

# **European Commission**



**Addendum**  
**VOLUME 3 – Annex B (A12115I)**

**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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April 2015	Initial version
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## 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.

The product A12115I is intended for the use as a nematicide as a soil drip (soilbound application) for the control of root-knot nematodes (*Meloidogyne* spp.) in tomato, eggplant, pepper, cucurbits - edible peel (cucumber, zucchini, etc), cucurbits - inedible peel (melon, watermelon, squash) and green beans. These representative uses are in protected cropping systems of walk-in tunnels. The product may only be used in walk-in tunnels which remain in place for at least 5 years.

### 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

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reference	: Hubbard et al. (2011)	species	: Bobwhite quail ( <i>Colinus virginianus</i> )
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		

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##### 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<sub>50</sub> (*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 <sup>1</sup>
Acute toxicity	<i>Anas platyrhynchos</i>	LD <sub>50</sub> : ≤ 77 mg/kg/bw/day	LD <sub>50</sub> : 26 mg/kg bw/day
Dietary toxicity (short-term)	<i>Anas platyrhynchos</i>	LC <sub>50</sub> : 48.6 mg/kg/bw/day	-
Reproductive toxicity (long-term)	<i>Anas platyrhynchos</i> (males)	NOEC: 1.33 mg/kg/bw/day	-

<sup>1</sup> 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 ( <i>Colinus virginianus</i> )	14 d LD <sub>50</sub>	>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 PEC<sub>sw</sub> 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:

$$PEC_{\text{fish}} = 21\text{-days TWA-}PEC_{\text{SW}} \times \text{BCF}.$$

Because the PEC<sub>sw</sub> 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	: Liedtke, A. (2011)	species	: Rainbow trout ( <i>Oncorhynchus mykiss</i> )
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	mortality
ug a.s./L	ug a.s./L	ug a.s./L	ug a.s./L	ug a.s./L	ug a.s./L	
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).

## Characteristics

reference	: Höger, S. (2010)	species	: <i>Daphnia magna</i>
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/w B1b 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 <math>\text{LOQ}\_{\text{bio}}</math> (=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 <math>\text{LOQ}\_{\text{bio}}</math> at the start of the test and <math>\text{LOQ}\_{\text{bio}}</math> 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<sub>50</sub> 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	t=0	t=2	%	%	
ug a.s./L	ug a.s./L	ug a.s./L	afname	immobility	geomean
					ug a.s./L
0.0039	<LOQ*	<b>0.00297**</b>		10	n.a.
0.0125	0.01	<b>0.00297**</b>	70	10	0.00545
0.039	0.0324	<b>0.00297**</b>	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	: <i>Pseudokirchneriella subcapitata</i>
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		

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## Methods

A 96-hour toxicity test on green algae (*Pseudokirchneriella subcapitata*) (treatment and the untreated controls each containing  $5 \times 10^3$  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  $\leq 35\%$ ). 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 mg a.s./L	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 aquatic 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
<b>Acute toxicity to fish</b>			
abamectin	<i>Oncorhynchus mykiss</i>	96 hr (static)	Mortality, LC <sub>50</sub> : 3.6 (nom)
abamectin	<i>Oncorhynchus mykiss</i>	96 hr (flow-through; modified exposure test)	Mortality, LC <sub>50</sub> : 8.7 (nom)

Test substance	Test species	Test Type/Duration	EU agreed endpoints (EFSA Scientific report No. 147 (2008))  µg as/L
Vertimec 018EC	<i>Oncorhynchus mykiss</i>	96 hr (flow-through)	Mortality, LC <sub>50</sub> : 2.3 (nom)
8,9-Z-avermectin B <sub>1a</sub> (NOA 427011)	<i>Oncorhynchus mykiss</i>	96 hr (flow-through)	Mortality, LC <sub>50</sub> : 5.1 (mm)
8a-hydroxyavermectin B <sub>1a</sub> (NOA 448112)	<i>Oncorhynchus mykiss</i>	96 hr (semi-static)	Mortality, LC <sub>50</sub> : 520 (mm)
<b>Chronic toxicity to fish</b>			
abamectin	<i>Oncorhynchus mykiss</i>	28 day (flow-through)	NOEC: 0.52 (mm)
<b>Acute toxicity to aquatic invertebrates</b>			
abamectin	<i>Daphnia pulex</i>	48 h (static)	Mortality, EC <sub>50</sub> : 0.12 (mm)
Vertimec 018 EC	<i>Daphnia magna</i>	48 h (flow-through)	Mortality, EC <sub>50</sub> : 0.59 (mm)
8,9-Z]-avermectin B <sub>1a</sub> (NOA 427011)	<i>Daphnia magna</i>	48 h (static)	Mortality, EC <sub>50</sub> : 0.082 (mm)
8a-hydroxyavermectin B <sub>1a</sub> (NOA 448112)	<i>Daphnia magna</i>	48 h (static)	Mortality, EC <sub>50</sub> : 1.6 (mm)
4"-oxoavermectin B <sub>1a</sub> (NOA 426289)	<i>Daphnia magna</i>	48 h (static)	Mortality, EC <sub>50</sub> : 0.28 (nom)
<b>Chronic toxicity to aquatic invertebrates</b>			
abamectin	<i>Daphnia magna</i>	21 day (flow-through)	Reproduction NOEC: 0.010 (nom)
<b>Toxicity to algae</b>			
Vertimec 018 EC	<i>Pseudokirchneriella subcapitata</i>	72 h (static)	Biomass: E <sub>b</sub> C <sub>50</sub> : >1590 (nom) Growth rate: E <sub>r</sub> C <sub>50</sub> : >1590 (nom)
8,9-Z]-avermectin B <sub>1a</sub> (NOA 427011)	<i>Pseudokirchneriella subcapitata</i>	72 h (static)	Biomass: E <sub>b</sub> C <sub>50</sub> : >9000 (nom) Growth rate: E <sub>r</sub> C <sub>50</sub> : >9000 (nom)
8a-hydroxyavermectin B <sub>1a</sub> (NOA 448112)	<i>Pseudokirchneriella subcapitata</i>	72 h (static)	Biomass: E <sub>b</sub> C <sub>50</sub> : >6100 (mm) Growth rate: E <sub>r</sub> C <sub>50</sub> : >6100 (mm)
<b>Toxicity to Higher Plants</b>			
<b>Insecticide - Not applicable 14 d EC<sub>50</sub>: 3900</b>			
<b>Microcosm or mesocosm tests</b>			
<b>NOEC: 1st study:</b> 0.2 µg as/L, nominal concentration after single application, recirculation; NOEAEC value not possible to derive. <b>2nd study:</b> 0.015 µg as/L; NOEAEC = 0.049 µg as/L, nominal		"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	

Test substance	Test species	Test Type/Duration	EU agreed endpoints (EFSA Scientific report No. 147 (2008))  µg as/L
		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 effects. The similarity between the chronic <i>Daphnia</i> NOEC and the EAC (SF of 3 on the NOEAEC value) indicates however that <i>Daphnia</i> will probably not be affected. The risk of metabolite [8,9-Z]-avermectin B <sub>1a</sub> to aquatic invertebrates is considered to be covered by the mesocosm studies. The risk to fish needs to be addressed.	streams. The experts recommended a safety factor of 2 to address uncertainty with regard to differences in species 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: aquatic organisms**

Test substance	Test species	Test Type/Duration	Endpoint  µg as/L
A12115I	<i>Oncorhynchus mykiss</i>	96 hr (static)	LC50: 5.86 (mean measured)
A12115I	<i>Daphnia magna</i>	48 h (static)	EC50: 0.00759 (mean measured)
A12115I	<i>Pseudokirchneriella subcapitata</i>	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 PEC<sub>sw</sub> < 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 0.016 µg/L, based on the NOEAEC of 0.049 µg/L with a safety factor of 3. This is much higher than the PEC<sub>sw</sub>. 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.

During the peer review EFSA set a data requirement to the applicant to provide PEC<sub>sw</sub> at step 3 and possibly step 4 for the soil metabolites NOA448111, NOA448112, NOA457464 and NOA457465 and present a consequent aquatic risk assessment.

Below the response of the applicant is given.

Syngenta acknowledge EFSA's request for new surface water modeling and consequent risk assessment based on revised soil DT<sub>50</sub> data for the soil metabolites.

Syngenta note that RMS has emphasized that the current use extension is a product assessment and not a substance assessment. Furthermore this issue was already addressed in the addendum on confirmatory data by showing the much lower surface water exposure of the metabolites compared to the parent. A STEP 3 assessment using the available drainage scenarios would be expected to show that the PEC of the soil metabolites in surface water will also be negligible (as is already shown for groundwater). This is based on the comparable sorption and degradation endpoints of the metabolites in combination with a lower "dose rate" compared to the parent.

For clarity, RMS has copied the relevant part of the confirmatory data addendum (final version April 2012) in the Fate and Behaviour part of the Addendum (see Addendum Volume 3 - Annex B (A12115I), B.8, February 2016).

From the calculations (see B.8) it appears that the PEC<sub>sw</sub> values of the soil metabolites are two orders of magnitude lower than respective abamectin PEC<sub>sw</sub>. Although the currently proposed GAP differs from the proposed uses at the original approval, a similar difference in exposure level between parent and metabolites can be expected. Hence, even when it is assumed that the toxicity of the metabolites is ten times higher than the parent, the risk from the soil metabolites to aquatic organisms is acceptable.

### 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: Toxicity 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 PEC<sub>sw</sub> 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:

$$PEC_{\text{fish}} = 21\text{-days TWA-}PEC_{\text{SW}} \times \text{BCF.}$$

Because the PEC<sub>sw</sub> 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 ( <i>apis mellifera</i> )
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 after 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 assessments 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
<i>Poecilus cupreus</i> (Ground beetle)	Laboratory, inert sand substrate	1.2 - 58 g a.s./ha	0% mortality at all rates LR <sub>50</sub> >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 2 <sup>nd</sup> application <50% effect on fecundity after 2 <sup>nd</sup> 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.

During the peer review EFSA set a data requirement to submit information to address the risk to non-target arthropods, especially to provide information on the toxicity of abamectin to *Folsomia candida* and *Hypoaspis aculeifer*.

The notifier has submitted these studies and they are summarised and evaluated below.

Study title : Abamectin SC (A13796I) – A laboratory test to determine the effects of fresh residues on the springtail *Folsomia candida* (Collembola, Isotomidae).

### Characteristics

reference	McCormac, A	species	<i>Folsomia candida</i>
type of study	Laboratory reproduction toxicity study	exposure duration	28 days
year of execution	2014	nom. conc.	189, 105, 58.3, 32.4, 18, 10, 5.6 and 3.1 mg test item/kg soil dry weight.
GLP statement	Yes	dosing method	Test substance was dissolved in water and mixed into the soil
guideline	OECD, No. 232 (2009)	acceptability	Acceptable
test substance	Abamectin SC (A13796I)- batch SMU1IP001	NOEC	18 mg test item/kg soil dw (0.31 mg a.s./kg soil dw), (5 % peat)
a.s. content	nominal: 18.0 g/L, measured 1.73 %ww (17.8 g/L)		

EC50 = 42.7 mg test item/kg soil dw (5 % CL 32.2-58.2), equivalent to 0.74 mg a.s./kg soil dw  
 NOEC is 18 mg test item/kg soil d.w., equivalent to 0.31 mg a.s./kg soil d.w.. (5% peat).

### Methods

In a 28-day reproduction study, 11 day old springtails (*Folsomia candida*) were exposed to Abamectin SC (A13796I)- batch SMU1IP001 (18g abamectin)/L 28 days in artificial soil prepared according to OECD 232 with 5% peat. A preliminary range-finding bioassay was carried out with 5 concentrations of the test item and a control. This was followed by a definitive bioassay at eight

concentrations, equivalent to 189, 105, 58.3, 32.4, 18, 10, 5.6 and 3.1 mg test item/kg soil dry weight. These variants were compared to a control treatment of purified water and a toxic reference treatment of treatment of Betosip 114 (nominally 114 g/L phenmedipham), applied at a concentration of 200 mg product/kg soil dry weight. The test substance was dissolved in water and mixed into the soil. The test arenas were glass jars (approximately 125 mL capacity and 4.5 cm in diameter), secured with a close-fitting lid containing 30 g of moist soil. For the range-finding test, there were two replicate arenas per treatment, each set up with 10 springtails (i.e. a total of 20 springtails per treatment).

For the definitive test, there were eight replicate arenas for the control treatment, five for the toxic reference treatment and four for each of test-item treatments, each set up with 10 springtails (i.e. a total of 40-80 springtails per treatment).

Containers were maintained at 19.9-21.4°C under a 12 h light regime (400-800 lux). Soil moisture was maintained at 50% of MWHC. The pH was 6.19 - 6.29 at the start and 5.49 - 5.84 at 28 days, when adult survival and the number of juveniles were assessed.

## Results

In the toxic reference treatment, 30% mortality (16% corrected mortality) was recorded. This result did not differ significantly from the control (Fisher's Exact Test,  $\alpha = 0.05$ ). The mean number of progeny produced per replicate was 7, a value that differed significantly from the control (t-test for independent samples,  $\alpha = 0.05$ ).

Other results are summarised in the table below. The validity criteria for the control group satisfied the guideline requirements:  $\leq 20\%$  adult mortality,  $\geq 100$  instar juveniles per test vessel, CV for reproduction  $\leq 30\%$ . For assessments of mortality, the NOEC was 189 mg test item/kg soil dry weight. The 28-day LC50 > 189 mg test item/kg soil dry weight, the maximum concentration tested. The most sensitive test endpoint, reproduction, as measured by the number of offspring, was significantly reduced at 32.4 mg test item/kg and above. The NOEC is 18 mg test item/kg soil d.w., equivalent to 0.31 mg a.s./kg soil d.w..

Table B.9.7-01 Effects of A137961 on *Folsomia candida*

Treatment	Concentration (mg test item/kg soil dry wt.)	% mortality at 28 DAT <sup>a)</sup>	Corrected % mortality at 28 DAT <sup>b)</sup>	Mean no. progeny per replicate <sup>c)</sup>	% change in numbers of progeny, relative to control <sup>d)</sup>
Control	-	16	-	511	-
A13796I	189.0	30	16	90 *	82
	105.0	5	0	185 *	64
	58.3	20	4	229 *	55
	32.4	20	4	235 *	54
	18.0	13	0	373	27
	10.0	10	0	424	17
	5.6	13	0	479	6
	3.1	10	0	467	9
Toxic ref.	-	30	16	7 *	99

Results of a Probit regression analysis on the numbers of progeny to determine EC<sub>x</sub> values, with their 95% confidence limits <sup>e)</sup>.

EC <sub>50</sub> value (and 95% CL)	mg test item/kg soil	42.7	(32.2 and 58.2)
EC <sub>20</sub> value (and 95% CL)	mg test item/kg soil	9.8	(5.7 and 14.2)
EC <sub>10</sub> value (and 95% CL)	mg test item/kg soil	4.6	(2.1 and 7.5)

- a) Mortality amongst the springtails originally introduced. Individual test-item treatments were compared to the control using Fisher's Exact Test ( $\alpha = 0.05$ ), but none differed significantly.
- b) Calculated using Abbott's formula.
- c) Fecundity data from test-item treatments were compared by one-way ANOVA and Dunnett's t-test ( $\alpha = 0.05$ ) or by Mann-Whitney U-test ( $\alpha = 0.05$ ). The toxic reference was compared to the control by t-test for unmatched pairs ( $\alpha = 0.05$ ), due to inequality of variances. Values marked with an asterisk (\*) differed significantly from the control.
- d) A positive value indicates a decrease in reproduction, relative to the control.
- e) Only data from what was considered to be the active part of the response curve were used for analysis. Therefore, the results from concentration of 3.1 mg/kg soil were excluded.

## Conclusion

EC<sub>10</sub> = 4.6 mg test item/kg soil dw (5 % CL 2.1-7.5), equivalent to 0.08 mg a.s./kg soil dw

EC<sub>50</sub> = 42.7 mg test item/kg soil dw (5 % CL 32.2-58.2), equivalent to 0.74 mg a.s./kg soil dw

NOEC is 18 mg test item/kg soil d.w., equivalent to 0.31 mg a.s./kg soil d.w.. (5% peat).

The study was conducted in agreement with OECD 232 and is valid without restrictions.

Study Title : Abamectin SC (A13796I) – A laboratory test to determine the effects of fresh residues on the predatory mite *Hypoaspis aculeifer* (Acari, Laelapidae)

## Characteristics

reference	Vinall, S	species	<i>Hypoaspis aculeifer</i>
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type of study	Laboratory reproduction toxicity study	exposure duration	14 days
year of execution	2014	nom. conc.	1000, 555.6, 308.6, 171.5 and 95.3 mg test item/kg soil dry weight.
GLP statement	Yes	dosing method	Test substance was dissolved in water and mixed into the soil
guideline	OECD 226	acceptability	Acceptable
test substance	Abamectin SC (A13796I)- batch SMU1IP001	NOEC	1000 mg test item/kg soil d.w. (17.3 mg a.s./kg soil d.w.), soil with 5% organic matter
a.s. content	nominal: 18.0 g/L, measured 1.73 %ww (17.8 g/L)		

## Methods

In a 14-day reproduction study, soil mites (*Hypoaspis aculeifer*) were exposed to 1.73% w/w (18 g/L) Abamectin SC (A13796I) at 1000, 555.6, 308.6, 171.5 and 95.3 mg test item/kg soil dry weight. for 14 days in artificial soil prepared according to OECD 226 (5% organic matter).

The mites were aged 35 days after start of egg laying (approximately 7-14 days from becoming adult). A range finding test was conducted before the definitive test.

The test substance was dissolved in water and mixed into the soil. The control was treated with water only. A test with toxic reference treatment of Perfekthion (nominally 400 g/L dimethoate), applied at a concentration of 32.07 mg product/kg soil dry weight (12 mg a.s./kg soil dry weight) was conducted simultaneously.

The test was conducted in glass containers (four for each treatment and eight for the control), covered with screw-top lids with a hole for ventilation and covered by nylon netting to prevent escape and containing 20 g d.w. treated soil and 10 mites. Containers were maintained at 18-22°C under a 16 h light regime (400-800 lux). Soil moisture was maintained at 50% of MWHC. The pH was 6.2 at the start and 4.94-5.35 at 14 days, when adult survival and the number of juveniles were assessed using an extraction method.

## Results

The positive control gave the expected response (>50% reduction of offspring compared to the control. Other results are summarised in the table below. The validity criteria for the test satisfied the guideline requirements (OECD 226, 2008): ≤20% adult mortality, ≥50 juveniles per test vessel, CV for reproduction ≤30%. None of the differences between the test item treatments and the control were statistically significant at the 5% level. The NOEC is 1000 mg test item/kg soil d.w., equivalent to 17.3 mg a.s./kg soil d.w..

Table B.9.7-04 Effects of A13796I on *Hypoaspis aculeifer*

Treatment	Concentration (mg test item/ kg soil dry wt.)	% mortality at 14 DAT <sup>a)</sup>	Corrected % mortality at 14 DAT <sup>b)</sup>	Mean no. progeny per replicate <sup>c)</sup>	% change in numbers of progeny, relative to control <sup>d)</sup>
Control	-	0	-	302	-
A13796I	1000.0	5	5	309	-2
	555.6	3	3	287	5
	308.6	0	0	323	-7
	171.5	3	3	303	0
	95.3	3	3	323	-7
Toxic ref.	-	100 *	100	6 *	98

- a) Mortality amongst mites originally introduced. Individual test-item treatments compared to the control using Fisher's Exact Test ( $\alpha = 0.05$ ). Treatments that differed significantly from the control are indicated with an asterisk (\*).
- b) Calculated using Abbott's formula.
- c) Fecundity assessment data from individual treatments were compared to the data from the control by one-way ANOVA and Dunnett's t-test ( $\alpha = 0.05$ ). Values marked with an asterisk (\*) differed significantly from the control.
- d) A positive value indicates a decrease and a negative value an increase in reproduction, relative to the control.

### Conclusion

NOEC 1000 mg test item/kg soil d.w., equivalent to 17.3 mg a.s./kg soil d.w. (soil with 5% organic matter).

The study was conducted in agreement with OECD 226 and is valid without restrictions.

Hence, the endpoints from the studies are the following:

Species	Endpoint
<i>Folsomia candida</i>	EC10 = 4.6 mg A13796I/kg dw soil (0.08 mg ai/kg dw soil) (28 days reproduction) NOEC = 18 mg A13796I/kg dw soil (0.31 mg ai/kg dw soil) (28 days reproduction)
<i>Hypoaspis aculeifer</i>	NOEC = 1000 mg A13796I/kg dw soil (17.3 mg ai/kg dw soil) (14 days reproduction)

In the technical report "Outcome of the pesticides peer review meeting on general recurring issues in ecotoxicology, published 22 December 2015 the following was concluded with respect to the use of an ECx or NOEC value in the risk assessment: Regarding the use of ECX in the risk assessment, the experts agreed that where a reliable median EC10 could be calculated, then the lower between this value and the NOEC should be used (unless a recent guidance document explicitly indicates a

preference - i.e. currently only EFSA PPR Panel (2013b)). Hence, in the risk assessment below the EC10 value from the test with *Folsomia candida* of 0.08 mg ai/kg soil will be used.

In the same technical report the following was concluded with respect to the correction factor of 2 for studies with soil organisms: Overall, it was agreed to retain the factor of 2 for all the first tier studies, when relevant (i.e. log Pow of >2). However, it was acknowledged that a correction factor of 2 may not be totally appropriate for studies when the level of OM is lowered. In these cases a smaller correction factor could be more appropriate. However, currently there is lack of evidence on how much the increase in bioavailability would be or whether the relationship would be linear.

It was noted that the application of the correction factor of 2 may not be totally appropriate for collembolan and soil mite studies where 5% OM is used in the test guideline. However, in the absence of any better data, it was agreed that the factor of 2 should be applied. It was also mentioned that agricultural soils in the EU normally have an OM content below 5%.

Hence, a correction factor of 2 will be applied on the endpoint values of the studies (LogPow > 2).

The TER values for the two soil species are presented below:

Test Organism	NOEC <sub>corr</sub> (mg/kg soil)	Abamectin PEC <sub>soil</sub> (mg/kg)	TER <sub>LT</sub>	Trigger value
<i>Folsomia candida</i>	0.04	0.136	0.3	5
<i>Hypoaspis aculeifer</i>	8.7	0.136	64	

The TER value for *Hypoaspis aculeifer* is above the trigger value of 5 and the risk is acceptable.

However, the TER value for *Folsomia candida* is below the trigger value of and hence a risk cannot be excluded for this species.

The notifier submitted the following argument: “at the NOEC 0.31 mg/kg there were no effects on adult mortality and 50% reduction in fecundity. As a result it is considered that there are likely to be negligible population level effects on *Folsomia* under field conditions. As a result, it is considered that the long term risk is acceptable according to the proposed use pattern.”

The RMS does not agree with this argument and is of the opinion that a risk cannot be excluded, because the TER, based on the EC10 value, is far below the relevant trigger value. Hence, the risk to *Folsomia candida* must be addressed.

However, not in all Member States the soil in protected systems 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. Therefore this issue may be considered as a Member State specific issue.

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

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 (mg/kg soil d.w.)	Mortality at 14 days (%)	Mean biomass change over 14 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<sub>1a</sub> (NOA 427011) and 8a-hydroxy-avermectin B<sub>1a</sub> (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
				[%]			
abamectin	<i>Eisenia fetida</i>	artificial	10	25	28	14-d LC <sub>50</sub>	33
abamectin	<i>Eisenia fetida</i>	artificial	10	25	28	14-d LC <sub>50</sub>	> 55
A12115I	<i>Eisenia fetida</i>	artificial	10	20	14	LC <sub>50</sub>	> 100
Vertimec 0.18 EC	<i>Eisenia fetida</i>	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
				[%]			
NOA 427011	<i>Eisenia fetida</i>	artificial	10	20	14	LC <sub>50</sub>	50
NOA 448112	<i>Eisenia fetida</i>	artificial	10	20	14	LC <sub>50</sub>	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<sub>50</sub>. 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<sub>50</sub> 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<sub>S</sub> of 0.136 mg/kg (see Section B.8.3). Because the log K<sub>OW</sub> of abamectin is > 2, the toxicity values are corrected to the default OM content of 5 % for agricultural soil. The lowest LC<sub>50</sub> 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  $\geq 0.72$  mg/kg at 10 % OM, which is equivalent to  $\geq 0.36$  mg/kg at 5 % OM. At the highest initial PEC<sub>S</sub> (0.136 mg/kg soil), the TER is  $\geq 2.6$ , indicating that there is a possible long-term risk for earthworms. This possible risk should be addressed by the notifier.

With regard to the chronic risk of the active substance the notifier submitted the following statement: "The long-term TER value for earthworms is below the trigger value of 5, however the endpoint used  $>0.36$  mg/kg is the highest rate tested in the long term study. No effects on body weight, reproduction and behaviour were observed at this rate. As a result, it is considered that little or no significant long term risk to earthworm populations."

#### Reaction RMS:

The RMS considers this statement not sufficient to address the long-term risk from abamectin to earthworms. Hence, further data are necessary.

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, requirement mentioned above can be considered as a requirement on Member State level.

#### Metabolites

For NOA 427011 and NOA448112, no PEC<sub>S</sub> was calculated. As a worst case, the maximum initial PEC<sub>S</sub> of abamectin can be used for risk assessment. The LC<sub>50</sub> 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 LC<sub>50</sub> 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<sub>50</sub> for NOA 448112, it can be assumed that toxicity of these metabolites will also not be higher than that of the parent. With PEC<sub>S</sub> for these compound being lower than the PEC<sub>S</sub> of NOA 448112, a risk is not expected and further information is not considered necessary.

During the peer review, EFSA set the following data requirement:

"Applicant to provide data to address the risk for soil metabolites of abamectin. The chronic risk for the soil metabolite NOA448112 should be addressed as well. As present data are not enough to demonstrate a low chronic risk for the active substance abamectin, further information to address this risk should be provided."

The notifier responded by saying that EFSA's request is acknowledged and that the RMS has performed indicative calculations for the 4 metabolites using the agreed endpoints and the worst-case application scheme (assuming two consecutive crop cycles with a minimum interval between the

cycles) of ten applications with an interval of 10 days. The parent dose rate was corrected for max observed % and relative molar weight for each metabolite. This results in the following PECsoil values:

Metabolite	Dose rate metabolites (g a.s./ha)	DT <sub>50</sub> (days)	PECsoil (mg/kg)
NOA448111	10	50.6	0.078
NOA448112	16	75.4	0.146
NOA457464	10	99.0	0.099
NOA457465	10	173.0	0.112

For all metabolites except NOA448112 this results in PEC values lower than the PEC for the parent substance (0.136 mg/kg).

The applicant submitted the following risk assessment:

For risk assessment an acute toxicity 10 times higher than the parent is assumed for a screening assessment, in case that no toxicity values are available. For TER values see table below.

Test substance	LC <sub>50</sub> * (mg/kg)	PEC <sub>soil</sub> (mg/kg)	TER <sub>A</sub> **	Trigger value
NOA448111	1.65	0.078	10.6	10
NOA448112	321	0.146	1100	
NOA457464	1.65	0.099	8.3	
NOA457465	1.65	0.112	7.4	

\* where no toxicity data available - 1/10 of parent value 16.5 mg/kg used

\*\* LC<sub>50</sub> reduced by a factor of 2

For earthworms the acute TERs for the metabolites NOA448111 and NOA448112 are above the relevant trigger values of 10 indicating a low risk to earthworms. For metabolites NOA457464 and NOA457465 the acute TERs are below the trigger.

The chronic risk for the soil metabolite NOA448112 was assessed using the a toxicity endpoint derived from the acute endpoint for NOA448112 divided by the acute to chronic ratio of the parent (x 45.8).

Test substance	NOEC (mg/kg)	PECsoil (mg/kg)	TERLT*	Trigger value
NOA448112	6.98	0.146	24	5

\* NOEC reduced by a factor of 2

For earthworms the chronic TER for the metabolite NOA448112 is above the relevant trigger values of 5 indicating a low risk to earthworms.

#### Reaction RMS

RMS can agree with the risk assessment of the notifier. With regard to the chronic endpoint for the metabolite NOA448112 there is some uncertainty, because it is an extrapolated value based on the

acute to chronic ratio of the parent. However, there is quite some margin of safety (about a factor of 5). Hence, the RMS can agree with the conclusion that there is a low chronic risk to earthworms from this metabolite.

Looking at the acute risk assessment the TER for the metabolites NOA457464 and NOA457465 is below the trigger. However, the TER is not far below the trigger of 10 and the risk assessment is worst-case (assumption that the metabolites are 10 times more toxic than the parent and a correction factor of 2 on the LC50 values). Therefore, the risk is considered to be acceptable.

However, from the data submitted for other soil macro-organisms (see B.9.5.2), it appears that *Folsomia candida* is considerably more sensitive for abamectin than earthworms. Hence, it must be shown that the risk from the soil metabolites to *Folsomia candida* is acceptable.

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.9.7 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<sup>3</sup>)

**NOA 427011 ([8,9-Z]-avermectin B<sub>1a</sub>):**  
< 25 % effect after 28 days at 0.40 mg/kg

**NOA 448112 (8a-hydroxy-avermectin B<sub>1a</sub>):**  
< 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<sup>3</sup>)

**NOA 427011 ([8,9-Z]-avermectin B<sub>1a</sub>):**  
< 25 % effect after 28 days at 0.40 mg/kg

**NOA 448112 (8a-hydroxy-avermectin B<sub>1a</sub>):**  
< 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.

During the peer review EFSA set the following data requirement: "Applicant to provide data to address the risk to soil micro-organisms for soil metabolites of abamectin."

The notifier responded with the following statement: "Syngenta acknowledge EFSA's request. With regard to the soil metabolites, NOA 448111, NOA 457464 and NOA 457465, no additional 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 PECS for these compounds being lower than the PECS of NOA 448112, a risk is not expected and further information is not considered necessary."

**Reaction RMS:**

RMS can agree with the statement of the notifier and the risk of the soil metabolites to soil micro-organisms is 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

- Literature search

A literature search on the active substance abamectin and its metabolites was submitted by the notifier.

The following STN databases were selected for the search:

MEDLINE  
EMBASE  
EMBAL  
ESBIOBASE  
AGRICOLA  
BIOSIS  
CABA  
CAPLUS  
FSTA  
FROSTI  
GEOREF  
TOXCENTER

PQSCITECH  
PASCAL  
SCISEARCH  
ANABST

Searches were limited to 2004 onwards.

Search results regarding ecotoxicology are presented below. The searches were conducted on the 3rd December 2013.

#### Reporting/Overview of the search process for Ecotoxicological studies

Data requirement(s) captured in the search	Number (2004-2013)
Total number of <i>summary records</i> retrieved after <i>all*</i> searches of peer-reviewed literature (excluding duplicates)	129
Number of <i>summary records</i> excluded from the search results after rapid assessment of title and/or abstract for relevance	127

\*both from bibliographic databases and other sources of peer-reviewed literature

#### Ecotoxicological studies considered relevant by the notifier:

Authors	Title	Source
Cabrera, J. A.; Menjivar, R. D.; Dababat, A. E. A.; Sikora, R. A.	Properties and nematicide performance of avermectins.	Journal of Phytopathology (2013), Volume 161, Number 2, pp. 65-69.
Sun YingJian; Diao XiaoPing; Zhang Qidi; Shen JianZhong; Sun, Y. J.; Diao, X. P.; Zhang, Q.; Shen, J. Z.	Bioaccumulation and elimination of avermectin B1a in the earthworms ( <i>Eisenia fetida</i> ).	Chemosphere (2005), Volume 60, Number 5, pp. 699-704.

The notifier did not submit a list of all captured studies. Also the two selected studies above were not submitted and no summary and evaluation of the two studies is available.

Furthermore the notifier did not provide the criteria considered for relevancy of studies.

Hence, based on the available data, it is not possible to check the selection process as performed by the notifier and also it is not possible to use the results of the two selected studies in the risk assessment.

The work done by the notifier regarding this issue is considered insufficient.

## Ecotoxicology data

Annex point	Author	Year	Title Source (where different from company) Company, Report No. GLP or GEP status (where relevant) Published or Unpublished	Data protection claimed Y/N	Owner (SYN = Syngenta)
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	Arcelin G.	2009	Abamectin SC (A12115I) - Acute Oral Toxicity Study in the Rat (Up and Down Procedure) Syngenta - Jealott's Hill, Bracknell, United Kingdom RCC Ltd., Füllinsdorf, Switzerland, C31684 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 <i>Apis mellifera</i> L. in the laboratory Syngenta Eurofins Agrosience Services GmbH, Niefem-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 <i>Aphidius rhopalosiphii</i> 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 <i>Typhlodromus pyri</i> 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 <i>Poecilus cupreus</i> 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

OECD point	Author	Year	Title Source / Company Report No GLP or GEP status (where relevant), Published or not		
10.6.6	McCormac, A	2014	Abamectin SC (A13796I) – A laboratory test to determine the effects of fresh residues on the springtail <i>Folsomia candida</i> (Collembola, Isotomidae), Syngenta Ltd., Report Number SYN-14-3, GLP, not published	Y	SYN
10.6.6	Vinall, S	2014	Abamectin SC (A13796I) – A laboratory test to determine the effects of fresh residues on the predatory mite <i>Hypoaspis aculeifer</i> (Acari, Laelapidae), Syngenta Ltd. Report Number SYN-14-2, GLP, Not published	Y	SYN

