: 80 organisms per concentration; 10 in each of 8 replicates
<b>Test substrate</b>	Artificial substrate (10% peat) according to OECD 222
<b>Max Water holding capacity</b>	62.8 g/100 g dw
<b>Environmental conditions</b>	
<b>Temperature</b>	18.2 – 21.9 °C
<b>Photoperiod</b>	16 hours light / 8 hours darkness 510 lx
<b>pH</b>	Test start: 6.07 – 6.10 Test end: 5.76 – 5.82
<b>Water content</b>	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 EC<sub>10</sub>, EC<sub>20</sub> and EC<sub>50</sub> 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 156:** 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;  $\geq 0.05$ , one-sided smaller)

Negative values indicate an increase, relative to control

**Table A 157:** 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 <sub>50</sub>	> 4.10 mg a.s./kg dw
EC <sub>10</sub> (95% CI)	0.90 mg a.s./kg dw (0.84 – 0.98 mg a.s./kg dw)
EC <sub>20</sub> (95% CI)	1.07 mg a.s./kg dw (1.01 – 1.14 mg a.s./kg dw)
EC <sub>50</sub> (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 158:** 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 $\geq 30$	$\geq 84$
The coefficient of variance (CV %) of reproduction should be $\leq 30$	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 EC<sub>10</sub>, EC<sub>20</sub> and EC<sub>50</sub> 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.

### 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 *Folsomia candida*

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<sub>mortality</sub> = 0.30 mg a.s./kg dws NOEC<sub>reproduction</sub> = 0.18 mg a.s./kg dws EC<sub>10</sub> = 0.41 mg a.s./kg dws</p> <p>According the conclusions presented in EFSA Supporting publication 2019:EN-1673, reliability of the derived EC<sub>10</sub> 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"> <li>• NW (normalised width) of 1.23 was calculated, which results with rating “poor” in line with Table E9,</li> <li>• median EC<sub>10</sub> is higher than EC<sub>20,low</sub>,</li> <li>• the dose-response curve is shallow with steepness of 0.28 (i.e. in range of 0.33-0.66).</li> </ul> <p>Based on above indications, the calculated EC<sub>10</sub> is considered to be not sufficiently reliable, but potentially the lower limit EC<sub>10</sub> 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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<b>Reference:</b>	KCP 10.4.2.1/01
<b>Report</b>	MCW-2222 - Effects on the reproduction of the collembolan <i>Folsomia candida</i> , Friedrich, S. 2014, R-33841
<b>Guideline(s):</b>	OECD 223 (2009), ISO 11267 (1999)
<b>Deviations:</b>	No
<b>GLP:</b>	Yes, certified laboratory
<b>Acceptability:</b>	Acceptable
<b>Duplication (if vertebrate study)</b>	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 EC<sub>10</sub>, EC<sub>20</sub> and EC<sub>50</sub> 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

<b>Test item</b>	MCW-2222
<b>Batch #</b>	611-280413-01
<b>Purity</b>	Acetamiprid 200 g/L (nominal); 202.7 g/L (analysed)
<b>Description</b>	Yellowish liquid
<b>Control</b>	Deionised water
<b>Toxic reference</b>	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

<b>Species</b>	<i>Folsomia candida</i> (Collembola), juveniles 9 – 12 days old
<b>Source</b>	In-house culture, originally obtained from the Biologische Bundesanstalt BBA, Berlin-Dahlem, Germany
<b>Food</b>	Granulated dry yeast, ~2 mg at test start and after 14 days

### Study design and methods

<b>Test duration and exposure</b>	4 weeks (28 days) The test item was mixed into the substrate containing 5 % peat
<b>Experimental dates</b>	04 March - 01 April 2014
<b>Test rates</b>	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
<b>Test units</b>	Glass container (approximately 150 mL) covered with a glass lid; surface area of soil: 18.9 cm <sup>2</sup>
<b>Group size/replicates</b>	Test rates: 40 organisms per concentration; 10 in each of 4 replicates Control: 80 organisms per concentration; 10 in each of 8 replicates
<b>Test substrate</b>	Artificial substrate (5% peat) according to OECD 223, crumbly structured in test vessel
<b>Max Water holding capacity</b>	43.1 g/100 g dw
<b>Environmental conditions</b>	
<b>Temperature</b>	18.2 – 21.0 °C
<b>Photoperiod</b>	16 hours light / 8 hours darkness 530 lx
<b>pH</b>	Test start: 6.06 – 6.10 Test end: 5.80 – 5.84
<b>Water content</b>	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 159: Effect of MCW-2222 on *Folsomia candida* mortality and reproduction after an exposure period of 28 days**

	Treatment rate [mg a.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 [%] <sup>a</sup>	2.5	2.5	2.5	12.5	40*	47.5*	50.0*	42.5*	50.0*
Mean number of juveniles <sup>b</sup>	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 (<sup>a</sup> Fisher-exact test for mortality,  $\alpha = 0.05$ , one-sided greater;

<sup>b</sup> Williams-t-test for reproduction;  $\alpha = 0.05$ , one-sided smaller)

Negative values = increase, relative to control

**Table A 160: Endpoints**

	Endpoints
NOEC (mortality)	0.30 mg a.s./kg dw
NOEC (reproduction)	0.18 mg a.s./kg dw
LC <sub>50</sub> (95% CI)	2.30 mg a.s./kg dw (0.7 – 5.86 mg a.s./kg dw)
EC <sub>10</sub> (95% CI)	0.41 mg a.s./kg dw (0.22 – 0.79 mg a.s./kg dw)
EC <sub>20</sub> (95% CI)	0.64 mg a.s./kg dw (0.35 – 1.17 mg a.s./kg dw)
EC <sub>50</sub> (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 161: 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 $\geq 100$	749
The coefficient of variance (CV %) of reproduction should be $\leq 30$	12.4%

### Conclusion

In a 28 day *Folsomia candida* reproduction study, in which collembolans were exposed to MCW-2222, the LC<sub>50</sub> value was calculated to be 2.03 mg a.s./kg soil dry weight. The EC<sub>10</sub>, EC<sub>20</sub> and EC<sub>50</sub> 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 EC<sub>10</sub> or LC<sub>10</sub> could be calculated based on the study results, as effects &gt;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 &lt;10% were observed. Following endpoints were thus agreed for purposes of re-evaluation of CA3573:</p> <p>NOEC<sub>mortality</sub> = 100 mg a.s./kg dws NOEC<sub>reproduction</sub> = 100 mg a.s./kg dws</p> <p>Reliable EC<sub>10</sub> could not be determined</p>
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<b>Reference:</b>	KCP 10.4.2.1/02
<b>Report</b>	Effects of MCW-2222 on the reproduction of the predatory mite <i>Hypoaspis aculeifer</i> , Schulz, L., 2014, R-33842
<b>Guideline(s):</b>	OECD 226 (2008)
<b>Deviations:</b>	Yes
<b>GLP:</b>	Yes, certified laboratory
<b>Acceptability:</b>	Acceptable
<b>Duplication (if vertebrate study)</b>	Not applicable

#### Executive Summary

In a 14 days study the effects of MCW-2222 on mortality and reproduction of the soil mite species *Hypoaspis aculeifer* were investigated. The LC<sub>50</sub> for mortality and the EC<sub>50</sub> for reproduction could not be calculated due to an absence of adverse effects. Hence it can be concluded that the LC<sub>50</sub> and the EC<sub>50</sub> 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

<b>Test item</b>	MCW-2222
<b>Batch #</b>	611-280413-01
<b>Purity</b>	Acetamiprid 200 g/L (nominal); 202.7 g/L (analysed)
<b>Description</b>	Yellowish liquid
<b>Control</b>	Deionised water
<b>Toxic reference</b>	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

<b>Species</b>	<i>Hypoaspis aculeifer</i> (Canestrini), adult age synchronised (≤ 3 days) females
<b>Source</b>	In-house culture, originally obtained from Bayer CropScience AG, Mohnheim, Germany
<b>Food</b>	Before and during the test, the predatory mites were fed every 2 - 3 days with <i>Tyrophagus putrescentiae</i> (Schrank)



## Study design and methods

<b>Test duration and exposure</b>	14 days. The test item was mixed into the substrate.
<b>Experimental dates</b>	25 Jul – 13 Aug 2014
<b>Test rates</b>	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
<b>Test units</b>	100 mL SCHOTT-bottles with screw cap (inside dimensions: 4 cm in diameter, 11 cm high). Bottle contained 20 g soil dry weight
<b>Group size/replicates</b>	Test rates: 40 organisms per concentration; 10 in each of 4 replicates Control: 80 organisms per concentration; 10 in each of 8 replicates
<b>Test substrate</b>	Artificial substrate containing 5% peat
<b>Max Water holding capacity</b>	36-09 g/100 g dw
<b>Environmental conditions</b>	
<b>Temperature</b>	19.7 - 21.2 °C
<b>Photoperiod</b>	16 hours light / 8 hours darkness 513 lx
<b>pH</b>	Test start: 5.6 – 5.7 Test end: 5.6 – 5.7
<b>Water content</b>	Test start: 50.34 - 52.82% of WHC Test end: 49.32 - 52.36 % of WHC
<b>Biological observations</b>	

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 162:** 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 163:** Endpoints

Endpoint	[mg a.s./kg dry weight]
NOEC (mortality)	200 <sup>1)</sup>
NOEC (reproduction)	>200 <sup>1)</sup>
LC <sub>50</sub>	>200
EC <sub>10</sub>	>200 <sup>2)</sup>
EC <sub>20</sub>	>200
EC <sub>50</sub>	>200

<sup>1)</sup> NOEC set by the zRMS to 100 mg a.s./kg dws due to >10% effects at 200 mg a.s./kg dws and no reliable EC<sub>10</sub>

<sup>2)</sup> Value not reliable, due >10% effects seen only at this maximum tested concentration

### ***Validity criteria***

As shown in the following table, all validity criteria were met.

**Table A 164:                      Validity criteria**

<b>Validity criteria according to OECD 226 (2008)</b>	<b>Observed in study</b>
The mortality in the control group should be below 20%	3.8%
The number of juveniles in the control group was $\geq 50$	201.1
The coefficient of variance (CV %) of reproduction should be $\leq 30$	12.4%

### **Conclusion**

In a 14 day *Hypoaspis aculeifer* reproduction study with MCW-2222, the LC<sub>50</sub> for mortality and the EC<sub>50</sub> for reproduction could not be calculated due to an absence of adverse effects. Hence it was concluded that the LC<sub>50</sub> and the EC<sub>50</sub> 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**

## 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 &lt;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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<b>Reference:</b>	KCP 10.5/01
<b>Report</b>	MCW-2222 - Effects on the activity of soil microflora (Nitrogen transformation test) Schulz, L. 2014, R-33843, 14 10 48 018 N
<b>Guideline(s):</b>	OECD 216 (2000)
<b>Deviations:</b>	No
<b>GLP:</b>	Yes, certified laboratory
<b>Acceptability:</b>	Yes
<b>Duplication (if vertebrate study)</b>	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 NO<sub>3</sub>-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 NO<sub>3</sub>-N-production) at the end of the 28-day incubation period.

### Materials and methods

#### Materials

<b>Test item</b>	MCW-2222
<b>Batch #</b>	611-280413-01
<b>Content of active substance</b>	Acetamiprid 200 g/L (nominal); 202.7 g/L (analysed)
<b>Description</b>	Yellowish liquid
<b>Control</b>	Deionised water
<b>Toxic reference</b>	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

<b>Species</b>	Microflora from an agricultural soil
<b>Source</b>	Wassergut Canitz, Canitz, Sachsen, Germany
<b>Food</b>	Lucerne meal (concentration in soil 0.5 %).

#### Study design and methods

<b>Test duration and exposure</b>	28 days. The test item was mixed into the soil.
<b>Experimental dates</b>	13 May – 10 June 2014
<b>Test rates</b>	2.27 mg/kg dw, 22.74 mg/kg dw, equivalent to 1.5 or 15 L test item/ha, respectively
<b>Test units</b>	Wide-mouth glass flasks (500 mL) per concentration, each filled with 200 g

<b>Group size/replicates</b>	soil dry weight 3 replicates per treatment group
<b>Soil</b>	Loamy sand (DIN 4220) / sandy loam (USDA), pH 6.6, 1.47 % C <sub>org</sub> , 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
<b>Environmental conditions</b>	
<b>Temperature</b>	19.8 - 21.4 °C
<b>Photoperiod</b>	None, conducted in darkness
<b>pH</b>	6.2 – 6.3
<b>Water content</b>	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 NH<sub>4</sub>-N, NO<sub>3</sub>-N and NO<sub>2</sub>-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 165: 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 <sub>3</sub> -N [mg/kg dry soil]	NO <sub>3</sub> -N [mg/kg dry soil]	Deviation from control [%] <sup>1)</sup>	NO <sub>3</sub> -N [mg/kg dry soil]	Deviation from control [%] <sup>1)</sup>
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

<sup>1)</sup> Based on NO<sub>3</sub>-nitrogen production; - = inhibition, + = stimulation

**Table A 166: 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 <sub>3</sub> -N [mg/kg dry soil]	NO <sub>3</sub> -N [mg/kg dry soil]	Deviation from control [%] <sup>1)</sup>	NO <sub>3</sub> -N [mg/kg dry soil]	Deviation from control [%] <sup>1)</sup>
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

<sup>1)</sup> Based on NO<sub>3</sub>-nitrogen production; - = inhibition, + = stimulation

### *Validity criteria*

As shown in the following table, all validity criteria were met.

**Table A 167: Validity criteria**

Validity criteria according to OECD 216	Observed in study
Variation between replicate control samples ≤ 15%	≤ 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 NO<sub>3</sub>-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

### A 2.6.2 KCP 10.6.2 Testing on non-target plants

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 &lt;10% on all tested species.</p> <p>Based on results of the study the NOER was determined to be <math>\geq 510</math> g a.s./ha.</p>
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<b>Reference:</b>	KCP 10.6.2/01
<b>Report</b>	Terrestrial plant test with MCW-2222: Vegetative vigour test, Friedrich, S., 2014, 14 10 48 002 P
<b>Guideline(s):</b>	OECD 227 (2006)
<b>Deviations:</b>	No
<b>GLP:</b>	Yes, certified laboratory
<b>Acceptability:</b>	Yes
<b>Duplication (if vertebrate study)</b>	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

<b>Test item</b>	MCW-2222
<b>Batch #</b>	611-280413-01
<b>Content of active substance</b>	Acetamiprid 200 g/L (nominal); 202.7 g/L (analysed)
<b>Description</b>	Yellowish liquid
<b>Control</b>	Deionised water
<b>Test organism</b>	
<b>Species</b>	Monocotyledones: oat ( <i>Avena sativa</i> ), perennial ryegrass ( <i>Lolium perenne</i> ) Dicotyledons: turnip ( <i>Brassica rapa</i> ), tomato ( <i>Lycopersicon esculentum</i> ), cucumber ( <i>Cucumis sativus</i> ), soybean ( <i>Glycine max</i> )
<b>Age</b>	2-4 leaf stage BBCH 12-14
<b>Source</b>	Commercial suppliers
<b>Study design and methods</b>	

<b>Test duration and exposure</b>	21 days, spray application at test start
<b>Experimental dates</b>	03 to 24 April 2014
<b>Test rates</b>	510 g a.s./ha in 400 L/ha of water
<b>Test units</b>	Non-porous plastic flower pot (ø 15 cm), capacity/pot: 1.6 kg fresh soil; actual used amount of soil/pot: 1.4 kg
<b>Group size/replicates</b>	30-32 plants per treatment; 2-4 plants per replicates in 8-15 replicates per treatment
<b>Test soil</b>	Agricultural soil (sandy loam) from site Gerichshain (batch G 01/2014) and stored for at least 1 year before used in the test
<b>Irrigation</b>	Daily bottom watering in pot saucers with tap water
<b>Environmental conditions</b>	
<b>Temperature</b>	14 - 31°C
<b>Photoperiod</b>	16 h light / 8 hours darkness 310 – 393 µE/m <sup>2</sup> /s
<b>Relative humidity</b>	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 tables below.

#### **Phytotoxic effects on day 21 after application**

Treatment group	Phytotoxic effects, effects on growth and effects on plant development (BBCH growth stage)					
	Plant species					
MCW-2222 (g a.i./ha)	<i>Avena sativa</i>	<i>Lolium perenne</i>	<i>Brassica rapa</i>	<i>Lycopersicon esculentum</i>	<i>Cucumis sativus</i>	<i>Glycine max</i>
Control	Necrosis (%) on day 21 after application (mean % per treatment group)					
	0	0	0	0	0	0
510	0	0	< 1*	< 1*	< 1*	0
Control	Chlorosis (%) on day 21 after application (mean % per treatment group)					
	0	0	0	0	0	0
510	0	0	0	0	0	0
Control	Deformations (%) on day 21 after application (mean % per treatment group)					
	0	0	0	0	0	0
510	0	0	0	0	0	0
Control	Growth inhibition (%) on day 21 after application (mean % per treatment group)					
	0	0	0	0	0	0
510	0	0	0	0	0	0
Control	BBCH growth stage on day 21 after application					
	31	25	17-18	61	61-63	21-22
510	31	25	17-18	61	61-63	21-22

\* Slight necrotic effects (< 1%) were observed at leaf edges

#### Effects on shoot fresh weight on day 21 after application

Species	Mean shoot fresh weight [g]		
	Control	510 g a.s./ha	% inhibition
<i>Lolium perenne</i>	12.52	11.92	5
<i>Brassica rapa</i>	32.20	29.46	9
<i>Lycopersicon esculentum</i>	39.56	39.01	1
<i>Cucumis sativus</i>	49.47	50.24	-2
<i>Glycine max</i>	12.71	13.15	-4
<i>Avena sativa</i>	21.58	31.02	2

negative value indicates stimulation

**Table A 168:** 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>
<b>Survival (on day 21 after application)</b>							
NOER	≥ 510	≥ 510	≥ 510	≥ 510	≥ 510	≥ 510	≥ 510
<b>Growth (shoot fresh weight on day 21 after application)</b>							
NOER	≥ 510	≥ 510	≥ 510	≥ 510	≥ 510	≥ 510	≥ 510

#### Validity criteria

As shown in the following table, all validity criteria were met.

**Table A 169:** Validity criteria

Validity criteria according to OECD 227	Observed in study
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.

<b>A 2.6.3</b>	<b>KCP 10.6.3</b>	<b>Extended laboratory studies on non-target plants</b>
<b>A 2.7</b>	<b>KCP 10.7</b>	<b>Effects on other terrestrial organisms (flora and fauna)</b>
<b>A 2.8</b>	<b>KCP 10.8</b>	<b>Monitoring data</b>