SEC Adviesrapport **12794A010**

Imidacloprid

Samenvatten en evalueren van milieustudies

5.1.2.e Woo

15 April 2011~~dd mmmmm jjjj~~

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Opdrachtgegevens

CAS-nummer:	13826-41-3
Engelse common name:	Imidacloprid
Nederlandse naam:	Imidacloprid
Engelse synoniemen:	
Type rapport:	RIVM/SEC adviesrapport; confidentieel
SEC rapportnummer	12794A00
Projectnummer	E/320201/16/GT
Opdrachtgever	Ctgb
Ctgb briefnummer	10/3274 5.1.2.e Woo
Ctgb opdrachtnummer	25698-mil
Datum opdracht	11-11-2010
Datum rapportage	~~dd mmmmm jjjj~~15 april 2011
Auteur(s)	5.1.2.e Woo
Toetser	5.1.2.e Woo ~~26 februari~~31 januari 2011
Opdracht	Samenvatten en evaluerenvan ecotoxicologische studies.

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Voorwoord

Kwaliteitsprocedures en beoordelingskader

De samenvattingen (Hoofdstuk 1) in dit rapport zijn opgesteld in overeenstemming met de vigerende SEC kwaliteitsprocedures.

Selectie van gegevens

Gegevens die in de samenvattingen met Reliability Index (Ri) 3 zijn aangeduid, zijn onbetrouwbaar en niet geschikt voor de risicobeoordeling.
Voor studies die worden aangeduid met een Ri 2 geldt dat de methodologie en/of de beschrijving niet geheel in overeenstemming zijn met de vereisten zoals beschreven in {Moncink, Smit, et al. 2008 #5970}[6].
Nadere informatie over afwijkende onderdelen of ontbrekende informatie is vermeld onder de kop 'Remarks' in de samenvatting van de desbetreffende studie.
Gegevens van studies met Ri 2 worden niet bij voorbaat ongeschikt geacht voor de risicobeoordeling. Het al dan niet gebruiken van gegevens met Ri 2 voor de risicobeoordeling wordt mede bepaald door de beschikbaarheid van studies met Ri 1, die volledig in overeenstemming zijn met de vereisten zoals beschreven in {Moncink, Smit, et al. 2008 #5970}[6]. Voor alle studies geldt dat de toetsomstandigheden relevant moeten zijn voor het doel van de risicobeoordeling.

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1 Summaries

1.1 Ecotoxicology

1.1.1 Effects on aquatic organisms

1.1.1.1 Effects on algae

1 Toxicity aquatic organisms

Substance	Species	Method	T [°C]	pH	Duration [h]	Criterion	Value [mg/L]	Value [mg as/L]	RI
KOHINOR 700 WG	*Desmodesmus subspicatus*	static	21 - 23	7.6 – 8.8	72	E_rC_{50}	175	122	1
						NOE_rC	80.7	56.1	1
						E_yC_{50}	88.1	61.2	1
						NOE_yC	31.8	22.1	1

Reference

[8][7]

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Guidelines

OECD Guideline No. 201 (2006).

Test substance

KOHINOR 700 WG (69.5% imidacloprid, analysed). Solubility in water: dispersible

Test organisms

Desmodesmus subspicatus, strain 86.81 CHODAT SAG (formerly known as *Scenedesmus subspicatus* strain 86.81 SAG).

Principle of method

Algae were exposed to the test substance for 72 h under static conditions, cell numbers were determined directly after application and after 24, 48 and 72 h via chlorophyll-a-fluorescence excitation at 436 nm, emission at 685 nm. Cells were microscopically examined at the start and at the end of the test. After 72 h exposure, algae from test concentration 243 and 729 mg/L were transferred into fresh medium and allowed to grow for a further 3 days to determine the reversibility of the effect.

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Application, concentrations, replicates

Control, 1.0, 3.0, 9.0, 27, 81, 243, and 729 mg test item/L. Three replicates per test concentration and six in the control, initial cell density 2 – 5 x 10^3 cells/mL.

Water

OECD test medium, hardness 0.24 mmol/L (Ca + Mg), pH 8.

Conditions

21 - 24 °C ± 2 °C, continuous light (60 - 120 µE/m^2·s), continuous shaking (70 rpm), no aeration.

Verification of concentrations

Water samples of all test concentrations and control at start and end of test, analysis by HPLC with DAD detection at 270 nm after dilution with 20% acetonitrile : 80% phosphoric acid (0.1%) (recovery 94 - 97%). LOQ: 0.09 mg test item/L (corresponding to 0.06 mg imidacloprid/L). LOD was 0.02 mg imidacloprid/L.

Calculations and statistics

EC_{50} , EC_{10} and 95% CI for growth rate and yield calculated by sigmoidal dose-response regression with computer program Graphpad Prism 4, SigmaPlot rel and Excel.

Results

Mean measured concentrations 89 – 96% of nominal. Average control cell density after 72 h 131.4861·10^4 cells/mL (>16 fold increase). No deviations of cell shape, no cell debris. The test item effect was observed to be reversible during the 3 days recovery period.

Table 1: EC10 and EC50 values.

	KOHINOR 700 WG [mg/L]	Imidacloprid [mg/L]
	Yield	
E_yC_{10} (95% CI)	31.8 (21.9 – 42.6)	22.1 (15.2 – 29.6)
E_yC_{50} (95% CI)	88.1 (79.7 – 97.9)	61.2 (55.4 – 68.0)
	Growth rate	
E_rC_{10} (95% CI)	80.7 (72.4 – 96.6)	56.1 (50.3 – 67.1)
E_rC_{50} (95% CI)	175 (165 – 184)	122 (115 – 128)
	Biomass	
E_bC_{10}	54.3	37.7
E_bC_{50}	176.6	122.7

Remarks by RMS

Water quality parameters within accepted range. The validation criteria of OECD 201 for the mean VC% and the VC% of average specific growth rates during the whole study in the control were ~~also~~ met (mean VC% 6.87%, average VC% 0.79%). Other validity criteria of OECD 201 were also met. The results E_rC_{10} 80.7 mg test item/L and E_yC_{10} 31.8 mg test tem/L (corresponding to 56.1 and 22.1 mg imidacloprid/L, respectively). E_rC_{50} 175 mg test item/L and E_yC_{50} 88.1 mg test item/L (corresponding to 122 mg and 61.2 mg imidacloprid/L, respectively), based on nominal concentrations, can be used for risk assessment. EC_{10} values can be considered as NOEC values.

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1.1.1.2 Effects on aquatic invertebrates

1.1.1.2.1 Acute toxicity

1 Toxicity aquatic organisms

Substance	Species	Method	T [°C]	pH	Duration [h]	Criterion	Value [mg/L]	Value [mg as/L]	RI
KOHINOR 700 WG	*Daphnia magna*	static	18 - 20	7.54-7.98	48	EC_{50}	53.6	37.3	1

Reference

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Guidelines

OECD Guideline No. 202, (2004).

Test substance

KOHINOR 700 WG (69.5% imidacloprid, analysed). Solubility in water: dispersible

Test organisms

Daphnia magna Straus (Clone 5), <24 h old. Acclimatisation at least 2 h.

Principle of method

Daphnids were exposed to the test substance for 48 h under static conditions, number of immobilised animals was determined after 24 and 48 h.

Application, concentrations, replicates

Control, 6.25, 12.5, 25.0, 50.0, 100, and 200 mg/L. Four replicates with 5 animals each per treatment.

Water

Reconstituted water, according to Directive 92/69/EEC method C2, hardness 271 mg/L as $CaCO_3$, conductivity 656 µS/cm, pH 7.8.

Conditions

18 - 22°C ± 1°C, 16 h fluorescent light (20 $\mu E.m^{-2}.s^{-1}$), no aeration, no feeding.

Verification of concentrations

Water samples of all test concentrations and control at start and end of test, analysis by HPLC with DAD detection at 270 nm after dilution with 20% acetonitrile: 80% phosphoric acid (0.1%) (recovery 94 - 97%). LOQ: 0.09 mg test item/L (corresponding to 0.06 mg imidacloprid/L). LOD was 0.02 mg imidacloprid/L.

Calculations and statistics

EC_{50} and 95% CI calculated by sigmoidal dose-response regression and according to Clopper and Pearson, graphical presentation of logarithmic concentration- effect relationship. Program used was: Graphpad Prism 4 and Excel.

Results

The test items at 100 and 200 mg/L were pale yellow. The test concentration 200 mg/L was slightly turbid at the start and clear after 24 hours. The 100 mg/L was clear. Other concentrations were colourless and clear throughout the whole test duration. Only a few white particles of the test substance were observed at 25.0 to 200 mg/L. At 50.0 mg/L and 100 mg/L particles of the test item were observed adhered to the antennae of the daphnids after 24 (50 and 100 mg/L) and 48 h (only at 50 mg/L). Mean measured concentrations 96 - 100% of nominal. Immobilisation: 10% at 25.0 mg/L~~, after 24 and 48 h,~~ 65% at 100 mg/L after 24 h~~, and 100% immobilisation at 100 mg/L after 48 h.~~ Immobilisation after 48h was: 0, 0, 0, 10, 40,100, and 100% at control, 6.25, 12.5, 25, 50, 100, and 200 mg/L nominal test item, respectively. 48-h EC_{50} reported as 53.6 mg test item/L (95% CI: 48.6 – 59.2 mg/L), corresponding to 37.3 mg imidacloprid/L (95% CI: 33.8 – 41.2 imidacloprid/L).

Remarks

Water quality parameters within accepted range.
The result nominal 48-h EC_{50} of 53.6 mg formulation/L (37.3 mg imidacloprid/L) can be used for risk assessment.

1.1.1.3 Effects on fish

1.1.1.3.1 Acute toxicity

1 Toxicity aquatic organisms

Substance	Species	Method	T [°C]	pH	Duration [h]	Criterion	Value [mg/L]	Value [mg as/L]	Ri
KOHINOR 700 WG	*Oncorhynchus mykiss*	static	15	6.9-7.7	96	LC_{50}	> 100	> 69.5	2

Reference

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Guidelines

OECD 203 (1992)

Test substance

KOHINOR 700 WG (69.5% imidacloprid, analysed). Solubility in water: dispersible.

Test organisms

Oncorhynchus mykiss, average: 5.09 cm, 1.31 g, obtained from a commercial provider. Acclimatisation at least 12 days.

Principle of method

Fish were exposed to the test substance for 96 h under static conditions, observations for mortality and sublethal effects after 24, 48, 72 and 96 h.

Application, concentrations, replicates

Preliminary test at 100 mg test item/L.

Definite test at 100 mg test item/L. One replicate per treatment with 7 fish each.

Water

Tap water of local origin, filtered and aerated to remove chlorine. Hardness 64 mg/L as $CaCO_3$, pH 6.9 – 7.7.

Environmental conditions

15°C, 12 h light (0.1 – 10 µmol photons.m^{-2}.s^{-1}), no aeration, no feeding.

Verification of concentrations

Water samples of all test concentrations and control at the start and end of the test, analysis by HPLC with DAD detection at 270 nm, after dilution with 20% acetonitrile : 80% phosphoric acid (0.1%) (recovery 94 - 97%). LOQ: 0.09 mg test item/L (corresponding to 0.0626 mg imidacloprid/L). LOD was 0.02 mg imidacloprid/L.

Calculations and statistics

Due to the results the LC_0 and LC_{50} were not calculated by sigmoidal dose-response regression but could be determined out of the results.

Results

Measured concentrations 91 - 94% of nominal. No mortalities in control and test concentration or sublethal effects at any time point. Nominal 96-h LC_{50} > 100 mg/L

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Remarks

Water quality parameters within accepted range.

According to OECD 203 the preliminary test should be conducted at 100 mg active ingredient/L. This preliminary test was performed at 100 mg test item/L equivalent to 69.5 mg imidacloprid/L. It can not be foreseen whether mortalities had occurred when the preliminary test had been conducted at 100 mg imidacloprid/L. If mortalities had occurred a definite test with at least 5 concentrations should have been performed.

The result nominal LC_{50} > 100 mg/L can be used for risk assessment but is less reliable.

1.1.2 Effects on bees

1 Toxicity (bumble)bees

Substance	Species	Method	Duration	Recovery	Criterion	Value	RI
			[h]	[h]		[µg as/bee]	
KOHINOR 700 WG	*Apis mellifera*	Contact		96	LD50	0.057	1
		Oral	96		LD50	0.037	1

Reference

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Guidelines

OECD 213 and 214 (1998).

Test substance

KOHINOR 700 WG (69.5% imidacloprid, analysed). Solubility in water: dispersible.

Test organisms

Apis mellifera L., young adult worker bees, from an in-house colony. Adaptation 1 hour.

Principle of method

The effect of the test item on honey bees after contact and oral exposure was determined for 96 hours. Bees were exposed to seven concentrations by contact and oral exposure. Mortality and behaviour of the bees were observed after 4, 24, 48, 72 and 96 hours.

Application, concentrations, replicates

Three replicates per treatment for both the contact and oral test, five replicates for the control. Each replicate with 10 bees. Control group was used both for the test item as the reference item.

Contact exposure:

Seven nominal concentrations were used: 0.002, 0.004, 0.008, 0.016, 0.032, 0.064, and 0.128 µg a.s/bee dispersed in Tween 85 (1%) and controls. The contact test was performed with a drop of 1 µL per bee. A Tween 85 (1%), an acetone and a demineralised water control were included. Reference item was dimethoate (425 g/L) dosed at 0.046, 0.10, 0.22, and 0.46 µg a.s/bee.

Oral exposure:

Seven nominal test concentrations were used: 0.002, 0.004, 0.008, 0.016, 0.032, 0.064, and 0.128 µg a.s/bee and controls. Bees were offered 200 µL of test item contaminated food solution containing 50% sucrose solution/10 bees. The amount of test solution consumed by each replicate was determined after 4 hours. Doses of reference item were 0.046, 0.10, 0.22, and 0.46 µg a.s/bee.

Environmental conditions

Controlled climatic room: 25 ± 2 ºC, 50 – 70% RH, continuous dark, except during observations. Feeding: a 50% sucrose solution in dechlorinated tap water *ad libitum*. In the oral test food was provided after 4 hours of exposure with contaminated food.

Verification of dosages

No verification.

Calculations and statistics

Mortality rates were corrected according to formula of Schneider-Orelli. LD50 was calculated by sigmoidal dose-response regression according to Clopper and Pearson (1934). Dunn's method for comparison of food uptake. Software programms used were GraphPad Prism 4, SigmaStat rel. SPSS and Excel.

Results

Contact test:

No mortality in any control (Tween 85, 1%), water and acetone). No mortality at any observation time-point up to 0.008 µg as/bee. No mortality at any concentration after 4 hours. Mortality was first observed

after 24 hours from 0.016 µg as/bee 3.33% and was at 0.128 µg as/bee 6.67%. Mortality rates increased during the observation period. After 96 hours mortality was ~~10% at 0.016 and 96.7% at µg as/bee~~0, 0, 0, 0, 0, 10, 20, 53.3, and 96.7% at tween control, water control, acetone control, 0.002, 0.004, 0.008, 0.016, 0.032, 0.064, and 0.128 µg as/bee, respectively.

Table 2: LD50 values after 48, 72, and 96 hours.:

Time point [hours]	LD50 [µg as/bee]	95% CI [µg as/bee]
48	0.086	0.053 – 0.139
72	0.057	0.044 – 0.075
96	0.057	0.044 – 0.073

Oral test:

The mean (of three replicates) consumed test item by bees was 0.002, 0.004, 0.007, 0.015, 0.029, 0.043, and 0.093 µg as/bee. Significant lower food uptake, compared to control, was observed in the nominal test concentrations 0.064 and 0.128 µg as/bee. No mortality in the control group. Mortality in the test concentrations were 3.33, 16.7, 6.67, 10.0, 33.3, 60.0, and 33.3% at mean real test concentrations of 0.002, 0.004, 0.007, 0.015, 0.029, 0.043, and 0.093 µg as/bee. An repellence effect was observed in the two highest test concentrations. 96-h LD50 is reported to be 0.110 µg as/bee (95% CI: 0.020 – 0.594 µg as/bee, based on actual test item concentrations.

Table 3: Treatment concentrations and mortality of the bees in the oral test.

Nominal dose provided [µg as/bee]	Food uptake [%]	Actual dose consumed [µg as/bee]	Mortality after 96 h [%]
Control	100	--	0
0.002	100	0.002	3.33
0.004	99.6	0.004	16.7
0.008	86.3	0.007	6.67
0.016	89.8	0.015	10.0
0.032	92.3	0.029	33.3
0.064	67.3 *	0.043	60.0
0.128	72.8 *	0.093	33.3

*: significant difference compared to control.

Remarks

Validity criteria were met. Percentage mortality for the test item concentrations in the oral test was not related to the dose consumed. 50% mortality was not reached at nominal 0.128 µg as/bee while at 0.064 µg as/bee 60% mortality was observed. In addition, food repellence was observed in the two highest test concentrations (food uptake was 67 and 73% compared to control at nominal 0.064 and 0.128 µg as/bee). It is uncertain whether the lower food uptake was caused by only a few bees, resulting in a lower mortality. Due to the fact above, the oral LD_{50} was recalculated by Spearman-Karber with omitting the highest test concentration. The recalculated 96-h oral LD_{50} is 0.037µg as/bee (95%CI: 0.035 – 0.069 µg as/bee), based on mean consumed test concentration. The 96-h contact and oral LD_{50} values of 0.057 and 0.037 µg as/bee can be used for risk assessment.

1.1.3 Effects on other arthropod species

1 Effects on arthropod species

Substance	Species	Method	Dose [g/ha]	Dose [g as/ha]	Exposure duration [d]	Parameter	Adverse effect [%]	RI
KOHINOR 700 WG	*Typhlodromus pyri*	Residues on glass	1.7	1.2	7 14	Mortality Reproduction		3
KOHINOR 700 WG	*Typhlodromus pyri*	Residues on glass	5	3.5	7 14	Mortality Reproduction		3
KOHINOR 700 WG	*Typhlodromus pyri*	Residues on glass	15	10.4	7 14	Mortality Reproduction		3
KOHINOR 700 WG	*Typhlodromus pyri*	Residues on glass	45	31.3	7 14	Mortality Reproduction		3
KOHINOR 700 WG	*Typhlodromus pyri*	Residues on glass	135	93.8	7 14	Mortality Reproduction		3
KOHINOR 700 WG	*Typhlodromus pyri*	Residues on glass	405	281.5	7 14	Mortality Reproduction		3
KOHINOR 700 WG	*Typhlodromus pyri*	Residues on glass	1215	844.4	7 14	Mortality Reproduction		3

Substance	Species	Method	Duration [d]	Criterion	Value [L/ha]	Value [g as/ha]	RI
KOHINOR 700 WG	*Typhlodromus pyri*	Residues on glass	7	LR_{50}			3

Reference

[3]

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Guidelines

IOBC, BART and EPPO, Blümel et al. (2000) and ESCORT 2, Candolfi (2001)

Test substance

KOHINOR 700 WG (69.5% imidacloprid, analysed). Solubility in water: dispersible.

Test organisms

Typhlodromus pyri (Scheuten), protonymphs < 24 h old, obtained from a commercial provider.

Principle of method

Mites were exposed to fresh dried residues on glass plates. Mortality and escaped mites were assessed after 4 and 7 days of exposure. Number of males and females were counted on day 7 and the number of eggs/female was determined at three assessment days from day 7 onwards. Eggs laid up to and including day 7 were removed and were not counted. Males, females, eggs and hatched juveniles were counted on day 10, 12, and 14 after exposure. Reproduction was not assessed in the toxic reference.

Application, concentrations, replicates

Control (water), 1.7, 5, 15, 45, 135, 405, and 1215 g product/ha (corresponding to 1.2, 3.5, 10.4, 31.3, 93.8, 281.5, and 844.4 g as/ha). Toxic standard Danadim Progress (dimethoate, 425 g/L) at 15 mL/ha. Spray volume was 200 L/ha. Five replicates for the control, each test concentration and reference substance. Each replicate contained 20 protonymphs. Pollen from apple and walnut were added as feed every 3 days during the whole study and tap water was supplied *ad libitum* by wet filter paper.

Environmental conditions

Controlled climate chamber, 23.0 – 27°C, 62 - 85% RH, 16 : 8 h L : D (444- 780 lux).

Verification of dosages

Calibration of the equipment by weighing of sprayed glass plates before and after spraying with water.

Calculations and statistics

Mortality was corrected using Schneider and Orelli's formula.

Dunnett's test to compare test concentration to control after performing a normality test and equal variance test. Due to the results the LD_{50} and EC_{50} were not calculated by sigmoidal dose-response regression but could be determined out of the results. Statistical programme used was SigmaStat and Excel.

Results

Results for mortality and fecundity are summarised in the table below

Table 4: Mortality and fecundity of *Typhlodromus pyri* after exposure to BAS 480 38 F.

Treatment [g product/ha]	Mortality At day 7 [%]	Control corrected mortality [%]	Escaped after 7 days [%]	Mean cumulative number of eggs/female	Fecundity reduction [%]
Control	10	-	8	5.70	-
1.7	26	17.8	23	3.69	35.3 *
5	46	40.0 *	42	3.67	35.6 *
15	76	73.3 *	69	n.d	n.d
45	99	98.9 *	81	n.d	n.d
135	32	24.4	27	4.08	28.4
405	63	58.9 *	52	n.d	n.d
1215	97	96.7 *	66	n.d	n.d
Reference substance	73	70.0	20	n.d	n.d

*: statistically significant difference compared to control.
n.d.: not determined

A significant effect on mortality was found at 5 out of the 7 the test concentrations. At 135 g product/ha no significant mortality was found (Dunnett, α=0.05). No clear dose-response was apparent. The same effects were found in the three preliminary tests. Most of the dead mites were found in the glue barrier (escaped). According to the author, this indicates a repellent effect of the test item. Reproduction was only tested at 1.7, 5 and 135 g product/ha. Reproduction was significant reduced at 1.7 and 5 g product/ha compared to control. The 7-days LR_{50} for mortality is conservatively reported to be > 5 g product/ha (equivalent to 3.5 g as/ha). The effect on fecundity at 1.7, 5, and 135 g product/ha was < 50%.

Remarks

Validity criteria were met (dead and escaped mites in the control on day 7 <20%, cumulative mean number of eggs/female in the control from day 7 to day 14 was ≥ 4 and the cumulative control corrected mortality on day 7 in the toxic reference was between 50% and 100%). A reason for the low mortality at 135 g product/ha could not be given by the author. The same dose-response effect was observed for the escaped mites, also few escaped mites were found at 135 g/product/ha and thereafter the number of escaped mites increased. No reason could be given by the more or less two separate response effects in the mortality test. The fact that very high numbers of escapees were found in the lowest concentrations, indeed indicates that the active substance is highly repellent. The escapees were included in the numbers of dead insects. Thus, it is expected that the mortality rates are too high at the lower concentrations. This

would explain the two seemingly separated into two “series”. Another way to determine to mortality is however not possible. The LD_{50} value can not be determined. The results can not be used for risk assessment.

2 Effects on arthropod species

Substance	Species	Method	Dose [g/ha]	Dose [g as/ha]	Duration [d]	Parameter	Adverse effect[1] [%]	Ri
KOHINOR 700 WG	*Aphidius rhopalosiphi*	Residues on glass plates	0.003	0.002	2	Mortality reproduction	0 +44	1
KOHINOR 700 WG	*Aphidius rhopalosiphi*	Residues on glass plates	0.01	0.007	2	Mortality reproduction	1.73 +22	1
KOHINOR 700 WG	*Aphidius rhopalosiphi*	Residues on glass plates	0.033	0.023	2	Mortality reproduction	22.4 + 46	1
KOHINOR 700 WG	*Aphidius rhopalosiphi*	Residues on glass plates	0.1	0.07	2	Mortality reproduction	71.6 n.d	1
KOHINOR 700 WG	*Aphidius rhopalosiphi*	Residues on glass plates	0.3	0.21	2	Mortality reproduction	100 n.d	1
KOHINOR 700 WG	*Aphidius rhopalosiphi*	Residues on glass plates	0.9	0.63	2	Mortality reproduction	100 n.d	1

1: as corrected for the control
a + sign for reproduction indicates a positive effect.
n.d : not determined.

Substance	Species	Method	Duration [d]	Criterion	Value [g/ha]	Value [g as/ha]	Ri
KOHINOR 700 WG	*Aphidius rhopalosiphi*	Residues on glass plates	2	LR_{50}	0.062	0.043	1

Reference

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Guidelines

IOBC, Mead-Briggs et al. (2000).

Test substance

KOHINOR 700 WG (69.5% imidacloprid, analysed). Solubility in water: dispersible.

Test organisms

Aphidius rhopalosiphi, adult wasps < 48 h old from a commercial provider. Adaptation at least 2 h.

Principle of method

Wasps were exposed to fresh residues on glass plates, mortality was assessed after 2, 24 and 48 h. Sublethal effects and the position of the wasps were recorded at the same time as the condition. Surviving females were transferred to untreated aphid infested barley plants (50 - 100 aphids/pot). Wasps were allowed to parasitise for 24 h and parasitised aphids were counted after 11 days. No reproduction phase in the toxic reference.

Application, concentrations, replicates

Control (water), 0.003, 0.01, 0.033, 0.1, 0.3, and 0.9 g product/ha (0.002, 0.007, 0.023, 0.070, 0.21, and 0.63 g as/ha). Toxic standard Danadim Progress (dimethoate 425 g/L), 0.12 g as/ha. Spray volume was 200 L/ha. Mortality assessment: four replicates per test and reference treatment, six replicates in the

control, each replicate with 10 adults with at least 5 females. Fecundity assessment: 15 replicates at 0.033 g product/ha and 25 replicates in the control and the other test concentrations, each with a single female wasp.

Environmental conditions

18 - 20 °C, 60 - 90% RH, 16 h light (823 - 1840 lux) for the mortality phase and 16.5 – 23 ºC and 16 h light (3000 – 6740 lux) for the reproduction phase. A honey water mixture (1:6 v/v) was provided as food in the mortality phase, no additional feeding in the reproduction phase.

Verification of dosages

Weighing of sprayed glass plates.

Calculations and statistics

Mortality was corrected according to the formula of Schneider-Orelli. ANOVA with Dunnett's test for comparison. LD_{50} for mortality was calculated by sigmoidal doe-response regression. Program used was SigmaStat and Excel.

Results

Mortality phase: control mortality 3.33%, control corrected mortality (Schneider-Orelli) 0%, 1.73%, 22.4%, 71.6%, 100%, and 100%.at 0.003, 0.01, 0.033, 0.1, 0.3, and 0.9 g product/ha, respectively. At 0.033 to 0.9 g product/ha effects were statistically significant compared to the control. Wasps at 0.01 and 0.033 g product/ha without coordination problems moved slower compared to the control. LD_{50} for mortality is calculated to be 0.062 g product/ha (95% CI: 0.050 – 0.076), corresponding to 0.043 g as/ha.
Reproduction phase: mean number of mummies per female in the control was: 17.6. The surviving females (without any coordination problems) at 0.003, 0.01, and 0.033 g product/ha were tested for reproduction. Mean number of mummies/female were: 25.4, 21.4 and 25.7 at 0.003, 0.01 and 0.033 g product/ha, respectively. No statistically significant reduction of the reproduction compared to control could be observed in these tested concentrations.

Remarks

Validity criteria were met (mortality in the control < 13%, > 5 mummies/female in the control and two wasps out of 25 which produced zero mummies in the control). The result LR_{50} for mortality of 0.062 g product/ha (0.043 g as/ha) and < 50% effect on reproduction at concentrations up to 0.033 g product/ha (0.002, 0.007, and 0.023 g as/ha) can be used for risk assessment.

1.1.4 Effects earthworms

1.1.4.1 Acute toxicity

1 Toxicity earthworms

Substance	Species	Soil type	pH	T [°C]	Duration [d]	Criterion	Value [mg/kg]	Value [mg as/kg]	RI
KOHINOR 700 WG	*Eisenia fetida fetida*	Artificial soil	5.4 – 6.1	18 - 20	14	LC_{50}	27.4	19.0	1

Reference

~~Insert temporary Procite marker~~[4]

Guidelines

OECD 207 (1984)

Test substance

KOHINOR 700 WG (69.5% imidacloprid, analysed). Solubility in water: dispersible.

Test organisms

Eisenia fetida fetida, adults (with clitellum). Acclimatisation for two days.

Principle of method

Earthworms were exposed to contaminated soil~~,~~. M~~m~~ortality and sublethal effects were determined after 7 and 14 d, weight of surviving worms was determined after 14 d.

Soil

OECD artificial soil: 69% industrial quartz sand, 20% kaolin clay, 10% sphagnum peat, ≤ 1% $CaCO_3$ to adjust pH to 6.

Application, concentrations, replicates

Control (water), 2.82, 5.63, 11.3, 22.5, 45.0, and 90.0 mg product/kg dwt. Substance was added mixed in the artificial soil. Four replicates per treatment with 10 worms each. Reference item: 2-chloroacetamide (99.8%) was tested within 7 months before at 15, 30, and 60 mg/kg dwt of soil.

Environmental conditions

18-22 °C, 16 h light (400 - 800 lux). Water content was 52% of the MWHC at the start of the test. No feeding.

Verification of dosages

No verification of dosages.

Calculations and statistics

Comparison of controls and treatments with ANOVA, Dunnett's test and Kruskal-Wallis ($\alpha = 0.05$). LC50 value was calculated by sigmoidal dose-response regression using procedures according to Clopper and Pearson (1934). Programs used were: Excel, SigmaStat, SigmaPlot and GraphPad Prism.

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Results

No mortality in the control and mortality in the test concentrations were: 2.5%, 2.5%, 2.5%, 12.5%, 97.5% and 100% after 14 days at 2.82, 5.63, 11.3, 22.5, 45.0, and 90.0 mg product/kg dwt. Mortality at the two highest test concentrations differed significantly from the control.
Sublethal effects were found at 22.5 mg product/kg dwt and and higher, after 7 days. Observed sublethal effects were: spontaneous segmentation and separation, ulcer and skin bleedings.
Average weight decreased 4% in control, 13, 15, 19 and 23% difference at 2.82 to 22.5 mg product/kg dwt (all significant). Only the body weight decrease of 23% at 22.5 mg product/kg dwt was found biological significant. At lower concentrations the biomass loss was below 20% and thus not biologically significant.
LC_{50} value is reported to be 27.4 mg product/kg dwt (95% CI: 26.0 – 28.8 mg product/kg dwt). LC_{50} of the reference item was reported to be 55.8 mg/kg dwt.

Remarks

Validity criteria were met.
The result LC_{50} 27.4 mg product/kg dwt of soil with 10% peat (corresponding to 19.0 mg as/kg dwt) can be used for risk assessment.

1.1.4.2 Sub-lethal toxicity

1 Toxicity earthworms

Substance	Species	Soil type	pH	T [°C]	Duration [d]	Criterion	Value [mg/kg]	Value [mg as/kg]	Ri
KOHINOR 700 WG	*Eisenia fetida*	artificial	6.0 – 6.9	17.0 22.5	56	NOEC	≥ 1.44	≥ 1.0	1

Reference

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Guidelines

OECD 222 (2004).

Test substance

KOHINOR 700 WG (69.5% imidacloprid, analysed). Solubility in water: dispersible.

Test organisms

Eisenia fetida Savigny 1826, in-house bred, adults 2 - 12 months old with clitellum, difference of age did not deviate by more than 1 month (0.30 -0.60 g at the start). Acclimatisation 2 days in artificial soil.

Principle of method

Earthworms were exposed to contaminated soil. Mortality, behaviour and weight of worms were determined after 4 weeks, when adult worms were removed from the treated substrate. Reproduction (number of juveniles) was determined after 8 weeks.

Soil

OECD artificial soil: 69% industrial quartz sand, 20% kaolin clay, 10% sphagnum peat, 0.40% $CaCO_3$ to adjust pH to 6. Water content was 52% of MWHC.

Application, concentrations, replicates

Control (water), 0.09, 0.18, 0.36, 0.72, and 1.44 mg product/kg dwt (corresponds to 0.0625, 0.125, 0.25, 0.5, and 1.0 mg as/kg dwt, respectively).Test item was thoroughly mixed into the soil. Four replicates per treatment with 10 worms each. Worms were placed on the contaminated soil. Reference substance was carbendazim (502 g/L) at 1.0, 2.5 and 5.0 mg as/kg dwt, tested at least once a year.

Environmental conditions

20 ± 2 ºC, 16 h light (400 – 800 lux). Test vessels were covered with perforated lids which allowed gas exchange. Feeding with cattle manure weekly with 5 g/vessel. Cattle manure was moistened to maintain the soil moisture.

Verification of dosages

No verification of dosages.

Calculations and statistics

Normality and equal variance test, ANOVA for comparison of control with treatments (α = 0.05). Statistical programs used were Excel, Sigma Plot and Sigma Stat.

Results

Moisture content of the soil was 30.8 – 32.8% of dwt at the start and 39.4 – 41.4% dwt at the end. No mortality was observed in the control and all test concentrations after 28 days of exposure. At 1.44 mg product/kg dwt one worm was found with skin bleeding. All other worms showed no pathological symptoms or changes in behaviour after 28 days of exposure. Body weight of the worms increased in the control and all other test concentrations throughout the first 28 days, biomass was not statistically significant different from control. Mean number of juveniles in the control was 284, average number of juveniles in the test concentrations were: 272, 291, 269, 284, and 233 at 0.0625, 0.125, 0.25, 0.5 and 1.0 mg as/kg dwt, respectively. No significant difference compared to control in any test concentration. The NOEC is reported to be ≥ 1.44 mg product/kg dwt (corresponds to ≥ 1.0 mg as/kg dwt) for mortality, body weight and reproduction.

Remarks

Validity criteria were met.

The result NOEC ≥ 1.44 mg product/kg dwt of soil containing 10% o.m. (corresponds to ≥ 1.0 mg a.s/kg dwt) for mortality, body weight change and reproduction can be used for risk assessment.

1.1.5 Effects on soil non-target micro-organisms

1 Effects on soil micro-organisms and processes

Substance	Soil type	Dose [mg as/kg]	Dose[1] [kg as/ha]	Duration [d]	OM [%]	pH	T [°C]	Process	Maximal effect [%]	After ... [d]	Effect at end > 25% [Y/N]	Ri
KOHINOR 700 WG	Loamy sand	0.20	0.15	28	1.6	7.5	20±2	C-mineralisation	-3	14	N	1
KOHINOR 700 WG	Loamy sand	1.0	0.75	28	1.6	7.5	20±2	C-mineralisation	-9	14	N	1

1: assuming 5 cm soil depth and soil bulk density 1500 kg/m^3
a negative sign represents an increase.

Reference

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Field Code Changed

Guidelines

OECD 217 (2000).

Test substance

KOHINOR 700 WG (69.5% imidacloprid, analysed). Solubility in water: dispersible.

Principle of method

Soil was applied with the test substance, short-term respiration was determined after addition of glucose (4 g/kg) after 0 – 2h, and 7, 14 and 28 d.

Soil

A loamy sand soil, (LUFA soil 2.3, sieved at 2 mm). MWHC 34.4 g/100 g soil dwt, 1.6% o.m., CEC: 80 mmol/kg, pH 6.4, microbial biomass 3.0% of total organic carbon. No history of pesticide use in the past five years, no application of organic fertilisers, use of inorganic fertilisers (two years), three years before sampling. Soil was stored for 14 days in the dark at 6 ºC in a climatic room and preincubated at room temperature for 19 days before use. Moisture content adjusted to 45% of MWHC prior to application.

Application, concentrations, replicates

Control (water), 0.29 mg product/kg dwt (0.20 mg as/kg dwt, 0.215 kg product/ha, 0.15 kg as/ha), 1.43 mg/kg dwt (1.0 mg as/kg dwt, 1.075 kg product/ha, 0.75 kg as/ha). Three replicates (3000 g dwt). Soil was adjusted to 50% of MWHC at the start of the test. Toxic standard: dinoterb (98.0% pure), 13.75 kg/ha)18.3 mg/kg dwt), performed 6 months before.

Environmental conditions

20 ± 2 °C, dark

Verification of dosages

No verification of dosages.

Analysis

Respiration: evolved CO_2 was measured continuously with a pressure sensor for 2424 h.

Calculations and statistics

Oxygen consumption was calculated based on the difference of pressure between the start and end of the measurement period. Mean respiration rates, standard deviations, and coefficients of variation were calculated for each treatment and assessment date. Difference from control was calculated.

Results

Coefficient of variation in the control 1 - 4%. Deviations from control at 0.15 kg as/ha: 1%, 0%, -3%, and -1% on days 0, 7, 14 and 28, respectively. Deviations at 0.75 kg as/ha: -6%, -5%, -9%, and -5%. Values with a negative sign represent an increase compared to control. Deviations in dinoterb treatment: -36 to -40%.

Remarks

Variation between the replicate control samples was less than 15%. Thus the study is regarded to be valid. CO2 production was measured for 24 consecutive h instead of 12 h according to the guideline. However, the control showed a proper response.

The result <25% effect on respiration at concentrations up to 1.0 mg as/kg after 28 d can be used for risk assessment.

2 Effects on soil micro-organisms and processes

Substance	Soil type	Dose [mg as/kg]	Dose[1] [kg as/ha]	Duration [d]	OM [%]	pH	T [°C]	Process	Maximal effect [%]	After ... [d]	Effect at end > 25% [Y/N]	Ri
KOHINOR 700 WG	Loamy sand	0.20	0.15	28	1.6	7.3	20±2	nitrification	30.5	14	N	1
KOHINOR 700 WG	Loamy sand	1.0	0.75	28	1.6	7.3	20±2	nitrification	13.6	14	N	1

1: assuming 5 cm soil depth and soil bulk density 1500 kg/m3

Reference

Insert temporary Procite marker[11][10]

Field Code Changed

Guidelines

OECD guideline 216 (2000).

Test substance

KOHINOR 700 WG (69.5% imidacloprid, analysed). Solubility in water: dispersible).

Principle of method

Soil was applied with the test substance and amended with. Lnitrification was determined after addition of Lucerne grass meal (0.5% of dwt), nitrification was determined after 0, 7, 14 and 28 d.

Soil

A loamy sand soil, (LUFA soil 2.3, sieved at 2 mm). MWHC 34.4 g/100 g soil dwt, 1.6% o.m., CEC: 80 mmol/kg, pH 6.4, microbial biomass 3.0% of total organic carbon. No history of pesticide use in the past five years, no application of organic fertilisers, use of inorganic fertilisers (two years), three years before

sampling. Soil was stored for 14 days in the dark at 6 ºC in a climatic room and preincubated at room temperature for 19 days before use. Moisture content adjusted to 45% of MWHC prior to application. Amendment with powdered Lucerne grass meal at 0.5% dwt.

Application, concentrations, replicates

Control (water), 0.29 mg product/kg dwt (0.20 mg as/kg dwt, 0.215 kg product/ha, 0.15 kg as/ha), 1.43 mg/kg dwt (1.0 mg as/kg dwt, 1.075 kg product/ha, 0.75 kg as/ha). Three replicates (450 g dwt). Soil was adjusted to 50% of MWHC at the start of the test. Toxic standard: cyanoguanidin (101% pure), 50 and 100 mg/kg dwt), performed about 6 months before.

Environmental conditions

20 ± 2 °C, dark

Verification of dosages

No verification of dosages.

Analysis

Nitrification: analysis of NO_2-N, NO_3-N and NH_4-N by photometric determination at 588 nm after extraction with 1M KCl and 2M KCl for 1 hour. The spectrophotometer was calibrated prior to the start and analyse method was validated on linearity and LOQ was determined, LOQ for nitrate was 1.90 mg NO_3-N/kg dwt.

Calculations and statistics

Average nitrate-N contents for each assessment date and treatment were calculated. Standard deviations and coefficient of variation were determined. Formation rates compared to day 0 and their deviation of the control were calculated for each sampling date. Software used was Excel (2003) and SigmaPlot (2002).

Results

Table 5: with the differences of the nitrate-N contents compared to control.

Application rate	Differences of nitrate-N content [%]			
[kg product/ha]	0 d	7 d	14 d	28 d
0.215	5	-1	-9	1
1.075	8	-3	-6	0

A negative sign represents an increase.

Table 6: with the differences of the nitrate-N formation rates compared to control.

Application rate	Differences of nitrate-N formation rates [%]		
[kg product/ha]	7 d	14 d	28 d
0.215	10	-6	4
1.075	10	-3	4
Reference 50 mg/kg dwt	61	66	20
Reference 100 mg/kg dwt	103	73	87

A negative sign represents an increase.

Remarks

Nitrate formation rates were recalculated by the reviewer conform OECD guidelines.

Table 7: Formation rates of NO_3^--N in soil [mg/kg dwt]

Dose [kg/ha]	Day 0-7	Day 7-14	Day 14-28
0.215	-10.4	30.5	-19.4
1.075	-10.4	13.6	-9.7

A negative sign represents an increase.

Variation between the replicate control samples was less than 15%. Thus the study is regarded to be valid.

The result <25% effect on nitrification at concentrations up to 1.0 mg as/kg after 28 d can be used for risk assessment.

Referenties

~~Op alfabetische volgorde van auteurs. Zie verder de ProCite manual van~~ 5.1.2.e

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~~3. Bruhnke, C. (2009) KOHINOR 700 WG Laboratory test on *Typhlodromus pyri* (Acari: Phytoseiidae). Dr. U. Noack-Laboratorien, Sarstedt, Germany, IRL12347.~~

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