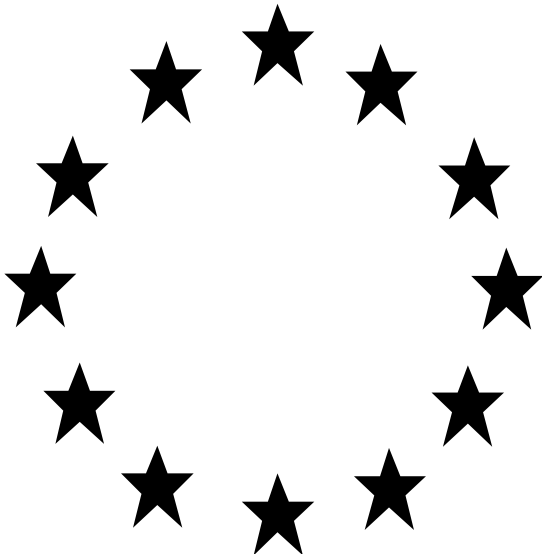

Draft Assessment Report (DAR)

- 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 1

January 2005

Draft Assessment Report

TOLCLOFOS-METHYL

Volume 3
Annex B.9
Ecotoxicology

Rapporteur Member State: Sweden

October 2003

KEMI
NATIONAL CHEMICALS INSPECTORATE

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B.9 Ecotoxicology

Ecotoxicological studies were performed with the active ingredient (tolclofos-methyl) and one metabolite that occurred in amounts > 10 % in soil, surface water and sediment. In addition, studies were carried out with one representative formulated product specified by the Notifier, Tolclofos-methyl 50WP containing 50 % w/w tolclofos-methyl as active ingredient. The Notifier also selected two representative crop scenarios with potatoes and protected lettuce with the maximum recommended application rates of 625 g a.s./ha and 2000 g a.s./ha, respectively.

B.9.1 Effects on birds (Annex IIA 8.1; Annex IIIA 10.1)

B.9.1.1 Acute oral toxicity

ACTIVE INGREDIENT

Reference:	Roberts, N.L., Hakin, B. (1981a) The acute oral toxicity (LD_{50}) of S-3349 to ring-necked pheasant
Guideline:	U. K. Pesticides Safety Precautions Scheme, Working Document D5 (revised 1979) U. S. EPA guidelines Part II 163 71.1 (10th July, 1978)
GLP:	Yes

Material and methods:

Test substance:	Tolclofos-methyl (Batch No.: 00304, Purity: 99.8%)
Species:	Ring-necked pheasant (*Phasianus colchicus*)
Treatments:	0 (vehicle control) and a limit dose of 5000 mg a.s./kg b.w., administered by oral gavage as a 40% w/v concentration of technical tolclofos-methyl in corn oil.
Number of animals:	5/sex/dose
Duration:	A 14-day settling in period was used. The single dose was followed by 14-day observation period.
Test conditions:	The test was performed at natural daylight with 7 hours of darkness, a temperature of 16 - 30°C and a mean relative humidity of 72%. Standard chicken diet and water was provided *ad libitum*.
Observations:	Bird health, body weights, food consumption, mortalities and *post mortem* examination for gross pathological changes.

Results:

No deaths related to the treatment occurred during the test. Body weights and food consumption were unaffected by treatment with tolclofos-methyl. From the results of this study it was not possible to obtain an LD_{50} of

technical tolclofos-methyl to ring-necked pheasant. LD_{50} was therefore concluded to be >5000 mg a.s./kg. The no-observed effect level (NOEL) was set to 5000 mg a.s./kg, the highest dose tested.

Comments:

The study was well performed and reported.

Reference:	Roberts, N.L., Hakin, B. (1981b) The acute oral toxicity (LD_{50}) of S-3349 to mallard duck
Guideline:	U. S. EPA guidelines Part II 163 71.1 (10th July, 1978)
GLP:	Yes

Material and methods:

Test substance:	Tolclofos-methyl, purity: 99.8%
Species:	Mallard duck (*Anas platyrhynchos*)
Treatments:	0 (vehicle control) and a limit dose of 5000 mg a.s. /kg b.w. administered by oral gavage as a 40% w/v concentration of technical tolclofos-methyl in corn oil.
Number of animals:	5/sex/dose
Duration:	A 14-day settling in period was used. The single dose was followed by 14-day observation period.
Test conditions:	The test was performed at natural daylight, a temperature of 13 - 26°C and a mean relative humidity of 70%. Standard chicken diet and water was provided *ad libitum*.
Observations:	Bird health, body weights, food consumption, mortalities and *post mortem* examination for gross pathological changes.

Results:

There were no mortalities or signs of toxicity and all birds remained in good health throughout the study period. Body weights and food consumption were unaffected by treatment with tolclofos-methyl. No abnormalities were detected in any of the birds at the *post mortem* examination.

From the results of this study it was not possible to obtain an LD_{50} of technical tolclofos-methyl to the mallard duck. LD_{50} was therefore concluded to be >5000 mg a.s./kg, the highest dose tested. The no-observed effect level (NOEL) was set to 5000 mg a.s./kg.

Comments:

The study was well performed and reported.

Reference:	Roberts, N.L., Hakin, B. (1982) The acute oral toxicity (LD_{50}) of S-3349 to bobwhite quail
Guideline:	U. S. EPA guidelines Part II 163 71.1 (10th July, 1978)
GLP:	Yes

Material and methods:

Test substance:	Tolclofos-methyl (Batch No.: 00304, Purity: 99.8%)
Species:	Bobwhite quail (*Colinus virginianus*)
Treatments:	0 (vehicle control) and a limit dose of 5000 mg a.s./kg b.w. administered by oral gavage as a 40% w/v concentration of technical tolclofos-methyl in corn oil.
Number of animals:	5/sex/dose
Duration:	A 14-day settling in period was used. The single dose was followed by 14-day observation period.
Test conditions:	The test was performed with 17 hours light and 7 hours dark at a temperature of 13 - 26°C and a mean relative humidity of 70%. Standard chicken diet and water was provided *ad libitum*.
Observations:	Bird health, body weights, food consumption, mortalities and *post mortem* examination for gross pathological changes.

Results:

There were no mortalities or signs of toxicity and all birds remained in good health throughout the study period. Body weights and food consumption were unaffected by treatment with tolclofos-methyl. No abnormalities were detected in any of the birds at the *post mortem* examination

From the results of this study it was not possible to obtain an LD_{50}. The acute oral toxicity (LD_{50}) of technical tolclofos-methyl to bobwhite quail was therefore concluded to be >5000 mg a.s./kg, the highest dose tested. The no-observed effect level (NOEL) was set to 5000 mg a.s./kg.

Comments:

The study was well performed and reported.

PLANT PROTECTION PRODUCT

Reference:	Ross, D.B., Roberts, N.L., Phillips, C.N.K. (1980a) The acute oral toxicity (LD_{50}) of S-3349 to the mallard duck
Guideline:	U. S. EPA guidelines, Working Group Draft April 27, 1977
GLP:	No

Material and methods:

Test substance:	Tolclofos-methyl 50WP (Batch No.: 61258, content of a.s. 50% w/w)
Species:	Mallard duck (*Anas platyrhynchos*)
Treatments:	0 (vehicle control), 7260 and 7554 mg/kg b.w. administered by oral gavage, 25 ml of a 40% w/v concentration in corn oil.

Number of animals:	5/sex/dose
Duration:	A 14-day settling in period was used. The single dose was followed by 14-day observation period.
Test conditions:	The test was performed at a temperature of approx. 20°C and a mean relative humidity of approx. 60%. Standard chicken diet and water was provided *ad libitum*.
Observations:	Bird health, body weights, food consumption, mortalities and *post mortem* examination for gross pathological changes.

Results:

There were no mortalities or signs of toxicity that could be attributed to dosing and all birds remained in good health throughout the study period. Body weight gain and food consumption were unaffected by treatment with Tolclofos-methyl 50WP. No abnormalities were detected in any of the birds at the *post-mortem* examination. The results are presented in Tables 9.1.1.j (mortality and clinical observations), 9.1.1.k (bodyweight changes) and 9.1.1.l (food consumption).

From the results of this study it was not possible to obtain an LD_{50}. The acute oral toxicity (LD_{50}) of tolclofos-methyl 50WP to mallard duck was therefore concluded to be >7554 mg product/kg (> 3777 mg a.s./kg), the highest concentration tested. The no-observed effect level (NOEL) was set to 7554 mg product/kg (3777 mg a.s./kg).

Comments:

The study was well performed and reported.

Reference:	Ross, D.B., Roberts, N.L., Phillips, C.N.K. (1980b) The acute oral toxicity (LD_{50}) of S-3349 to the bobwhite quail
Guideline:	U. S. EPA guidelines, Working Group Draft April 27, 1977
GLP:	No

Material and methods:

Test substance:	Tolclofos-methyl 50WP (Batch No.: 61258, content of a.s. 50% w/w).
Species:	Bobwhite quail (*Colinus virginianus*)
Treatments:	0 (vehicle control), 11173 and 11364 mg /kg b.w. administered by oral gavage, 5 ml of a 40% w/v concentration in corn oil.
Number of animals:	5/sex/dose
Duration:	A 14-day settling in period was used. The single dose was followed by 14-day observation period.
Test conditions:	The test was performed at a temperature of approx. 23°C and a mean relative humidity of 50 - 60%. Standard chicken diet and water was provided *ad libitum*.
Observations:	Bird health; body weights; food consumption; mortalities; *post mortem* examination for gross pathological changes.

Results:

There were no mortalities or signs of toxicity that could be attributed to dosing and all birds remained in good health throughout the study period. All groups showed a weight loss during the settling-in period and the two test groups continued to lose weight until Day 3 (Group 1) and Day 7 (Group 2). In the second week both groups regained weight. Food consumption was unaffected by treatment with Tolclofos-methyl 50WP. No abnormalities were detected in any of the birds at the *post- mortem* examination

From the results of this study it was not possible to obtain an LD_{50}. The acute oral toxicity (LD_{50}) of tolclofos-methyl 50WP to bobwhite quail was therefore concluded to be >11,364 mg product/kg bw (>5682 mg a.s./kg bw), the highest dose tested. The no-observed effect level (NOEL) was set to 11,364 mg product/kg bw (5682 mg a.s./kg bw).

Comments:

The study was acceptably performed and well reported.

B.9.1.2 Short term dietary toxicity

ACTIVE INGREDIENT

Reference:	Beavers, J.B. (1985a)
	Technical Rizolex: A dietary LC_{50} study with the mallard
Guideline:	U. S. EPA Pesticide Assessment Guideline 71.2 (October 1982)
	ASTM Standard E857-81 (1982)
GLP:	Yes (Self certification by the laboratory)

Material and methods:

Test substance:	Tolcofos-methyl (Batch No.: 40810, Purity: 97.7%)
Species:	Mallard duck chicks (*Anas platyrhynchos*)
Treatments:	0 (2 % corn oil), 562, 1000, 1780, 3160 and 5620 mg a.s./kg food, mixed into feed with corn oil.
Number of animals:	10 per group; 5 test groups and 5 control groups
Duration:	8 days (5 days test diet + 3 days with normal feed)
Test conditions:	The test was performed with a photoperiod of 17 hours light and 7 hours dark at a temperature of 27±1°C and a mean relative humidity of 57% after a 9 days acclimatization period of the of birds.
Observations:	Bird health, body weights, food consumption, mortalities.

Results:

There were no mortalities or signs of toxicity and all birds remained in good health throughout the study period. Body weights and food consumption were unaffected by treatment with tolclofos-methyl.

The short term dietary toxicity (LC_{50}) of tolclofos-methyl to mallard duck was concluded to be >5620 mg a.s./kg food, the highest dose tested. The no-observed effect level (NOEC) was set to 5620 mg a.s./kg food.

Comments:

The study was well performed and reported. From the available data on bird body weight and food consumption RMS converted toxicity figures to daily dose as recommended in the Guidance Document on Risk Assessment for Birds and Mammals Under Council Directive 91/414/EEC (SANCO/4145/2000 25 September 2002). For this conversion group food consumption (expressed as g per bird per day) for the 5-day exposure period and the average of day 0 and day 5 group mean body weight was used. Daily dose was then calculated by the formula: daily dose (mg/kg bw/day) = concentration in food (mg/kg) x daily food consumption (g/bird/day)/bw. The so calculated values of daily dose were in the range of 168 – 1624 mg/kg bw/day and hence the LC_{50} was concluded to be > 1624 mg a.s /kg bw/day and the NOEL was set to 1624 mg a.s./kg bw/day. These values were used for the risk assessment.

Reference:	Beavers, J.B. (1985b)
	Technical Rizolex: A dietary LC_{50} study with the bobwhite
Guideline:	U. S. EPA Pesticide Assessment Guideline 71.2
	ASTM Standard E857-81
GLP:	Yes (Self certification by the laboratory)

Material and methods:

Test substance:	Tolcofos-methyl (Batch No.: 40810, Purity: 97.7%
Species:	Bobwhite quail chicks (*Colinus virginianus*)
Treatments:	0 (2 % corn oil), 562, 1000, 1780, 3160 and 5620 mg a.s./kg food, mixed into feed with corn oil.
Number of animals:	10 per group; 5 test groups and 5 control groups
Duration:	8 days (5 days test diet + 3 days with normal feed)
Test conditions:	The test was performed with a photoperiod of 17 hours light and 7 hours dark at a temperature of 27±1°C and a mean relative humidity of 57% after a 9 days acclimatization period of the of birds.
Observations:	Bird health, body weights, food consumption and mortalities.

Results:

There was 20% mortality at the 1000 mg a.s./kg food test concentration and 50% and 10% mortality in two of the control groups. These mortalities appeared to be incidental and unrelated to treatment. At all other test

concentrations there were no overt signs of toxicity and all birds remained in good health throughout the study period. Body weights and food consumption were unaffected by treatment with tolclofos-methyl.

The short term dietary toxicity (LC_{50}) of tolclofos-methyl to bobwhite quail was concluded to be >5620 mg a.s./kg food, the highest dose tested. The no-observed effect level (NOEC) was set to 5620 mg a.s./kg food.

Comments:

According to the study report, deaths in control group 1 were attributed to aggression. Deaths among treated birds only occurred in the group treated with 1000 mg a.s./kg food and as there were no other signs of illness, decreased weight gain or food consumption among treated birds there is no reason to believe that these deaths were related to exposure to tolclofos-methyl. The study was well performed and reported. From the available data on bird body weight and food consumption RMS converted toxicity figures to daily dose as recommended in the Guidance Document on Risk Assessment for Birds and Mammals Under Council Directive 91/414/EEC (SANCO/4145/2000 25 September 2002). For this conversion group food consumption (expressed as g per bird per day) for the 5-day exposure period and the average of day 0 and day 5 group mean body weight was used. Daily dose was then calculated by the formula: daily dose (mg/kg bw/day) = concentration in food (mg/kg) x daily food consumption (g/bird/day)/bw. The so calculated values of daily dose were in the range of 339 – 2248 mg/kg bw/day and hence the LC_{50} was concluded to be > 2248 mg a.s /kg bw/day and the NOEL was set to 2248 mg a.s./kg bw/day. These values were used for the risk assessment.

B.9.1.3 Subchronic toxicity and reproduction

ACTIVE INGREDIENT

Reference:	Beavers, J.B., Jaber, M. (1987a) Rizolex Technical: A one-generation reproduction study with the mallard (*Anas platyrhynchos*)
Guideline:	U. S. EPA Pesticide Assessment Guideline, FIFRA 71-4 (October 1982)
GLP:	Yes (Self certification by the laboratory)

Material and methods:

Test substance:	Tolclofos-methyl (Batch No.: 40810, Purity: 97.7%)
Species:	Mallard ducks (*Anas platyrhynchos*)
Treatments:	0 (0.09 % corn oil), 500, 2500 and 7500 mg a.s./kg food, mixed into feed with corn oil.
Number of animals:	16 pairs/group
Exposure duration:	19 weeks (10 weeks prior to the start of egg production and 9 weeks of egg production)
Test conditions:	**Adults:** Birds were 41 weeks old. A photoperiod of 8 hours light and 16 hours darkness was used until the start of Week 9, followed thereafter by 17 hours light

and 7 hours darkness for the rest of the study. Light intensity was 12 footcandles, temperature 20 ± 6°C and relative humidity 90%.

Eggs: Eggs were stored at 13 ± 1°C and 88% relative humidity during a 7-day period, candled and any broken or cracked eggs were recorded and discarded. Remaining eggs were incubated for 24 days at 37.4°C and 54% relative humidity. After 24 days incubation, the eggs were transferred to a hatcher at 37.1°C; any unhatched eggs remaining after three days were classified as dead in the shell.

Observations: **Adults:** body weights, feed consumption, mortalities, signs of toxicity and abnormal behaviour and necropsy at end of study.

Reproductive parameters: eggs laid, eggs set, eggs cracked, viable embryos, live three-week embryos, hatchlings, body weight of hatchlings, 14-day old survivors, body weight of 14-day survivors and egg-shell thickness.

Results:

Samples of feed analysed for residues showed from 89 to 127 % of nominal concentrations. There were no treatment-related effects on adult birds and reproductive parameters exposed to a nominal dietary concentration of 500 mg a.s./kg food. At 2500 and 7500 mg a.s./kg food, there were treatment-related effects on adult survival and body weight changes. At 2500 mg a.s./kg food, one male and two female birds had died by the end of the study and the surviving females showed a marked weight loss over this period compared to the controls. At 7500 mg a.s./kg food, eight male and two female birds had died by the end of the study and all surviving birds showed a marked weight loss compared to the controls. Clinical signs of toxicity first appeared during week 5 in the 7500-ppm group and during week 7 in the 2500-ppm group. Signs of toxicity were lower limb weakness, lethargy and loss of coordination and reduced reaction to external stimuli. Gross necropsy showed a dose-response increase in the number of drakes and hens with regressing or regressed testes or ovaries at 2500 and 7500 ppm. In addition, at 2500 mg a.s./kg food, there was a treatment-related reduction in the number of eggs laid as a percentage of the maximum number of eggs laid. This reduction was also reflected in the number of hatchlings and 14-day old survivors of the maximum number of eggs set. At 7500 mg a.s./kg food, there was a severe treatment-related effect upon egg production, with only 14 eggs being laid by hens in the seven pens where no mortality occurred. The results for the effects on adult birds and the main reproduction parameters are presented in Tables 9.1.3.a to 9.1.3.d.

Table 9.1.3.a. Subchronic and reproductive toxicity of tolclofos-methyl to mallard duck: Cumulative mortality of adult birds

Treatment (mg a.s./kg food)		Week of study																		
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	Total
Control	Males	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0/16
	Females	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0/16
500	Males	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0/16
	Females	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0/16
2500	Males	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1	1/16
	Females	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1	1	2	2/16
7500	Males	0	0	0	0	1	4	5	5	5	5	6	6	6	6	7	7	7	8	8	8/16
	Females	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1	1	2	2	2/16

Table 9.1.3.b. Subchronic and reproductive toxicity of tolclofos-methyl to mallard duck: Group mean body weight changes

Treatment (mg a.s./kg food)		Mean body weight change (g)						
		Weeks 0 to 2	Weeks 2 to 4	Weeks 4 to 6	Weeks 6 to 7	Weeks 7 to 8	Week 8 to Termination	Total Change
Control	Males	52	9	24	-11	-5	21	90
	Females	29	6	14	-4	4	179	228
500	Males	40	-3	18	29	-3	22	102
	Females	36	8	12	15	13	164	247
2500	Males	30	37	29	31	0	-4	136
	Females	15	21	7	35	-15	-81	-3**
7500	Males	-14	-123	-11**	63**	82	-23	-43
	Females	-1	-14	-22	28	-9	-141	-126**

** Statistically different from control group (ANOVA and Dunnett's test, p<0.01)

Table 9.1.3.c. Subchronic and reproductive toxicity of tolclofos-methyl to mallard duck: Group mean food consumption (g)

Treatment (mg a.s./kg food)	Week of study																		
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
Control	167	172	182	106	118	132	155	142	161	183	189	204	175	210	211	345	329	293	193
500	153	180	185	113	105	133	153	143	155	172	193	180	131	207	195	275**	250**	229*	155**
2500	150	191	209	134	129	127	156	134	143	166	162	193	139	198	186	226**	247**	280	172
7500	134	178	136	111	99	100	148	132	142	167	173	183	113*	179	148**	184**	206**	279**	124**

* Statistically different from control (ANOVA and Dunnet's test, p<0.05)
** Statistically different from control (ANOVA and Dunnet's test, p<0.01)

Table 9.1.3.d. Subchronic and reproductive toxicity of tolclofos-methyl to mallard duck: Reproductive parameters

	Treatment (mg a.s./kg food)			
	Control	500	2500	7500
Eggs laid	561	525	245	14
Eggs cracked	34	15	3	1
Eggs set	475	454	211	8
Viable Embryos	395	391	159	2
Live 3-Week embryos	385	372	157	2
Hatchlings	290	269	114	1
14-Day old survivors	280	257	107	1
Eggs laid / hen	35	33	19	2
Eggs laid / hen / day	0.63	0.59	0.34	0.04
14-Day old survivors / hen	18	16	8	0
Egg shell thickness	0.368	0.390	0.372	0.327
Body weight (Hatchlings)	39	41	41	50
Body weight (14-Day old survivors)	230	209	218	258
Eggs laid / Max. laid (%)[1]	64	60	34**	4**
Eggs cracked / Eggs laid (%)	7	2	1	2
Viable embryos / Eggs set (%)	86	88	72	50
Live 3-Week embryos / Viable embryos (%)	96	89	99	100
Hatchlings / Live 3-Week embryos (%)	68	71	68	50
14-Day old survivors / Hatchlings (%)	97	94	94	100
14-Day old survivors / Eggs set (%)	57	52	48	17
Hatchlings / Max. set (%)	38	35	18	0*
14-Day old survivors / Max. set (%)	36	33	17	0**

* Statistically different from control (ANOVA of arcsine transformed data followed by Dunnet's test p<0.05)
** Statistically different from control (ANOVA of arcsine transformed data followed by Dunnet's test p<0.01)
[1] Max laid = maximum number of eggs laid by any one hen in the study

Based on the results of this study the long-term no-observed effect concentration (NOEC) was set to 500 mg a.s./kg food.

Comments:

The study was well performed and reported.

From the available data on bird body weight and food consumption RMS converted toxicity figures to daily dose as recommended in the Guidance Document on Risk Assessment for Birds and Mammals Under Council Directive 91/414/EEC (SANCO/4145/2000 25 September 2002). For this conversion average group food consumption (expressed as g per bird per day) for the whole exposure period and the average body weight of both sexes over the exposure period was used. Daily dose was then calculated by the formula: Daily dose (mg/kg bw/day) = concentration in food (mg/kg) x daily food consumption (g/bird/day)/bw. The resulting values of daily dose were 76, 382 and 1041mg/kg bw/day and hence the NOEL was set to 76 mg a.s./kg bw/day. These values were used for the risk assessment.

Reference:	Beavers, J.B., Jaber, M. (1987b) Rizolex Technical: A one-generation reproduction study with the bobwhite (*Colinus virginianus*)
Guideline:	U. S. EPA Pesticide Assessment Guideline, FIFRA 71-4
GLP:	Yes (Self certification by the laboratory)

Material and methods:

Test substance:	Tolclofos-methyl (Batch No.: 40810, Purity: 97.7%)
Species:	Bobwhite quail (*Colinus virginianus*)
Treatments:	0 (0.09 % corn oil), 500, 2500 and 7500 mg a.s./kg food mixed into feed with corn oil.
Number of animals:	16 pairs/group
Exposure duration:	21 weeks (10 weeks prior to the start of egg production and 11 weeks of egg production).
Test conditions:	**Adults:** Birds were approx. 22 weeks old. A photoperiod of 8 hours light and 16 hours of darkness was used until the start of Week 9, followed by 17 hours light and 7 hours darkness for the rest of the study. Light intensity was 12 footcandles, temperature 23 ± 2°C and relative humidity 69%. **Eggs:** Eggs were stored at 13 ± 1°C and 76% relative humidity during a 7-day period, candled and any broken or cracked eggs were recorded and discarded. Remaining eggs were incubated for 21 days at 37.4°C, 54% relative humidity. After 21 days incubation, embryo survival of eggs was determined and then the eggs were transferred to a hatcher at 37.1°C. Any unhatched eggs remaining after five days were classified as dead in the shell.
Observations:	**Adults:** body weights, feed consumption, mortalities, signs of toxicity and abnormal behaviour and necropsy at end of study. **Reproductive parameters:** eggs laid, eggs set, eggs cracked, viable embryos, live three-week embryos, hatchlings, body weight of hatchlings, 14-day old survivors, body weight of 14-day survivors and egg-shell thickness.

Results:

In the control group, one male and three female birds died during the course of the study and in the 2500 mg a.s./kg food treatment group, two females died. There were no apparent treatment-related effects upon body weight or feed consumption among adults at concentrations of 500 and 2500 mg a.s./kg food. However, a trend towards lower bodyweight gain with increasing dose was observed, and at 7500 mg a.s./kg food, there was a slight reduction in feed consumption and a significant effect on body weight among hens. Although some statistical differences were noted, there was no clear treatment-related effect on the body weight of hatchlings or of 14-day old survivors at any test concentration. When compared to the control group, there appeared to be a slight reduction in the number of eggs laid at both 2500 and 7500 mg a.s./kg food. This was reflected in the number of surviving hatchlings and 14-day old survivors as a percentage of the maximum number of eggs set. There were no statistical effects on cracked eggs, viable embryos, live 3-week embryos, hatchability or survivors to 14 days of age. The results for the effects on adult birds and the main reproductive parameters are presented in Tables 9.1.3.e to 9.1.3.h.

Table 9.1.3.e. Subchronic and reproductive toxicity of tolclofos-methyl to bobwhite quail: Cumulative mortality of adult birds

Treatment		Week of study																				
(mg as/kg food)		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	Total
Control	Males	0	0	0	0	0	0	0	0	0	0	1[a]	1	1	1	1	1	1	1	1	1	1	1/15[b]
	Females	0	0	0	0	0	0	0	0	0	0	1	1	1	1	2	2	2	2	3	3	3	3/17[b]
500	Males	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0/16
	Females	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0/16
2500	Males	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0/16
	Females	0	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	2	2	2	2	2/16
7500	Males	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0/16
	Females	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0/16

a: One male was sacrificed following pen mate mortality.
b: While phenotypically a cock, and treated as such during the study, one bird from the control group was found to be a hen at necropsy. The total number of cocks was therefore 15, not 16 and the total number of females was 17, not 16.

Table 9.1.3.f. Subchronic and reproductive toxicity of tolclofos-methyl to bobwhite quail: Group mean bodyweight changes

Treatment		Mean body weight change (g)						
(mg a.s./kg food)		Weeks 0 to 2	Weeks 2 to 4	Weeks 4 to 6	Weeks 6 to 7	Weeks 7 to 8	Week 8 to Termination	Total Change
Control	Males	-3	-3	12	3	0	6	15
	Females	-4	-2	10	1	-1	38	42
500	Males	-2	0	7	1	1	8	16
	Females	-3	-3	7	1	1	32	35
2500	Males	-3	-2	9	1	4	1	10
	Females	0	-2	9	2	3	25	36
7500	Males	-8	-3	12	-5	6	7	8
	Females	-6	0	11	-5	4*	27	32

* Statistically different from control (ANOVA and Dunnet's test, $p<0.05$)

Table 9.1.3.g. Subchronic and reproductive toxicity of tolclofos-methyl to bobwhite quail: Group mean food consumption (g)

Treatment (mg as/kg food)	Week of study																				
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
Control	17	16	15	21	21	20	21	20	16	17	21	25	24	24	23	28	29	27	24	28	32
500	16	17	15	20	20	18	20	18	16	17	18*	21**	22	25	21	24*	26	24	25	27	33
2500	18	17	15	19	22	19	21	20	17	19	19	22*	21	22	23	27	24**	25	24	28	34
7500	14*	15	16	15**	20	19	19	18	17	17	19	21*	20**	25	22	27	25*	24	24	24*	31

* Statistically different from control (ANOVA and Dunnet's test, $p<0.05$)
** Statistically different from control (ANOVA and Dunnet's test, $p<0.01$)

Table 9.1.3.h. Subchronic and reproductive toxicity of tolclofos-methyl to bobwhite quail: Reproductive parameters

	Treatment (mg a.s./kg food)			
	Control	500	2500	7500
Eggs laid	474	584	370	459
Eggs cracked	16	36	13	36
Eggs set	403	477	301	359
Viable Embryos	350	445	279	314
Live 3-Week embryos	346	445	278	307
Hatchlings	336	426	259	284
14-Day old survivors	328	373	227	249
Eggs laid/hen	40	37	26	29
Eggs laid/hen/day	0.63	0.58	0.42	0.46
14-Day old survivors/hen	27	23	16	16
Egg shell thickness	0.228	0.225	0.237	0.229
Body weight (Hatchlings)	7	7	7	7
Body weight (14-day old survivors)	31	28	27*	27*
Eggs laid/Max. laid (%)[1]	68	63	46*	49
Eggs cracked/Eggs laid (%)	7	7	4	9
Viable embryos/Eggs set (%)	89	86	93	87
Live 3-Week embryos/Viable embryos (%)	99	100	100	96
Hatchlings/Live 3-Week embryos (%)	97	96	92	93
14-Day old survivors/Hatchlings (%)	98	87*[a]	88	89*
14-Day old survivors/Eggs set (%)	82	72	76	70
Hatchlings/Max. set (%)	54	51	36	34
14-day old survivors/Max. set (%)	53	45	31*	30*

* Statistically different from control (ANOVA of arcsine transformed data followed by Dunnet's test $p<0.05$)
*[a] Due to a malfunction of a heater
[1] Max laid = maximum number of eggs laid by any one hen in the study

Based on the results of this study the long term no-observed effect concentration (NOEC) was set to 500 mg a.s./kg food.

Comments:

The study was well performed and reported. No signs of regressed ovaries or testes were reported among bobwhites. From the available data on bird body weight and food consumption RMS converted toxicity figures to daily dose as recommended in the Guidance Document on Risk Assessment for Birds and Mammals Under Council Directive 91/414/EEC (SANCO/4145/2000 25 September 2002). For this conversion average group food consumption (expressed as g per bird per day) for the whole exposure period and the average body weight of both sexes over the exposure period was used. Daily dose was then calculated by the formula: daily dose (mg/kg bw/day) = concentration in food (mg/kg) x daily food consumption (g/bird/day)/bw. The resulting values

of daily dose were 49, 255 and 743 mg/kg bw/day and hence the NOEL was set to 49 mg a.s./kg bw. This value was used for the risk assessment

B.9.1.4 Summary of the toxicity studies on birds

The studies indicate that tolclofos-methyl and the formulated plant protection product tested have low acute and short-term dietary toxicity to birds. There were no overt signs of toxicity and all birds were normal in appearance and behaviour throughout the test. No treatment related effects on body weight or feed consumption was detected at any test concentration.

Avian reproduction studies were carried out with mallard duck and bobwhite quail. In the mallard study, there were treatment-related effects on adult survivability and body weight gain. A dose-response increase in number of hens and drakes with regressing or regressed ovaries or testes was observed. There was also a treatment-related reduction in the number of eggs laid and in the number of hatchlings and 14-day old survivors at 2500 and 7500 mg a.s./kg food. In particular, at 7500 mg a.s./kg food, there was a severe treatment related effect upon egg production. In the bobwhite study, there was a slight reduction in feed consumption and an effect on body weight among hens at 7500 mg a.s./kg food. There appeared to be a slight reduction in the number of eggs laid at both 2500 and 7500 mg a.s./kg food, also reflected in the number of surviving hatchlings and 14-day old survivors as a percentage of the maximum number of eggs set, but no effects were apparent on cracked eggs, viable embryos, live 3-week embryos, hatchability and survivors to 14 days of age. No signs of regressed ovaries or testes were reported among bobwhites. A summary of the studies of the toxicity of tolclofos-methyl to birds is presented in Table 9.1.4.a.

Table 9.1.4.a. Summary of the studies on effects on birds treated with tolclofos-methyl.

Species	Test duration	Dose range	Results (Daily dose)	Reference
ACUTE ORAL				
Ring-necked pheasant	Acute oral	0 and 5000 mg as/kg bw	LD_{50} >5000 mg a.s./kg bw	Roberts, N.L., *et al*., 1981a
Mallard duck	Acute oral	0 and 5000 mg as/kg bw	LD_{50} >5000 mg a.s./kg bw	Roberts, N.L., *et al*., 1981b
Bobwhite quail	Acute oral	0 and 5000 mg as/kg bw	LD_{50} >5000 mg a.s./kg bw	Roberts, N.L., *et al*., 1982
Mallard duck	Acute oral (formulation)	0, 7260 and 7554 mg product/kg bw	LD_{50} >7554 mg product/kg bw (>3777 mg a.s./kg bw)	Ross, D.B., *et al.,* 1980a
Bobwhite quail	Acute oral (formulation)	0, 11173 and 11364 mg product/kg bw	LD_{50} >11364 mg product/kg bw (>5682 mg a.s./kg bw)	Ross, D.B., *et al*., 1980b
SHORT TERM DIETARY TOXICITY				
Mallard duck	5 days dietary	0, 562, 1000, 1780, 3160 and 5620 ppm	LC_{50} >5620 ppm (>1624 mg a.s./kg bw/ day)	Beavers, J.B., 1985a
Bobwhite quail	5 days dietary	0, 562, 1000, 1780, 3160 and 5620 ppm	LC_{50} >5620 ppm (>2248 mg a.s./kg bw/ day)	Beavers, J.B., 1985b
REPRODUCTIVE TOXICITY				
Mallard duck	Reproduction, 19 weeks	0, 500, 2500 and 7500 ppm	NOEC 500 ppm (76 mg a.s./kg bw/day)	Beavers, J.B., *et al*., 1987a
Bobwhite quail	Reproduction, 21 weeks	0, 500, 2500 and 7500 ppm	NOEC 500 ppm (49 mg a.s./kg bw/day)	Beavers, J.B., *et al*., 1987b

B 9.1.5 Risk assessment for birds

Notifiers risk assessment

Residue levels of tolclofos-methyl in food items of birds (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. Assuming that there will be no significant exposure to birds from the use of tolclofos-methyl on glasshouse lettuce, the Notifier provided a risk assessment based on daily dose estimated from initial residues on various categories of avian feed items (calculated according to the method of Hoerger and Kenaga (1972)), and the equivalent field rate for potato tuber treatment (625 g a.s./ha). The result is presented in Table 9.5.1.a.

Table 9.1.5.a. Notifiers risk assessment for birds 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	>3777	649	<10
		Short-term	5.82	>1624	>279	<10
		Long-term	5.82	49	8	<5
Small insects	625 (potato tuber dressing)	Acute	5.43	>3777	>696	<10
		Short-term	5.43	>1624	>299	<10
		Long-term	5.43	49	9	<5
Seed, large insects	625 (potato tuber dressing)	Acute	0.51	>3777	>7406	<10
		Short-term	0.51	>1624	>3184	<10
		Long-term	0.51	49	96	<5
Fruits	625 (potato tuber dressing)	Acute	0.24	>3777	>15738	<10
		Short-term	0.24	>1624	>6767	<10
		Long-term	0.24	49	204	<5

[1] *All TER values calculated using worst-case toxicity values and daily intake (30% for 10 g bird)*

All TER values are above the trigger values given in Annex VI of Directive 91/414, hence indicating an acceptable risk for birds.

RMS comments

Since limited exposure of birds is expected from the proposed areas of use, the risk assessment provided by the Notifier is accepted. Tolclofos-methyl is not systemic and residue levels in food items (e.g. insects and vegetation) are expected to be low following the proposed areas of use. There may be a limited concern for birds following the use of tolclofos-methyl as a potato tuber dressing e.g. with cranes feeding on potatoes. It might therefore be necessary to carry out more detailed risk assessments in cases were there are specific local concerns.

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

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 an avian risk assessment, 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) 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_{worm} 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 1.1 (100 g bird eating 113 g per day) = 5.5 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 8.9. Hence, the trigger value of 5 recommended in the Guidance Document on Risk Assessment for Birds and Mammals Under Council Directive 91/414/EEC (SANCO/4145/2000 25 September 2002) is not met. However, since tolclofos-methyl degrades rapidly in soil (DT_{50} = 3.95 days) exposure is not expected to continue for longer periods and secondary poisoning to birds is therefore considered acceptable.

In view of the use pattern of tolclofos-methyl within the EU, including also spray application to wheat, RMS 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. For this assessment see Appendix B at the end of Volume3, Annex B.9.

B.9.2 Effects on aquatic organisms (Annex IIA 8.2; Annex IIIA 10.2)

B.9.2.1 Acute toxicity to fish

ACTIVE INGREDIENT

Reference:	Miyamoto, J.,Takimoto, Y., Kagoshima, M., Ashida, S. (1982) The acute toxicity of S-3349 to rainbow trout (*Salmo gairdneri*)
Guideline:	In-house method
GLP:	No

Material and methods:

Test substance:	Tolclofos-methyl (Batch No.: 10101, Purity: 99.8%) emulsified with 10 parts

	dimethylsulfoxide and 10 parts polyoxyethylene hydrogenated castor oil and diluted with distilled water.
Species:	Rainbow trout (*Oncorhynchus mykiss*; formerly *Salmo gairdneri*)
Treatments:	0, solvent control, 0.05, 0.10, 0.18, 0.32, 0.56, 0.75, 1.00, 1.80 and 3.20 mg/L
Number of animals:	10/treatment
Duration:	96-hours
Test conditions:	Juvenile fish (weight 0.85 ± 0.14 g and length 3.75± 0.24 cm) were used. The test was performed in 20 L glass aquaria under semi-static conditions at a temperature of 15±1°C, a pH of 7.18-7.74 and dissolved oxygen concentration of 7.4-9.5 ppm. The test media was renewed daily. Samples were taken from the new and expired solutions at 0.56mg/L level for analysis. Sodium pentachlorophenolate monohydrate ($PCP{\cdot}Na{\cdot}H_2O$) at 0.056, 0.075, 0.100 and 0.180 ppm was used as a positive control:
Observations:	Mortality, abnormal respiration, lethargy, loss of equilibrium, darkening.

Results:

Measured concentrations at the nominal concentration of 0.56 mg/L ranged from 0.52-0.58 mg/L in fresh media and remained at 71-95% of this nominal value after 24 hours (in the expired media). Symptoms of intoxication for tolclofos-methyl were in the order of occurrence: abnormal respiration, lethargy, loss of equilibrium, darkening and death. At 0.05 mg/L, no adverse effects were observed. The results are presented in Table 9.2.1.a.

Table 9.2.1a. Cumulative mortality data for rainbow trout exposed to tolclofos-methyl for 96 hours

Nominal concentration (mg/l)	Cumulative mortality (initial population: 10)			
	24 hours	48 hours	72 hours	96 hours
Control	0	0	0	0
Solvent Control	0	0	0	0
0.05	0	0	0	0
0.10	0	0	0	0
0.18	0	0	0	0
0.32	0	0	0	0
0.56	0	0	0	0
0.75	0	1	3	3
1.00	3	6	7	7
1.80	5	9	9	9
3.20	9	9	9	9
$PCP{\cdot}Na{\cdot}H_2O$ 0.056	0	0	0	0
$PCP{\cdot}Na{\cdot}H_2O$ 0.075	0	1	1	2
$PCP{\cdot}Na{\cdot}H_2O$ 0.100	4	8	8	9
$PCP{\cdot}Na{\cdot}H_2O$ 0.180	9	10	10	10

Based on the results of this study the acute toxicity (96-hour LC_{50}, probit analysis) of technical tolclofos-methyl to rainbow trout was 0.87 mg/L (95 % confidence limits 0.31- 1.25). The no-observed effect concentration (NOEC) was 0.05 mg/L.

Comments:

Measured concentrations of tolclofos-methyl varied during the test and were only 72 % of the nominal value at 24 hours, which indicates that actual test concentrations were lower than nominal values during the test. Additionally, chemical analysis was only carried out in one of the test concentrations and calculated LC_{50} and

NOEC seem to be based on nominal concentrations. It is not clear from the study report at which concentrations sublethal effects occurred or in which frequencies. The calculated LC_{50}, and NOEC based on nominal values are therefore unreliable and the study is not accepted.

Reference:	McAllister, W.A. (1989) Acute flow-through toxicity of Rizolex® technical to bluegill (*Lepomis macrochirus*)
Test substance:	Tolclofos-methyl (Batch No.: 40810, Purity: 97.2%) dissolved in acetone
Guideline:	Methods for Acute Toxicity Tests with Fish, Macroinvertebrates and Amphibians. U. S. EPA, Ecological Research Series EPA-660/3-75-009 (April, 1975)
GLP:	Yes (Self certification by the laboratory)

Material and methods:

Species:	Bluegill (*Lepomis macrochirus*)
Treatments:	Nominal concentrations of 0, solvent control, 0.046, 0.093, 0.190, 0.370 and 0.740 mg/L
Number of animals:	20/treatment
Duration:	96-hours
Test conditions:	Juvenile fish was used. The test was performed in a flow through system at a temperature of 21-22°C, pH of 7.7-8.0 and dissolved oxygen concentration of 7.3-7.9 mg/L. Samples were taken for analysis of test substance concentration at 0 and 96 hours.
Observations:	Mortality, sublethal behavioural effects

Results:

Mean measured concentrations (0.043, 0.093, 0.179, 0.360 and 0.720 mg/L) ranged from 93-100% of the nominal concentrations. No mortality or sublethal/behavioural responses were observed among the fish in any of the test concentrations. Based on the results from this study a 96-hour LC_{50} for technical tolclofos-methyl to bluegill could not be determined and was therefore concluded to be >0.720 mg/L, the highest concentration tested. The no-observed effect concentration (NOEC) was set to 0.720 mg/L.

Comment:

The concentrations used in the study were too low to enable calculations of LC_{50}. However, since 0.72 mg/L was at compound solubility when the maximum solvent amount allowed was used (0.10 mL/L) the study is accepted for the purpose of risk assessment.

Reference:	Sousa, J.V. (2003) Tolclofos-methyl - Acute toxicity to rainbow trout (*Oncorhynchus mykiss*) under flow-through conditions
Guideline:	FIFRA guideline 72-1, OPPTS draft guideline 850.1075, OECD 203 (17 July 1992), EEC C.1

GLP: Yes (Self certification by the laboratory)

Material and methods:

Test substance: Tolclofos-methyl (Batch No.: 00668G, Purity: 97.8%

Species: Rainbow trout (*Oncorhynchus mykiss*

Treatments: Nominal concentrations of 0, solvent control, 0.21, 0.36, 0.59, 0.99 and 1.6 mg/L

Number of animals: 10/treatment

Duration: 96-hours

Test conditions: Juvenile fish (length: 46-58 mm, weight: 0.80-1.9 g) were exposed to tolclofos-methyl in a flow-through system. The loading rate was 0.36 g of fish/L of solution per day. The test substance was dissolved with acetone and diluted with the dilution water having a pH of 7.8 and total hardness and alkalinity (as $CaCO_3$) of 42 and 33-34 mg/L, respectively. The test solutions were analysed at 0 and 96 hours. All test solution samples were centrifuged prior to extraction and analysis. The test conditions were (range of daily measurements): temperature of 11-13°C; pH 6.5-7.6; dissolved oxygen: 6.5-9.1 mg/L (60-84% saturation). .

Observations: Mortality and sublethal responses after 0, 3, 6, 24, 48, 72 and 96 hours of exposure

Results:

Mean measured concentrations (0.17, 0.25, 0.35, 0.61 and 0.80 mg/L) ranged from 50-82% of the nominal concentrations. The results are presented in Table 9.2.1.b.

Table 9.2.1.b Cumulative mortality data and sublethal responses for rainbow trout exposed to tolclofos-methyl for 96 hours

Mean measured concentration (mg/L)	Cumulative mortality: Observations (initial population: 20)					
	3 hours	6 hours	24 hours	48 hours	72 hours	96 hours
Control	0 : 20N	0 : 20N	0 : 20N	0 : 20N	0 : 20N	0 : 20N
Solvent control	0 : 20N	0 : 20N	0 : 20N	0 : 20N	0 : 20N	0 : 20N
0.17	0 : 20N	0 : 20N	0 : 20N	0 : 20N	0 : 20N	0 : 20N
0.25	0 : 20N	0 : 20N	0 : 20N	0 : 20N	0 : 20N	0 : 20N
0.35	0 : 20N	0 : 20N	0 : 20N	0 : 20N	0 : 20N	0 : 20N
0.61	0 : 20N	0 : 18 N, 2PLC	1 : 14 N, 3PLC,2CLE	3 : 11LC, 6CLE	3 : 4L, 2PLE, 11CLE	4 : 1L, 2PLE, 13CLE
0.80	0 : 20N	3 : 10 N, 3L, 1PLC, 1CLE	6 : 9 L 3PLE, 2CLE	14 : 3L, 3CLE	17 : 3CLE	17 : 3CLE

N= Normal; L=Lethargic; PLC=Partial Loss of Equilibrium; CLE=Complete Loss of Equilibrium

Based on the results of this study the acute toxicity (96-hour LC_{50}) of technical tolclofos-methyl to rainbow trout was 0.69 mg/L, with 95% confidence intervals of 0.64 – 0.74 mg/L. The no-observed effect concentration (NOEC) was 0.35 mg/L.

Comments:

The study was well performed and reported.

METABOLITE

Reference: Dionne, E. (1998a)

	Desmethyl-tolclofosmethyl - Acute toxicity to rainbow trout (*Oncorhynchus mykiss*) under static acute conditions
Guideline:	OECD 203 (17 July 1992) - equivalent to EEC C.1
GLP:	Yes (Self certification by the laboratory)

Material and methods:

Test substance:	DM-TM (Batch No.: CTS98009G; Purity: 98.9%)
Species:	Rainbow trout (*Oncorhynchus mykiss*)
Treatments:	Nominal concentrations of 0, 18, 30, 50, 84 and 140 mg/L
Number of animals:	10/treatment
Duration:	96-hours
Test conditions:	A static test system with glass aquaria (one per test concentration) containing 15 L of test solution was used. The temperature was 13-15°C, pH 6.8-7.3;and dissolved oxygen concentration 6.2-9.4 mg/L (60-91% saturation).
Observations:	Mortality and sublethal responses after 0, 3, 6, 24, 48, 72 and 96 hours of exposure.

Results:

Mean measured concentrations (14, 24, 39, 64 and 110 mg/L) ranged from 77-79% of the nominal concentrations. Following 96 hours of exposure, no mortality or sublethal effects were observed among fish exposed to any of the treatment levels or to the control.

Based on the results from this study a 96-hour LC_{50} for the metabolite DM-TM to rainbow trout could not be determined and was therefore concluded to be >110 mg/L, the highest concentration tested. The no-observed effect concentration (NOEC) was set to 110mg/L.

Comment:

The study was well performed and reported.

PLANT PROTECTION PRODUCT

Reference:	Christopher, D.H., Pell, I.B. (1979) The acute toxicity of S 3349 to bluegill sunfish (*Lepomis macrochirus*) and rainbow trout (*Salmo gairdneri*)
Guideline:	Methods for Acute Toxicity Tests with Fish, Macroinvertebrates and Amphibians. U. S. EPA, Ecological Research Series EPA-660/3-75-009 (April, 1975)
GLP:	No

Material and methods:

Test substance:	Tolclofos-methyl 50WP (Batch No.: 61258, Contents: 50% w/w.
Species:	Bluegill (*Lepomis macrochirus* and rainbow trout (*Oncorhynchus mykiss*)

Treatments:	Nominal concentrations of 34, 67, 125, 250 and 375 mg/L
Number of animals:	20/dose/species
Duration:	96-hours
Test conditions:	**Bluegill:** A static test system with 25 L glass aquaria was used. The temperature was 20.8 - 22.3°C, dissolved oxygen concentration 47-96% of saturation and photoperiod 16 hours light and 8 hours dark. There was no analysis of the test concentrations. Mortality and other observations were made after 24, 48, 72 and 96 hours. **Rainbow trout:** A static test system with 55 L glass aquaria was used. The temperature was 11.3 - 13.0°C, dissolved oxygen concentration 44-98% of saturation and photoperiod 16 hours light and 8 hours dark. There was no analysis of the test concentrations.
Observations:	Mortality and sublethal observations were made after 24, 48, 72 and 96 hours.

Results:

Bluegill exhibited respiratory distress and excess mucus production from the gill area (numbers and concentrations not specified). Rainbow trout exhibited respiratory distress, loss of equilibrium torpor and excess mucus production. In two fishes at 125 mg/L and two fishes at 67 mg/L a marked bilateral exophthalmos was seen after 72 hours exposure. Results on mortality are presented in Table 9.2.1.c (Bluegill sunfish) and Table 9.2.1.d (Rainbow trout).

Table 9.2.1.c. Cumulative mortality data for bluegill exposed to tolclofos-methyl 50WP for 96 hours

Nominal Concentration	Cumulative mortality (initial population: 20)			
(mg/L)	24 hours	48 hours	72 hours	96 hours
Control	0	0	0	0
34	0	0	2	3
67	0	2	4	5
125	2	5	7	8
250	3	8	11	15
375	7	12	18	20

Table 9.2.1.d. Cumulative mortality data for rainbow trout exposed to tolclofos-methyl 50WP for 96 hours

Nominal Concentration	Cumulative mortality (initial population: 20)			
(mg/L)	24 hours	48 hours	72 hours	96 hours
Control	0	0	0	0
34	2	3	3	7
67	5	8	9	11
125	4	12	6	17
250	6	19	19	20
375	20	20	20	20

Based on the results of this study the acute toxicity (96-hour LC_{50}) of Tolclofos-methyl 50WP to bluegill was 118 mg product/L (95% confidence limits: 90 - 156), equivalent to 59 mg a.s./L based on 50% a.s. content. The no-observed effect concentration (NOEC) was <34 mg product/L (<17 mg a.s./L).

The acute toxicity (96-hour LC_{50}) of Tolclofos-methyl 50WP to rainbow trout was 52 mg product/L (95% confidence limits: 34 - 78), equivalent to 26 mg a.s./L based on 50% a.s. content.

The no-observed effect concentration (NOEC), based on nominal values, was <34 mg product/L (<17 mg a.s. /L).

Comments:

Since no chemical analysis of test concentrations were performed the actual concentration of a.s. is not known and the validity of the test is questionable. However, since the nominal concentrations were much higher than the median lethal levels seen in the studies on the active ingredient it can be concluded that the formulation is less toxic than the active ingredient. Mortality occurred at the lowest concentration tested for both species and thus a NOEC could not be obtained.

Reference:	Sayers, L.E. (2003c) Tolclofos-methyl 50WP - Acute toxicity to rainbow trout (*Oncorhynchus mykiss*) under static-renewal conditions
Guideline:	OECD 203 (17 July 1992), EEC C.1
GLP:	Yes (Self certification by the laboratory)

Material and methods:

Test substance:	Tolclofos-methyl 50WP (Batch No.: B2190010, Contents: 51.03% w/w
Species:	Rainbow trout (*Oncorhynchus mykiss*)
Treatments:	Nominal concentrations of 0.96, 1.9, 3.8, 7.6, 15 and 30 mga.s./L
Number of animals:	10/dose
Duration:	96-hours
Test conditions:	Juvenile fish (total length: 46-58 mm, weight: 0.80-1.9 g) were exposed in a static-renewal system (renewal at 24, 48 and 72 hours of exposure). The test was conducted in 20.8 L glass aquaria (one per test concentration) containing 15 L of test solution. The test substance was dosed directly into the water (no solvent required), having a pH of 7.4 and total hardness and alkalinity (as $CaCO_3$) of 42 and 33 mg/L, respectively. There was a dilution water control. The test solutions were analysed at 0 (new solutions), at 24, 48, 72 (aged and new solutions) and at 96 hours (aged solutions). The temperature was 12-15°C, pH 6.6-7.3 and dissolved oxygen 5.3-9.8 mg/L (51-95% saturation) (range of daily measurements).
Observations:	Mortality and sublethal observations were made after 0, 3, 6, 24, 48, 72 and 96 hours of exposure.

Results:

Mean measured concentrations (0.62, 1.1, 2.2, 4.6, 9.0 and 20 mg a.s./L) ranged from 58-68% of the nominal concentrations. Undissolved test material was present at 2.2 mg a.s./Land all higher concentrations during the study. The results are presented in Table 9.2.1.e.

Table 9.2.1.e. Cumulative mortality data and sublethal responses for rainbow trout exposed to tolclofos-methyl 50WP for 96 hours

Mean measured concentration (mg a.s./L)	Cumulative mortality: Observations (initial population: 10)					
	3 hours	6 hours	24 hours	48 hours	72 hours	96 hours
Control	0 : 10N	0 : 10N	0 : 10N	0 : 10N	0 : 10N	0 : 10N
0.62	0 : 10N	0 : 10N	0 : 10N	0 : 9N, 1CLE	0 : 10N	0 : 10N
1.1	0 : 10N	0 : 10N	0 : 10N	0 : 9N, 1CLE	0 : 8N, 2CLE	0 : 8N, 2CLE
2.2	0 : 10N	0 : 10N	0 : 10N	0 : 10N	0 : 8N, 1PLE, 1CLE	0 : 8N, 1PLE, 1CLE
4.6	0 : 10N	0 : 10N	1 : 9N	1 : 7N, 2OB	1 : 3N, 2PLE, 4CLE	1 : 2N, 3PLE, 4CLE
9.0	0 : 10N	0 : 10N	1: 7N, 1PLE, 1OB	3 : 4N, 3OB	3 : N, 2OB, 3PLE, 2CLE	3 : 2OB, 1PLE, 4CLE
20	0 : 10N	0 : 10N	0 : 2OB, 2LC, 1PLE, 1CLE	3 : 3N, 2OB, 2CLE	3: 2PLE, 5CLE	3 : 7CLE

N= Normal; OB=On the Bottom; L=Lethargic; PLC=Partial Loss of Equilibrium; CLE=Complete Loss of Equilibrium

Based on the results of this study the acute toxicity (96-hour LC_{50}) of tolclofos-methyl 50WP to rainbow trout was >20 mg a.s./L. The no-observed effect concentration (NOEC) was 0.62 mg a.s./L

Comments:

The study was well performed and reported.

Reference: Sayers, L.E. (2003d)
Tolclofos-methyl 50WP - Acute toxicity bluegill sunfish (*Lepomis macrochirus*) under static-renewal conditions

Guideline: OECD 203 (17 July 1992), EEC C.1

GLP: Yes (Self certification by the laboratory)

Material and methods:

Test substance: Tolclofos-methyl 50WP (Batch No.: B2190010, Contents: 51.03% w/w

Species: Bluegill (*Lepomis macrochirus*)

Treatments: Nominal concentrations of 1.9, 4.4, 9.6, 21.1, 46.4 and 102 mg a.s./L

Number of animals: 10/dose

Duration: 96-hours

Test conditions: The fish had a total length of 40-55 mm and a weight of 0.87-2.4 g. The test was conducted in 20.8 L glass aquaria (one per test concentration) containing 15 L of test solution. The test substance was dosed directly into the water (no solvent required), having a pH of 7.3-7.5 and total hardness and alkalinity as $CaCO_3$ of 42 and 33-34 mg/L, respectively. There was a dilution water control. The test solutions were analysed at 0 (new solutions), at 24, 48, 72 (aged and new solutions) and at 96 hours (aged solutions). Temperature was 20-23°C, pH 6.7-7.7 and dissolved oxygen 5.6-8.6 mg/L (64-99% saturation) (range of daily measurements).

Observations: Mortality and sublethal observations were made after 0, 3, 6, 24, 48, 72 and 96 hours exposure.

Results:

Mean measured concentrations (1.2, 2.5, 6.2, 13, 27 and 54 mg a.s./L) ranged from 53-65% of the nominal concentrations. Undissolved test material was present at 6.2 mg a.s./L and all higher concentrations during the study. The results are presented in Table 9.2.1.f.

Table 9.2.1.f. Cumulative mortality data and sublethal responses for bluegill exposed to tolclofos-methyl 50WP for 96 hours

Mean measured concentration (mg a.s./l)	Cumulative mortality: Observations (initial population: 10)					
	3 hours	6 hours	24 hours	48 hours	72 hours	96 hours
Control	0 : 10N	0 : 10N	0 : 10N	0 : 10N	0 : 10N	0 : 10N
1.2	0 : 10N	0 : 10N	0 : 10N	0 : 10N	0 : 10N	0 : 10N
2.5	0 : 10N	0 : 10N	0 : 10N	0 : 10N	0 : 10N	0 : 10N
6.2	0 : 10N	0 : 10N	0 : 10N	0 : 10N	0 : 10N	1 : 9N
13	0 : 10N	0 : 10N	0 : 10N	0 : 10N	1 : 9N	1 : 9N
27*	0 : 10N	0 : 10N	0 : 10N	0 : 10N	0 : 10N	0 : 10N
54*	0 : 10N	0 : 10N	0 : 10N	1 : 9N	2 : 8N	2 : 8N

* test media were very cloudy, making the fish difficult to see, N=Normal

Based on the results of this study the acute toxicity (96-hour LC_{50}) of tolclofos-methyl 50WP to bluegill was >54 mg a.s./L. The no-observed effect concentration (NOEC) was 27 mg a.s./L.

Comments:

The study was well performed and reported.

B.9.2.2 Fish early life stage toxicity test

Reference:	Cohle, P. (1991) Early life stage toxicity of Rizolex® technical to rainbow trout (*Oncorhynchus mykiss*) in a flow-through system
Guideline:	U. S. EPA. Proposed Recommended Bioassay Procedure for Egg and Fry Stages of Freshwater Fish. Unpublished manuscript (January 1972) American Society for Testing and Materials. Proposed New Standard Practice for Conducting Fish Early Life Stages Toxicity Tests. Draft No. 7, December 1983, ASTM Committee E-47.01.
GLP:	Yes (Self certification by the laboratory)

Material and methods:

Test substance:	Tolclofos-methyl (Batch No.: 40810, Purity: 97.6%) dissolved in dimethylformamide (max. conc. 0.0125 mL/L).
Species:	Rainbow trout (*Oncorhynchus mykiss*)
Treatments:	Nominal concentrations of 0, solvent control, 0.013, 0.025, 0.050, 0.100 and 0.200 mg/L
Number of animals:	4 replicates of 35 eggs (fertilised < 4 hours before study initiation) per treatment

Duration: 97 days

Test conditions: A flow-through system (flow rate approx. 92 L/day/replicate) with glass chambers of 11.7 L was used. The temperature was 8.6-11.2°C, pH 8.0-8.7 and dissolved oxygen concentration 8.1-10.8 mg/L. Biomass loading levels ranged from 0.00231 to 0.198 g/L and eggs were shielded from excess light. At 8 days post-hatch a 16 hour light/8 hour dark photoperiod was applied. On day 40, a reduction to 15 fry per replicate chamber was made. The fry were initially (from day 53) fed live brine shrimp nauplii (*Artemia*) and salmon starter mash, as the fry grew the mash was replaced by salmon starter in the small pellet form. Test solutions were analysed weekly.

Observations: Egg viability, time to hatch, hatchability, time to swim-up, morphological and behavioural abnormalities, fry survival and fry growth (length and weight). The standard length of the fry was determined by the photographic method on 37 days post-hatch. At termination (Day 97, 60 days post-hatch), standard length and wet weight of the fry were measured.

Results:

Mean measured concentrations (0.012, 0.028, 0.053, 0.110 and 0.200 mg/L ranged from 92-112% of the nominal concentrations. The results on hatchability, fry survival and length from the study are presented in Table 9.2.2a.

There did not appear to be any dose-related delay with regard to time to hatch. Swim-up progressed somewhat slower at test concentrations of 0.028 and 0.053 mg/L, but only at concentrations of 0.110 and 0.200 mg/L did there appear to be a biologically significant delay in swim-up. The incidence of sublethal abnormalities (e.g. dark discoloration, swimming vertically in chamber and surfacing) was much greater in the two highest test concentrations and was considered to be dose-related.

Table 9.2.2.a. Egg hatchability, fry survival, standard length and wet weight of rainbow trout (*Oncorhynchus mykiss*) exposed to tolclofos-methyl during the early life stage toxicity test

Mean Measured Concentration (mg/l)	Egg Hatchability (%)	37-Day Post-Hatch		60-Day Post-Hatch		
		Survival (%)	Standard length (mm)	Survival (%)	Standard length (mm)	Wet weight (g)
Control	100	92	33.0	92	46.6	1.677
Vehicle Blank	100	100	32.3	100	45.1	1.541
0.012	99	98	31.6	98	44.0	1.440
0.028	99	97	30.9*[2]	95	43.2	1.359*[2]
0.053	99	100	29.2*[2]	100	39.0*[2]	1.036*[2]
0.110	99	90	25.2*[2]	85*[1]	31.3[a]	0.511[a]
0.200	100	18*[1]	20.5[a]	17*[1]	25.2[a]	0.274[a]

a: Data not included in growth analysis since a survival effect had been determined at this level.
*[1]: Statistically significant reduction compared to Vehicle Blank (Fisher's exact test, $P \leq 0.05$).
*[2]: Statistically significant reduction compared to Vehicle Blank (Nested ANOVA and Dunnett's test, $P \leq 0.05$).

Based on the observed effects on growth, the 97-day no-observed effect concentration (NOEC) for technical tolclofos-methyl to the early life-stages of rainbow trout was 0.012 mg/L. The lowest-observed effect concentration (LOEC) was 0.028 mg/L.

Comments:

The study was well performed and reported.

B.9.2.3 Bioconcentration in fish

Reference:	**In-life phase** Forbis, A.D., Bunch, B. (1986) Uptake, depuration and bioconcentration of ^{14}C-Rizolex by bluegill sunfish (*Lepomis macrochirus*) **Analytical phase** Yu, C.C., Guirguis, A.S. (1986) Metabolism of tolclofos-methyl in bluegill sunfish (*Lepomis macrochirus*) **BIOFAC calculation** 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
Guideline:	U. S. EPA, 1979. Toxic Substances Control, Discussion of Premanufacture Testing Policy and Technical Issues; Request for Comment. Federal Register, Vol. 44, No. 53, March 16, 1979
GLP:	Yes (Self certification by the laboratory)

Material and methods:

Test substance:	[Phenyl-^{14}C]tolclofos-methyl (Batch No.: 1-C-59-2, Radiochemical purity: >99%, Specific radioactivity: 1.31 GBq/mmol). Non-labelled: Batch no.: 40810, purity: 97.7%
Species:	Bluegill sunfish (*Lepomis macrochirus*)
Treatments:	Nominal concentrations of 0 (acetone solvent control) and 0.030 mg/L
Number of animals:	120 fish/concentration
Duration:	28 days of exposure followed by 14 days of depuration
Test conditions:	A flow-through system with glass aquaria containing 70 L was used. Flow rate was 350 to 420 mL/minute/aquarium resulting in replacement of the test volume 7.2 to 8.6 times per 24-hour period. The fish was acclimatized during a 14-days period to test conditions of 16 hours light and 8 hours dark, a temperature of 22 ± 2°C, pH of 8.0-8.2 and dissolved oxygen concentration of 7.0-8.8 mg/L. Fish initial mean weight was 3.4 ± 0.92 g and initial mean standard length was 47 ± 3.7mm. The fish were fed commercial fish food daily equivalent to 3 % of their initial body weight.
Analysis:	Water and fish samples were taken at 0 (immediately prior to adding the fish), 0.17, 1, 3, 7, 14, 21 and 28 days of the exposure phase and 1, 3, 7, 10 and 14 days of the depuration phase. A 500 ml sample of the test water was taken to measure the concentrations of radiocarbon and tolclofos-methyl in water.

On the sampling dates, three fish were collected from both the control and treated aquaria. The control and treated fish were dissected into fillet/edible (body, muscle, skin and skeleton) and viscera/non-edible (fins, head and internal organs). Three additional fish from each aquarium were used for whole fish analysis. The weights of dissected parts and whole fish were measured. In addition, supplemental fish were taken for metabolite identification on certain sampling dates.

For calculation of the BCF (bioconcentration factor) and ^{14}C concentration (ppm) in fish, individual samples were homogenized with dry ice in a grinder and aliquots were subjected to combustion analysis for radioassay. The uptake rate constant (K_1) and depuration rate constant (K_2) were determined by the BIOFAC computer program.

For metabolite identification, 5 g of each treated samples (21- and 28-day viscera, 21- and 28-day fillet) and 5 g of each control sample were separately homogenized with distilled water using a homogeniser. The homogenate was adjusted to pH 1 and extracted with diethyl ether. The remaining aqueous and solid fractions were refluxed for 1 hr and extracted with ether. Each extract was radioassayed. The remaining solid was combusted to measure unextractable radioactivity. Extractable radioactive substances were identified by TLC and GC co-chromatography.

Results:

The ^{14}C-radioactivity calculated as mg/L of ^{14}C-tolclofos-methyl in test water during the 28-day exposure period averaged 0.027± 0.0079) mg/L. During the depuration period, the concentration of radioactivity was negligible. Behavioural observations during the study indicated no adverse effects on control and treated fish. Two treated fish died on day 12 of the study. These deaths were considered incidental.

Under flow-through conditions at a nominal concentration of 0.030 mg/L, a steady state concentration was attained approximately 7 days after exposure. The BCF values at 28 days of exposure for fillet, viscera and whole fish were 100, 1300 and 670, respectively, Table 9.2.3.a.

Table 9.2.3.a. Concentration of radioactivity (tolclofos-methyl equivalents) in fish and BCF values calculated for fillet, viscera and whole fish (pools of 3 fish per sample) based on fresh weight.

Exposure (days)		Concentration of radioactivity					
		Fillet		Viscera		Whole fish	
		ppm	BCF	ppm	BCF	ppm	BCF
Exposure	0.17	0.94	39	4.9	200	3.1	130
	1	1.3	53	14	570	7.6	310
	3	1.8	69	28	1100	16	610
	7	3.2	120	30	1100	20	750
	14	1.1	45	25	1000	12	490
	21	2.8	110	31	1200	20	770
	28	2.7	100	36	1300	18	670
Depuration	1	0.87		25		13	
	3	0.14		0.90		0.93	
	7	0.058		0.28		0.22	
	10	0.057		0.28		0.15	
	14	0.043		0.28		0.18	

The clearance times (CT_{50}, CT_{90} and CT_{95}) were also calculated using a non-linear two-compartment kinetic modelling computer program (BIOFAC). The results of these calculations are shown in Table 9.2.3.b. The Uptake Rate Constant $K_{1,}$ and the Clearance Rate Constant $K_{2,}$ for whole fish were 410 day^{-1} and 0.63 day^{-1}, respectively.

Table 9.2.3.b. The clearance time (CT_{50}, CT_{90} and CT_{95}) of radioactivity in fish during depuration phase

	Clearance time (days)		
	Fillet	Viscera	Whole fish
CT_{50}	0.66	1.1	1.1
CT_{90}	2.2	3.7	3.6
CT_{95}	2.9	4.8	4.7

The distribution of metabolites in fish is shown in Table 9.2.3.c. The majority of the accumulated radioactivity was tolclofos-methyl in both fillet (79 to 81%) and viscera (39 to 46%). Of the metabolites TMO and ph-COOH (fillet) and TM-CHO, ph-COOH, TMO and ph-CH_2OH (viscera) were detected at levels greater than 5% of total radioactive residue in tissue. Additionally, one unknown metabolite was detected at levels of approximately 6 % in viscera. Tolclofos-methyl in fish was metabolized by oxidation of the para-methyl group (35-39% of total radiocarbon in the viscera and 4 to 8% in the fillet). Another pathway was via cleavage of the P-O-aryl linkage to form phenolic metabolites (4 to 8% in the fillet and 12 to 27% in the viscera). Of the total radiocarbon in tissues, the desulfuration reaction and the formation of an oxon analogue accounted for 4 to 5% in the viscera and 1 to 9% in the fillet. The chemical name and abbreviation of the metabolites are shown in Table 9.2.3.d. The proposed metabolic pathways of tolclofos-methyl are shown in Figure 9.2.3.a.

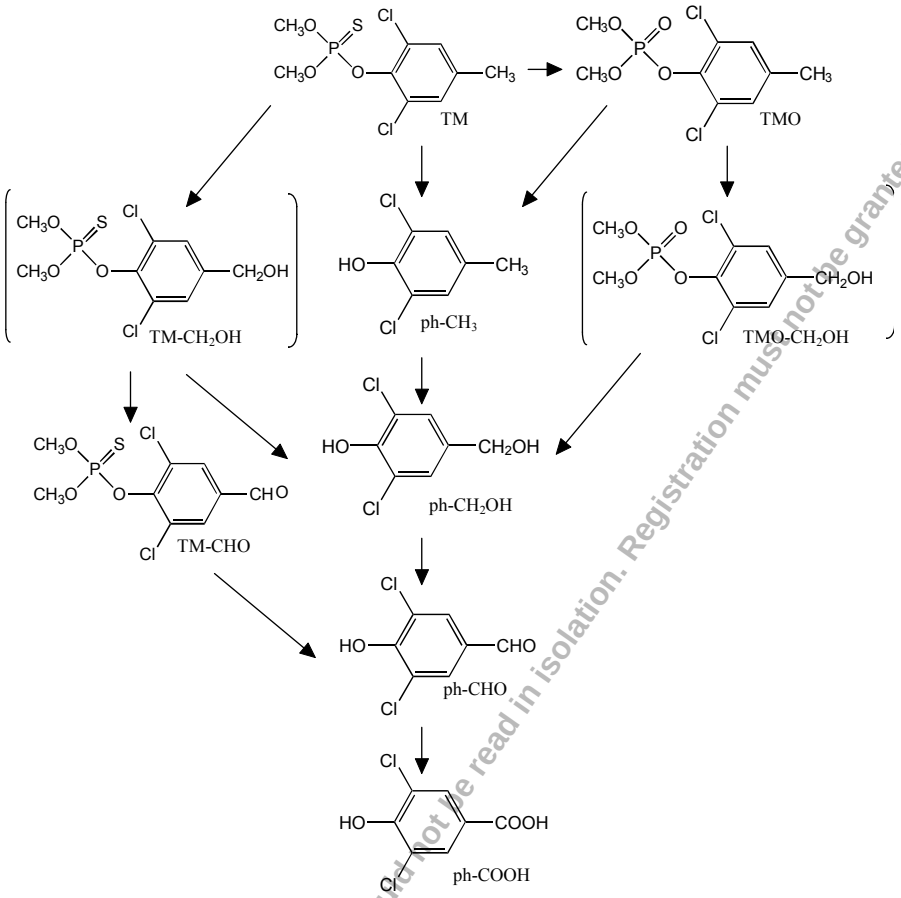
Table 9.2.3.c. Metabolites and percentage of total radioactivity in fillet and viscera

	Percentage of total radioactive residue in tissue			
	Fillet		Viscera	
	Day 21	**Day 28**	**Day 21**	**Day 28**
TM (parent compound)	79.14	81.17	45.85	38.79
TM-CHO	0.00	0.00	22.37	12.30
ph-CHO	0.00	1.35	2.78	2.23
ph-COOH	6.66	1.95	3.81	18.76
TMO	9.32	1.01	4.06	5.20
ph-CH_2OH	1.82	0.83	5.84	6.16
Unknown-1	0.42	1.54	6.07	5.51
Unknown-2	0.64	0.97	0.43	0.84
Unknown-3	1.82	2.15	0.29	0.94
Unknown-4	0.00	0.00	0.32	1.00
Unknown-5	1.35	0.71	2.81	3.65
Unknown-6	2.33	1.89	2.16	3.13
Unknown-7	1.14	2.62	1.99	1.92
Unextractable	5.01	7.25	6.40	5.60

Table 9.2.3.d. Identity of metabolites

Designation	**Chemical name**
TM (parent compound)	*O,O*-dimethyl *O*-(2,6-dichloro-4-methylphenyl) phosphorothioate
TMO	*O,O*-dimethyl *O*-(2,6-dichloro-4-methylphenyl) phosphate
TM-CHO	*O,O*-dimethyl *O*-(2,6-dichloro-4-formylphenyl)phosphorothioate
ph-CHO	3,5-dichloro-4-hydroxybenzaldehyde
ph-COOH	3,5-dichloro-4-hydroxybenzoic acid
ph-CH_2OH	3,5-dichloro-4-hydroxybenzyl alcohol

Figure 9.2.3.a. Proposed metabolic pathway of tolclofos-methyl in fish



Comments:

The study was performed with only one test concentration, which also was comparatively high (3.4% of LC_{50}). According to the Directive 91/414/EEC bioconcentration studies should be performed in accordance with OECD guideline 305, which states that at least two concentrations should be tested, and that the highest concentration should be 1% of LC_{50}. Measured concentration in water at day 14 was only 0.012 mg/L (45 % of mean concentration for the whole exposure period), which also resulted in a lower concentration in fish the same day. No measurements of TOC were performed. Since TOC might significantly influence BCF of lipophilic compounds, this is a drawback of the study. Furthermore, BCF for compounds with log P_{ow} >3 should preferable be expressed in relation to lipid content in addition to whole body weight.

For the proposed uses, as potato tuber dresser and for glasshouse lettuce, the risk of contamination of the aquatic environment is considered to be low based to the low mobility and relatively rapid degradation and the study is therefore accepted as an indication of the BCF. However for extended uses a new study in accordance with the OECD guideline is required.

B.9.2.4 Acute toxicity to aquatic invertebrates: *Daphnia magna*

ACTIVE INGREDIENT

Reference: Murrell, H. (1994)
Acute toxicity of Rizolex to *Daphnia magna*
Guideline: EPA FIFRA, 40 CFR, Part 158.145, Guideline 72-2
GLP: Yes (Self certification by the laboratory)

Material and methods:

Test substance: Tolclofos-methyl (Batch No.: 30909G, Purity: 98.8%
Species: *Daphnia magna*
Treatments: Nominal concentrations of 0, solvent control, 15, 24, 38, 62 and 100 mg/L emulsified with dimethylformamide and HCO-40 (1 g/L test solution of each)
Number of animals: 2 replicates of 10 animals per concentration
Duration: 48 hours
Test conditions: The test was performed in a static system with 250 mL glass beakers containing 200 mL test volume at a 16-hour daylight photoperiod. Temperature was 20-21°C, pH 7.9-8.1 and dissolved oxygen 6.3-8.4 mg/L.
Observations: Immobility and other abnormal effects at 24 and 48 hours

Results:

Mean measured concentrations (8.4, 17, 26, 41 and 64 mg/L) ranged from 56-71% of the nominal values. Individual immobility and behavioural observations are presented in Table 9.2.4.a. Based on the results of this study the acute toxicity (48-hour EC_{50}) of technical tolclofos-methyl to *Daphnia magna* was 48 mg/L. The no-observed effect concentration (NOEC) was 17 mg/L.

Table 9.2.4.a. Immobility and behavioural observations during the acute toxicity test of tolclofos-methyl to *Daphnia magna*

Mean measured concentration (mg/L)	Cumulative immobility: Observations (initial population: 20)	
	24 hours	48 hours
Control	0 : 20 Normal	0 : 20 Normal
Solvent control	0 : 20 Normal	0 : 20 Normal
8.4	0 : 20 Normal	0 : 20 Normal
17	0 : 20 Normal	0 : 20 Normal
26	0 : 20 Normal	0 : 7 Normal, 13 On bottom
41	0 : 13 Normal, 7 On bottom	5 : 2 Normal, 13 On bottom
64	0 : 15 Normal, 5 On bottom	18 : 2 On bottom

Comments:

Measured test concentrations were only 56-71% of the nominal values. All calculations were however based on mean measured test concentrations and the test is therefore accepted. No confidence limits were reported for the EC_{50}value. Otherwise the study was well performed and reported.

METABOLITE

Reference: Dionne, E. (1998b)
Desmethyl-tolclofosmethyl - Acute toxicity to daphnids (*Daphnia magna*) under

	static conditions
Guideline:	OECD 202 (4 April 1984) - equivalent to EEC C.2
GLP:	Yes (Self certification by the laboratory)

Material and methods:

Test substance:	DM-TM (Batch No.: CTS98009G; Purity: 98.9%)
Species:	*Daphnia magna*
Treatments:	Nominal concentrations of 0, 16, 27, 45, 75 and 125 mg/L
Number of animals:	4 replicates of 5 animals per test concentration
Duration:	48 hours
Test conditions:	The test was performed in a static system with 250 mL glass beakers containing 200 mL test volume at a 16-hour daylight photoperiod. Temperature was 20-21°C, pH 8.0-8.2, dissolved oxygen 8.1-9.5 mg/L (89-105% saturation), total hardness and alkalinity as $CaCO_3$ in dilution water 170 and 120 mg/L, respectively.
Observations:	Immobility and other abnormal effects at 24 and 48 hours

Results:

Mean measured concentrations (12, 20, 34, 50 and 95 mg/L) ranged from 67-76% of the nominal values.
Individual immobility and behavioural observations are presented in Table 9.2.4.b.

Table 9.2.4.b. Immobility and behavioural observations during the acute toxicity test of DM-TM to *Daphnia magna*

Mean measured concentration (mg/L)	Cumulative immobility: Observations (initial population: 20) 24 hours	48 hours
Control	0 : 20 Normal	0 : 20 Normal
12	0 : 20 Normal	0 : 20 Normal
20	0 : 20 Normal	0 : 20 Normal
34	0 : 20 Normal	0 : 20 Normal
50	0 : 20 Normal	0 : 20 Normal
95	0 : 20 Normal	0 : 20 Normal

Based on the results of this study a 48-hour EC_{50} of DM-TM to *Daphnia magna* could not be obtained and was therefore concluded to be >95 mg/L.
The no-observed effect concentration (NOEC) was set to 95 mg/L, the highest concentration tested.

Comments:

According to the test guideline (OECD 202) a maximum test concentration of 1 g/L can be used. Even though a lower concentration was used in the study, it can be concluded that the metabolite is less toxic than the parent compound. Hence, for the purpose of risk assessment the results can be used and the study is accepted.

PLANT PROTECTION PRODUCT

Reference: Christopher, D.H., Fraser, W.D. (1978)
The toxicity of S 3349 to *Daphnia magna*, Straus

Guideline:	AFNOR protocol (I'Association Francaise de Normalisation) (May, 1977)
GLP:	Yes (Self certification by the laboratory)

Material and methods:

Test substance:	Tolclofos-methyl 50WP (Batch No.: 61258, Contents: 50% (w/w)
Species:	*Daphnia magna*
Treatments:	Nominal concentrations of 1, 2, 4, 8, 16, 32, 64, 128 and 256 mg/L
Number of animals:	2 replicates of 10 animals per test concentration
Duration:	24 hours
Test conditions:	Static system; 300 mL glass beakers containing 200 mL test volume; 21±1°C; pH 8.0; dissolved oxygen: 69%; 16-hour daylight photoperiod. Potassium dichromate was used (0.45, 0.68, 1.00, 1.50 and 2.25 mg/L) to test the sensitivity of the *Daphnia* strain
Observations:	Immobility and other abnormal effects at 24 hours

Results:

The results are presented in Table 9.2.4.c. The 24-hour EC_{50} (immobilisation) for *Daphnia* obtained with potassium dichromate was 1.2 mg/L (95% confidence limits: 0.8 - 1.6), which is the mean value for the acceptable limits of sensitivity.

Table 9.2.4.c. Percentage of *Daphnia* immobilised after exposure for 24 hours to Tolclofos-methyl 50WP

Nominal Concentration (mg/l)	**% of immobilised *Daphnia* (24 hours)**		
	Replicate 1	Replicate 2	Mean*
Control	10	0	5
1	20	20	20
2	20	30	25
4	50	40	45
8	30	20	25
16	60	30	45
32	60	50	55
64	70	60	65
128	90	90	90
256	100	100	100

* Mean of two replicates (n = 10)

Based on the results of this study the acute toxicity (24-hour EC_{50}) of Tolclofos-methyl 50WP to *Daphnia magna* was 10.3 mg product/L (95% confidence limits: 4.8 - 22.2), equivalent to 5.2 mg a.s./L based on 50% a.s. content. The no-observed effect concentration (NOEC) was <1 mg product/L (< 0.5 mg a.s./L).

Comments:

Calculated EC_{50} and NOEC were based on nominal values. Since the concentrations of test solutions were not analysed the calculated EC_{50} is not reliable. The test was only run for 24 hours and not for 48 hours as recommended. Since the results indicate that the formulated product is more toxic to *Daphnids* than the a.i. a new study is required in order to determine a correct EC_{50}.

Reference:	Sayers, L.E. (2003b) Tolclofos-methyl 50WP - Acute toxicity to water fleas, *Daphnia magna*, under static conditions.
Guideline:	OECD 202 (4 April 1984), EEC C.2
GLP:	Yes (Self certification by the laboratory)

Material and methods:

Test substance:	Tolclofos-methyl 50WP (Batch No.: B2190010, Contents: 51.03% (w/w)
Species:	*Daphnia magna*
Treatments:	Nominal concentrations of 1.9, 4.4, 9.6, 21.1, 46.4 and 102 mg a.s./L
Number of animals:	4 replicates of 5 animals per test concentration
Duration:	48 hours
Test conditions:	The test was conducted in a static system with 250 mL glass beakers containing 200 mL of test solution. Animals were not fed during the exposure. The test substance was dosed directly into the water (no solvent required), with the dilution water having a pH of 8.0 and total hardness and alkalinity (as $CaCO_3$) of 160 and 110 mg/L, respectively. There was a dilution water only control. The test solutions were analysed at 0 and 48 hours. Test conditions: temperature of 20-21°C; pH 7.9-8.2; dissolved oxygen: 8.0-8.6 mg/L (90.6-97.4% saturation) (range of daily measurements).
Observations:	Immobility and other abnormal effects at 24 and 48 hours.

Results:

Mean measured concentrations (1.2, 2.4, 8.2, 18, 38 and 91 mg a.s./L) ranged from 54-89% of the nominal values. Undissolved test material was observed at all treatment levels. The results are presented in Table 9.2.4.d.

Table 9.2.4.d. Immobility observations during the acute toxicity test of tolclofos-methyl 50WP to *Daphnia magna*

Mean measured concentration (mg a.s./L)	**Cumulative immobility: Observations (initial population: 20)**	
	24 hours	**48 hours**
Control	0 : 20N	0 : 20N
1.2	0 : 20N	0 : 20N
2.4	0 : 20N	0 : 20N
8.2	0 : 20N	1 : 19N
18	0 : 20N	0 : 20OB
38	4 : 16N	16 : 4OB
91	18 : 2N	20 : 0

N=Normal; OB= On the Bottom

Based on the results of this study the acute toxicity (48-hour EC_{50}) of tolclofos-methyl 50WP to *Daphnia magna* was 30 mg a.s./L with 95% confidence limits of 18 to 38 mg a.s./L. The no-observed effect concentration (NOEC) was 8.2 mg a.s./L.

Comments:

The study was well performed and reported.

B.9.2.5 Chronic toxicity to aquatic invertebrates: *Daphnia magna*

ACTIVE INGREDIENT

Reference:	Burgess, D. (1989) Chronic toxicity of Rizolex to *Daphnia magna* under flow-through test conditions
Guideline:	ASTM: Proposed Standard Practice for Conducting *Daphnia magna* Chronic Toxicity Test in a Flow-Through System. Draft No. 3, March 1981, ASTM Committee E-47.01.
GLP:	Yes (Self certification by the laboratory)

Material and methods:

Test substance:	Tolclofos-methyl (Batch No.: 80410, Purity: 97.6%) diluted in acetone
Species:	*Daphnia magna*
Treatments:	Nominal concentrations of 0, solvent control, 0.024, 0.048, 0.10, 0.20 and 0.40 mg/L
Number of animals:	4 replicates of 10 animals per test concentration
Duration:	21 days
Test conditions:	The test was performed by use of a flow-through system at a 16-hour daylight photoperiod. Temperature was 20-21°C, pH 7.4-8.1, dissolved oxygen 5.4-8.2 mg/L and loading rate ~1 daphnid per 100 mL. Test solutions were analysed at 0, 4, 7, 10, 14, 17 and 21 days.
Observations:	Survival, abnormal effects, time to first brood, number of offspring produced during the study, length of adult daphnids at day 21.

Results:

Mean measured concentrations (0.026, 0.062, 0.089, 0.19 and 0.39 mg/L) ranged from 89-129% of the nominal values. All results were based on mean measured concentrations. The results from the study are presented in Table 9.2.5.a.

Table 9.2.5.a. Survival, number of young/adult reproduction day, time to first brood and adult length of *Daphnia magna* exposed to tolclofos-methyl during a 21 days life cycle test

Mean Measured Concentration (mg/l)	Mean Survival (%)	Adult Mean Length (mm)	Time to First Brood (days)	Mean Young/Adult Reproduction Day
Control	93	3.5	9.0	3.8
Solvent Control	95	3.6	8.5	4.7
Pooled control	94	-	8.8	-c
0.026	98	3.5	8.8	4.2
0.062	95	3.3*	8.5	3.2*
0.089	80	3.2*	9.0	3.1*
0.19	35*	2.7*	9.0	0.8*
0.39	0*	-	-	-

* Values significantly different ($P \leq 0.05$) from the pooled controls using one-way analysis of variance (ANOVA) and Dunnett's t-test or Turky's HSD Multiple Means Comparison Test.
c: A significant difference ($P < 0.05$) was identified between the control and the solvent control so the values were not pooled.

Based on the results of this study the 21 days no-observed effect concentration (NOEC) of tolclofos-methyl to *Daphnia magna* in the 21-day chronic toxicity test was 0.026 mg/L. The lowest-observed effect concentration (LOEC) was 0.062 mg/L.

Comments:

The study was well performed and reported.

B.9.2.6 Effects on algal growth and growth rate

ACTIVE INGREDIENT

Reference:	Takimoto, Y., Yasutaniya, T. (1983) The effect of tolclofos-methyl on the growth of the green alga*, Scenedesmus quadricauda*
Guideline:	Dutch draft Standard Method of NEN 6506 (1979) and OECD 201 (1981) Deviations: The Closterium medium was used in place of OECD medium, because the growth of algae was found to be better in the former medium than the latter.
GLP:	No

Material and methods:

Test substance:	Tolclofos-methyl (Batch No.: 10901, Purity: 98.3%)
Species:	*Scenedesmus quadricauda*
Treatments:	Nominal concentrations of 0.01, 0.032, 0.1, 0.32, 1.0 and 5.6 mg/L dissolved in dimethylsulfoxide (0.5 mL/L) and a solvent control.
Number of animals:	3 replicate cultures per treatment
Duration:	7 days
Test conditions:	The test was performed at 20± 2°C with continuous illumination at about 5000 lux and shaking at 100 rpm. Potassium dichromate was used as a reference compound.
Observations:	Number of colonies/mL at 24-hour intervals; morphological abnormalities at start and end of study. There was no analysis of the test concentrations.

Results:

The results are presented in Table 9.2.6.a. No morphological abnormalities were observed for algae treated with tolclofos-methyl at the start and end of the test.

Table 9.2.6.a. Colony counts and growth parameters (specific growth rate and mean relative growth rate) of the algal growth inhibition test of tolclofos-methyl with *Scenedesmus quadricauda*

Nominal Test Concentrations (mg/l)	Colony counts (x10^4 colonies/mL)						Specific Maximum Growth Rate (/day)	Mean Relative Growth Rate (/day)
	Day 0	Day 1	Day 2	Day 3	Day 4	Day 7	[% inhibition]	[% inhibition]
Solvent control	1.06	1.35	6.46	14.2	34.1	97.9	1.57	1.56
0.01	1.06	1.25	6.27	13.6	30.2	88.8	1.61 [-2.55%]	1.61 [-3.21%]
0.032	1.06	1.49	6.33	13.7	28.0	79.4	1.45 [7.64%]	1.44 [7.69%]
0.1	1.06	1.24	6.21	12.4	19.6*	79.2	1.61 [-2.55%]	1.61 [-3.21%]
0.32	1.06	1.21	6.17	12.3	18.0*	69.6*	1.63 [-3.82%]	1.64 [-5.13%]
1.0	1.06	1.27	6.16	12.4*	17.5*	65.7*	1.58 [-0.64%]	1.58 [-1.28%]
5.6	1.06	1.28	6.28	11.8*	17.1*	64.0*	1.59 [-1.27%]	1.60 [-2.56%]

*: P<0.05 determined by Student's t-test of data from treated culture versus control

Based on the results of this study the 7-day acute toxicity (EC_{50}) of tolclofos-methyl to the alga *Scenedesmus quadricauda* could not be obtained, and was therefore concluded to be >5.6 mg/L. The no-observed effect concentration (biomass, NOEC) was 0.032 mg/L. Both EC_{50} and NOEC were based on nominal concentrations.

Comments:

Concentrations of tolclofos-methyl in test solutions were not determined and therefore the NOEC is not reliable. Growth rate seem to level off before end of test that was run for 7 days instead of recommended 72 hours. Although available studies indicate that the substance is relatively stable in aqueous solutions the actual concentrations in the test is not known. Furthermore, the statistical method used to determine NOEC (Student' s t-test) should not be used for multiple comparisons. The study is not of acceptable quality and a new study is required.

Reference: Sayers, L.E. (2003)
Tolclofos-methyl - Toxicity to the freshwater green alga, *Scenedesmus subspicatu*
Guideline: OECD 201 (7 June 1984), EEC C.3
GLP: Yes (Self certification by the laboratory)

Material and methods:

Test substance: Tolclofos-methyl, Batch No.: 00668G, Purity: 97.8%
Species: *Scenedesmus subspicatus*
Treatments: Nominal concentrations of 0.13, 0.25, 0.50, 1.0 and 2.0 mg a.s./L
Number of animals: 3 replicate cultures per treatment
Duration: 72 hours
Test conditions: The culture medium was Algal Assay Procedure (AAP) medium prepared with sterile deionised water. Untreated AAP medium was used to culture the control population and there was also a solvent control containing acetone (0.1 mL/L). The initial (0 hour) cell density was 1.0 x 10^4 cells/mL. The test solutions were analysed at 0 and 72 hours. The test conditions were: temperature of 23-24°C (range of daily measurements); pH 7.3 at initiation and 8.6-9.7 at termination; light intensity 7000-

8900 lux; shaking at 100 rpm.

Observations: Cell counts (cells/ml) were made at 24-hour intervals using a haemocytometer and observations on the health of the algal cells were also made at each 24-hour interval.

Results:

Mean measured concentrations (0.13, 0.22, 0.39, 0.69 and 1.1 mg/L) ranged from 53-97% of the nominal concentrations. The results are presented in Table 9.6.2.b.

Table 9..2.6.b. Cell density and growth parameters of the algal growth inhibition test of tolclofos-methyl with *Scenedesmus subspicatus*

Mean measured concentration (mg/L)	Cell density ($x10^4$ cells/mL)[1]			72-hour biomass ($x10^4$ cells.days/mL)		0-72 hour growth rate ($days^{-1}$)	
	24-hour	48-hour	72-hour	Total area[1]	[% inhibition]	Growth rate[1]	[% inhibition]
Control	3.75(0.66)	18.42(3.01)	55.2(2.7)	43.8(1.9)	-	1.37(0.02)	-
Solvent control	5.00(1.64)	16.25(2.95)	67.9(14.3)	49.0(8.4)	-	1.44(0.07)	-
Pooled control				46.4(6.1)	-	1.41(0.06)	-
0.13	4.75(0.25)	22.08(0.88)	56.3(4.3)	48.7(1.5)	-5	1.38(0.03)	2
0.22	4.17(0.38)	19.25(3.70)	52.7(7.8)	43.9(1.4)	5	1.36(0.05)	4
0.39	4.00(1.09)	13.67(3.22)	40.8(5.6)	33.1(6.2)[2]	29	1.27(0.05)[2]	10
0.69	3.17(0.95)	11.75(1.75)	30.3(4.2)	25.6(4.3)[2]	45	1.17(0.05)[2]	17
1.1	2.00(0.50)	8.83(3.39)	18.1(3.2)	16.1(4.1)[2]	65	0.99(0.06)[2]	30

[1]: Mean (SD)
[2]: Significantly reduced compared to the control (Williams' Test, $P \leq 0.05$)

The 72 hour E_bC_{50} for biomass was calculated to be 0.78 mg a.s./L, with 95% confidence limits of 0.62 to 0.92 mg a.s./L. The 72 hour E_rC_{50} for growth rate was calculated to be >1.1 mg a.s./L. The no-observed effect concentration (NOEC) for both of biomass and growth rate was 0.22 mg/L.

Comments:

The study was well performed and reported.

METABOLITE

Reference: Hoberg, J.R. (1998)
Desmethyl-tolclofosmethyl - Toxicity to the freshwater green alga, *Scenedesmus subspicatus*

Guideline: OECD 201 (adopted 7 June 1984) - equivalent to EEC C.3

GLP: Yes (Self certification by the laboratory)

Material and methods:

Test substance: DM-TM (Batch No.: CTS98009G; Purity: 98.9%)

Species: *Scenedesmus subspicatus*

Treatments: Nominal concentrations of 0, 8.0, 16, 31, 63 and 125 mg/L

Number of animals: 3 replicate cultures per treatment

Duration: 72 hours

Test conditions: Initial (0 hour) cell density) was 1.0 x 10^4 cells/ml. Algal Assay Procedure (AAP) medium was used. The test was performed with a light intensity 4100-5000 lux at continuous shaking at 100 ± 10 rpm. Temperature was 24-25°C and pH 7.4 at initiation and 9.6-9.7 at termination. The test solutions were analysed at 0 and 72 hours.

Observations: Cell counts (cells/mL) at 24-hour intervals using a haemocytometer; observations on the health of the algal cells at each 24-hour interval.

Results:

Mean measured concentrations (6.0, 12, 23, 34, 48 and 97 mg/L) ranged from 75-77% of the nominal values. Results are therefore based on mean measured concentrations and are presented in Table 9.2.6.c. For the 72-hour biomass results, test concentrations of 23 mg/L and above were significantly reduced as compared with the control. The 0-72 hour growth rates at test concentrations of 48 mg/L and above were significantly reduced compared with the control.

Table 9.2.6.c Cell density and growth parameters of the algal growth inhibition test of DM-TM with *Scenedesmus subspicatus*

Mean measured concentration (mg/l)	Cell density (x10^4 cells/ml)[1]			72-hour biomass (x10^4 cells.days/ml)		0-72 hour growth rate (days^{-1})	
	24-hour	48-hour	72-hour	Total area[1]	[% inhibition]	Growth rate[1]	[% inhibition]
Control	9.5(0.25)	98(1.0)	139(3.4)	165(2.2)	-	1.67(0.008)	-
6.0	9.6(0.63)	99(2.2)	142(1.4)	167(3.2)	-1.0	1.68(0.003)	-0.48
12	7.6(0.38)	98(1.3)	138(2.4)	162(1.6)	1.9	1.67(0.006)	0.12
23	6.5(0.43)	95(2.1)	136(1.6)	158(2.9)[2]	4.6	1.66(0.004)	0.42
48	7.1(1.0)	94(1.5)	133(0.72)	156(1.8)[2]	5.9	1.65(0.003)[2]	0.90
97	6.5(1.4)	91(0.8)	131(1.2)	151(1.0)[2]	8.6	1.65(0.003)[2]	1.3

[1]: Mean (SD)
[2]: Significantly reduced compared to the control (Williams' Test, P≤0.05)

Based on the results of this study the acute toxicity (72-hour EbC_{50} and ErC_{50}) of DM-TM to the alga *Scenedesmus subspicatus* could not be obtained and was therefore concluded to be >97 mg/L.

The no-observed effect concentration (NOEC) for biomass and growth rate was 12 and 23 mg/L, respectively.

Comments:

The study was well performed and reported.

PLANT PROTECTION PRODUCT

Reference: Sayers, L.E. (2003a)
Tolclofos-methyl 50WP - Toxicity to the freshwater green alga, *Scenedesmus subspicatus*

Guideline: OECD 201 (7 June 1984), EEC C.3

GLP: Yes (Self certification by the laboratory)

Material and methods:

Test substance: Tolclofos-methyl 50WP (Batch No.: B2190010, Contents: 51.03% w/w

Species: *Scenedesmus subspicatus*

Treatments: Nominal concentrations of 0.20, 0.39, 0.78, 1.6 and 3.1 mg a.s./L

Number of animals: 3 replicate cultures per treatment

Duration: 72 hours

Test conditions: The culture medium was Algal Assay Procedure (AAP) medium prepared with sterile deionised water. Untreated AAP medium was used to culture the control population. The initial (0 hour) cell density was 1.0 x 10^4 cells/ml. The test solutions were analysed at 0 and 72 hours. Test conditions: temperature of 20-24°C (range of daily measurements); pH 7.1-7.3 at initiation and 7.6-9.2 at termination; light intensity 7300-8600 lux; shaking at 100 rpm.

Observations: Cell counts (cells/mL) were made at 24-hour intervals using a haemocytometer and observations on the health of the algal cells were also made at each 24-hour interval.

Results:

Mean measured concentrations (0.15, 0.31, 0.55, 1.1 and 1.7 mg a.s./L) ranged from 56-80% of the nominal concentrations. The results are presented in Table 9.2.6.d.

Table 9.2.6.d. Cell density and growth parameters of the algal growth inhibition test of tolclofos-methyl 50WP with *Scenedesmus subspicatus*

Mean measured concentration (mg a.s./L)	Cell density (x10^4 cells/mL)[1]			72-hour biomass (x10^4 cells.days/mL)		0-72 hour growth rate (days^{-1})	
	24-hour	48-hour	72-hour	Total area[1]	[% inhibition]	Growth rate[1]	[% inhibition]
Control	1.58(0.72)	7.67(1.61)	59.8(8.2)	41.8(6.63)	-	1.33(0.04)	-
0.15	0.83(0.38)	9.17(1.01)	54.8(20.17)	39.9(12.03)	5	1.29(0.11)	3
0.31	0.83(0.29)	8.0(1.09)	62.1(17.51)	42.8(10.01)	-2	1.33(0.1)	0
0.55	1.08(0.29)	5.42(1.26)	33.4(8.66)	23.6(5.44)[2]	44	1.13(0.09)[2]	15
1.1	0.50(0.25)	3.67(0.14)	10.5(3.93)	7.9(2.06)[2]	81	0.75(0.12)[2]	44
1.7	0.50(0.25)	3.83(0.8)	8.9(3.66)	7.2(2.7)[2]	83	0.69(0.15)[2]	48

[1]: Mean (SD)
[2]: Significantly reduced compared to the control (Williams' Test, P≤0.05)

The 72 hour E_bC_{50} for biomass was calculated to be 0.65 mg a.s./l, with 95% confidence limits of 0.50 to 0.76 mg a.s./L.

The 72 hour E_rC_{50} for growth rate was calculated to be >1.7 mg a.s./L. The no-observed effect concentration (NOEC) for both of biomass and growth rate was 0.31 mg a.s./L.

Comments:

The study was well performed and reported.

B.9.2.7 Chronic effects on sediment dwelling organisms: *Chironomus riparius*

Reference:	Putt, A.E. (2002a) Tolclofos-methyl - The full life-cycle toxicity to midge (*Chironomus riparius*) under static conditions
Guideline:	OECD Draft Guideline No. 219
GLP:	Yes (Self certification by the laboratory)

Material and methods:

Test substance:	Tolclofos-methyl (Non-radiolabelled - Batch No.: 90437G, Purity: 98.0%,; Radiolabelled - Batch No.: CP-2427, Radiochemical purity: 98.9%.
Species:	*Chironomus riparius*
Treatments:	Nominal test concentrations of 0.063, 0.13, 0.25, 0.50 and 1.0 mg a.s./L
Number of animals:	20 midge larvae (3 days old) per test vessel
Duration:	28 days
Test conditions:	Eight replicate test vessels (600 ml glass beakers) were established for each treatment level and the controls (solvent and blank): four were for monitoring the biological results and the other four were to determine exposure concentrations in the overlying water. Overlying water used was laboratory well water (total hardness and alkalinity as $CaCO_3$ of 42 and 30 mg/L, respectively, and a pH of 7.0), together with field-collected sediment (2.4% organic carbon, 94% sand, 6% silt and 0% clay and a pH of 6.2). The ratio of sediment to water was 1: 4. Food was added at introduction and daily during the test. The test substance was introduced by adding the appropriate dosing stock solution to the overlying water and then gently stirring. Temperature: 19-21°C; pH: 5.5-7.7; dissolved oxygen: 7.6-9.6 mg/L (60% of air saturation at 20°C = 5.4 mg/L); photoperiod: 16 hours light (970 – 1080 lux): 8 hours dark.
Observations:	Midge emergence; abnormal behaviour; sex and number of adult midges that emerged daily were recorded. Overlying water and sediment in all test levels were analysed at each interval (day 0 (+1hour), 7, 14 and 28) for total ^{14}C by LSC. The pore water samples in the 0.063, 0.25 and 1.0 mg a.s./L treatment levels were analysed at each interval for total ^{14}C by LSC. In addition, the 0.063, 0.25 and 1.0 mg a.s./L treatment levels were analysed at each interval for the concentration of tolclofos-methyl in water and sediment by high performance liquid chromatography using radiochemical detection (HPLC/RAM). A DM-TM (metabolite) reference standard was analysed by HPLC/UV on three occasions to establish its retention time.

Results:

The summarised results of LSC analysed for total ^{14}C in the overlying water, pore water and sediment are presented in Table 9.2.7.a. The results of the HPLC/RAM analysis established that any test substance that remained in the water column during the study degraded to DM-TM (0 to 9 % of applied ^{14}C) or other minor

degradates (0 to <7% of applied ^{14}C) by the end of the 28-day exposure. The test substance that portioned to the sediment generally remained as parent material. Approximately 80% (23 to 46% of applied ^{14}C) remained as tolclofos-methyl, and the remaining 20% (7 to 11% of applied ^{14}C) was associated with DM-TM. A summary of the mean percentage emergence and mean development rate at test termination is presented in Table 9.2.7.b.

Table 9.2.7.a. Concentrations of total [^{14}C] residue measured by liquid scintillation counting (LSC) in overlying water, pore water and sediment samples during the 28-day exposure of *Chironomus riparius* to tolclofos-methyl

Nominal Concentration (mg a.s./L)	Measured Concentration, mg a.s./L (mg a.s./kg for sediment) (% of nominal concentration)			
	1-Hour	Day 7	Day 14	Day 28
Overlying water				
Control	<LOQ	<LOQ	<LOQ	<LOQ
Solvent Control	<LOQ	<LOQ	<LOQ	<LOQ
0.063	0.064(100%)	0.0067(11%)	0.0074(12%)	<LOQ
0.13	0.13(100%)	0.022(17%)	0.019(15%)	0.0099(7.6%)
0.25	0.23(94%)	0.035(14%)	0.031(12%)	0.019(7.6%)
0.50	0.47(94%)	0.11(22%)	0.10(20%)	0.070(14%)
1.0	0.76(76%)	0.16(15%)	0.12(12%)	0.16(16%)
Pore water				
0.063	0.00037	0.0029	0.0062	0.0048
0.25	0.00068	0.013	0.021	0.019
1.0	0.0057	0.059	0.079	0.16
Sediment				
Control	<LOQ	<LOQ	<LOQ	<LOQ
Solvent Control	<LOQ	<LOQ	<LOQ	<LOQ
0.063	0.020(15%)	0.056(42%)	0.071(54%)	0.075(57%)
0.13	0.024(8.8%)	0.22(81%)	0.20(73%)	0.13(48%)
0.25	0.063(12%)	0.34(65%)	0.18(34%)	0.16(31%)
0.50	0.10(10%	0.72(69%)	0.86(82%)	0.88(84%)
1.0	0.26(12%)	1.5(72%)	1.2(57%)	0.87(41%)

LOQ =0.00468 mg/L and 0.0180 µg/g sediment

Table 9.2.7.b. Mean percentage emergence and mean development rates calculated at test termination (Day 28) of the midge (*Chironomus riparius*) full life-cycle exposure with tolclofos-methyl

Nominal concentration (mg a.s./L)	Mean Percent Emerged	Mean Development Rates		
		Males	Females	Males/Females (pooled data)
Control	86	0.0649	0.0553	0.0597
Solvent control	85	0.0664	0.0555	0.0600
Pooled control	86	0.0657	0.0554	0.0599
0.063	86	0.0654	0.0570	0.0613
0.13	86	0.0630	0.0558	0.0593
0.25	78	0.0615	0.0538	0.0577
0.50	78	0.0592	0.0528	0.0561*
1.0	79	0.0521	0.0481	0.0501*

* significantly different from the pooled control data (Williams' test, $p < 0.05$)

Based on the nominal concentrations applied and the midge development rate the 28-day no-observed effect concentration (NOEC) of tolclofos-methyl to *Chironomus riparius* in the chronic toxicity test was 0.25 mg a.s./L. The lowest-observed effect concentration (LOEC) was 0.50 mg a.s./L. The 28-day EC_{50} based on midge emergence, was concluded to be >1.0 mg a.s./L.

Comments:

The study was well performed and reported.

B.9.2.8 Summary of the toxicity studies on aquatic organisms

Fish

Tolclofos-methyl is of high to moderate acute toxicity to fish. Signs of toxicity for rainbow trout included abnormal respiration, lethargy, loss of equilibrium and darkening, while no signs of toxicity were observed with the bluegill sunfish.

The metabolite DM-TM is of low toxicity to fish, and it is concluded that it is less toxic than the parent compound.

From the submitted test it can be concluded that the formulated product, Tolclofos-methyl 50 WP, is less toxic than the active ingredient.

The chronic toxicity of tolclofos-methyl to fish was investigated in an early life-stage study with rainbow trout. No adverse effects were observed on egg hatchability at any concentration, while fry survival was adversely affected at concentrations greater than or equal to 0.11 mg/L. Growth of the fry (wet weight at 60 days post-hatch and standard length at 37 days post-hatch) was the most sensitive test endpoint, with a lowest-observed effect concentration (LOEC) of 0.028 mg/L and no-observed effect concentration (NOEC) of 0.012 mg/L.

Tolclofos-methyl shows a potential for bioaccumulation in fish under flow-through exposure system at a nominal concentration of 0.030 mg/L. The steady state of BCF was attained approximately after 7 days exposure. The BCF at 28 days of exposure for whole fish was 670. However, tolclofos-methyl is quickly cleared away from fish tissues with a CT_{90} value calculated to be 3.6 days for whole fish.

A summary of the studies of the toxicity of tolclofos-methyl to fish is presented in Table 9.2.8.a. Results from studies that were not accepted for the purpose of risk assessment are indicated with *.

Table 9.2.8.a Summary of the studies on effects on fish treated with tolclofos-methyl, the metabolite DM-TM and the formulated product 50WP.

Test substance	Test species (duration)	Test concentrations	Result	Reference
ACUTE TOXICITY				
Tolclofos-methyl	Rainbow trout *Salmo gairdneri* = *Oncorhynchus mykiss* (96 hours)	0.05, 0.10, 0.18, 0.32, 0.56, 0.75, 1.00, 1.80, 3.20 mg/L (nominal)	LC_{50} : 0.87 mg/L* NOEC: 0.05 mg/L*	Takimoto, Y., *et al*., 1982
	Rainbow trout *Oncorhynchus mykiss* (96 hours)	0.17, 0.25, 0.35, 0.61, 0.80 mg/L (mean measured)	LC_{50} : 0.69 mg/L NOEC: 0.35 mg/L	Sousa, J.V., 2003
	Bluegill *Lepomis macrochirus* (96 hours)	0.043, 0.093, 0.179, 0.360, 0.720 mg/L (mean measured)	LC_{50} : >0.720 mg/L NOEC : 0.720 mg/L	McAllister, W.A., 1989
DM-TM	Rainbow trout *Oncorhynchus mykiss* (96 hours)	14, 24, 39, 64 and 110 mg/L (mean measured)	LC_{50} : >110 mg/L NOEC : 110 mg/L	Dionne, E., 1998a
Tolclofos-methyl 50WP	Bluegill sunfish *Lepomis macrochirus* (96 hours)	34, 67, 125, 250 and 375 mg/L (nominal)	LC_{50} : 118 mg/L (59 mg a.s./L) NOEC: <34 mg/L (<17 mg a.s./L	Christopher, D.H., Pell, I.B. (1979)
	Rainbow trout *Salmo gairdneri* = *Oncorhynchus mykiss* (96 hours)	34, 67, 125, 250 and 375 mg/L (nominal)	LC_{50} : 52 mg/L (26 mg a.s./L) NOEC: <34 mg/L (<17 mg a.s/L)	Christopher, D.H., Pell, I.B. (1979)
	Rainbow trout *Oncorhynchus mykiss* (96 hours)	0.62, 1.1, 2.2, 4.6, 9.0 and 20 mg a.s/L (mean measured)	LC_{50} : >20 a.s./L NOEC: 0.62 mg/L mg a.s/L	Sayers, L.E. (2003a)
	Bluegill *Lepomis macrochirus* (96 hours)	1.2, 2.5, 6.2, 13, 27 and 54 mg a.s./L (mean measured)	LC_{50} : >54 mg a.s./L NOEC: 27 mg a.s/L)	Sayers, L.E. (2003b)
CHRONIC TOXICITY/ELS				
Tolclofos-methyl	Rainbow trout *Oncorhynchus mykiss*= *Oncorhynchus mykiss* (97 days, 60 days post-hatch)	0.012, 0.028, 0.053, 0.110, 0.200 mg/L (measured)	NOEC : 0.012 mg/L LOEC : 0.028 mg/L	Cohle, P., 1991
BIOCONCENTRATION				
Tolclofos-methyl	Bluegill sunfish *Lepomis macrochirus* (28 days exposure and 14 days depuration)	0.030 mg/L (Nominal)	BCF: 670 (whole fish) CT_{50}: 1.1 (whole fish) CT_{90}: 3.6 (whole fish)	Forbis, A., *et al*., 1986 (in-life phase) Yu, C.C., *et al*., 1986 (analytical phase) Fujisawa, T., *et al*., 1999 (BIOFAC calculation)

***** result from study not accepted for risk assessment

Aquatic invertebrates

Tolclofos-methyl and its formulated product Tolclofos-methyl 50 WP is of slight toxicity to aquatic invertebrates and the metabolite DM-TM is of low toxicity.

The long-term toxicity of tolclofos-methyl to aquatic invertebrates was investigated in a 21-day *Daphnia magna* flow-through chronic toxicity study. The growth of adult daphnids (length) and production of offspring were the most sensitive endpoints, with a lowest-observed effect concentration (LOEC) of 0.062 mg/L and no-observed effect concentration (NOEC) of 0.026 mg/L.

The chronic (life-cycle) toxicity of tolclofos-methyl to a sediment-dwelling insect, the midge *Chironomus riparius*, was investigated in a 28-day study in which larvae were exposed in a water-sediment system to which the test substance was introduced by addition to the overlying water. Midge emergence was unaffected at all test concentrations (up to 1.0 mg a.s./L) but development rates were reduced compared to the pooled data for males and females at 0.50 and 1.0 mg a.s./L, resulting in no-observed effect concentration (NOEC) of 0.25 mg a.s./L.

A summary of the studies of the toxicity of tolclofos-methyl to aquatic invertebrates is presented in Table 9.2.8.b. Results from studies that were not accepted for the purpose of risk assessment are indicated with *.

Table 9.2.8.b Summary of the studies on effects on aquatic invertebrates treated with tolclofos-methyl, the metabolite DM-TM and the formulated product 50WP.

Test substance	Test species (duration)	Test concentrations	Result	Reference
ACUTE TOXICITY				
Tolclofos-methyl	*Daphnia magna* (48 hours)	8.4, 17, 26, 41, 64 mg/L (mean measured)	EC_{50}: 48 mg/L NOEC: 17 mg/L	Murrell, H., 1994
DM-TM	*Daphnia magna* (48 hours)	12, 20, 34, 50 and 95 mg/L (measured)	EC_{50}: >95 mg/L NOEC: 95 mg/L	Dionne, E., 1998b
Tolclofos-methyl 50 WP	*Daphnia magna* (24 hours)	1, 2, 4, 16, 32, 64, 128 and 256 mg/L (nominal)	EC_{50}: 10.3 mg/L (5.2 mg a.s./L)* NOEC: <1mg/L (<0.5 mg a.s./L)*	Christopher, D.H., Fraser, W.D. (1978)
Tolccofos-methyl 50 WP	*Daphnia magna* (48 hours)	1.2, 2.4, 8.2, 18, 38, 91 mg a.s./L (mean measured)	EC_{50}: 30 mg a.s./L NOEC: 8.2 mg a.s./L	Sayers, L.E., 2003
CHRONIC TOXICITY				
Tolclofos-methyl	*Daphnia magna* (21 days)	0.026, 0.062, 0.089, 0.19, 0.39 mg/L (mean measured)	NOEC: 0.026 mg/L LOEC: 0.062 mg/L	Burgess, D., 1989
Tolclofos-methyl	*Chironomus riparius* (28 days)	0.063, 0.13, 0.25, 0.50, 1.0 mg/L (nominal)	NOEC: 0.25 mg/L LOEC: 0.50 mg/L	Putt, A.E., 2002a

* result from study not accepted for risk assessment

<u>*Algae*</u>

Tolclofos-methyl and the formulated product Tolclofos-methyl 50 WP is of high to moderate acute toxicity to the green alga, *Scenedesmus subspicatus*. The metabolite DM-TM is of low toxicity to the green alga.
A summary of the studies of the toxicity of tolclofos-methyl to algae is presented in Table 9.2.8.c.

Table 9.2.8.c Summary of the studies on effects on green algae treated with tolclofos-methyl, the metabolite DM-TM and the formulated product 50WP

Test substance	Test species (duration)	Test concentrations	Result	Reference
ACUTE TOXICITY				
Tolclofos-methyl	*Scenedesmus quadricauda* (7 days)	0.01, 0.032, 0.1, 0.32, 1.0, 5.6 mg/L (nominal)	EC_{50}: >5.6 mg/L* NOEC: 0.032 mg/L*	Takimoto, Y., *et al*., 1983
Tolclofos-methyl	*Scenedesmus subspicatus* (72 hours)	0.13, 0.22, 0.39, 0.69 and 1.1 mg a.s/L (mean measured)	E_bC_{50}: 0.78 mga.s./L NOEC: 0.22 mg a.s./L	Sayers, L.E., 2003
DM-TM	*Scenedesmus subspicatus* (72 hours)	6.0, 12, 23, 34, 48 and 97 mg/L (measured)	EC_{50}: >97 mg/L NOEC: 12 mg/L	Hoberg, J.R., 1998
Tolclofos-methyl 50 WP	*Scenedesmus subspicatus* (72 hours)	0.15, 0.31, 0.55, 1.1 and 1.7 mg a.s./L	E_bC_{50}: 0.65 mg a.s./L NOEC: 0.31 mg a.s./L	Sayers, L.E., 2003d

* result from study not accepted for risk assessment

B.9.2.9 Risk assessment for aquatic organisms

Notifiers assessment

The Notifier, based on the uses as potato tuber dressing or applied to lettuce in glasshouses, proposed estimated predicted concentrations of tolclofos-methyl and the major metabolite DM-TM. Maximum recommended application rate was assumed, i.e. 625 g a.s./ha for potatoes (0.25 kg a.s./tonne potato x 2.5 tonne potato/ha) and 2000 g a.s./ha for lettuce. From the suggested areas of use, there will be no exposure of surface waters via spray drift. However, PEC_{sw}s and PEC_{sed}s were calculated using the Dutch national model, which assumes 0.1% "spray drift" exposure from glasshouse uses. A static 30 cm deep water body was assumed, with a 5 cm deep sediment layer, with uniform distribution within both. As this is considered a "worst case", PEC values for moving water bodies were not estimated. A single application of 2000 g/ha was assumed with 0.1% of this drifting to an adjacent surface water body. Worst case DT_{50}s for tolclofos methyl from the sediment/water study, within the water and sediment phases were 1.6 and 27 days respectively (Notifiers calculations, both 1st order, see Section B.8.4.3.2). A maximum of 73% of the applied tolclofos-methyl partitioned to sediment.

The metabolite DM-TM was detected in the sediment/water study at respective maximum levels of 10.7 and 12.7% of applied radioactivity in the water and sediment phases. "Worst case" DT_{50}s for DM-TM from the sediment/water study within the water and sediment phases were 42 and 43 days respectively (both 1st order, see Section B.8.4.3.2)

Acute TER values were calculated by dividing acute LC_{50} values (mg a.s./L) by the initial PEC_{sw} (mg a.s./L). In the case of tolclofos-methyl, the initial surface water PEC value provides the worst-case aquatic exposure estimate.

The long-term TER values were calculated by dividing the chronic NOEC value (mg a.s./L) by the appropriate time-weighted average PEC_{sw} (mg a.s./L). The fish early life-stage toxicity test lasted for 97 days, and for the worst-case scenario the 42-day time-weighted average surface water PEC value (Table 8.6.2.a) instead of 97-

day's one was chosen by the Notifier for the maximum recommended application rate of 2000 g a.s./ha for lettuce with 0.1% drift from glasshouses. RMS followed the recommendations in the Guidance Document on Aquatic Ecotoxicology in the context of the Directive 91/414/EEC /SANCO/3268/2001 rev. 4, 17 October 2002) and added a first tier assessment for long term toxicity to fish based on the initial PEC value.

The *Daphnia* chronic toxicity study lasted for 21 days. The Notifier used the 21-day time-weighted average surface water PEC value (Table 8.6.2.a) for the maximum recommended application rate of 2000 g a.s./ha for lettuce with 0.1% drift from glasshouses for the TER-calculations. RMS followed the recommendations in the Guidance Document on Aquatic Ecotoxicology in the context of the Directive 91/414/EEC /SANCO/3268/2001 rev. 4, 17 October 2002) and added a first tier assessment for long term toxicity to *Daphnia* based on the initial PEC value.

The long-term TER for sediment dwelling insects was calculated by dividing the chronic NOEC value (mg a.s./L) for *Chironomus* by the intial PECsw (mg a.s./L) because of the design of test (spiked water test). The short and long term effect concentrations for each species, the predicted environmental concentrations and the toxicity to exposure ratios are given in Table 9.2.9a.

Table 9.2.9.a. Notifiers calculated Toxicity to Exposure Ratios (TER) for tolclofos-methyl and the major metabolite DM-TM in surface water assuming 0.1% drift from glasshouses at 2000 g a.s./ha and a maximum level for DM-TM of 10.7% 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	0.67	1030	<100
Fish - Bluegill sunfish	Tolclofos-methyl	>0.72	0.67	>1075	<100
Fish - Rainbow trout	Tolclofos-methyl 50WP	26	0.67	38806	<100
Fish - Bluegill sunfish	Tolclofos-methyl 50WP	59	0.67	88060	<100
Fish - Rainbow trout	DM-TM	>110	0.07	>1.57 x 10^6	<100
Aquatic invertebrates -*Daphnia magna*	Tolclofos-methyl	48	0.67	71642	<100
	Tolclofos-methyl 50WP	30	0.67	44,776	<100
	DM-TM	>95	0.07	>1.36 x 10^6	<100
LONG TERM					
Fish - Rainbow trout	Tolclofos-methyl	0.012	0.67 (initial)**	18	<10
		0.012	0.04 (42 d TWA)	300	<10
Aquatic invertebrates -*Daphnia magna*	Tolclofos-methyl	0.026	0.67 (initial)**	39	<10
		0.026	0.07 (21 d TWA)	371	<10
Aquatic invertebrates (insects) and Sediment dwelling organisms *Chironomus riparius*	Tolclofos-methyl*	0.25	0.67	373	<10
Algae *Scenedesmus subspicatus*	Tolclofos-methyl	0.78	0.67	1164	<10
	DM-TM	>97	0.07	>1.39 x 10^6	<10
	Tolclofos-methyl 50WP	0.65	0.67	970	<10

* The metabolite DM-TM was observed in the test media.
** RMS calculation for first tier assessment

All the TER values calculated for aquatic organisms are well above Annex VI trigger values. On the basis of these results, it was concluded that the proposed uses of tolclofos-methyl as potato tuber dressing or in glasshouses would present an acceptable risk to aquatic organisms.

RMS assessment

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. For this assessment see Appendix B at the end of Volume 3, Annex B.9.