REGISTRATION REPORT

Part B

Section 9

Ecotoxicology

Detailed summary of the risk assessment

Product name: ASAHI MAX

Product code: ARY-0469-04

Chemical active substance:

0.9% w/w sodium p-nitrophenolate

0.6% w/w sodium o-nitrophenolate

0.3% w/w sodium 5-nitroguaiacolate

Central Zone

Zonal Rapporteur Member State: Poland

CORE ASSESSMENT

(authorization)

Applicant: Asahi Chemical Europe s.r.o.

Submission date: June 2022

MS Finalisation date: April 2023 (Initial Core Assessment)

June 2023 (final Core Assessment)

Version history

When	What
June 2022	Version 1 Applicant
April 2023	Initial zRMS assessment The report in the dRR format has been prepared by the Applicant, therefore all comments, additional evaluations and conclusions of the zRMS are presented in grey commenting boxes. Minor changes are introduced directly in the text and highlighted in grey. Not agreed or not relevant information are ~~struck through and shaded for transparency~~.
June 2023	Final report (Core Assessment updated following the commenting period) No additional information or assessments after the commenting period.

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9 Ecotoxicology (KCP 10)

This document reviews the ecotoxicological studies and risk assessment for the product ASAHI MAX containing the active substances sodium 5-nitroguaiacolate (Na 5-NG), sodium *ortho*-nitrophenolate (Na *o*-NP) and sodium *para*-nitrophenolate (Na *p*-NP) which were included into Annex I of Directive 91/414/EEC (Commission Directive 2009/11/EC). All active substances included into Annex I of Directive 91/414 have been approved under Regulation 1107/2009 by Commission Implementing Regulation (EU) No. 540/2011 of 25 May 2011. A full risk assessment is provided which demonstrates that the product is safe for non-target organisms.

The SANCO report for the active substances sodium 5-nitroguaiacolate, sodium *ortho*-nitrophenolate and sodium *para*-nitrophenolate (SANCO/210/08 – 02 December 2008) and the EFSA Conclusion on the peer review of sodium 5-nitroguaiacolate, sodium *ortho*-nitrophenolate and sodium *para*-nitrophenolate (EFSA Journal 2008; 191, 1-130) are considered to provide the relevant review information or a reference to where such information can be found. Each section will begin with a table providing the EU endpoints to be used in this evaluation.

Ecotoxicological studies have been carried out with Sodium 5-Nitroguaiacolate, Sodium o-Nitrophenolate and Sodium p-Nitrophenolate as well as with the Atonik MUP, the formulation Atonik Plus (identical to ASAHI MAX) and the representative formulation Atonik. For details of the respective composition please refer to Part C (KCP 1.4.3).

9.1 Critical GAP and overall conclusions

Table 9.1-1: Table of critical GAPs

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
Use-No. *	Member state(s)	Crop and/or situation (crop destination / purpose of crop)	F, Fn, Fpn G, Gn, Gpn or I **	Pests or Group of pests controlled (additionally: developmental stages of the pest or pest group)	**Application**				**Application rate**			PHI (days)	Remarks: e.g. g 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)	kg or L product/ha a) max. rate per appl. b) max. total rate per crop/season	g or kg as/ha a) max. rate per appl. b) max. total rate per crop/season	Water L/ha min/max			Birds	Mammals	Aquatic organisms	Bees	Non-target arthro-	Soil organisms	Non-target plants
Zonal uses (field or outdoor uses, certain types of protected crops)																				
1	PL	Winter oilseed rape	F	Plant growth regulator, number of pods per plant, number of seeds per plant, higher lignification of pods	Spray	BBCH 29-69 (spring)	2	7	0.2	0.6 1.2 1.8	200-500	28		A	A	A	A	A	A	A
2	PL	Winter wheat	F	Plant growth regulator, number of tillers and ears, portion Aabove the sieves, germination energy	Spray	BBCH 21-49 (spring)	1	-	0.2	0.6 1.2 1.8	200-300	28		A	A	A	A	A	A	A
3	PL	Sugar beet	F	Plant growth regulator, effect on higher yield of sugar, lower content of unwanted Sodium	Spray	BBCH 12-49 (spring-summer)	2	7	0.2	0.6 1.2 1.8	200-500	15		A	A	A	A	A	A	A
Interzonal uses (use as seed treatment, in greenhouses (or other closed places of plant production), as post-harvest treatment or for treatment of empty storage rooms)																				
None																				
Minor uses according to Article 51 (field uses)																				
4	PL	Mustard, spring rape, turnip rape, camelina, garden radish, poppy, linseed,	F	Plant growth regulator, number of pods per plant, number of seeds per plant, higher lignification of pods	Spray	BBCH 29-69 (spring)	2	7	0.2	0.6 1.2 1.8	200-500	28	Extrapolation from winter osr.	A	A	A	A	A	A	A

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
		hemp, sun-flower, borage.																		
5	PL	Spring rye, spelt, emmer wheat, small spelt, du-rum wheat.	F	Plant growth regula-tor, number of tillers and ears, portion above the sieves, germination energy	Spray	BBCH 21-49 (spring)	1	-	0.2	0.6 1.2 1.8	200-300	28	Extrapo-lation from winter wheat.	A	A	A	A	A	A	A
6	PL	Fodder beet, red beet, swede, turnip.	F	Plant growth regula-tor, effect on higher yield.	Spray	BBCH 12-49 (spring-summer)	2	7	0.2	0.6 1.2 1.8	200-500	15	Extrapo-lation from sugar beet	A	A	A	A	A	A	A
Minor uses according to Article 51 (interzonal uses)																				
None																				

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

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

Explanation for column 15 – 21 "Conclusion"

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

Remarks table:

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

9.1.1 Overall conclusions

zRMS comments:

Conclusions presented in points 9.1.1.1 to 9.1.1.7 below were checked by the zRMS and amended where necessary.

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)

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.

A safe use for birds and mammals has been concluded at screening step. There is no risk for birds and mammals from drinking water consumption and the risk from secondary poisoning for fish-eating and worm-eating birds and mammals is acceptable.

Therefore, it is concluded that the intended use of ASAHI MAX does not pose any potential risk for birds and mammals.

No relevant data on amphibians and reptiles are available. ~~According to Chapter 7.2.4 in the Aquatic guidance Document, EFSA, 2013, the rainbow trout is a good surrogate test species for predicting the toxicity of formulated products for larval stages of amphibian species living in the aquatic compartment of the environment. The terrestrial life stages of amphibians and reptiles can be considered covered by the risk assessment for birds and mammals.~~

9.1.1.2 Effects on aquatic organisms (KCP 10.2)

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).
An acceptable risk is concluded for aquatic organisms from the use of ASAHI MAX and the intended GAP.
No risk mitigation measures are required.

9.1.1.3 Effects on bees (KCP 10.3.1)

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).

An acceptable risk is concluded for bees from the use of ASAHI MAX and the intended GAP.

9.1.1.4 Effects on arthropods other than bees (KCP 10.3.2)

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).

An acceptable risk is concluded for non-target arthropods from the use of ASAHI MAX and the intended GAP. No risk mitigation measures are required.

9.1.1.5 Effects on non-target soil meso- and macrofauna (KCP 10.4), Effects on soil microbial activity (KCP 10.5)

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).

An acceptable risk is concluded for soil organisms from the use of ASAHI MAX and the intended GAP.

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

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).

An acceptable risk is concluded for non-target plants from the use of ASAHI MAX and the intended GAP. No risk mitigation measures are required.

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

No further information is required.

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 ASAHI MAX grouped application pattern

Grouping according to application pattern			
Group	**Intended uses**	**relevant use parameters for grouping**	**relevant parameter or value for sorting**
Birds and mammals			
Oilseed rape Sugar beet Minor crops included in the GAP	Oilseed rape Winter wheat Sugar beet Minor crops included in the GAP	Same screening group Max application number Max application rate	Same screening group 2 x 0.2 L/ha (7d)
Aquatic organisms			
Oilseed rape	Oilseed rape	Not grouped	2 x 0.2 L/ha (7d) Specific scenario
Winter wheat	Winter wheat	Not grouped	0.2 L/ha Specific scenario
Sugar beet	Sugar beet	Not grouped	2 x 0.2 L/ha (7d) Specific scenario
Sunflower	Sunflower	Sunflower	Sunflower
Leafy vegetables	Leafy vegetables	Leafy vegetables	Leafy vegetables
Bees			
All	Oilseed rape	Max application rate	0.2 L/ha

Grouping according to application pattern			
Group	**Intended uses**	**relevant use parameters for grouping**	**relevant parameter or value for sorting**
	Winter wheat Sugar beet Minor crops included in the GAP		
NTA			
Oilseed rape Sugar beet	Oilseed rape Winter wheat Sugar beet Minor crops included in the GAP	Max application number Max application rate	2 x 0.2 L/ha
Earthworms and other macro-organisms			
All	Oilseed rape Winter wheat Sugar beet	Max PECsoil	Max PECsoil
Soil microorganisms			
All	Oilseed rape Winter wheat Sugar beet Minor crops included in the GAP	Max PECsoil	Max PECsoil
NTP			
All	Oilseed rape Winter wheat Sugar beet Minor crops included in the GAP	Max application rate Same drift rate	0.2 L/ha 2.77%

9.1.3 Consideration of metabolites

The assessment of metabolites is considered covered by the risk assessment of the active substances and de formulated product. No further assessment of metabolites is considered necessary.

zRMS comments:

The metabolites listed as ecotoxicologically relevant in the EFSA conclusion are the photolytic metabolites which have been addressed as part of the confirmatory data procedure.
Therefore, no further assessment of metabolites is required for product registration.

9.2 Effects on birds (KCP 10.1.1)

9.2.1 Toxicity data

Avian toxicity studies have been carried out with the active substances and representative formulation. Full details of these studies are provided in the respective EU DAR and related documents.

Effects on birds of ASAHI MAX were not evaluated as part of the EU assessment. However, the provision of further data on the formulation is not considered essential, because the risk assessment can be based on data from the active substances.

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

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

Study	Test species	Test substance	EU agreed endpoints (mg/kg/bw/day) EFSA Scientific report. (2008) 191, 1-130
Acute toxicity	*Colinus virginianus*	Na 5-NG	LD_{50} 2067
		Na *o*-NP	LD_{50} 1046
		Na *p*-NP	LD_{50} >1670
Acute toxicity		Mixture toxicity (finney formula) - ATONIK	$LD_{50\ (mix)}$ = 238536 mg product /kg/bw/day
Acute toxicity	Rat	Asahi Max	LD_{50}>2000 mg product/kg bw
Short-term	*Colinus virginianus*	Na 5-NG	LC_{50} 1830[1]
		Na *o*-NP	LC_{50} >2698
		Na *p*-NP	LC_{50} >1412
	Mallard duck	Na *o*-NP	LC_{50} > 2539
Reproductive toxicity (long-term)	*Colinus virginianus*	MUP of ATONIK[2]	NOEL 95 mg product/kg/bw/d (1000 ppm mg/kg feed) Equivalent to 73.5 mg a.s./kg bw/day[3]

***** Based on a total active substance purity of 0.6% as ATONIK contains Na 5-NG 0.1%, Na *o*-NP 0.2%, Na *p*-NP 0.3%
[1] Although the EFSA Conclusion states an LC_{50} of 1830 mg a.s./kg bw it should be noted that the true LC_{50} is >1830 mg a.s./kg bw as there were no mortalities at the highest concentration tested of 5620 ppm (equivalent to 1830 mg a.s./kg bw)
[2] Atonik Manufacture Use Product containing Sodium 5-nitroguaiacolate 11.6%, Sodium ortho-nitrophenolate 23.2%, Sodium para-nitrophenolate 42.6%. Measured concentration 50% of the nominal, but the tested preparation is 100 times more concentrate than the representative
[3] Based on a total active substance purity of 77.4% as MUP of ATONIK contains Na 5-NG 11.6 %, Na *o*-NP 23.2%, Na *p*-NP 42.6 %

zRMS comments:

Avian toxicity data provided in Table 9.2-1 above were checked by zRMS and then confirmed that they are in line with EU agreed endpoints reported in EFSA EFSA Scientific Report (2008) 191.

For Na 5-NG and Na *p*-NP the short-term dietary LC_{50} values are lower than the acute oral LD_{50} values. For Na 5-NG the short-term dietary LC_{50} value is 1830 mg a.s./kg bw which is lower than the acute oral LD_{50} of 2067 mg a.s./kg bw. However, it should be noted that although the EFSA Conclusion (EFSA Scientific Report (2008) 191, 1-130) states that the dietary LC_{50} for Na 5-NG is 1830 mg a.s./kg bw, the true LC_{50} is in fact >1830 mg a.s./kg bw. This is because there were no mortalities at the highest concentration tested of 5620 ppm (equivalent to 1830 mg a.s./kg bw) in the study (Long *et al*., 1991). Thus, the lower endpoint derived in the dietary study is "greater than" value and there is no evidence to suggest that short-term dietary exposure is a worse case than acute exposure.
Likewise, for Na *p*-NP the short-term dietary LC_{50} value is >1412 mg a.s./kg bw which is technically lower than the acute oral LD_{50} of >1670 mg a.s./kg bw. However, it should be noted that in the short-term dietary study (Long *et al*., 1991) there were no mortalities or sub-lethal effects at the highest concentration tested of 5620 ppm (equivalent to 1412 mg a.s./kg bw). Thus, the lower endpoint derived in the dietary study is "greater than" value and there is also no evidence here to suggest that short-term dietary exposure is worse case than acute exposure.
For both Na 5-NG and Na *p*-NP the short-term dietary LC_{50} values are lower than the acute oral LD_{50} values, however the short-term endpoints have been determined in tests in which there were no or very limited mortalities therefore the LC_{50} has been set as greater than the highest dose tested in each study. Thus, although the LC_{50} values appear lower than the acute oral LD_{50} values, this does not indicate that short-term dietary exposure leads to greater mortality than acute exposure. In fact, greater levels of

mortality were observed in the acute oral studies than in the associated short-term dietary studies therefore acute oral exposure is still considered to be the worst-case route of exposure. However, in order to conduct a conservative risk assessment, the lowest LD_{50}/LC_{50} value in each case for Na-5-NG, Na *o*-NP and Na *p*-NP has been used in the risk assessment below.

zRMS comments:

zRMS agrees with the Applicant's position of using dietary endpoints in the acute risk assessment, where necessary.
In the same time, it should be noted that at product authorisation the agreed endpoints in LoEP should be not changed and for this reason not corrected value are taken into account by zRMS in the risk assessment:

Na-5-NG = 1830 mg a.s./kg bw (dietary endpoint)
Na p-NP = 1412 mg a.s./kg bw (dietary endpoint)
Na o-NP= 1046 mg a.s./kg bw (acute endpoint)

Mixture toxicity

Acute studies with ASAHI MAX have not been conducted; toxicity of the formulation can be predicted from the toxicity of the active substances as toxicity is not enhanced by formulation. In order to assess the toxicity of the mixture, a surrogate $LD_{50\ mixture}$ can be calculated based on the Finney Equation and the relative toxicity of the different substances in the mixture.

$LD_{50\ mix} = (\Sigma(X(a.s._i)/LD_{50}\ (a.s._i))^{-1}$
Where:

X, fraction of the active ingredient i (in weight) in the formulation

LD_{50i}, the toxicity of the active ingredients i for each organism

Active substance	LD_{50} (mg a.s./kg bw/d)	Nominal content (%)	Fraction in the mixture*	Calculated LD_{50} mix (mg a.s./kg bw/d)
Na-5-NG	≥1830	0.3%	0.17	1311.5
Na *o*-NP	1046	0.6%	0.33	
Na *p*-NP	≥1412	0.9%	0.50	

The calculated $LD_{50\ mix}$ is 1311.5 mg a.s./kg bw/d, corresponding to 72861 mg ASAHI MAX/kg bw/d.

zRMS comments:

Acute combined toxicity

zRMS checked calculations of LD_{mix} value. The slight difference in comparison of the Applicant's LD_{50} mix value was noted by zRMS.

The relevant calculations are presented below :

Avian LD_{50} (mix) for bird when combined all active substances in Asahi Max (step 1 in EFSA GD 2009, Appendix B).

	NA -5 -NG	Na-o-NP	Na p-NP
Relative amount of a.s. (%) (g/L)	0.3 3	0.6 6	0.9 9
Fraction in the a.s. mixture	0.17	0.33	0.50
LD_{50} of a.s. mg/kg bw]	1830	1046	1412
Fraction / LD_{50}	0.000093	0.000315	0.000354
Sum	0.000762		

1/ sum = predicted LD_{50} (mix)	**1312.33 mg a.s./kg bw**

Finally, LD_{50} mix of 1312 mg a.s./kg bw was considered by zRMS in the acute mixture risk assessment.
Long-term combined toxicity**:**

zRMS agrees that for the approach assuming dose additivity of the active substances, reliable results would only be expected for combinations of effect levels with a defined x, e.g. a LD_{50}, but not for NOELs since the latter effect indicators may represent varying risk or response levels for different compounds depending on dose-spacing according to GD B&M, EFSA Journal 2009; 7(12): 1438, Appendix B. Therefore, for the risk assessment based on long-term effects it is not recommended to consider the use of predicted toxicity values.
It should be noted that according to recommendation given in Appendix B of the Guidance Document 2009 for the evaluation of sublethal effects the use of the lowest NO(A)EL of the actives in the formulation, along with the combined exposure estimate from both active substances provides a conservative representation of long-term risks to birds.
It should be pointed out that the long-term toxicity endpoints for each of the active substances are not available in LoEP, EFSA 2008.
For this reason, the long-term mixture toxicity assessment could not be performed by zRMS.

9.2.1.1 Justification for new endpoints

Not relevant, EU-agreed endpoints have been used in the risk assessment.

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.

The risk envelope approach is applied: the screening step for oilseed rape and sugar beets covers the risk assessment for winter wheat.

Table 9.2-2: First-tier assessment of the acute and long-term/reproductive risk for birds due to the use of ASAHI MAX

Intended use	**Oilseed rape, sugar beets**
Active substance/product	Na 5-NG = 2 x 0.6 g a.s/ha (7d) Na o-NP = 2 x 1.2 g a.s/ha (7d) Na p-NP = 2 x 1.8 g a.s/ha (7d) ASAHI MAX = 2 x 3.6 g a.s/ha (7d)
Application rate (g/ha)	
Acute toxicity (mg/kg bw)	Na 5-NG >1830 Na o-NP = 1046 Na p-NP >1412 ASAHI MAX = 1312.33 ~~1311.5~~
TER criterion	10

Crop scenario Growth stage	**Indicator/generic focal species**	**SV_{90}**	**MAF_{90}**	**DDD_{90} (mg/kg bw/d)**	**TER_a**
Bulbs and onion like	Small omnivorous	158.8	1.4	Na 5-NG = 0.13	Na 5-NG = 13719.0

crops, cereals, fruiting vegetables, leafy vegetables, legume forage, maize, oilseed rape, potatoes, pulses, root and stem vegetables, strawberries, sugar beet, and sunflower	bird			Na o-NP = 0.27 Na p-NP = 0.40 ASAHI MAX = 0.80	Na o-NP = 3920.8 Na p-NP = 3528.4 ASAHI MAX = 1640.41 ~~1638.7~~
Reprod. toxicity (mg/kg bw/d)	73.5 (sum of active substances)				
TER criterion	5				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_m**	**MAF_m × TWA**	**DDD_m (mg/kg bw/d)**	**TER_{lt}**
Bulbs and onion like crops, cereals, fruiting vegetables, leafy vegetables, legume forage, maize, oilseed rape, potatoes, pulses, root and stem vegetables, strawberries, sugar beet, and sunflower	Small omnivorous bird	64.8	2.0 x 0.53	ASAHI MAX = 0.20	ASAHI MAX = 371.5

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.

The acute and chronic TER values are above of the the respective trigger values. Therefore, a safe use for birds can be concluded for the intended uses of ASAHI MAX.

zRMS comments:

Screening step in the acute and long-term risk assessment

The acute and long-term screening step risk assessment for all active substances is validated by zRMS.
It should be noted that the long-term endpoint was obtained from formulation MUP of ATONIK expressed in terms of the total active substances content (73.5 mg sum of a.s./kg bw/day).
In zRMS's opinion this approach is justified as % active substances in MUP of ATONIK is higher; Na 5-NG: 11.6 %, Na o-NP: 23.2 %, Na p-NP: 42.6 % in comparison to Asahi Max (Na 5-NG: 0.3%, Na o-NP: 0.6%, Na p-NP: 0.9 %).

Based on the calculations performed in the Table 9.2-2, TER_A and TER_{Lt} are above trigger 10 and 5, respectively, indicating an acceptable risk to birds from exposure Asahi Max in main and minor crops included in the GAP Table.

9.2.2.2 Higher-tier risk assessment

Not relevant.

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

Not relevant.

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 463.4 mL/g (Na 5-NG), 156.1 mL/g (Na o-NG) and 288.1 mL/g (Na p-NG), the active substances belong to the group of less sorptive substances. To achieve a concise risk assessment, the risk envelope approach is applied. Here, the assessment for oilseed rape and sugar beets covers the risk assessment for winter wheat.

Crop	Effective application rate (g/ha)	Acute toxicity (mg/kg bw)	Quotient	Effective application rate (g/ha)	Reprod. toxicity (mg/kg bw/d)	Quotient	Trigger 50 Risk?
Na 5-NG	0.6 x 1.4	~~>~~1830	0.00046	-	-	-	No
Na o-NG	1.2 x 1.4	1046	0.00161	-	-	-	No
Na p-NG	1.8 x 1.4	~~>~~412	0.00178	-	-	-	No
ASAHI MAX	3.6 x 1.4	1312.33 ~~1311.5~~	0.00384	3.6 x 2.0	73.5	0.09796	No

There is no risk for birds from drinking water consumption.

zRMS comments:

The evaluation of the risk resulting from uptake of contaminated water in Puddle scenario was not required since ratio between effective application rate and endpoint relevant for acute risk and long-term assessment is <500.

9.2.2.4 Effects of secondary poisoning

According to the 'EFSA Guidance Document on Risk Assessment for Birds and Mammals (Anonymous 2009)[1] substances with a log P_{OW} greater than 3 have potential for bioaccumulation and should be assessed for the risk of biomagnification in terrestrial food chains.

Each active substance Na 5-NG, Na *o*-NP and Na *p*-NP has a log P_{OW} value of <3. It was therefore not necessary to consider the risk from secondary poisoning further. Therefore, based on the log P_{OW} values, the risk from secondary poisoning to fish-eating and worm-eating birds is acceptable.

Risk assessment for earthworm-eating birds via secondary poisoning

Not required.

Risk assessment for fish-eating birds via secondary poisoning

Not required.

zRMS comments:

[1]European Food Safety Authority; Guidance Document on Risk Assessment for Birds and Mammals on request from EFSA. EFSA journal 2009; 7(12):1438. [139 pp.]

The Applicants' approach in evaluation of the risk of secondary poisoning is in line with EFSA (2009).. Evaluation was not triggered for active substances and their metabolites due to their log Pow <3.

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 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.

A safe use for birds has been concluded at screening step. There is no risk for birds from drinking water consumption and the risk from secondary poisoning for fish-eating and worm-eating birds is acceptable.

Therefore, it is concluded that the intended use of ASAHI MAX does not pose any potential risk for birds.

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 active substances and representative formulation ATONIK and the MUP of ATONIK. Full details of these studies are provided in the respective EU DAR and related documents.
In addition, the acute toxicity study is available in Section 6 for formulation Asahi Max.
Effects on mammals of ASAHI MAX were not evaluated as part of the EU assessment of active substances.
However, the provision of further data on the formulation is not considered essential, because the risk assessment can be based on data from the active substances.

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

	Active substance	Test species	EU agreed endpoints (mg/kg bw/d) (EFSA Scientific report (2008)
Acute	5-NG	Rat	LC_{50} 716
	o-NP	Rat	LC_{50} 960.1
	p-NP	Rat	LC_{50} 345.5
	~~ATONIK~~	~~Rat~~	~~LC_{50} >5000 mg product/kg bw Equivalent to >30 mg a.s./kg bw[1]~~
Acute	Asahi Max	Rat	LC_{50} >2000 mg product/kg bw Equivalent to **>32 mg a.s./kg bw [1]**
Long term	MUP of ATONIK*	Rat	NOAEL 300 mg product/kg bw/day Equivalent to 232.2 mg a.s./kg bw/day[2]

~~[1] Based on a nominal total active substance content of 0.6% (Na 5-NG 0.1 %, Na o-NP 0.2 %, Na p-NP 0.3 %)~~

[1] New study evaluated in Section Toxicology.

[2] Based on a total active substance purity of 77.4% as MUP of ATONIK contains Na 5-NG 11.6 %, Na o-NP 23.2 %, Na p-NP 42.6 %

* Atonik Manufacture Use Product (MUP): Sodium 5-nitroguaiacolate 11.6 %, Sodium ortho-nitrophenolate 23.2 %, Sodium para-nitrophenolate 42.6 %

zRMS comments:

Avian toxicity data provided in Table 9.3-1 above were checked by zRMS and then confirmed that they are in line with EU agreed endpoints reported in EFSA EFSA Scientific Report (2008) 191.

Product MUP of ATONIK was prepared for the toxicological studies purpose only.
The test material in the studies submitted for Annex I inclusion was a red powder identified as: ATONIK MUP powder had a reported purity of 11.6% sodium 5-nitroguaiacolate, 23.2% sodium ortho-nitrophenolate and 42.6% sodium para-nitrophenolate·dihydrate.

This was done because concentration of active substances in the representative product ATONIK was very low, it was decided to prepare an artificial product from ATONIK by drying. The increased concentration was intended to show the toxicological effect. The tested preparation is 100 times more concentrate than the representative product ATONIK.

The NOEL = 232.2 mg sum of a.s./kg bw was used in the current risk assessment.

9.3.1.1 Justification for new endpoints

Not relevant, EU-agreed endpoints have been used in the risk assessment.

9.3.2 Risk assessment for spray applications

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

To achieve a concise risk assessment, the risk envelope approach is applied. Here, the screening step for oilseed rape and sugar beets covers the risk assessment for winter wheat.

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 ASAHI MAX

Intended use	**Oilseed rape, sugar beet**				
Active substance/product **Application rate (g/ha)**	Na 5-NG = 2 x 0.6 g a.s/ha (7d) Na o-NP = 2 x 1.2 g a.s/ha (7d) Na p-NP = 2 x 1.8 g a.s/ha (7d) ASAHI MAX = 2 x 3.6 g a.s/ha (7d)				
Acute toxicity (mg/kg bw)	Na 5-NG = 716 Na o-NP = 960.1 Na p-NP = 345.5 ATONIK > 32 ~~9~~ (sum of active substances)				
TER criterion	10				
Crop scenario **Growth stage**	**Indicator/generic focal species**	**SV_{90}**	**MAF_{90}**	**DDD_{90}** **(mg/kg bw/d)**	**TER_a**
Bulbs and onion like crops, cereals, oilseed rape, potatoes, root and stem vegetables, strawberries, sugar beet, and sunflower	Small herbivorous mammal	118.4	1.4	Na 5-NG = 0.10 Na o-NP = 0.20 Na p-NP = 0.30 ASAHI MAX = 0.60	Na 5-NG = 7199.2 Na o-NP = 4826.8 Na p-NP = 1158.0 ASAHI MAX = 53.33 ~~50.3~~
Reprod. toxicity (mg/kg bw/d)	232.2 (sum of active substances)				
TER criterion	5				
Crop scenario **Growth stage**	**Indicator/generic focal species**	**SV_m**	**MAF_m × TWA**	**DDD_m** **(mg/kg bw/d)**	**TER_{lt}**
Bulbs and onion like crops, cereals, oilseed rape, potatoes, root and stem vegetables, strawberries, sugar beet, and sunflower	Small herbivorous mammal	48.3	1.6 x 0.53	ASAHI MAX = 0.15	ASAHI MAX = 1574.77

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 chronic TER values are above of the the respective trigger values. Therefore, a safe use for mammals can be concluded for the intended uses of ASAHI MAX.

zRMS comments:

Screening step in the acute and long-term risk assessment

The acute and long-term screening step risk assessment for all active substances is validated by zRMS.
It should be noted that the long-term endpoint was obtained from formulation MUP of ATONIK (expressed in terms of total active substance content (232.2 mg a.s./kg bw/day).
In zRMS's opinion this approach is justified as % active substances in MUP of ATONIK is higher; Na 5-NG: 11.6 %, Na o-NP: 23.2 %, Na p-NP: 42.6 % in comparison to Asahi Max (Na 5-NG: 0.3%, Na o-NP: 0.6%, Na p-NP: 0.9 %). In addition, the acute mixture toxicity assessment to mammals was considered acceptable.

Acute combined toxicity assessment:

The acute toxicity study for mammals for formulation Asahi Max is available.

According to the evaluation in Toxicology Section the LD_{50}>2000 mg Asahi Max/kg bw value is confirmed to be correct and use in the risk assessment.

Calculation of LD_{50} mix value according to EFSA for mammals.

	NA -5 -NG	Na-o-NP	Na p-NP
Relative amount of a.s. (%) g/L	0.3 3	0.6 6	0.9 9
Fraction in the a.s. mixture	0.17	0.33	0.50
LD_{50} of a.s. mg/kg bw]	716	960.1	345.5
Fraction / LD_{50}	0.000237	0.000343	0.00144
Sum	0.00202		
1/ sum = predicted LD_{50} (mix)	**495.05 mg a.s./kg bw**		

According to evaluation by zRMS experimentally derived endpoint for the formulated product (LD_{50}>2000 mg a.s./kg bw) expressed in terms of the sum of active compounds would be greater than 32 mg a.s./kg bw which in comparison with estimated $LD50_{mix}$ of 495.05 mg a.s./kg bw indicates that the formulated product may be more toxic than the particular substances.
Taking this into account the acute risk assessment for the formulation presented in the Table 9.3-2 was considered acceptable with corrected LD_{50} >32 mg a.s./kg bw value.

Long-term combined toxicity risk assessment risk

In LoEP in EFSA Conclusion (2008) the long-term toxicity endpoints for each a.s. is not given.
For this reason, the combined long-term risk is not provided in this case.
The long-term risk was based on a total active substance purity of 77.4% as MUP of ATONIK contains Na 5-NG 11.6 %, Na o-NP 23.2 %, Na p-NP 42.6 %.

9.3.2.2 Higher-tier risk assessment

Not relevant.

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 463.4 mL/g (Na 5-NG), 156.1 mL/g (Na o-NG) and 288.1 mL/g (Na p-NG), the active substances belong to the group of less sorptive substances. To achieve a concise risk assessment, the risk envelope approach is applied. Here, the assessment for oilseed rape and sugar beets covers the risk assessment for winter wheat.

Crop	Effective application rate (g/ha)	Acute toxicity (mg/kg bw)	Quotient	Effective application rate (g/ha)	Reprod. toxicity (mg/kg bw/d)	Quotient	Trigger 50 Risk?
Na 5-NG	0.6 x 1.4	716	0.00117	-	-	-	No
Na o-NG	1.2 x 1.4	960.1	0.00175	-	-	-	No
Na p-NG	1.8 x 1.4	345.5	0.00729	-	-	-	No
ASAHI MAX	3.6 x 1.4	32 ~~30~~	0.1575 ~~0.16800~~	3.6 x 1.6	232.2	0.02481	No

There is no risk for mammals from drinking water consumption.

zRMS comments:

The evaluation of the risk resulting from uptake of contaminated water in Puddle scenario was not required since ratio between effective application rate and endpoint relevant for acute risk and long-term assessment is <500.

9.3.2.4 Effects of secondary poisoning

According to the 'EFSA Guidance Document on Risk Assessment for Birds and Mammals (Anonymous 2009)[2] substances with a log P_{OW} greater than 3 have potential for bioaccumulation and should be assessed for the risk of biomagnification in terrestrial food chains.

Each active substance Na 5-NG, Na *o*-NP and Na *p*-NP has a log P_{OW} value of <3. It was therefore not necessary to consider the risk from secondary poisoning further. Therefore, based on the log P_{OW} values, the risk from secondary poisoning to fish-eating and worm-eating mammals is acceptable.

Risk assessment for earthworm-eating mammals via secondary poisoning

Not required.

Risk assessment for fish-eating mammals via secondary poisoning

Not required.

zRMS comments:

The Applicants' approach in evaluation of the risk of secondary poisoning is in line with EFSA (2009).. Evaluation was not triggered for active substances and their metabolites due to their log Pow <3.

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.

[2]European Food Safety Authority; Guidance Document on Risk Assessment for Birds and Mammals on request from EFSA. EFSA journal 2009; 7(12):1438. [139 pp.]

9.3.4 Overall conclusions

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.
A safe use for mammals has been concluded at screening step. There is no risk for mammals from drinking water consumption and the risk from secondary poisoning for fish-eating and worm-eating mammals is acceptable. Therefore, it is concluded that the intended use of ASAHI MAX does not pose any **potential** risk for mammals.

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 Na 5-NG, Na *o*-NP and Na *p*-NP. Regarding assessment of potential effects on reptiles and amphibians neither guidance documents nor testing guidelines are available at present.

According to Chapter 7.2.4 in the Aquatic guidance Document, EFSA, 2013, the rainbow trout is a good surrogate test species for predicting the toxicity of formulated products for larval stages of amphibian species living in the aquatic compartment of the environment.
The terrestrial life stages of amphibians and reptiles can be considered covered by the risk assessment for birds and mammals.

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 active substances and representative formulation. Full details of these studies are provided in the respective EU DAR and related documents.

Effects on aquatic organisms of ASAHI MAX were not evaluated as part of the EU assessment of active substances. New data with ATONIK is submitted with this application are listed in Appendix 1 and summarised in Appendix 2.

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

Active substance	Test species[1]	EU agreed endpoints (mg/L) (EFSA Scientific report (2008) 191, 1-139)	Endpoints used in risk assessment (mg /L)
Acute toxicity to fish			
Na 5-NG	Rainbow trout	96 h LC_{50} = 37.4	96 h LC_{50} = 37.4
Na *o*-NP	Rainbow trout	96 h LC_{50} = 69	96 h LC_{50} = 69
Na *p*-NP	Rainbow trout	96 h LC_{50} = 25.0	96 h LC_{50} = 25.0
ATONIK PLUS[5]	*Cyprinus carpio*	96 h LC_{50} = 6800 mg product / L	96 h LC_{50} = 6800 mg product / L ($1^2$2.4 mg a.s/L)
Chronic toxicity to fish			
MUP of ATONIK[3]	Zebra fish	NOEC = 10 mg a.s/L	NOEC = 7.74 mg a.s/L[6]
Acute toxicity to aquatic invertebrates			
Na 5-NG	*Daphnia magna*	48 h LC_{50} = 71.1	48 h LC_{50} = 71.1
Na *o*-NP	*Daphnia magna*	48 h LC_{50} > 68.8	48 h LC_{50} > 68.8
Na *p*-NP	*Daphnia magna*	48 h LC_{50} = 27.7	48 h LC_{50} = 27.7
ATONIK PLUS[5]	*Daphnia magna*	48 h LC_{50} = 2000 mg product / L	48 h LC_{50} = 2000 mg product / L (36 mg a.s/L)
Chronic toxicity to aquatic invertebrates			
MUP of ATONIK[3]	*Daphnia magna*	NOEC = 1.0	NOEC = 0.774 mg a.s./L[6/7]
Toxicity to green algae			
Na 5-NG	*Scenedesmus subspicatus*	72 h E_bC_{50} = 6.2 72 h E_rC_{50} = >21 $NOEC_b$ = 0.46 $NOEC_r$ = 1.0	72 h E_bC_{50} = 6.2
Na *o*-NP	*Scenedesmus subspicatus*	72 h E_bC_{50} = 4.8 72 h E_rC_{50} = >10 $NOEC_b$ = 0.21 $NOEC_r$ = 0.46	72 h E_bC_{50} = 4.8
Na *p*-NP	*Scenedesmus subspicatus*	72 h E_bC_{50} = 2.5 72 h E_rC_{50} = 4.6 NOECb = 0.21 NOECr = 0.46	72 h E_bC_{50} = 2.5
ATONIK [4]	*Scenedesmus subspicatus*	72 h EC_{50} = >100 72 h NOEC = 100	72 h EC_{50} = >100 mg product/L (>0.6 mg a.s/L)
ATONIK [4]	*Anabaena flos-aq-uae*	-	72 h E_bC_{50} = 1720 mg product / L (10.32 mg a.s./L) 72 h E_rC_{50} = 6990 mg product / L (41.940 mg a.s./L) NOErC = 300 mg product / L

Toxicity to aquatic plants			
			7d EC_{50} = 7820 mg product/L (46.92 mg a.s./L) (yield, frond number) $E_rC_{10\ (frond\ number)}$= 2850 mg/L NOEC = 370 mg/L
ATONIK [4]	*Lemna gibba*	-	ErC_{10} (frond number)= 28570 mg test item/L ErC_{50} (frond number) = 25700 mg test item/L ErC_{10} (dry weight)= 5005 mg test item/L ErC_{50} (dry weight) = 21200 mg test item/L

[1] end-point for the critical species only
[2] Since Annex I inclusion new studies on the active substance have been performed and as a result there are new end-points which are used in the risk assessment.
[3]MUP (Manufacture Use Product) of ATONIK containing Sodium 5-nitroguaiacolate 11.6 %, Sodium ortho-nitrophenolate 23.2 %, Sodium para-nitrophenolate 42.6 %
[4] ATONIK : Sodium 5-nitroguaiacolate 0.1%, Sodium ortho-nitrophenolate 0.2 %, Sodium para-nitrophenolate 0.3%
[5] ATONIK PLUS : Sodium 5-nitroguaiacolate 0.3%, Sodium ortho-nitrophenolate 0.6 %, Sodium para-nitrophenolate 0.9%
[6] based on sum of purity of active substances.
[7] reported from Addendum 1 to Annex B9 (NOEC daphnia = 1.0 mg product/L = 0.774 mg a.s/L since sum of purity of active substances is 77.4% for MUP of ATONIK). An error has been reported in EFSA journal (NOEC = 0.0774 mg a.s./L.)

zRMS comments:

Aquatic toxicity data provided in Table 9.5-1 above were checked by zRMS and then confirmed that they are in line with EU agreed endpoints reported in EFSA EFSA Scientific Report (2008) 191.

Further studies on a second algal species, Anabaena flos-aquae and aquatic plants, Lemna gibba for ATONIK, were provided by the Applicant in the current dossier (data gap was identified in EFSA conclusions) and was considered acceptable by zRMS.

It should be indicated that in case of formulation studies for Asahi Max (other name ATONIK PLUS, ATONIK 1.8%), the Applicant referred to different formulations such as: MUP of ATONIK, ATONIK, ATONIK solution. All these formulations were assessed at EU level and in EFSA Conclusion 2008.

In case of acute formulation studies for Asahi Max (ATONIK PLUS) for fish and aquatic invertebrates the toxicity endpoints from ATONIK SOLUTION (from LoEP) was used. It is justified due to the same % of active substances and water in both formulations.

However, in case of algae (Scenedesmus subspicatus, Anabaena flos-aquae) and aquatic macrophytes (Lemna gibba) the endpoints from representative formulation ATONIK containing lower % of each active substances than in Asahi Max such as: Sodium 5-nitroguaiacolate 0.1%, Sodium ortho-nitrophenolate 0.2 %, Sodium para-nitrophenolate 0.3% was used by the Applicant (Please refer to PART C) was used by the Applicant.

By comparison of Part C of the two products there is three times more of each active substances in Asahi Max than in ATONIK, but the proportion of each active substances in both products are the same (ATONIK: 1g, 2g, 3 g/L and Asahi Max: 3 g, 6 g, 9 g/L). In the same time no differences in toxicity due to the adjuvants is expected (water only).

Therefore, in this case toxicity of Asahi Max formulation for algae and aquatic macrophytes it is based on the the toxicity of the formulation of ATONIK divided by 3 with assumption of three times higher amount of a.s. in Asahi Max and the same proportion of the a.s. in the products.
It should be pointed out that the final toxicity expressed in mg sum of a.s./L is the same for both of products ATONIK and Asahi Max (ATONIK PLUS). Therefore, it can be concluded that toxicity endpoints for ATONIK expressed in mg sum of a.s./L are protective also for Asahi Max.

This approach was approved by zRMS Greece in the evaluation of the product in Southern Zone for Asahi Max 2014, in case of lack data for the most sensitive organism (algae and aquatic macrophytes) for formulation Asahi Max being plant growth regulator. In this specific case this approach is also considered acceptable by zRMS-PL.
In addition, the combined mixture toxicity was presented based on available data for the active substances and product data for ATONIK Plus (Asahi Max) and ATONIK.

9.5.1.1 Justification for new endpoints

Not relevant, EU-agreed endpoints have been used in the risk assessment.

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 PEC_{SW} for risk assessments covering the proposed use pattern and the resulting PEC/RAC ratios are presented in the table below.
The risk assessment for metabolites is considered covered with the risk assessment of active substances.
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.

Table 9.5-2: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for Na 5-NG for each organism group based on FOCUS Steps 1-2 calculations

Group		**Fish acute**	**Inverteb. acute**	**Algae**
Test species		***Oncorhynchus mykiss***	***Daphnia magna***	***Scenedesmus subspicatus***
Endpoint		LC_{50}	EC_{50}	E_bC_{50}
(µg/L)		37 400	71 100	6 200
AF		100	100	10
RAC (µg/L)		374	711	620
FOCUS Scenario	**PEC $_{gl-max}$ (µg/L)**			
Step 1				
Winter Oilseed rape	0.26	0.0007	0.0004	0.0004
Summer Oilseed rape	0.26	0.0007	0.0004	0.0004
Sunflower	0.26	0.0007	0.0004	0.0004
Leafy vegetables	0.26	0.0007	0.0004	0.0004
Winter wheat	0.13	0.0003	0.0002	0.0002
Sugar beet	0.26	0.0007	0.0004	0.0004

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 9.5-4: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for Na o-NG for each organism group based on FOCUS Steps 1-2 calculations

Group		**Fish acute**	**Inverteb. acute**	**Algae**
Test species		***Oncorhynchus mykiss***	***Daphnia magna***	***Scenedesmus subspicatus***
Endpoint		LC_{50}	EC_{50}	E_bC_{50}
(µg/L)		69 000	68 800	4 800
AF		100	100	10
RAC (µg/L)		690	688	480
FOCUS Scenario	**PEC $_{gl\text{-}max}$ (µg/L)**			
Step 1				
Winter Oilseed rape	0.34	0.0005	0.0005	0.0007
Summer Oilseed rape	0.34	0.0005	0.0005	0.0007
Sunflower	0.34	0.0005	0.0005	0.0007
Leafy vegetables	0.34	0.0005	0.0005	0.0007
Winter wheat	0.34	0.0005	0.0005	0.0007
Sugar beet	0.34	0.0005	0.0005	0.0007

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 9.5-5: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for Na p-NG for each organism group based on FOCUS Steps 1-2 calculations

Group		**Fish acute**	**Inverteb. acute**	**Algae**
Test species		***Oncorhynchus mykiss***	***Daphnia magna***	***Scenedesmus subspicatus***
Endpoint		LC_{50}	EC_{50}	E_bC_{50}
(µg/L)		25 000	27 700	2 500
AF		100	100	10
RAC (µg/L)		250	277	250
FOCUS Scenario	**PEC $_{gl\text{-}max}$ (µg/L)**			
Step 1				
Winter Oilseed rape	0.90	0.0036	0.0032	0.0036
Summer Oilseed rape	0.90	0.0036	0.0032	0.0036
Sunflower	0.90	0.0036	0.0032	0.0036
Leafy vegetables	0.90	0.0036	0.0032	0.0036
Winter wheat	0.45	0.0018	0.0016	0.0018
Sugar beet	0.90	0.0036	0.0032	0.0036

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 9.5-6: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for ASAHI MAX for each organism group based on FOCUS Steps 1-2 calculations

Group		**Fish acute**	**Fish prolonged**	**Inverteb. acute**	**Inverteb. prolonged**	**Algae**	**Algae**	**Aquatic plants**
Test species		***Oncorhynchus mykiss***	***Oncorhynchus mykiss***	***Daphnia magna***	***Daphnia magna***	***Scenedesmus subspicatus***	***Anabaena flos-aquae***	***Lemna gibba***
Endpoint		LC_{50}	NOEC	EC_{50}	NOEC	E_bC_{50}	E_bC_{50}	EC_{50}
(µg a.s./L)		122 400	7 740	36 000	774	600	10 320	46 920
AF		100	10	100	10	10	10	10
RAC (µg/L)		1224	774	360	77.4	60	1032	4692
FOCUS Scenario	**PEC $_{gl\text{-}max}$ (µg/L)**							
Step 1								
Winter Oilseed rape	1.5	0.0001	0.0019	0.0042	0.0194	0.0250	0.0015	0.0003
Summer Oilseed rape	1.5	0.0001	0.0019	0.0042	0.0194	0.0250	0.0015	0.0003
Sunflower	1.5	0.0001	0.0019	0.0042	0.0194	0.0250	0.0015	0.0003
Leafy vegetables	1.5	0.0001	0.0019	0.0042	0.0194	0.0250	0.0015	0.0003
Winter wheat	0.92	0.0000	0.0012	0.0026	0.0119	0.0153	0.0009	0.0002
Sugar beet	1.5	0.0001	0.0019	0.0042	0.0194	0.0250	0.0015	0.0003

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

zRMS comments:

Generally, zRMS validated the risk assessment provided by the Applicant with some remarks provided below:

Acute risk assessment:

According to the 'Guidance Document on tiered Risk Assessment for plant protection products for aquatic organisms in edge-of-field surface waters' (EFSA, 2013), growth rate is preferred endpoint.

However, the Applicant in case of algae and aquatic macrophyte (Lemna sp.) used yield values lower than growth rate value in a worst-case approach. This value is kept for the risk assessment and PEC/RAC ratio were not corrected by zRMS with E_rC_{50} as risk was already acceptable in step 1 with such worst-case endpoints.

Long term risk assessment

There is no long-term fish and daphnia toxicity value for each active substances as such studies were directly conducted with MUP of ATONIK. Thus, MUP of ATONIK endpoint are converted into sum of active substances endpoints and PECsw are calculated for sum of active substances in order to conduct risk assessment as worst case.

In addition, the ratio PEC/RAC for formulation was based on toxicity endpoints using E_bC_{50} values expressed in mg sum of a.s./L and with consideration sum of PECsw values.
It should be noted that toxicity mixture assessment for formulation required according to AGD 2013 for all aquatic organism are provided under Point 9.5.2.

In addition, it was noted that the Central Zone GAP presented several minor crops is included. The following surrogate crops were considered by the Applicant in simulations of PEC_{sw} calculations in Section 8.

1. Winter wheat (major crop) for spring rye, spelt, emmer wheat, small spelt and durum wheat. This is agreed by the zRMS e fate expert, as all minor crops in this group belong to cereals.
2. Sugar beet (major crop) for fodder beet, red beet, swede and turnip. Although some Member States consider root or leafy vegetables as relevant surrogate crops for this group, the zRMS e-fate expert is of the opinion that sugar beet is much more relevant for fodder beet and red beet as all these crops are just various cultivars of the same species, *Beta vulgaris* and their morphology and physiology are comparable. Swede and turnip are leafy vegetables which seem thus to be the relevant surrogate crop. Nevertheless, the morphology of these crops as well as their cultivation are much more comparable with sugar beet than with leafy vegetables and for this reason the zRMS e-fate expert agrees that the surface water exposure following application to swede and turnip is covered by simulations performed for sugar beet.
3. Winter oilseed rape (major crop) for mustard, spring rape, turnip rape, camelina, garden radish, poppy, linseed, hemp, sunflower and borage. The zRMS e fate expert agrees that winter OSR is most suitable surrogate crop for mustard, spring rape, turnip rape, camelina, poppy, linseed, hemp and borage. However, it is not relevant **for garden radish and sunflower**. Based on FOCUS crop scenarios and crop morphology, **leafy vegetables**, are in opinion of the zRMS e fate expert more relevant for garden radish, while for sunflower there is no need to consider surrogate crop as sunflower is available as crop scenario in FOCUS.

Since the surrogate crops selected by the Applicant for garden radish and sunflower were not agreed, additional surface water modelling was performed in Section 8 by zRMS e - fate expert with consideration of application to sunflower and leafy vegetables at BBCH 29-69, with assumption of two applications with 7 days interval.
Crop interception was set as an average crop cover as representing a worst case for the intended application period.
Therefore, zRMS in added in the Tables from 9.5-3 to 9.5-5 above the additional crops group (Sunflower and leafy vegetables).
Based on performed calculations of the risk assessment for each active substances the PEC/RAC ratio is below 1 indicating an acceptable risk to aquatic organism.

Mixture toxicity presented by the Applicant.

The content of the active substances in ASAHI MAX is 0.3% Na 5-NG, 0.6% Na *o*-NP and 0.9% NA *p*-NP. The theoretical toxicity mixture for ASAHI MAX is calculated and afterwards compared (MDR)

to the toxicity for fish and daphnids from the formulation ATONIK PLUS and for algae from the formulation ATONIK.

Measured toxicities:

Endpoints and effect values relevant for the aquatic risk assessment – plant protection products

Species	Substance	Exposure System	Results	Reference
Cyprinus carpio	ATONIK PLUS[1]	96h,ss	LC_{50} = 6800 mg/L Corresponding to 122.4 mg a.s.(sum)/L	EFSA Scientific Report (2008) 191, 1-130
Daphnia magna	ATONIK PLUS[1]	48 h, s	LC_{50} = 2000 mg a.s./L Corresponding to 36 mg a.s.(sum)/L	EFSA Scientific Report (2008) 191, 1-130
Scenedesmus subspicatus	ATONIK[2]	72 h ,s	EC_{50} = >100 mg/L Corresponding to >0.6 mg a.s.(sum)/L	EFSA Scientific Report (2008) 191, 1-130

[1] ATONIK PLUS : Sodium 5-nitroguaiacolate 0.3%, Sodium ortho-nitrophenolate 0.6 %, Sodium para-nitrophenolate 0.9%
[2] ATONIK: Sodium 5-nitroguaiacolate 0.1%, Sodium ortho-nitrophenolate 0.2 %, Sodium para-nitrophenolate 0.3%

Calculated LD_{50} for the mixture ASAHI MAX:

The toxicity values of the active substances used for the calculation of the mixture toxicity are:

Endpoints and effect values relevant for the risk assessment for aquatic organisms – Na 5-NG, Na *o*-NP and Na *p*-NP.

Species	Substance	Exposure System	Results	Reference
Oncorhynchus mykiss	Na 5-NG	96 h, f	LC_{50} = 37.4 mg a.s./L	EFSA Scientific Report (2008) 191, 1-130
Oncorhynchus mykiss	Na *o*-NP	96h,f	LC_{50} = 69 mg a.s./L	EFSA Scientific Report (2008) 191, 1-130
Oncorhynchus mykiss	Na *p*-NP	96h,f	LC_{50} = 25.0 mg a.s./L	EFSA Scientific Report (2008) 191, 1-130
Daphnia magna	Na 5-NG	48 h, f	LC_{50} = 71.1 mg a.s./L	EFSA Scientific Report (2008) 191, 1-130
Daphnia magna	Na *o*-NP	48 h, f	LC_{50} > 68.8 mg a.s./L	EFSA Scientific Report (2008) 191, 1-130
Daphnia magna	Na *p*-NP	48 h, f	LC_{50} = 27.7 mg a.s./L	EFSA Scientific Report (2008) 191, 1-130

Species	Substance	Exposure System	Results	Reference
Scenedesmus subspicatus	Na 5-NG	72 h ,s	E_bC_{50} = 6.2 mg/L **E_rC_{50} = >21 mg**/L	EFSA Scientific Report (2008) 191, 1-130
Scenedesmus subspicatus	Na *o*-NP	72 h ,s	E_bC_{50} = 4.8 mg/L **E_rC_{50} = >10 mg/L**	EFSA Scientific Report (2008) 191, 1-130
Scenedesmus subspicatus	Na *p*-NP	72 h ,s	E_bC_{50} = 2.5 mg/L **E_rC_{50} = 4.6 mg/L**	EFSA Scientific Report (2008) 191, 1-130

A calculation of the surrogate EC_{50} value for effects of the mixture was estimated using the Concentration Addition Model (CA model):

LD_{50} mix = $(\Sigma(X(a.s._i)/LD_{50}\ (a.s._i))^{-1}$

With X ($a.s._i$) = fraction of the active substance [*i*] in the mixture (sum =1)
LD_{50} ($a.s._i$) = acute toxicity for active substance [*i*]

Calculation of mixture toxicity for each group.

Active substance	LC_{50} (mg/L)	Nominal content (g/L)	Fraction in the mixture	Calculated LC_{50} mix (mg sum a.s./L)
Fish - acute				
Na 5-NG	37.4	3	0.17	34.10
Na o-NP	69.0	6	0.33	
Na p-NP	25.0	9	0.50	
Daphnia - acute				
Na 5-NG	71.1	3	0.17	39.62
Na o-NP	68.8	6	0.33	
Na p-NP	27.7	9	0.50	
Algae (E_bC_{50})				
Na 5-NG	6.2	3	0.17	3.38
Na o-NP	4.8	6	0.33	
Na p-NP	2.5	9	0.50	
Algae (E_rC_{50})				
Na 5-NG	21	3	0.17	6.68
Na o-NP	10	6	0.33	
Na p-NP	4.6	9	0.50	

Model Deviation Ratio: comparison of the calculated mixture toxicity with the measured mixture toxicity

The calculated mixture toxicity for ASAHI MAX has been compared with the available measured toxicity value for ATONIK PLUS (identical formulation as ASAHI MAX) and ATONIK:

Calculation of the Model Deviation Ratio (MDR) for each group

Organisms	Test type	Calculated mixture toxicity (expressed as sum of mg a.s./L)	Measured mixture toxicity (expressed as sum of mg a.s./L)	MDR	Type of mixture toxicity
Cyprinus carpio	96h,ss	34.10	122.4	0.279	Additive
Daphnia magna	48 h, s	39.62	36	1.101	Additive

Scenedesmus subspicatus	72 h ,s	3.38 (E_bC_{50}) **6.68 (E_rC_{50})**	>0.6	<5.633 <11.133	Possibility to be synergistic

For fish and daphnids, the Model Deviation Ratio (MDR) is between the trigger values of 0.2 and 5, which indicates that the active substances show additive behavior and the predicted and measured toxicities can be considered equivalent.

For algae, a synergistic behavior cannot be excluded. Therefore, the risk assessment carried out with the formulation measured toxicity in the dRR represents worse case and no further calculation would be necessary with the calculated mixture toxicity.

Nevertheless, the risk assessment with the calculated toxicity value for the mixture for aquatic organisms has been also performed for completeness:

Risk assessment for the mixture ASAHI MAX

1. PECsw were calculated for the formulated product in Point 8.9.2.1 of the Section B8 based in based on drift entries and the total annual rate:

PECsw of ASAHI MAX according to Point 8.9.2.1 (Section B8)

Formulated product	Crop	Application rate (g/ha)	Drift	PECsw (µg/L)
Asahi Max	Oilseed rape Sugar beet	2 x 0.2 L FP/ha (2 x 200 g/ha)	2.77%	3.693 Corresponding to 0.066 µg a.s.(sum)/L
Asahi Max	Winter wheat	0.2 L FP/ha (200 g/ha)	2.77%	1.847 Corresponding to 0.033 µg a.s.(sum)/L

The PECsw/RAC values were calculated:

PECsw/RAC values for ASAHI MAX according to Point 8.9.2.1 (Section B8)

Group		**Fish acute**	**Inverteb. acute**	**Algae**
Test species		***Cyprinus carpio***	***Daphnia magna***	***Scenedesmus subspicatus***
Endpoint **(µg sum of a.s. /L)**		LC_{50} 34 100	EC_{50} 39 620	E_bC_{50} 3 380
AF		100	100	10
RAC (µg/L)		341	396	338
FOCUS Scenario	**PEC $_{gl-max}$ (µg/L)**			
Step 1				
Winter Oilseed rape	0.066	0.0002	0.0002	0.0002
Winter wheat	0.033	0.0001	0.0001	0.0001
Sugar beet	0.066	0.0002	0.0002	0.0002

2. Furthermore, PECsw used in the aquatic risk assessment on Point 9.5.2 of the Section B9 were based on the sum of the Step1-PECsw of the active substances:

PECsw of ASAHI MAX (sum of the PECsw of a.s.)

FOCUS Scenario	**PEC $_{sw}$ (µg/L) – sum of PECsw of active substances**

Step 1	
Winter Oilseed rape	1.5
Summer Oilseed rape	1.5
Winter wheat	0.92
Sugar beet	1.5

The PECsw/RAC values were calculated:

PECsw/RAC values for ASAHI MAX (sum of the PECsw of a.s.)

Group		**Fish acute**	**Inverteb. acute**	**Algae**
Test species		***Cyprinus carpio***	***Daphnia magna***	***Scenedesmus subspicatus***
Endpoint		LC_{50}	EC_{50}	E_bC_{50}
(µg/L)		34 100	39 620	3 380
AF		100	100	10
RAC (µg/L)		341	396	338
FOCUS Scenario	**PEC $_{gl\text{-}max}$ (µg/L)**			
Step 1				
Winter Oilseed rape	1.5	0.0044	0.0038	0.0044
Summer Oilseed rape	1.5	0.0044	0.0038	0.0044
Winter wheat	0.92	0.0027	0.0023	0.0027
Sugar beet	1.5	0.0044	0.0038	0.0044

*Based on the total amount of 1.8% of active substances

Conclusion

For the intended uses of ASAHI MAX, in all cases calculated PEC/RAC ratios indicated an acceptable risk for the most sensitive group of aquatic organisms with big safety margin considering the calculated mixture toxicity.

RMS comments:

zRMS checked the mixture risk assessment according to recommendation given in EFSA GD 2013 using aquatic mixtox assessment (v.1.15) - according to decision scheme 10.3.11 in the AGD.
For algae (S. subscapitatus, as the most sensitive species) value was included in the calculations provided below. The max PEC_{sw} values for each active substances were included in the risk assessment as the worst case.

The calculations are provided below:

Product data

Product data	
Product name	**Asahi Max**
Density of product [g/cm³]	1,000
LC_{50}fish [mg prod./L]	6800,000
LC_{50}fish a.s. based [mg sum of a.s/L]	122,400
EC_{50}invertrebrates [mg prod./L]	2000,000
LC_{50}invertrebrates a.s. based [mg sum of a.s/L]	36,000
EC_{50}algae [mg prod./L]	33,330
EC_{50}algae a.s. based [mg sum of a.s/L]	0,600
EC_{50}macrophytes [mg prod./L]	2606,660
EC_{50}macrophytes a.s. based [mg sum of a.s/L]	46,92

Calculated mixture toxicity (Eq. 13) based on Tier 1 data only	[mg/L]
$ECx_{mix\text{-}ca}$fish	34,14
$ECx_{mix\text{-}ca}$invertrebrates	39,62
$ECx_{mix\text{-}ca}$algae	6,67
$ECx_{mix\text{-}ca}$macrophytes	

Calculated mixture toxicity (Eq. 13) based also on additional data	[mg/L]
$ECx_{mix\text{-}ca}$fish	34,14
$ECx_{mix\text{-}ca}$invertrebrates	39,62
$ECx_{mix\text{-}ca}$algae	6,67
$ECx_{mix\text{-}ca}$macrophytes	

Active substance data

Active Substance (a.s.) standard data (Tier 1 EP)				
Active substance names	**Na 5-NG**	**Na o-NP**	**Na p-NP**	
Concentration in Product [g a.s./L or g a.s./kg]	3	6	9	
p(X) (fraction in product)	0,17	0,33	0,50	
LC_{50}fish [mg a.s./L]	37,40	69	25	
LC_{50}invertrebrates [mg a.s./L]	71,1	68,8	27,7	
EC_{50}algae [mg a.s./L]	21	10	4,6	
EC_{50}macrophytes [mg a.s./L]				
Additional a.s. data (i.e. most sensitive species tested as Tier 1 data or refinements Tier 2A/B EP)				
LC_{50}fish [mg a.s./L]				
LC_{50}invertrebrates [mg a.s./L]				
EC_{50}algae [mg a.s./L]				
EC_{50}macrophytes [mg a.s./L]				
AF for RAC				
Fish	100	100	100	100
Invertrebrates	100	100	100	100
Algae	10	10	10	10
Macrophytes	10	10	10	10
RAC				
Fish	0,374	0,69	0,25	
Invertrebrates	0,711	0,688	0,277	
Algae	2,1	1	0,46	
Macrophytes				

*Toxicity of product Asahi Max is based on the toxicity for ATONIC divided/3 in case of algae.

STEP 1

Step 1: data available?

Question	Available options	Chose option	Conclusion
1. Are measured toxicity data (ECx) available for the given endpoint (typically chronic data available only for a.s.)?	Option 1: Endpoints available for a.s. and the PPP, **go to 2.** Option 2: Endpoints only available for the a.s., **go to 7.**	**Fish**	
		Option 1	Endpoints available for a.s. and the ppp, go to 2.
		Invertebrates	
		Option 1	Endpoints available for a.s. and the ppp, go to 2.
		Algae	
		Option 1	Endpoints available for a.s. and the ppp, go to 2.
		Macrophytes	

Next Step:	EP for a.s. and ppp	Go to 2
	EP only for a.s.	Go to 7

Data overview (from input sheet)

Relevant endpoints for mixtox assessment:	Standard data						Additional data (optional)			
	Na 5-NG	Na o-NP	Na p-NP		Asahi Max	Asahi Max	Na 5-NG	Na o-NP	Na p-NP	
	ECx (a.s.)	ECx (a.s.)	ECx (a.s.)		ECx (sum a.s.)	ECx (ppp)	ECx (a.s.)	ECx (a.s.)	ECx (a.s.)	
LC50fish [mg a.s./L]	37,4	69	25		122,40	6800,00				
LC50invertrebrates [mg a.s./L]	71,1	68,8	27,7		36,00	2000,00				
EC50algae [mg a.s./L]	21	10	4,6		0,60	33,33				
EC50macrophytes [mg a.s./L]					46,92	2606,66				

Option 1 is chosen.

Go to STEP 2

STEP 2

Step 2: MDR calculation; i.e. apparent synergism or antagonism?

Question	Explanation	Available options	Chose option	Conclusion
2. Check the plausibility of the measured formulation toxicity (ECxPPP) against the calculated mixture toxicity ECxmix-CA (assuming CA, Equation 13) for exactly the mixture composition of the a.s. in the formulation (ECxPPP) by means of the model deviation ratio (MDR = ECxmix-CA/ECxPPP).	In this step the **calculated mixture toxicity (ECxmix-CA)** is calculated according to equation 13 based, on the endpoints from the a.s. and the relative proportion of these a.s. in the plant protection product (PPP). In addition, the **MDR (model deviation ratio)** is calculated according to equation 15. The MDR is the ratio between the **calculated mixture toxicity (ECxmix-CA)** and the **measured mixture toxicity** (studies performed with the plant protection product, **ECxPPP**). The **MDR** determines how to proceed in the decision scheme. This MDR calculation is done to check if CA (concentration addition) holds true or if antagonism or synergism is indicated. The MDR calculation should be based on the **same species of a taxonomic group** (at least if possible). Further, it should refer to the **same calculation basis** (i.e. **ECxPPP expressed as "sum of mg a.s./L" has to be used** and not ECxPPP expressed as "mg product/L" ("mg product/L" is usually delivered); cf. AGD, 10.3.4.). The tool automatically takes the ECxPPP as "sum of mg a.s./L".	**MDR result:** **Option 1:** Concentration addition (CA) approximately holds for the mixture **(MDR 0.2-5), go to 3** **Option 2:** The mixture is more toxic than CA **(MDR >5), go to 10** **Option 3:** The mixture is less toxic than CA **(MDR < 0,2), go to 9**	**Fish** MDR 0.2-5 **Invertebrates** MDR 0.2-5 **Algae** MDR >5 **Macrophytes**	The MDR is between 0.2-5. No antagonism or synergism is indicated. Thus, the "concentration addition" concept holds, go to 3. The MDR is between 0.2-5. No antagonism or synergism is indicated. Thus, the "concentration addition" concept holds, go to 3. The MDR is >5. Thus, synergism is indicated, go to 10.

Equation 13:

$$EC_{X_{mix-CA}} = \left(\sum_{i=1}^{n} \frac{p_i}{EC_{X_i}} \right)^{-1}$$

Result in [mg sum a.s./L]
n: number of mixture components
i: index from 1...n mixture components
pi: the ith component as a relative fraction of the mixture composition (note: Σ pi must be 1)
ECxi: concentration of component i provoking x % effect (pragmatically, NOECi may be inserted, too).

Equation 15:

$$MDR = \frac{EC_{X_{mix-CA}}\,(calculated\ mixture\ toxicity)}{EC_{X_{PPP}}\,(measured\ mixture\ toxicity)}$$

Next Step:					
	MDR_{Fish}	✓ 0,28	Go to 3		
	$MDR_{Daphnids}$	✓ 1,10	Go to 3		
	MDR_{Algae}	✗ 11,11		Go to 10	
	$MDR_{Makrophytes}$	#DZIEL/0!	#DZIEL/0!	#DZIEL/0!	#DZIEL/0!

Use optional data

MDR ratio is in range (0.2-5) for fish and aquatic invertebrates indicating additivity

Go to STEP 3

MDR ratio > 5 ratio for algae suggests that synergism cannot be excluded

Go to STEP 10

STEP 10 (algae)

Question	Explanation and guiding questions			Available options	Chose option	Conclusion
10. Carefully recheck the apparent synergism as observed in the measured mixture toxicity data (ECxPPP) regarding potential impacts of heterogeneous input data (a.s.) and of co-formulants ignored in the CA calculation. Does the apparent synergism remain?	Following points can be used as guidance: **Control for possible effects of co-formulants:** 1) Check composition of product (typically given part C of the registration report). In addition, investigate if any toxic metabolites may contribute to the toxicity in the product study. 2) Investigate co-formulants/metabolites. Are any of the co-formulants/metabolites known toxicants? You may check safety data sheets provided by the applicant, C&L inventory, classification proposed in the QSAR-toolbox or study derived endpoints if available. 3) If yes, and if reliable endpoints exist, include the co-formulant in the calculation of the mixture toxicity. 4) Does the apparent synergism remain? **Check data:** Regarding heterogeneous input data, please make sure, that all data (for product, a.s., metabolites and co-formulants) are as homogeneous as possible, i.e. all species are the same and that the testing conditions (study design, expoure design, e.g. static vs. flow through) are as similar as possible.	**Question**	**Answer**	**After controlling metabolites/co-formulants and testing conditions, does the apparent synergism remain?** Option 1: Yes (synergism indicated in Step 2 confirmed): **Go to 3** Option 2: Yes (synergism indicated in Step 2 confirmed), but not similar according to Step 3, **Go to 8*** Option 3: No (synergism indicated in Step 2 not confirmed): **Go to 3** *in AGD 10.3.4 it is noted that a **modified trigger could be used** in this case (e.g. ETR-trigger/MDR = AF*MDR -> Go to 8b)	**Fish**	
		Is it possible that **co-formulants or metabolites** contributed to the toxicity measured in the product study?	*no*			
		If yes, which co-formulants/ metabolites? Are there reliable endpoints, which can be included in the calculation of mixture toxicity?	*none*		**Invertebrates**	
		When included in the mixture toxicity scheme, does the apparent synergism remain?				
					Algae	
		Were the same species tested?	*yes*		Option 1	Measured mixture toxicity plausible: Go to 3
		Were the testing conditions similar?	*yes*		**Macrophytes**	
		Can different testing conditions, different species etc. explain the synergism?	*no*			

Next Step:		
	Yes: synergism indicated in Step 2 confirmed, i.e. true synergism detected	Go to 3
	if measured data are not available (see AGD section 7.5.2., e.g. for all species/endpoints) or if the assessment in Step 3 indicates that the mixtures are not similar	Go to 8
	No: synergism indicated in Step 2 not confirmed, i.e. no true synergism detected	Go to 3

Useful links	
C&L inventory:	https://echa.europa.eu/information-on-chemicals/cl inventory-database
QSAR-toolbox:	https://www.qsartoolbox.org/

Option 1 is chosen for algae.

Go to STEP 3

STEP 3 (Fish and aquatic invertebrates and algae)

Step 3: are mixture composition in PPP test and at PECmix similar or not?

Question	Explanation	Available options	Chose option	Conclusion
3. Check whether the mixture composition in the formulation study giving the measured mixture toxicity (ECxPPP) in terms of the relative proportions of the individual a.s. is similar to the mixture composition at the PECmix. As a direct comparison on the basis of the relative proportions of the a.s. at the ECxPPP with the relative proportion at the PECmix is not informative as such, the comparison is done based on calculated mixture toxicity (assuming CA) for both mixture compositions. **Therefore, calculate ECxmix-CA (see Equation 13) for the mixture composition of the a.s. at the PECmix and compare with the estimate calculated for the formulation (as already done in step 2 above).**	This step is conducted for each PECmix to **check if product data can be used for assessment, when CA applies (i.e. MDR ratio is between 0.2 and 5).*** If the mixture in the environment is different from the initial mixture in the product (i.e. ECxmix-CA (a.s. in PPP)/ECxmix-CA (a.s. in PECmix) is outside the range of 0.8–1.2), then only calculated ECxmix-CA can be used (in fact ECxmix-CA can be used in every case if CA holds true). The Step 3 calculation is performed as: 1) **PECmix** is calculated using the PECsw;max values (calculated in input tox sheet). 2) Calculated mixture toxicity (**ECx mix-CA (a.s. in PECmix)**) based on the endpoints from the a.s. and the relative proportion of the a.s. at PECmix (according to equation 13 with pi as in equation 19; calculated in the input tox sheet (Calc 3a)). 3) Calculated mixture toxicity (**ECx mix-CA (a.s. in PPP)**) based on the endpoints from the a.s. and the relative proportion of the a.s. in the product (according to equation 13 with pi as in the product; calculated in input tox sheet (termed "Calculated mixture toxicity (Eq. 13) based on Tier 1 data") and also used in step 2). 4) The **calculated values from 2) and 3) are compared** in the tables below. *Note: This Step can also be conducted in the cases where Step 9 or Step 10 suggest to use measured data. If there is synergism and product data cannot be utilised because the mixture in the environment (PECmix) is different than the mixture in the product, than a modified ETR-trigger may be applied Step 8a (e.g. ETR-trigger/MDR; cf. AGD 10.3.4.).	ECx mix-CA (a.s. in PPP)/ ECx mix-CA (a.s. in PECmix): Option 1: between 0.8-1.2 (mixture similar), **go to 4** Option 2: not between 0.8 and 1.2 (mixture not similar), **go to 5** Option 3: mixture similar, however, **different assessment factor or additional data** available, **go to 5 or 8**	Fish Mixture similar (in all scenarios) Invertebrates Mixture similar (in all scenarios) Algae Mixture similar (in all scenarios) Macrophytes	Mixture similar every scenario. All scenarios can be assessed via product test, go to 4. Mixture similar every scenario. All scenarios can be assessed via product test, go to 4. Mixture similar every scenario. All scenarios can be assessed via product test, go to 4.

Next Step:		
	Option 1: Mixture similar	Go to 4
	Option 2: Mixture not similar	Go to 5
	Option 3: mixture similar, but different AF or additional data	Go to 8

Fish	
ECxmix-CA (a.s. in PPP)/ ECxmix-CA (a.s. in PECmix)	
Step 1	1,09
Step 2	

Invertebrates	
ECxmix-CA (a.s. in PPP)/ ECxmix-CA (a.s. in PECmix)	
Step 1	1,09
Step 2	

Algae	
ECxmix-CA (a.s. in PPP)/ ECxmix-CA (a.s. in PECmix)	
Step 1	1,08
Step 2	

Macrophytes	
ECxmix-CA (a.s. in PPP)/ ECxmix-CA (a.s. in PECmix)	
Step 1	#DZIEL/0!
Step 2	

The mixture was similar.

Go to STEP 4

STEP 4

Step 4: Measured risk assessment (ETRmix-PPP based on pr		
Question	**Explanation**	**Assessment**
Conduct a mixture RA based n measured mixture toxicity, ith the exposure-toxicity tio (ETRmix) being defined as e PECmix divided by the easured ECxPPP and mpare the outcome with the ceptability criterion (trigger lue) decisive for the specific dpoint/exposure scenario mbination.	In this step, the **ETRmix-PPP** is calculated,i.e. the ratio between the **PECmix** (calculated in the input sheet), and the **measured mixture toxicity** (i.e. endpoint from studies conducted with the PPP and expressed as ECxPPP [mg sum a.s./L]). The ETRmix-PPP is then compared with the **ETR-trigger-values**. Important: please note that performing the risk assessment based on the measured mixture toxicity is only **applicable if the study conducted with the PPP was performed with the most sensitive species tested.** If not, this could lead to an underestimation of the risk. In the cases where additional data (sensitive species, Tier 2A/B) has been entered a warning will appear below. Notes: 1) The ETR is the inverse of the TER. This was chosen, to get an assessment similar to other regulations, where the ratio PEC/PNEC is compared to a trigger (TER would be PNEC/PEC). Therefore, the trigger values are defined as 1/AF (and not as AF from the TER approach). For example, for fish (acute) the TER trigger of 100 would correspond to an ETR trigger of 1/100 = 0.01. 2) In case the mixture is similar in the product and in the environment (and CA holds true), an assessment based on the calculated mixture toxicity (Step 8) or based on a driver (Step 5) could also be performed (and would lead to the same outcome as with the product).	**Compare ETR with trigger:** **Green:** ETRmix-PPP is lower than trigger, indicate low risk **Red:** ETRmix-PPP is higher than the trigge risk is not demonstrated, check refineme options* *On the effect side a.s. refinements coul adressed in Step 8b.

Final CONCLUSION	low risk
	high risk

Fish	
ETRmix-PPP	
Step 1	0,00001

Invertebrates	
ETRmix-PPP	
Step 1	0,000042

Algae	
ETRmix-PPP	
Step 1	0,00250

Based on the results the mixture toxicity (ETRmix-PPP) assessment indicated an acceptable risk based on measured toxicity data expressed in mg sum of a.s./L for fish, aquatic invertebrates and algae.

Since for aquatic macrophytes only data for the formulated product are available which could be used for mixture toxicity, the product endpoint expressed in active substances content - E_bC_{50} of 46 920 µg sum of a.s./L as the worst case was used in the risk assessment.

ETRmix for aquatic macropyte- Lemna gibba

Group		**Aquatic plants**
Test species		***Lemna gibba***
Endpoint		**E_bC_{50}**
(µg a.s./L)		46 920
AF		10
RAC (µg/L)		4692
FOCUS Scenario	**PEC $_{gl\text{-}max}$ (µg/L)**	
Step 1		
Winter Oilseed rape	1.5	0.0003
Summer Oilseed rape	1.5	0.0003
Sunflower	1.5	0.0003
Leafy vegetables	1.5	0.0003
Winter wheat	0.92	0.0002
Sugar beet	1.5	0.0003

Overall, an acceptable risk is concluded for aquatic organisms from the use of Asahi Max for all intended uses in the GAP.

For the intended uses of ASAHI MAX, calculated PEC/RAC ratios indicated an acceptable risk for the most sensitive group of aquatic organisms with big safety margin.

9.5.3 Overall conclusions

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).
An acceptable acute risk is concluded for aquatic organisms from the use of ASAHI MAX and the intended GAP.

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 active substances. Full details of these studies are provided in the respective EU DAR and related documents.

Effects on bees of ASAHI MAX were not evaluated as part of the EU assessment of active substances. However, the provision of further data on the formulation is not considered essential, because the risk assessment can be based on the data from the active substances.

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

Table 9.6-1: Endpoints and effect values relevant for the risk assessment for bees

Active substance	EU agreed endpoints (µg/bee)	Reference
Na 5-NG	Oral (48 h) LD_{50} = 131 Contact (48 h) LD_{50} = >100	EFSA Scientific report (2008) 19, 1-130
Na *o*-NP	Oral (48 h) LD_{50} = 123.5 Contact (48 h) LD_{50} = >100	EFSA Scientific report (2008) 19, 1-130
Na *p*-NP	Oral (48 h) LD_{50} = 61.2 Contact (48 h) LD_{50} = 111	EFSA Scientific report (2008) 19, 1-130
MUP of ATONIK*	Oral (48 h) LD_{50} = 57.12 µg product/bee (44.27 µg a.s/bee) Contact (48 h) LD_{50} = >100 (>77.5 µg a.s/bee)	EFSA Scientific report (2008) 19, 1-130
New chronic studies	**Endpoints**	**Reference**
AMP*	10 d NOEDD (oral) = 45.67 µg AMP/bee/day, 10 d LDD_{50} (oral) > 55.18 µg AMP/bee/day	Harkin (2020),
AMP**	22 d LDD_{10} = 16.65 µg AMP/larva 22 d LDD_{50} = 112.2.2 µg AMP/larva 22 d NOED = 28.4 µg AMP/larva	Couture (2020
AMP*	8 d LDD_{50} = 560.19 µg AMP/larva 8 d NOED = 350 µg AMP/larva	Harkin (2020),

*MUP of ATONIK containing 11.6% Na 5-NG, 23.3% Na o-NP, 42.6% Na p-NP
** ATONIK MUP powder. containing: 13.0% Na 5-NG, 25.8% Na o-NP, 46.5 % Na p-NP.

Chronic toxicity studies on bees and larvae are submitted for AIR and had not yet been previously evaluated. Please find the study summaries in Appendix II (A 2.3.1.2, 2.3.1.3).

zRMS comments:

Bee toxicity data provided in Table 9.6-1 above were checked by zRMS and then confirmed that they are in line with EU agreed endpoints reported in EFSA EFSA Scientific Report (2008) 191.

The acute oral and contact risk assessment for product Asahi Max was based on toxicity endpoints for formulation MUP of ATONIK included in LoEP, containing higher % of active substances: 11.6% Na 5-NG, 23.3% Na o-NP, 42.6% Na p-NP.
To fulfil the data requirements as set by Commission Regulation (EU) No 284/2013, studies on acute toxicity to adult bees and chronic and larvae toxicity to bees were submitted by the Applicant.
It should be pointed out that the chronic 10 d study for adult bees for formulation for Asahi Max was based on formulation MUP powder of ATONIK containing: 13.3% Na 5-NG, 26.2% Na o-NP, 48 % Na p-NP and toxicity 22 d study for larvae for ATONIK MUP powder containing: 13.0% Na 5-NG, 25.8% Na o-NP, 46.5 % Na p-NP.
Due to that these all formulations contains much higher amount of active substance than Asahi Max this approach is accepted by zRMS.
In addition, 8 d study for larvae was submitted for current evaluation based on formulation of MUP powder of ATONIK used also in 10 d chronic study for adult bees.
Studies on chronic effects of the formulated product to bees listed in Table above were evaluated by the zRMS and considered acceptable. The reported endpoints are confirmed.
It should be noted that only 10 d for adult bees and 22 d study for larvae bees is considered as appropriate to use in the risk assessment by zRMS. The remaining 8 d study is considered as additionally information.
Summary of the performed studies together with zRMS evaluation may be found in Appendix 2.

9.6.1.1 Justification for new endpoints

Not relevant, the EU-agreed endpoints have been used for the risk assessment.

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).

9.6.2.1 Hazard quotients for bees

Table 9.6-2: First-tier assessment of the risk for bees due to the use of ASAHI MAX

	Intended use	All uses		
	Active substance **Application rate (g/ha)**	Na 5-NG 0.6 g a.s/ha Na o-NP 1.2 g a.s/ha Na p-NP 1.8 g a.s/ha ASAHI MAX 3.6 g a.s/ha		
Test design		**LD_{50} (lab.) (µg/bee)**	**Single application rate (g/ha)**	**Q_{HO}, Q_{HC} criterion: $Q_H \leq 50$**
Oral toxicity	Na 5-NG	131	0.6	0.00458
	Na o-NP	123.5	1.2	0.00972
	Na p-NP	61.2	1.8	0.02941
	ASAHI MAX *	44.27	3.6	0.08132
Contact toxicity	Na 5-NG	>100	0.6	0.00600
	Na o-NP	>100	1.2	0.01200
	Na p-NP	111	1.8	0.01622
	ASAHI MAX *	>77.5	3.6	0.04645

Q_{HO}, Q_{HC}: Hazard quotients for oral and contact exposure. Q_H values shown in bold breach the relevant trigger.
*****Based on MUP of ATONIK containing :11.6% Na 5-NG, 23.3% Na o-NP, 42.6% Na p-NP.

An acceptable risk is concluded for bees.

zRMS comments:

Acute risk assessment:

The acute risk assessment for bees presented in Table 9.6-2 is validated by the zRMS.
HQoral, contact values for the active substances and the formulated product (MUP of ATONIK formulation) are below the trigger of 50, indicating a low acute risk for bees.

Please note that the evaluation has been performed in line with SANCO/10329/2002 rev 2 final.

Chronic risk assessment:

To fulfil the data requirements as set by Commission Regulation (EU) No 284/2013, studies on acute toxicity to adult bees and chronic and larvae toxicity to bees were submitted by the Applicant (please refer to commenting box under Point 9.6.1.).

The chronic and larvae risk assessment is not required according to SANCO/10329/2002 rev 2 final.

Overall, acceptable risk to bees may be concluded from the intended uses of Asahi Max.

9.6.2.2 Higher-tier risk assessment for bees (tunnel test, field studies)

Not relevant.

9.6.3 Effects on bumble bees

No information available.

9.6.4 Effects on solitary bees

No information available.

9.6.5 Overall conclusions

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).

An acceptable risk is concluded for bees from the use of ASAHI MAX and the intended GAP.

9.7 Effects on arthropods other than bees (KCP 10.3.2)

9.7.1 Toxicity data

Studies on the toxicity to non-target arthropods have been carried out with the representative formulation ATONIK. Full details of these studies are provided in the respective EU DAR and related documents.

Effects on non-target arthropods of ASAHI MAX were not evaluated as part of the EU assessment of active substances. However, the provision of further data on the formulation is not considered essential, because the risk assessment can be based on the data of ATONIK.

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

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

	Active substance	EU agreed endpoints (EFSA Scientific report (2008) 191, 1-130)
~~Acute~~	ATONIK*	*Amblyseius californicus* LR_{50} >2 L/ha (12 g a.s/há), 2D lab.
		Aphidius colemani LR_{50} >2 L/ha (12 g a.s/há), 2D lab.
		Poecilus cupreus LR_{50} >2 L/ha (12 g a.s/há), 2,D extended lab.
		Coccinella septenpunctata LR_{50} >2 L/ha (12 g a.s/há), 2D extended lab.
Chronic	ATONIK	*Amblyseius californicus* NOEC >2 L/ha (12 g a.s/há)
		Aphidius colemani NOEC >2 L/ha (12 g a.s/há)
		~~*Poecilus cupreus* NOEC >2 L/ha (12 g a.s/há)~~
		~~*Coccinella septenpunctata* NOEC >2 L/ha (12 g a.s/há)~~

*ATONIK solution containing Na 5-Ng 0.11%, Na o-NP 0.21%, Na p-NP 0.33 %

zRMS comments:

NTA toxicity data provided in Table 9.7-1 above were checked by zRMS and then confirmed that they are in line with EU agreed endpoints reported in EFSA EFSA Scientific Report (2008) 191.
The risk assessment for product Asahi Max was based on toxicity endpoints for formulation of ATONIK solution evaluated at EU level containing lower % of active substances: 0.1% Na 5-NG, 0.2% Na o-NP, 0.3% Na p-NP in comparison to Asahi Max.
By comparison of Part C of the two products there is three times more of each active substances in Asahi Max than in ATONIK solution, but the proportions of each active substances in both products are the same (ATONIK solution: 1g, 2g, 3 g/L and Asahi Max: 3 g, 6 g, 9 g/L). In the same time no differences in toxicity due to the adjuvants is expected (Water only). For both formulations it was considered that any toxic effects are attributed to the active substances alone.
The maximum application rate of Asahi Max (ATONIK PLUS), containing 1.8% of a.s/L)) is 0.2 L product/ha correspond to 0.6 L product ATONIK/ha (containing 0.6 % of a.s./L).
The differing concentrations of active substances within the formulations combined with the two different rates of application, result in the same application rate of the active substances from application of both formulations.
The toxicity endpoints presented at the Point 9.7-1 based on ATONIK solution endpoints but expressed in g sum of the a.s./ha is considered as acceptable for Asahi Max.

9.7.1.1 Justification for new endpoints

Not relevant. The EU-agreed endpoints have been used for the risk assessment.

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. The risk envelope is applied for the risk assessment: the evaluation of oilseed rape and sugar beet covers the assessment for winter wheat. The risk assessment for soil exposure also covers the assessment for foliar exposure.

9.7.2.1 Risk assessment for in-field exposure

Table 9.7-2: First- and higher-tier assessment of the in-field risk for non-target arthropods due to the use of ASAHI MAX

Intended use	Oilseed rape / Sugar beet/ Cereals/MINOR CROPS uses		
Active substance/product	ASAHI MAX		
Application rate (g/ha)	2 x 3.6 g a.s/ha (sum of active substances/ha)		
MAF	1.7 ~~1.9 (soil, as worst case)~~		
Test species Tier I	**LR_{50} (lab.) (g/ha)**	**$PER_{in\text{-}field}$ (g/ha)**	**$HQ_{in\text{-}field}$ criterion: HQ ≤ 2**
Amblyseius californicus	>12 g a.s/ha	6.12 ~~6.84~~	0.51~~7~~
Aphidius colemani	>12 g a.s/ha		0.51~~7~~
Test species Tier-1	**Rate with ≤ 50 % effect (g a.s/ha)**	**$PER_{in\text{-}field}$ (g /ha)**	**$HQ_{in\text{-}field}$ < 2?**
Poecilus cupreus	>12 g a.s/ha	6.12	0.51
Coccinella septenpunctata	>12 g a.s/ha		0.51

MAF: Multiple application factor; PER: Predicted environmental rate; HQ: Hazard quotient; DALT: Days after last treatment. Criteria values shown in bold breach the relevant trigger.
If an LR_{50} or ER_{50} 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 presented in Table 9.7-2 has been amended by the zRMS using foliar MAF of 1.7 according to recommendation given in ESCORT 2 instead of MAF_{soil} used by the Applicant.

Based on calculations performed with consideration of the Tier I laboratory and extended laboratory studies data, acceptable in-field risk to non-target arthropods from all intended uses of Asahi Max may be concluded.

9.7.2.2 Risk assessment for off-field exposure

Not necessary, a safe use has been concluded for in-field exposure, thus a safe use is also concluded for off-field areas.

zRMS comments:

The off -field risk assessment was added by zRMS and presented in the Table below.

First- and higher-tier assessment of the in-field risk for non-target arthropods due to the use of ASAHI MAX

Intended use	Oilseed rape / Sugar beet/ Cereals/MINOR CROPS uses
Active substance/product	ASAHI MAX
Application rate (g/ha)	2 x 3.6 g a.s/ha (sum of active substances)
MAF	1.7, VDF 10 (2D), VDF=1 (3D)

Test species Tier I	**LR_{50} (lab.) (g/ha)**	**% Drift**	**PER off field (g/ha)**	**HQ off -field criterion: HQ ≤ 2**
Amblyseius californicus	>12 g a.s/ha	2.38	0.14	0.012
Aphidius colemani	>12 g a.s/ha			
Poecilus cupreus	>12 g a.s/ha		0.07	0.0060
Coccinella septenpunctata	>12 g a.s/ha			

Based on calculations performed with consideration of the Tier I laboratory data and extended laboratory studies acceptable off-field risk to non-target arthropods from all intended uses of Asahi Max may be concluded with no need for risk mitigation measures.

9.7.2.3 Additional higher-tier risk assessment

Not relevant.

9.7.2.4 Risk mitigation measures

No risk mitigation needed.

9.7.3 Overall conclusions

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.
An acceptable risk is concluded for non-target arthropods from the use of ASAHI MAX and the intended GAP.

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 the MUP of ATONIK. Full details of these studies are provided in the respective EU DAR and related documents.
Effects on earthworms and other non-target soil organisms (meso- and macrofauna) of ASAHI MAX were not evaluated as part of the EU assessment of active substances. However, the provision of further data on the formulation is not considered essential, because the risk assessment can be based on the data from the MUP of ATONIK. The selection of studies and endpoints for the risk assessment is in line with the results of the EU review process.

Table 9.8-1: Endpoints and effect values relevant for the risk assessment for earthworms and other non-target soil organisms (meso- and macrofauna)

		Active substance	EU agreed endpoints (mg product/kg soil) (EFSA Scientific report No.(2008) 191, 1-130)
~~Earthworms~~	~~Acute~~	~~MUP of ATONIK*~~	~~14-d LC_{50} >101.8 (78.895 mg as/kg)~~
Earthworms	Chronic	MUP of ATONIK*	8 weeks NOEC= 37.0 (28.675 mg a.s/kg) – 4.3 mg Na-5 NG, 8.6 mg NA0P, 15.8 mg NA-pNP/kg dws)

* ATONIK Manufacture Use Product (MUP) containing Na 5-NG 11.6 %, Na *o*-NP 23.2 %, Na *p*-NP 42.6 %

According to Regulation 284/2013, for foliar applications, the chronic studies on *Folsomia candida* and *Hypoaspis aculeifer* are not necessary when an acceptable risk has been demonstrated for non-target arthropods. Therefore, no studies on *Folsomia candida* and *Hypoaspis aculeifer* are submitted.

zRMS comments:

Earthworms toxicity data provided in Table 9.8-1 above were checked by zRMS and then confirmed that they are in line with EU agreed endpoints reported in EFSA EFSA Scientific Report (2008) 191.
The toxicity endpoint for MUP of ATONIK containing Na 5-NG 11.6 %, Na *o*-NP 23.2 %, Na *p*-NP 42.6% expressed in mg a.s./kg dws was used in the risk assessment for Asahi Max.
This approach was considered acceptable due to much higher % of the a.s./kg dws in this formulation in comparison to Asahi Max.

9.8.1.1 Justification for new endpoints

Not relevant, EU-agreed endpoints have been used in the risk assessment.

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).

9.8.2.1 First-tier risk assessment

The relevant PEC_{soil} for risk assessments covering the proposed use pattern are taken from Section 8 (Environmental Fate), Chapter 8.7. According to the assessment of environmental-fate data, multi-annual accumulation in soil does not need to be considered for active substances.

To achieve a concise risk assessment, the risk envelope approach is applied. Here, the maximum PECsoil covers the risk assessment for all intended uses.

Table 9.8-2: First-tier assessment of the acute and chronic risk for earthworms and other non-target soil organisms (meso- and macrofauna) due to the use of ASAHI MAX

Intended use	All uses		
Acute effects on earthworms			
Product/active substance	**LC_{50} (mg/kg dw)**	**PEC_{soil} (mg/kg dw)**	**TER_a (criterion TER ≥ 10)**
ASAHI MAX	78.895	0.0040*	19723.75
Chronic effects on earthworms			
Product/active substance	**NOEC (mg/kg dw)**	**PEC_{soil} (mg/kg dw)**	**TER_{lt} (criterion TER ≥ 5)**
ASAHI MAX	28.675	0.0040*	7168.75
Na 5-NG	4.3	0.0006	7166.66
Na o-NP	8.6	0.0013	6615.38
Na p-NP	15.8	0.0021	7523.80

TER values shown in bold fall below the relevant trigger.
*sum of PECs for each active substance

zRMS comments:

The risk assessment for earthworms for product Asahi Max has been validated the zRMS.
It should be indicated that the risk for Asahi Max was based on the formulation MUP of ATONIK containing expressed in mg sum of a.s./mg kg dws and this approach was considered acceptable.
In addition, the calculations of the risk assessment for active substances were amended by zRMS.

All TER_{LT} values for earthworms for active substances and formulation are greater than the trigger of 5, indicating an overall acceptable risk.

9.8.2.2 Higher-tier risk assessment

Not relevant.

9.8.3 Overall conclusions

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).

An acceptable risk is concluded for earthworms and other meso- and macro-organisms from the use of ASAHI MAX and the intended GAP.

9.9 Effects on soil microbial activity (KCP 10.5)

9.9.1 Toxicity data

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

Effects on soil microorganisms of ASAHI MAX were not evaluated as part of the EU assessment of active substances. However, the provision of further data on the formulation is not considered essential, because the risk assessment can be based on the data from ATONIK.

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

process.

Table 9.9-1: Endpoints and effect values relevant for the risk assessment for soil microorganisms

Active substance	Test design[1]	EU agreed endpoints (EFSA Scientific Report (2008) 191, 1-130)
ATONIK*	~~C~~	~~NOEC (<25% effects) Day 28 – 4 mg product/kg d.w. soil (0.024 mg a.s/kg)~~
	N	NOEC (<25% effects) Day 28 – 4 mg product/kg d.w. soil (0.024 mg sum of a.s/kg dws)

*ATONIK solution containing Na 5-Ng 0.11%, Na o-NP 0.21%, Na p-NP 0.33 %

zRMS comments:

Soil microorganism toxicity data provided in Table 9.8-1 above were checked by zRMS and then confirmed that they are in line with EU agreed endpoints reported in EFSA EFSA Scientific Report (2008) 191.

Information regarding effects on carbon mineralisation is no longer a data requirement and for this reason is struck through in tables above.

9.9.1.1 Justification for new endpoints

Not relevant, the EU-agreed endpoints have been used for the risk assessment.

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_{soil} for risk assessments covering the proposed use pattern are taken from Section 8 (Environmental Fate), Chapter 8.7 and were already used in the risk assessment for earthworms and other non-target soil organisms (meso- and macrofauna) (see 9.8).
To achieve a concise risk assessment, the risk envelope approach is applied. Here, the assessment the highest PECsoil covers the risk assessment for all intended uses.

Table 9.9-2: Assessment of the risk for effects on soil micro-organisms due to the use of ASAHI MAX

Intended use	All intended crops in the GAP		
N-mineralisation			
Product/active substance	**Max. conc. with effects ≤ 25 % (mg/kg dw)**	**PEC_{soil} (mg/kg dw)**	**Risk acceptable?**
~~ASAHI MAX~~	~~0.024 mg a.s/kg~~	~~0.0040*~~	~~yes~~
~~**C-mineralisation**~~			
Product/active substance	**Max. conc. with effects ≤ 25 % (mg/kg dw)**	**PEC_{soil} (mg/kg dw)***	**Risk acceptable?**
ASAHI MAX	0.024 mg a.s/kg*	0.004	yes

*sum of PECs for each active substance

zRMS comments:

The risk assessment in Table 9.9-2 above based toxicity endpoints NOEC expressed in mg sum of a.s/kg dws for Asahi Max is in general agreed by the zRMS.

In this case toxicity of Asahi Max formulation NOEC value is based on the the toxicity of the formulation of ATONIK solution divided by 3 with assumption of three times higher amount of a.s. in Asahi Max and the same proportion of the a.s. in the both products.

Endpoint	NOEC (mg ATONIKsolution/kg dws)	Equivalent NOEC (mg Asahi Max/kg dws)
N transformation	4 (0.024 mg sum of a.s./kg dws	1.33 (0.024 mg a.s./kg dws)

Therefore, assessment presented at the Point 9.9-2 based on ATONIK solution expressed mg sum of a.s./kg dws is therefore considered acceptable for Asahi Max.

The effects on the nitrogen transformations are acceptable (<25%) at concentration which is higher than the maximum relevant PECs for the maximum application rate of active substances and the product Asahi Max.

Overall, no unacceptable effects on soil microbial activity are expected following application of Asahi Max.

9.9.3 Overall conclusions

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).

An acceptable risk is concluded for soil micro-organisms from the use of ASAHI MAX and the intended GAP.

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

9.10.1 Toxicity data

Studies on the toxicity to non-target terrestrial plants have been carried out with the representative product ATONIK. Full details of these studies are provided in the respective EU DAR and related documents.

Effects on non-target terrestrial plants of ASAHI MAX were not evaluated as part of the EU assessment of active substances. However, the provision of further data on the formulation is not considered essential, because the risk assessment can be based on the data from ATONIK.

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

Table 9.10-1: Endpoints and effect values relevant for the risk assessment for non-target terrestrial plants

Substance	Test type	Endpoint (EFSA Scientific report 2008, 191 1-130)
ATONIK*	vegetative vigour	ER50 > 5L/ha (30 g a.s./ha)
ATONIK*	seedling emergence	ER50 > 5L/ha (30 g a.s./ha)

* ATONIK solution containing Na 5-Ng 0.11%, Na o-NP 0.21%, Na p-NP 0.33 %

zRMS comments:

NTPs toxicity data provided in Table 9.10-1 above were checked by zRMS and then confirmed that they are in line with EU agreed endpoints reported in EFSA EFSA Scientific Report (2008) 191.
The toxicity endpoints for Atonik solution expressed in g sum of a.s./ha can be used for Asahi Max.
The maximum application rate of Asahi Max (ATONIK PLUS), containing 1.8% of a.s/L)) is 0.2 L product/ha correspond to 0.6 L product ATONIK/ha (containing 0.6 % of a.s./L).
The differing concentrations of active substances within the formulations combined with the two different rates of application, result in the same application rate of the active substances from application of both formulations.
The toxicity endpoints presented at the Point 9.7-1 based on ATONIK solution endpoints but expressed in g sum of the a.s./ha is considered as acceptable for Asahi Max.

9.10.1.1 Justification for new endpoints

Not relevant, the EU-agreed endpoints have been used in the risk assessment.

9.10.2 Risk assessment

9.10.2.1 Tier-1 risk assessment (based screening data)

Not relevant.

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

The risk assessment is based on the "Guidance Document on Terrestrial Ecotoxicology", (SANCO/10329/2002 rev.2 final, 2002). It is restricted to off-field situations, as non-target plants are non-crop plants located outside the treated area.

Table 9.10-2: Assessment of the risk for non-target plants due to the use of ASAHI MAX

Intended use	All uses			
Active substance/product	ASAHI MAX			
Application rate (g/ha)	1 x 3.6 g a.s./ha			
Test	**ER_{50} (g/ha)**	**Drift rate**	**$PER_{off\text{-}field}$ (g/ha)**	**TER criterion: TER ≥ 5**
Vegetative vigour	> 30 g a.s/ha	2.77%	0.09972	>300.84
Seedling emergence	> 30 g a.s/ha	2.77%	0.09972	>300.84

MAF: Multiple application factor; PER: Predicted environmental rate; TER: toxicity to exposure ratio. TER values shown in bol fall below the relevant trigger.d

*sum of PECs for each active substance

zRMS comments:

The risk assessment-based toxicity endpoints E_rC_{50} obtained from formulation study ATONIK Solution expressed in g sum of a.s./ha, presented in Table 9.10-2 above is in general agreed by the zRMS.

Overall, no unacceptable effects for NTP are expected following application of Asahi Max.
No risk mitigation measures is required.

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 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).

An acceptable risk is concluded for non-target plants from the use of ASAHI MAX and the intended GAP.

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

No further information is necessary.

9.12 Monitoring data (KCP 10.8)

Not relevant.

9.13 Classification and Labelling

ASAHI MAX did not classify for environmental aspects, according Regulation (EC) No 1272/2008.
Please, refer to Part C for the composition of ASAHI MAX.
No mitigation measures are necessary for ecotoxicological section.

zRMS comments:

zRMS agrees that ASAHI MAX is not classified for environmental aspects, according Regulation (EC) No 1272/2008.

Appendix 1 Lists of data considered in support of the evaluation

List of data submitted by the applicant and relied on

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
KCP 10.2.1/01	Hasler, T.	2011	ATONIK: Growth inhibition test with *Anabaena flos-aquae* under static conditions. Report No. 1094.005.430 Arysta Life Science S.A.S GLP, Unpublished	N	Asahi Chemical Europe s.r.o.
KCP 10.2.1/02	Biester, M.	2011	ATONIK: Growth inhibition test with the freshwater duckweed (*Lemna gibba*) under semi static conditions. Report No 1094005410 Arysta Life Science S.A.S GLP, Unpublished	N	Asahi Chemical Europe s.r.o.
KCP 10.3.1/01	Harkin, S.	2020	ATONIK: 10 day chronic oral toxicity test (repeated doce) for adult honeybees (*Apis mellifera* L.) (Amended Final Report) Report No.: FR/000623 Fera Science Ltd., Centre for Chemical Safety & Stewardship, Sand Hutton, United Kingdom GLP, Unpublished	N	Asahi Chemical Europe s.r.o.
KCP 10.3.1/02	Harkin, S.	2016	ATONIK: in vitro 8 day toxicity test – repeated exposure to larval stage honeybee (*Apis mellifera* L.) Report No.: FR/000624 Fera Science Ltd., Centre for Chemical Safety & Stewardship, Sand Hutton, United Kingdom GLP, Unpublished	N	Asahi Chemical Europe s.r.o.
KCP 10.3.1/03	Couture, E.	2020	AMP (ATONIK MUP POWDER) – a laboratory study to determine the chronic effects on the honey bee *Apis mellifera* L. (Hymenoptera: Apidae) 22-day larval toxicity test with repeated exposure Report No.: 516SRFR18C05 SynTech Research France SAS, La Chapelle de Guinchay, France GLP, Unpublished	N	Asahi Chemical Europe s.r.o.

Appendix 2 Detailed evaluation of the new studies

zRMS comments:

In Appendix 2 there are summarised only new studies for product which were evaluated by zRMS for the current evaluation of Asahi Max.
The studies for the a.s. and representative formulations evaluated in DAR were not provided by the Applicant. For this reason, please refer to relevant endpoints in DAR for details of evaluation by RMS.

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

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

Comments of zRMS:	The study was conducted in line with OECD 201 with no deviations. All the validity criteria were met and the study is considered acceptable with the following endpoints relevant for the risk assessment: Yield: E_yC_{50} = 1.72 g test item/L nom $NOEC_y$ = 0.3 g test item/L nom Growth rate: E_rC_{50} = 6.99 g test item/L nom $NOEC_r$ = 0.3 g test item/L nom

Report:	KCP 10.2.1/01, Hasler. T. (2011)
Title:	ATONIK: Growth inhibition test with *Anabaena flos-aquae* under static conditions.
Document No:	1094.005.430
Guidelines:	Commission Directive 92/69/EEC. Annex Part C. C.3: "Algal Inhibition Test". Official Journal of the European Communities No. L 383 A. dated December 29. 1992. OECD Guideline for Testing of Chemicals. Section 2. No. 201: "Alga. Growth Inhibition Test". Adopted June 7. 1984. Deviations: none
GLP	Yes

Executive summary

An algal growth inhibition test (dose response test, 72 hours) was performed in order to evaluate the effect of ATONIK on the growth of *Anabaena flos-aquae* in a static test system. The test included five treatment groups exposed to ATONIK at nominal dose levels of 0.3, 1.0, 3.0,10 and 30 g/L and a control.

The E_yC_{50} was determined to be 1.72 g/L and the E_rC_{50} 6.99 g/L based on geometric mean measured concentrations. The corresponding NOEC value was 0.3g/L for both yield and growth rate.

E_yC_{50} 1.72 g/L
E_rC_{50} 6.99 g/L
ATONIK was shown to be toxic to *Anabaena flos-aquae* at concentrations greater than 0.3g/L.

Materials and method

Materials

Test material:	ATONIK
Lot/Batch #	052DO
Expiry date	19 April 2013
Vehicle and/or positive control	no positive control, only water control
Test organisms	
Species:	*Anabaena flos-aquae*
Strain:	not applicable
Age	not applicable
Initial cell density	1.0 x 10^4 cells/mL
Test containers:	250 mL-Erlemeyer flasks with each 100 ml test medium
Test conditions:	
Test medium	Algal assay growth medium (OECD 2006)
Temperature:	24 - 26°C
pH	7.91 – 8.34
Conductivity	not available

Illumination	3955 to 4497 Lux (continuos illumination)
Shaking rate	110 rpm (orbital shaker)

Study design and method

Dates of experimental work	20 June 2011– 23 June 2011

Methods

The test was based on five treatment groups exposed to ATONIK at nominal dose levels of 0.3, 1.0, 3.0,10 and 30 g/L and a control. Three replicate, sterile 250-mL Erlenmeyer flasks per treatment level were prepared per test concentration, with six replicates flasks used for the control. An aliquot of 100 mL of the appropriate test solution was placed in each replicate flask. The cell density in each flask was made up to be 1.0×10^4 cells/mL. All test vessels were fitted with stainless steel caps which permitted gas exchange. An aliquot of 100 mL of the 3.0 mg/L solution was used as an abiotic control and was not inoculated with algae. In order to estimate the impact of the presence of algal biomass on test item concentration, this abiotic control was analysed after 72 hours of exposure for ATONIK. Every 24 hours, cell counts were conducted on each replicate vessel of the treatment levels and the control using a hemocytometer (Neubauer Improved) and a Leica DMLS microscope.

Temperature was measured continuously. Minimum and maximum temperatures were recorded daily and ranged from 24-26°C. Light intensity was measured every 24 hours and ranged from 3955-4497 Lux. The pH of the test solutions and control was measured at test initiation and at the end of the 72 hour exposure and ranged from 7.91-7.97 during the test.

Statistics

The data were initially checked for normality using Shapiro-Wilks' Test and for homogeneity of variance using Bartlett's test (Bartlett, 1937). Both yield and growth rate data were found to be normally distributed. ANOVA and Bonferroni t-Test were used to determine the NOEC and LOEC values. All statistical determinations were made at the 95% level of certainty, except in the case of Shapiro-Wilks' and Bartlett's Tests, where the 99% level of certainty was applied.

Results and discussion

Analytical results

Analytical data on concentrations in test media:
After 72 hours, the recovery of the test substance ranged from 91.5% to 97.8% of the nominal concentrations. The 72-hour recovery of the abiotic control at test termination was 91.5-97.8% indicating that the algal biomass did not influence the concentration of the active ingredients. Based on these results, nominal concentrations were used for the evaluation of the biological data. Details of the analysis are given in Tables 1 to 3 below.

Table 1: Concentrations of Na 5-NG measured in the exposure solutions during the 72-hour exposure of *Anabaena flos-aquae*

Nominal concentration (g test item/L)	Nominal concentration (mg/L)	Measured Concentration (mg./L)[a] (% Recovery)	
		Hour 0 (new)	Hour 72 (aged)
Control	Control	< LOQ	< LOQ
0.3	0.289	0.271 (93.5)	0.266 (91.9)
1.0	0.965	0.913 (94.7)	0.876 (90.8)
3.0	2.89	2.70 (93.3)	2.63 (91.0)
10	9.65	9.15 (94.8)	8.95 (92.8)
30	28.9	28.1 (97.2)	28.0 (96.8)
3.0 (abiotic control)	2.89	NA	2.65 (91.5)
QC # 1 (0.201)	QC # 1 (0.194)	0.191 (98.4)	0.195 (101)
QC # 2 (4.01)	QC # 2 (3.87)	3.72 (96.0)	3.72 (96.1)
QC # 3 (40.1)	QC # 3 (38.7)	35.4 (91.4)	37.3 (96.4)

LOQ Limit of quantification. Defined as 0.199 mg a.i./L
Note: Percent recovery was calculated from original raw data, not from the rounded values presented in this table
NA Not Applicable
QC Quality Control
[a] Measured concentrations were calculated for Na 5-NG

Table 2 Concentrations of Na o-NP measured in the exposure solutions during the 72-hour exposure of *Anabaena flos-aquae*

Nominal concentration (g test item/L)	Nominal concentration (mg a.i./L)	Measured Concentration (mg a.i./L)[a] (% Recovery)[a]	
		Hour 0 (new)	Hour 72 (aged)
Control	Control	< LOQ	< LOQ
0.3	0.847	0.787 (92.9)	0.818 (96.5)
1.0	2.82	2.94 (104)	2.77 (98.1)
3.0	8.47	8.19 (96.7)	8.27 (97.7)
10	28.2	27.7 (98.3)	28.0 (99.0)
30	84.7	85.1 (100)	84.7 (100)
3.0 (abiotic control)		NA	8.29 (97.8)
QC # 1 (0.201)	QC # 1 (0.567)	0.586 (103)	0.605 (107)
QC # 2 (4.01)	QC # 2 (11.3)	11.6 (102)	11.6 (102)
QC # 3 (40.1)	QC # 3 (113)	106 (93.5)	113 (99.7)

LOQ Limit of quantification. Defined as 0.582 mg a.i./L
Note: Percent recovery was calculated from original raw data, not from the rounded values presented in this table
NA Not Applicable
QC Quality Control
[a] Measured concentrations were calculated for Na o-NP

Table 3 Concentrations of Na p-NP measured in the exposure solutions during the 72-hour exposure of *Anabaena flos-aquae*

Nominal concentration (g test item/L)	Nominal concentration (mg a.i./L)	Measured Concentration (mg a.i./L)[a] (% Recovery)[a]	
		Hour 0 (new)	Hour 72 (aged)
Control	Control	< LOQ	< LOQ
0.3	0.554	0.511 (92.1)	0.568 (102)
1.0	1.85	2.00 (108)	1.82 (98.4)
3.0	5.54	5.39 (97.2)	5.32 (96.0)
10	18.5	18.3 (99.0)	18.1 (98.1)
30	55.4	56.8 (102)	55.4 (100)
3.0 (abiotic control)		NA	5.36 (96.7)
QC # 1 (0.201)	QC # 1 (0.371)	0.404 (109)	0.441 (119)
QC # 2 (4.01)	QC # 2 (7.42)	7.65 (103)	7.66 (103)
QC # 3 (40.1)	QC # 3 (74.2)	70.7 (95.3)	75.4 (102)

LOQ Limit of quantification. Defined as 0.582 mg a.i./L
Note: Percent recovery was calculated from original raw data, not from the rounded values presented in this table
NA Not Applicable
QC Quality Control
[a] Measured concentrations were calculated for Na p-NP

Biological results

The cell densities and yield at each observation interval are presented in Table 10.2.2.3.5 and growth rates in Table 10.2.2.3.6. Algal cells appeared normal throughout the test at all treatment levels. The 72-hour cell densities in the control averaged 247 $x10^4$ cells/mL. Cell density in the nominal 0.3, 1.0, 3.0 10 and 30 g test item/L treatment levels averaged 223, 159, 70.3 5.42 and 0.00 x 10^4 cells/mL, respectively.

The 0 to 72 hour yield in the control and the 0.3, 1.0, 3.0 10 and 30 g/L treatment levels averaged 246, 222, 158, 69.3, 4.42 and -1.00 x 10^4 cells/mL, respectively. Statistical analysis (Bonferroni t-Test, $p < 0.05$) demonstrated a significant difference between the control and the 0.3, 1.0, 3.0, 10 and 30 g test item/L treatment levels. Therefore, the 72-hour NOEC and LOEC values for yield were determined to be 0.3 and 1.0 g test item/L, respectively.

The 0 to 72 hour growth rate in the control averaged 1.87 day^{-1}. The 0 to 72 hour growth rate in the nominal 0.3, 1.0, 3.0 10 and 30 g/L treatment levels averaged 1.82, 1.71, 1.43, 0.56 and 0.00 day^{-1}, respectively. Statistical analysis (Bonferroni t-Test, $p < 0.05$) demonstrated a significant difference between the control and the 0.3, 1.0, 3.0, 10 and 30 g test item/L treatment levels. Therefore, the 72-hour NOEC and LOEC values for growth were determined to be 0.3 and 1.0 g test item/L, respectively and the EC_{10}, EC_{20} and EC_{50} were 1.18, 2.59 and 6.99 g test item/L

The acceptance criteria were met for the control as a 16 fold increase in cell growth within 72 hours was reported, the coefficient of variation of daily growth rates in the control cultures during the course of the test did not exceed 35% and average growth rates in replicate control cultures did not exceeding 7%. Based on these criteria the study conditions are considered acceptable.

Table 4 Cell density and yield of *Anabaena flos-aquae* after 24, 48 and 72 hours exposure to ATONIK

Nominal Concentration (g test item/L)	Mean Cell Density (x 10^4 cells/mL) Observation interval (hours)			72 Hour Inhibition (%)[a]	Mean Yield (x 10^4 cells/mL) Observation interval (hours)			72 Hour Inhibition (%)[a]
	24 hours (SD)	48 hours (SD)	72 hours (SD)		24 hours (SD)	48 hours (SD)	72 hours (SD)	
Control	5.29 (± 4.65)	28.4 (±8.42)	247 (± 12.0)	NA	4.29 (±4.65)	27.4 (±8.42)	2446 (±12.0)	NA
0.3	12.7 (± 5.39)	22.7 (±10.1)	223 (± 61.7)	10.0	11.7 (±5.39)	21.7 (±10.1)	222 (±61.7)	10.0
1.0	9.92 (± 6.63)	18.1 (±4.54)	159 (±40.6)	35.8	8.92 (±6.63)	17.1 (±4.54)	158* (±40.6)	36.0
3.0	10.7 (± 8.05)	7.25 (±10.5)	70.3 (± 18.2)	71.6	9.67 (±8.05)	6.25 (±10.5)	69.3* (±18.2)	71.9
10	13.5 (±5.02)	4.08 (±4.89)	5.42 (±2.16)	97.8	12.5 (±5.02)	3.08 (±4.89)	4.42* (±2.16)	98.2
30	2.33 (±2.52)	0.00 (± 0.00)	0.00 (± 0.00)	100	1.33 (±2.52)	-1.00 (±0.00)	-1.00* (±0.00)	100

Note Mean, standard deviation (SD) and percent inhibition were calculated from original raw data, not from the rounded values presented in this table.
NA Not Applicable.
SD Standard deviation.
* Statistically significantly reduced when compared with the control, based on Bonferroni t-Test ($p < 0.05$)

Table 5 Growth rate of *Anabaena flos-aquae* after 24, 48 and 72 hours of exposure to ATONIK

Nominal Concentration (g test item/L)	Growth Rate (day^{-1}) Observation interval (hours)			72 Hour Inhibition (%)[a] (SD)
	0 -24 (SD)	0- 48 (SD)	0-72 (SD)	
Control	1.46 (± 0.97)	1.70 (±0.15)	1.87 (± 0.02)	NA
0.3	2.60 (± 0.54)	1.56 (±0.27)	1.82 (± 0.10)	2.40
1.0	2.27 (± 0.66)	1.47 (±0.14)	1.71* (± 0.08)	8.41
3.0	2.27 (± 0.87)	0.66 (±0.78)	1.43* (± 0.09)	23.3
10	2.69 (± 0.43)	0.56 (±0.58)	0.56* (± 0.14)	70.3
30	0.81 (± 0.85)	0.00 (±0.00)	0.00* (± 0.00)	100

Note: Mean, standard deviation (SD) and percent inhibition were calculated from original raw data, not from the rounded values presented in this table.
NA Not Applicable.
SD Standard deviation.
* Statistically significantly reduced when compared with the control, based on Bonferroni t-Test ($p < 0.05$)

Deficiencies

There were no deficiencies.

Conclusion

After 72 hours of exposure to ATONIK, the following endpoints were determined for *Anabaena flos-aquae*:

- Yield: E_yC_{50} = 1.72 g test item/L
  $NOEC_y$ = 0.3 g test item/L

- Growth rate: E_rC_{50} = 6.99 g test item/L
  $NOEC_r$ = 0.3 g test item/L

Comments of zRMS:	The study was conducted in line with OECD 221 with no deviations. All the validity criteria were met and the study is considered acceptable with the following endpoints relevant for the risk assessment: Yield, frond number 7d EC_{50} = 7820 mg ATONIK/L (46.92 mg a.s./L) E_rC_{10} = 2850 mg ATONIK/L NOEC = 370 mg ATONIK/L Yield, dry weight 7d EC_{50} = 8720 mg ATONIK/L E_rC_{10} = 1510 mg ATONIK/L NOEC = 370 mg ATONIK/L Growth rate: ErC_{10} (frond number) = 2850 mg ATONIK/L ErC_{50} (frond number) = 2570 mg ATONIK/L E_rC_{10} (dry weight) = 5050 mg ATONIK/L E_rC_{50} (dry weight) = 2120 mg ATONIK/L

Report:	KCP 10.2.1/02, Biester, M. A. (2011)
Title:	ATONIK: Growth inhibition test with the freshwater duckweed (*Lemna gibba*) under semi-static conditions ArystraLifeScience S.A.S
Document No:	1094 005 410. February 17, 2011
Guidelines:	OECD Guideline for Testing of Chemicals #221, *Lemna* sp., Growth Inhibition Test (OECD, 2006). Deviations: none
GLP	Yes

Executive summary

A *Lemna* growth inhibition test (dose-response test, 7 days) was performed in order to evaluate the effect of ATONIK on the growth of duck weed, *Lemna gibba* in a semi-static test system. The test included treatment groups exposed to ATONIK at the nominal dose levels of 0.37, 1.11, 3.33, 10 and 30 g test item/L. The lowest NOEC and LOEC values were both determined to be 0.37 g test item/L. The EC_{10}, EC_{20}, and EC_{50} values for 7-day yield (frond number) were estimated to be <0.37, 1.12 and 7.82 g test item/L, respectively.
ATONIK is of low toxicity to *Lemna gibba*.

Materials and method

Materials:

Test material:	482-HA
Lot/Batch #	052DO and 055I0
Content of active ingredient:	Sodium 5-nitroguaiacolate 0.109% Sodium ortho-nitrophenolate 0.214% Sodium para-nitrophenolate 0.327%
Expiry date	052DO :19 April 2013 055I0: 03 September 2013
Vehicle and/or positive control	no positive control, only water control
Test organisms	
Species:	*Lemna gibba*
Strain:	Source – University of Waterloo, Canada
Age	not applicable
Test containers:	500 mL-crystallizing dishes with each 200 mL test medium
Test conditions:	
Test medium	20X Algal Assay Procedure (AAP) medium
Sediment	None
Temperature:	23-24°C
pH	7.60 – 9.24
Illumination	6667 – 9424 Lux

Study design and method

Dates of experimental work	24 September to 02 October 2010

Methods

Triplicate cultures of duckweed (*Lemna gibba*) were exposed to ATONIK at the nominal concentration of 0.37, 1.11, 3.33, 10.0, 30.0 g test item/L for 7 days in a semi-static water system. A control group (triplicate cultures) was also tested. 200 mL of the test solution was placed in each replicate vessel and at test initiation, 12 fronds were added to each vessel. At test initiation, a sample of 199 fronds was taken from the stock culture for dry weight determination. The average dry weight of this sample was 0.15 mg per frond. The test solutions were analysed at 0 and 3 days. On days 3, 5 and 7, fronds were counted and observations were made. At test termination, fronds were counted and removed from each vessel, then dried in an oven at 60°C for 24 hours days prior to dry weight determinations. Temperature was recorded daily and ranged between 24 and 26°C. pH was measured on days 0, 3, 5, and 7 in one replicate of each treatment level and ranged between 7.60 and 9.24. Light intensity was measured at test initiation and every 24 hours thereafter and ranged from 6667 to 9424 lux.

Statistics

The data were first checked for normality using Shapiro-Wilks' Test and for homogeneity of variance using Bartlett's Test. ANOVA and Dunnett's Test were used to determine statistical significant differences between the treatments and the control. TOXSTAT® version 3.5 was used to perform these calculations.

Results and discussion

Analytical data on concentrations in test media:

After preparation of the test solutions on day 0, recovery of 87.2% and 103% of the nominal concentrations were found. After 3 days, the recoveries ranged from 88.3 to 102% and from 82.8 to 106% on day 7. Based on these results, the nominal concentrations of 0.37, 1.11, 3.33, 10.0 and 30.0 g test item/L were used for the evaluation of the biological data.

Biological results

At test completion, fronds exposed to the control, 0.37, 1.11 and 3.33 g test item/L were observed to be normal. Fronds of the 10.0 g test item/L treatment level were curved and the roots were observed to be smaller compared to the fronds in the control. The fronds of the 30.0 g test item/L treatment level were observed to be smaller and fewer roots were counted when compared to the control. These observations are reported for completeness.

The 7-day frond number in the control and the 0.37, 1.11, 3.33, 10.0 and 30.0 g test item/L treatment levels averaged 435, 379, 345, 301, 186 and 56 fronds per replicate, respectively. Statistical analysis demonstrated a significant reduction in number among fronds exposed to all treatment levels when compared to the control.

Raw data of frond number of aquatic plant *Lemna gibba* and the percentage of inhibition after 7 days are presented below.

Table 1: Frond number of *Lemna gibba* after 3, 5 and 7 days of exposure to ATONIK

Nominal Concentration (g test item/L)		Frond number (Fronds/Replicate[a])			
		Day 3	Day 5	Day 7	7-day Inhibition (%)
Control	A	65	171	397	
	B	59	177	460	
	C	56	164	448	
	Mean	**60**	**171**	**435**	**NA**
	SD	**4.6**	**6.5**	**33.5**	
0.37	A	55	143	390	
	B	57	153	352	
	C	56	160	395	
	Mean	**56**	**152**	**379***	**12.9**
	SD	**1.0**	**8.5**	**23.5**	
1.11	A	55	143	352	
	B	59	157	338	
	C	61	161	346	
	Mean	**58**	**154**	**345***	**20.6**
	SD	**3.1**	**9.5**	**7.0**	
3.33	A	56	125	311	
	B	52	127	281	
	C	50	130	310	
	Mean	**53**	**127**	**301***	**30.9**
	SD	**3.1**	**2.5**	**17.0**	
10.0	A	47	92	182	
	B	44	94	190	
	C	48	95	185	
	Mean	**46**	**94**	**186***	**57.3**
	SD	**2.1**	**1.5**	**4.04**	
30.0	A	25	46	55	
	B	22	44	54	
	C	23	44	59	
	Mean	**23**	**45**	**56***	**87.1**
	SD	**1.5**	**1.2**	**2.7**	

[a] Initial number of fronds/replicate = 12.
NA Not Applicable
Note Mean, standard deviation (SD) and percent inhibition were calculated from original raw data, not from the rounded values presented in this table.
SD Standard deviation.
* At test termination statistically significantly reduced when compared to the control, based on Dunnett'sTest ($p < 0.05$).

The 7-day yield for the control and the 0.37, 1.11, 3.33, 10.0 and 30.0 g test item/L treatment levels averaged 423, 367, 333, 289, 174 and 44 fronds/replicate, respectively. Statistical analysis demonstrated a significant reduction in yield among fronds exposed to all treatment levels when compared to the control. Raw data of

yield for frond number of aquatic plant *Lemna gibba* and the percentage of inhibition after 7 days are presented below.

Table 2: Yield (frond number) of *Lemna gibba* after 3, 5 and 7 days of exposure to ATONIK

Nominal Concentration (g test item/L)		Yield (Fronds/Replicate[a]) Day 3	Day 5	Day 7	7-day Inhibition (%)
Control	A	53	159	385	
	B	47	165	448	
	C	44	152	436	
	Mean	**48**	**159**	**423**	**NA**
	SD	**4.6**	**6.5**	**33.5**	
0.37	A	43	131	378	
	B	45	141	340	
	C	44	148	383	
	Mean	**44**	**140**	**367***	**13.2**
	SD	**1.0**	**8.5**	**23.5**	
1.11	A	43	131	340	
	B	47	145	326	
	C	49	149	334	
	Mean	**46**	**142**	**333***	**21.2**
	SD	**3.1**	**9.5**	**7.0**	
3.33	A	44	113	299	
	B	40	115	269	
	C	38	118	298	
	Mean	**41**	**115**	**289***	**31.8**
	SD	**3.1**	**2.5**	**17.0**	
10.0	A	35	80	170	
	B	32	82	178	
	C	36	83	173	
	Mean	**34**	**82**	**174***	**58.9**
	SD	**2.1**	**1.5**	**4.0**	
30.0	A	13	34	43	
	B	10	32	42	
	C	11	32	47	
	Mean	**11**	**33**	**44***	**89.6**
	SD	**1.5**	**1.2**	**2.6**	

[a] Initial number of fronds/replicate = 12.
NA Not Applicable
Note Mean, standard deviation (SD) and percent inhibition were calculated from original raw data, not from the rounded values presented in this table.
SD Standard deviation.
* At test termination statistically significantly reduced when compared to the control, based on Dunnett's Test ($p < 0.05$).

At test completion, the average growth rate in the control and the 0.37, 1.11, 3.33, 10.0 and 30.0 g test item/L treatment levels averaged 0.51, 0.49, 0.48, 0.46, 0.39 and 0.22 days- 1, respectively. Statistical analysis demonstrated a significant reduction in growth rate among fronds exposed to all treatment levels when compared to the control. Raw data of growth rate for frond number of aquatic plant *Lemna gibba* and the percentage of inhibition after 7 days are presented below.

Table 3: Growth rate (frond number) of *Lemna gibba* after 3, 5 and 7 days of exposure to ATONIK

Nominal Concentration (g test item/L)		Growth Rate ($days^{-1}$) Observation Interval (Days)				
		Day 0-3	Day 3-5	Day 5-7	Day 0-7	7-day Inhibition (%)
Control	A	0.56	0.48	0.42	0.50	
	B	0.53	0.55	0.48	0.52	
	C	0.51	0.54	0.50	0.52	
	Mean	**0.54**	**0.52**	**0.47**	**0.51**	**NA**
	SD	**0.03**	**0.03**	**0.04**	**0.01**	
0.37	A	0.51	0.48	0.50	0.50	
	B	0.52	0.49	0.42	0.48	
	C	0.51	0.52	0.45	0.50	
	Mean	**0.51**	**0.50**	**0.46**	**0.49***	**3.82**
	SD	**0.01**	**0.02**	**0.04**	**0.01**	
1.11	A	0.51	0.48	0.45	0.48	
	B	0.53	0.49	0.38	0.48	
	C	0.54	0.49	0.38	0.48	
	Mean	**0.53**	**0.48**	**0.41**	**0.48***	**6.38**
	SD	**0.02**	**0.01**	**0.04**	**0.00**	
3.33	A	0.51	0.40	0.46	0.46	
	B	0.49	0.45	0.40	0.45	
	C	0.48	0.48	0.43	0.46	
	Mean	**0.49**	**0.44**	**0.43**	**0.46***	**10.3**
	SD	**0.02**	**0.04**	**0.03**	**0.01**	
10.0	A	0.46	0.34	0.34	0.39	
	B	0.43	0.38	0.35	0.39	
	C	0.46	0.34	0.33	0.39	
	Mean	**0.45**	**0.35**	**0.34**	**0.39***	**23.7**
	SD	**0.02**	**0.02**	**0.01**	**0.00**	
30.0	A	0.24	0.30	0.09	0.22	
	B	0.20	0.35	0.10	0.21	
	C	0.22	0.32	0.15	0.23	
	Mean	**0.22**	**0.33**	**0.11**	**0.22***	**57.1**
	SD	**0.02**	**0.02**	**0.03**	**0.01**	

NA Not Applicable
Note Mean, standard deviation (SD) and percent inhibition were calculated from original raw data, not from the rounded values presented in this table.
SD Standard deviation.
* At test termination statistically significantly reduced when compared to the control, based on Dunnett's Test ($p < 0.05$).

The 7-day dry weight for the control and the 0.37, 1.11, 3.33, 10.0 and 30.0 g test item/L treatment levels averaged 50.7, 47.6, 44.1, 43.0, 22.4 and 4.81 mg, respectively. Statistical analysis demonstrated a significant reduction in dry weight among fronds exposed to the 1.11, 3.33, 10.0 and 30.0 g test item/L treatment levels when compared to the control. Raw data of dry weight (frond number, yield and growth rate) of aquatic plant *Lemna gibba* and the percentage of inhibition after 7 days for each parameters are presented below.

Table 4: Dry weight (frond number, yield and growth rate) of *Lemna gibba* after 3, 5 and 7 days of exposure to ATONIK

Dry Weight							
Nominal Concentration (g test item/L)		Frond number (mg)		Yield (mg)		Growth Rate (days^{-1})	
		Day 7	7-day Inhibition (%)	Day 7	7-day Inhibition (%)	Day 7	7-day Inhibition (%)
Control	A	44.1		42.3		0.46	
	B	52.7		50.9		0.49	
	C	55.4		53.6		0.49	
	Mean	**50.7**	**NA**	**48.9**	**NA**	**0.48**	**NA**
	SD	**5.90**		**5.90**		**0.02**	
0.37	A	47.8		46.0		0.47	
	B	46.1		44.3		0.47	
	C	49.0		47.2		0.47	
	Mean	**47.6**	**6.11**	**45.8**	**6.33**	**0.47**	**1.75**
	SD	**1.46**		**1.46**		**0.00**	
1.11	A	48.4		46.6		0.47	
	B	42.7		40.9		0.46	
	C	41.3		39.5		0.45	
	Mean	**44.1***	**13.0**	**42.3***	**13.5**	**0.46**	**4.09**
	SD	**3.76**		**3.76**		**0.01**	
3.33	A	45.0		43.2		0.46	
	B	40.2		38.4		0.45	
	C	44.0		42.2		0.46	
	Mean	**43.0***	**15.1**	**41.3***	**15.7**	**0.46***	**4.78**
	SD	**2.53**		**2.53**		**0.01**	
10.0	A	20.7		18.9		0.35	
	B	23.7		21.9		0.37	
	C	22.8		21.0		0.37	
	Mean	**22.4***	**54.2**	**20.6***	**56.1**	**0.36***	**24.3**
	SD	**1.54**		**1.54**		**0.01**	
30.0	A	4.48		2.72		0.13	
	B	5.08		3.32		0.15	
	C	4.88		3.12		0.15	
	Mean	**4.81***	**90.6**	**3.05***	**93.8**	**0.14***	**70.1**
	SD	**0.31**		**0.31**		**0.01**	

NA Not Applicable
Note Dry weight of representative 199-frond sample at experimental starting = 0.15 mg.
Mean, standard deviation (SD) and percent inhibition were calculated from original raw data, not from the rounded values presented in this table.
SD Standard deviation.
* At test termination statistically significantly reduced when compared to the control, based on Dunnett's Test ($p < 0.05$).

The 7-day dry weight yield for the control and the 0.37, 1.11, 3.33, 10.0 and 30.0 g test item/L treatment levels averaged 48.9, 45.8, 42.3, 41.3, 20.6 and 3.05 mg, respectively. Statistical analysis demonstrated a significant reduction in yield among fronds exposed to the 1.11, 3.33, 10.0 and 30.0 g test item/L treatment levels when compared to the control.

At test completion, dry weight growth rate for the control and the 0.37, 1.11, 3.33, 10.0 and 30.0 g test item/L treatment levels averaged 0.48, 0.47, 0.46, 0.46, 0.36 and 0.14 days^{-1}, respectively. Statistical analysis demonstrated a significant reduction in growth rate among fronds exposed to the 3.33, 10.0 and 30.0 g test item/L treatment levels when compared to the control.

Table 5: EC_{10}, EC_{20} and EC_{50} values (95% Confidence Intervals), No-Observed-Effect Concentration (NOEC) and Lowest-Observed-Effect Concentration (LOEC) for ATONIK after 7 days of exposure with *Lemna gibba*

Biological parameter	Based on nominal concentration (g test item/L)				
	NOEC	LOEC	EC_{10} (95% C.I.)	EC_{20} (95% C.I.)	EC_{50} (95% C.I.)
7 day (frond number)	<0.37	0.37	NA	NA	NA
7 day yield (frond number)	<0.37	0.37	<0.37	1.12 (0.44-2.37)	7.82 (6.93-8.72)
7 day growth rate (frond number)	<0.37	0.37	2.85 (2.11-4.00)	7.95 (7.26-8.95)	25.7 (24.7-26.7)
7 day dry weight	0.37	1.11	NA	NA	NA
7 day yield (dry weight)	0.37	1.11	1.51 (0.39-3.87)	3.65 (0.90-5.30)	8.72 (7.84-9.60)
7 day growth rate (dry weight)	1.11	3.33	5.05 (4.18-6.07)	8.51 (7.60-9.60)	21.2 (20.2-22.3)

C.I.: Confidence Interval
NA: Not applicable according to study plan

Deficiencies

No deficiencies

Conclusions

The lowest reported NOEC and LOEC values were both determined to be 0.37 g test item/L. The EC_{10}, EC_{20}, and EC_{50} values for 7-day yield (based on frond number) were estimated to be <0.37, 1.12 and 7.82 g test item/L, respectively.

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 2.3 KCP 10.3 Effects on arthropods

A 2.3.1 KCP 10.3.1 Effects on bees

A 2.3.1.1 KCP 10.3.1.1 Acute toxicity to bees

A 2.3.1.1.1 KCP 10.3.1.1.1 Acute oral toxicity to bees

A 2.3.1.1.2 KCP 10.3.1.1.2 Acute contact toxicity to bees

A 2.3.1.2 KCP 10.3.1.2. Chronic toxicity to bees

The following study was submitted for Active substance renewal and was not yet previously evaluated. The study was conducted with AMP (MUP powder of Atonik). The study is provided in support of the risk assessment and has not been previously evaluated. The study was performed in order to comply with the new data requirements.

Comments of zRMS:	The study was conducted in accordance to OECD No. 245 (2017). All the validity criteria were met and the study is considered acceptable with the following endpoints relevant for the risk assessment: 10 d NOEDD (oral) = 45.67 µg AMP/bee/day, 10 d LDD_{50} (oral) > 55.18 µg AMP/bee/day, corresponding to 7.34 µg Na 5 NG/bee/d, 14.46 µg Na o-NP/bee/d, 21.65 µg Na p NP/bee/d

Data point addressed:	CA 8.3.1.2/01
Author (year):	Harkin, S. (2020)
Title:	ATONIK: 10 DAY CHRONIC ORAL TOXICITY TEST (REPEATED DOSE) FOR ADULT HONEYBEES (APIS MELLIFERA L.) (Amended Final Report)
Laboratory report / project Number (Doc. No.):	FR/000623
Testing facility:	Fera Science Ltd., Centre for Chemical Safety & Stewardship, Sand Hutton, United Kingdom
Published:	No
Test guideline used:	CEB No. 230 (2014), current recommendations of the ring test group (2014) for a proposal for a new OECD Guideline for 10 day chronic adult honey bee toxicity test
Deviations:	None
Previous evaluation:	No
GLP:	Yes; certified by the Department of Health of the Government of the United Kingdom
Acceptability/Reliability:	Yes

Executive Summary

The chronic oral toxicity of AMP (MUP powder of Atonik) on honey bee workers was determined by offering test item treated sucrose solution. The nominal concentrations were 312.5, 625, 1250, 2500 and 500 mg AMP/kg 50 % (w/v) aqueous sucrose solution. The effective doses, based on definitive consumptions, were 12.49, 27.30, 45.67, 55.18 and 52.78 µg AMP/bee/day.
The NOEDD was determined as 45.67 µg AMP/bee/day. The reduced feed uptake in the highest test item concentration (5000 mg AMP/kg sucrose solution) actually resulted in a lower dose at this concentration of 52.78 µg AMP/bee/day, compared to the uptake dose of 55.18 µg AMP/bee/day calculated for the lower concentration of 2500 mg AMP/kg feed. As mortality was not affected > 50 % in all tested groups, the LDD_{50} was estimated to be > 55.18 µg AMP/bee/day.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material:

Test Material:	AMP (MUP powder of Atonik)
Active components and content of components:	Sodium 5-Nitroguaiacolate 13.3 %, Sodium *o*-Nitrophenolate 26.2 %, Sodium *p*-nitrophenolate dihydrate 48.0 %
Description:	Brown crystal powder

2. Vehicle and/or positive control:

Vehicle:	Test item was dissolved in 50 % (w/v) aqueous sucrose solution
Positive control:	1.5 mg dimethoate/kg sucrose solution corresponding to 0.06 µg dimethoate/bee/day

3. Test animals

Species:	Honey bees (*Apis mellifera* L.)
Source:	Fera National Bee Unit, UK

Age:	Adult, newly emerged (not more than 48 h old)
Feeding	During the first two days after emergence, worker bees from all treatments were fed *ad libitum* with 50 % (w/v) aqueous sucrose solution. From test start onwards, honey bees were fed with 50 % (w/v) aqueous sucrose solution incorporating the test compound at appropriate concentrations for 10 consecutive days.
Environmental conditions:	
Temperature:	33 ± 2 °C
Humidity:	60 ± 5 %
Photoperiod:	Constant darkness

4. In-life dates 09 August, 2016 - 27 September, 2016

B. STUDY DESIGN AND METHODS

1. Experimental treatments

The study consisted of seven treatments: a control, five test item concentrations and one reference item concentration. Three replicate units, each consisting of 10 worker bees, were established for each treatment. Two-day-old worker bees were fed ad libitum with a 50 % (w/v) sucrose solution incorporating the test treatment at appropriate concentrations for 10 consecutive days. The main test was run as a dose response test at five concentrations of 312.5, 625, 1250, 2500 and 5000 mg AMP/kg 50 % (w/v) aqueous sucrose solution. The toxic reference group was dosed at 1.5 mg a.s./kg 50 % (w/v) aqueous sucrose solution.
Test item stock solutions were made up in deionised water on Day 0 and 4 and stored at 0-10 °C for the duration of the test. Toxic reference stock solutions were made up in deionised water on Day 0 and stored at 0-10 °C for the duration of the test. The dosed feed solutions were prepared on Days 0, 4 and 7 by adding 50 µL of each stock solution per g of 50 % (w/v) aqueous sucrose solution. Samples of the feed solutions for each of the test item treated groups were taken on Days 0, 4 and 7. These samples were analysed by liquid chromatography with diode-array UV detection (LC-DAD) in order to confirm the levels of the test item by summing up the areas of each individual peak to give total Atonik mixture powder response.
For dosing, a modified 1.5 mL micro-centrifuge tube was used. The feeders were labelled and filled with approximately 1.5 mL of dosing solution dilution (or control solution) using a syringe. Once filled, the feeder and contents were weighed before being placed in the appropriate test unit. Feeders were exchanged for new full feeders every day. Once removed, the feeders were reweighed to allow calculation of the feed uptake. To account for weight loss due to evaporation, cages were set up in exactly the same manner as the test units but without any bees. Three evaporation controls were run for each of the lowest (312.5 mg AMP/kg), and highest (5000 mg AMP/kg) dose rates and for the untreated control.

2. Observations

Mortality was assessed once a day. Bees were recorded as moribund when they were on their back or side, still twitching, but unable to up-right themselves. Bees were noted as dead when no reaction to a tactile stimulation was observed. Dead bees were removed from the cages. Behavioral abnormalities, e.g., uncoordinated movement, trembling, tumbling, abnormal movements of legs or wings, etc., were recorded once a day to assess sub-lethal effects of the test item.

3. Statistics

The data were statistically analysed using Code.R.

II. RESULTS AND DISCUSSION

Analytical results
Samples of the feed solutions for the test item treated groups were taken on Days 0, 4 and 7 and analysed by liquid chromatography with diode-array UV detection (LCDAD). The results are displayed in the table below.

Table 1: Results of analysis of stock solutions

Treatment group [mg AMP/kg]	Calculated expected concentration* [µg AMP/mL]	Analysed concentration [µg AMP/mL]						Mean of all days	% dev.
		Days 0-3	% dev	Day 4-6	% dev	Day 7-9	% dev		
312.5	352.125	344.26	-2.5	354.57	0.4	350.16	-0.8	349.66	-1.0
625	706.25	669.14	-5.3	709.98	0.5	686.85	-2.7	688.66	-2.5
1250	1412.5	1355.78	-4.0	1404.76	-0.5	1360.16	-3.7	1373.57	-2.8
2500	2825	2669.2	-5.5	2846.05	0.7	2762.65	-2.2	2759.30	-2.3
5000	5650	5396.7	-4.5	5754.3	1.8	5631.8	-0.3	5595.27	-1.0

* Based on an approximate weight of 1 mL 50 % aqueous sucrose solution of 1.13 g.
% dev = deviation from nominal value [%]

The results of the analysis confirm that the levels of the test item present in the feed were in the range of ± 20 % of nominal. Therefore, the calculations were based on nominal concentrations.

Biological results

After 10 days of chronic oral exposure, mortality of honey bee workers in the control treatment was 0.0 %. Mortality in the reference item was 100.0 %. The validity criteria for the control (≤ 15 % mortality) and the reference item treatment (≥ 50 % mortality) were met and therefore, the study can is valid.
Based on the measured uptake of the bees, the mean daily doses of 12.49, 27.30, 45.67, 55.18 and 52.78 µg AMP/bee per day were achieved. The mortality at the three lowest doses, 12.49, 27.30 and 45.67 µg AMP/bee/day did not differ significantly from to the control. The mortality at 55.18 and 52.78 µg AMP/bee/day was 16.7 and 100 %, respectively. The mortality in the reference item treatment 100.0 %, demonstrating the sensitivity of the test system.

The NOEDD was determined as 45.67 µg AMP/bee/day. The reduced feed uptake in the highest test item concentration (5000 mg AMP/kg sucrose solution) actually resulted in a lower dose at this concentration of 52.78 µg AMP/bee/day, compared to the uptake dose of 55.18 µg AMP/bee/day calculated for the lower concentration of 2500 mg AMP/kg feed. As effects of > 50 % were not reported at the highest dose of 55.1 µg AMP/bee/day, the LDD_{50} can therefore be said by observation to be > 55.18 µg AMP/bee/day.
No behavioural abnormalities were recorded in the control treatment and the test item treatments throughout the study.

Table 2: Mortality of honey bees in the chronic oral toxicity test at test termination (Day 10)

Treatment	Nominal concentration [mg/kg sucrose solution]	Final tested dose [µg/bee/day]	Mortality at Day 10 [%]
Control	0	-	0.0
AMP	312.5	12.49	6.7
	625	27.30	0.0
	1250	45.67	0.0
	2500	55.18	16.7
	5000	52.78	100.0
Dimethoate	1.5	0.06	100.0

Table 3 Endpoints of the chronic oral toxicity test (repeated exposure) with honey bees toxicity after exposure to AMP

Endpoint	Mortality Day 10 [µg AMP/bee/day]
LDD_{50}	> 55.18
NOEDD	45.67

III. CONCLUSIONS

The NOEDD was determined as 45.67 µg AMP/bee/day. The reduced feed uptake in the highest test item concentration (5000 mg AMP/kg sucrose solution) actually resulted in a lower dose at this concentration of 52.78 µg AMP/bee/day, compared to the uptake dose of 55.18 µg AMP/bee/day which was calculated for the lower concentration of 2500 mg AMP/kg feed. As effects of > 50 % were not reported at the highest

dose of 55.1 µg AMP/bee/day, the LDD_{50} can therefore be said by observation to be > 55.18 µg AMP/bee/day.

Table 4 summarises the results of all available chronic toxicity studies conducted with sodium nitrocompounds Sodium 5-Nitroguaiacolate, Sodium *o*-Nitrophenolate and Sodium *p*-Nitrophenolate on bees.

Table 4: Summary of chronic adult honey bee toxicity endpoints of sodium nitrocompounds

Test Substance	Endpoint	Value	Reference
AMP	10 d NOEDD (oral) 10 d LDD_{50} (oral)	45.67 µg AMP/bee/day, > 55.18 µg AMP/bee/day (corresponding to 7.34 µg Na 5-NG/bee/d, 14.46 µg Na *o*-NP/bee/d, 21.65 µg Na *p*-NP/bee/d)	Harkin (2020), CA 8.3.1.2/01

A 2.3.1.3 KCP 10.3.1.3 Effects on honey bee development and other honey bee life stages

The following studies was submitted for Active substance renewal and was not yet previously evaluated. The study was conducted with AMP (MUP powder of Atonik). The study is provided in support of the risk assessment and has not been previously evaluated. The study was performed in order to comply with the new data requirements.

Comments of zRMS:	The study was conducted in accordance to OECD Series Testing & Assessment No. 239 (2014). All the validity criteria were met and the study is considered acceptable with following endpoints: 8 d LDD_{50} = 560.19 µg AMP/larva 8 d NOED = 350 µg AMP/larva The chronic 22 d study for larvae bees according to OECD 239 (2016) is currently required as most appropriate in the chronic risk assessment.

Data point addressed:	CA 8.3.1.3/01
Author (year):	Harkin, S. (2020)
Title:	ATONIK: IN VITRO 8 DAY TOXICITY TEST - REPEATED EXPOSURE TO LARVAL STAGE HONEYBEE (APIS MELLIFERA L.) (Amended Final Report)
Laboratory report / project Number (Doc. No.):	FR/000624
Testing facility:	Fera Science Ltd., Centre for Chemical Safety & Stewardship, Sand Hutton, United Kingdom
Published:	No
Test guideline used:	Draft OECD Series on Testing & Assessment No. 239 (2014), Aupinel, F. et al. (2007)
Deviations:	None
Previous evaluation:	No
GLP:	Yes; certified by the Department of Health of the Government of the United Kingdom
Acceptability/Reliability:	Yes, as additional information

Executive Summary

A bee larval toxicity test was conducted with AMP (MUP powder of Atonik), in order to assess the effects to the honey bee (*Apis mellifera* L.) larvae. The test item was offered as treated diet on four consecutive days (Day 3 to 6). The larval mortality on Day 8 was assessed. The doses had been 43.75, 87.5, 175, 350 and 700 µg AMP/larva per developmental period, corresponding to 312.5, 625, 1250, 2500 and 5000 mg AMP/kg larval diet. An untreated control and a toxic reference were included in the study.
The LC_{50} for AMP was calculated to be 4001.4 mg AMP/kg larval diet (95% CI = 2500 – 5000). This is equal to a LDD_{50} of 560.19 µg AMP/larva per developmental period (95% CI = 350 – 700) (nominal). The NOEC was found to be 2500 mg AMP/kg larval diet, equal to a NOED of 350 µg AMP/larva per developmental period (nominal). The LOEC was found to be 5000 mg AMP/kg larval diet, equal to a LOED of 700 µg AMP/larva per developmental period (nominal). These values are based on an assumed uptake of the complete offered dose within 140 µL of diet over the exposure period and an assumed weight of 1 µL of larval diet of 1 mg.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material:

Test Material AMP (MUP powder of Atonik)

Active components and content of components:	Sodium 5-Nitroguaiacolate 13.3 %, Sodium *o*-Nitrophenolate 26.2 %, Sodium *p*-nitrophenolate dihydrate 48.0 %
Description:	Brown crystal powder

2. Vehicle and reference item:

Vehicle:	Deionised water
Reference item:	10 mg dimethoate/kg diet

3. Test animals

Species:	Honey bee (*Apis mellifera* L.)
Source:	Fera National Bee Unit, UK. The colonies were queen-right and, healthy, disease-free and with known history and physiological status.
Age:	1st stage larvae
Feeding	The larvae received different diets according to OECD Guidance Document No. 239, composed of a solution of royal jelly, yeast extract, glucose and fructose in different proportions, adapted to the needs of the larvae at different stages. The diets contained either the test item (test item treatment group), the reference item (reference item group), or the pure diet (control group).

4. Environmental conditions:

Temperature:	33 ± 2 °C
Humidity:	About 95 %
Photoperiod:	Constant darkness

B. STUDY DESIGN AND METHODS

1. Experimental phase 10 August 2016 – 27 September 2016

2. Experimental Treatments:
The test item AMP was mixed in the food for honeybee larvae starting with Day 3. In addition, a control treatment and the reference item (dimethoate) at a nominal dose of 10 mg dimethoate/kg diet were included. In total, seven treatment groups were set up for the study; one control, five test item doses and one reference treatment group. Three replicates per treatment were established from three different colonies consisting of 16 larvae from one hive and 14 from each of the other two hives. Each larva was placed in one cell of a multiwell plate. All larvae from the same treatment were placed in the same plate.
According to the OECD Guidance Document, the food was composed of three different diets, adapted to the needs of the larvae at different stages of development (diet A, B and C). The test item was mixed in an appropriate amount to diet B and diet C.

3. Observations
Treatment-related mortality checks were made each day at feeding. An immobile larva or one which did not respond when touched (if necessary), was recorded as dead and removed.

4. Analytics
Test item stock solutions were analysed using LC-DAD analysis in order to confirm the levels of the test item by summing up the areas of each individual peak to give total Atonik mixture powder response.

5. Statistics
The data were statistically analysed using Code.R.3.2.2.

II. RESULTS AND DISCUSSION

A. Validity criteria

According to the OECD Guidance Document No. 239, the test is considered valid as in the control plate the cumulative larval mortality from Day 4 to Day 8 was ≤ 15 % across the replicates (exact value 6.8 %). In addition, in the reference item group the larval mortality was ≥ 50 % at Day 8 (exact value: 100.00 %).

B. Analytical test results

The analytical results show that the dilutions were made correctly and the concentrations were within an acceptable range of the nominal concentration (± 20 %). Effect concentrations were therefore calculated based on nominal concentrations of the test item.

Table 1 LC-DAD analysis results

Treatment group [mg AMP/kg diet]	Calculated stock concentration [µg/µL]	Measured concentration [µg/µL]	% difference
312.5	6.25	6.12	-2.08
625	12.5	11.97	-4.24
1250	25	24.34	-2.64
2500	50	47.64	-4.72
5000	100	89.88	-10.12

C. Biological test results

The mortality of the larvae was statistically significantly different from control at the highest dose, 700 µg AMP/larva per developmental period (Table 8.3.1.3-2). Therefore, the NOED was determined to be 350.0 µg AMP/larva per developmental period. The LDD_{50} was calculated as 560.19 µg AMP/larva per developmental period.

Table 2 Mortality of honey bee larvae exposed to AMP in a repeated larval toxicity test

Treatment	Nominal dose [µg AMP/larva per developmental period]	Number of dead larvae	Cumulative mortality [%]	Corrected mortality[1] [%]
Control	-	3	6.8	-
AMP	43.75	10	20.9	17.1
	87.5	3	6.8	0.0
	175	4	4.8	2.4
	350	3	6.8	0.0
	700	31	70.5	68.3
Reference item (Dimethoate)	-	44	100.0	-

[1] mortality in test item and reference item treatment groups corrected for control mortality according to Abbott (1925), - not relevant.

Table 3 Endpoints of the larvae toxicity test (repeated exposure) after exposure to AMP

Endpoint	Mortality Day 8 [µg AMP/larva per developmental period]
LDD_{50} (95% confidence limit)	560.19 (350 – 700)
NOED	350
LOED	700

III. CONCLUSIONS

The LC_{50} for AMP was calculated to be 4001.4 mg AMP/kg larval diet (95% CI = 2500 – 5000). This is equal to a LDD_{50} of 560.19 µg AMP/larva per developmental period (95% CI = 350 – 700) (nominal). The NOEC was found to be 2500 mg AMP/kg larval diet, equal to a NOED of 350 µg AMP/larva per developmental period (nominal). The LOEC was found to be 5000 mg AMP/kg larval diet, equal to a LOED of 700 µg AMP/larva per developmental period (nominal). These values are based on an assumed uptake of

the complete offered dose within 140 µL of diet over the exposure period and an assumed weight of 1 µL of larval diet of 1 mg.

Comments of zRMS:	The study was conducted in accordance to OECD Series Testing & Assessment No. 239 (2016). All the validity criteria were met and the study is considered acceptable with the following endpoints relevant for the risk assessment: 22 d LDD_{10} = 16.65 µg AMP/larva corresponding to 2.16 µg Na 5 NG/larva, 4.30 µg Na o-NP/larva, 6.33 µg Na p NP/larva 22 d LDD_{20} =33.70 µg AMP/larva 22 d LDD_{50} =112.2 µg AMP/larva 22 d NOED =28.4 µg AMP/larva

Data point addressed:	CA 8.3.1.3/02
Author (year):	Couture, E. (2020)
Title:	AMP (ATONIK MUP POWDER) - A LABORATORY STUDY TO DETERMINE THE CHRONIC EFFECTS ON THE HONEY BEE APIS MELLIFERA L. (HYMENOPTERA: APIDAE) 22-DAY LARVAL TOXICITY TEST WITH REPEATED EXPOSURE
Laboratory report / project Number (Doc. No.):	516SRFR18C05
Testing facility:	SynTech Research France SAS, La Chapelle de Guinchay, France
Published:	No
Test guideline used:	OECD No. 239 (2016)
Deviations:	None
Previous evaluation:	No, not previously evaluated
GLP:	Yes; certified by Group Interministeriel des Produits Chimiques
Acceptability/Reliability:	Yes

Executive Summary

A bee larval toxicity test was conducted with AMP (MUP powder of Atonik), in order to assess the effects to the honey bee (*Apis mellifera* L.) larvae. The test item was offered as treated diet on four consecutive days (Day 3 to 6). The larval mortality on Day 8, the pupal mortality and adult emergence on Day 22 were assessed. The doses had been 5.36, 12.3, 28.4, 65.2 and 150 µg AMP/larva per developmental period, corresponding to 34.805, 79.870, 184.4155, 423.377 and 974.026 mg AMP/kg larval diet. An untreated control and a toxic reference were included in the study.
The NOEC for adult emergence was determined to be 184.4 mg AMP/kg larval diet, equivalent to a NOED of 28.4 µg AMP/larva per developmental period. Consequently, the LOEC was set to be 423.4 mg AMP/kg larval diet, equivalent to a LOED of 65.2 µg AMP/larva per developmental period. The LC_{50} for adult emergence was calculated to be 726.9 mg AMP/kg larval diet (95% CI = 439.8 - 1297) corresponding to a LDD_{50} of 112.2 µg AMP/larva per developmental period (95% CI = 67.73 – 206.7) (nominal). These values are based on an assumed uptake of the complete offered dose within 140 µL of diet over the exposure period as no uneaten food in the cup cells was recorded at Day 8. No non-lethal biological effects (as malformations or behavioural effects) were observed in any of the test item treatment groups.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material:

Test Material	AMP (Atonik MUP powder)
Active components and content of components:	Sodium 5-Nitroguaiacolate 13.0 %, Sodium *o*-Nitrophenolate 25.8 %, Sodium *p*-Nitrophenolate dihydrate 46.5 %

Description:	Red/orange powder

2. Vehicle and reference item:

Vehicle:	Destilled water
Reference item:	47.99 mg dimethoate/kg diet

3. Test animals

Species:	Honey bee (*Apis mellifera* L.)
Source:	Honey bee colonies reared at SynTech Research France The colonies were queen-right and, healthy, disease-free and with known history and physiological status. No sanitary treatment, such as antibiotics, anti-varroa, etc., was used on the colonies for at least four weeks prior to the test (first application).
Age:	1st stage larvae
Feeding	The larvae received different diets according to OECD Guidance Document No. 239, composed of a solution of royal jelly, yeast extract, glucose and fructose in different proportions, adapted to the needs of the larvae at different stages. The diets contained either the test item (test item treatment group), the reference item (reference item group), or the pure diet (control group).

4. Environmental conditions:

Temperature:	34.0 – 35.0 °C
Humidity:	64.9 - 99.7 % depending of the developmental stage
Photoperiod:	Constant darkness

B. STUDY DESIGN AND METHODS

1. Experimental phase 17 July 2019 – 05 August 2019

2. Experimental Treatments:

The test item AMP was mixed in the food for honeybee larvae starting with Day 3. In addition, a control treatment and the reference item (dimethoate) at a dose of 47.99 mg dimethoate/kg diet were included.
In total, seven treatment groups were set up for the study; one control, five test item doses and one reference treatment group. The test item doses were 5.36, 12.3, 28.4, 65.2 and 150 µg AMP/larva per developmental period, corresponding to 34.8, 79.9, 184.4, 423.4 and 974.0 mg AMP/kg larval diet. Three replicates per treatment were established from three different colonies, each containing 12 larvae. Each larva was placed in one cell of a multiwell plate. All larvae from the same treatment were placed in the same plate.
According to the OECD Guidance Document, the food was composed of three different diets, adapted to the needs of the larvae at different stages of development (diet A, B and C). The diet was warmed in a climatic chamber to approximately 35 °C before the larvae were fed. The test item was mixed in an appropriate amount to diet B and diet C.
The corresponding stock solution and test item dilutions were freshly prepared every day. The volume of application solution in the diet did not exceed 10 % of the final diet volume. The diet was homogenized using a mixer.

3. Observations

Larval mortality assessments were done on Days 4, 5, 6, 7, and 8. The presence of uneaten food was qualitatively recorded on day 8. Assessments of mortality during pupation phase were done on Days 15 and 22. Emergence rate was recorded on Day 22.

4. Analytics

Since the analytical measurement of the test chemical concentration in the larval diet is more difficult because of the presence of royal jelly, the validation of the method for quantification of the components of AMP in feeding solution has not been achieved for technical reasons. Hence, the dose verification was achieved by analysis of the solution used to prepare the diet (stock solution). Test item stock solutions were taken on every each day of application. The specimens were analysed using LC-MS/MS analysis in order to confirm the levels of the test item.

5. Statistics

Results were analysed with the statistical software R 3.3.2 version. All statistical analysis were performed at $\alpha = 0.05$.

Monotonicity was tested on mortality and emergence data with a Spearman's correlation test. In order to determine any significant differences between treatment and untreated control diet, a Cochran-Armitage trend step-down procedure was used on mortality data and emergence data (monotonic effect on mortality). LDD_{50}/LC_{50} and 95 % credible intervals for mortality data were not calculated because there was no adverse effect exceeding 50 %, for any of the tested doses. The EDD_{50}/EC_{50} and 95 % credible intervals for emergence data were calculated with a Bayesian inference model (Log-logistic binomial model with 3 parameters).

Behavioural observations were not evaluated for statistical significance due to the non-quantitative nature of the observations.

II. RESULTS AND DISCUSSION

A. Validity criteria

According to the OECD Guideline No. 239, Series on Testing & Assessment, the test is considered valid as the cumulative larval mortality on the control plate from Day 3 to Day 8 was ≤ 15 % across the replicates (exact value 8.33 %). The adult emergence rate on Day 22 was ≥ 70 % across all control replicates (actual value 88.8 %). In addition, in the reference item group the larval mortality was ≥ 50 % on Day 8 (exact value: 94.4 %).

B. Analytical test results

The analytical results show that the solutions were made correctly and the concentrations were within an acceptable range of the nominal concentration (± 20 % of AMP). Effect concentrations were therefore calculated based on nominal concentrations of the test item AMP.

Table 1 LC-MS/MS analytical results

Analytical samples	Recovery [%]			Geometric mean of daily recovery[1]
	Sodium 5-nitroguaiacolate	Sodium *o*-nitrophenolate	Sodium *p*-nitrophenolate dihydrate	
SS-T106-D3 A	82	89	91	87.25
SS-T106-D4 A	89	95	101	94.87
SS-T106-D5 A	92	98	101	96.93
SS-T106-D6 A	79	88	87	84.57
SS-T106-D3 A	82	89	91	87.25

[1] Average recoveries of the test item per day.

C. Biological test results

The mortality of the larvae on day 8 was not statistically significantly different from control at any test item concentration (Table 8.3.1.3-5). The emergence at Day 22 was statistically significantly different from control at the two highest doses, 65.2 and 150 µg AMP/larva per developmental period (Table 8.3.1.3-5). Therefore, the NOED was determined to be 28.4 µg AMP/larva per developmental period. The LDD_{50} was calculated as 112.2 µg AMP/larva per developmental period (Table 8.3.1.3-6). No non-lethal biological effects (as malformations or behavioural effects) and no uneaten food were recorded in any of the test item treatment groups.

Table 2 Mortality of honey bee larvae exposed to AMP in a repeated larval toxicity test

Treatment	Nominal dose [µg AMP/larva per developmental period]	Cumulative mortality [%]	Mortality [% deviation from control[1]]	Emergence [%]	Emergence: % deviation from control[2]
Control	0	8.33	-	77.8	-
AMP	5.36	8.33	0.0	80.6	+3.71
	12.3	5.56	-3.03	77.8	0.0
	28.4	13.9	+6.06	61.1	-21.43
	65.2	13.9	+6.06	58.3*	-25.00
	150	16.7	+9.09	33.3*	-57.14
Reference item (Dimethoate)	47.99				

[1] Value corrected from the untreated control diet results, according to Abbott (1925). Negative/positive values mean lower/higher mortality compared to the untreated control diet results

[2] Negative/positive values mean lower/higher emergence compared to the untreated control diet results

* significantly different from the untreated control diet (Cochran-Armitage trend step-down procedure for the 8-day mortality data and the 22-day emergence data)

Table 3 Endpoints of the larvae toxicity test (repeated exposure) after exposure to AMP

Endpoint	Mortality Day 8 [µg AMP/larva per developmental period]	Emergence Day 22 [µg AMP/larva per developmental period]
LDD_{10} (95% confidence limit)	> 150	16.65 (2.929 – 64.35)
LDD_{20} (95% confidence limit)	> 150	33.70 (10.74 – 87.90)
LDD_{50} (95% confidence limit)	> 150	112.2 (67.73 – 206.7)
NOED	≥ 150	28.4
LOED	> 150	65.2

III. CONCLUSIONS

The effects of AMP (MUP powder of Atonik) to larvae of honey bees (*Apis mellifera* L.) were assessed in a chronic larvae toxicity test following repeated exposure. The NOEC was determined to be 184.4 mg AMP/kg larval diet, equivalent to a NOED of 28.4 µg AMP/larva per developmental period. Consequently, the LOEC was set to be 423.4 mg AMP/kg larval diet, equivalent to a LOED of 65.2 µg AMP/larva per developmental period. The LC_{50} for AMP was calculated to be 726.9 mg AMP/kg larval diet (95% CI = 439.8 - 1297) corresponding to a LDD_{50} of 112.2 µg AMP/larva per developmental period (95% CI = 67.73 – 206.7) (nominal).

Toxicity to honeybee development – overall summary and conclusion

Table 4 summarises the results of all available toxicity studies conducted with sodium nitrocompounds Sodium 5-Nitroguaiacolate, Sodium *o*-Nitrophenolate and Sodium *p*-Nitrophenolate on honeybee development.

Table 8.3.1.3-7: Summary of honey bee larval toxicity endpoints of sodium nitrocompounds

Test Substance	Endpoint	Value	Reference
AMP	8 d LDD_{50} 8 d NOED	560.19 µg AMP/larva 350 µg AMP/larva (corresponding to 46.6 µg Na 5-NG/larva, 91.7 µg Na *o*-NP/larva, 137.3 µg Na *p*-NP/larva)	Harkin (2020), CA 8.3.1.3/01
AMP	22 d LDD_{10} 22 d LDD_{50} 22 d NOED	16.65 µg AMP/larva (corresponding to 2.16 µg Na 5-NG/larva, 4.30 µg Na *o*-NP/larva, 6.33 µg Na *p*-NP/larva) 112.2.µg AMP/larva 28.4 µg AMP/larva	Couture (2020), CA 8.3.1.3/02

A 2.3.1.4 KCP 10.3.1.4 Sub-lethal effects

A 2.3.1.5 KCP 10.3.1.5 Cage and tunnel tests

A 2.3.1.6 KCP 10.3.1.6 Field tests with honeybees

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

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 2.4.2.2 KCP 10.4.2.2 Higher tier testing

A 2.5 KCP 10.5 Effects on soil nitrogen transformation

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

A 2.6.3 KCP 10.6.3 Extended laboratory studies on non-target plants

A 2.7 KCP 10.7 Effects on other terrestrial organisms (flora and fauna)

A 2.8 KCP 10.8 Monitoring data