	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*30-10-2007*
Materials and Methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	
	COMMENTS FROM ...
Date	
Materials and Methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

98/8 Doc IIIA section No.	**7.4.1.2 / 08**	**Acute toxicity to invertebrates**
91/414 Annex Point addressed	**II 8.2.4 / 07**	**Acute toxicity to invertebrates**

		Official use only
Reference point (location) in dossier	**7.4.1.2/08**	
Title:	Acute toxicity test of NOA448112 (metabolite of MK 936) to *Daphnia magna* under static conditions	
Project/Report number:	808751	
Author(s):	Peither, A.	
Date of report:	30/05/2001	
Published:	Not published.	
Testing facility:	RCC AG, Itingen, Switzerland	

Study dates	2001	
GLP:	Yes	
Deficiencies:	None	
Reliability indicator	1	

Reference/notifier	:	Peither, A. (2001c)	GLP statement	:	yes
Type of study	:	Daphnia, acute toxicity	Guideline	:	OECD 202 US EPA FIFRA 72-2
Year of execution	:	2001	Acceptability	:	acceptable
Test substance	:	8a-hydroxy-avermectin B_{1a} (NOA 448112), batch [redacted] appearance clear liquid			

Substance	Species	Method	T [°C]	pH	Duration [h]	Criterion	Value [µg/L]
8a-hydroxy avermectin B_{1a}	*Daphnia magna*	static	20	8.0	48	EC_{50}	1.6

Description

Methods. Acute toxicity of 8a-hydroxy avermectin B_{1a} (NOA 448112) to *Daphnia magna* (6 - 24 h old) was tested under static conditions. Nominal concentrations 0.13, 0.25, 0.5, 1.0, 2.0 and 4.0 µg/L control, solvent control (DMF, 100 µL/L). Dilution with artificial freshwater, hardness 250 mg $CaCO_3$/L, pH 8.0, 200 mL per test unit. Four replicates with five organisms each.
Conditions. Temperature 20 °C, 16:8 h L:D (200 - 1200 lux), no aeration, no feeding.
Chemical analysis. Actual concentrations determined of all treatments at start and end. Analysis by HPLC-MS after extraction with dichloromethane, LOQ < 0.13 µg/L, recovery 105 %.
Calculations and statistics. EC_{50}-value was calculated using the binomial probability.

Results

Actual concentrations were 85 – 108% of nominal at start and 70 – 135 % of nominal at termination. Mean measured concentrations were 0.16, 0.23, 0.41, 0.78, 2.0 and 4.0 µg/L (78 - 123 % of nominal). No immobilisation in control, solvent control, and at 0.13 - 0.5 µg/L. Immobilisation was 20, 45 and 100 % at 1.0, 2.0 and 4.0 µg/L. EC_{50} reported as 1.6 µg/L (95 % CL 1.3 – 2.1 µg/L), based on nominal concentrations.

Remarks by RMS

Water quality parameters within accepted range. Recalculation with Spearman-Kärber yielded similar results. The result EC_{50} 1.6 µg/L, based on mean measured concentrations, is used for risk assessment.

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*30-10-2007*
Materials and Methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	
	COMMENTS FROM ...
Date	
Materials and Methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

98/8 Doc IIIA section No.	**7.4.1.2 / 09**	**Acute toxicity to invertebrates**
91/414 Annex Point addressed	**II 8.2.4 / 07**	**Acute toxicity to invertebrates**

		Official use only
Reference point (location) in dossier	**7.4.1.2/09**	
Title:	Acute Toxicity Test of MK936 tech. to *Simocephalus sp.*, *Daphnia longispina* and *Daphnia pulex*under Static Conditions	
Project/Report number:	2012505	
Author(s):	Knauer, K	
Date of report:	14/05/2001	
Published:	Not published.	

Testing facility:	Syngenta Crop Protection AG, Basel, Switzerland	
Study dates	9 - 28 January 2001	
GLP:	Yes	
Deficiencies:	None	
Reliability indicator	1	

Reference/notifier	:	Knauer, K. (2001b)	GLP statement	:	yes
Type of study	:	water fleas, acute toxicity	Guideline	:	OECD 202 US EPA
Year of execution	:	2001	Acceptability	:	acceptable
Test substance	:	abamectin technical, batch [redacted] chemical purity [redacted], white powder			

Substance	Species	Method	T [°C]	pH	Duration [h]	Criterion	Value [µg/L]
abamectin	*Daphnia longispina*	static	20	8.0	48	EC_{50}	0.38
	Daphnia pulex	static	20	8.0	48	EC_{50}	0.12
	Simocephalus sp.	static	20	8.0	48	EC_{50}	0.30

Description

Methods. Acute toxicity test with abamectin on waterfleas *Daphnia longispina*, *D. pulex* and *Simocephalus* sp.. Filtered natural pond water, 200 mL per test vessel, pH 8.0 – 8.3, total hardness 100 mg $CaCO_3$/L, 200 µS/cm. Nominal concentrations 0.035 (*Simocephalus* sp. and *D. pulex*), 0.088, 0.22, 0.55, 1.4 and 3.4 µg/L, control, solvent controls (DMF). Four replicates per treatment with five organisms each.
Conditions. Temperature 20 ± 1 °C, 16:8 h L:D (19 µE/m^2.sec), no feeding.
Chemical analysis. Samples taken at start and end of test, analysis by HPLC-UV, LOQ 0.01 µg/L after SPE, recovery 98 – 125%.
Calculations and statistics. EC_{50}-values calculated using maximum likelihood method, probit model.

Results

Daphnia pulex. Actual concentrations 0.0498, 0.0941, 0.179, 0.529, 1.24 and 2.86 µg/L at start (81 – 142 % of nominal) and 0.0280, 0.0151, 0.0424, 0.190, 0.557 and 1.78 µg/L at end (17 – 80 % of nominal). Mean measured concentrations 0.039, 0.055, 0.11, 0.36, 0.90 and 2.3 µg/L (50 – 111 % of nominal). No immobilisation in control and 0.039 µg/L, 5 % in solvent control and concentration related immobilisation of 15, 35, 100, 100 and 100 % at 0.055 µg/L and higher, 48-hours EC_{50} reported as 0.12 µg/L (95 % CL 0.095 – 0.16 µg/L), based on mean measured concentrations.
D. longispina. Actual concentrations same as for *D. pulex* (lowest not tested). Control immobilisation 5 %, no immobilisation in solvent control and at 0.055 and 0.11 µg/L, and 30, 100 and 100 % immobilisation at 0.36, 0.90 and 2.3 µg/L, respectively. EC_{50} 0.38 µg /L (no CL)), based on mean measured concentrations.
Simocephalus sp. Actual concentrations 0.0308, 0.0663, 0.336, 0.649, 1.94 and 3.34 µg/L at start (75 – 153 % of nominal), and 0.144, 0.049, 0.120, 0.424, 1.16 and 1.56 µg/L at end (46 – 411 % of nominal). Average concentrations 0.087, 0.058, 0.23, 0.54, 1.6 and 2.5 µg/L (66 - 250 % of nominal). No immobilisation in control, 5 % in solvent control, immobilisation of 10, 55, 35, 60, 60 and 95 % at 0.087 µg/L and higher. EC_{50} 0.30 µg/L (no CL), based on mean measured concentrations.

Remarks by RMS

Water quality parameters within accepted range. High initial concentrations at lowest level indicate problems with analytical method at lower concentrations, lowest test concentration for *Simocepahlus* seems to be an error and EC_{50} is recalculated without this concentration as 0.33 µg/L (95 % CL 0.14 - 0.79 µg/L) with Spearman-Kärber. The results 48-hours EC_{50} 0.38 µg/L for *Daphnia longispina*, 0.12 µg/L for *Daphnia pulex* and 0.30 µg/L for *Simocephalus* sp. are used for risk assessment.

	Evaluation by Competent Authorities
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	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*30-10-2007*
Materials and Methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	
	COMMENTS FROM ...
Date	
Materials and Methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

98/8 Doc IIIA section No.	**7.4.1.2 / 10**	**Acute toxicity to invertebrates**
91/414 Annex Point addressed	**II 8.2.4 / 05**	**Acute toxicity to invertebrates**

		Official use only
Reference point (location) in dossier	**7.4.1.2/10**	
Title:	Acute Toxicity Test of MK 936 tech. to the Cladoceran *Daphnia galeata* under Static Conditions	
Project/Report number:	2002535	
Author(s):	Knauer, K.	
Date of report:	27/04/2001	
Published:	Not published.	
Testing facility:	Syngenta Crop Protection AG, Basel, Switzerland	
Study dates	03 – 05 July 2000	
GLP:	Yes.	

Deficiencies:	No analytical measurements of the test concentrations were performed.	
Reliability indicator	1.	X

Reference/notifier	:	Knauer, K. (2001a)	GLP statement	:	yes
Type of study	:	Daphnia, acute toxicity	Guideline	:	OECD202 US EPA
Year of execution	:	2001	Acceptability	:	acceptable
Test substance	:	abamectin technical, batch [redacted], chemical purity [redacted] appearance white powder			

Substance	Species	Method	T [°C]	pH	Duration [h]	Criterion	Value [µg/L]
abamectin	*Daphnia galeata*	static	20	8.3	48	EC_{50}	0.55

Description

Methods. Daphnids exposed to abamectin solutions prepared in pond water to expose the organisms through the water phase and through grazing. Water passed through 90 µm mesh, total hardness 86 mg $CaCO_3$/L, 220 µS/cm, pH 8.3. Nominal concentrations 0.63, 1.3, 2.5, 5.0 and 10 µg/L, control. Four replicates per treatment and controls, with five daphnids each, 100 mL per test vessel.
Conditions. Temperature 21 - 22 °C, 16:8 h L:D, no feeding.
Calculations and statistics. EC_{50}-value was calculated using maximum likelihood method and probit model

Results

No immobilisation in controls, 60 and 85% immobilisation at 0.63 and 1.3 µg/L and 100% at higher concentrations. EC_{50} reported as 0.55 µg/L (95% CL 0.22 – 0.75 µg/L), based on nominal concentrations.

Remarks by RMS

Water quality parameters within accepted range. According to report, analytical determination of test concentrations was not possible due to heterogeneity of test solution. Assumed that is meant that pond water contained too much particulate matter after sieving. The result 48-hours EC_{50} 0.55 µg/L, based on nominal concentrations, is used for risk assessment.

Addendum to the RMS remarks after evaluation under the BPD:

[redacted]

	Evaluation by Competent Authorities
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	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*30-10-2007*
Materials and Methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	
	COMMENTS FROM ...
Date	
Materials and Methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

98/8 Doc IIIA section No.	**7.4.1.2 / 11**	**Acute toxicity to invertebrates**
91/414 Annex Point addressed	**II 8.2.4 / 07**	**Acute toxicity to invertebrates**

		Official use only
Reference point (location) in dossier	**7.4.1.2/11**	
Title:	Acute Toxicity Test of MK 936 tech. to Individual Invertebrate Species from a Natural Pond Assemblage (Schöhsee) under Static Conditions	
Project/Report number:	2001831	
Author(s):	Knauer, K.	
Date of report:	27/04/2001	
Published:	Not published.	
Testing facility:	Syngenta Crop Protection AG, Basel, Switzerland	

Study dates	25 – 27 July 2001	
GLP:	Yes.	
Deficiencies:	No analytical measurements of the test concentrations were performed.	
Reliability indicator	1.	X

Reference/notifier	:	Knauer, K. (2001c)	GLP statement	:	yes
Type of study	:	crustaceans, acute toxicity	Guideline	:	OECD202 US EPA
Year of execution	:	2001	Acceptability	:	acceptable
Test substance	:	abamectin technical, batch [redacted], chemical purity [redacted] appearance white powder			

Substance	Species	Method	T [°C]	pH	Duration [h]	Criterion	Value [µg/L]
abamectin	*Diaphanosoma* sp.	static	20	8.5	48	EC_{50}	0.53
	Daphnia pulex	static	20	8.5	48	EC_{50}	0.28

Description

Methods. Acute static toxicity test with abamectin on crustaceans *Diaphanosoma* sp. and *Daphnia pulex*. Dilution water was pond water filtered through 90 µm mesh but still containing particulate material to mimic realistic exposure of grazing organisms, pH 8.3 – 9.2, 220 µs/cm, total hardness 84 mg $CaCO_3$/L, 100 mL solution per test unit. Nominal concentrations 0.16, 0.31, 0.63, 1.3, 2.5, 5.0 and 10 µg/L, control. Four replicates with five organisms each.
Conditions. Temperature 20 ± 1°C °C, 16:8 h L:D (19 µE/m^2.sec), no feeding.
Calculations and statistics. EC_{50}-values were calculated using maximum likelihood method and probit model

Results

Diaphanosoma sp.. No immobilisation in control, immobilisation 10, 40, 60, 75, 85, 100 and 100 % at 0.16 µg/L and higher. Reported 48-hours EC_{50} 0.53 µg /L (95% CL 0.37 – 0.72 µg/L), based on nominal concentrations.
Daphnia pulex. No immobilisation in control, immobilisation of 30, 65, 65, 85, 100, 100 and 100 % at 0.16 - 10 µg/L. Reported 48-hours EC_{50} 0.28 µg/L (95% CL 0.17 – 0.40 µg/L), based on nominal concentrations.

Remarks by RMS

Water quality parameters within accepted range. According to report, analytical determination of test concentrations was not possible due to heterogeneity of test solution. Assumed that is meant that pond water contained too much particulate matter after sieving. The results 48-hours EC_{50} 0.53 µg/L for *Diaphanosoma* sp. and 0.28 µg/L for *Daphnia pulex*, based on nominal concentrations, are used for risk assessment.

Addendum to the RMS remarks after evaluation under the BPD:

[redacted]

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	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*30-10-2007*
Materials and Methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	
	COMMENTS FROM ...
Date	
Materials and Methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

98/8 Doc IIIA section No.	**7.4.1.2 / 12**	**Acute toxicity to invertebrates**
91/414 Annex Point addressed	**II 8.2.4 / 08**	**Acute toxicity to invertebrates**

		Official use only
Reference point (location) in dossier	**7.4.1.2/12**	
Title:	Acute Toxicity Test of MK 936 tech. to *Brachionus calyciflorus* and *Thamnocephalus platyurus* under Static Conditions	
Project/Report number:	2002609	
Author(s):	Knauer, K.	
Date of report:	27/04/2001	
Published:	Not published.	

Testing facility:	Syngenta Crop Protection AG, Basel, Switzerland	
Study dates	14 – 15 February 2000	
GLP:	Yes.	
Deficiencies:	Analytical determinations of the test concentrations were not performed.	
Reliability indicator	1.	X

Reference/notifier	:	Knauer, K. (2001d)	GLP statement	:	yes
Type of study	:	invertebrates, acute toxicity	Guideline	:	OECD202 US EPA
Year of execution	:	2001	Acceptability	:	partly acceptable
Test substance	:	abamectin technical, batch [redacted], chemical purity [redacted] appearance white powder			

Substance	Species	Method	T [°C]	pH	Duration [h]	Criterion	Value [µg/L]
abamectin	*Brachionus calyciflorus*	static	25	8.2	24	EC_{50}	3600
	Thamnocephalus platyurus	static	25	8.2	24	EC_{50}	30

Description

Methods. Acute toxicity of abamectin to the pelagic rotifer *Brachionus calyciflorus* and the fairy shrimp *Thamnocephalus platyurus* was tested under static conditions. Nominal concentrations 0.50, 1.0, 2.0, 4.0, 8.0 and 16 mg/L for *B. calyciflorus* and 0.032, 0.064, 0.13, 0.25 and 0.50 mg/L for *T. platyurus*, control. Reconstituted water, pH 8.2 ± 0.1, 335 µS/cm; 0.5 mL solution, six replicates with five organisms each for *B. calyciflorus*, 1 mL solution, three replicates with 10 organisms each for *T. platyurus*.
Conditions. Temperature 25 °C, darkness, no feeding.
Calculations and statistics. EC_{50}-values were calculated using maximum likelihood method and probit model.

Results

B. calyciflorus. No immobilisation in control and 0.50 mg/L, immobilisation of 7, 10, 23, 27 and 30 % at 1.0 – 16 mg/L. EC_{50} reported as 36 mg/L (95 % CL 16 – 340 mg/L), based on nominal concentrations.
T. platyurus. Immobilisation in control 3 %, 73 % at 0.032 mg/L and 100 % at 0.064 mg/L and higher. EC_{50} reported as 0.030 mg/L (no 95 % CL), based on nominal concentrations.

Remarks by RMS

Water quality parameters within accepted range. Due to heterogeneity of test solution analytical determination of test concentrations was not possible. Tested concentrations for *Brachionus calyciflorus* exceeded test compound's solubility (1.21 mg/L at 25 °C) and no solvent was used. EC_{50} is extrapolated. Therefore, only the nominal 24-hours EC_{50}-value of 30 µg/L for *Thamnocephalus platyurus* is used for risk assessment. Recalculation of *Th. platyurus* EC_{50}-value with Spearman-Kärber yielded similar results.

Addendum to the RMS remarks after evaluation under the BPD:

[redacted]

	Evaluation by Competent Authorities
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	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*30-10-2007*
Materials and Methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	
	COMMENTS FROM ...
Date	
Materials and Methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

98/8 Doc IIIA section No.	**7.4.1.2 / 13**	**Acute toxicity to invertebrates**
91/414 Annex Point addressed	**II 8.2.4 / 09**	**Acute toxicity to invertebrates**

		Official use only
Reference point (location) in dossier	**7.4.1.2/13**	
Title:	Acute toxicity test of MK 936 tech. to *Thamnocephalus platyurus* and *Brachionus calyciflorus* under static conditions	
Project/Report number:	2012545	
Author(s):	Knauer, K.	
Date of report:	14/05/2001	
Published:	Not published.	
Testing facility:	Syngenta Crop Protection AG, Basel, Switzerland	

Study dates	27 – 28 June 2000	
GLP:	Yes.	
Deficiencies:	None	
Reliability indicator	1.	

Reference/notifier	:	Knauer, K. (2001e)	GLP statement	:	yes
Type of study	:	invertebrates, acute toxicity	Guideline	:	OECD202 US EPA
Year of execution	:	2001	Acceptability	:	acceptable
Test substance	:	abamectin technical, batch [redacted], chemical purity [redacted] appearance white powder			

Substance	Species	Method	T [°C]	pH	Duration [h]	Criterion	Value [µg/L]
abamectin	*Brachionus calyciflorus*	static	24	8.2	24	EC_{50}	4000
	Thamnocephalus platyurus	static	24	8.2	24	EC_{50}	2.8

Description

Methods. Acute toxicity of abamectin to *Brachionus calyciflorus* and *Thamnocephalus platyurus* was tested under static conditions. Nominal concentrations 0.3, 0.6, 1.2, 2.3 and 4.6 mg/L for *B. calyciflorus*, and 2.4, 4.8, 10, 19 and 38 µg/L for *T. platyurus*, control, solvent control (DMF). Reconstituted water, pH 8.2 ± 0.1, 305 µS/cm; 0.5 mL solution, six replicates with five organisms each for *B. calyciflorus*, 1 mL solution, three replicates with 10 organisms each for *T. platyurus*.
Conditions. Temperature, 24 ± 1°C, darkness, no feeding.
Chemical analysis. Actual concentrations of all test units determined at start and end. Samples were diluted with acetonitrile and analysed by HPLC-UV, LOQ 4 µg/L, recovery 94 % for the test with *B. calyciflorus* and LOQ 0.5 µg/L, recovery 100 % for the test with *T. platyurus*.
Calculations and statistics. EC_{50}-values were calculated using maximum likelihood method and probit model.

Results

B. calyciflorus. Measured concentrations were 53 - 88% of nominal at start and 86 – 102 % at end, mean measured concentrations were 0.23, 0.43, 1.0, 2.0 and 4.4 mg/L (72 – 96 % of nominal). No immobilisation in control, solvent control and at 0.23 – 2 mg/L, 83 % at 4.4 mg/L. EC_{50} reported as 4.0 mg/L (no 95 % CL), based on mean measured concentrations
T. platyurus. Measured concentrations were 80 - 111% of nominal at start and 19 – 34 % of nominal at end, mean measured concentrations were 1.6, 3.0, 6.2, 11 and 21 µg/L (55 – 67 % of nominal). No immobilisation in control, solvent control and at 1.6 µg/L, 80 % at 3.0 µg/L and 100 % at 6.2 µg/L and higher. EC_{50} reported as 2.8 µg/L (no 95 % CL), based on mean measured concentrations.

Remarks by RMS

Water quality parameters within accepted range. Test concentrations for *B. calyciflorus* all above water solubility (1.21 mg/L at 25 °C). However, actual concentrations were measured at start and termination and no flocculation was reported. Recalculation of EC_{50}-values with Spearman-Kärber yielded similar results. The results EC_{50} 4000 µg/L for *Brachionus calyciflorus* and 2.8 µg/L for *Thamnocephalus platyurus*, based on mean measured concentrations, are used for risk assessment.

	Evaluation by Competent Authorities
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	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*30-10-2007*
Materials and Methods	
Results and discussion	
Conclusion	[redacted]
Reliability	
Acceptability	
Remarks	
	COMMENTS FROM ...
Date	
Materials and Methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

98/8 Doc IIIA section No.	**7.4.1.2 / 14**	**Acute toxicity to invertebrates**
91/414 Annex Point addressed	**II 8.2.4 / 10**	**Acute toxicity to invertebrates**

		Official use only
Reference point (location) in dossier	**7.4.1.2/14**	
Title:	Acute Toxicity Test of MK 936 tech. to *Chaoborus* sp. under Static Conditions	
Project/Report number:	2012532	
Author(s):	Knauer, K.	
Date of report:	27/04/2001	
Published:	Not published.	
Testing facility:	Syngenta Crop Protection AG, Basel, Switzerland	
Study dates	24 – 26 July 2000	
GLP:	Yes.	
Deficiencies:	None	

Reliability indicator	1.	

Reference/notifier	:	Knauer, K. (2001f)	GLP statement	:	yes
Type of study	:	Chaoboridae, acute toxicity	Guideline	:	OECD202 US EPA
Year of execution	:	2001	Acceptability	:	acceptable
Test substance	:	abamectin technical, batch [redacted], chemical purity [redacted] appearance white powder			

Substance	Species	Method	T [°C]	pH	Duration [h]	Criterion	Value [µg/L]
abamectin	*Chaoborus* sp.	static	20	8.2	48	EC_{50}	190

Description

Methods. Acute toxicity of abamectin to *Chaoborus* sp. was tested under static conditions. Natural pond water filtered over 0.20 µm mesh, 225 µS/cm, hardness 110 mg $CaCO_3$/L, pH 8.2 – 8.3, 200 mL solution per test unit. Nominal concentrations 0.063, 0.13, 0.25 and 1.0 mg/L, control, solvent control (DMF, 21.5 mg/L). Four replicates with five organisms each. *Conditions*. Temperature 20 °C, 16:8 h L:D (15 – 20 µE/m^2.sec), no feeding *Chemical analysis*. Actual concentrations of all test units determined at start and end. Samples diluted with acetonitrile and analysed by HPLC-UV-detection, LOQ 4 µg/L, mean recovery 110 %.
Calculations and statistics. EC_{50}-values were calculated using maximum likelihood method and probit model..

Results

Measured concentrations between 91 – 118 % of nominal at beginning and 81 – 103% of nominal at end of test. Mean measured concentrations 0.069, 0.125, 0.24, 0.45 and 0.87 mg/L (87 – 109 % of nominal). No immobilisation in control, solvent control and at 0.069 mg/L, concentration related immobilisation of 35, 75, 85, 100 and 100 % at 0.13 – 0.87 mg/L. Calculated EC_{50} for 48 hours exposure 0.19 mg/L (95 % CL 0.15 – 0.24 mg/L), based on mean measured concentration.

Remarks by RMS

Water quality parameters within accepted range. The result 48-hours actual EC_{50} 190 µg/L is used for risk assessment.

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*30-10-2007*
Materials and Methods	
Results and discussion	
Conclusion	[redacted]
Reliability	
Acceptability	
Remarks	
	COMMENTS FROM ...
Date	
Materials and Methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

98/8 Doc IIIA section No.	**7.4.1.2 / 15**	**Acute toxicity to invertebrates**
91/414 Annex Point addressed	**II 8.2.4 / 11**	**Acute toxicity to invertebrates**

		Official use only
Reference point (location) in dossier	**7.4.1.2/15**	
Title:	Acute Toxicity Test of MK 936 tech. to Individual Invertebrate Species from a Natural Pond Assemblage Under Static Conditions	
Project/Report number:	2002610	
Author(s):	Knauer, K.	
Date of report:	27/04/2001	
Published:	Not published.	
Testing facility:	Syngenta Crop Protection AG, Basel, Switzerland	
Study dates	11 – 20 July 2000	
GLP:	Yes.	
Deficiencies:	None.	
Reliability indicator	1.	X

Reference/notifier	:	Knauer, K. (2001g)	GLP statement	:	yes
Type of study	:	arthropods, acute toxicity	Guideline	:	OECD202 US EPA
Year of execution	:	2001	Acceptability	:	partly acceptable
Test substance	:	abamectin technical, batch [redacted], chemical purity [redacted] appearance white powder			

Substance	Species	Method	T [°C]	pH	Duration [h]	Criterion	Value [µg/L]
abamectin	*Chaoborus* sp.	static	20	8.5	48	EC_{50}	41
	Ostracoda	Static	20	8.5	48	EC_{50}	55
	Cloeon sp.	static	20	8.5	48	EC_{50}	2.9

Description

Methods. Acute toxicity of abamectin to *Chaoborus* sp., Ostracoda and *Cloeon* sp. was tested under static conditions. Organisms were collected from mesocosms of Syngenta, Stein, Switzerland. Nominal concentrations 4.1, 12, 37, 110, 330 and 1000 µg/L for *Chaoborus* sp., 0.63, 1.3, 2.5, 5.0, 10 and 20 µg/L for Ostracoda and 0.50, 1.0, 2.0, 4.0 and 8.0 µg/L for *Cloeon* sp., control. Dilution with pond water (pH 8.5), filtered over 90 µm mesh for *Chaoborus* sp. and Ostracoda (780 µS/cm, hardness 410 mg $CaCO_3$/L), 0.2 µm mesh for *Cloeon* sp. (210 µS/cm and 88 mg $CaCO_3$/L); water contained particulate material after filtration, thereby mimicking exposure of grazing organisms. Test units with 200 mL solution for *Chaoborus* sp. and *Cloeon* sp. and 100 mL solution for Ostracoda. Four replicates with five organisms each. *Conditions*. Temperature 21 °C, 16:8 h L:D (18 – 20 µE/m^2sec), no aeration, no feeding.
Chemical analysis. Actual concentrations of all test units determined at start. Samples diluted with acetonitrile and analysed by HPLC-UV, LOQ and recovery not reported.
Calculations and statistics. EC_{50}-values were calculated using maximum likelihood method and probit model.

Results

Results of chemical analysis not given because of analytical problems due to heterogeneity of the test solutions. Effect concentrations were calculated on basis of nominal concentrations.
Chaoborus sp. No immobilisation in control and 4.1 µg/L, concentration related immobilisation of 20, 55, 65, 100 and 100 % at 12 - 1000 µg/L nominal. Nominal EC_{50} 41 µg/L (95 % CL 28 – 60 µg/L).
Ostracoda. Control immobilisation 5 %, 10, 0, 15, 5, 20 and 45% in respective test concentrations. Nominal EC_{50} 55 µg/L (95 % CL 19 – 3000 µg/L).
Cloeon sp. No immobilisation in control, concentration related immobilisation of 25, 30, 45, 50 and 70 % at 0.50 - 8.0 µg/L nominal. Nominal EC_{50} 2.9 µg/L (95 % CL 1.6 – 8.7 µg/L).

Remarks by RMS

Water quality parameters within accepted range. The highest immobilisation rate for Ostracoda was 45 % and immobilisation rate showed an irregular pattern. Therefore, the EC_{50} for Ostracoda is not used for risk assessment. The results 48-hours nominal EC_{50} 41 µg/L for *Chaoborus* sp. and 2.9 µg/L for *Cloeon* sp. Are used for risk assessment.

Addendum to the RMS remarks after evaluation under the BPD:

[redacted]

	Evaluation by Competent Authorities
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	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*30-10-2007*
Materials and Methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	
	COMMENTS FROM ...
Date	
Materials and Methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

98/8 Doc IIIA section No.	**7.4.1.2 / 16**	**Acute toxicity to invertebrates**
91/414 Annex Point addressed	**II 8.2.4 / 12**	**Acute toxicity to invertebrates**

		Official use only
Reference point (location) in dossier	**7.4.1.2/16**	
Title:	Acute Toxicity Test of MK 936 tech. to *Gammarus* sp. under Static Conditions	
Project/Report number:	2002611	
Author(s):	Knauer, K.	
Date of report:	27/04/2001	
Published:	Not published.	
Testing facility:	Syngenta Crop Protection AG, Basel, Switzerland	
Study dates	25 – 27 July 2000	

GLP:	Yes.	
Deficiencies:	No analytical measurements of the test substance performed.	
Reliability indicator	1.	X

Reference/notifier	:	Knauer, K. (2001h)	GLP statement	:	yes
Type of study	:	Gammarus sp., acute toxicity	Guideline	:	OECD202 US EPA FIFRA 72-2
Year of execution	:	2001	Acceptability	:	acceptable
Test substance	:	abamectin technical, batch [redacted], chemical purity [redacted] appearance white powder			

Substance	Species	Method	T [°C]	pH	Duration [h]	Criterion	Value [µg/L]
abamectin	*Gammarus* sp.	static	16	8.5	48	EC_{50}	6.2

Description

Methods. Acute toxicity of abamectin to *Gammarus* sp. was tested under static conditions. Organisms were collected from the Kastelbach River, Switzerland. Nominal concentrations 0.31, 0.63, 1.3, 2.5, 5.0 and 10 µg/L, control. Dilution with filtered pond water (90 µm mesh) to mimic exposure of grazing organisms, 730 µS/cm, hardness 440 mg $CaCO_3$/L, pH 8.5. Four replicates with five organisms each, 200 mL solution per test unit.
Conditions. Temperature 16 °C, 16:8 h L:D (5 µE/m^2.sec), no aeration, no feeding.
Chemical analysis. Actual concentrations of all test units determined at start. Samples diluted with acetonitrile and analysed by HPLC-UV, LOQ and recovery not reported.
Calculations and statistics. EC_{50}-value was calculated using maximum likelihood method and probit model.

Results

Results of chemical analysis not given because of analytical problems due to heterogeneity of the test solutions. Therefore, effect concentrations were calculated on basis of nominal concentrations.
Immobilisation in control 5 %, 0, 10, 15, 15, 20 and 85% at repective dose levels. EC_{50} for 48 hours exposure reported as 6.2 µg/L (95% CL 2.5 – 60 µg/L), based on nominal concentrations.

Remarks by RMS

Water quality parameters within accepted range. The result EC_{50} 6.2 µg/L, based on nominal concentrations, is used for risk assessment.

Addendum to the RMS remarks after evaluation under the BPD:

[redacted]

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*30-10-2007*
Materials and Methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	
	COMMENTS FROM ...
Date	
Materials and Methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

98/8 Doc IIIA section No.	**7.4.1.2 / 17**	**Acute toxicity to invertebrates**
91/414 Annex Point addressed	**II 8.2.4 / 13**	**Acute toxicity to invertebrates**

		Official use only
Reference point (location) in dossier	**7.4.1.2/17**	
Title:	Acute Toxicity Test of MK 936 tech. to *Gammarus* sp. under Static Conditions	
Project/Report number:	2012543	
Author(s):	Knauer, K.	
Date of report:	27/04/2001	
Published:	Not published.	
Testing facility:	Syngenta Crop Protection AG, Basel, Switzerland	
Study dates	05 – 08 February 2000	
GLP:	Yes.	

Deficiencies:	None	
Reliability indicator	1.	

Reference/notifier	:	Knauer, K. (2001i)	GLP statement	:	yes
Type of study	:	Gammarus sp., acute toxicity	Guideline	:	OECD202 US EPA FIFRA 72-2
Year of execution	:	2001	Acceptability	:	acceptable
Test substance	:	abamectin technical, batch [redacted], chemical purity [redacted] appearance white powder			

Substance	Species	Method	T [°C]	pH	Duration [h]	Criterion	Value [µg/L]
abamectin	*Gammarus* sp.	static	10	8.5	48	EC_{50}	8.6

Description

Methods. Acute toxicity of abamectin to *Gammarus* sp. was tested under static conditions. Organisms were collected from the Frenke River, Switzerland. Nominal concentrations 2.5, 5.0, 10, 20 and 41 µg/L, control, solvent control (DMF, 0.3 µg/L). Dilution with filtered pond water (0.20 µm mesh), 200 µS/cm, hardness 100 mg $CaCO_3$/L, pH 8.5. Four replicates with five organisms each, 200 mL solution per test unit.
Conditions. Temperature 16 °C, 16:8 h L:D (5 µE/m^2.sec), no aeration, no feeding.
Chemical analysis. Actual concentrations of all units determined at start and end. Samples diluted with acetonitrile and analysed by HPLC-UV, LOQ 0.5 µg/L, recovery 98 %.
Calculations and statistics. EC_{50}-value was calculated using maximum likelihood method and probit model.

Results

Actual concentrations were 2.91, 6.33, 11.3, 19.3 and 38.5 µg/L at start (94 – 127 % of nominal), and 2.3, 3.58, 6.88, 13.3 and 27.5 µg/L at end (67 – 92 % of nominal). Mean measured concentrations 2.96, 4.96, 9.09, 16.3 and 33.0 µg/L (81 – 104 % of nominal).
No immobilisation in control, solvent control and at 2.96 and 4.96 µg/L, 75, 100 and 100 % immobilisation at 9.09 - 33 µg/L. EC_{50} for 48 hours exposure reported as 8.6 µg/L (95 % CL not determinable), based on mean measured concentrations.

Remarks by RMS

Water quality parameters within accepted range. The result EC_{50} 8.6 µg/L, based on mean measured concentrations, is used for risk assessment.

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*30-10-2007*
Materials and Methods	
Results and discussion	
Conclusion	[redacted]
Reliability	
Acceptability	
Remarks	
	COMMENTS FROM ...
Date	
Materials and Methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

98/8 Doc IIIA section No.	**7.4.1.2 / 18**	**Acute toxicity to invertebrates**
91/414 Annex Point addressed	**II 8.2.4 / 14**	**Acute toxicity to invertebrates**

		Official use only
Reference point (location) in dossier	**7.4.1.2/18**	
Title:	Acute Toxicity Test of MK 936 tech. to *Lymnaea stagnalis* under Static Conditions	
Project/Report number:	2012547	
Author(s):	Knauer, K.	
Date of report:	27/04/2001	
Published:	Not published.	
Testing facility:	Syngenta Crop Protection AG, Basel, Switzerland	
Study dates	27 February - 01 March 2000	
GLP:	Yes.	
Deficiencies:	None	
Reliability indicator	1.	

Reference/notifier	:	Knauer, K. (2001j)	GLP statement	:	yes
Type of study	:	Lymnaea, acute toxicity	Guideline	:	OECD202 US EPA FIFRA 72-2
Year of execution	:	2001	Acceptability	:	acceptable
Test substance	:	abamectin technical, batch [redacted], chemical purity [redacted] appearance white powder			

Substance	Species	Method	T [°C]	pH	Duration [h]	Criterion	Value [µg/L]
abamectin	*Lymnaea stagnalis*	static	20	8.0	48	EC_{50}	55

Description

Methods. Acute toxicity of abamectin to *Lymnaea stagnalis* (phyllum Mollusca, class Gastropoda), obtained from an in-house culture, was tested under static conditions. Nominal concentrations 43, 94, 210, 450 and 1000 µg/L, control, solvent control (DMF, 24 µg/L). Dilution with filtered pond water (0.20 µm mesh), 200 µS/cm, hardness 100 mg $CaCO_3$/L, pH 8. Four replicates with five organisms each, 200 mL solution per test unit.
Conditions. Temperature 20 ± 2 °C, 16 h L:D, 20 µE/m^2.sec), no aeration, no feeding.
Chemical analysis. Actual concentrations of all test units determined at start and end. Samples diluted with acetonitrile and analysed by HPLC-UV, LOQ 4 µg/L, recovery 97 %.
Calculations and statistics. LC_{50}-value was calculated using maximum likelihood method and probit model.

Results

Oxygen saturation values at 350 and 880 µg/L were < 30% after 48 h, DO remained > 80% in the lower concentrations. Actual concentrations were 42, 82, 190, 430 and 990 µg/L at start (87 - 99% of nominal) and 20, 46, 76, 270 and 770 µg/L at end (36 – 77 % of nominal). Mean measured concentrations 31, 64, 133, 350 and 880 µg/L (63 - 88 % of nominal). No immobilisation in control and solvent control, concentration related immobilisation of 5 - 100 % at 31 µg/L and higher. EC_{50} for 48 hours exposure reported as 55 µg/L (95 % CL 44 – 66 µg/L), based on mean measured concentrations.

Remarks by RMS

Lowered oxygen concentrations only occurred in the two highest test concentrations. The result EC_{50} 55 µg/L, based on mean measured concentrations, is used for risk assessment.

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*30-10-2007*
Materials and Methods	
Results and discussion	
Conclusion	[redacted]
Reliability	
Acceptability	
Remarks	
	COMMENTS FROM ...
Date	
Materials and Methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

98/8 Doc IIIA section No.	**7.4.1.2 / 19**	**Acute toxicity to invertebrates**
91/414 Annex Point addressed	**II 8.2.4 / 15**	**Acute toxicity to invertebrates**

		Official use only
Reference point (location) in dossier	**7.4.1.2/19**	
Title:	Acute toxicity of MK-936 technical to mysid shrimp (*Mysidopsis bahia*)	
Project/Report number:	33624	
Author(s):	Forbis, A.D., Burgess, D	
Date of report:	18/09/1985	
Published:	Not published.	
Testing facility:	ABC Analytical Bio-Chemistry Lab. Inc., Columbia, United States.	
Study dates	26 – 30 August 1985	
GLP:	Yes.	
Deficiencies:	None	
Reliability indicator	1.	X

Reference/notifier	:	Forbis, A.D. and Burgess, D. (1985)	GLP statement	:	yes
Type of study	:	mysid shrimp, acute toxicity	Guideline	:	EPA 1975 APHA 1980
Year of execution	:	1985	Acceptability	:	acceptable
Test substance	:	abamectin technical, batch [redacted], chemical purity [redacted] appearancewhite powder			

Substance	Species	Method	T	pH	Duration	Criterion	Value
			[°C]		[h]		[µg/L]
abamectin	*Mysidopsis bahia*	static	22	8.0	96	LC_{50}	0.21

Description

Methods. Acute toxicity of abamectin to commercially obtained juvenile *Mysidopsis bahia* was tested under static conditions. Nominal concentrations 0.10, 0.18, 0.32, 0.57 and 1.0 µg/L, control, solvent control (acetone). Dilution with aged artificial salt water, salinity 22 ‰, pH 8.0 – 8.5. Single vessels with 300 mL solution and 10 organisms.
Conditions. Temperature 20 ± 2 °C, 16:8 h L:D, no aeration, daily feeding with 2 mL brine shrimp/test unit.
Calculations and statistics. LC_{50}-values were calculated using binomial probability method of Stefan (1977).

Results

No mortality in control, solvent control and 0.10 µg/L, 30 % at 0.18 µg/L and 100 % at 0.32 µg/L and higher. LC_{50} for 96 hours exposure reported as 0.21 µg/L (95 % CL 0.10 – 0.32 µg/L), based on nominal concentrations.

Remarks by RMS

Water quality parameters within accepted range. Recalculation of LC_{50} with Spearman-Kärber yielded similar results. The result LC_{50} 0.21 µg /L, based on nominal concentrations, is used for risk assessment.

Addendum to the RMS remarks after evaluation under the BPD:

[redacted]

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*30-10-2007*
Materials and Methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	
	COMMENTS FROM ...
Date	
Materials and Methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

98/8 Doc IIIA section No.	**7.4.1.2 / 20**	**Acute toxicity to invertebrates**
91/414 Annex Point addressed	**II 8.2.4 / 16**	**Acute toxicity to invertebrates**

		Official use only
Reference point (location) in dossier	**7.4.1.2/20**	
Title:	Acute toxicity of 3H-Avermectin B1 to Mysid shrimp (*Mysidopsis bahia*) under flow-through conditions	
Project/Report number:	88-3-2684	
Author(s):	Surprenant, D.C.	
Date of report:	24/06/1988	
Published:	Not published.	
Testing facility:	Springborn Life Science Inc., Wareham, United States.	
Study dates	18 – 22 January 1988	
GLP:	Yes.	

Deficiencies:	None	
Reliability indicator	1.	

Reference/notifier	:	Surprenant, D.C. (1988a)	GLP statement	:	yes
Type of study	:	mysid shrimp, acute toxicity	Guideline	:	EPA 1975 APHA 1980
Year of execution	:	1988	Acceptability	:	acceptable
Test substance	:	^{3}H-abamectin, B_{1a}:B_{1b} 7.95:1, batch [redacted] appearance clear solution			

Substance	Species	Method	T [°C]	pH	Duration [h]	Criterion	Value [µg/L]
^{3}H-abamectin	*Mysidopsis bahia*	flow-through	25	7.7	96	LC_{50}	0.022

Description

Methods. Acute toxicity of ^{3}H-abamectin to commercially obtained juvenile *Mysidopsis bahia* was tested under flow-through conditions. ^{3}H-abamectin, 82.8 mCi/L, 7.95:1 B_{1a}:B_{1b} tritium only present in avermectin B_{1a}. Nominal concentrations 4.5, 6.9, 11, 16 and 25 ng/L, control, solvent control (acetone, 2.2 µL/L). Dilution with filtered natural seawater (5 µm mesh), salinity 30 ‰, pH 7.7. Two replicates with 10 organisms each, 1.2 L solution per unit flow-rate seven volume renewals/day.
Conditions. Temperature 25 ± 1 °C, 16:8 h L:D (215 - 968 lux), no aeration, daily feeding with brine shrimp.
Chemical analysis. Daily samples to determine actual concentrations. Samples extracted with ethylether and dried. Dry residues analysed by LSC, recovery 103 %, LOQ not reported.
Calculations and statistics. LC_{50}-value was calculated using nonlinear interpolation with 95% CL calculated by binomial probability method of Stefan (1977).

Results

Mean measured concentrations 4.2, 7.7, 10, 16 and 29 ng/L (91 - 116 % of nominal). No control mortality, 10 % mortality in solvent control, 5, 5, 15, 15 and 80 % mortality at 4.5 - 25 ng/L. LC_{50} for 96 hours exposure reported as 0.022 µg/L (95 % CL 0.016 – 0.029 µg/L), based on mean measured concentrations.

Remarks by RMS

Water quality parameters within accepted range. Recalculation of LC_{50} with Spearman-Kärber yielded similar results. The result LC_{50} 0.022 µg/L, based on mean measured concentrations, is used for risk assessment.

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*30-10-2007*
Materials and Methods	
Results and discussion	
Conclusion	[redacted]
Reliability	
Acceptability	
Remarks	
	COMMENTS FROM ...
Date	
Materials and Methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

98/8 Doc IIIA section No.	**7.4.1.2 / 21**	**Acute toxicity to invertebrates**
91/414 Annex Point addressed	**II 8.2.4 / 17**	**Acute toxicity to invertebrates**

		Official use only
Reference point (location) in dossier	**7.4.1.2/21**	
Title:	Acute toxicity of 3H-Avermectin B1 to Mysid shrimp (*Mysidopsis bahia*) of different ages under flow-through conditions	
Project/Report number:	88-4-2695	
Author(s):	Surprenant, D.C.	
Date of report:	24/06/1988	
Published:	Not published.	
Testing facility:	Springborn Life Science Inc., Wareham, United States.	
Study dates	17 – 21 March 1988	
GLP:	Yes.	
Deficiencies:	None	
Reliability indicator	1.	

Reference/notifier	:	Surprenant, D.C. (1988b)	GLP statement	:	yes
Type of study	:	mysid shrimp, acute toxicity	Guideline	:	EPA 1975 APHA 1980
Year of execution	:	1988	Acceptability	:	acceptable
Test substance	:	3H-abamectin, 11.8:1 B1a:B1b, batch [redacted], appearance clear solution			

Substance	Species	Method	T [°C]	pH	Duration [h]	Criterion	Value [µg/L]
^{3}H-abamectin	*Mysidopsis bahia*	flow-through	25	7.9	96	LC_{50}	0.020

Description

Methods. Acute toxicity of abamectin to commercially obtained juvenile *Mysidopsis bahia* of different ages, was tested under flow-through conditions. The test was performed with animals of ≤1, 4, 10 and 21 d old. ^{3}H-abamectin, 527 mCi/L, 11.8:1 B_{1a}:B_{1b}, tritium only present in avermectin B_{1a}. Nominal concentrations 2.6, 6.4, 16, 40, 100 and 250 ng/L, control, solvent control (acetone, 19 µL/L). Dilution with filtered natural seawater (5 µm mesh), salinity 31 ‰, pH 7.9. Two replicates with 10 organisms each, 1.2 L solution per unit flow-rate 11 volume renewals/day.
Conditions. Temperature 25 ± 1 °C, 16:8 h L:D (215 - 968 lux), no aeration, feeding with brine shrimp twice daily plus commercial feed 3 times per week.
Chemical analysis. Daily samples to determine actual concentrations. Samples extracted with ethylether and dried. Dry residues analysed by LSC, recovery 103 %, LOQ not reported.
Calculations and statistics. LC_{50}-value was calculated using moving average angle method

Results

Mean measured concentrations 1.3, 4.3, 11, 21, 52 and 98 ng/L (39 - 69 % of nominal). Mortality for different ages is given in the table below.

Table: Cumulative mortality of *Mysidopsis bahia* of different age after 96 h exposure to abamectin

Mean measured concentration	Mortality [%]			
[ng/L]	≤ 1 d	4 d	10 d	21 d
control	0	0	0	0
solvent	0	5	0	0
1.3	0	0	0	0
4.3	15	20	20	15
11	20	20	10	0
21	20	20	15	24
52	90	65	65	74
98	100	100	100	100

LC_{50} for 96 hours exposure reported as 0.020 µg/L (95 % CL 0.015 – 0.027 µg/L) for ≤ 1 d old mysids, 0.023 µg/L (95 % CL 0.017 – 0.033 µg/L) for 4 d old mysids, 0.026 µg/L (95 % CL 0.019 – 0.037 µg/L) for 10 d old mysids and 0.026 µg/L (95 % CL 0.020 – 0.037 µg/L) for 21 d old mysids, all based on mean measured concentrations.

Remarks by RMS

Water quality parameters within accepted range. LC_{50} tends to increase with increasing age, the lowest value is used. The result LC_{50} 0.020 µg/L, based on mean measured concentrations, is used for risk assessment.

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*30-10-2007*
Materials and Methods	
Results and discussion	
Conclusion	[redacted]
Reliability	
Acceptability	
Remarks	
	COMMENTS FROM ...
Date	
Materials and Methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

98/8 Doc IIIA section No.	**7.4.1.2 / 22**	**Acute toxicity to invertebrates**
91/414 Annex Point addressed	**II 8.2.4 / 18**	**Acute toxicity to invertebrates**

		Official use only
Reference point (location) in dossier	**7.4.1.2/22**	
Title:	Acute toxicity of MK-936 technical to embryos-larvae of eastern oysters (*Crassostrea virginica*)	
Project/Report number:	BP-83-7-76	
Author(s):	Ward, G.	
Date of report:	06/07/1983	
Published:	Not published.	
Testing facility:	Bionomics Inc., Florida, United States.	
Study dates	14 – 16 June 1983	
GLP:	Yes.	
Deficiencies:	None	
Reliability indicator	1.	X

Reference/notifier	:	Ward, G.S. (1983a)	GLP statement	:	yes
Type of study	:	oyster embryo, acute toxicity	Guideline	:	BMRL 1982
Year of execution	:	1983	Acceptability	:	acceptable
Test substance	:	abamectin technical, batch [redacted], purity [redacted] appearance white powder			

Substance	Species	Method	T [°C]	pH	Duration [h]	Criterion	Value [µg/L]
abamectin	*Crassostrea virginica*	static	21	8.0	48	EC_{50}	430

Description

Methods. Acute toxicity of abamectin to oyster embryos of *Crassostrea virginica* was tested under static conditions. Larvae were obtained by induced spawning of mature oysters. Nominal concentrations 50, 100, 200, 400, 800, 1600 and 3200 µg/L, control, solvent control (acetone 0.35 mL/L). Dilution with filtered natural seawater (5 µm mesh), salinity 24 ‰, pH 8.0. Three replicates with 26 each, 900 mL solution per test unit. Oysters were collected after 48 h and number of normal developed larvae was determined.
Conditions. Temperature 21 ± 1 °C, 16:8 h L:D, no aeration, no feeding.
Calculations and statistics. EC_{50}-value was calculated using moving average angle method.

Results

Normal larval development in control and solvent control, reduction in normal embryos was 13, 7, 12, 8, 91, 100 and 100% at 50 - 3200 µg/L. EC_{50} reported as 430 µg/L (95 % CL 280 – 580 µg/L), based on nominal concentrations.

Remarks by RMS

Water quality parameters within accepted range. The highest test concentrations above water solubility in freshwater (1.21 mg/L at 25 °C), but no flocculation reported for these concentrations. Recalculation with Spearman-Kärber yielded a somewhat higher EC_{50}-value (563 µg/L with 95% CL of 543 – 583 µg/L). The result EC_{50} 430 µg/L, based on nominal concentrations, is used for risk assessment.

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*30-10-2007*
Materials and Methods	
Results and discussion	
Conclusion	[redacted]
Reliability	
Acceptability	
Remarks	
	COMMENTS FROM ...
Date	
Materials and Methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

98/8 Doc IIIA section No.	**7.4.1.2 / 23**	**Acute toxicity to invertebrates**
91/414 Annex Point addressed	**II 8.2.4 / 19**	**Acute toxicity to invertebrates**

		Official use only
Reference point (location) in dossier	**7.4.1.2/23**	
Title:	Acute toxicity of MK-936 technical to pink shrimp (*Penaeus duorarum*)	
Project/Report number:	BP-83-8-92	
Author(s):	Ward, G.	
Date of report:	22/08/1983	
Published:	Not published.	

Testing facility:	Bionomics Inc., Florida, United States.	
Study dates	30 July - 03 August 1983	
GLP:	Yes.	
Deficiencies:	None	
Reliability indicator	1.	X

Reference/notifier	:	Ward, G.S. (1983b)	GLP statement	:	yes
Type of study	:	shrimp, acute toxicity	Guideline	:	BMRL 1982
Year of execution	:	1983	Acceptability	:	acceptable
Test substance	:	abamectin technical, batch [redacted], purity [redacted] appearance white powder			

Substance	Species	Method	T [°C]	pH	Duration [h]	Criterion	Value [µg/L]
abamectin	*Penaeus duorarum*	static	22	8.2	96	LC_{50}	1.6

Description

Methods. Acute toxicity of abamectin to pink shrimp *Penaeus duorarum* was tested under static conditions. Animals obtained from Big Lagoon, 33 mm long. Nominal concentrations 0.5, 1.0, 2.0, 4.0, 8.0 and 16 µg/L, control, solvent control (acetone 0.11 mL/L). Dilution with filtered natural seawater (5 µm mesh), salinity 28 ‰, pH 8.2. Four replicates with three organisms each, 15 L solution.
Conditions. Temperature 22 °C, 16:8 h L:D, aeration, no feeding.
Calculations and statistics. LC_{50}-value was calculated using binomial probability.

Results

No mortality in control and solvent control, mortality 17, 42, 25, 58, 50 and 100 % at 0.5 - 16 µg/L. LC_{50} 1.6 µg/L (95 % CL 0.5 – 16 µg/L), based on nominal concentrations.

Remarks by RMS

Water quality parameters within accepted range. Recalculation with Spearman-Kärber yielded a somewhat higher LC_{50}-value (3.5 µg/L with 95% CL of 2.8 – 4.3 µg/L). The result LC_{50} 1.6 µg/L, based on nominal concentrations, is used for risk assessment.

Addendum to the RMS remarks after evaluation under the BPD:

[redacted]

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*30-10-2007*
Materials and Methods	
Results and discussion	
Conclusion	[redacted]
Reliability	
Acceptability	
Remarks	
	COMMENTS FROM ...
Date	
Materials and Methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

98/8 Doc IIIA section No.	**7.4.1.2 / 24**	**Acute toxicity to invertebrates**
91/414 Annex Point addressed	**II 8.2.4 / 20**	**Acute toxicity to invertebrates**

		Official use only
Reference point (location) in dossier	**7.4.1.2/24**	
Title:	Acute toxicity of MK-936 technical to blue crabs (*Callinectes sapidus*)	
Project/Report number:	BP-83-7-85	
Author(s):	Ward, G.	
Date of report:	22/08/1983	
Published:	Not published.	
Testing facility:	Bionomics Inc., Florida, United States.	
Study dates	04 – 08 July 1983	

GLP:	Yes.	
Deficiencies:	None	
Reliability indicator	1.	X

Reference/notifier	:	Ward, G.S. (1983c)	GLP statement	:	yes
Type of study	:	crabs, acute toxicity	Guideline	:	BMRL 1982
Year of execution	:	1983	Acceptability	:	acceptable
Test substance	:	abamectin technical, batch [redacted], purity [redacted] appearance white powder			

Substance	Species	Method	T [°C]	pH	Duration [h]	Criterion	Value [µg/L]
abamectin	*Callinectes sapidus*	static	22	7.8	96	LC_{50}	153

Description

Methods. Acute toxicity of abamectin to blue crab *Callinectes sapidus* was tested under static conditions. Animals commercially obtained, 48 mm long. Nominal concentrations 31, 62, 125, 250, 500 and 1000 µg/L, control, solvent control (acetone 0.07 mL/L). Dilution with filtered natural seawater (5 µm mesh), salinity 18 ‰, pH 7.5 – 7.8. Four replicates with three organisms each, 15 L solution per test unit.
Conditions. Temperature 22 °C, 16 h L:D, aeration, no feeding.
Calculations and statistics. LC_{50}-value was calculated using the moving average angle method.

Results

No mortality in control and solvent control, mortality 8, 25, 33, 58, 92 and 100 % at 31 - 1000 µg/L. LC_{50} reported as 153 µg/L (95 % CL 119 – 251 µg/L), based on nominal concentrations.

Remarks by RMS

Water quality parameters within accepted range. The result LC_{50} 153 µg/L, based on nominal concentrations, is used for risk assessment.

Addendum to the RMS remarks after evaluation under the BPD:

[redacted]

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*30-10-2007*
Materials and Methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	
	COMMENTS FROM ...
Date	
Materials and Methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

98/8 Doc IIIA section No.	**7.4.1.2 / 25**	**Acute toxicity to invertebrates**
91/414 Annex Point addressed	**II 8.2.4 / 24**	**Acute toxicity to invertebrates**

		Official use only
Reference point (location) in dossier	**7.4.1.2/25**	
Title:	Acute toxicity of delta 8,9-cis Avermectin B1a to *Daphnia magna*	
Project/Report number:	32979	
Author(s):	Forbis, A.D., Georgie, L, Burgess, D	
Date of report:	22/10/1985	
Published:	Not published.	
Testing facility:	ABC Analytical Bio-Chemistry Lab. Inc., Columbia, United States	
Study dates	19 – 21 April 1985	
GLP:	Yes.	

Deficiencies:	Test concentrations were not confirmed by analysis	
Reliability indicator	1.	X

Reference/notifier	:	Forbis, A.D., Georgie, L. and Burgess, D. (1985b)	GLP statement	:	yes
Type of study	:	Daphnia, acute toxicity	Guideline	:	US EPA 1975 APHA 1980
Year of execution	:	1985	Acceptability	:	acceptable
Test substance	:	[8,9-Z]-avermectin B_{1a} (NOA 427011), batch [redacted]3, purity [redacted] appearance clear liquid			

Substance	Species	Method	T [°C]	pH	Duration [h]	Criterion	Value [µg/L]
[8,9-Z]-avermectin B_{1a}	*Daphnia magna*	static	18	8.5	48	EC_{50}	14

Description

Methods. Acute toxicity of [8,9-Z]-avermectin B_{1a} (NOA 427011) to *Daphnia magna* (< 24 h old) was tested under static conditions. Nominal concentrations 3.2, 5.6, 10, 18 and 32 µg/L, control, solvent control (ethanol 1.6 mL/L). Dilution with artificial freshwater, hardness 250 mg $CaCO_3$/L, pH 8.5, 200 mL solution per test unit. Duplicate vessels with 10 organisms each.
Conditions. Temperature 18 °C, 16:8 h L:D (538 - 753 lux), no aeration, no feeding.
Calculations and statistics. EC_{50}-value was calculated using the probit method.

Results

No immobilisation in control, solvent control, 3.2 and 5.6 µg/L treatments, 10, 85 and 100 % immobilisation at 10, 18 and 32 µg/L. EC_{50} reported as 14 µg/L (95 % CL 12 – 16 µg/L), based on nominal concentrations.

Remarks by RMS

Water quality parameters within accepted range. The result EC_{50} 14 µg/L, based on nominal concentrations, is used for risk assessment.

Addendum to the RMS remarks after evaluation under the BPD:

[redacted]

	Evaluation by Competent Authorities
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	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*30-10-2007*
Materials and Methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	
	COMMENTS FROM ...
Date	
Materials and Methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

98/8 Doc IIIA section No.	**7.4.1.2 / 26**	**Acute toxicity to invertebrates**
91/414 Annex Point addressed	**II 8.2.4 / 26**	**Acute toxicity to invertebrates**

		Official use only
Reference point (location) in dossier	**7.4.1.2/26**	
Title:	Acute Toxicity Test of NOA426289 (Metabolite of Abamectin) to *Daphnia magna* in a 48-hour Immobilization Test	
Project/Report number:	847403	

Author(s):	Bätscher R	
Date of report:	25/07/2003	
Published:	Not published.	
Testing facility:	RCC Ltd., Itingen, Switzerland.	
Study dates	16 – 23 June 2003	
GLP:	Yes.	
Deficiencies:	None	
Reliability indicator	1.	X

Reference/notifier	:	Bätscher, R. (2003b)	GLP statement	:	yes
Type of study	:	Daphnia, acute toxicity	Guideline	:	OECD 202 US EPA
Year of execution	:	2003	Acceptability	:	acceptable
Test substance	:	4"-oxo-avermectin B_{1a} (NOA 426289), batch [redacted], purity [redacted] appearence white powder			

Substance	Species	Method	T [°C]	pH	Duration [h]	Criterion	Value [µg/L]
4"-oxo-avermectin B_{1a}	*Daphnia magna*	static	20	7.8	48	EC_{50}	0.28

Description

Methods. Acute toxicity of 4"-oxo-avermectin B_{1a} (NOA 426289) to *Daphnia magna* was tested under static conditions. Nominal concentrations 0.025, 0.050, 0.10, 0.20, 0.40, 0.80 and 1.6 µg/L, control. Dilution with artificial freshwater, hardness 250 mg $CaCO_3$/L, pH 7.84, 500 mL solution per test unit. Four replicates with five organisms each.
Conditions. Temperature 20 °C, 16:8 h L:D (460 - 670 lux), no aeration, no feeding.
Chemical analysis. Samples of each test concentration at start and termination of test, analysis by HPLC-UV/VIS detection, recovery 101 %.
Calculations and statistics. EC_{50}-value was calculated using the probit method.

Results

Actual concentrations could not be determined due to low test concentrations. Actual concentrations in stock solutions were 95 % of nominal and effect concentrations were based on nominal concentrations. No immobilisation in control, 10, 25, 20, 25, 65, 70 and 95 % immobilisation at 0.025 - 1.6 µg/L. EC_{50} reported as 0.28 µg/L (95 % CL 0.067 – 0.10 µg/L), based on nominal concentrations.

Remarks by RMS

Water quality parameters within accepted range. The result EC_{50} 0.28 µg/L, based on nominal concentrations, is used for risk assessment.

Addendum to the RMS remarks after evaluation under the BPD:

[redacted]

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*30-10-2007*
Materials and Methods	
Results and discussion	
Conclusion	[redacted]
Reliability	
Acceptability	
Remarks	
	COMMENTS FROM ...
Date	
Materials and Methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

98/8 Doc IIIA section No.	**7.4.1.3 / 01**	**Growth inhibition test on algae**
91/414 Annex Point addressed	**II 8.2.6/01**	**Effects on algal growth**

		Official use only
Reference point (location) in dossier	**7.4.1.3/01**	
Title:	The effect of avermectin B1 to the freshwater alga *Selenastrum capricornutum*	
Project/Report number:	BP-81-7-127	
Author(s):	Hollister, T.A.	
Date of report:	24/07/1981	
Published:	Not published.	
Testing facility:	EG & G Bionomics, Marine Research Laboratory, Florida, USA	
Study dates	06 to 15 July 1981.	
GLP:	Yes.	
Deficiencies:	No analysis to confirm nominal a.s. concentrations and demonstrate stability during exposure.	
Reliability indicator	2.	X

Reference/notifier	:	Hollister, T.A. (1981a)	GLP statement	:	no
Type of study	:	algae, growth inhibition	Guideline	:	not reported
Year of execution	:	1981	Acceptability	:	not acceptable
Test substance	:	abamectin, batch [redacted], chemical purity [redacted] appearance white powder			

Substance	Species	Method	T [°C]	pH	Duration [d]	Criterion	Value [mg/L]
abamectin	*Pseudokirchneriella subcapitata*	static	24		9	E_bC_{10}	48

Description

Methods. Static growth inhibition test with abamectin, nominal concentrations 6, 12, 25, 50 and 100 mg/L, control, solvent control (acetone, 1 mL/L). Three replicates for test substance and controls, 50 mL test solution per test unit, initial cell density 1 x 10^4 cells/mL. Cells were counted after 9 days.
Conditions. Temperature 24°C, continuous light (4200 lux).
Calculations and statistics. ANOVA and Williams' test.

Results

Dry cell weight on day 9 was 107 ± 7 mg dwt/L in the controls. Dry cell weight was only significantly lower in the highest treatment. Calculated EC_{10}-value was 48 mg/L (95 % CL 40 – 58 mg/L).

Remarks by RMS

Test concentrations above water solubility (1.21 mg/L at 25 °C), actual concentrations not determined, visible precipitate reported in 25, 50 and 100 mg/L treatments. Dilution water not defined. The result is not used for risk assessment.

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*30-10-2007*
Materials and Methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	
	COMMENTS FROM ...
Date	
Materials and Methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

98/8 Doc IIIA section No.	**7.4.1.3 / 02**	**Growth inhibition test on algae**
91/414 Annex Point addressed	**II 8.2.6/02**	**Effects on algal growth**

		Official use only
Reference point (location) in dossier	**7.4.1.3/02**	
Title:	The Effect of Avermectin B1 to the freshwater alga *Selenastrum capricornutum*	
Project/Report number:	1047.056.430	
Author(s):	Gries, T.	
Date of report:	21/07/1999	
Published:	Not published.	
Testing facility:	Springborn Laboratories (Europe) AG, Horn, Switzerland	
Study dates	1999	
GLP:	Yes.	
Deficiencies:	None.	
Reliability indicator	1.	X

Reference/notifier	:	Gries, Th. (1999)	GLP statement	:	yes
Type of study	:	algae, growth inhibition	Guideline	:	OECD 201
Year of execution	:	1999	Acceptability	:	not acceptable
Test substance	:	abamectin, batch [redacted] chemical purity [redacted] appearance white powder			

Substance	Species	Method	T [°C]	pH	Duration [h]	Criterion	Value [mg/L]
abamectin	*Pseudokirchneriella subcapitata*	static	24	8	72	E_rC_{50} E_bC_{50}	

Description

Methods. Static growth inhibition test with abamectin, nominal concentrations 20, 30, 44, 67 and 100 mg/L, control. Three replicates for test compound and controls, 100 mL test solution per test unit, initial cell density 1 x 10^4 cells/mL. Medium pH 8, conductivity 225 µS/cm. Cells counted daily.
Conditions. Temperature 24 °C, continuous light (6200 - 6500 lux), continuous shaking.
Chemical analysis. Samples were taken at start and end of test. Analysis by HPLC-UV after addition of acetonitrile. Recovery 107 %, LOQ 1.22 - 1.26 mg/L.
Calculations and statistics. Area under the growth curve, specific growth rate per day and percentage inhibition were calculated from cell counts.

Results

Actual concentrations were 18.6, 29.8, 44.4, 67.4, 97.8 mg /L at start (93 - 101 % of nominal) and 16.8, 24.0, 40.1, 52.7 and 78.3 mg/L at end (78 - 91 % of nominal). Mean measured concentrations 17.7, 26.9, 42.3, 60.1 and 88.1 mg/L (88 - 96 % of nominal). Cell numbers in control increased by factor of 75 after 72 h, cell numbers 74.8, 75.8, 74.7 and 66.8 x 10^4 cells/mL at 20, 30, 44, 67 and 100 mg/L, respectively. Growth rate significantly reduced at 100 mg/L. Area under the growth curve not significantly reduced. E_bC_{50} and E_rC_{50} reported as >100 mg/L, based on nominal concentrations.

Remarks by RMS

The pH maximally deviated 2.43 units. On basis of the high growth rates of the algae, this deviation was considered not to have any impact on the results of the study. Test concentrations all > 10 times above water solubility (1.21 mg/L at 25 °C). LOQ at level of water solubility. Actual concentrations determined after addition of acetonitrile to the test flasks, thereby dissolving any precipitate or adsorbed compounds. Therefore, actual concentrations are not representative for dissolved abamectin concentrations. The results are not used for risk assessment.

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*30-10-2007*
Materials and Methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	
	COMMENTS FROM ...
Date	
Materials and Methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

98/8 Doc IIIA section No.	**7.4.1.3 / 03**	**Growth inhibition test on algae**
91/414 Annex Point addressed	**II 8.2.6/02**	**Effects on algal growth**

		Official use only
Reference point (location) in dossier	**7.4.1.3/03**	
Title:	Toxicity of NOA 448112 (Metabolite of MK936) to *Pseudokirchneriella subcapitata* (formerly *Selenastrum capricornutum*) in a 72-hour Algal Growth Inhibition Test	
Project/Report number:	812215	
Author(s):	Peither, A	
Date of report:	06/07/2001	
Published:	Not published.	
Testing facility:	RCC Ltd, Environmental Chemistry &Pharmanalytics Division, CH–4452 Itingen / Switzerland	
Study dates	11 May - 13 June 2001	
GLP:	Yes.	
Deficiencies:	None.	
Reliability indicator	1.	

Reference/notifier	:	Peither, A. (2001d)	GLP statement	:	yes
Type of study	:	algae, growth inhibition	Guideline	:	OECD 201
Year of execution	:	2001	Acceptability	:	acceptable
Test substance	:	8a-hydroxy-avermectin B_{1a} (NOA 448112), batch [redacted], chemical purity [redacted] appearance white powder			

Substance	Species	Method	T [°C]	pH	Duration [h]	Criterion	Value [mg/L]
8a-hydroxy-avermectin B_{1a}	*Pseudokirchneriella subcapitata*	static	22	7.5	72	E_rC_{50} E_bC_{50}	> 6.1 > 6.1

Description

Methods. Limit test with supersaturated dispersion of 8a-hydroxy-avermectin B_{1a} (NOA 448112). Nominal concentration 100 mg/L, prepared by ultrasonic treatment for 15 min. Three replicates for test compound, six controls, 15 mL test solution per test unit, initial cell density 1 x 10^4 cells/mL. Medium pH 7.5, total hardness 24 mg $CaCO_3$/L. Daily cell counting.
Conditions. Temperature 22 °C, continuous light (8400 lux), continuous shaking.
Chemical analysis. Samples taken at start and end. Analysis by HPLC-UV/VIS after dilution with acetonitrile. Recovery 102 %, and LOQ 0.055 mg/L.
Calculations and statistics. Area under the growth curve and specific growth rate per day were calculated from cell densities.

Results

Actual concentration 7.4 mg/L at start and 4.9 mg/L at end, mean measured concentration 6.1 mg/L (6.1 % of nominal). Average control cell density increased by factor of 11.6, cell density in saturated dispersion 10.3 x 10^4 cells/mL. Growth rate and biomass of controls and treated units were not significantly different.

Remarks by RMS

Water quality parameters within accepted range. Growth in control does not meet validity criterion of increase in cell numbers by factor of 16, but result is considered acceptable. The results E_rC_{50} and E_bC_{50} > 6.1 mg/L, based on mean measured concentrations, are used for risk assessment.

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*30-10-2007*
Materials and Methods	
Results and discussion	
Conclusion	[redacted]
Reliability	
Acceptability	
Remarks	
	COMMENTS FROM ...
Date	
Materials and Methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

98/8 Doc IIIA section No.	**7.4.1.3 / 04**	**Growth inhibition test on algae**
91/414 Annex Point addressed	**II 8.2.6/03**	**Effects on algal growth**

		Official use only
Reference point (location) in dossier	**7.4.1.3/04**	
Title:	A 72 hour toxicity test with the freshwater Alga (*Selenastrum capricornutum*)	
Project/Report number:	108A-215	
Author(s):	Sutherland, C.A., Kendall, T.Z, Krueger, H.O	
Date of report:	11/01/2000	
Published:	Not published.	
Testing facility:	Novartis Crop Protection Inc., Greensboro, United States Wildlife International Ltd., Easton, MD, United States	
Study dates	19 – 24 November 1999	
GLP:	Yes.	
Deficiencies:	None	
Reliability indicator	1.	X

Reference/notifier	:	Sutherland, C.A., Kendall, T.Z. and Krueger, H.O. (2000b)	GLP statement	:	yes
Type of study	:	algae, growth inhibition	Guideline	:	OECD 201
Year of execution	:	2000	Acceptability	:	acceptable
Test substance	:	[8,9-Z]-avermectin B (NOA 427011), batch no. not reported, chemical purity [redacted] appearance white powder			

Substance	Species	Method	T	pH	Duration	Criterion	Value
			[°C]		[h]		[µg/L]
[8,9-Z]-avermectin B_{1a}	*Pseudokirchneriella subcapitata*	static	24	7.5	72	E_rC_{50}	> 10
						E_bC_{50}	> 10

Description

Methods. Static growth inhibition test on algae with [8,9-Z]-avermectin B_{1a} (NOA 427011). Nominal concentrations 0.63, 1.3, 2.5, 5.0 and 10.0 mg/L, control, solvent control (DMF). Three replicates for test compound and controls, 100 mL test solution per test unit, initial cell density 1 x 10^4 cells/mL. Medium pH 7.5, conductivity 225 µS/cm. Daily cell counting.
Conditions. Temperature 24°C, continuous light (8000 lux), continuous shaking.
Chemical analysis. Samples were taken at start and end of test. Analysis by HPLC-UV after addition of methanol. Recovery 103 %, LOQ 0.40 mg/L.
Calculations and statistics. Area under the growth curve, specific growth rate per day and percentage inhibition were calculated from cell counts.

Results

Solutions at 5 and 10 mg/L appeared slightly cloudy white. Actual concentrations 0.622, 1.28, 2.29, 4.89 and 9.5 mg/L at start and 0.489, 0.933, 1.75, 4.15 and 8.43 mg/L at end, mean measured concentrations 0.56, 1.1, 2.0, 4.5 and 9.0 mg/L (80 - 90 % of nominal). Cell numbers in control increased by factor of 53 and by factor of 57 in solvent control. Cell numbers 50.9, 52.8, 52.8, 52.8 and 54.4 x 10^4 cells/mL at 0.56, 1.1, 2.0, 4.5 and 9.0 mg/L, respectively. Growth rate and area under the growth curve were not significantly reduced at any concentration. E_bC_{50}, E_rC_{50} and NOEC's reported as > 9.0 mg/L, based on actual concentrations.

Remarks by RMS

Water quality parameters within accepted range. Solubility of [8,9-Z]-avermectin B_{1a} not known, but assumed to be in the same order of magnitude as solubility of abamectin (1.21 mg/L at 25 °C). Solubility apparently exceeded at 5 and 10 mg/L nominal. Actual concentrations determined after addition of methanol, which may explain recovery of > 80 % in test solutions. As test concentrations do not exceed solubility by more than factor of 10, result is considered acceptable. The results nominal E_bC_{50} and E_rC_{50} > 10 mg/L are used for risk assessment.

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*30-10-2007*
Materials and Methods	
Results and discussion	
Conclusion	[redacted]
Reliability	
Acceptability	
Remarks	
	COMMENTS FROM ...
Date	
Materials and Methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

		Official use only
Reference point in dossier	**7.4.1.3/05**	
Title:	Toxicity OF A-8612 A to green algae (growth inhibition test)	
Project/Report number:	G 536 17 30	
Author(s):	Maetzler, P.	
Date of report:	30/10/1998	
Published:	Not published	
Testing facility:	Novartis Services AG	
Study dates	1998	
GLP:	Yes	
Reliability indicator	1	

Reference/notifier	:	Maetzler, P. (1998)	GLP statement	:	yes
Type of study	:	algae, growth inhibition	Guideline	:	OECD 201; EC L383 A, C.3
Year of execution	:	1998	Acceptability	:	acceptable
Test substance	:	A-8612, batch [redacted] purity 1.9 % abamectin, appearance yellow liquid			

Substance	Species	Method	T [°C]	pH	Duration [h]	Criterion	Value product [mg/L]	Value [mg as/L]
A-8612	*Pseudokirchneriella subcapitata*	static	22.5 - 24.0	7.6 - 9.9	72	E_bC_{50}	> 82	> 1.59
						E_rC_{50}	> 82	> 1.59

Description

Methods. Static growth inhibition test with A-8612 (Vertimec 0.18 EC, 1.946 % abamectin). Stock solution prepared in test medium and diluted to nominal concentrations of 4.3, 9.4, 21, 45 and 100 mg product/L (0.08, 0.18, 0.41, 0.88 and 1.9 mg as/L), and a control, additional vessel with 100 mg/L without algae. Artificial medium, pH 8. Three replicates per concentration, initial cell density 1 x 10^4 cells/mL. Cell density determined after 24, 48 and 72 hours with an electronic particle counter. Water quality parameters monitored.
Conditions. Temperature 22.5 - 24.0 °C, continuous light (104 µE/m^2.s), shaking.
Chemical analysis. Water samples control and 100 mg/L (with and without algae) at start and end. Samples diluted in methanol, analysis by HPLC-UV (244 nm). Concentrations calculated from avermectin B_{1a} and B_{1b}-peaks. LOD 0.02 mg abamectin/L.
Calculations and statistics. Specific growth rate and area under the curve calculated according to guideline, NOEC and EC_{50} not applicable.

Results

Recovery of fortified test medium 97.4 and 97.6 % for avermectin B_{1a} and 101.3 and 101.7 % for avermectin B_{1b}, both at fortification levels 0.071 and 0.879 mg/L. Actual concentration in highest test concentration with and without algae was 1.74 and 1.73 mg abamectin/L at start (89.4 and 88.9 % of nominal) and 1.43 and 1.38 mg abamectin/L at end (73.7 and 71.1 % of nominal). Mean measured concentration in vessel with algae 81.6 mg product/L.
Cell numbers in control increased by factor of 681. Growth rate at 100 mg product/L inhibited by 2.9 % as compared to control after 72 hours, slight stimulation (2.0 - 4.9 %) at other concentrations. Area under the curve inhibited by 6.3 and 17.6 % at 4.3 and 100 mg product/L, stimulation by 3.6 - 5.2 % at other levels. E_bC_{50} and E_rC_{50} reported as > 82 mg product/L, NOE_bC and NOE_rC as 82 mg product/L, all based on mean measured concentrations.

Remarks by RMS

The results E_bC_{50} and E_rC_{50} > 82 mg product/L (> 1.59 mg as/L) are used for risk assessment.

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*30-10-2007*
Materials and Methods	
Results and discussion	
Conclusion	[redacted]
Reliability	
Acceptability	
Remarks	
	COMMENTS FROM ...
Date	
Materials and Methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

98/8 Doc IIIA section No.	**7.4.1.4 / 01**	**Inhibition of microbiological activity**
91/414 Annex Point addressed	**II 8.7**	**Effects on biological methods for sewage treatment**

		Official use only
Reference point (location) in dossier	**7.4.1.4/01**	
Title:	Report on the test for activated sludge inhibition of abamectin (MK 936 A)	
Project/Report number:	982522	
Author(s):	Grade, R.	
Date of report:	22/04/1999	
Published:	Not published.	
Testing facility:	Novartis Crop Protection AG, Basel, Switzerland	
Study dates	04 September 1998.	
GLP:	Yes.	
Deficiencies:	None.	
Reliability indicator	1.	X

Reference/notifier	:	Grade, R. (1999b)	GLP statement	:	yes
Type of study	:	activated sludge respiration	Guideline	:	OECD 209
Year of execution	:	1999	Acceptability	:	acceptable
Test substance	:	abamectin, batch [redacted] chemical purity [redacted] %, appearance white powder			

Substance	Sludge source/type	Contact time [h]	T [°C]	Process	Criterion	Value [mg/L]
abamectin	municipal seawage treatment plant	3	20 ± 2	BOD	EC_{50}	> 100
					EC_{20}	> 100
					EC_{80}	> 100

Description

The effect of abamectin on respiration of activated sludge was determined according to OECD 209.
Test system. Stock solution prepared in dechlorinated tap water by 30 min. ultrasonic treatment and stirring for 1 hour. Stock added to dechlorinated tap water with nutrient solution, final concentrations 1.0, 3.2, 10.0, 32.0 and 100 mg/L. Activated sludge added to bottles, suspended solids concentration 1.75 g/L, pH 8.0. Two controls, and reference compound 3,5-dichlorophenol at 3.2, 10.0 and 32.0 mg/L. Biological oxygen demand measured during incubation for 3 hours.

Results

Oxygen consumption rate in controls 47.6 and 49.2 mg/L.h. Inihibtion in abamectin treatments 3 to 11 %, not related to concentration. Dose related inhibition in reference of 14, 57 and 85 % as compared to control. EC_{20}, EC_{50} and EC_{80} > 100 mg/L.

Remarks by RMS

Test concentrations above reported solubility of abamectin, likely that major part was adsorbed to suspended solids. The result EC_{20}, EC_{50} and EC_{80} > 100 mg/L is used for risk assessment.

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*01-11-2007*
Materials and Methods	
Results and discussion	
Conclusion	[redacted]
Reliability	
Acceptability	
Remarks	
	COMMENTS FROM ...
Date	
Materials and Methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

98/8 Doc IIIA section No.	**7.4.2/01**	**Bioconcentration**
91/414 Annex Point addressed	**II 8.2.3**	**Bioconcentration in fish**

		Official use only
Reference point (location) in dossier	**7.4.2/01**	
Title:	Uptake, depuration and bioconcentration of ^{3}H-avermectin B_{1a} by bluegill sunfish (*Lepomis macrochirus*).	
Project/Report number:	30261	
Author(s):	[redacted]	
Date of report:	08/08/1983	
Published:	Not published	
Testing facility:	[redacted]	
Study dates	1983	
GLP:	Yes.	
Deficiencies:	None.	

Reliability indicator	1.	X

Reference/notifier	:	[redacted] (1983)	GLP statement	:	yes
Type of study	:	fish, bioconcentration	Guideline	:	ASTM 1978 Hamelink 1977
Year of execution	:	1983	Acceptability	:	acceptable
Test substance	:	5-^{3}H-avermectin B_{1a}-, batch [redacted]			

Substance	Species	Duration [d]	Method	BCF [L/kg wwt]	Based on
^{3}H-avermectin B_{1a}	*Lepomis macrochirus*	28 uptake 14 depuration	flow-through	69 30 110	total ^{3}H in whole fish total ^{3}H in fillet total ^{3}H in viscera

Description

Methods. Bluegill sunfish were exposed to ^{3}H-avermectin B_{1a} for 28 days to measure uptake of the compound and then placed in clean water for 14 days to determine elimination rate. Fish were commercially obtained, acclimated for 14 days, mean weight 6.2 g and mean length 55 mm at test initiation. Flow-through system, continuous aeration, 70 L test solution per system, 110 fish per system, one control and one exposure level 0.1 µg/L. Daily observations. Fish and water were sampled weekly or biweekly.
Chemical analysis. Determination of ^{3}H-avermectin B_{1a} by LSC water samples, and in fish homogenates after combustion of fillet, viscera and whole fish. LOQ 0.043 – 0.045 µg/kg for fish samples and LOQ 0.0019 µg/L for water samples. Recovery 106 – 109 % for fish samples.
Calculations and statistics. Uptake and elimination rate constants were graphically determined. Bioconcentration factors were determined by calculating the ratio of the total radioactive residues in fish (plateau values) and the average concentration of the test substance in the water.

Results

No mortality or abnormal behaviour in control and treatment. Actual water concentrations in the treated system were 0.099 ± 0.019 µg/L during uptake period. Concentrations of ^{3}H-avermectin B_{1a} in fish increased until day 10. Plateau concentrations were 6.8 µg/kg wwt for whole fish, 3.0 µg/kg wwt for fillet and 11 µg/kg for viscera, resulting in bioconcentration factors of 69, 30 and 110 L/kg wwt, respectively. During the elimination phase of 14 days, ^{3}H-avermectin B_{1a} decreased to 0.32 µg/kg wwt in whole fish. Uptake rate constant was 11 L/kg wwt.d and elimination rate constant 0.21/d.

Remarks by RMS

Test criteria were met. BCF based on total radioactivity, transformation may have taken place and values are *worst case*. The results BCFs of 69, 30 and 110 L/kg wwt for whole fish, fillets and viscera, respectively, are used for risk assessment.

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*01-11-2007*
Materials and Methods	
Results and discussion	
Conclusion	[redacted]
Reliability	
Acceptability	
Remarks	
	COMMENTS FROM ...
Date	
Materials and Methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

98/8 Doc IIIA section No.	**7.4.3 / 01**	**Effects on aquatic organisms, further studies**
91/414 Annex Point addressed	**8.2.9**	

		Official use only
Reference point (location) in dossier	**7.4.3/01**	
Title:	Assessment of the potential biological effects of Abamectin MK 936, 018 EC (A-8612 A) exposures on aquatic ecosystems as measured in an outdoor microcosm tank system	
Project/Report number:	982570	
Author(s):	Rufli, H.	
Date of report:	20/12/1999	
Published:	Not published	
Testing facility:	Novartis Crop Protection AG, Basel, Switzerland	
Study dates	1999	
GLP:	Yes.	
Deficiencies:	None.	
Reliability indicator	1.	

Comment of CA: The summary and conclusions below in shaded *italics* were prepared by the notifier. The summary as copied from the DAR can be found on page 207 and following pages.	Official use only
Test system: *The purpose of this study was to assess the potential biological effects of abamectin (MK 936) administered as an 018 EC formulation (A-8612 A, VERTIMEC® 018 EC) on the structure and function of aquatic ecosystems as measured in field microcosms and to follow its chemical fate.* ***Description of Field Microcosm Installation:*** *Individual microcosm pond tanks fabricated in high-density polyethylene, each of 10 m^3 volume were employed for the study. Tanks were installed in the ground and were surrounded by soil in order to minimise rapid temperature fluctuations, and were individually connected to a communal supply pond, referred to as the "biotope". The dimensions and contents of the ponds were, as follows: dimensions - 3.0 m internal diameter, height 1.5 m; contents - 0.05 m of clay overlain with 0.10 m of sediment, overlain by approximately 1.35 m of water.* ***Preparation of Field Microcosms for Study:*** *A total of twenty-one microcosms were employed. The ponds were set-up three months in advance of the application date to allow a period of acclimitisation. After all microcosms were filled, water recirculation was initiated between all tanks in order to maximise the inter-tank water homogeneity. The recirculation/homo-genisation step was accomplished by pumping water into each microcosm from the nearby supply pond. Water from the "biotope" acted as the source matrix of algae, plankton, zooplankton and other organisms. Three different species of macrophytes (Haloragaceae (Myriophyllum verticullatum), Potamogetonaceae (Potamogeton crispus),*	

[2] Ganzelmeier *et al.* (1995): Studies on the spray drift of plant protection products, 111 pp.

Charophyceae (Chara gracilis)) were introduced into the system. Macroinvertebrates entered the ponds via aerial colonisation and egg deposition from adult insects native to the study site and by introducing sediment.

Treatment Rates and Application Details: *The 21 ponds were divided into triplicate controls and triplicate replicates treated with A-8612 A (VERTIMEC® 018 EC formulation, Batch No. HC 04200, 19.46 g/L, density 0.968 g/cm^3) to provide six different treatment levels. The formulation was dissolved and applied in bidistilled water. The specific dose levels were selected to bracket a theoretical PEC_{SW} burden range which may arise from calculated spray drift deposition inputs Ganzelmeier et al. (1995) [2]. Specific information concerning the preparation and application rates for the individual treatment rates is given in the table below.*

Concentrations of A-8612 A applied to outdoor microcosms

Treatment Group	Site Position No. of Mesocosm	No. of Applications	Application Details			
			Total per Pond (g)		Nominal Concn. * (µg/L)	
			A-8612 A	abamectin	A-8612 A	abamectin
Control	2 / 5 / 8	NA	0	0	0	0 (< 0.1)
1	6 / 9 / 17	1	0.033	0.00066	3.3	0.066 (< 0.1)
2	10 / 23 / 24	1	0.10	0.0020	10	0.20 (0.198)
3	15 / 19 / 21	1	0.31	0.0062	31	0.62 (0.52)
4	16 / 18 / 20	1	0.92	0.018	92	1.8 (1.00)
5	4 / 7 / 22	1	2.78	0.056	278	5.6 (3.05)
6	3 / 11 / 13	1	8.33	0.17	833	17 (8.78)

* *Based on a total water volume of 10,000 L in field microcosm.*
Values in brackets indicate mean measured abamectin concentrations for triplicate samples.

A re-circulation step was initiated in the ponds on Day 14 after treatment, where each pond underwent an exchange of approximately 1.7 m^3 per day.

Sampling and Monitoring: *Sampling intervals for the biological and analytical sampling and monitoring program related to this study varied with respect to the different parameters being monitored. Sampling commenced 14 days prior to application of the test substance (16 June, 1998) and continued until 91 days after last application (29 September, 1999).*

Water Quality Measurements – Dissolved O_2, pH, conductivity, temperature and NO_3 were measured.

Biological Monitoring – Sampling of representative water samples involved dividing each pond into quadrants, and sampling 4 sub-samples from each quadrant sector. The 16 samples per pond were then pooled (approx. 21 L in total), mixed and filtered through a 55 µm mesh - zooplankton species were retained on the mesh, collected, preserved in a mixture of ethylene glycol/water (80:20) and a sub-sample used for taxanomic identification.

Zooplankton density was analysed by counting under microscope. Phytoplankton loadings were quantified via use of a Delayed Fluorescent-Kinetics-Photometer to measure the chlorophyll a content and also via counting under microscope from sub-samples of the filtrate fraction of water samples. A further aliquot of the filtered sample was preserved and employed for the determination of total numbers and identification of individual taxa of phytoplankton via microscopic observation. Emergent insects were collected in the headspace of floating traps (one per tank). The overall surface area sampled for emerging insects was equivalent to 0.25m^2. Trapped macroinvertebrates were identified and abundances expressed

as the number of individuals emerging per m^2. *Biological data evaluation comprised of developing criteria and end-point definitions. The relevant end-points to be established for the phytoplankton, zooplankton and macroinvertebrate communities were defined as: total abundance; abundance of major taxonomic groups; abundance of individual taxa; taxa richness - based on lowest taxonomic level identified.* *Statistical evaluation was employed to analyse the dynamics of the biological communities in the treated and control systems.* ***Abamectin Analysis:** Water samples from the control and all of the treatment levels were sampled and analysed on Day 0 (2 hours after application) by HPLC-UV to establish actual water loadings of abamectin compared with nominal. The analytically determined (recovery corrected) water concentrations of abamectin are given in parentheses on Table 8.2.9-1. Only sediment and water samples from the highest treatment level ponds were sampled and analysed thereafter.* ***Findings:** Weather conditions during the test period were in the normal seasonal range expected for the area in which the test site is situated. Water temperature ranged from 14 to 27 ^{0}C over the test period. Oxygen saturation, pH, conductivity and redox potential were comparable at all timepoints across all treatment levels. Analysis of water samples for abamectin residues indicated a water-phase DT_{50} for abamectin of approximately 5 days. Detectable concentrations in sediment were limited to Day 0 (2 hours after application - average of 60.1 µg abamectin/kg sediment dry weight) for triplicate samples at the highest dose concentration. On Day 6, the average abamectin sediment concentration at the highest treatment level had fallen below the limit of quantification of 20 µg/kg sediment (dry weight). Based on this information the sedimentary DT_{50} for abamectin under semi-realistic outdoor field conditions are < 6-days.* ***Phytoplankton** – In total, 152 different groups of taxa were classified in the water samples. Approximately 12 individual taxa could be analysed by univariate statistics. Prior to application, phytoplankton abundance ranged from 3000 to 8000 cells/mL. Principal response curve (PRC) analysis indicated effects on the phytoplankton community at nominal concentrations of 5.6 and 17 µg ai/L treatment levels, between Day 6 and Day 49, where an increase in overall abundance compared to that in the control systems was observed. This result was confirmed by chlorophyll a analyses which showed increased abundances at treatment levels > 1.8 µg/L (Day 3 through Day 13). The relatively enhanced biomass reflects a corresponding reduction in grazing zooplankton over the acute time-window. The phytoplankton communities at treatment levels of < 1.8 µg/L were unaffected. Behaviour and abundance of phytoplankton communities were comparable to those in the control systems from Day 63 on.* ***Zooplankton** – Zooplankton were characterised into 34 different taxanomic groups. Four dominant groups of zooplankton taxa were identified: Brachionidae, Ostracoda, Cyclopidae, Daphnidae. At the start of the study zooplankton abundance ranged from 400 to 1000 organisms/mL. PRC was initially used to identify any deviations from the control and treated microcosms. These observations were then applied to univariate (Dunnett's Test) analysis by segmenting the study period into 3 different assessment periods:, Day -7 to 0 (pre-application), Day 1 to 49 (effect window), and Day 63 to 91 (recovery window). The initial PRC step indicated clear changes in zooplankton community structure with dose-related effects on total zooplankton abundances following application in the Day 1 to Day 49 time window at nominal treatment levels of 5.6 and 17 µg/L. Concentrations of 0.62 and 1.8 µg/L were less affected and results from the 0.066 and 0.20 µg/L levels were comparable to controls. Diversity analysis reveals significant effects at 17 µg/L. The observation was further analysed by similarity analysis which indicated community differences at the 5.6 and 17 µg/L treatment levels. Analysis of taxanomic data suggested that Ostracoda may be used as a good*	

representative of the overall zooplankton community, based on PRC determination. The most sensitive organisms belonged to the order of Cyclopoida.species and abundances were negatively influenced at a concentration of 0.2 µg ai/L. The abundance of Ostracoda was statistically significantly reduced at dose levels of 5.6 and 17 µg ai/L. However, concentrations of 0.2 µg ai/L enhanced the denisty of the Ostracoda from Day 20 to Day 49. No statistically significant effects on this subclass were determined from Day 63 on. The EC_{50} value for Cyclopoida was determined to be 0.067 µg ai/L (during the effect window, Day 1 through Day 49). Employing this taxa as the model for zooplankton communities and combining results from univariate analysis, principal response curve, and diversity and similarity indices yields an $NOEC_{community}$ of 0.066 µg/L during the acute effect window. Complete recovery of most taxa was apparent at treatment levels up to, and including, 5.6 µg/L.

Emergent Insects *– PRC analysis indicated that reductions in abundance of emerging insects were apparent at treatment levels ≥ 0.62 µg/L. Dominant emergent insects were Chironomidae, Chaoboridae and Baetidae which contributed substantially to the total abundance of emergent insects (38 species identified). Recovery of the community was observed from Day 63 on. PRC analysis suggests that the different insect sub-groups all reacted in a similar manner indicating that these groups were representative species for the insect community. Chironomidae had the highest abundance during the study and underwent a significant reduction at the 5.6 and 17 µg/L treatment levels. Chaoborus was affected significantly down to 0.62 µg/L, whereas no decline of Baetidae was observed at any of the test concentrations. Statistical evaluation of the data for emergent insects, at a community basis, proposes an EC_{50} of 0.14 µg/L during the acute effect time (Day 6 through Day 49). The overall NOEC for emergent insects was 0.066 µg ai/L.*

Further to the derivation of community level NOEC values, the study has also considered the attenuation of recovery between species and individual taxa. Based on the biological data, an EAC (Ecological Acceptable Concentration) value can be derived where within a given time-frame, all of the various species and taxa studied recover and re-populate within their relevant test system. From this an EAC of 5.6 µg abamectin/L is calculated for the system following one single application of A-8612 A.

EC_{50} values derived from the field microcosm study for a variety of zooplankton and emergent insect species are compiled below.

EC_{50} values for zooplankton and aquatic insects following application of A-8612 A to outdoor microcosms

Class	*Species*	*Assessment Time-Window*	*EC_{50} (95% confidence limits) (µg abamectin/L)*
Zooplankton / Crustacea	*Ostracoda*	*Day 1 – Day 49*	*0.708 (0.029 – 1.592)*
	Simocephalus vetulus	*Day 1 – Day 49*	*0.990 (0.001 – 2.589)*
	Cyclopoida	*Day 1 – Day 49*	*0.067 (0.001 – 0.307)*
	Keratella quadrata	*Day 1 – Day 49*	*4.690 (-)*
	Lecane sp.	*Day 1 – Day 49*	*- (-)*
	Daphnia longispina	*Day 1 – Day 49*	*0.284 (0.000 – 0.735)*
Insects	*Arthropoda*	*Day 6 – Day 49*	*0.218 (0.000 – 1.511)*
	Chaoburus	*Day 6 – Day 49*	*0.137 (0.022 – 0.322)*
	Chironomidae	*Day 6 – Day 49*	*0.180 (0.000 – 1.898)*
	Ephemeroptera	*Day 6 – Day 49*	*0.5525 (-)*

Reference/notifier	:	Rufli, H. (1999)	GLP statement	:	Yes
Type of study	:	outdoor microcosm, 22 w, single application	Guideline	:	Guidance document on testing procedures for Pesticides in Freshwater Mesocosm (1991); Workshop on Aquatic Microcosms for Ecological Assessment of Pesticides (1991); EWOFFT (1992); Draft OECD-Guidelines for Testing of Chemicals (1993).
Year of execution	:	1998	Acceptability	:	acceptable
Test substance	:	A 8612 A, (Vertimec 0.18 EC), batch [redacted] purity 19.46 g as/L, appearance light yellow liquid			

Substance	Species	Method	T [°C]	pH	Duration [d]	Criterion	Value [µg as/L]
A 8612 A	phytoplankton, zooplankton, emerging insects	outdoor microcosm	14-27	7.5-9.8	91	$NOEC_{community}$	0.066
						NOEAEC	1.8

Description

Outdoor microcosm study with single substance application of A 8612 A (Vertimec 018 EC).
Guidelines. The test is conducted in accordance with the Guidance document on testing procedures for Pesticides in Freshwater Mesocosm (1991), the report of the Workshop on Aquatic Microcosms for Ecological Assessment of Pesticides (1991), the European Workshop on Freshwater Field Tests (EWOFFT) (1992) and the Draft OECD-Guidelines for Testing of Chemicals (1993).
Test design. Polyethylene tanks (depth 1.5 m, diameter 3 m, volume 10 m^3), located at the test site of Syngenta in Stein, Aargau, CH, had been established in Spring 1996 and used for experiments since then. Sediment type sandy loam, layer depth ca. 10 cm at time of construction, on a 5 cm clay layer. Cosms were set up 3 months before application. The macrophytes (*Myriophyllum verticulatum* and *Potamogeton crispus*) were planted. Water was circulated from a supply pond until 1 day before application. Algae, zooplankton and other organisms were introduced with the water of the supply pond, macroinvertebrates were introduced via aerial colonisation and by adding sediment from the natural supply pond. Zoo- and phytoplankton were analyzed 14 days, 7 days before application and just before application, emergent insects 14 days before application and just before application. No statistical significant differences in species composition were present between cosms of the different treatments, before treatment. Recirculation was started again 14 days after application.
Application, concentrations, replicates. Test substance diluted in double distilled water and sprayed on the water surface in a single application on June 30, 1998. Six dose levels, 3.3, 10. 31. 92, 278 and 833 µg product/L, equivalent to 0.066, 0.20, 0.62, 1.8, 5.6 and 17 µg as/L. Three replicate tanks per dose and three controls.
Biological observations. Effects on zooplankton and phytoplankton were assessed on day 1, 3, 6, 13, 21, 28, 35, 49, 63, 77 and 91 after application. Zooplankton species were identified and counted. For phytoplankton, chlorophyll *a* was measured and number of cells was counted, and individual species of algae were determined. Emerging insects were sampled 6, 13, 21, 35, 49, 63, 77 and 91 days after application. Emergent insects were captured using 1 floating trap per tank that was left in place for 1 week to allow emerging insects to be trapped, and identified. Traps were made of a submerged PVC cylinder, covered with a gauze pyramid (base area = 0.25 m^2).
Environmental conditions. Weather conditions on the day of application: wind speed 0.5-4.5 m/s, 24.5 °C, no rain. During the test period temperature varied between 14 and 27 °C and pH between 7.5 and 9.8. From day 0 to 28, oxygen concentration ranged between 105 and 260 % of saturation. From day 35 to 91, oxygen concentration ranged between 50 to 246 % of saturation.
Verification of concentrations. Water and sediment samples were taken from the control and highest concentration 7 days before application and at regular time intervals after application, additional sampling from all tanks 2 hours after application. Water analysed according to analytical method AM98-07. Water samples were concentrated by SPE, columns were eluted with acetonitrile. Eluate was made up to volume with bidistilled water and analysed by HPLC-UV (245 nm). LOQ 0.1 µg/L, recovery 89.6 %. Application solutions were analysed according to method AM98-07a without pre-concentration, LOQ 0.5 µg/L. Sediment analysed according to method AM99-03. Sediment and interstitial water were separated by centrifugation. Water was cleaned up by SPE, elution with water/acetonitrile 7/3 (v/v), analysis by HPLC-UV (245 nm). Sediment was extracted by shaking with methanol, extracts were diluted with water and cleaned up by SPE, analysis by HPLC. LOQ for sediment 1 mg/kg, for interstitial water 0.2 mg/L, recovery for both 82 %.

Calculations and statistics. For phytoplankton, zooplankton and emergent insects, the community response was studied using multivariate (PRC) and univariate (Dunnett's test) statistics. For all three organism groups, the Shannon diversity index and the Bray-Curtis similarity index was calculated and compared between treatments. For zooplankton and emergent insects, the effects on the dominant groups were assessed separately by univariate analysis. These tests were performed for two aggregated time intervals: day 1-49 and day 63-91 (and the period before application).

Results

Chemical analysis. Concentrations in control and in interstitial water were always < LOQ. Concentrations two hours after application were < LOQ at test concentration 0.066 µg as/L, and on average 99, 84, 54, 55 and 53 % of nominal at test concentrations 0.20, 0.62, 1.8, 5.6 and 17 µg as/L, respectively. Measured concentrations in water and sediment of the highest test concentration (17 µg as/L) during the test are given in the table below.

Table: Measured concentrations in dose level 17 µg as/L (240 g as/ha)

	measured concentrations of abamectin					
	water [µg/L]			sediment [µg/kg dwt]		
Time	tank 1	tank 2	tank 3	tank 1	tank 2	tank 3
2 h	7.00	14.6	4.74	58.4	72.5	49.4
1	12.7	10.4	10.6			
3	9.45	10.1	9.49			
6	7.83	7.87	8.60	< LOQ	23.1	< LOQ
13	5.46	6.18	4.56	< LOQ	< LOQ	< LOQ
21	0.858	0.844	0.853			
28	0.201	0.234	0.208	< LOQ	< LOQ	< LOQ
35	< LOQ	< LOQ	0.100			
49	0.234	< LOQ	< LOQ			

Phytoplankton.

The Principal Response Curves (PRC) showed an increase of phytoplankton from day 6 in the 1.8, 5.6 and 17 µg as/L treatments, probably due to reduced consumption as a result of a decrease of zooplankton foraging on algae. Tested with a crossvalidaton/Jackknife methodology, these differences were statistically significant at day 13 and 28 for the 1.8 µg as/L treatment, at day 6, 13, 28 and 49 for the 5.6 µg as/L treatment and at day 6, 21 and 49 for the 17 µg as/L treatment. In the lower treatments, the 0.066 µg as/L treatment showed no effect, the 0.2 µg as/L treatment showed a non significant decrease and a significant increase on day 63. The 0.62 µg as/L treatment showed a significant increase at day 63 and a significant decrease of abundancy at day 91. Univariate analysis was applied for 12 species, but results were not given in the report. The effects were mentioned to be supported by chlorophyll *a* measurements, although recovery was faster (recovery at day 28 in all treatments) for chlorophyll. The Shannon Diversity index was significantly lower (Dunnett's test) for the 0.62 µg as/L treatment only at days 49 and 91. The Bray-Curtis Index showed some significant differences between treatments and control. However, these differences did not seem to be dose-related.

Zooplankton.

The PRC showed a decrease of zooplankton from day 3 onwards in all concentrations. For the lowest concentration, these differences were not statistically significant. For the 0.2 µg as/L treatment, the difference was significant only at day 13. For the 0.62 µg/L treatment, differences were significant from day 1 until day 21 and for 1.8 µg as/L from day 3 until day 21. For 5.6 and 17 µg as/L, the effect lasted from day 1 to day 49. For the 17 µg as/L treatment, a significant difference is also found at day 77, due to an increase in algae abundance. A further detailed analysis of the dominant zooplankton taxa was made for Rotatoria and Arthropoda. *Keratella quadrata* abundance was significant lowered in the period from day 1 to 49 at the two highest and the two lowest concentrations. For 0.62 and 1.8 µg as/L, no significant effects were found. At later time points no significant effects appeared at any of the test concentrations.
The dominant Arthropoda groups were the Cladocera, the Ostracoda and the Cyclopoida. Within the Cladocera, *Daphnia longispina* and *Simocephalus vetulus* were the dominant species. Significant decreases for the period day 1 to 49 were found at 1.8, 5.6 and 17 µg as/L for *D. longispina* and at 5.6 and 17 µg as/L for *S. vetulus*. In the lowest two concentrations (0.066 and 0.2 µg as/L), a significant increase of *S. vetulus* abundance compared to the controls was found, possibly due to abundance reduction by the competitive grazer *D. longispina*.

The Ostracoda showed a significant decrease at 5.6 and 17 µg as/L during the period of day 1 to day 49. At 0.2 µg as/L, a significant increase of Ostracoda abundance was found from day 20 to day 49. For the Cyclopoida a decrease is found for all concentrations ≥ 0.2 µg as/L for the period day 1 – 49. From day 63 on, only a significant increase in Cyclopoida is found for the highest treatment.
Emergent insects.

PRC analysis showed that abundance of emergent insects was affected by concentrations ≥ 0.62 µg as/L during the timeframe of day 6 - 49. The effects appeared to be caused dominantly by *Chironomus* and *Chaoborus*. Diversity and similarity indexes only showed some significant differences at the highest treatment levels but after day 49 no significant differences between treatments and controls were indicated by the 2 indexes. Significant effects were found for the 2 highest concentrations for the total abundance and *Chironomus* for the period day 1 - 49. For *Chaoborus*, effects were significant at levels ≥ 0.62 µg as/L for the period day 6 - 49. For Ephemeroptera, no significant differences were found.
Macrophytes.

Macrophytes were not monitored during the test period.

Conclusion

From the results, the author established a $NOEC_{community}$ of 0.066 µg as/L. Based on the recovery of the community an EAC (Ecological Acceptable Concentration) of 5.6 µg as/L was established. According to the author at this concentration complete recovery of the affected communities within the period of the experiment (91 days), indicating no long term effect for the zooplankton assemblage.

Remarks by RMS

Fate part of study was evaluated in Document IIIA reference point 7.1.2.2.2/04. Author reported total recovery just after application as 83 %, 69 % in water and 14 % in sediment. As was pointed out in Document IIIA reference point 7.1.2.2.2/04 the mass balance for the highest dose level is 64, 126 and 65 % (average 85 %), mass balances for the other levels cannot be calculated. Metabolites were not analysed.
According to the EU guidance document on Aquatic Ecotoxicology, effects in micro- or mesocosm studies may be classified according to Brock *et al.*, (2000). For this study an adapted classification is used, in which especially the duration of the effects is taken as an extra criterion:

- Class 1: *no observed effects*
- Class 2: *slight effects*: (a) slight and transient, and (b) short-term and/or quantitatively limited response of sensitive endpoints, and (c) on individual time-points;
- Class 3: *large, acute/short-term effects, lasting < 8 weeks*: (a) a very clear response of sensitive endpoints, but a total recovery within 8 weeks after the last application, and (b), the total time span of effects does not exceed 8 weeks, and (c) "temporary effects on more sensitive species", or "temporary elimination of sensitive species", or "temporary effects on less sensitive species or endpoints", and (d) on some consecutive time-points;
- Class 4: *large, acute/short-term effects, lasting > 8 weeks, but full recovery within 8 weeks post last application.* a very clear response of sensitive endpoints, but a total recovery within 8 weeks after the last application, but the total time span of effects is larger than 8 weeks and on some consecutive time-points; or
  large effects in short-term test: very clear effects (e.g. large reductions of functional endpoints and elimination of sensitive species) during the whole test, though the test duration is too short to demonstrate a complete recovery within 8 weeks after the (last) application of the pesticide;
- Class 5: *large, long-term effects, without recovery*: (a) very clear response of sensitive species and recovery of species after >8 weeks after the last application, and (b) "long-term effects on more sensitive

species", or "elimination of sensitive species", or "effects on less sensitive species or endpoints", and (c) on various consecutive time-points.

Phytoplankton.

Univariate analysis of phytoplankton species were not given in the report. The principal response curves showed an increase of phytoplankton from day 6, lasting until day 77 for the 1.8, 5.6 and 17 µg as/L treatment. Significant differences are however found on a number of individual points in time, not in an unbroken series. Therefore these effects are classified as class 3 effects. For the 0.62 µg as/L treatment, a smaller and shorter lasting effect is found (two time-points), on day 63 and 91 significant, as calculated with the Jackknife method. The 0.62 µg/L treatment were classified as class 2 effects, because significant effects computed with community parameters took place on only 2 not connected points in time. For the 0.066 µg/L treatment the Bray-Curtis similarity index shows some significant differences. Since these differences are not dose related and only found for this index, they are not taken into account for the classification of the effects. For the 0.2 µg as/L treatment a siginificant increase was found after 63 days, and these effects were classified as class 2 effects.

Zooplankton.

The PRC analyses of the zooplankton and the testing with the Jacknife method showed no significant effects at 0.066 µg as/L (class 1), effects at one point in time at 0.2 µg as/L (class 2), a significant decrease at 4 underbroken points in time for the 0.62 and 1.8 µg as/L treatment (class 3) and a significant effect from day 1 to 49 at 5.6 and 17 µg as/L. These treatments appear to be recovered at day 63. Since the 8 weeks limit is between 49 and 63 days, the effects are classified as class 4 effects.
For the Rotatoria (*Keratella quadrata)* a significant decrease was found in the period 1 – 49 days in the two lowest and highest treatments. These effects are classified as class 3 effects, since the PRC shows a clear recovery at day 49. Individual points of time were not tested, but at tue 0.61 and 1.8 µg as/L treatment clear effects appeared to be visable at individual time points (class 2). Since the effects would thus not be dose related, but clear effects nevertheles appear to be present, it is proposed to give all treatments class 2-3 effects.
For the Cladocera a clear significant decrease of abundance was found in the three highest treatments, however with recovery within 8 weeks (Class 3). In the two lowest treatments an increase of *Simocephalus vetulus* was found for a number of consequetive points in time (class 3).
For the Ostracoda the significant decrease at 5.6 and 17 µg as/L during the period of day 1 to day 49 is classified as class 3. Although not significant for the aggregated period 1-49 days, the 1.8 µg as/L treatment shows a clear effect in the PRC and is classified as class 2. At 0.2 µg as/L, a significant increase of Ostracoda abundance was found from day 20 to day 49, since these effect is found at this concentration only, it is not taken into account for the classification.
For the cyclopoida, significant short term effect were found for all treatments $\geq$ 0.2 µg as/L (class 3). In the highest treatment in the second period analysed, a significant increase is found in the 17 µg as/L treatment. This effect does not seem to be correlated with the decreasing effect in the first period analysed, so effects in this treatment are classified as class 3 effects.
Emergent insects.

PRC analysis showed that abundance of emergent insects was affected by concentrations $\geq$ 0.62 µg as/L during the timeframe of day 6 – 49. However, the Jackknife method showed that the macroinvertebrate community is significantly different from the controls on day 91 in the 1.8, 5.6 and 17 µg as/L treatments. Univariate analyses for individual species showed no significant differences between treatments and controls on day 91. At this date, numbers of emerging insects were relatively low, both in the treated as in the untreated cosms, although in the treated cosms more often zero emerging individuals were found for one of the emerging families. Since number of emerging individuals were also very low in the lowest treatments, in which no significant effects were found at all, differences are considered not to be caused by the application. Moreover, the life cycle of the organisms present in the cosms is completed within 2 months and a second generation of emerging insects is probably not affected due to very low compound levels. Furthermore, in some of the higher treatments, emergence of some Notonecta individuals was found, probably increasing the difference with the control as calculated with the Jackknife method. In the second mesocosm experiment, described hereafter, no indications for a second effect on emergence after recovery was found, and effects on the larvae of the organisms considered only suffered from

shorter term effects. The combination of these considerations results in the classification of effects in the 0.62, 1.8, 5.6 and 17 µg as/L treatments as class 3 effects.
For the Nematocera signifcant effects were found in the case of *Chaoborus* for treatments of 0.620 µg as/L and higher for a number of points in time, but with recovery within 8 weeks.

Macrophytes.

Macrophytes were not monitored during the test period. However, taking the mode of action of the substance into consideration, no great direct effect of the substance on macrophytes is expected.
A summary of the effects according to this classification is given in the table below.

Table: Summary of the effect classes observed for several endpoints in the outdoor microcosm study with Vertimec 018 EC

	Nominal concentration [µg as/L]					
	0.066	0.2	0.62	1.8	5.6	17
Phytoplankton	1	2↑	2↑↓	2↑	3↑↓	3↑
Zooplankton	1	2↓	3↓	3↓	4↓	4↓
Rotatoria	2-3↓	2-3↓	2-3↓	2-3↓	2-3↓	2-3↓
Cladocera	3↑	3↑	1	3↓	3↓	3↓
Ostracoda	1	1	1	2↓	3↓	3↓
Cyclopoida	1	3↓	3↓	3↓	3↓	3↓↑
Emerging insects	1	1	3↓	3↓	3↓	3↓
Nematocera	1	1	3↓	3↓	3↓	3↓

From the table above it can be concluded that the NOEC for some individual species < 0.066 µg as/L. For community parameters it can be concluded that the $NOEC_{community}$ is 0.066 µg as/L. According to the Guidance Document on Aquatic Ecotoxicology, the No Observed Ecologically Adverse Effect Concentration (NOEAEC) is the concentration at or below which no long-lasting adverse effects were observed in a particular higher-tier study (e.g. mesocosm). No long-lasting effects are defined as those effects on individuals that have no or only transient effects on populations and communities and are considered of minor ecological relevance (e.g. effects that are not shown to have long-term effects on population growth, taking into account the life-history characteristics of the organisms concerned). According to this definition, class 3 effects are judged as ecologically acceptable. From the table above it can be derived that the NOEAEC can be set at 1.8 µg as/L.
A summary of the endpoints as derived from this study is given below in the table below.

Table: Summary of the several endpoints in the outdoor microcosm study with Vertimec 018 EC, values based on nominal concentrations.

	NOEC [µg product/L]	NOEAEC [µg product/L]	NOEC [µg as/L]	NOEAEC [µg as/L]
Phytoplankton	3.3		0.066	
Zooplankton	3.3		0.066	
Emerging insects	10		0.2	
Community	3.3	92	0.066	1.8

Because 14 days after application recirculation was allowed, recovery was accelerated by offering the possibility for organism to migrate and recolonise the affected systems. Therefore, the study setup is applicable to a single dose application in a flowing water body, and it is expected that effects in a static system with lower recolonisation potential would be more pronounced. Water circulation rate was 1.7 m^3/d, corresponding residence time is 5.9 days. Measured concentrations at the level of the NOEAEC within 2 hours after application were 54 % of nominal. This may be due either to insufficient mixing at the time of sampling, or to the fact that rapid initial sorption has occurred. The former explanation is favoured by the analysis results of the highest treatment level, where concentrations in the water phase reached their maximum 1 - 3 days after application. Given the fact that the mass balance at 17 µg as/L was > 80 %, and assuming that substance application in other treatments was comparable, the nominal NOEAEC is considered acceptable for risk assessment. The nominal NOEAEC of 1.8 µg as/L is used for risk assessment in (slowly) flowing water bodies.

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*01-11-2007*
Materials and Methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	
	COMMENTS FROM ...
Date	
Materials and Methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

98/8 Doc IIIA section No.	**7.4.3 / 02**	**Effects on aquatic organisms, further studies**
91/414 Annex Point addressed	**8.2.9**	

		Official use only
Reference point (location) in dossier	**7.4.3/02**	
Title:	Assessment on the effects of abamectin 018 EC (A8612A) in outdoor microcosms	
Project/Report number:	2002590	
Author(s):	Knauer, K.	
Date of report:	10/04/2002	
Published:	Not published	
Testing facility:	Syngenta Crop Protection AG, Basel, Switzerland	
Study dates	17 April 2000 – 15 March 2001	
GLP:	Yes.	
Deficiencies:	None.	
Reliability indicator	1.	

Comment of CA: The summary and conclusions below in shaded *italics* were prepared by the notifier. The summary as copied from the DAR can be found on page 219 and following pages.	Official use only
Material and methods: ***Test system:*** *Twenty-one outdoor aquatic microcosms were treated with an emulsifiable concentrate (EC) formulation (VERTIMEC® 018 EC ; A-8612A) containing 19.6 g abamectin/L, batch. No. HC 04200, to determine the potential ecological effects on freshwater populations and communities. Each microcosm contained sediment and approximately 10 m^3 water which was sourced from a nearby semi-natural pond. The test substance was applied by hand-held sprayer to simulate spray drift entry into surface water. Three replicate microcosms were used for the control and each treatment level.* ***Treatment rates:*** *Six treatments that comprised applications at 0.071, 0.21, 0.64, 1.9, 5.8 and 17 g abamectin/ha were made three times at weekly intervals on May 9th, 16th and 23rd, 2000. Assuming total mixing of the application solution with the pond water, nominal treatment concentrations were 0.245, 0.736, 2.21, 6.63, 19.9 and 59.9 µg VERTIMEC® 018 EC/L. These were equivalent to nominal concentrations of 0.005, 0.015, 0.045, 0.135, 0.405 and 1.22 □g abamectin/L in pond water.*	

Treatment rates

Dose Level	*Amount of added VERTIMEC® 018 EC per pond (mg/pond)*	*Treatment rates of VERTIMEC® 018 EC (g/ha)*	*Treatment rates of abamectin (g/ha)*	*Nominal Conc. Of VERTIMEC® 018 EC (µg/L)*	*Nominal Conc.of abamectin (µg/L)*
D 0	*0.00*	*0.00*	*0.000*	*0.000*	*0.000*
D 1	*2.45*	*3.47*	*0.071*	*0.245*	*0.005*
D 2	*7.36*	*10.4*	*0.21*	*0.736*	*0.015*
D 3	*22.1*	*31.3*	*0.64*	*2.21*	*0.045*
D 4	*66.3*	*93.8*	*1.9*	*6.63*	*0.135*
D 5	*199*	*282*	*5.8*	*19.9*	*0.405*
D 6	*599*	*847*	*17*	*59.9*	*1.220*

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The test substance solutions were applied by spraying on the water surface using a Birchmeier Super Star 1.25 with 2 bar pressure tank containing the relevant concentrations of the test substance. Applications were performed in sequence from the lowest to the highest treatment level. All surface applications for a given treatment level were completed before switching to the next higher treatment level. The application solutions contained nominal concentrations of 2.45, 7.36, 22.1, 66.3, 199 and 599 mg VERTIMEC® 018 EC/L. ***Physico-chemistry and biological evaluation:*** *The microcosms were monitored from May to September 2000. Water physico-chemistry such as dissolved oxygen, pH, conductivity and temperature were always measured once on each sampling date. Samples for nitrate analysis were taken as a subsample from the water specimens for residue monitoring.* *Abundances of phytoplankton, zooplankton, macroinvertebrate and emergent insect taxa were measured before, during and after treatment to assess the responses of the aquatic community to the test substance. Data were analyzed using several statistical methods. Effects at the community level were analyzed using the Principal Response Curves (PRC) method of van den Brink (1999). Univariate statistical methods were also used to identify effects at the population level.* ***Findings:*** ***Test substance analysis*** *of the application solutions demonstrated that for all treated ponds, applications were on average made at 102 ± 8.0%, 104 ± 9.4% and 96 ± 5.3% of the nominal concentrations for the first, second and third application, respectively. This demonstrated that suitable treatments had been prepared and applied to the microcosms (apart from one replicate at the 3 x 0.21 g abamectin/ha treatment which did not receive any of the test substance on the second application – this replicate was excluded from subsequent analyses).* *Following applications,* ***water residue samples*** *were analyzed for abamectin for up to 91 days. During the first six hours after application, measured concentrations in water samples were on average 51, 59 and 128% of nominal after the first, second and third application for the three highest treatments of 0.135, 0.405 and 1.22 µg abamectin/L (initial nominal concentrations assuming complete mixing of the water body), respectively. In the lower treatment levels concentrations of the test substance were highly variable and thus recovery values were not calculated. This variability was not unexpected due to the complex matrix of the samples and the very high affinity of abamectin to adsorb to solids and organic matter. Using concentrations determined from water samples collected after the third application the*	

median dissipation time (DT_{50}) was estimated to be 4.9 days (range 4.3 – 5.8 days).

The analytical results of the sediment samples were not reported because of technical difficulties with the HPLC/MS-equipment during analysis and because only minor adverse effects on the macrozoobenthos were observed.

Summary of observed effects by endpoint:

*There were no changes in the **physico-chemical** characteristics of the microcosm water that could be attributed directly or indirectly to abamectin treatments. The trends in temperature, dissolved oxygen content, pH, conductivity and redox potential were those expected for small water bodies in the region.*

*The **phytoplankton** was only affected after the third application, with increases in biomass observed at treatment rates of ≥ 3 x 5.8 g abamectin/ha from day 15 to 133. This considered to be an indirect effect of abamectin due to the reduction in zooplankton grazing pressure. The $NOEC_{community}$ for the phytoplankton was 3 x 1.9 g abamectin/ha (initial nominal concentrations assuming complete mixing 0.135 µg abamectin/L). By Day 63 of exposure (49 d after the last application) and until the end of the study the abundance of the phytoplankton in the 3 x 5.8 g abamectin/ha treatment was similar to the control, probably because the zooplankton community had recovered and normal grazing pressure had resumed. The highest treatment rate had greater phytoplankton biomass than the control until the end of the study.*

*The **zooplankton** was directly affected at treatment rates ≥3 x 0.64 g abamectin/ha, but there was recovery in all affected treatments before the end of exposure. There were effects on populations of Cyclopoida. Among the Cladocera, Chydorus sphaericus was adversely affected but effects on Daphnia longispina were less pronounced. The overall NOEC for the most sensitive zooplankton populations and the $NOEC_{community}$ was 3 x 0.21 g ai/ha (initial nominal concentration assuming complete mixing 0.015 µg abamectin/L). The zooplankton community recovered at the treatments of 3 x 0.64, 3 x 1.9, 3 x 5.8 and 3 x 17 g abamectin/ha within 65, 77, 119 and 119 days after the last application, respectively.*

*There were no major effects of treatment on the **macrozoobenthos**. Differences in community structure were observed on Day 16 and 29 but only at the highest treatment. Effects were observed once in addition at an application rate ≥ 3 x 0.64 g abamectin/ha on Day 70. There were no further effects during the remainder of the study. The $NOEC_{community}$ for the macrozoobenthos was 3 x 0.21 g abamectin/ha (initial nominal concentration assuming complete mixing of 0.045 µg abamectin/L). Recovery was observed on Day 85 of exposure, 71 days after the last application.*

*The **emergent insect** community was affected after the third application from day 21 onwards for a period of 70 days. Adverse effects were observed at the three highest treatments of 3 x 1.9, 3 x 5.8 and 3 x 17 g abamectin/ha. The NOECcommunity was 3 x 0.64 g abamectin/ha (initial nominal concentration assuming complete mixing of 0.045 µg abamectin/L). The community recovered fully on Day 105 of exposure, 91 days after the last application at all treatment rates.*

Summary of community-level treatment effects

Communities	*$NOEC_{community}$ (3 x g ai/ha)*	*$LOEC_{community}$ (3 x g ai/ha)*	*Time to Recover (in days after the last application)*
Phytoplankton	*1.9*	*5.8*	*>119*

Zooplankton	*0.21*	*0.64*	*119*
Macrozoobenthos	*0.21*	*0.64*	*71*
Emergent Insects	*0.64*	*1.9*	*91*

Summary of effects by treatment rate:

In treatments of 3 x 0.071 and 3 x 0.21 g abamectin/ha, there were no adverse effects on the aquatic community structure and component populations of the aquatic system. Enhanced abundance of the zooplankton community at the treatment 3 x 0.21 g abamectin/ha between Day 3 and Day 14 of exposure could not be clearly related to the test substance and was therefore considered not to be ecologically relevant. Indirect effects on the phytoplankton community, which led to a reduction in the phytoplankton biomass between Day 14 and 63, were linked to the enhanced grazing capacity in the same treatment 3 x 0.21 g abamectin/ha.

In treatments of 3 x 0.64 g and 3 x 1.9 g abamectin/ha there were initial effects on the zooplankton and macrozoobenthos later on during the exposure period but recovery proceeded rapidly, with conditions returning to control levels within 1–3 months. Reductions in zooplankton resulted in a lower food supply for Chaoborus cristallinus leading to reduced abundance but recovery was also observed within 3 months of exposure.

In treatments of 3 x 5.8 g and 3 x 17 g abamectin/ha, there were substantial effects on the zooplankton, which also resulted in indirect effects (increased abundance of the phytoplankton). The zooplankton and the phytoplankton recovered within 3–4 months with the exception of the phytoplankton at the highest treatment rate. In the highest treatment, certain macrozoobenthos taxa, namely Chaoborus sp., Baetis sp., the Tanytarsini and Chironomus sp., were adversely affected by the test substance. However, they recovered quickly and no long-term effects were observed. At the highest treatment rate, the emergent insects recovered within 91 days of the last application.

Conclusion: *The overall NOEC for the most sensitive zooplankton populations and the $NOEC_{community}$ was 3 x 0.21 g abamectin/ha. The zooplankton community recovered at the treatments of 3 x 0.64, 3 x 1.9, 3 x 5.8 and 3 x 17 g abamectin/ha within 65, 77, 119 and 119 days of the last application, respectively. At the end of exposure, i.e. within the same season, recovery of the affected communities was observed in all treatments, with the exception of the phytoplankton in the highest treatment indicating no unacceptable long-term effects of abamectin in treatments of ≤ 3 x 5.8 g abamectin/ha.*

Reference/notifier	:	Knauer, K. (2002)	GLP statement	:	Yes
Type of study	:	outdoor microcosm study, 20 w 3 applications	Guideline	:	Monks Wood workshop (1991); EWOFFT (1992); draft OECD (issued by SETAC, 1993); HARAP (1998); CLASSIC (1999)
Year of execution	:	2000	Acceptability	:	acceptable
Test substance	:	A 8612A (Vertimec EC 018), batch [redacted] purity 19.5 g abamectin/L, appearance light yellow liquid			

Substance	Species	Method	T [°C]	pH	Duration [d]	Criterion	Value abamectin [µg as/L]
A 8612 A	phytoplankton, zooplankton, macroinvertebrates, emerging insects	outdoor microcosm,	15-25	8.8-10.3	140	$NOEC_{community}$ NOEAEC	3 x 0.015 3 x 0.045

Description

Outdoor microcosm study submitted under Document IIIA section No. 7.4.3.

Guidelines. The test is conducted in accordance with the Guidance document on testing procedures for Pesticides in Freshwater Mesocosm (1991), the report of the Workshop on Aquatic Microcosms for Ecological Assessment of Pesticides (1991), the European Workshop on Freshwater Field Tests (EWOFFT) (1992) and the Draft OECD-Guidelines for Testing of Chemicals (1993), the Guidance Document on Higher-tier Aquatic Risk Assessment for Pesticides HARAP (1998) and the proceedings of the CLASSIC workshop (Community Level Aquatic System Studies-Interpretation Criteria) (1999).
Test design. Polyethylene tanks (depth 1.5 m, diameter 3 m, volume 10 m^3), located at the test site of Syngenta in Stein, Aargau, CH, had been established in Spring 1996 and used for experiments since then. Sediment type loam, layer depth ca. 15 cm at time of construction. Water was renewed on March 8, 2000 (1.5 months before application), sediment was not replaced but did not contain residues of the product and metabolites tested in 1999. Water circulation was started on March 21, 2000, and stopped on the day of first application (May 9, 2000). The macrophytes present (*Myriophyllum verticullatum* and *Potamogeton crispus*) were planted in 1998; the macrophytes were dominated by *Elodea canadensis* which had entered the ponds via natural colonisation. Algae, zooplankton and other organisms were introduced with the water of the supply pond, macroinvertebrates were introduced via aerial colonisation and by introducing sediment from the natural supply pond.
Application, concentrations, replicates. Test substance diluted in double distilled water and sprayed on the water surface three times with a 7-days interval, on May 9, 16 and 23, 2000. Six dose levels, 3.47, 10.4, 31.3, 93.8, 282 and 847 g product/ha, equivalent to 0.071, 0.21, 0.64, 1.9, 5.8 and 17 g as/ha. Assuming complete mixing, application rates are corresponding to 0.245, 0.736, 2.21, 6.63, 19.9 and 59.9 µg product/L or 0.005, 0.015, 0.045, 0.135, 0.405 and 1.22 µg as/L. Three replicate tanks per dose and three controls. Water samples were taken 7 days before application and at regular time intervals after application. Sediment samples were collected, but not analysed due to technical failure.
Biological observations. Effects on zooplankton and phytoplankton were assessed a few hours and 1 and 3 days after the first, second and third application and on day 7, 21, 35, 49, 65, 77, 91, 105 and 119 after the third application. Emerging insects were assessed 7 days after the first, second and third application and 21, 35, 49, 65, 77, 91, 105 and 119 days after the third application. Macroinvertebrates were sampled 2 days after the first, second and third application and 15, 28, 43, 56, 71, 98 and 126 days after the last application.
For phytoplankton assessment, chlorophyll *a* was measured, number of cells were counted and individual species of algae were determined; 4 whole column samples of 2.4 L each were taken per sample. Zooplankton species were identified and counted. Two depth integrated samples were taken using a 25 cm plankton net with 55 µm mesh. The water volume sampled per sample was approximately 50 L. Emergent insects were captured using one floating trap of 0.25 m^2 per cosm, identified and counted. Macroinvertebrates were sampled using plastic cylinders of 5 cm diameter x 5 cm length as artificial substrate. Six cylinders were used per sample. Substrates were left for 4 weeks in the cosm to be colonised.
Environmental conditions.Weather condition on the days of application: day 1: wind speed 0.431 m/s, 18.2°C, day 2: wind speed 0.605 m/s. 20.7°C, day 3: wind speed 0.527 m/s, 14.9 °C. No rain on all three days. During the test period the average water temperature varied between 15 and 25°C. Oxygen concentration ranged between 99 and 242% saturation. The pH varied between 8.8-10.3 during the study.
Verification of concentrations. Water samples were taken from ponds with highest application rate and from the control ponds, 6 hours and 1, 3 and 7 days after each application and on day 14, 21, 35, 49, 65 and 77 after the third application. Also, water samples were taken of all ponds 6 hours and 1 day after each application. Dose levels 0.135, 0.405 µg as/L, and 1.22 µg/L up to day 29 were analysed by diluting water samples with acetonitrile (acetonitrile volume 6 %) and passing them over a C_{18}-column. Columns were eluted with acetonitrile, the eluate was made up to volume and analysed by HPLC-UV (245 nm), LOQ 0.1 µg abamectin /L, recovery 87.4 – 144 %, average 104.1 % (n = 4, RSD 25.7 %).
Dose levels 0.005 – 0.045 µg abamectin/L, 1.22 µg/L from day 29 onwards were analyzed by extraction on SPE columns with solvent water/acetonitrile 1/1 (v/v). Samples were filtered before analysis by HPLC-MS, LOQ 1 ng/L, recovery 78 – 133 %, average 104 % (n = 9, RSD 19 %).
Calculations and statistics. For phytoplankton, zooplankton, macrozoobenthos and emergent insects the community response is studied using multivariate (PRC) and univariate (Dunnett's test) statistics. Endpoints studied: total abundance, abundance of major taxonomic groups, abundance of individual taxa and taxon richnes. One replicate of the 3 x 0.015 µg as/L was left out of the analysis because this pond received only two applications.

Results

In the three highest dose levels, mean measured concentrations of 51 % (35 – 71 %), 59 % (49 - 71) and 128 % (58 - 270%) of nominal were found 6 h after the first, second and third application, respectively. One day after the first, second and third application, mean actual concentrations were 69 %, 60 % and 60 % of nominal, respectively. For the lowest three dose levels the measured rates were very variable, 17 - 162% of nominal 6 h after the first application, 7 - 257% of nominal 6 h after the second application and 29 – 228 % of nominal 6 h after the third application. In the highest treatment concentrations, actual water concentrations were 71 – 113 % of nominal 6 h and 58 – 75 % 1 day after third application. Actual concentrations in the highest treatment steadily declined to 0.2 % of nominal by day 91. A DT_{50} value of 4.9 days was calculated for the water compartment. Measured concentrations of abamectin in control and treatments are given in the table below (dose levels 0.071 - 5.8 g as/ha) and in the table further below (control and 17 g as/ha). All values are corrected for recovery.

Table: Measured concentrations of abamectin in outdoor microcosms: dose levels 1 to 5.

Time [days]	Nominal dose level of abamectin [g as/ha] (Corresponding nominal concentration in µg as/L)														
	3 x 0.071[1] (3 x 0.005 µg as/L)			3 x 0.21[1] (3 x 0.015 µg as/L)			3 x 0.64[1] (3 x 0.045 µg as/L)			3 x 1.9[2] (3 x 0.135 µg as/L)			3 x 5.8[2] (3 x 0.405 µg as/L)		
	tank 1	tank 2	tank 3	tank 1	tank 2	tank 3	tank 1	tank 2	tank 3	tank 1	tank 2	tank 3	tank 1	tank 2	tank 3
0.25[3]	0.0081	0.0031	0.0058	0.0063	0.0098	0.0060	0.0321	< LOD	< LOD	0.0957	0.0780	0.047	0.207	0.228	0.117
1	0.0057	0.0043	0.0036	0.0081	0.0071	0.0043	0.0206	0.0178	0.0208	0.0907	0.0957	0.0818	0.233	0.282	0.279
7.25[3]	0.0128	< LOD	0.0013	0.0287	< LOD	0.0141	0.0298	0.0049	0.0030	0.125	0.0866	< LOD	0.299	0.322	0.323
8	0.0170	0.0077	0.0165	0.0278	< LOD	0.0246	0.0377	0.0226	0.0395	0.102	0.103	0.0912	0.413	0.306	0.282
14.25[3]	0.0114	0.0061	0.0022	0.0056	0.0071	0.0043	0.0581	0.0316	0.0220	0.120	0.503	0.467	0.527	0.686	0.323
15	0.0072	0.0069	0.0083	0.0260	0.0090	0.0130	0.0368	0.0372	0.0292	0.104	0.134	0.0826	0.436	0.451	0.110

1: Solvias data
2: Syngenta data
3: 6 h after application

Table: Measured concentrations of abamectin in outdoor microcosms: control and dose level 6.

Time [days]	Nominal dose level of abamectin [g as/ha] (Corresponding nominal concentration in µg as/L)					
	control[1,2]			3 x 17[1,2] (3 x 1.22 µg as/L)		
	tank 1	tank 2	tank 3	tank 1	tank 2	tank 3
0.25[1,3]	0.0018	0.0011	0.0011			
0.25[2,3]	< LOD	< LOD	< LOD	0.603	0.802	0.535
1[2]				0.920	0.980	0.929
3[2]	< LOD			0.592	0.631	0.549
7[2]		< LOD		0.386	0.426	0.282
7.25[2,3]	< LOD	< LOD	< LOD	0.836	1.08	0.790
8[2]			< LOD	1.01	< LOD	0.942
10[2]	< LOD			0.653	0.540	0.537
14[2]			< LOD	0.606	0.452	0.742
14.25[2,3]	< LOD	< LOD	< LOD	1.33	1.96	2.07
15[2]	< LOD			1.36	1.10	1.06
17[2]		< LOD		1.32	1.07	0.852
21[2]			< LOD	0.790		0.970
29[2]	< LOD			0.278	0.217	0.326
29[1]				0.1066	0.1592	0.1875
35[1]				< LOD	0.0579	0.0291
49[1]				0.0785	0.0242	0.0456
63[1]				0.0119	0.0107	0.0122
79[1]				0.0062	0.0075	0.0078
91[1]				0.0033	0.0023	0.0039

1: Solvias data
2: Syngenta data
3: 6 h after application

Phytoplankton.

The PRC showed a statistically significant increase of phytoplankton from day 15 to 133 treatment with 1.22 µg as/L and from day 15 until 49 after the third treatment with 0.405 µg as/L, probably due to reduced consumption as a result of a decrease of zooplankton foraging on algae. These increases of phytoplankton were significant compared to the controls, as calculated using taxa weights per treatment replicate. In the 0.015 µg as/L treatment from day 14 to 63, the abundance of the phytoplankton community was decreased, probably due to an enhanced abundance of zooplankton. At 0.045 µg/L an increase on a few individual points in time is found, and at 0.135 µg/L a decrease on some points in time. Univariate analysis were applied for 35 species. A number of species

(*Anabaena* sp., *Aocystis naegelii, Cosmarium meneghinii, Spirogyra* sp., *Tetraedron minimum, Staurastrum* sp.1, *Nitzschia palea* and *Chroomonas acuta*) significantly increased in the two highest treatments of 0.405 and 1.220 µg as/L. In the 0.015 µg as/L treatment, *Chlamydomonas* sp., *Chlorophyceae* and *Tetrachlorella ornate* showed significantly reduced abundances on one occasion (35, 7 and 7 days after third treatment, respectively) or in the case of *Coelastrum* sp. on five sampling dates (1, 3, 7, 105 and 119 days after third treatment). *Chroomonas acuta* showed reduced growth in the 0.135 µg as/L treatment and higher dosages from day 8 to 21. Based on these results (leaving indirect growth enhancing effects out of consideration) the author concluded that the NOEC for phytoplankton was 0.045 µg as/L.

Zooplankton.

The PRC showed a decrease of zooplankton in all concentrations. For the 2 lowest concentrations, (0.005 and 0.015 µg as/L), these differences were only seen at one point in time (21 days after third application), being significant for the 0.005 µg as/L treatment (calculated with taxa weights). The 0.045 and 0.135 µg as/L treatments showed effects after the second and third treatment, and at the two highest dosages (0.405 and 1.220 µg as/L) zooplankton community reacted after the first treatment. The 0.015 µg as/L treatment showed a short-term significant *increase* of the abundance of zooplankton shortly after the applications, which could not be explained. Time to recover (in days after last application until no significant effects were observed) was 65 and 77 days for the 0.045 and 0.135 µg as/L treatments, respectively. At 0.405 and 1.220 µg as/L, recovery time was > 119 days and 119 days, respectively. The author concluded that without considering recovery, the $NOEC_{zooplankton}$ is 3 x 0.015 µg as/L based on PRC analysis.

According to the author, recovery can be taken into account and the $NOEC_{community/time}$ can be seen as time dependent. Following this reasoning the $NOEC_{community}$ varied depending on the sampling date from 0.015 to 1.22 µg as/L. The author concluded that all treatments had recovered after 119 days. However, significant differences are still found in the 0.405 µg as/L treatment.

Univariate analysis was performed with the species which contributed most to the variance explained within PRC. These species were Cyclopoida, *Chydorus spaericus* and *Daphnia longispina*. Furthermore, the Crustacea and Copepoda were analysed as a group. For the Cyclopoida, the NOEC was 0.015 µg as/L. Recovery time was dose related: 119 days for 1.22 µg as/L, 105 days for 0.405 µg as/L, 84 days for 0.135 µg as/L, and at 0.045 µg as/L significant effects were found on the day of treatment only. For *Chydorus spaericus*, the NOEC was 0.015 µg as/L. In the highest treatment, effects were found from day 3 – 119, at 0.405 µg as/L from day 10 – 49, at 0.135 µg as/L from day 17 - 49 and at 0.045 µg as/L on days 17 and 35. For *Daphnia longispina*, comparable trends were visible, but the abundance was low (0 - 4 organisms). Therefore, statistically significant differences were not found. For the Crustacea-Copepoda, a NOEC of 0.015 µg as/L was found. Crustacea-Copepoda densities had recovered in all treatments 35 days after last exposure. Among the Rotifera, *Lecane* sp. abundance significantly increased at treatments ≥ 0.405 µg as/L, with recovery from day 91 onwards (77 days after last application). The results are summarised in the table below.

Table: Summary of the NOECs [µg as/L] of the affected taxa of zooplankton and the changes of the NOECs over time.

Zooplankton taxa	NOEC on day of exposure after first application											
	14.25	15	17	21	35	49	63	79	91	105	119	133
Cyclopoida	1.220	0.045	0.135	0.405	0.015	1.220	1.220	1.220	0.045	1.220	0.405	1.220
C. sphaericus	1.220	1.220	0.015	0.135	0.015	0.045	1.220	1.220	1.220	1.220	0.405	1.220
D. longispina	1.220	1.220	1.220	1.220	1.220	1.220	1.220	1.220	1.220	1.220	1.220	1.220
Copepoda	0.015	0.015	1.220	1.220	0.135	1.220	1.220	1.220	1.220	1.220	1.220	1.220
Lecane sp.	0.135	0.405	0.405	0.135	0.405	1.220	0.405	0.405	1.220	1.220	1.220	1.220

Macroinvertebrates.

PRC analysis showed significant effects on community structure on day 70 for treatments of 0.045 µg as/L and higher, as calculated with taxa weights. The NOEC for macroinvertebrates is 0.015 µg as/L. Only the highest treatment showed significant effects on day 16 and 29. Univariate analysis of the most dominant macroinvertebrate group, the Zygoptera (Odonata) showed no treatment related effects at all. The dominant species which showed an effect were *Chaoborus* sp., *Beatis* sp., Tanytarsini and *Chironomus* sp. as indicated by the univariate analysis. *Chaoborus* and *Baetis* showed significant effects on day 16 and 42 only in the highest treatment (1.22 µg as/L). For the Tanytarsini, the highest treatment showed a significant effect at day 29. On day 57 and 70, effects were found at 0.135 µg as/L and 0.405 µg as/L. For *Chironomus* sp., a significant effect was only found on day 29 in the highest treatment.

Emergent insects.

PRC analysis showed that abundance of emergent insects was significantly affected from day 21 - 91 at 1.22 µg as/L, on day 70 and 91 at 0.405 µg as/L, from day 63 - 105 at 0.135 µg as/L and on day 105 at 0.045 µg as/L. The author concluded that the NOEC for emergent insects was 0.045 µg as/L. Full recovery was observed at the end of the experiment for all treatments. The effects appeared to be caused dominantly by *Chaoborus,* the Diptera-Nematocera and the Baetidae. For *Chaoborus* significant effects were seen in the 1.22 µg as/L treatment on day 63 and 79 and for treatments ≥ 0.135 µg as/L on day 79. The NOEC for *Chaoborus* was 0.045 µg as/L. For the Diptera-Nematocera significant effects were found at day 35 in the highest treatment and at day 63, 91 and 105 in the 0.135 µg as/L treatment. The author concludes the NOEC for Diptera-Nematocera to be 0.405 µg as/L. For the Baetidae, significant effects were found on one sampling date (day 35) in the highest treatment (1.22 µg as/L) only.

Macrophytes.

Macrophytes were not monitored during the test period.

Conclusion

From the study the author concluded that the $NOEC_{community}$ was 0.015 µg as/L, based on multivariate analyses of the most sensitive groups (zooplankton and macrozoobenthos). Because recovery was seen within the period of the test (3 - 4 months) an ecological acceptable concentration EAC of 3 x 0.405 µg as/L was proposed, on basis of nominal concentrations.

Remarks by RMS

Fate part of study was evaluated in Document IIIA reference point 7.1.2.2.2/05.
Because one replicate at 0.015 µg as/L did receive two applications instead of three, this cosm was left out of the analysis. Since this treatment in a number of cases was estimated to be the NOEC, it is not clear if statistical differences would have been different with three replicates instead of two replicates.

Phytoplankton.

At 0.05 no effects were found, so effects are classified as class 1. At 0.015 µg as/L clear effects were found, but recovery took place within 8 weeks, so effects are classified as class 3 effects. At 0.045 µg/L some positive effects were found and at 0.135 µg/L a negative effect was found at one date, and effects are classified as class 2 effects. At 0.405 µg as/L a significant increase was found, but within 8 weeks no differences were found with the untreated control, so the effects are classified as class 3 effects. The highest treatment (1.22 µg as/L) showed a significant (increase) effect till the end of the experiment and effects are classified as class 5 effects. As stated in the results section, effects are deemed to be indirect effects. For this reason, no NOEC for phytoplankton is given.

Zooplankton. For a number of organisms, effects lasting more than 8 weeks were found. In most cases at the last sampling date, no significant effects were found, and the author concluded that total recovery was observed. For the community, at treatments of 3 x 0.405 and 3 x 1.22 µg as/L recovery did not take place within 8 weeks after the last treatment, so the effects are classified as class 5 effects. At the 3 x 0.135 effects were found from day 7 after first application until day 79 (65 days after third application), but not in un underbroken range, so effect are classified as class 4 effects. At the lowest treatment a significant increase of abundancy was found, and effects are classified as class 2 effects. At 0.015 µg as/L a significant increase was found found (class 2) and at 0.045 µg as/L effects are found on fiver timepoints scattered over time. These effects are classified as class 3 effects. Since an increase found at the lower dosages cannot be explained as a direct effect, and a decrease is found at the lowest treatment only, and not at the 0.015 µg as/L treatment, the NOEC is set at 0.015 µg as/L.
For the Copepoda, the analyses show a short-term decrease direct after treatment in the for highest treatments (class 2).
For the Cyclopoida an significant increase is found in the 0.005 and 0.015 µg as/L treatment direct after the third application (class 2), at 0.045 µg as/L a decrease is found at one point of time (class 2), at 0.135 µg as/L at several scattered time points (class 3), and at the two highest treatments at a number of time points between day 1 and 119 (class 4). For *Daphnia* numbers were too low to derive reliable statistical results.

Macrozoobenthos.