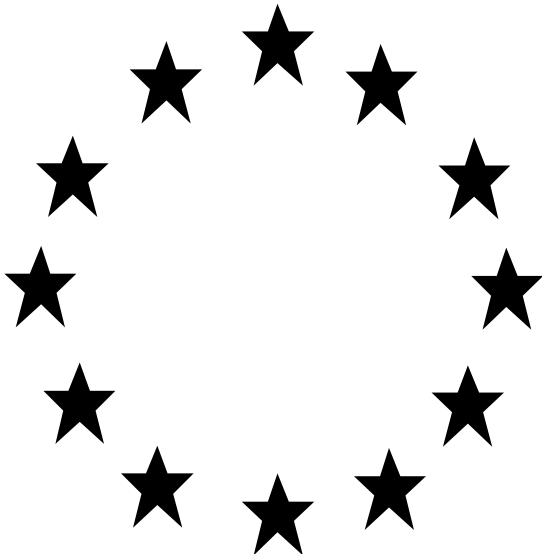

Draft Assessment Report (DAR)

- public version -

Initial risk assessment provided by the rapporteur Member State Sweden for the existing active substance

TOLCLOFOS-METHYL

of the second stage of the review programme referred to in Article 8(2) of Council Directive 91/414/EEC

Volume 3, Annex B, B.9, part 2

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B.9.3 Effects on other terrestrial vertebrates (Annex IIIA 10.3)

B.9.3.1 Acute oral and long term toxicity

In studies on acute oral toxicity of tolclofos-methyl to mammals, LD_{50} values were found to be >5000 mg/kg bw for rats and > 3500 mg/kg bw for mice, indicating a low toxicity. The value for mice was used in the acute risk assessment. Reproduction toxicity was only studied in rats and did not reveal evidence of adverse reproductive toxicity. For the long-term risk assessment, the NOAEL from the available data was selected from a 3-generation reproduction study in rats (Pence et al., 1985b), where the NOAEL was concluded to be 70.6 mg/kg bw/day, (see Section B.6.6.1.1).

B.9.3.2 Risk assessment for mammals

Notifiers assessment

Residue levels of tolclofos-methyl in food items of mammals (e.g. insects, fruit and vegetation) are expected to be relatively low due to the recommended usages as soil application to greenhouse lettuce or as potato tuber dressing. The Notifier provided a risk assessment based on estimated initial residues on various categories of feed items calculated according to the method of Hoerger and Kenaga (1972). The result is presented in Table 9.3.2.a.

Table 9.3.2.a. Notifiers risk assessment for mammals exposed to tolclofos-methyl.

Feed item category	Application rate (g a.s./ha)	Time scale (Acute/Short-term/Long-term)	Estimated daily intake (mg a.s./kg bw/day)	$LD_{50}/LC_{50}/NOEC$ as Daily Dose (mg a.s./kg bw/day)	Toxicity exposure ratio (TER)	TER assessment trigger
Leafy foliage	625 (potato tuber dressing)	Acute	5.82	3500	601	<10
		Long-term	5.82	70.6	12	<5
Small insects	625 (potato tuber dressing)	Acute	5.43	3500	645	<10
		Long-term	5.43	70.6	13	<5
Seed, large insects	625 (potato tuber dressing)	Acute	0.51	3500	6863	<10
		Long-term	0.51	70.6	138	<5
Fruits	625 (potato tuber dressing)	Acute	0.24	3500	14583	<10
		Long-term	0.24	70.6	294	<5

RMS assessment

Following the proposed uses (on potato tubers and in glasshouses) the exposure on vegetation will be limited and the risk to mammals low. In accordance with the risk assessment performed for birds and in view of current uses within the EU, RMS also considered spray application for protection of wheat as help for further assessment of extended uses at the MS level. In the case of wheat, spray application is carried out just after tillering, on bare soil. RMS therefore considers exposure of terrestrial mammals via residues in food items to be limited and mainly caused by secondary poisoning.

Secondary poisoning

The main route of secondary poisoning will be via soil-dwelling organisms e.g. earthworms. In order to assess the potential for bioaccumulation and to consider the estimated residue levels in earthworms for a mammalian risk assessment, the approach recommended in the "Guidance Document on Risk Assessment for Birds and Mammals Under Council Directive 91/414/EEC" (Working Draft February 2001, SANCO/4145/2000) has been followed.

A simple worst-case assessment was conducted according to the following steps:

The 3-week time-weighted soil PEC of 0.99 mg/kg was used (application rate of 2kg/ha and a DT_{50} in soil at 20ºC = 3.95 days). The bioconcentration factor BCF (C_{worm}/C_{soil}) was estimated according to the formula:

$$BCF = (0.84 + 0.01\ K_{ow}) / f_{oc}\ K_{oc}$$

where tolclofos methyl K_{oc} = 3620 (Section B.8.2.1); K_{ow} = 36308 (log P_{ow} = 4.56); f_{oc} (organic carbon content of soil) = 0.02 as default value. On this basis, BCF was estimated to 5.03.

The estimated residue level in earthworms = 0.99 mg a.s./kg x 5.03 = 5.0 mg a.s./kg.

Earthworm residue level was then converted to daily dose by multiplying by 1.4 (10 g mammal eating 14 g per day) = 7 mg a.s./kg b.w./day. The daily dose value was compared with long term NOAEL value of >70.6 mg/kg b.w./day which resulted in a TER value of 10.

Hence, the risk for secondary poisoning is considered acceptable.

B.9.4 Effects on bees (Annex IIA 8.3.1; Annex IIIA 10.4)

B.9.4.1. Acute toxicity

ACTIVE INGREDIENT

Reference:	Winter, P.A., Hoxter, K.A., Smith, G.J. (1990) Rizolex: An acute contact toxicity study with the honey bee
Guideline:	US EPA FIFRA 141-1 (1982, October draft)
GLP:	Yes (Self certification by the laboratory)

Material and methods:

Test substance:	Tolclofos-methyl (Batch No.: 40810, Purity: 97.7% dissolved in acetone
Species:	*Apis mellifera*
Treatments:	0, 13, 22, 36, 60 and 100 µg a.s./bee.
Number of animals:	2 replicates of 25 bees/treatment
Duration:	48 hours
Test conditions:	Worker honey bees 1 to 4 days of age were used. Bees were anaesthetised with nitrogen and then dosed with a 2 µL droplet of the test solution applied to the thorax and/or abdomen using a micro-pipette. Photoperiod was 8 hours light and 16 hours dark, temperature 22-23°C and mean relative humidity 82%. A 50 % sugar/water solution was provided *ad libitum*. The test was carried out in August.
Observations:	Mortality and signs of toxicity were observed twice on the day of test initiation (Day 0) and once each on Day 1 and Day 2.

Results:

Environmental parameters remained within acceptable limits throughout the study. Mortality at test termination (48 hours after application) in the negative and solvent control groups was 2 and 10%, respectively. No significant treatment-related effects were observed in any of the treatment groups (up to 4% mortality with no dose-response).

Based on the results of this study the 24- and 48-hour contact LD_{50} could not be determined and was therefore estimated to be >100 µg a.s./bee, the highest dose tested. The corresponding NOEC was set to 100 µg a.s./bee.

Comments:

The study was well performed and reported. No positive control was included in the test, however this is not a requirement in the guideline referred to, and the study is accepted.

No study on oral toxicity was submitted. This is not considered necessary since oral and contact toxicity of the formulated product tolclofos-methyl 50WP was investigated.

PLANT PROTECTION PRODUCT

Reference:	Londzin, W. (1997) The toxicity of Rizolex 50WP to the honey-bee
Guideline:	The Hygienic-Toxicological Requirements for Pesticides Registration in Poland and German Democratic Republic, 1976, Method # 12

GLP: No

Material and methods:

Test substance:	Rizolex 50WP (Batch No.: Not specified, Contents: 50% (nominal
Species:	*Apis mellifera*
Treatments:	**Oral test:** 0, 125, 250, 500 and 1000 µg test substance/bee (nominally, 62.5, 125, 250 and 500 µg a.i./bee) **Contact test:** 0, 100, 200, 400 and 800 µg test substance/bee (nominally, 50, 100, 200 and 400 µg a.i./bee
Number of animals:	150 bees per treatment level (3 replicates of 50 bees each) and test type
Duration:	24 hours
Test conditions:	**Oral test:** The test substance was applied as a suspension in sugar solution to individual bees using a micropipette. Each bee received 10µl of test solution. **Contact test:** The test substance was applied individually as suspension in water on the upper thorax of each bee, using a micro-applicator. The application volume was 1µL/bee
Observations:	Mortality

Results:

The results from the oral and contact toxicity tests on honeybees are presented in Tables 9.4.1.b and 9.4.1.c. respectively.

Table 9.4.1.b. Mortality data for honeybees exposed to tolclofos-methyl 50WP by oral administration

Treatment/nominal concentration (µg product/bee)	Mortality (%)
0 (control)	0
125	5
250	30
500	95
1000	95

Table 9.4.1.c. Mortality data for honeybees exposed to tolclofos-methyl 50WP by contact administration

Treatment/nominal concentration (µg product/bee)	Mortality (%)
0 (control)	0
100	2
200	14
400	54
800	62

Based on the results of this study the 24-hour acute oral LD_{50} was 300.9 µg product/bee (150.5 µg a.s./bee). The 24-hour acute contact LD_{50} was 508.5 µg product/bee (254.3 µg a.s./bee).

Comments:

Very limited information on test conditions was submitted. Mortality was only observed after 24 hours. Only four doses were tested and no positive control was used. The statistical method used for the calculation of LD_{50} was not reported and no confidence limits were given in the report. However, since both the active ingredient

and the formulated product seem to be of low toxicity and the proposed uses for tolclofos-methyl are as a tuber dressing for potatoes and for application to lettuce in glasshouses it is not expected that tolclofos-methyl will present a high risk to bees. Thus no additional studies are required.

B.9.4.2 Summary and risk assessment for honeybees

The available data from the studies carried out on bees indicate that tolclofos-methyl has low acute contact and oral toxicity to bees. Due to the proposed use pattern of tolclofos-methyl (greenhouse lettuce and as a potato tuber dressing), the potential for exposure to honeybee by spraying or via contaminated flowers is considered to be negligible and no further testing is required, although the studies did not fulfil modern test guidelines. The results from the studies on bees are summarised in Table 9.4.2.a.

Table 9.4.2.a Summary of the studies of tolclofos-methyl toxicity to honeybees

Type of study	Test substances	Dose range tested	Results	Reference
Acute contact	Tolclofos-methyl	13, 22, 36, 60, 100 µg a.s./bee	24- and 48-hour LD_{50}: > 100 µg a.s./bee	Winter, P.A., *et al*., 1990
Acute oral	Rizolex 50 WP	125, 250, 500, 1000 µg product/bee	24-hour LD_{50}: 300.9 µg product/bee (150.5 µg a.s./bee).	Londzin, W., 1997
Acute contact	Rizolex 50 WP	100, 200, 400, 800 µg product/bee	LD_{50} (24 hours): 508.5 µg product/bee (254.3 µg a.s./bee).	Londzin, W., 1997

The Notifier conducted the standard risk assessment (i.e. hazard quotient evaluation) and calculated the oral exposure Q_{HO} by dividing the oral LD_{50} value (µg a.s./bee) by the dose (application rate, g a.s./ha) using the maximum recommended application rate from the range of recommended application rates and the results of the acute oral toxicity studies. In the same way contact exposure Q_{HC} for bees was calculated by dividing the contact LD_{50} value (µg a.s./bee) by the dose (application rate, g a.s./ha). The results are presented in Table 9.4.2.b.

Table 9.4.2.b. Oral and contact exposure hazard quotient for bees exposed to tolclofos-methyl

Field rate (g as/ha)	Exposure route				Hazard quotient assessment trigger
	Oral		Contact		
	LD_{50} (µg a.s./bee)	Hazard quotient	LD_{50} (µg a.s./bee)	Hazard quotient	
2000	150.5	13.3	>100	<20	>50
2000			254.3	7.9	>50

The hazard quotients for the contact and oral routes of exposure are all <50. On the basis of this information, it is concluded that the proposed uses of tolclofos-methyl will present a low risk to bees.

B.9.5 Effects on other arthropod species (Annex IIA 8.3.2; Annex IIIA 10.5)

B.9.5.1 Laboratory tests

PLANT PROTECTION PRODUCT

Reference:	Kollman, S.I. (2002a) Tolclofos-methyl 50WP: Dose response toxicity test with the parasitic wasp, *Aphidius rhopalosiphi* (Hymenoptera: Braconidae)
Guideline:	Based on Mead-Briggs *et al*. (2000)
GLP:	Yes

Material and methods:

Test substance:	Tolclofos-methyl 50WP (Batch No.: B000002, Contents: 503 g a.s./kg
Species:	*Aphidius rhopalosiphi*
Treatments:	0 (deionised water), 8, 16, 32, 64 and 128 g a.s./ha
Number of animals:	**Exposure phase:** 3 replicates of 10 wasps/treatment **Fecundity phase:** 10-15 females/treatment
Duration:	48 hours exposure + 11 days for fecundity
Test conditions:	A preliminary range-finding test was performed with 16, 80, 400, 2000, and 10000 g a.s./ha. **Glass plate test:** Adult females less than 48 h old was used. The glass plates were sprayed with calibrated spray application equipment and left to air dry. Perfecthion (0.3 mL/ha) was used as toxic standard. Feed was supplied in the form of a honey: water solution (1:2). Temperature was 19 -21ºC, relative humidity 74-88% and photoperiod 16 hours light/8 hours dark with a light intensity of 800-1531 lux. **Fecundity test:** Surviving females from the three lowest treatment rates were offered aphids on barley plants for oviposition for 24 hours before surviving wasps were removed. Temperature was 19 -21ºC, relative humidity 74-88% and photoperiod 16 hours light/8 hours dark with a light intensity of 2020-2800 lux. Dimethoate was applied as a toxic standard at a rate of 0.12 g a.s./ha.
Observations:	**Exposure phase:** Healthy, affected, moribund and dead wasps at 24 and 48 hours. **Fecundity phase:** Alive, dead and missing females at 24 h; number of aphid mummies per surviving female at 10 days.

Results:

The test was considered valid since the mean mortality (including dead and moribund wasps) in the control was ≤13% (0%) after 48 hours, the mean mortality in the toxic standard was ≥50% (100%) after 48 hours, the minimum control parasitation rate was >5 aphid per surviving female and no more than 2 females in the control group failed to produce mummies. The summarised results for the test substance are presented in Table 9.5.1.a (mortality) and Table 9.5.1.b (fecundity).

Table 9.5.1.a. Percent healthy, affected, moribund and dead *Aphidius rhopalosiphi* after 48 hours exposure to tolclofos methyl

Treatment (g a.s./ha)	Wasps (%) (mean ± SD)				
	Healthy	Affected	Moribund	Dead	Moribund + dead
0 (control)	100 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
8	100 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
16	93.3 ± 11.5	0 ± 0	0 ± 0	6.7 ± 11.5	6.7 ± 11.5
32	100 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
64	0 ± 0	6.7 ± 5.8	13.3 ± 15.3	80.0 ± 20.0	93.3 ± 5.8
128	0 ± 0	0 ± 0	0 ± 0	100 ± 0	100 ± 0
Toxic standard	0 ± 0	0 ± 0	0 ± 0	100 ± 0	100 ± 0

Table 9.5.1.b. Female mortality and fecundity during the fecundity phase

Treatment (g a.s./ha)	% female mortality (mean ± SD)	Aphid mummies/female (mean ± SD)	% reduction compared to control	Aphid mummies/surviving female (mean ± SD)	% reduction compared to control
0 (control)	0 ± 0 (n = 15)	28.6 ± 7.6 (n = 15)	-	28.6 ± 7.6	-
8	13.3 ± 35.2 (n = 15)	14.4 ± 9.0** (n = 15)	49.7	16.4 ± 7.9** (n = 13)	42.7
16	0 ± 0 (n = 15)	15.5 ± 9.1** (n = 15)	45.7	15.5 ± 9.1** (n = 15)	45.7
32	28.6 ± 46.9* (n = 14)	6.8 ± 6.1** (n = 14)	76.3	8.7 ± 6.1** (n = 10)	69.6

* Statistically significantly different from the control (Fisher's exact test: $p = 0.042$)
** Statistically significantly different from the control (ANOVA: $p < 0.001$; Dunnett's test: $p < 0.001$).

Based on the results of this study the LR_{50} of Tolclofos-methyl (50 WP) to *Aphidius rhopalosiphi* was calculated to be 43.7 g a.s./ha after 48 hours exposure. The corresponding 95 % confidence interval was 19.6 to 154.3 g a.s./ha. Statistically significant effects on the reproduction were observed at all rates tested from 8 to 32 g a.s./ha, hence no NOEL could be established.

Comments:

The standard deviation in some test groups was very large. However, an extended laboratory study is available and can be used to improve the risk assessment.

Reference: Kollman, S.I. (2002b)
Tolclofos-methyl 50WP: Dose response toxicity test with the predatory mite, *Typhlodromus pyri* Scheuten (Acari: Phytoseiidae)
Guideline: Based on Blümel *et al*. (2000)
GLP: Yes

Material and methods:

Test substance: Tolclofos-methyl 50WP (Batch No.: B000002, Contents: 503 g a.s./kg
Species: *Typhlodromus pyri*
Treatments: 0 (deionised water), 1.56, 3.13, 6.25, 12.5 and 25 kg a.s./ha
Number of animals: 4 replicates of 20 mites per treatment

Duration:	14 days (7 days for mortality)
Test conditions:	**Preliminary test:** A range-finding test was performed with 0.016, 0.08, 0.4, 2, and 10 kg a.s./ha for 7 days. No increased mortality compared to the control was observed. **Definitive test:** Protonymphs (differing no more than 24 hours in age) was used as test organisms. Glass cover slips were sprayed with the test substance by use of a calibrated spraying device. Pollen (walnut and apple) as feed and drinking water was supplied *ad libitum*. At least 1:5 males to females were used per replicate. Temperature was 23.5-25.5ºC, relative humidity 63-86 % and photoperiod 16 hours light/8 hors dark with a light intensity of 915-1350 lux. From day 7 through to day 14 the reproduction rate of the surviving mites was determined in the control and all treatment rates (at least three surviving females were found on day 7 in all cases). On days 7, 9, 11 and 14 the sex of the mites and numbers of eggs and, if present, hatched larvae was determined. Eggs and larvae were removed after each assessment. In order to confirm the efficacy of the test system, dimethoate was applied as a toxic standard (at a rate of 4.8 g a.s./ha).
Observations:	Number of alive, dead and missing mites and mites stuck in the barrier at days 7,9,11 and 14 (mortality was considered as the sum of dead and missing mites, including mites stuck in the sticky barrier. Sex of adult mites and number of larvae and eggs for all treatments with more than 50 % surviving mites and at least 3 females on day 7. Mortality in the treated groups was corrected for control mortality according to Abbott (1925), as modified by Schneider-Orelli (1947).

Results:

The test was considered valid since the mean mortality (including dead and missing mites) in the control was <20% (2.5%) on day 7, the mean mortality with the toxic standard was greater than 50% (98.8%) on day 7and the cumulative number of offspring per female was >4 eggs in the control. The summarised results for the test substance are presented in Table 9.5.1.c (mortality) and Table 9.5.1.d (reproduction).

Due to less than 50% mortality at all treatment rates, an LR_{50} value could not be calculated, and LR_{50} of tolclofos-methyl (50 WP) to *Typhlodromus pyri* was estimated to be >25 kg a.s./ha after 48 hours exposure. Statistically significant effects on the reproduction were observed at all rates tested, from 1.56 to 25 kg a.s./ha, but this reduction exceeded 50% only at 6.25 and 25 kg a.s./kg, thus not showing a clear dose-response.

Table 9.5.1.c. Percent mortality for *Typhlodromus pyri* after 7 days exposure to tolclofos methyl

Treatment (kg a.s./ha)	N	% mortality on day 7 (mean ± SD)	% corrected mortality*
0 (control)	4	2.5 ± 5.0	-
1.56	4	6.3 ± 6.3	3.8
3.13	4	15 ± 14.1	12.8
6.25	4	5.0 ± 10.0	2.6
12.5	4	5.0 ± 5.8	2.6
25	4	0 ± 0	-2.6
Toxic standard	4	98.8 ± 2.5	98.7

* Mortality in the treated groups was corrected for control mortality according to Abbott (1925), as modified by Schneider-Orelli (1947).

Table 9.5.1.d. Effects on reproduction for *Typhlodromus pyri*

Treatment (kg a.s./ha)	N	No. eggs/female (mean ± SD)	% reduction in reproduction (compared to control)
0 (control)	4	9.9 ± 0.2	-
1.56	4	6.2 ± 1.2*	37.4
3.13	4	5.0 ± 0.6*	49.6
6.25	4	4.9 ± 1.6*	50.4
12.5	4	5.0 ± 0.4*	49.3
25	4	4.7 ± 0.7*	52.9

* Statistically significantly different from the control (ANOVA: $p < 0.001$; Dunnett's test: $p < 0.001$)

Comments:

The study was well performed and reported.

Reference: Shono, Y. (1998)
Toxicity test of Rizolex (tolclofos-methyl) to two beneficial insects, *Orius sauteri* and *Chrysoperla carnea*

Guideline: In-house method

GLP: No

Material and methods:

Test substance: Tolclofos-methyl 50WP (Contents: 50% (w/w)

Species: *Orius sauteri* and *Chrysoperla carnea*

Treatments: 500 and 1000 ppm

Number of animals: 3 replicates/5 animals/treatment

Duration: 48 hours

Test conditions: Acute toxicity to foliage dwelling predatory species was tested on two species.
Orius sauteri: Five female adults were placed in a glass tube (20 ml) and the testing solution was poured on top of the test insects. A toxic standard, Malathion 50EC was tested at 500 ppm. After 10 seconds immersion, the insects were taken out from the tube and placed in a polyethylene cup (200 ml) in which there was cotton wool soaked with water and a small amount of *Ephestia* eggs for food.
Chrysoperla carnea: Five 2nd instar larvae of testing insects were placed on a small sheet of nylon gauze and dipped into the testing solutions. A toxic standard,

Malathion 50EC was tested at 500 ppm. After 10 seconds immersion, the insects were taken out and each one placed separately in a polyethylene cup (100 ml) in which there was a filter paper disk together with a small amount of *Ephestia* eggs for food.

Observations: Mortality at 2, 24 and 48 hours after treatment.

Results:

No mortality was observed in the tolclofos-methyl treatment groups for either *Orius sauteri* or *Chrysoperla carnea*. The results of the toxic standard showed moderate to high levels of toxicity. The results are presented in Table 9.5.1.e.

Table 9.5.1.e. Cumulative mortality data for *Orius sauteri* and *Chrysoperla carnea* exposed to tolclofos-methyl for 48 hours

Treatment/nominal concentration (ppm)		**Cumulative mortality (%)**					
		Orius sauteri			***Chrysoperla carnea***		
		2 hours	**24 hours**	**48 hours**	**2 hours**	**24 hours**	**48 hours**
Control		0	0	0	0	0	0
Malathion 50EC	500	93.3	93.3	100	26.7	33.3	33.3
Tolclofos-methyl 50WP	500	0	0	0	0	0	0
	1000	0	0	0	0	0	0

Based on the results of this study the 48-hour contact LC_{50} for both *Orius sauteri* and *Chrysoperla carnea* was estimated to be >1000 ppm, the highest dose tested. The corresponding NOEC was set to 1000 ppm in both cases.

Comments:

It is not clear from the study protocol how the test solutions were prepared and there is no guideline or reference given for this type of study. However, the test concentrations seem to correspond well to recommended spray concentrations for lettuce (0.08-0.2 kg a.s./hL, Table B.7.4.2) and the extreme exposure situation can be considered as a worst case exposure. The study is accepted.

B.9.5.2 Extended laboratory tests

Reference: Nienstedt, K.M. (2003)
Tolclofos-methyl 50WP: An extended aged residue test with the parasitic wasp, *Aphidius rhopalosiphi* (Hymenoptera: Braconidae)

Guideline: Based on the draft *Aphidius* ring-test-guideline (Mead-Briggs, Longley *et al*., 2000)
Deviations: In the first bioassay, in one of the control replicates, erroneously two insects were introduced at the start of the reproduction phase. For the statistical analysis, the number of mummies were divided by two and rounded up, i.e. 16 mummies/female were used instead of 31 mummies/two females

GLP: Yes

Material and methods:

Test substance:	Tolclofos-methyl 50WP (Batch No.: B000002, Contents: 503 g a.s./kg
Species:	*Aphidius rhopalosiphi*
Treatments:	2.0 kg a.s./ha; deionized water control and a toxic standard treatment (Perfekthion applied at 15 mL/ha, equivalent to 6 g dimethoate/ha)
Number of animals:	**Exposure:** 6 replicates of 5 wasps per treatment **Fecundity:** 15 replicates containing 1 wasp per treatment
Duration:	48 h exposure + 11 days fecundity test (1 day oviposition and 10 days mummy preparation)
Test conditions:	**Exposure phase:** Adult female wasps less than 48 hours old were exposed to potted barley plants (10 plants, approximately 10 cm tall, per pot). Treatments were applied in a volume of 400 L/ha using a calibrated precision laboratory sprayer. The plants were sprayed with 10% fructose solution and left to dry prior to the bioassays, which served as a food supply for the test wasps and encouraged them to be active on the surface of the plants. After about 30 minutes of exposure, the location of the wasps was monitored to ensure that they were exposed to the test treatments and to detect any possible repellent effects. The number of wasps on the plants, the cage wall and the sand was recorded in 5 successive assessments over 2 hours during which the replicates of each treatment were observed in turn (each assessment taking 20 to 25 minutes). **Fecundity phase:** The fecundity test was conducted with 15 females randomly selected from each treatment group except the toxic standard. The females were individually offered aphids on barley plants for oviposition for 24 hours before the surviving wasps were removed (the number of live, missing and dead wasps was recorded). Counting of parasitised aphid mummies was carried out after a further 10 days. Two bioassays were conducted, the first on fresh, dried residues and the second after a 7-day ageing period during which the plants were kept in a controlled environment room (19.5 to 22.5^{o}C, 59 to 83% relative humidity and with a photoperiod of 16 hours light: 8 hours darkness at 10220 to 17090 lux). The 2nd bioassay was conducted in order to confirm the survival results of the 1st bioassay and because the reproduction results of the 1st bioassay were not yet available. During the exposure and fecundity phases, the temperature and relative humidity ranged from 17.5 to 21.5^{o}C and 60 to 87%, respectively, and with a photoperiod of 16 hours light: 8 hours darkness at 6790 to 11250 lux (exposure) and 4350 to 7000 lux (reproduction). Both mortality (dead plus moribund wasps) and fecundity were compared separately to that of the control: Fisher's exact test was used to analyse mortality and the location of the wasps while the reproduction results were analysed

by t-tests. No corrected mortality was calculated, as control mortality was 0% in both bioassays.

Observations: Alive and healthy, affected, moribund or dead wasps after 2, 24 and 48 hours exposure; number of aphid mummies after a further 10 days.

Results:

In the behavioural assessments for the 1st bioassay (5 assessments conducted over 2 hours, starting after about 30 minutes of exposure), the mean percentage (± SD) of wasps observed on the treated plants for all assessments in the control and tolclofos-methyl treatments were 48.7 (±20.8) and 32.0 (±22.0)%, respectively. The percentage observed on the control plants was significantly higher than on the tolclofos-methyl plants (Fisher's exact test, p=0.002), thus indicating a possible repellent effect of the test substance on the female wasps. In the 2nd bioassay, the mean percentage (± SD) of wasps observed on the treated plants for all assessments in the control and tolclofos-methyl treatments were 87.3 (±14.4) and 89.3 (±16.4)%, respectively. There was no significant difference between these two treatments (Fisher's exact test, p>0.05), confirming the lack of any treatment effects after the 7-day ageing period. The higher percentage of wasps observed on the plants in the 2nd bioassay compared to the 1st was attributed to the increased size of the plants during the 7-day ageing period.

The results of the study are considered valid, as all the validity criteria were met. Thus, control mortality in bioassays 1 and 2 did not exceed 17%, the mortality with the toxic standard was greater than 50% (bioassay 1 only) and the mean number of mummies per female in the control was greater than 5 and no more than 2 females produced zero mummies.

There was no treatment-related mortality or effect on parasitation rate of the female wasps. The results are summarized in Table 9.5.2.a.

Table 9.5.2.a. Mean mortality and parasitisation rates after exposure of *Aphidius rhopalosiphi* adults to fresh and aged residues of Tolclofos-methyl 50 WP on barley plants

Treatment (kg a.s./ha)	**48-hour mortality (%) (mean ± SD)**		**Mummies per female (mean ± SD)**	
	Fresh residues	**7-day aged residues**	**Aged residues**	**7-day aged residues**
Control (0)	0.0 ± 0.0	0.0 ± 0.0	9.5 ± 7.4	12.9 ± 7.6
2.0	3.3 ± 8.2^{NS1}	0.0 ± 0.0	13.9 ± 7.2^{NS2}	10.1 ± 6.1^{NS3}
Toxic standard	66.7 ± 32.7*	NA	NA	NA

NS1 – not significantly different from control (Fisher's exact test, p=0.05)
NS2 – not significantly different from control (Student's t-test, p=0.110)
NS3 – not significantly different from control (Student's t-test, p=0.287)
* - significantly different from the control (Fisher's exact test, p<0.001)
NA – not applicable

Comments:

The study was well performed and reported.

Reference: Nienstedt, K.M. (2002a)
Tolclofos-methyl 50WP: An extended laboratory test with *Poecilus cupreus* L. (Coleoptera: Carabidae)

Guideline:	Based on Heimbach *et al.* (2000)
GLP:	Yes

Material and methods:

Test substance:	Tolclofos-methyl 50WP (Batch No.: B000002, Contents: 503 g a.s./kg
Species:	*Poecilus cupreus*
Treatments:	0.625 kg a.s./ha (equivalent to the maximum rate used on potatoes) and 2.0 kg a.s./ha (the maximum recommended rate on glasshouse lettuce, deionized water control and a toxic standard treatment (Perfekthion applied at 390 g dimethoate/ha).
Number of animals:	5 replicates/3 female and 3 male beetles/treatment
Duration:	14 days
Test conditions:	Adult beetles, six to seven weeks old at the start of the test, were exposed to fresh, dried residues of the test treatments applied to a natural soil, LUFA 2.1 (sand; 0.9% organic C content; pH 5.2; 30 g/100 g dry weight maximum water holding capacity). The beetles were kept in test cages comprising a 1 L plastic vessel filled with test soil to a depth of about 1 cm (equivalent to 231.8 g moist soil). The treatments were applied in a volume of 400 L/ha using a calibrated precision laboratory sprayer, except in the case of the 0.625 kg a.s./ha tolclofos-methyl treatment that was mixed directly into the soil. Fly pupae (*Lucilia* sp.) were added to the test cages as food. At each assessment, the number of pupae consumed (including those partly eaten) was recorded and all remaining pupae were removed and replaced by fresh ones (1 pupae per surviving beetle). The temperature was 19.5-20.5°C and the relative humidity ranged from 75 - 86%, A photoperiod of 16 hours light : 8 hours dark with a light intensity of 553 to 741 lux was used. The moisture of the soil substrate was controlled on days 2, 4, 7, 10 and 14 by weighing each replicate and adding water as necessary to achieve the starting weight (equivalent to 53% of the maximum water holding capacity).
Observations:	Mortality and sub-lethal effects (alive and healthy, affected, moribund or dead) were assessed after 2 and 24 hours, then 2, 4, 7, 10 and 14 days after application. The number of beetles dead or alive, as well as their behaviour, was recorded without disturbing the substrate (beetles which did not respond to touching were considered dead). Beetles showing no movements or on their backs with only slight movements were moved to a corner of the test cage and if still there at the following assessment were considered to be dead. Dead beetles were sexed and removed from the test cages. On day 14, all beetles were dug out of the soil and their condition noted.

Results:

After 14 days exposure, there was no mortality in the control or in the tolclofos-methyl treatments at either rate tested (0.625 and 2.0 kg a.s./ha). Mortality in the toxic standard treatment (dimethoate) at this time was 76.7%. In the control and tolclofos-methyl treatments, all beetles were showing normal behaviour while in the toxic standard treatment, first effects on behaviour (e.g. uncoordinated movements and quivering legs) were observed 24 hours after application and lasted for up to 4 days. The differences in feeding activity over the 14-day exposure period between the control and tolclofos-methyl treatments was not significant, while the toxic standard showed a marked reduction of about 90%, which was significantly different compared to the control.

The results of the study are considered valid, as all the validity criteria were met. Thus, control mortality did not exceed 6.7% and the mortality in the toxic standard treatment was within the required range of 65 ± 35%. The mortality and feeding activity results for the study are summarized in Table 9.5.2.b.

Table 9.5.2.b. Mean mortality and feeding activity after exposure of *Poecilus cupreus* adults to fresh residues of Tolclofos-methyl 50WP applied to natural (LUFA 2.1) soil

Treatment (kg a.s./ha)	Mortality (%) (mean ± SD)	Feeding activity	
		Sum of pupae consumed per surviving beetle (mean ± SD)	Mean of consumed fly pupae per surviving beetle per day (% reduction compared to control)**
Control (0)	0.0 ± 0.0	1.3 ± 0.1	0.13 (-)
0.625 (mixed)	0.0 ± 0.0	1.6 ± 0.5^{NS}	0.14 (-14.2%)
2.0 (sprayed)	0.0 ± 0.0	1.5 ± 0.6^{NS}	0.13 (-1.4%)
Toxic standard	76.7 ± 34.6	0.3 ± 0.4*	0.01 (90.4%)

NS – not significantly different from control (ANOVA: $p<0.001$, Dunnett's test $p>0.05$)
* - significantly different from the control (ANOVA: $p<0.001$, Dunnett's test $p<0.05$)
** - a negative value indicates an increase when compared to the control

Comments:

The study was well performed and reported.

Reference: Nienstedt, K.M. (2002b)
Tolclofos-methyl 50WP: An extended aged residue test with *Aleochara bilineata* Gyll. (Coleoptera: Staphylinidae)

Guideline: Based on Grimm *et al.* (2000) with minor deviations.

GLP: Yes

Material and methods:

Test substance: Tolclofos-methyl 50WP (Batch No.: B000002, Contents: 503 g a.s./kg

Species: *Aleochara bilineata*

Treatments: 0.625 kg a.s./ha (equivalent to the maximum rate used on potatoes) and 2.0 kg a.s. /ha (the maximum recommended rate on glasshouse lettuce), deionised water control and a toxic standard treatment (Perfekthion applied at 390 g dimethoate/ha).

Number of animals: 4 replicates per treatment, each containing 10 female and 10 male beetles.

Duration: 28 days + 5 weeks reproduction study

Test conditions: **Exposure phase:** Adult staphylinid rove beetle, *Aleochara bilineata*, 0-3 (1st bioassay) and 0-7 (2nd bioassay) days old were allowed to mate and then mated pairs were exposed to fresh, dried residues of the test treatments applied to a natural soil, LUFA 2.1 (sand; 0.9% organic C content; pH 5.2; 30 g/100 g dry weight maximum water holding capacity). The beetles were kept in test cages comprising a plastic vessel (17 x 12.5 cm area and 11.5 cm height) filled with test soil to a depth of about 5 cm (equivalent to 1000 g dry soil) and fed *ad libitum* with chironomid larvae. Pupae of *Delia antiqua* were mixed into the test soil as hosts for parasitisation (approximately 500 pupae on days 7, 14 and 21). The treatments were applied in a volume of 400 L/ha using a calibrated precision laboratory sprayer, except in the case of the 0.625 kg a.s./ha tolclofos-methyl treatment that was mixed directly into the soil to simulate the use on potato tubers.

Reproductive phase: For each bioassay, on day 28 the content of each test vessel was emptied out and the number of surviving, dead and missing beetles was recorded. After removing the beetles, the test soil was returned to the vessels for a further week after which the host pupae were recovered by sieving the soil and they were then placed in the reproductive phase test units. These comprised plastic pipes (approximate diameter 7.5 cm and length 7 cm) with stainless steel netting on the bottom. Each pipe was positioned above a gauze covered plastic vessel into which the emerging beetles were collected.

Two bioassays were conducted, the first on fresh, dried residues and the second after a 7-day ageing period during which the test cages were kept in a controlled environment room (19.0 to 21.0°C, 43 to 57% relative humidity and with a photoperiod of 16L : 8D at 12610 to 18670 lux). During the exposure and reproduction phases, the temperature and relative humidity ranged from 18.0 to 24.5°C and 39 to 88%, respectively, and with a photoperiod of 16L: 8D at 407 to 574 lux. The moisture of the soil substrate was checked weekly (twice weekly in the ageing period) by weighing each replicate and adding water as necessary to achieve the starting weight (moisture based on dry weight was between 8.81 and 11.20% at the start of the test).

Fisher's exact test was used to compare treatment mortality on day 28 with that for the control and in addition, mortality in the treated groups was corrected for control mortality. The sum of the hatched beetles for all observations was calculated for each replicates and these were tested for normal distribution with Kolmogorov-Smirnov test. As the assumption of normality was met, ANOVA followed by Dunnett's t-test was used to test for significances between the treatment means.

Observations: Number of surviving, dead and missing beetles on Day 28. During the reproductive phase the number of hatching beetles was counted and removed 4 times per week during the main emergence phase (approximately 2 weeks). In the following 3 weeks, the counting intervals were extended as the hatching declined

Results:

In the 1st bioassay, conducted with fresh, dried residues, there was some mortality of the adult beetles at the end of the 28-day exposure phase, with control corrected mortality of 33.9 and 39.0% in the tolclofos-methyl treatments (0.625 and 2.0 kg a.s./ha, respectively). At the same time, there was 83.1% corrected mortality in the toxic standard treatment (dimethoate). All of these mortality results were significantly different from the control. However, in the 2nd bioassay, conducted after the 7-day ageing period, there was no treatment-related mortality in either of the tolclofos-methyl treatments, with control corrected mortality of –20.0 and 25.0% at 0.625 and 2.0 kg a.s./ha, respectively. In the 2nd bioassay, there was 95.0% corrected mortality in the toxic standard treatment (dimethoate) and only this mortality was significantly different compared to the control.

No significant effects of tolclofos-methyl treatments on number of hatched beetles were detected in either the 1st bioassay, conducted with fresh, dried residues, or in the 2nd bioassay, conducted after the 7-day ageing period. . The mortality and parasitisation rates for the study are summarized in Table 9.5.2.c.

The results of the study are considered valid as at least 400 beetles hatched in the control and reproduction was reduced by at least 50% in the toxic standard when compared to the control.

Table 9.5.2.c. Mean mortality and parasitisation rates after exposure of *Aleochara bilineata* adults to fresh and aged residues of Tolclofos-methyl 50 WP applied to natural (LUFA 2.1) soil

Treatment (kg a.s./ha)	Mortality		Parasitisation		
	Mortality (%) (mean ± SD)	Corrected mortality (%)[1] (mean ± SD)	Sum of hatched beetles (mean ± SD)	Parasitisation (%) (mean ± SD)	Percentage effect[2]
	1st bioassay (fresh, dried residues)				
Control (0)	26.3 ± 17.5	-	564 ± 141	37.6 ± 9.4	-
0.625 (mixed)	51.3 ± 20.2*[1]	33.9	649 ± 73[NS]	43.3 ± 4.9	-15.0
2.0 (sprayed)	55.0 ± 18.7*[1]	39.0	647 ± 132[NS]	43.1 ± 8.8	-14.6
Toxic standard	87.5 ± 10.4*[1]	83.1	155 ± 77*	10.3 ± 5.1	72.5
	2nd bioassay (7-day old residues)				
Control (0)	50.0 ± 26.5	-	644 ± 64	42.9 ± 4.3	-
0.625 (mixed)	40.0 ± 16.8[NS1]	-20.0	596 ± 107[NS]	39.8 ± 7.1	7.4
2.0 (sprayed)	62.5 ± 34.3[NS1]	25.0	670 ± 113[NS]	44.7 ± 7.5	-4.0
Toxic standard	97.5 ± 2.9*[1]	95.0	190 ± 136*	12.7 ± 9.1	70.5

NS - not significantly different from control (Fisher's exact test: p>0.05)
NS - not significantly different from control (ANOVA: p <0.001, Dunnett's test p>0.05)
*[1] - significantly different from the control (Fisher's exact test: p<0.05)
*[2] - significantly different from control (ANOVA: p <0.001, Dunnett's test p<0.001)
[1] - Schneider-Orelli, 1947
[2] - a negative value indicates an increase when compared to the control

Comments:

Mortality in the control groups was high, especially in the 2nd bioassay. However, since number of beetles emerging from the pupae is the critical endpoint, and all quality criteria of the test were met, the study is accepted.

B.9.5.3 Summary and risk assessment for non-target arthropod species other than bees.

A summary of the results from the laboratory studies of effects of tolclofos-methyl on non-target arthropods is shown in Table 9.5.3.a.

Table 9.5.3.a. Summary of laboratory tests on arthropods

Species	Test substance	Dose	Result	Reference
LABORATORY TESTS				
Aphidius rhopalosiphi (adult)	Tolclofos-methyl 50WP	0, 8, 16, 32, 64 and 128 g a.s./ha	48-hour LR_{50}: 43.7 g a.s./ha	Kollman, S.I., 2002a
Typhlodromus pyri (protonymph)	Tolclofos-methyl 50WP	0, 1.56, 3.13, 6.25, 12.5 and 25 kg a.s./ha	7-day LR_{50}: >25 kg a.s./ha	Kollman, S.I., 2002b
Orius sauteri (Adult female)	Tolclofos-methyl 50WP	500 and 1000 ppm	LC_{50} (48 hour): > 1000 ppm	Shono, Y., 1998
Chrysoperla carnea (Larvae)	Tolclofos-methyl 50WP	500 and 1000 ppm	LC_{50} (48 hour): > 1000 ppm	Shono, Y., 1998
EXTENDED LABORATORY AND AGED RESIDUE TESTS				
Aphidius rhopalosiphi	Tolclofos-methyl 50WP	2.0 kg a.s./ha (fresh and 7-day aged substrates)	No treatment related mortality and <50% reduction in parasitisation rate	Nienstedt, K.M., 2003
Poecilus cupreus	Tolclofos-methyl 50WP	0.625 kg a.s./ha (mixed into the soil) and 2.0 kg a.s./ha (sprayed onto the soil surface)	No treatment related mortality and <50% effect on feeding activity	Nienstedt, K.M., 2002a
Aleochara bilineata	Tolclofos-methyl 50WP	0.625 kg a.s./ha (mixed into the soil, fresh and 7-day aged substrates) and 2.0 kg a.s./ha (sprayed onto the soil surface, fresh and 7-day aged substrates)	Fresh substrates: Some treatment related mortality (<50%) with no effect on parasitisation rate (i.e. <50% reduction) 7-day aged substrates: No treatment related mortality or effect on parasitisation rate (i.e. <50% reduction)	Nienstedt, K.M., 2002b

The LR_{50} of tolclofos-methyl to the predatory mite *Typhlodromus pyri* and to the parasitic wasp, *Aphidius rhopalosiphi* indicate a clear difference in the susceptibility of these two standard susceptible species to tolclofos-methyl.

The toxicity of tolclofos-methyl was also estimated for two foliage dwelling predators, *Orius sauteri* and *Chrysoperla carnea*, resulting in a 48hr-LC_{50} >1000 ppm. Although the testing conditions were severe by the dipping method compared to a realistic situation, the results indicate that no significant effects of tolclofos-methyl under laboratory conditions were observed to those species.

First tier risk assessment

The Notifier performed a tier 1 risk assessment based on the ESCORT 2 workshop guidance and calculated the hazard quotients by comparing the LR_{50} values of the laboratory standard tests for parasitoid and predatory mites with the application rates.

Based on the limited use patterns, as potato tuber dressing and for glasshouse lettuce, off-field exposure is negligible and the off-field HQ calculation is not applicable. However, for the greenhouse lettuce use, a worst-case off-field risk assessment was performed on the basis of the exposure calculations using the Dutch national model, which assumes 0.1% "spray drift" exposure from glasshouse uses. The result is presented in Table 9.5.3.b.

Table 9.5.3.b Tier 1 risk assessment for non-target arthropods exposed to tolclofos-methyl

Crop, Application	**Application rate**	**Test species**	**LR_{50}**	**In-field HQ**	**Off-field HQ**	**HQ Trigger**
Potato, tuber dressing	0.625 kg a.s./ha	*Aphidius rhopalosiphi*	43.7 g a.s./ha	14.3	Not applicable	≥2
		Typhlodromus pyri	>25 kg a.s./ha	<0.025	Not applicable	≥2
Lettuce, soil application	2 kg a.s./ha	*Aphidius rhopalosiphi*	43.7 g a.s./ha	45.8	0.0458*	≥2
		Typhlodromus pyri	>25 kg a.s./ha	<0.08	$<8x10^{-5}$*	≥2

*Based on the Dutch national model, this assumes 0.1% "spray drift" exposure from glasshouse uses.

The first tier risk assessment based on laboratory studies, indicated unacceptable in-field risk to non-target arthropods, therefore extended laboratory tests and tests with aged residues were conducted in order to refine the assessment.

Refined risk assessment

When considering the proposed uses for tolclofos-methyl, the use as a potato tuber dressing involves direct incorporation into the soil i.e. there will only be sub-surface exposure to residues. In the case of the use on glasshouse grown lettuce, there may be a sprayed application up to 7 days after transplant, which means that there may be limited foliar residues although the main area of exposure will be on the soil surface. By considering these intended uses, which are relevant primarily for soil exposure, two extended laboratory aged residue tests were conducted with ground-dwelling predators, the staphylinid rove beetle *Aleochara bilineata* Gyll. and the carabid ground beetle *Poecilus cupreus*. In addition, an extended laboratory aged residue test with *A. rhopalosiphi* was conducted as a higher tier test, as this was the tier 1 species for which the initial risk assessment had indicated an unacceptable in-field risk. This species can also be seen as representative of foliar-dwelling insects, appropriate for the use on lettuce.

With all test species, the levels of lethal and sublethal effects at both treatment rates (where appropriate) were found to be below the 50% threshold (ESCORT 2 workshop guidance trigger value), even on fresh, dried residues (see Table 9.5.3.a.).

It is concluded, taking the low exposure levels into account, that the proposed uses of Tolclofos-methyl 50WP in lettuce and potato will present an acceptable risk to non-target arthropod populations and no further higher tier testing is necessary. For extended uses, the risk to non-target arthropods should be considered at MS level.

B.9.6 Effects on earthworms (Annex IIA 8.4; Annex IIIA 10.6.1)

B.9.6.1 Acute toxicity

ACTIVE INGREDIENT

Reference:	Candolfi, M.P. (1997) Tolclofos methyl: 14-day acute toxicity test with the earthworm (*Eisenia foetida*) based on OECD Guideline # 207 and the Commission Directive 88/302/EEC
Guideline:	OECD 207 (1984) Directive 88/302/EEC, Part C, Toxicity for earthworms: Artificial soil test
GLP:	Yes

Material and methods:

Test substance:	Tolclofos-methyl (Batch No.: 60105G, Purity: 97.9%)
Species:	*Eisenia foetida andrei*
Treatments:	0 (deionised water), 0 (carrier control), 62.5, 125, 250, 500 and 1000 mg a.s./kg soil
Number of animals:	4 replicates of 10 earthworms/treatment
Duration:	14 days
Test conditions:	Two months old mature animals with clitellum weighing 300-600 mg were placed in 1.5 L glass beakers containing 750 g of artificial soil (70% industrial sand; 20% kaolin clay; 10% sphagnum peat moss). Temperature was 19.0 – 21.5°C, soil moisture content 37.2 - 39.4% (test initiation) and 35.8 – 37.8% (test termination), soil pH 5.53 – 5.71 (test initiation) and 5.51 - 5.59 (test termination). The test was carried out with continuous illumination (624 lux).
Observations:	Mortality and biological observations (e.g. colour changes, lethargy, lesions and burrowing time) of the earthworms were recorded on Days 0, 7 and 14. Body weights were measured on Days 0 and 14.

Results:

There was no mortality observed in the control and any of the tolclofos-methyl treatment groups (up to and including 1000 mg a.s./kg) during the 14-day exposure test. Burrowing time on day 7 (after the worms had been removed for the 7-day assessment) was significantly increased compared to the acetone (carrier) control at a test concentration of 1000 mg a.s./kg (a significant increase at 250 mg a.s./kg on day 0 was not considered to be biologically significant). The burrowing times for the earthworms are presented in Table 9.6.1.a. Also, there was

a significant difference in weight loss between the acetone (carrier) control and the 1000 mg a.s./kg soil group. The group mean weights of earthworms are presented in Table 9.6.1.b.

Table 9.6.1.a. Burrowing time of earthworms

Treatment (mg a.s./kg soil)		Mean burrowing time (minutes) ± SD	
		Day 0	Day 7
Blank control		2.9 ± 0.7	1.4 ± 0.4
Acetone control		3.9 ± 0.5	1.7 ± 0.3
Technical tolclofos-methyl	62.5	4.1 ± 0.5	2.0 ± 0.8
	125	3.5 ± 0.3	1.5 ± 0.4
	250	5.3 ± 0.3*	1.6 ± 0.4
	500	4.6 ± 0.7	1.6 ± 0.1
	1000	4.3 ± 2.0	2.9 ± 0.2*

* Significant difference between test substance treatment and carrier control (t-test, $p<0.05$)

Table 9.6.1.b. Mean weight and mean weight differences of earthworms

Treatment (mg a.s./kg dry soil)		Mean weight (mg) ± SD	
		Day 0	Weight difference Day 0 - Day 14[1]
Blank control		344.1 ± 39.1	8.8 ± 18.4
Acetone control		337.3 ± 44.0	19.4 ± 23.0
Technical tolclofos-methyl	62.5	343.7 ± 40.8	22.9 ± 23.8
	125	351.1 ± 42.5	28.9 ± 25.5
	250	339.7 ± 35.1	40.1 ± 22.6
	500	344.5 ± 38.1	49.0 ± 30.4
	1000	350.2 ± 41.0	75.6 ± 17.7*

[1] Weight difference= weight Test Day 0-weight Test Day 14 (positive values indicate a weight loss)
* Significant difference between test substance treatment and carrier control (t-test, $p<0.05$)

Based on the results of this study the acute toxicity (14-day LC_{50}) to earthworms was estimated to be >1000 mg a.s./kg soil. The NOEC was 500 mg a.s./kg soil, based on mean burrowing time and weight loss compared to the solvent control.

Comments:

The study was well performed and reported. However, Student's t-test should not be used to analyse differences between control and multiple test substance treatments.

PLANT PROTECTION PRODUCT

Reference:	Leimgruber, R. (1984a)
	Determination of the acute toxicity of Rizolex 25% flowable in earthworm
Guideline:	OECD Draft Guideline for Testing of Chemicals, "Earthworm Acute Toxicity Test", ET 82.5
GLP:	No

Material and methods:

Test substance:	Tolclofos-methyl 25SC (Batch No.: C1208, Contents: 26.0%)
Species:	*Eisenia foetida foetida*

Treatments:	Untreated control, 10, 100 and 1000 mg a.s./kg dry weight soil
Number of animals:	4 replicates of 10 earthworms/treatment
Duration:	14 days
Test conditions:	Artificial soil was prepared according to the guidelines. Earthworms were 300-500 mg in weight and had an average age of 3.5 months. The soil moisture content was 35% (initially) and 30% (at end), and temperature was 20°C during the day and 17-18°C during the night. The test was performed with continuous illumination. pH of the soil was not determined. No details regarding the stock solution were provided but the test solutions were applied to the artificial soil as a fine spray in an aqueous solution. Chloracetamide was tested as a reference substance at concentrations of 0.1, 1.0, 10 and 100 mg/kg soil.
Observations:	Mortality and other physical or pathological symptoms after 7 and 14 days exposure.

Results:

There were no mortalities during the study. The earthworms were described as very agile and showed no physical or pathological symptoms. The 14-day LC_{50} for chloracetamide was between 10 and 100 mg/kg soil.

Based on the results of this study the acute toxicity (14-day LC_{50}) to earthworms was concluded to be >1000 mg a.s./kg soil. The NOEC was set to 1000 mg a.s./kg soil, the highest concentration tested.

Comments:

A different formulation than the one selected as the representative formulation was used in this study. However, since toxicity of the formulated product to earthworm is not formally required, the study is accepted.

Reference:	Leimgruber, R. (1984b) Determination of the acute toxicity of Rizolex 10% dust in earthworm
Guideline:	OECD Guideline for Testing of Chemicals, "Earthworm Acute Toxicity Test", Draft ET 82.5
GLP:	No

Material and methods:

Test substance:	Tolclofos-methyl 10% Dust (Batch No.: 49489, Contents: 10.6%
Species:	*Eisenia foetida foetida*
Treatments:	Untreated control, 10, 100 and 1000 mg a.s./kg dry weight soil
Number of animals:	4 replicates of 10 earthworms/treatment
Duration:	14 days
Test conditions:	Artificial soil was prepared according to the OECD guideline (ET 82.5). Earthworms were 300-500 mg in weight and had an average age of 3.5 months. The soil had a moisture content of 35% (initially) and 30% (at end). Temperature was 20°C during the day and 17-18°C during the night. The test was carried out with

continuous illumination. pH of the soil was not determined. No details regarding the stock solution were provided but the test solutions were applied to the artificial soil as a fine spray in an aqueous solution Chloracetamide was tested as a reference substance at concentrations of 0.1, 1.0, 10 and 100 mg/kg soil.

Observations: Mortality and other physical or pathological symptoms after 7 and 14 days exposure.

Results:

There were two dead earthworms at the highest concentration tested, 1000 mg a.s./kg soil, after 7 days. The remaining living earthworms were described as very agile and showed no physical or pathological symptoms. After 14 days, there were no more mortality, and the worms were normal in appearance and behaviour. The 14-day LC_{50} for chloracetamide was between 10 and 100 mg/kg soil.

Based on the results of this study the acute toxicity (14-day LC_{50}) to earthworms was concluded to be >1000 mg a.s./kg soil. The NOEC was 100 mg a.s./kg soil.

Comments:

A different formulation than the one selected as the representative formulation was used in this study. However, since toxicity of the formulated product to earthworm is not formally required, the study is accepted.

B.9.6.2. Summary and risk assessment for earthworms

Tolclofos-methyl shows low acute toxicity to earthworms in soil, with no mortality detected at concentrations up to 1000 mg a.s./kg soil. A summary of the available studies on effects of the active substance and formulated products is presented in Table 9.6.2.a. Since the metabolite DM-TM was only detected at levels above 10% (up to a maximum of 13%) of applied radioactivity on two occasions in one of eight trials and was found to degrade rapidly with DT_{50} values <1 day and DT_{90} values <3 days, no studies on the metabolite are necessary as this is considered to be covered by studies on the parent compound. The DT_{90} values for parent compound and the metabolite are less than 100 days and the number of applications is less than 3, thus chronic studies are not required.

Table 9.6.2.a. Summary of acute toxicity of tolclofos-methyl to earthworms

Test substance	Dose rate (mg a.s./kg soil)	LC_{50} (14 days) (mg as/kg soil)	NOEC (14 days) (mg as/kg soil)	Reference
Tolclofos-methyl	0, 62.5, 125, 250, 500, 1000	>1000	500	Candolfi, M.P., 1997
Tolclofos-methyl 25SC	10, 100, 1000	>1000	1000	Leimgruber, R., 1984a
Tolclofos-methyl 10Dust	10, 100 1000	>1000	100	Leimgruber, R., 1984b

The acute TER for earthworms is calculated by dividing the acute LC_{50} value (mg a.s./kg) by the initial soil PEC (mg a.s./kg). The $logK_{ow}$ for tolclofos-methyl is > 2, thus LC_{50} values were divided by 2 in order to correct for the higher f_{oc} of the artificial substrate used in the test compared to natural soil (according to the Guidance Document on Terrestrial Ecotoxicology Under Council Directive 91/414/EEC (SANCO/10329/2002 17 October

2002 rev2)). The calculated acute TERs for earthworm, using the initial soil PEC values (Tables 8.3.a and 8.3.b) for the range of recommended application rates and the corrected LC_{50} values, are presented in Table 9.6.2.b.

Table 9.6.2.b. Risk assessment for earthworms exposed to tolclofos-methyl.

Crop	Application rate (kg a.s./ha)	LC_{50corr} (mg as/kg soil)	PEC_{soil} (mg as/kg soil)	TER	Annex VI trigger
Potato, tuber dressing	0.625	>500	0.83	>602	>10
Lettuce, soil application	2	>500	2.67	>187	>10

The short-term TER values of tolclofos-methyl to earthworms were well above the Annex VI trigger of 10 for short-term effects. The risk for harmful effects to earthworms is thus considered to be acceptable.

B.9.7 Effects on other soil non-target macro-organisms (Annex IIIA 10.6.2)

Tolclofos-methyl is applied once per year as a potato tuber dressing or by soil application to green house lettuce and it has a short persistence in soil: DT_{50} values of 2 to 5.4 days and DT_{90} values of 6.9 to 20.1days in four EU soils at 20°C (2-phase exponential model, see Section B.8.1.4). Also, data obtained on toxicity to earthworms and soil non-target microorganisms indicates that the toxicity to soil-dwelling organisms is low. It is thus considered that the risk to soil non-target macro-arthropods following the recommended uses of tolclofos-methyl will be acceptable.

B.9.8 Effects on soil non-target micro-organisms (Annex IIA 8.5; Annex IIIA 10.7)

B.9.8.1. Nitrogen transformation

ACTIVE INGREDIENT

Reference: Barug, D., Vogelaar, J.C. (1983a)
Effect of tolclofos-methyl on nitrogen transformations in loam soil

Guideline: Form A, *Commissie Toelating Bestrijdingsmiddelen* (Dutch Commission for Registering Pesticides) modified Appendix H.4.1 (May 1983)

GLP: No

Material and methods:

Test substance: Tolclofos-methyl (Batch No.: Not specified, Purity: 97.9%)

Treatments: Control, 2.5 and 25 mg a.s./kg soil

Duration: 12 weeks

Test conditions: The soil characteristics are presented in Table 9.8.1.a. Incubation was carried out in flasks containing 100 g of soil (wet weight) in darkness at a temperature of 20±1°C.

Soil was amended with 0.5% lucerne (as a nitrogen source).

Analysis: Nitrogen transformation was followed by measuring the amounts of NH_4^+, NO_2^- and NO_3^-. Two flasks were analysed at 0, 1, 2, 3, 4, 5, 6, 9 and 12 weeks after treatment: extraction was carried out by shaking with 2 M KCl for one hour and then analysing the filtrate using an auto-analyser.

Table 9.8.1.a. Soil characteristics

Texture class		Loam soil
pH		7.3
Moisture content (pressure, pF)		2.5
Clay (< 2 µm)	[%]	25.2
Silt (2-50 µm)	[%]	45.1
Sand (> 50 µm)	[%]	29.7
Organic matter	[%]	2.1
$CaCO_3$	[%]	7.9

Results:

The results showed that nitrogen transformation in the loam soil treated with 2.5 and 25 mg tolclofos-methyl/kg soil was not seriously affected by tolclofos-methyl. In comparison with the control, a lower amount of NO_3^- - N was observed at a rate of 25 mg a.s./kg after 5 weeks incubation only, but the effect was small and not considered significant. The deviations in measured activity throughout the incubation period were less than 25% for nitrate formation. The nitrite concentrations in the soil were negligible, with less than 0.06 mg NO_2^- - N/kg soil throughout the incubation period. Based on the results of this study, tolclofos-methyl can be considered to have no significant effects (<25% difference compared to the control throughout a 12 week incubation period) on nitrogen transformation in a loam soil at concentrations of up to 25 mg a.s./kg (18.8 kg a.s./ha).

Comments:

The study was well performed and reported.

Reference: Barug, D., Vogelaar, J.C. (1984)
Effect of tolclofos-methyl on nitrogen transformations in humic sandy soil

Guideline: Form A, *Commissie Toelating Bestrijdingsmiddelen* (Dutch Commission for Registering Pesiticides) modified Appendix H.4.1 (May 1983)

GLP: No

Material and methods:

Test substance: Tolclofos-methyl (Batch No.: Not specified, Purity: 97.9%)

Treatments: Control, 2.5 and 25 mg a.s./kg soil

Duration: 12 weeks

Test conditions: The soil characteristics are presented in Table 9.8.1.b. Incubation was carried out in flasks containing 100 g of soil (wet weight) at a temperature of 20±1°C and in the dark soil amended with 0.5% lucerne (as a nitrogen source).

Analysis: Nitrogen transformation was followed by measuring the amounts of NH_4^+, NO_2^- and

NO_3^-. Two flasks were analysed at 0, 1, 2, 3, 4, 6, 9 and 12 weeks after treatment: extraction was carried out by shaking with 2 M KCl for one hour and then analysing the filtrate using an auto-analyser.

Table 9.8.1.b. Soil characteristics

Texture class		Humic sandy soil
pH		5.4
Moisture content (pressure, pF)		2.5
Clay (< 2 µm)	[%]	3.3
Silt (2-50 µm)	[%]	8.3
Sand (> 50 µm)	[%]	88.5
Organic matter	[%]	4.5
$CaCO_3$	[%]	0.1

Results:

The results showed that nitrogen transformation in the humic sandy soil treated with 2.5 and 25 mg tolclofos-methyl/kg soil was not seriously affected by tolclofos-methyl. The deviations in measured activity throughout the incubation period were less than 25% for nitrate formation. The nitrite concentrations in the soil were negligible, with less than 0.15 mg NO_2^- - N/kg soil being found throughout the incubation period. Based on the results of this study, tolclofos-methyl can be considered to have no significant effects (<25% difference compared to the control throughout a 12 week incubation period) on nitrogen transformation in a humic sandy soil at concentrations of up to 25 mg a.s./kg (18.8 kg a.s./ha).

Comments:
The study was well performed and reported.

B.9.8.2. Carbon mineralization

ACTIVE INGREDIENT

Reference: Barug, D., Vogelaar, J.C. (1983b)
Effect of tolclofos-methyl on soil respiration

Guideline: Form A, *Commissie Toelating Bestrijdingsmiddelen* (Dutch Commission for Registering Pesiticides) modified Appendix H.4.1 (May 1983)

GLP: No

Material and methods:

Test substance: Tolclofos-methyl (Batch No.: Not specified, Purity: 97.9%)

Treatments: Control, 2.5 and 25 mg a.s./kg soil, with and without 0.5% lucerne.

Duration: 21 days

Test conditions: Four replicate flasks containing 100 g of soil (wet weight); temperature: 20±1°C; darkness. The soil characteristics are presented in Table 9.8.2.a.

Analysis: Soil respiration was followed by measuring carbon dioxide production in the soils every two hours.

Table 9.8.2.a. Soil characteristics

Texture class		**Humic sandy soil**	**Loam soil**
pH		5.4	7.3
Moisture content (pressure, pF)		2.5	2.5
Clay (< 2 µm)	[%]	3.3	25.2
Silt (2-50 µm)	[%]	8.3	45.1
Sand (> 50 µm)	[%]	88.5	29.7
Organic matter	[%]	4.5	2.1
$CaCO_3$	[%]	0.1	7.9

Results:

The evolution of carbon dioxide from the humic sandy soil treated with tolclofos-methyl at rates of 2.5 and 25 mg a.s./kg was similar to the control. Differences between the treated and control soils were less than 10% throughout the incubation period (up to 19 days).

The evolution of carbon dioxide from the loam soil treated with tolclofos-methyl at rates of 2.5 and 25 mg a.s./kg showed some differences compared to the control. At the higher rate, after an initial decrease the evolution of carbon dioxide was higher than the control, reaching a 25% relative increase after 8 days incubation. However, from 10 days onwards carbon dioxide levels were similar to the control. This effect was much less pronounced at the lower rate. In both cases, differences between the treated and control soils were less than 10% by the end of the incubation period (21 days).

Based on the results of this study, tolclofos-methyl can be considered to have either transient or no significant effects (<25% difference compared to the control after a 19-21 day incubation period) on soil respiration at concentrations of up to 25 mg a.s./kg.

Comments:

The study was well performed and reported.

B.9.8.3 Summary and risk assessment for soil micro-organisms

A summary of the available laboratory studies is presented in Table 9.8.3.a. The Annex VI trigger for acceptable effects to microorganisms is <25% effect. The results from available laboratory studies performed on tolclofos-methyl indicate that the effects on soil microorganisms are acceptable at the recommended application rates. At rates up to 10 times the recommended, only transient effects reaching 25 % were observed on carbon mineralization. Thus, the risk is considered to be acceptable at the recommended dose rates of tolclofos-methyl.

Table 9.8.3.a. Effects of tolclofos-methyl on nitrogen transformation and carbon mineralization

Type of study	Test soils & Time scale	Dose range tested	Results	Reference
Nitrogen transformation	Loam soil (12 weeks)	2.5 and 25 mg a.s./kg soil (19 and 1.9 kg a.i./ha)	No significant effects (<25% deviation from control)	Barug, D., *et al*., 1983a (QW-31-0020)
	Humic sandy soil (12 weeks)	2.5 and 25 mg a.s./kg soil (19 and 1.9 kg a.i./ha)	No significant effects (<25% deviation from control)	Barug, D., *et al*., 1984 (QW-31-0021)
Carbon mineralization	Humic sandy soil Loam soil (19-21 days)	2.5 and 25 mg a.s./kg soil (19 and 1.9 kg a.i./ha)	No significant effects (<25% deviation from control)	Barug, D., *et al*., 1983b (QW-31-0019)

B.9.9 Effects on other non-target organisms (flora and fauna) believed to be at risk (Annex IIA 8.6)

B.9.9.1 Insecticidal activity

PLANT PROTECTION PRODUCT

Reference: Umeda, K. (2001)
Evaluation of insecticidal activity of tolclofos-methyl

Guideline: In-house method

GLP: No

Material and methods:

Test substance: Tolclofos-methyl 50% wettable powder (Batch No.: L703H11, Content: 50% a.s. w/w)

Species: Small brown planthopper (*Laodelphax striatellus*), two-spotted spider mite (*Tetranychus urticae*), common mosquito (*Culex pipiens pallens*).

Test conditions: 1. Small brown planthopper (*Laodelphax striatellus*)
20 ml of the predetermined concentration of the test chemicals (500 ppm a.s.) was sprayed onto a potted rice plant. After drying, 10 adult small brown planthoppers were released onto the plant. Mortality was assessed two days after treatment.
2. Two-spotted spider mite (*Tetranychus urticae*)
20-30 individuals (nymphs and adults) were released onto a potted kidney bean plant. 20 ml of the predetermined concentration of the test chemicals (500 ppm a.s.) was sprayed onto the potted plant. Leaf damage was assessed seven days after treatment.
3. Common mosquito (*Culex pipiens pallens*)
0.7 ml of the predetermined concentration of the test chemical (500 ppm a.s.) was added to 100 ml of deionized water and two replicates of 20 last-instar larvae were released into this. Mortality was assessed one day after treatment.

Results:

Tolclofos-methyl did not show any marked insecticidal activity to the three insect species tested. The results are summarised in Table 9.9.1.a.

Table 9.9.1.a. Insecticidal activity of tolclofos-methyl

Treatment	Small brown planthopper	Two-spotted spider mite	Common mosquito
	Mortality (%)*	Leaf damage*	Activity*
Tolclofos-methyl	0	C	C
Control	0	C	C

* Index and criteria
Small brown planthopper: Mortality
Two-spotted spider mite: A: No leaves damaged, B: 50% of leaves damaged, C: >80% of leaves damaged
Common mosquito: A: >90% mortality, B: 10-90% mortality, C: <10% mortality

Comment:

On the basis of the results obtained, tolclofos-methyl is not expected to show any significant insecticidal activity.

B.9.9.2 Herbicidal activity

PLANT PROTECTION PRODUCT

Reference: Mito, N. (2001)
Evaluation of herbicidal activity of tolclofos-methyl

Guideline: In-house method

GLP: No

Material and methods:

Test substance: Test material: tolclofos-methyl 50% wettable powder (Batch No.: L703H11, Content: 50% a.s. w/w)

Species: Barnyardgrass (Echinochloa crus-galli), cucumber (Cucumis sativus) and radish (Raphanus sativus)

Test conditions: The test plants were cultivated in a greenhouse at 30°C (by day) and 25°C (by night). The seeds were sown in plastic pots (10 cm diameter) containing a sandy loam soil. The chemical was applied at a rate of 2000 g a.s./ha to the test plants (one pot per species with no replication) at post-emergence (a foliar application 10 days after sowing). Herbicidal activity was visually evaluated on a scale of 0 (no injury) to 10 (complete kill). This evaluation was made at 19 days after treatment.

Results:

Tolclofos-methyl did not show any marked herbicidal activity to the three plant species tested. The results are presented in Table 9.9.2.a.

Table 9.9.2.a. Herbicidal activity of tolclofos-methyl

Application rate (g a.s./ha)	Herbicidal activity (Index*)		
	Barnyardgrass	Cucumber	Radish

2000	0	0	0
Control	0	0	0

* Index and criteria: 0 - No injury; 10 - Complete kill

Comments:

No replication of treatments was performed. However, the results from this preliminary test indicate that tolclofos-methyl is not expected to show any significant herbicidal activity.

B9.9.3. Summary and risk assessment for non-target flora and fauna believed to be at risk

The insecticidal and herbicidal activities of tolclofos-methyl were evaluated against three species of insects and three species of plants. On the basis of the results obtained from these preliminary tests, no marked insecticidal or herbicidal activity can be expected.

B.9.10 Effects on biological methods of sewage treatment (Annex IIA 8.7)

B.9.10.1 Effect study

Reference: L'Haridon, J. (2000)
Activated sludge, respiration inhibition test

Guideline: OECD 209 (4 April, 1984)

GLP: Yes

Material and methods:

Test substance: Tolclofos-methyl (Batch No.: 90437G, Purity: 98.0%

Treatments: 1.0, 3.16, 10.0, 31.6 and 100 mg/L

Duration: 3 hours

Test conditions: A sample of activated sludge was obtained from a water treatment works whose wastewater catchment is predominantly domestic (27000 Evreux, France). The inoculum was filtered, washed and then diluted with dechlorinated water to provide nominal suspended solids concentration of 4 g/L. The inoculum was maintained under agitation for one day before the test and 50 mL/L of sewage feed was added just before agitation. 3,5-dichlorophenol (3,5-DCP) was used as a reference inhibitor. Two controls were prepared, at the beginning and end of the test, to verify its quality. All mixtures comprised 16 mL of sewage feed diluted to 300 mL with water alone (controls), a nominal mixture of water and test substance (test substance treatments), or with water and the 3,5-DCP stock solution. Dechlorinated tap water (hardness of 280 ± 20 mg/L as $CaCO_3$) was used. Inoculation entailed addition of 200 mL activated sludge followed by 3 h incubation at a temperature in the range

21-22°C and with aeration throughout at a rate of 0.5 – 1 L/min. At the end of the incubation, a sample was taken and the oxygen concentration was determined for a period of approximately 10 minutes.

Results:

No significant inhibition was observed at the highest tolclofos-methyl concentration tested, 100 mg/L. The respiration rate at this level was equivalent to the respiration rate of the first control (i.e. these rates were within 15% of each other). Accordingly, the oxygen consumption rate of the first four test solutions (1 to 31.6 mg/L) was not determined. The results for the reference inhibitor, 3,5-DCP, were subjected to Probit analysis and an EC_{50} value of 13.0 mg/L (95% confidence limits, 10.8 – 15.7) was obtained. The study was considered valid as the following criteria were satisfied: control respiration rates were within 15% of each other and the EC_{50} of the reference inhibitor, 3,5-DCP, was between 5 and 30 mg/L. The results of the assessment are presented in Table 9.10.a.

Based on the results of this study, the effect (3-hour EC_{50}) of tolclofos-methyl on activated sludge was estimated to be >100 mg/L.

Table 9.10.a. Respiration rate of tolclofos-methyl and reference inhibitor (3,5-DCP)

Treatment	**Respiration rate mg O_2/L/h**	**Percentage inhibition**
Control 1 (test start)	54.0	-
Control 2 (test end)	54.0	
Tolclofos-methyl (mg/L)		
100.0	54.0	0
3,5-DCP (mg/L)		
4	46.5	14
12	27.0	50
36	10.8	80

ND: not determined

Comments:

The study was well performed and reported.

B.9.10.2 Risk assessment for biological methods of sewage treatment

For the proposed uses of tolclofos-methyl contamination of sewage treatment plants is not considered likely. No adverse effects were seen in the laboratory test at the highest concentration tested (100 mg a.s./L) and the risk for harmful effects on biological methods of sewage treatment is therefore considered to be acceptable.

B.9.11 References relied on

Annex point / reference number	Author(s)	Year	Title Source (where different from company) Company, Report No GLP or GEP status (where relevant) Published or not	Data Protection Claimed Y/N	Owner **
Annex II Data and Information					
IIA, 8.1.1/01	Roberts, N.L., Hakin, B.	1981a	The acute oral toxicity (LD_{50}) of S-3349 to the ring-necked pheasant Sumitomo Chemical Co., Ltd. Report No. QW-11-0012 GLP, Unpublished	N	SUM
IIA, 8.1.1/02	Roberts, N.L., Hakin, B.	1981b	The acute oral toxicity (LD_{50}) of S-3349 to the mallard duck Sumitomo Chemical Co., Ltd. Report No. QW-11-0013 GLP, Unpublished	N	SUM
IIA, 8.1.1/03	Roberts, N.L., Hakin, B.	1982	The acute oral toxicity (LD_{50}) of S-3349 to the bobwhite quail Sumitomo Chemical Co., Ltd. Report No. QW-11-0014 GLP, Unpublished	N	SUM
IIA, 8.1.2/01	Beavers, J.B.	1985a	Technical Rizolex: A dietary LC_{50} study with the mallard [redacted] Report No. 107-207 Sumitomo Chemical Co., Ltd. Report No. QT-51-0080 GLP, Unpublished	N	SUM
IIA, 8.1.2/02	Beavers, J.B.	1985b	Technical Rizolex: A dietary LC_{50} study with the bobwhite [redacted] Report No. 107-206 Sumitomo Chemical Co., Ltd. Report No. QT-51-0081 GLP, Unpublished	N	SUM
IIA, 8.1.3/01	Beavers, J.B., Jaber, M.	1987a	Rizolex Technical: A one-generation reproduction study with the mallard (*Anas platyrhynchos*) [redacted] Report No. 107-214 Sumitomo Chemical Co., Ltd. Report No. QW-71-0027 GLP, Unpublished	Y*	SUM

Annex point / reference number	Author(s)	Year	Title Source (where different from company) Company, Report No GLP or GEP status (where relevant) Published or not	Data Protection Claimed Y/N	Owner **
IIA, 8.1.3/02	Beavers, J.B., Jaber, M.	1987b	Rizolex Technical: A one-generation reproduction study with the bobwhite (*Colinus virginianus*) [redacted] Report No. 107-213 Sumitomo Chemical Co., Ltd. Report No. QW-71-0028 GLP, Unpublished	Y*	SUM
IIA, 8.2.1/01	Takimoto, Y., Kagoshima, M., Ashida, S.	1982	The acute toxicity of S-3349 to rainbow trout (*Salmo gairdneri*) Sumitomo Chemical Co., Ltd. Report No. QW-20-0015 Not GLP, Unpublished	N	SUM
IIA, 8.2.1/02	McAllister, W.A.	1989	Acute flow-through toxicity of Rizolex® technical to bluegill (*Lepomis macrochirus*) [redacted] Report No. 37795 Sumitomo Chemical Co., Ltd. Report No. QW-91-0036 GLP, Unpublished	Y*	SUM
IIA, 8.2.1/03	Dionne, E.	1998a	Desmethyl-tolclofosmethyl - Acute toxicity to rainbow trout (*Oncorhynchus mykiss*) under static acute conditions Sumitomo Chemical Co., Ltd. Report No. QW-0055 GLP, Unpublished	Y*	SUM
IIA, 8.2.1/04	Sousa, J.V.	2003	Tolclofos-methyl - Acute toxicity to rainbow trout (*Oncorhynchus mykiss*) under flow-through conditions Sumitomo Chemical Co., Ltd. Report No. QW-0071 GLP, Unpublished	Y*	SUM
IIA, 8.2.2.2/01	Cohle, P.	1991	Early life stage toxicity of Rizolex® technical to rainbow trout (*Oncorhynchus mykiss*) in a flow-through system [redacted] Report No. 38586 Sumitomo Chemical Co., Ltd. Report No. QW-11-0040 GLP, Unpublished	Y*	SUM

Annex point / reference number	Author(s)	Year	Title Source (where different from company) Company, Report No GLP or GEP status (where relevant) Published or not	Data Protection Claimed Y/N	Owner ** **
IIA, 8.2.3/01	Forbis, A.D., Bunch, B.	1986	Uptake, depuration and bioconcentration of ^{14}C-Rizolex by bluegill sunfish (*Lepomis macrochirus*) [redacted], Report No. Not allocated Sumitomo Chemical Co., Ltd. Report No. QM-51-0019 GLP, Unpublished	N	SUM
IIA, 8.2.3/02	Yu, C.C., Guirguis, A.S.	1986	Metabolism of tolclofos-methyl in bluegill sunfish (*Lepomis macrochirus*) [redacted] Report No. 411968-7 Sumitomo Chemical Co., Ltd. Report No. QM-61-0029 GLP, Unpublished	Y*	SUM
IIA, 8.2.3/03	Fujisawa, T., Nambu, K., Nishioka, K., Takimoto, Y.	1999	Calculation of ^{14}C-tolclofos-methyl clearance time (CT_{50}, CT_{90}, CT_{95}) in bluegill sunfish Sumitomo Chemical Co., Ltd. Report No. QM-0045 Not GLP, Unpublished	Y*	SUM
IIA, 8.2.4/01	Murrell, H.	1994	Acute toxicity of Rizolex to *Daphnia magna* Sumitomo Chemical Co., Ltd. Report No. QW-41-0046 GLP, Unpublished	Y*	SUM
IIA, 8.2.4/02	Dionne, E.	1998b	Desmethyl-tolclofosmethyl - Acute toxicity to daphnids (*Daphnia magna*) under static conditions Sumitomo Chemical Co., Ltd. Report No. QW-0054 GLP, Unpublished	Y*	SUM
IIA, 8.2.5/01	Burgess, D.	1989	Chronic toxicity of Rizolex to *Daphnia magna* under flow-through test conditions Sumitomo Chemical Co., Ltd. Report No. QW-91-0031 GLP, Unpublished	N	SUM
II A, 8.2.5/02	Putt, A.E.	2000	Tolclofos-methyl - The full life-cycle toxicity to midge (*Chironomus riparius*) under static conditions Sumitomo Chemical Co., Ltd., Report No. QW-0063 GLP, Unpublished	Y*	SUM

Annex point / reference number	Author(s)	Year	Title Source (where different from company) Company, Report No GLP or GEP status (where relevant) Published or not	Data Protection Claimed Y/N	Owner ** **
IIA, 8.2.6/01	Takimoto, Y., Yasutaniya, T.	1983	The effect of tolclofos-methyl on the growth of the green alga, *Scenedesmus quadricauda* Sumitomo Chemical Co., Ltd. Report No. QW-30-0017 Not GLP, Unpublished	N	SUM
IIA, 8.2.6/02	Hoberg, J.R.	1998	Desmethyl-tolclofosmethyl - Toxicity to the freshwater green alga, *Scenedesmus subspicatus* Sumitomo Chemical Co., Ltd., Report No. QW-0053 GLP, Unpublished	Y*	SUM
IIA, 8.2.6.1/03	Sayers, L.E.	2003	Tolclofos-methyl - Toxicity to the freshwater green alga, *Scenedesmus subspicatus* Sumitomo Chemical Co., Ltd. Report No. QW-0072 GLP, Unpublished	Y*	SUM
IIA, 8.2.7/01	Putt, A.E.	2000	Tolclofos-methyl: The full life-cycle toxicity to midge (*Chironomus riparius*) under static conditions Sumitomo Chemical Co., Ltd., Report No. QW-0063 GLP, Unpublished	Y*	SUM
IIA, 8.3.1.1/01	Londzin, W.	1997	The toxicity of Rizolex 50WP to the honey-bee Sumitomo Chemical Co., Ltd., Report No. QW-0057 Not GLP, Unpublished	Y	SUM
IIA, 8.3.1.1/02	Winter, P.A., Hoxter, K.A., Smith, G.J.	1990	Rizolex: An acute contact toxicity study with the honey bee [redacted] Report No. Not allocated Sumitomo Chemical Co., Ltd. Report No. QW-01-0039 GLP, Unpublished	Y*	SUM
IIA, 8.3.2.1/01	Kollman, S.I.	2002a	Tolclofos-methyl 50WP: Dose response toxicity test with the parasitic wasp, *Aphidius rhopalosiphi* (Hymenoptera: Braconidae) Sumitomo Chemical Co., Ltd. Report No. QW-0062 GLP, Unpublished	Y	SUM
IIA, 8.3.2.1/02	Kollman, S.I.	2002b	Tolclofos-methyl 50WP: Dose response toxicity test with the predatory mite, *Typhlodromus pyri* Scheuten (Acari: Phytoseiidae) Sumitomo Chemical Co., Ltd., Report No. QW-0061 GLP, Unpublished	Y	SUM

Annex point / reference number	Author(s)	Year	Title Source (where different from company) Company, Report No GLP or GEP status (where relevant) Published or not	Data Protection Claimed Y/N	Owner **
IIA, 8.3.2.1/03	Shono, Y.	1998	Toxicity test of Rizolex (tolclofos-methyl) to two beneficial insects, *Orius sauteri* and *Chrysoperla carnea* Sumitomo Chemical Co., Ltd. Report No. QW-0050 Not GLP, Unpublished	Y	SUM
IIA, 8.3.2.1/04	Nienstedt, K.M.	2003	Tolclofos-methyl 50WP: An extended aged residue test with the parasitic wasp, *Aphidius rhopalosiphi* (Hymenoptera: Braconidae) Sumitomo Chemical Co., Ltd. Report No. QW-0067 GLP, Unpublished	Y	SUM
IIA, 8.3.2.1/05	Nienstedt, K.M.	2002a	Tolclofos-methyl 50WP: An extended laboratory test with *Poecilus cupreus* L. (Coleoptera: Carabidae) Sumitomo Chemical Co., Ltd. Report No. QW-0066 GLP, Unpublished	Y	SUM
IIA, 8.3.2.1/06	Nienstedt, K.M.	2002b	Tolclofos-methyl 50WP: An extended aged residue test with *Aleochara bilineata* Gyll. (Coleoptera: Staphylinidae) Sumitomo Chemical Co., Ltd. Report No. QW-0065 GLP, Unpublished	Y	SUM
IIA, 8.4.1/01	Candolfi, M.P.	1997	Tolclofos-methyl: 14-day acute toxicity test with the earthworm (*Eisenia foetida*) Sumitomo Chemical Co., Ltd. Report No. QW-0047 GLP, Unpublished	Y*	SUM
IIA, 8.5/01	Barug, D., Vogelaar, J.C.	1983a	Effect of tolclofos-methyl on nitrogen transformations in loam soil Sumitomo Chemical Co., Ltd. Report No. QW-31-0020 Not GLP, Unpublished	N	SUM
IIA, 8.5/02	Barug, D., Vogelaar, J.C.	1984	Effect of tolclofos-methyl on nitrogen transformations in humic sandy soil Sumitomo Chemical Co., Ltd. Report No. QW-31-0021 Not GLP, Unpublished	N	SUM
IIA, 8.5/03	Barug, D., Vogelaar, J.C.	1983b	Effect of tolclofos-methyl on soil respiration Sumitomo Chemical Co., Ltd. Report No. QW-31-0019 Not GLP, Unpublished	N	SUM

Annex point / reference number	Author(s)	Year	Title Source (where different from company) Company, Report No GLP or GEP status (where relevant) Published or not	Data Protection Claimed Y/N	Owner **
IIA, 8.6/01	Umeda, K.	2001	Evaluation of insecticidal activity of tolclofos-methyl Sumitomo Chemical Co., Ltd., Report No. QG-0020 Not GLP, Unpublished	Y*	SUM
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IIA, 8.7/01	L'Haridon, J.	2000	Activated sludge, respiration inhibition test Sumitomo Chemical Co., Ltd. Report No. QM-0048 GLP, Unpublished	Y*	SUM
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IIIA, 10.1.1/01	Ross, D.B., Roberts, N.L., Phillips, C.N.K.	1980a	The acute oral toxicity (LD_{50})of S-3349 to the mallard duck Sumitomo Chemical Co., Ltd. Report No. QW-91-0001 Not GLP, Unpublished	N	SUM
IIIA, 10.1.1/02	Ross, D.B., Roberts, N.L., Phillips, C.N.K.	1980b	The acute oral toxicity (LD_{50}) of S-3349 to the bobwhite quail Sumitomo Chemical Co., Ltd. Report No. QW-01-0007 Not GLP, Unpublished	Y	SUM
IIIA, 10.2.1/01	Christopher, D.H., Pell, I.B.	1979	The acute toxicity of S 3349 to bluebill sunfish (*Lepomis macrochirus*) and rainbow trout (*Salmo gairdneri*) Sumitomo Chemical Co., Ltd. Report No. QW-91-0002 Not GLP, Unpublished	N	SUM
IIIA, 10.2.1/02	Christopher, D.H., Fraser, W.D.	1978	The toxicity of S 3349 to *Daphnia magna*, Straus Sumitomo Chemical Co., Ltd. Report No. QW-81-0004 GLP, Unpublished	N	SUM
IIIA, 10.2.1/03	Sayers, L.E.	2003a	Tolclofos-methyl 50WP - Toxicity to the freshwater green alga, *Scenedesmus subspicatus* Sumitomo Chemical Co., Ltd. Report No. QW-0076 GLP, Unpublished	Y*	SUM

Annex point / reference number	Author(s)	Year	Title Source (where different from company) Company, Report No GLP or GEP status (where relevant) Published or not	Data Protection Claimed Y/N	Owner **
IIIA, 10.2.1/04	Sayers, L.E.	2003b	Tolclofos-methyl 50WP - Acute toxicity to water fleas, *Daphnia magna*, under static conditions Sumitomo Chemical Co., Ltd. Report No. QW-0073 GLP, Unpublished	Y*	SUM
IIIA, 10.2.1/05	Sayers, L.E.	2003c	Tolclofos-methyl 50WP - Acute toxicity to rainbow trout (*Oncorhynchus mykiss*) under static-renewal conditions Sumitomo Chemical Co., Ltd. Report No. QW-0074 GLP, Unpublished	Y*	SUM
IIIA, 10.2.1/06	Sayers, L.E.	2003d	Tolclofos-methyl 50WP - Acute toxicity bluegill sunfish (*Lepomis macrochirus*) under static-renewal conditions Sumitomo Chemical Co., Ltd. Report No. QW-0075 GLP, Unpublished	Y*	SUM
IIIA, 10.4.1/01	Londzin, W.	1997	The toxicity of Rizolex 50WP to the honey-bee Sumitomo Chemical Co., Ltd., Report No. QW-0057 Not GLP, Unpublished	Y	SUM
IIIA, 10.5.1/01	Kollman, S.I.	2002a	Tolclofos-methyl 50WP: Dose response toxicity test with the parasitic wasp, *Aphidius rhopalosiphi* (Hymenoptera: Braconidae) Sumitomo Chemical Co., Ltd. Report No. QW-0062 GLP, Unpublished	Y	SUM
IIIA, 10.5.1/02	Kollman, S.I.	2002b	Tolclofos-methyl 50WP: Dose response toxicity test with the predatory mite, *Typhlodromus pyri* Scheuten (Acari: Phytoseiidae) Sumitomo Chemical Co., Ltd., Report No. QW-0061 GLP, Unpublished	Y	SUM
IIIA, 10.5.1/03	Shono, Y.	1998	Toxicity test of Rizolex (tolclofos-methyl) to two beneficial insects, *Orius sauteri* and *Chrysoperla carnea* Sumitomo Chemical Co., Ltd. Report No. QW-0050 Not GLP, Unpublished	Y	SUM
IIIA, 10.5.1/04	Nienstedt, K.M.	2003	Tolclofos-methyl 50WP: An extended aged residue test with the parasitic wasp, *Aphidius rhopalosiphi* (Hymenoptera: Braconidae) Sumitomo Chemical Co., Ltd. Report No. QW-0067 GLP, Unpublished	Y	SUM

Annex point / reference number	Author(s)	Year	Title Source (where different from company) Company, Report No GLP or GEP status (where relevant) Published or not	Data Protection Claimed Y/N	Owner **
IIIA, 10.5.1/05	Nienstedt, K.M.	2002a	Tolclofos-methyl 50WP: An extended laboratory test with *Poecilus cupreus* L. (Coleoptera: Carabidae) Sumitomo Chemical Co., Ltd. Report No. QW-0066 GLP, Unpublished	Y	SUM
IIIA, 10.5.1/06	Nienstedt, K.M.	2002b	Tolclofos-methyl 50WP: An extended aged residue test with *Aleochara bilineata* Gyll. (Coleoptera: Staphylinidae) Sumitomo Chemical Co., Ltd. Report No. QW-0065 GLP, Unpublished	Y	SUM
IIIA, 10.6.1.1/01	Leimgruber, R.	1984a	Determination of the acute toxicity of Rizolex 25% flowable in earthworm Sumitomo Chemical Co., Ltd. Report No. QW-41-0023 Not GLP, Unpublished	N	SUM
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*: Protection for 5 years claimed from date of decision concerning listing in Annex I - the study report has not been submitted any of the Member States in support of an application for authorization, or (though the study report has been submitted) has not been used any of the Member States as the basis for decision on the initial authorization, or to maintain a given authorization, of a plant protection product before the date of submission of the dossier to Rapporteur Member State.

**: Owners' code identifications and names (Code identification: SUM, Name: Sumitomo Chemical

TOLCLOFOS-METHYL

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Annex B.9
Ecotoxicology

APPENDIX B

Risk assessment for birds and aquatic organisms following spray application to field crops

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Risk assessment for birds following spray application to field crops

In view of the use pattern of tolclofos-methyl within the EU, including also spray application to wheat, RMS also considered spray application for protection of wheat as help for further assessment of extended uses at the MS level. In the case of wheat, spray application is carried out just after tillering, on bare soil. Exposure of birds via residues in food items is therefore considered to be limited and mainly caused by secondary poisoning.

Secondary poisoning

In view of the use pattern of tolclofos-methyl within the EU, including also spray application to wheat, RMS also considered spray application for protection of wheat as help for further assessment of extended uses at the MS level. In case of spray application to field crops contamination of surface water cannot be excluded, and therefore the risk of secondary poisoning of fish-eating birds was estimated. The approach recommended in the "Guidance Document on Risk Assessment for Birds and Mammals Under Council Directive 91/414/EEC" (SANCO/4145/2000 25 September 2002) was followed.

A simple worst-case assessment was conducted according to the following steps:

The 3-week time-weighted PEC_{water} of 1.01 µg a.s./L (see section B.8.6.2) was used based on an application rate of 1kg a.s./ha and DT_{50} in surface water of 1.6 days. A bioconcentration factor (BCF) for fish of 670, obtained in the study by Forbis and Bunch (1986) was used and the residue in fish calculated according to the formula:

$PEC_{fish} = PEC_{water} \times BCF$

The estimated residue level in fish = 1.01 µg a.s./L x 670 = 0.68mg/kg

The residue level in fish was converted to daily dose by multiplying with 0.21 (1000-g bird eating 206 g per day) = 0.14 mg a.s./kg bw/day. The daily dose value was compared with long term NOEC value of 49 mg/kg bw/day, which resulted in a TER value of 350. Hence, the risk for fish-eating birds can be neglected.

Risk assessment for aquatic organisms following spray application to field crops.

In accordance with the risk assessment performed for birds, and in the light of the current use of tolclofos-methyl within EU, RMS considered also spray application to field crops. Although not required for the proposed areas of use this was performed as help for further assessment of extended uses at the MS level.

A maximum application rate of 1 kg a.s./ha was assumed, and the loading via spray drift was estimated based on Rautmann 90th percentile drift data (2.77% spray drift at 1 m distance from the field). The contribution from drainage and run-off to the total loading was not taken into account. The FOCUS surface water report was not implemented for active substances on the second list and therefore not formally correct to use at this stage, but could be so in future MS level assessments of the total loading to surface water. A static 30 cm deep water body

was assumed, with a 5 cm deep sediment layer, with uniform distribution within both. Worst case DT_{50}s for tolclofos methyl and the metabolite DM-TM from the sediment/water study, within the water phase were 1.6 and 42 days respectively (both 1st order, see Table B.8.4.3.2.d).

The short and long term effect concentrations of the most sensitive species in each taxonomic group, the predicted environmental concentrations and the toxicity to exposure ratios are given in the table below.

Calculated Toxicity to Exposure Ratios (TER) for tolclofos-methyl (TM) and the major metabolite (DM-TM) in surface water following spray drift at 1 m distance from a field treated at 1 kg a.s./ha and a maximum level for DM-TM of 11 % of applied radioactivity.

Species	Test substance	LC_{50}/NOEC (mg a.s./L)	PEC* (µg a.s./L)	TER value	Annex VI trigger
SHORT TERM					
Fish - Rainbow trout	Tolclofos-methyl	0.69	9.2	75	<100
Fish - Bluegill sunfish	Tolclofos-methyl	>0.72	9.2	>78	<100
Fish - Rainbow trout	Tolclofos-methyl 50WP	26	9.2	2826	<100
Fish - Bluegill sunfish	Tolclofos-methyl 50WP	59	9.2	6413	<100
Fish - Rainbow trout	DM-TM	>110	1.0	>110,000	<100
Aquatic invertebrates -*Daphnia magna*	Tolclofos-methyl	48	9.2	5217	<100
	Tolclofos-methyl 50WP	30	9.2	3261	<100
	DM-TM	>95	1.0	>95,000	<100
LONG TERM					
Fish - Rainbow trout	Tolclofos-methyl	0.012	9.2 (initial)	1.30	<10
Fish - Rainbow trout	Tolclofos-methyl	0.012	0.51 (42 d TWA)	24	<10
Aquatic invertebrates -*Daphnia magna*	Tolclofos-methyl	0.026	9.2 (initial)	2.8	<10
Aquatic invertebrates -*Daphnia magna*	Tolclofos-methyl	0.026	1.0 (21 d TWA)	26	<10
Aquatic invertebrates (insects) and Sediment dwelling organisms *Chironomus riparius*	Tolclofos-methyl	0.25	9.2	27	<10
Algae* – *Scenedesmus quadricauda*	Tolclofos-methyl	0.78	0.67	85	<10
Scenedesmus subspicatus	DM-TM	>97	1.0	>97,000	<10
	Tolclofos-methyl 50WP	0.65	0.67	71	

The calculated TER values for aquatic organisms indicate that spray application of tolclofos-methyl to field crops may imply an acute risk to fish. Also the long-term/reproduction TER values for fish and aquatic invertebrates were below the Annex VI trigger for Tier 1 assessment when initial PEC values were used. In conclusion this means that risk to aquatic organisms has to be considered at MS level for formulated products containing tolclofos-methyl intended for spray application to field crops.