European Commission

Addendum

VOLUME 3 – Annex B (A12115l)

Abamectin

B.9 Ecotoxicology data

Rapporteur Member State: The Netherlands

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Draft Assessment Report and Proposed decision of the Netherlands prepared in the context of the possible extension of the approval conditions of abamectin under Regulation (EC) 1107/2009

Version history page

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Contents

B.9 Ecotoxicology

A12115I is a suspension concentrate (SC) containing 20 g/l abamectin for use on vegetables grown indoors. This formulation was not the representative formulation in the original EU review of abamectin.

B.9.1 Effects on birds

The notifier has submitted an acute toxicity study with the formulation A12115I. This study has been summarised and evaluated below.

B.9.1.3 Acute toxicity of the formulation (IIIA 10.1.6)

Characteristics

reference	:	[redacted] (2011)	species	:	Bobwhite quail (*Colinus virginianus*)
type of study	:	Acute toxicity study	exposure duration	:	14 days
year of execution	:	2011	nominal conc.	:	2000 mg A12115I/kg
GLP statement	:	Yes	dosing method	:	Oral by gavage
guideline	:	OECD 223	acceptability	:	Acceptable
test substance	:	A12115I (abamectin:)	LD50	:	>2000 mg A12115I/kg bw
purity	:	1.74% w/w abamectin, corresponding to 20.6 g/L			

Methods

Test Substance: A12115I

Test species : Colinus virginianus

Sex, Weight, age : mixed sex, 186-240 g, approximately 38 weeks old

Number of animals : one control group of 5 birds, one test group of 5 birds

Applied concentrations : one control, 2000 mg A12115I /kg body weight;

Type of application : oral by gavage using corn oil

Time of exposure : one single application, monitoring during 14 days

Results

There were no mortalities in the control group and all birds in the control group were normal in appearance and behaviour for the duration of the test. There were no mortalities in the treatment group and no regurgitation was observed after dosing.

Conclusion

LD_{50} (*Colinus virginianus*) > 2000 mg A12115I/kg bw

NOEL (*Colinus virginianus*) = 2000 mg A12115I/kg bw

Guidelines and limitations

The study was performed according to OECD 223 and is acceptable.

B.9.1.4 Summary of toxicity data

Acute, short-term and (sub-)chronic dietary toxicity data

An overview of avian toxicity data for abamectin and the formulation A12115I is given in Table B.9.1.4-01 and -02.

Table B.9.1.4-01: EU Conclusions - Toxicity to birds of abamectin

Study	Test species	EU agreed endpoints (EFSA Scientific report No. 147 (2008))	Proposed endpoint[1]
Acute toxicity	*Anas platyrhynchos*	LD_{50} : ≤ 77 mg/kg/bw/day	LD_{50} : 26 mg/kg bw/day
Dietary toxicity (short-term)	*Anas platyrhynchos*	LC_{50} : 48.6 mg/kg/bw/day	-
Reproductive toxicity (long-term)	*Anas platyrhynchos* (males)	NOEC: 1.33 mg/kg/bw/day	-

[1] Since Annex I submission new calculations have been performed and as a result there is a new endpoint which is agreed by the EU experts and used in the risk assessment.

Table B.9.1.4-02: Summary of avian toxicity endpoints for A12115I

Study type	Test substance	Species	Endpoint	Value
Acute oral toxicity	A12115I	Northern Bobwhite *(Colinus virginianus)*	14 d LD_{50}	>2000 mg A12115I/kg bw

B.9.1.5 Risk assessment for birds

Since the application is indoors, no exposure of birds through consumption of residues on food items is expected. Exposure is possible by surface water after emission of the active substance from indoors to the surface water. Furthermore secondary poisoning by consuming fish is a possible route of exposure.

B.9.1.5.1 Exposure via drinking water (surface water)

The risk from exposure through drinking surface water is calculated for a small bird with body weight 10 g and a DWI (daily water intake) of 2.7 g/d. Surface water concentrations are calculated in section B.8. The PECsw is very low: < 0.0001 µg/L. Hence, a low risk is expected via this route.

B.9.1.5.2 Indirect exposure via contaminated fish

The concentration in fish is calculated according to the Guidance Document on risk assessment for birds and mammals (2009) as:

PEC_{fish} = 21-days TWA-PEC_{SW} x BCF.
Because the PECsw is very low (< 0.0001 µg/L) a low risk is expected via this route.

B.9.2 Effects on aquatic organisms

The notifier has submitted acute toxicity studies with the formulation A12115I for fish, invertebrates and algae. These studies have been summarised and evaluated below.

B.9.2.1.3 Acute toxicity of the formulated product (IIIA 10.2.2)

IIIA, 10.2.2.1/01

Characteristics

reference	:	[redacted] (2011)	species	:	Rainbow trout (*Oncorhynchus mykiss*)
type of study	:	Acute toxicity study	exposure duration	:	96 hours
year of execution	:	2011	nominal concn.	:	0.13, 0.28, 0.60, 1.3 and 2.8 mg product/L
GLP statement	:	Yes	dosing method	:	Static
guideline	:	EEC C.1, OECD 203	acceptability	:	Acceptable
test substance	:	A12115I	96-h LC50	:	0.337 mg product/L (mm) (= 5.86 µg a.s./L (mm))
a.s. content	:	1.74 % w/w abamectin, corresponding to 20.6 g/L, containing 0.03% w/wB1b and 1.71% w/w B1a	96-h NOEC		0.23 mg product/L (mm)

Methods

A 96-hour acute toxicity test in juvenile rainbow trout (*Oncorhynchus mykiss*) (1 replicate of 7 fish for each test concentration, not fed for 48 hours prior to testing) was conducted under static conditions with A12115I formulation (1.74% abamectin) at a nominal test concentration of 0.13, 0.28, 0.60, 1.3 and 2.8 mg product/L. A concentrated stock solution of nominal 100 mg/L was freshly prepared by mixing 100.1 mg of the test item into one litre of test water using stirring for 15 minutes at room temperature. This stock solution was used to prepare an intermediate stock solution of 10 mg/L and the two highest test concentrations. The second stock solution of 10 mg/L was used to prepare the three lowest test media. The test included an untreated control, tested also in 1 replicate of 7 fish. The loading rate was 0.53 g fish wet weight per litre test medium. Thus, the guideline requirement of a loading rate not exceeding 0.8 g fish/L was fulfilled. The test duration was 96 hours and the fish were not fed during the test. The concentrations of abamectin B1a were determined in samples of all test solutions taken at the start and end of exposure.

Results

The measured concentrations of the active ingredient abamectin B1a of the formulation A12115I in the test media of all test concentrations were between 80 and 105% of the nominal values at the start of the test. After the respective exposure time (until all fish were dead) the concentrations of abamectin B1a were between 53 and 96% of the nominal values.
The reported biological results were based on the nominal concentrations of the test item A12115I. In the control and at the lowest test concentration of 0.13 mg/L, all fish survived until the end of the test

and no visible abnormalities were observed in the test fish. At the next higher test concentration of 0.28 mg/L, all fish showed one or two visible abnormalities. However, at the end of the test all fish survived at this test concentration. At the three highest test concentrations of 0.60, 1.3 and 2.8 mg/L, all fish were dead before test end. The 96-hour NOEC (mortality) of A12115I to rainbow trout was determined to be 0.28 mg/L (nominal). The 96-hour LOEC was 0.60 mg/L (nominal).
The 96-hour LC50 of A12115I was calculated to be 0.41 mg/L (nominal) with a 95% confidence interval of 0.28-0.60 mg/L. Thus, the concentration-effect relationship was rather steep.
The pH value in the test media and in the control was 7.1. The oxygen concentration was at least 9.3 mg/L, corresponding to 88% oxygen saturation. The water temperature was 13-14 °C during the test.

Conclusions

The biological results can be summarized as follows (on the basis of nominal concentrations of the test item):

- 96 h LC50: 0.41 mg product/L
- 96 h NOEC: 0.28 mg product/L

Evaluation by RMS

The endpoints as derived by the authors are based on nominal concentrations. However, the concentration of the active substance is lower than 80% in the course of the test. Hence, the endpoints should be based on geometric mean concentrations. The RMS has recalculated the endpoints based on measured concentrations. See the table below:

nom	t=0	t=1	t=3	t=4	geomean	
ug a.s./L	ug a.s./L	ug a.s./L	ug a.s./L	ug a.s./L	ug a.s./L	mortality
2.22	2.33			1.55	1.90	0/7
4.79	4.93			4.59	4.76	0/7
10.3	9.54		5.45		7.21	7/7
22.2	22.2	18.2			20.1	7/7
47.9	38.2	36.2			37,2	7/7

The probit analysis is based on the yellow marked data (the rest is not relevant). The endpoint is:
- 96 h LC50: 5.86 µg a.s./L (= 337 µg formulation/L). The NOEC based on mean measured concentrations is 0.23 mg product/L (= 4.0 µg a.s./L).

IIIA, 10.2.2.2/01

Characteristics

reference	:	Höger, S. (1997)	species	:	*Daphnia magna*
type of study	:	Acute toxicity study	exposure duration	:	48 hours
year of execution	:	2010	nominal concn.	:	0.25, 0.80, 2.5, 8.0, 25 and 80 µg product/L
GLP statement	:	Yes	dosing method	:	Static
guideline	:	EEC C.2, OECD 202	acceptability	:	Acceptable
test substance	:	A12115I	48 h-EC50	:	0.477 µg product/L (mm) (= 0.00759 µg a.s./L (mm))
a.s. content	:	1.59 % w/w abamectin,			

	corresponding to 19.0 g/L, containing 0.03% w/wB1b and 1.56% w/w B1a

Methods

A 48-hour acute toxicity test in *Daphnia magna* (<24 h old, 4 replicates of five daphnia each for each of the treatments and the control) was conducted under static conditions with A12115I formulation (1.59% abamectin) at nominal test concentrations of 0.25, 0.80, 2.5, 8.0, 25 and 80 µg product/L. The test included an untreated control. The concentration of the active ingredient abamectin B1a was analyzed in the duplicate test media samples of all concentrations from both sampling times (0 and 48 hours). The second active ingredient abamectin B1b which is present in the formulation at a fixed concentration ratio relative to the first active ingredient was not analyzed.

Results

Water quality parameters (pH, oxygen concentration and temperature) were in accordance with the OECD 202 guideline. No mortality/immobility and signs of intoxication were observed in the control.

The measured concentrations of A12115I (based on the active ingredient abamectin B1a) in the test media of the test concentrations of 0.80 to 25 µg/L were between 69 and 105% of the nominal values at the start of the test (see analytical results and Table 2 in Appendix 3). During the test period of 48 hours, a decrease of test item concentration occurred in these test media. At the end of the test, the measured values were between $<LOQ_{bio}$ (=0.00594 µg/L) and 14% of nominal.

At the lowest and highest test concentrations (nominal 0.25 and 80 µg/L, respectively) the measurements were $<LOQ_{bio}$ at the start of the test and $<LOQ_{bio}$ and 7% at the end of the test, respectively. The low recoveries for the highest tested concentrations are considered to be the result of a sampling mistake. Both concentrations were not essential for the calculation of the EC values and were excluded from the statistical analysis.

During the first 24 hours of the test no immobilized test organisms were determined in the control. At the two lowest test item concentrations 10% of test organisms were found to be immobile at the observation after 24 hours. This low immobilization rate was estimated as a significant toxic effect of the test item because abamectin is known to cause flat concentration-effect relationships in *Daphnia magna.* At the next higher initial measured test concentration of 2.1 µg/L (nominal 2.5 µg/L), 70% of daphnids were immobile. From the initial measured concentration of 5.5 µg/L (nominal 8.0 µg/L) onwards, all test organisms were found to be immobile after 24 hours.

The 24-hour EC50 of the test item was calculated to be 1.7 µg/L with 95% confidence limits of 1.2 and 2.7 µg/L.

After 48 hours of exposure no immobilized test organisms were determined in the control. At the two lowest test concentrations, the immobilization remained at the level of 24 hours. At the initial measured concentration of 2.1 µg/L (nominal of 2.5 µg/L), the immobilization increased to 90%.

The 48-hour EC_{50} was calculated to be 1.2 µg/L with 95% confidence limits of 0.86 and 1.6 µg/L (based on initial measured test concentrations). The 48-hour NOEC of A12115I could not be

determined, since an immobilization of 10% was observed at the lowest test concentration of 0.25 µg/L (based on the nominal test item concentration).

Conclusions

The biological results can be summarized as follows (on the basis of initial measured concentrations of the test item):

- 48 h EC50: 1.2 µg product/L.
- 48 h NOEC: n.d.

Evaluation by RMS

The endpoints as derived by the authors are based on nominal concentrations. However, the concentration of the active substance is lower than 80% in the course of the test. Hence, the endpoint should be based on geometric mean concentrations. The RMS has recalculated the endpoint based on measured concentrations. See the table below:

nom ug a.s./L	t=0 ug a.s./L	t=2 ug a.s./L	% afname	% immobility	geomean ug a.s./L
0.0039	<LOQ*	**0.00297****		10	n.a.
0.0125	0.01	**0.00297****	70	10 µg	0.00545
0.039	0.0324	**0.00297****	91	90	0.00981
0.125	0.0867	0.0134	85	100	0.0341
0.39	0.411	0.0553	87	100	0.15076
1.25	<LOQ*	0.093		100	

* lack of measurement because of a sampling mistake is not essential

** the values in bold < LOQ, put on 0.5LOQ

The probit analysis is based on the yellow marked data (the rest is not relevant). The endpoint is:

- 48-h EC50: 0.00759 µg a.s./L (= 0.477 µg formulation/L).

IIIA, 10.2.2.3/03

Characteristics

reference	:	Liedtke, A. (2011)	species	:	*Pseudokirchneriella subcapitata*
type of study	:	Algae growth inhibition	exposure duration	:	96 hours
year of execution	:	2011	nominal concn.	:	6.25, 12.5, 25. 50 and 100 mg product/L
GLP statement	:	Yes	dosing method	:	Static
guideline	:	EEC C.3, OECD 203	acceptability	:	Acceptable
test substance	:	A12115I	ErC50, EbC50, EyC50		>45 mg product/L (mm) (>0.781 mg a.s./L (mm))
purity	:	1.74 % w/w abamectin, corresponding to 20.6 g/L, containing 0.03% w/w B1b and 1.71% w/w B1a			

Methods

A 96-hour toxicity test on green algae (*Pseudokirchneriella subcapitata*) (treatment and the untreated controls each containing 5 x 10^3 cells/mL at the start) was conducted with A12115I formulation (1.74% abamectin) at nominal test concentrations 6.25, 12.5, 25. 50 and 100 mg product/L. The test solutions were prepared by adding an aliquot of a stock solution in test medium to the test water. Small volumes of all test concentrations and the control were taken from all test flasks after 24, 48, 72 and 96 hours of exposure. The algal cell densities in these samples were determined by counting with an electronic particle counter. In addition, after 96 hours exposure, a sample was taken from the control and from a test concentration with reduced algal growth (nominal 100 mg A12115I/L). The shape of the algal cells was examined microscopically in these samples.
The pH was measured at the start and at the end of the test in each test concentration and the control. The water temperature was measured daily in a flask incubated under the same conditions as the test flasks.
The test concentrations were verified by chemical analysis of abamectin B1a at 0 and 96 hours, using high performance liquid chromatography.

Results

The concentrations of the active ingredient abamectin B1a of the formulation A12115I in the test media of the two highest test concentrations of 50 and 100 mg/L were 83 and 94% of nominal respectively, measured at the start of the test. At the end of the test the values found at these concentrations were below LOQana (<0.0202 mg/L) and 28% of nominal, respectively. Since no toxic effect of the test item A12115I on the algal growth was observed up to the highest test concentration, and the correct dosage was confirmed at the two highest concentrations, the reported biological results were related to nominal concentrations of the test item A12115I.
The 72-hour and 96-hour EbC50, EyC50 and ErC50 values could not be determined because of the absence of a significant inhibitory effect.
There were no abnormalities, observed microscopically, in the control or 100 mg/L test culture at 96 hours.
At the start of the test, the pH measured in the treatments was between 8.1 and 8.3. At the end of the test, pH values between 8.7 and 8.8 were measured. The water temperature during the test was between 21 and 22 °C.

Conclusion

Based on nominal concentrations of the formulation the 72 and 96-hour ErC50, EyC50 and EbC50 were >100 mg product/L, the highest concentration tested.

Guidelines and limitations

The algal biomass in the control increased by a factor of 100 over 72 hours (must be least 16).
The mean coefficient of variation of the daily growth rates in the control cultures was 12.9%

over 96 hours (must be ≤ 35%). The coefficient of variation of average specific growth rates during the whole test period in replicate control cultures was 2.5 % (must be <7%). Hence, all validity criteria were met.

Evaluation by RMS

The endpoints as derived by the authors are based on nominal concentrations. However, the concentration of the active substance is lower than 80% in the course of the test. Hence, the endpoint should be based on geometric mean concentrations. The RMS has recalculated the endpoint based on measured concentrations. See the table below:

nom	t=0	t=4	t=3	% immobility	0-4 d	0-3 d
mg a.s./L	mg a.s./L	mg a.s./L	mg a.s./L		mg a.s./L	
0.107	n.a.					
0.214	n.a.					
0.428	n.a.					
0.855	n.a.					
1.71	1.61	0.234	0.3790		0.61	0.781

Only measured data are available for days 0 and 4. The concentration on day 3 is estimated based on exponential degradation. Results:

- EC50 > 0.781 mg a.s./L (= >45 mg formulation/L).

B.9.2.4 Summary of aquatic toxicity data

An overview of avian toxicity data for abamectin is given in Table B.9.2.4-01 (active substance and metabolites) and -02 (formulation A12115I).

Table B.9.2.4-01: EU Conclusions - Toxicity to aquatic organisms of abamectin, metabolite NOA 427011, metabolite NOA 448112 and metabolite NOA 426289

Test substance	Test species	Test Type/Duration	EU agreed endpoints (EFSA Scientific report No. 147 (2008)) µg as/L
Acute toxicity to fish			
abamectin	*Oncorhynchus mykiss*	96 hr (static)	Mortality, LC_{50}: 3.6 (nom)
abamectin	*Oncorhynchus mykiss*	96 hr (flow-through; modified exposure test)	Mortality, LC_{50}: 8.7 (nom)
Vertimec 018EC	*Oncorhynchus mykiss*	96 hr (flow-through)	Mortality, LC_{50}: 2.3 (nom)
8,9-Z-avermectin B_{1a} (NOA 427011)	*Oncorhynchus mykiss*	96 hr (flow-through)	Mortality, LC_{50}: 5.1 (mm)
8a-	*Oncorhynchus mykiss*	96 hr (semi-static)	Mortality, LC_{50}: 520 (mm)

Test substance	Test species	Test Type/Duration	EU agreed endpoints (EFSA Scientific report No. 147 (2008)) µg as/L
hydroxyavermectin B_{1a} (NOA 448112)			
Chronic toxicity to fish			
abamectin	*Oncorhynchus mykiss*	28 day (flow-through)	NOEC: 0.52 (mm)
Acute toxicity to aquatic invertebrates			
abamectin	*Daphnia pulex*	48 h (static)	Mortality, EC_{50}: 0.12 (mm)
Vertimec 018 EC	*Daphnia magna*	48 h (flow-through)	Mortality, EC_{50}: 0.59 (mm)
8,9-*Z*]-avermectin B_{1a} (NOA 427011)	*Daphnia magna*	48 h (static)	Mortality, EC_{50}: 0.082 (mm)
8a-hydroxyavermectin B_{1a} (NOA 448112)	*Daphnia magna*	48 h (static)	Mortality, EC_{50}: 1.6 (mm)
4”-oxoavermectin B_{1a} (NOA 426289)	*Daphnia magna*	48 h (static)	Mortality, EC_{50}: 0.28 (nom)
Chronic toxicity to aquatic invertebrates			
abamectin	*Daphnia magna*	21 day (flow-through)	Reproduction NOEC: 0.010 (nom)
Toxicity to algae			
Vertimec 018 EC	*Pseudokirchneriella subcapitata*	72 h (static)	Biomass: E_bC_{50}: >1590 (nom) Growth rate: E_rC_{50}: >1590 (nom)
8,9-*Z*]-avermectin B_{1a} (NOA 427011)	*Pseudokirchneriella subcapitata*	72 h (static)	Biomass: E_bC_{50}: >9000 (nom) Growth rate: E_rC_{50}: >9000 (nom)
8a-hydroxyavermectin B_{1a} (NOA 448112)	*Pseudokirchneriella subcapitata*	72 h (static)	Biomass: E_bC_{50}: >6100 (mm) Growth rate: E_rC_{50}: >6100 (mm)
Toxicity to Higher Plants			
Insecticide - Not applicable 14 d EC_{50}: 3900			
Microcosm or mesocosm tests			
NOEC: 1st study: 0.2 µg as/L, nominal concentration after single application, recirculation; NOEAEC value not possible to derive. **2nd study**: 0.015 µg as/L; NOEAEC = 0.049 µg as/L, nominal concentration after three applications, no recirculation. It should be noted that Daphnids did not have sufficient abundance for statistical analysis and that it is thus not known whether at the NOEAEC-level Daphnids will be able to recover from possible			“It was concluded by the meeting that the NOEC from this study (0.2 µg a.s./L) can be used for risk assessment of ditches and streams. The experts recommended a safety factor of 2 to address uncertainty with regard to differences in species

Test substance	Test species	Test Type/Duration	EU agreed endpoints (EFSA Scientific report No. 147 (2008)) µg as/L
effects. The similarity between the chronic *Daphnia* NOEC and the EAC (SF of 3 on the NOEAEC value) indicates however that *Daphnia* will probably not be affected. The risk of metabolite [8,9-Z]-avermectin B_{1a} to aquatic invertebrates is considered to be covered by the mesocosm studies. The risk to fish needs to be addressed.			sensitivity. Frequency of application should not be of influence on the NOEC-value and the endpoint of the study with one application can also be used for the risk assessment of uses with three applications. The NOEAEC of 0.049 µg a.s./L from the study with a conventional static system (3 applications of the test substance) together with a safety factor of 3 was agreed upon and should be used for ponds and the glasshouse applications."

* Since Annex I submission/inclusion new studies/calculations for the active substance have been performed and as a result there are new end-points which are used in the risk assessment.
Nom: Nominal
mm : mean measured concentrations

Table B.9.2.4-02: Endpoints for formulation A12115I

Test substance	Test species	Test Type/Duration	Endpoint µg as/L
A12115I	*Oncorhynchus mykiss*	96 hr (static)	LC50: 5.86 (mean measured)
A12115I	*Daphnia magna*	48 h (static)	EC50: 0.00759 (mean measured)
A12115I	*Pseudokirchneriella subcapitata*	72 h (static)	EC50 > 0.781 (mean measured)

B.9.2.5 Risk assessment for aquatic organisms

The exposure to surface water is very low: The PECsw < 0.0001 µg/L. The lowest endpoint available is the endpoint of the formulation A12115I for *Daphnia magna*: EC50 = 0.00759 µg as/L. With a safety factor of 100 the first tier RAC = 0.0000759 µg/L. The TER is then > 0.759, possibly below 1. The higher tier RAC from the mesocosm study is 0.049 µg/L with a safety factor of 3 is 0.016 µg/L. This is much higher than the PECsw. Hence, there is an acceptable risk for aquatic organisms.
The risk of metabolites which are formed in water are covered by the risk assessment of the parent.

B.9.3 Effects on other terrestrial vertebrates

An overview of mammalian toxicity data for abamectin and the formulation A12115I is given in Table B.9.3.1-01 and -02.

Table B.9.3.1-01: EU Conclusions - Toxicity to mammals of abamectin

Study	Test species	EU agreed endpoints (EFSA Scientific report No. 147 (2008))
Acute toxicity	Rat	LD50 = 8.7 mg/kg bw
Long-term toxicity	Rat	NOEC = 0.12 mg/kg bw/d

Table B.9.3.1-02: Toxiciy to mammals of formulation A12115I

Study	Test species	EU agreed endpoints (EFSA Scientific report No. 147 (2008))
Acute toxicity	Rat	LD50 = 1086 mg A12115I /kg bw

B.9.1.5 Risk assessment

Since the application is indoors, no exposure of mammals through consumption of residues on food items is expected. Exposure is possible by surface water after emission of the active substance from indoors to the surface water. Furthermore secondary poisoning by consuming fish is a possible route of exposure.

B.9.1.5.1 Exposure via drinking water

The risk from exposure through drinking from surface water is calculated for a small mammal with body weight 10 g and a DWI (daily water intake) of 1.57 g/d. Surface water concentrations are calculated in section B.8. The PECsw is very low: < 0.0001 µg/L. Hence, a low risk is expected via this route.

B.9.1.5.2 Indirect exposure via contaminated fish

The concentration in fish is calculated according to the Guidance Document on risk assessment for birds and mammals (2009) as:

PEC_{fish} = 21-days TWA-PEC_{SW} x BCF.

Because the PECsw is very low (< 0.0001 µg/L) a low risk is expected via this route.

B.9.4 Effects on bees (IIA 8.7, IIIA 10.4)

B.9.4.1 Toxicity

The notifier has submitted an acute oral and contact toxicity study for bees with the formulation A12115I. This study has been summarised and evaluated below.

B.9.4.1.2 Acute toxicity of the formulated product (IIIA 10.4.2.1 and 10.4.2.2)

IIIA 10.4.2.1/01 & IIIA 10.4.2.2/01

Characteristics

reference	:	Kling, A. (2000e)	dose		Nominal: Oral and contact: 0.4, 0.8, 1.5, 3 and 6 ng a.s./bee; Actual: 0.44, 0.90, 1.74, 3.42 and 6.82 ng a.s./bee
type of study	:	Acute oral and contact toxicity	species	:	Honey bee (*apis mellifera*)
year of execution	:	2011	exposure duration	:	72 hours: oral toxicity test; 96 hours: contact toxicity test
GLP statement	:	Yes	dosing method	:	Contact: application solution in tap water; Oral: mixture with 50% sucrose solution
guideline	:	OECD 213 and OECD 214	acceptability	:	Acceptable
test substance	:	A12115I	LD50 (oral)	:	5.28 ng a.s./bee
a.s. content	:	1.74 % w/w abamectin, corresponding to 20.6 g/L	LD50 (contact)	:	1.98 ng a.s./bee

Methods

A 96-hour acute oral and contact toxicity test on honeybees (*Apis mellifera*) was conducted with A12115I formulation (1.74% abamectin). In the oral and contact toxicity test 5 replicates of ten bees each per concentration were exposed to a nominal dose of 0.4, 0.8, 1.5, 3 and 6 ng a.s./bee (actual: 0.44, 0.90, 1.74, 3.42 and 6.82 ng a.s./bee), with control (50% aqueous sucrose solution) and positive control (dimethoate, 0.06, 0.08, 0.11 and 0.15 µg a.s./bee in the oral toxicity test and 0.10, 0.15, 0.23 and 0.34 µg a.s./bee in the contact toxicity test).

Results

In the control group of the oral toxicity test fed with 50 % aqueous sucrose solution a mortality of 4.0 % had occurred during the 72 hours observation period. In the water treated control group of the contact toxicity test a mortality of 2.0 % was observed at the final assessment after 96 hours.

The 24-hour oral and contact LD50 values for the reference item were 0.12 and 0.16 µg dimethoate/bee, respectively. Consequently, validity criteria for both control and reference item mortality were met and the test was deemed valid.

In the oral toxicity test, up to the highest nominal dose level of 6 ng a.i./bee of A12115I (actual tested dose: 6.82 ng a.i./bee of A12115I) a maximum mortality of 72.0 % (corrected: 70.8 %) was observed after 72 hours.

In the contact toxicity test, a maximum mortality of 100 % (corrected 100 %) had occurred at

the highest tested dose level of 6 ng a.i./bee of A12115I during the 96 h observation period. The 72-hour oral LD50 value for A12115I is 5.28 ng a.i./bee and the 96-hour contact LD50 is 1.98 ng a.i./bee, based on actual concentrations.

In the oral toxicity test sublethal effects (apathetic and affected bees) were mainly observed at the assessments 24 and 48 hours after start of feeding in the two highest dose levels. At the final assessment 72 hours aftrer feeding no remarkable sublethal effects were observed.

In the contact toxicity test severe and long lasting sublethal effects (affected, apathetic, cramped and moribund bees) were observed in all test item treatment levels throughout the whole observation period but mainly at the assemments 4, 24 and 48 hours after application. At the 72 and 96-h assessments some bees still showed sublethal effects in the dose levels 1.5 and 3 ng a.i./bee of A12115I.

Table B.9.4.1.2-01 Oral toxicity of A12115I to honey bees

nominal dose (ng a.s./bee)	consumed dose (ng a.s./bee)	% mortality 24h	% mortality 48h	% mortality 72h
control	control	4	4	4
0.4	0.44	2	2	2
0.8	0.90	4	4	4
1.5	1.74	0	0	0
3	3.42	4	14	20
6	6.82	30	64	72

Table B.9.4.1.2-02 Contact toxicity of A12115I to honey bees

dose (ng a.s./bee)	% mortality 24h	% mortality 48h	% mortality 72h	% mortality 96h
control	0	0	0	2
0.4	2	4	4	6
0.8	2	8	10	14
1.5	10	16	30	40
3	6	48	58	62
6	26	94	96	100

Conclusion

The 72-hour oral LD50 value for A12115I is 5.28 ng a.i./bee and the 96-hour contact LD50 is 1.98 ng a.i./bee, based on actual concentrations.

Guidelines & Limitations

The study is acceptable.

B.9.4.2 Risk assessment for bees

The exposure of bees is predominantly through contact and oral ingestion of the residues on crops. The proposed soil drip use indoors will result in negligible exposure to bees. Hence, the risk to bees is considered to be acceptable.

B.9.5 Effects on arthropods other than bees

No additional data for the formulation A12115I regarding arthropods other than bees has been submitted.

The notifier argues that the proposed indoor drip use will result in negligible exposure through overspray and through contact with residues on plants. Hence, the risk for foliar non-target arthropods is negligible and only the risk to soil dwelling non-target arthropods is relevant. The RMS can agree with this argument.
Below the risk by exposure to soil is assessed.

B.9.5.1. Summary of toxicity data

Only the toxicity data to soil dwelling arthropods are relevant. For abamectin only toxicity data for the beetle *Poecilus cupreus* are available (see table below).

Table B.9.5.1-1: Toxicity of abamectin to *Poecilus cupreus*

Species	Test Type	Vertimec 018EC Treatment Rates	Endpoints
Poecilus cupreus (Ground beetle)	Laboratory, inert sand substrate	1.2 - 58 g a.s./ha	0% mortality at all rates LR_{50}>58 g a.s./ha
	Lab/semi-field	2 x 5.4 g a.s./ha 2 x 27 g a.s./ha	<50% corrected mortality after 2nd application <50% effect on fecundity after 2nd application

B.9.5.2 Risk assessment other non-target arthropods

A12115I is applied at a maximum rate of 100 g as/ha. This dose rate must be multiplied with the MAF. For soil residues, a DT50 of 0.65 days is used. That means that there will be no build-up of the active substance between the applications. Hence the MAF is assumed to be 1.

The LR50 for *Poecilus cupreus* is >58 g as/ha. That means that it is not clear if at a the dose of 100 gas/ha there will be effects >50% on this species or not. Also the available extended lab test has been done with too low dose rates. Hence, it is not clear yet if the risk for *Poecilus cupreus* is acceptable. Furthermore it is not considered sufficient to test only one soil species, which is also known as fairly insensitive, when the use is directly on soil. A test on *Folsomia candida* or *Hypoaspis aculeifer* is required.

Not in all Member States the soil in glasshouses is considered as a natural soil with a natural soil community. E.g. in The Netherlands no risk assessment is performed for soil organisms regarding glasshouse uses, because management practice includes regular sterilisation of the soil, which prevents the formation of a natural soil organism community within glasshouses. Hence, the requirement mentioned above can be considered as a requirement on Member State level.

B.9.6 Effects on earthworms and other soil non-target macro-organisms

B.9.6.1 Toxicity

The notifier has submitted an acute toxicity study with earthworms with the formulation A12115I. This study has been summarised and evaluated below.

B.9.6.2 Acute toxicity to earthworms of the formulated product (IIIA 10.6.2)

Characteristics

reference	:	Friedrich, S. (2011)		:	
type of study	:	Acute toxicity	species	:	*Eisenia foetida*
year of execution	:	2011	exposure duration	:	14 days
GLP statement	:	Yes	nominal conc.	:	1.0, 3.2, 10, 32 and 100 mg as/kg soil dw
Guideline	:	OECD 207 (1984), ISO 11268-1, EC C.8	dosing method	:	Soil was treated with solution of test substance in acetone mixed with quartz sand
test substance	:	A12115I (1.74% w/w, corresponding to 20.6 g/L)	LC50	:	>100 mg as/kg soil d.w. (10% organic matter)

In an acute toxicity study, earthworms (*Eisenia fetida*, adults, weight in range 301-460 mg) were exposed to the formulation A12115I for 14 days in artificial soil (OECD 207 substrate, with 10% organic matter). The test was conducted in 1 L glass jars covered with glass lids allowing ventilation, containing 556 g d.w. artificial soil. Treatment rates were 1.0, 3.2, 10, 32 and 100 mg as/kg soil d.w.. Each treatment and the controls were tested in 4 replicates of 10 worms each. Test soils were prepared by mixing the artificial soil with quartz sand treated with 1 mL of a solution of the test substance in acetone. The control soil was mixed with quartz sand. Jars were maintained at 20±2°C under continuous light (720 lux). Soil moisture was in the range 34.8 - 35% of soil dry weight at the start and the end of the test. The soil pH was 5.9 at the start of the test and 6.13 at the end. A reference product (2-chloroacetamide) was tested in a separate test <1 year earlier and gave an acceptable result.

Results

Test results are summarised in the table below. The results of the control group satisfied the guideline requirements (OECD 207, 1984): ≤10% mortality. The 14-day LC50 was >100 mg/kg soil d.w..

Table B.9.6.1-01 Acute toxicity to *Eisenia fetida*

Nominal concentration	Mortality at 14 days	Mean biomass change

(mg/kg soil d.w.)	(%)	over14 days (%)
Control	0	-6.5
1.0	0	-9.6
3.2	0	-11.4
10	0	-15.7*
32	2.5	-19.2*
100	5.0	-27.1*

* statistically significant difference from control at 5% level.

Conclusion

14-day LC50 >100 mg as/kg soil d.w. (soil with 10% organic matter). NOEC = 3.2 mg as/kg d.w.

Guidelines and limitations

The study was performed according to OECD 207 (1984) and is acceptable.

B.9.6.3 Risk assessment for earthworms

B.9.6.3.1 Summary of toxicity data

Acute earthworm studies have been performed with abamectin, the formulation A12115I and metabolites [8,9-Z]-avermectin B_{1a} (NOA 427011) and 8a-hydroxy-avermectin B_{1a} (NOA 448112). A sub-lethal test was performed with the formulation Vertimec 0.18 EC. For A12115I no chronic test is available.The results of the studies are summarised in Table B.9.6.3.1-1 and B.9.6.3.1-2.

Table B.9.6.3.1-1. Summary of earthworm toxicity studies with (formulations of) abamectin

Test substance	Species	Soil type	OM	T	Duration	Criterion	Value
			[%]	[°C]	[d]		[mg as/kg]
abamectin	*Eisenia fetida*	artificial	10	25	28	14-d LC_{50}	33
abamectin	*Eisenia fetida*	artificial	10	25	28	14-d LC_{50}	> 55
A12115I	*Eisenia fetida*	artificial	10	20	14	LC_{50}	> 100
Vertimec 0.18 EC	*Eisenia fetida*	artificial	10	20	56	NOEC	≥ 0.72

Table B.9.6.3.1-2. Summary of earthworm toxicity studies with metabolites of abamectin

Test substance	Species	Soil type	OM	T	Duration	Criterion	Value
			[%]	[°C]	[d]		[mg/kg]
NOA 427011	*Eisenia fetida*	artificial	10	20	14	LC_{50}	50
NOA 448112	*Eisenia fetida*	artificial	10	20	14	LC_{50}	321

The two studies with abamectin were 28-day studies that were not performed according to OECD 207 or equivalent guidelines. The mortality figures after 14 days have been used to estimate the LC_{50}. Actual concentrations were measured in the second study with abamectin. The results indicate that degradation in artificial soil is slower than in natural soil with 62 - 72 % of nominal present after 28 days at 25 °C. The toxicity of abamectin in the second study was less than expected on the basis of the first study where an LC_{50} of 33 mg/kg was found. Degradation and a delay in burrowing time may have caused differences in actual exposure between studies. Abamectin caused a delay in burrowing time at levels of 23 mg/kg (nominal) and higher, a similar effect was observed for the [8,9-Z]-isomer, where burrowing was delayed at 12 mg/kg and higher.

Risk assessment

Abamectin

The acute risk assessment for earthworms is based on the highest initial PEC_S of 0.136 mg/kg (see Section B.8.3). Because the log K_{OW} of abamectin is > 2, the toxicity values are corrected to the default OM content of 5 % for agricultural soil. The lowest LC_{50} is 33 mg/kg at 10 % OM, equivalent to 16.5 mg/kg at 5 % OM. The resulting TER is 121, which is higher than the trigger of 10 and an acute risk is not expected.
The long-term NOEC is ≥ 0.72 mg/kg at 10 % OM, which is equivalent to ≥ 0.36 mg/kg at 5 % OM. At the highest initial PEC_S (0.136 mg/kg soil), the TER is **≥2.6**, indicating that there is a possible long-term risk for earthworms. This possible risk should be addressed by the notifier.

Not in all Member States the soil in glasshouses is considered as a natural soil with a natural soil community. E.g. in The Netherlands no risk assessment is performed for soil organisms regarding glasshouse uses, because management practice includes regular sterilisation of the soil, which prevents the formation of a natural soil organism community within glasshouses. Hence, the requirement mentioned above can be considered as a requirement on Member State level.

Metabolites

For NOA 427011 and NOA448112, no PEC_S was calculated. As a worst case, the maximum initial PEC_S of abamectin can be used for risk assessment. The LC_{50} for NOA 427011 is 50 mg/kg at 10 % OM, equivalent to 25 mg/kg at 5 %. The resulting TER is 184, indicating a low risk for earthworms.
The LC50 for NOA 448112 is 321 mg/kg at 10 % OM, equivalent to 161 mg/kg at 5 % OM. The TER is 1184, indicating a low risk.
For the other metabolites, NOA 448111, NOA 457464 and NOA 457465, no toxicity data are available. In view of the LC_{50} for NOA 448112, it can be assumed that toxicity of these metabolites will also not be higher than that of the parent. With PEC_S for these compound being lower than the PEC_S of NOA 448112, a risk is not expected and further information is not considered necessary.

B.9.7 Effects on soil micro-organisms

B.9.7.1 Summary of effect data

Nitrogen mineralisation	**abamectin**: < 25 % effect after 28 days at 0.347 mg/kg (equivalent to 216 g as/ha at 5 cm depth assuming soil bulk density 1500 kg/m^3)

	NOA 427011 ([8,9-Z]-avermectin B_{1a}): < 25 % effect after 28 days at 0.40 mg/kg **NOA 448112 (8a-hydroxy-avermectin B_{1a})**: < 25 % effect after 28 days at 0.66 mg/kg
Carbon mineralisation	**abamectin**: < 25 % effect after 28 days at 0.347 mg/kg (equivalent to 216 g as/ha at 5 cm depth assuming soil bulk density 1500 kg/m^3) **NOA 427011 ([8,9-Z]-avermectin B_{1a})**: < 25 % effect after 28 days at 0.40 mg/kg **NOA 448112 (8a-hydroxy-avermectin B_{1a})**: < 25 % effect after 28 days at 0.66 mg/kg

B.9.7.2 Risk assessment for soil micro-organisms

Abamectin was found to have no unacceptable effects (< 25% compared to the control group) on soil nitrification at up to 0.347 mg product/kg. This concentration is 2.5 times higher than the maximum soil PEC of 0.136 mg a.s./kg, indicating an acceptable level of risk; no further evaluation is considered necessary.

For NOA 427011 and NOA448112, no PEC_S was calculated. As a worst case, the maximum initial PEC_S of abamectin can be used for risk assessment.
Metabolite NOA 427011 was found to have no unacceptable effects (< 25% compared to the control group) on soil nitrification at up to 0.40 mg a.s./kg, respectively. This concentration is nearly 3 times higher than the maximum soil PEC of 0.136 mg a.s./kg for the parent, indicating an acceptable level of risk.

Metabolite NOA448112 was found to have no unacceptable effects (< 25% compared to the control group) on soil nitrification at up to 0.66 mg a.s./kg, respectively. This concentration is nearly 5 times higher than the maximum soil PEC of 0.136 mg a.s./kg for the parent, indicating an acceptable level of risk.

Although the toxicity of soil metabolites NOA448111, NOA457464 and NOA457465 to soil micro-organisms has not been measured, the soil PECs were lower than for NOA448112 and exposure to the soil of these metabolites of abamectin will clearly be far lower than that for the parent due to their formation percentages.
Furthermore, although the toxicity of these metabolites to soil microorganisms has not been measured, the toxicity to aquatic organisms of those investigated is significantly lower than that of abamectin (see Point IIIA 10.2) and it is very unusual that the toxicity of a metabolite is more than ten times greater than that of the parent. On this basis, the risk from metabolites NOA448111, NOA457464 and NOA457465 is also considered to be acceptable.

B.9.8 Effects on non-target plants

B.9.8.1 Summary of toxicity data

herbicide profiling test	**Vertimec 0.18 EC**: seedling emergence: no effect on maize, wild oat, onion, sugar beet, oilseed rape and soybean at 50.6 g as/ha vegetative vigour: no effect on of maize, wild oat, onion, sugar beet and oilseed rape at 50.6 g as/ha slight effect on vegetative vigour of soybean at 25.3 and 50.6 g as/ha (rating 8.5 and 8 out of 9)

B.9.8.2 Risk assessment for terrestrial non-target plants

Effects on non-target plants are of concern in the off-field environment, where they may be exposed to drift. The proposed uses of A12115I as an indoor soil drip will result in negligible exposure to the off-field environment. Hence, the risk to terrestrial non-target plants is acceptable.

B.9.9 Effects on biological methods of sewage treatment (Annex IIA 8.7)

Emission of abamectin to sewage treatment plants may occur following the proposed use of A12115I in glasshouses. Based on the results of the activated sludge respiration test, with EC_{20}, EC_{50} and EC_{80} > 100 mg/L, no effect on biological methods of sewage treatment are expected.

B.9.10 References relied on

Author(s)	Annex point/ reference number	Year	Title Sponsor/Source Test Facility, Report No Indication of the reason not submitted Published or not Syngenta File No.	Data Protection Claimed Y/N	Owner (SYN = Syngenta)
KIIIA1 10.1.6 / 01	[redacted]	2011	Abamectin SC (A12115I) - An acute oral toxicity study with the northern bobwhite using a sequential testing procedure Syngenta [redacted] GLP, not published Syngenta File No A12115I_10034	Y	SYN
KIIIA1 10.2.2.1 / 01	[redacted]	2011	Abamectin SC (A12115I) - Acute toxicity to rainbow trout (Oncorhynchus mykiss) in a 96-hour test Syngenta [redacted] GLP, not published Syngenta File No A12115I_10041	Y	SYN
KIIIA1 10.2.2.2 / 01	Hoger S	2010	Abamectin SC (A12115I) - Acute toxicity to Daphnia magna in a 48-hour immobilization test Syngenta - Jealott's Hill, Bracknell, United Kingdom Harlan Laboratories Ltd., Zelgliweg 1, 4452 Itingen, Switzerland, C86663 GLP, not published Syngenta File No A12115I_10025	Y	SYN
KIIIA1 10.2.2.3 / 01	Liedtke A.	2011a	Abamectin SC (A12115I) - Toxicity to Pseudokirchneriella subcapitata in a 96-hour algal growth inhibition test Syngenta Harlan Laboratories Ltd., Itingen, Switzerland, D36398 GLP, not published Syngenta File No A12115I_10038	Y	SYN

KIIIA1 10.2.3 / 01	Rufli H.	1999	Assessment of the potential biological effects of Abamectin (MK936, 018 EC) (A-8612 A) exposures on aquatic ecosystems as measured in an outdoor microcosm tank system Novartis Crop Protection AG, Basel, Switzerland Novartis Crop Protection AG, Basel, Switzerland, 982570 GLP, not published Syngenta File No MK936/0638	Y	SYN
KIIIA1 10.2.3 / 02	Knauer K.	2002	Assessment of the Effects of Abamectin 018 EC (A8612A) in Outdoor Microcosms Syngenta Crop Protection AG, Basel, Switzerland Syngenta Crop Protection AG, Basel, Switzerland, 2002590 GLP, not published Syngenta File No MK936/0817	Y	SYN
KIIIA1 10.3.2.1 / 01	[redacted]	2009	Abamectin SC (A12115I) - Acute Oral Toxicity Study in the Rat (Up and Down Procedure) Syngenta - Jealott's Hill, Bracknell, United Kingdom [redacted] GLP, not published Syngenta File No A12115I_10020	Y	SYN
KIIIA1 10.4.2.1 / 01	Kling A.	2011	Abamectin SC (A12115I) - Acute oral and contact toxicity to the honeybee Apis mellifera L. in the laboratory Syngenta Eurofins Agroscience Services GmbH, Niefern-Öschel., Germany, S11-02876 GLP, not published Syngenta File No A12115I_10035	Y	SYN

KIIIA1 10.5.2 / 01	Fussell S.	2004	MK936 (abamectin): a rate-response extended laboratory test to determine the effects of an 18 g/L EC formulation (A8612AB) on the parasitic wasp Aphidius rhopalosiphi Syngenta Crop Protection AG, Basel, Switzerland Mambo-Tox. Ltd., Southampton, United Kingdom, SYN-04-1 2032631 GLP, not published Syngenta File No MK936/1105	Y	SYN
KIIIA1 10.5.2 / 02	Waterman L.	2004	MK936 (abamectin): A rate-response extended laboratory test to determine the effects of an 18 g/L EC formulation (A8612AB) on the predatory mite Typhlodromus pyri Syngenta Crop Protection AG, Basel, Switzerland Mambo-Tox. Ltd., Southampton, United Kingdom, SYN-04-2 GLP, not published Syngenta File No MK936/1106	Y	SYN
KIIIA1 10.5.2 / 03	Reber B.	1999	Acute toxicity of MK 936 EC 018 (A-8612 A) to the predatory ground beetle Poecilus cupreus L. (Coleoptera: carabidae) Novartis Crop Protection AG, Basel, Switzerland Novartis Crop Protection AG, Basel, Switzerland, 982611 GLP, not published Syngenta File No MK936/0626	Y	SYN