Draft Renewal Assessment Report
under Regulation (EC) 1107/2009

CLOPYRALID

Volume 3 – B.9 (AS)

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B.9. ECOTOXICOLOGY DATA

Most of the ecotoxicological data on the active substance clopyralid were evaluated during the Annex I inclusion process in 2002-2005. This dRAR therefore focuses on those studies which were not evaluated in the DAR and its addenda, because the conclusions of the on clopyralid (EFSA Scientific Report 2005: 50) are still valid. However, a number of new ecotoxicological studies were submitted by the Notifier to fulfil the current data requirements, and because the representative formulation was changed.

The representative uses of clopyralid include two uses evaluated during the first approval (cereals and pasture) which also reflect changes in dosage of clopyralid containing products as doses have been reduced since the first approval. The representative formulation includes a different product (GF-1374) compared to the product evaluated for the first approval (Lontrel 100 Herbicide). GF-1374 is an emulsifiable concentrate containing the active substance clopyralid at 80 g ae/L and 2 mixing partners namely Fluroxypyr mepthyl 144 g/l (100 g ae/L) and Florasulam 2.5 g/L.

This evaluation only reviews data (Annex II or Annex III) and additional information that has not previously been considered within the EU review process, as part of the Annex I inclusion decision of clopyralid. Studies which have already been evaluated during the Annex I process are only briefly summarised. New Annex II data is only included if they are considered essential for the evaluation of clopyralid and a full study summary is provided. A risk envelope approach is being applied for the risk assessment of fluroxpyr and florasulam. Within the scope of this assessment, one application of fluroxypyr at 200 g a.s./ha was determined to be a safe use on both cereals and grasslands. Also, one application of florasulam at 6.25 g a.s./ha was determined to be a safe use on cereals and grasslands. Therefore, for the below assessments, it is justified to refer to fluroxypyr and florasulam data wherever appropriate. Specific risk assessments for these two active substances are not necessary to defend the Annex I listing of clopyralid since the proposed use rate falls within their safe use. Please refer to the EFSA Scientific reports of florasulam and fluroxypyr to review their associated risk assessments.

Details of the active substances, the Annex I inclusion Directive and Commission Review Report of each active substance are provided in Table 9.0.1.

Table 9.0.1. Details for the active substances

Active Substance	Annex I Inclusion Directive	SANCO Review Report	EFSA Scientific Report
Fluroxypyr	736/2011	SANCO/11019/2011	*EFSA Scientific Report* (2011) 9, (3), 2091
Clopyralid	06/64/EC	SANCO/10012/2006	*EFSA Scientific Report* (2005) 50, 1–65
Florasulam	02/64/EC	SANCO/1406/2001	*EFSA Scientific Report* (2015) 13(1), 3984

For a better overview, existing data and their evaluation resulting from the process of Annex I inclusion of clopyralid are briefly mentioned and amended by new data generated in order to fulfil current requirements. The numbering and the headlines correspond to latest EU requirements.

In addition to AIR3 data generated and owned by the Notifier, also several studies from published literature have been evaluated in this section.

B.9.1. EFFECTS ON BIRDS AND OTHER TERRESTRIAL VERTEBRATES

B.9.1.1. Effects on birds

The Notifier submitted adequate data on the effects of clopyralid to birds in the dossier for the first Annex I inclusion in 2002, as evaluated during the Annex I inclusion process in 2002-2005, and concluded by the EFSA (EFSA Scientific Report 2005: 50). Therefore the original studies are not re-evaluated here again.The studies submitted on the active substance were of acceptable quality and valid, and the outcomes are still valid for the risk assessment. A summary of the critical endpoints that were used in the avian risk assessment is presented in Table 9.1.1 below.

Table 9.1.1. Clopyralid – Summary of effects on birds

Data point	Test organism	Test substance	Study type	EU Agreed endpoint	Reference
CA 8.1.1.1/1	Mallard duck (*Anas platyrhynchos*)	Clopyralid	Acute oral	LD_{50} = 1465 mg a.s./kg bw	[redacted]. 1980; DAS report No. GH-RC 164; Ref. J01
CA 8.1.1.3/1	Mallard duck (*Anas platyrhynchos*)	Clopyralid	One-generation reproduction	NOEC = 1000 mg/kg diet (118 mg/kg bw/day)	[redacted]. 1985; DAS report No. 103-235; Ref. J31

New data and risk assessments were included in the supplementary dossier to reflect changes in legal requirements and changes in scientific and technical knowledge since the approval. It was also necessary to reflect the presence of two other active substances in the new representative formulation GF-1374 with an acute oral toxicity study on birds, not evaluated before in the context of the approval of clopyralid. Most relevant data and endpoints for the two other actives (mixing partners) fluroxypyr and florasulam are briefly summarized in the supplementary dossier.

B.9.1.1.1. Acute oral toxicity to Birds

Data to address this point were presented in the dossier submitted on 30 April 2002 for the Approval of clopyralid and were deemed acceptable following evaluation and peer review at EU level, and therefore the study details are presented here only very briefly. These data are still valid for decision making and are used in the avian acute risk assessment of clopyralid.

CA 8.1.1.1/1 - Acute Oral LD50 – Mallard Duck – DOWCO 290

Report	[IIA 8.1.1/01], [redacted] 1980
Report title	Acute Oral LD50 – Mallard Duck – DOWCO 290
DAS Study number	GH-RC 164; Ref. J01.
Guidelines	US EPA FIFRA 71-1, assessed to correspond SETAC 1995
GLP	No

Methodology:

Clopyralid (termed 'DOWCO 290' in the report), Batch No. AGR 124095, Purity 96.9%. The acute toxicity of clopyralid to the Mallard duck was assessed after oral dosing.

Groups of ten Mallard ducks, *Anas platyrhynchos*, (5 male, 5 female), approximately 9 months old, weighing 831 to 1235 g, were administered clopyralid suspended in corn oil by oral intubation directly into the crop. Each group received a single dose of corn oil only (control group) or one of five treatment levels (nominal clopyralid doses of 398, 631, 1000, 1590 or 2510 mg/kg body weight).

Birds were housed indoors in pens, according to dose group at 18 to 24°C and 30 to 80% relative humidity with a photoperiod of 14 hours. Body weights were recorded individually at initiation and by pen on days 3, 7, 14 and 21. Group mean food consumption was estimated for days 1 to 7, 8 to 14 and 15 to 21.

No macropathology was carried out on any birds *post-mortem*. This deviation is not considered to have affected the outcome of the study.

Findings:

There were no mortalities or signs of toxicity in the control birds or in birds dosed with 398 mg clopyralid/kg. Mortality at the end of the observation period ranged from 10% at 1000 mg/kg to 90% in the highest dose level of 2510 mg/kg (Table 9.1-1). Because of the pattern of continued mortality at these dose levels the observation period was extended to 21 days. Signs of toxicity were observed at 631 mg/kg and above. At 631 mg/kg, one hen was lethargic on day 1 and had a reduced reaction to external stimuli and loss of co-ordination on day 2. All birds at this dose level were asymptomatic on day 3 onwards. At 1000 mg/kg, some birds exhibited lethargy, reduced reaction to external stimuli and regurgitation after dosing. Lethargy, intermittent wing droop and a loss of co-ordination continued through day 3. On day 4, all birds except one were asymptomatic; this hen exhibited a loss of co-ordination and lower limb weakness. On day 8 to 10 a few birds were lethargic and one hen was found dead. All survivors were asymptomatic on day 11 to 21.

Immediately after dosing in the 1590 mg/kg dose group, symptoms were observed similar to those recorded in the lower dose group, with the addition of lacrimation and salivation. On day 1, additional symptoms in two birds were prostrate posture, loss of righting reflex, lower limb rigidity, reflexed head and tail and convulsive activity. All birds exhibited lethargy and loss of co-ordination on day 2 and one hen exhibited lower limb weakness on day 3. On day 5 to 7, all surviving birds were asymptomatic with lethargy reappearing on day 8 to 18 in most birds. In the 2510 mg/kg group, symptoms prior to death included those described at lower dose groups plus depression, prostrate posture and loss of righting reflex. One drake was noted with a prolapsed penis on day 1 and one drake exhibited clonic convulsions on day 2. The single surviving bird was asymptomatic by day 19 at this dose level.

There was a dose-related reduction in body weight at 1000 mg/kg to 2510 mg/kg on days 3, 7 and 14 (Table 9.1-2). Food consumption was also reduced in a dose-related manner at all doses for the first 7 days of the study, remaining reduced at 1590 mg/kg until the end of the study. The one survivor at 2510 mg/kg showed an increase in body weight and food consumption during the last 7 days of the study. The results are summarised in Table 9.1-3.

Table 9.1-1. Cumulative mortality of Mallard duck dosed orally with clopyralid

Nominal clopyralid dose (mg/kg bw)	**Cumulative Mortality (out of ten birds)**						
	1 d[a]	**3 d**	**7 d**	**10 d**	**14 d**	**18d**	**21 d**
Control	0	0	0	0	0	0	0
398	0	0	0	0	0	0	0
631	0	0	0	0	0	0	0
1000	0	0	0	1	1	1	1
1590	0	2	2	5	7	7	7
2510	3	4	5	8	8	9	9

[a] days after dosing.

Table 9.1-2. Body weights and food consumption of Mallard duck dosed orally with clopyralid

Nominal clopyralid dose (mg/kg bw)	**Average body weight (g)**					**Estimated food consumption (g/bird/day)**		
	Day 1	**Day 3**	**Day 7**	**Day 14**	**Day 21**	**Day 1-7**	**Day 8-14**	**Day 15-21**
Control	1137	1200	1187	1284	1305	121	160	161
398	1077	1126	1129	1207	1229	110	158	178
631	1060	1080	1093	1149	1136	99	135	137
1000	1105	1114	1096	1188	1212	83	123	132
1590	1052	970	896	943	940	20	54	69
2510	1091	974	880	910	1231	5	64	139

Table 9.1-3. Summary of results with mallard duck

Endpoint	**Clopyralid (nominal) mg/kg body weight**
LD_{50} (95% C.L.)	1465 (1220 to 1760)
NOEL	< 398

Conclusions:

The acute oral LD_{50} value for Mallard duck, dosed orally with clopyralid, was calculated to be 1465 mg/kg body weight (with 95% confidence limits of 1220 to 1760 mg/kg). The NOEL was < 398 mg/kg, since there was a consistent reduction in body weight throughout the post-dosing period, compared to the control group (not determined statistically).

Comments

The study was well performed and reported, and acceptable, however not in compliance with GLP. The NOEL could not be determined as there were dose-related sublethal symptoms even in the lowest treatment group.

The acute toxicity of technical clopyralid (purity of 96.9 %) to the Mallard Duck was assessed after oral dosing for 21 days. The acute oral LD50 value was 1465 mg/kg bw with confidence limit of 1220 to

1760 mg/kg. The NOEL was <398 mg/kg, since there was a consistent reduction in body weight throughout the post-dosing period, compared to control group (not determined statistically).

RMS comments and evaluation:

The study was well performed and reported, and acceptable, however not in compliance with GLP. The study was conducted prior to the formal introduction of GLP but was assessed to appropriately follow the GLP principles.

[redacted]

The NOEL could not be determined as there were dose-related sublethal symptoms even in the lowest treatment group. No macropathology was carried out on any birds *post-mortem*. This deviation was not considered to have affected the outcome of the study. As this study gave the lowest endpoint value (LD_{50} 1465 mg a.s./kg bw), it was considered as critical for the avian risk assessment. To avoid unnecessarily repeating vertebrate studies, this study is still considered as adequate, and the previous conclusion is still valid to use this endpoint in the avian risk assessment.

The data requirement is considered as fulfilled, and no further data on the acute toxicity of clopyralid on birds is required.

Additionally, the Notifier submitted another study on the effects of the new representative formulation GF-1374 to bobwhite quail (CP 10.1.1.1; [redacted] 2005; DAS Study ID 040261). The study is reviewed under Chapter B.9.1.1 of Vol. 3 CP of the formulation GF-1374. The submission of this vertebrate study was justified since it was conducted after the Annex I inclusion of clopyralid and using the new lead formulation, although its outcome is not a critical endpoint and therefore not used for the avian risk assessment. Overall, the endpoint for the formulated product does not change the overall conclusion of low risk to avian species from exposure to clopyralid.

B.9.1.1.2. Short-term dietary toxicity to birds

This is no longer required under Regulation 1107/2009. However, in the DAR (2003) two studies were available and assessed, as presented below.

Study 1 – Mallard duck – J65

Report: [redacted] (2001b): Clopyralid technical: Dietary toxicity (LC_{50}) to the Mallard duck. Dow AgroSciences, unpublished report No. GHE-T-1098. HLS Study ID: DOS 163/003537, 15 March 2001. Ref. J65

Guidelines: OECD 205 (1984)

GLP: Yes. Laboratory certified by United Kingdom Good Laboratory Practice Monitoring Authority, [redacted].

Methodology:

Clopyralid technical, Batch No. RMM 2373, Purity 95.8% w/w. The short-term dietary toxicity of clopyralid to the Mallard duck was assessed.

Groups of ten Mallard ducks, *Anas platyrhynchos*, approximately 8 days old, were fed clopyralid in the diet (standard chick diet) for five consecutive days at the following nominal concentrations: 0 (control, chick diet only), 156, 313, 625, 1250, 2500 and 5000 ppm (mg clopyralid/kg diet). Clopyralid was added directly to a pre-mix and diluted with the diet.

Birds were housed indoors in floor pens, according treatment group at 22 to 28°C and 53% relative humidity with a photoperiod of 14 hours. Samples from the 156 and 5000 ppm diet preparations were taken for analysis of homogeneity and stability of clopyralid by UV-HPLC. Birds were observed daily and at frequent intervals during the treatment and post-treatment periods. Group mean body weights were recorded on days -3, 0 (immediately before introduction of the test diets), 5 and 8. Group mean food consumption was measured over days –3 to 0, 1 to 5 (daily) and 6 to 8. Any bird which died during the study, and all birds from the highest treatment group and one control group, were macroscopically examined *post-mortem*.

Findings:

There were no mortalities and all birds remained in good health throughout the test. The excreta of each group appeared normal.

There was no evidence of any treatment-related effects on body weight or food consumption. There were no abnormalities in any bird examined *post mortem*.

The measured concentrations of clopyralid in the test diets deviated in average 5.5 % from the nominal concentrations. The diets were considered to be homogeneous and stable during ambient storage for eight days following fresh preparation and one day following freezer storage for five days, representing the maximum time from preparation to completion of use. The results of the study are summarised in Table 9.1-6.

Table 9.1-4. Body weights of Mallard duck fed clopyralid in the diet for 5 days

Nominal concentration of clopyralid in the diet (ppm)	**Group mean body weight (g)**				**Group mean body weight change (g)**		
	Day -3	**Day 0**	**Day 5**	**Day 8**	**Day -3 to 0**	**Day 0 to 5**	**Day 5 to 8**
Control	65	108	220	310	43	112	90
Control	66	108	227	315	42	119	88
156	65	106	221	309	41	115	88
313	66	111	240	328	45	129	88
625	66	115	244	336	49	129	92
1250	65	108	221	310	43	113	89
2500	65	113	228	316	48	115	88
5000	66	113	229	322	47	116	93

Table 9.1-5. Food consumption of Mallard duck fed clopyralid in the diet for 5 days

Nominal concentration of clopyralid in the diet (ppm)	**Group mean food consumption (g/bird/day)**							
	Day –3 to -1	**Day 1**	**Day 2**	**Day 3**	**Day 4**	**Day 5**	**Day 1 to 5**	**Day 6 to 8**
Control	26	37	43	48	53	55	47	69
Control	29	38	44	51	54	61	50	74
156	28	34	43	47	56	58	48	69
313	34	45	50	58	57	65	55	75
625	30	39	46	54	58	58	51	72
1250	28	34	43	49	49	56	46	70
2500	32	41	51	61	66	61	56	75
5000	29	38	50	53	62	57	52	72

Table 9.1-6. Results

Endpoint	**Clopyralid (nominal) ppm**
LC_{50} (95% C.L.)	> 5000
NOEC	5000

Conclusions:

The short-term dietary LC_{50} value of clopyralid to mallard duck was greater than 5000 ppm. The NOEC was 5000 ppm, since at this concentration level there were no mortalities, symptoms of toxicity or effects on body weight or food consumption.

Comments

The study appeared well performed and reported and it was in compliance with GLP. The concentrations in the diet were verified by analysis. The study is acceptable. The dietary toxicity of clopyralid to mallard duck is low.

Study 2 – Bobwhite quail – J67

Report: [redacted] (2001a): Clopyralid technical: Dietary toxicity (LC_{50}) to the Bobwhite quail. Dow AgroSciences, unpublished report No. GHE-T-1097. HLS Study ID: DOS 162/003536, 17 May 2001. Ref. J67

Guidelines: OECD 205 (1984)

GLP: Yes. Laboratory certified by United Kingdom Good Laboratory Practice Monitoring Authority, [redacted].

Methodology:

Clopyralid technical, Batch No. RMM 2373, Purity 95.8% w/w. The short-term dietary toxicity of clopyralid to the Bobwhite quail was assessed.

Groups of ten Bobwhite quail, *Colinus virginianus*, approximately 14 days old, were fed clopyralid in the diet (standard chick diet) for five consecutive days at the following nominal concentrations: 0 (control, chick diet only), 156, 313, 625, 1250, 2500 and 5000 ppm (mg clopyralid/kg diet). Clopyralid was added directly to a pre-mix and diluted with the diet.

Birds were housed indoors in floor pens, according treatment group at 27 to 29°C and 45% relative humidity with a photoperiod of 14 hours. Samples from the 156 and 5000 ppm diet preparations were taken for analysis of homogeneity and stability of clopyralid by UV-HPLC. Birds were observed daily and at frequent intervals during the treatment and post-treatment periods. Group mean body weights were recorded on days -3, 0 (immediately before introduction of the test diets), 5 and 8. Group mean food consumption was measured over days –3 to 0, 1 to 5 (daily) and 6 to 8. Any bird which died during the study, and all birds from the highest treatment group and one control group, were macroscopically examined *post-mortem*.

Findings:

There were two mortalities, one a control bird on day 2 and the other bird from the 156 ppm treatment group which appeared subdued on day 3 and was sacrificed. There were no treatment-related mortalities and all other birds remained in good health throughout the test. The excreta of each group appeared normal.

There was no evidence of any treatment-related effects on body weight or food consumption (Tables 9.1-7 and 9.1-8). There were no abnormalities in any bird examined *post mortem*.

The measured concentrations of clopyralid in the test diets deviated in average less than 3% of nominal concentrations. The diets were considered to be homogeneous and stable during ambient storage for eight days following fresh preparation and one day following freezer storage for five days, representing the maximum time from preparation to completion of use. The results of the study are summarised in Table 9.1-9.

Table 9.1-7. Body weights of Bobwhite quail fed clopyralid in the diet for 5 days

Nominal concentration of clopyralid in the diet (ppm)	**Group mean body weight (g)**				**Group mean body weight change (g)**		
	Day -3	**Day 0**	**Day 5**	**Day 8**	**Day -3 to 0**	**Day 0 to 5**	**Day 5 to 8**
Control	16.1	21.5	30.6	38.0	5.4	9.1	7.4
Control	16.2	20.1	29.3	35.4	3.9	9.2	6.1
156	15.9	20.5	29.3	36.9	4.6	8.8	7.6
313	15.8	20.4	29.2	36.9	4.6	8.8	7.7
625	15.8	20.7	29.2	36.5	4.9	8.5	7.3
1250	15.6	20.4	29.5	35.9	4.8	9.1	6.4
2500	15.9	20.9	28.8	35.3	5.0	7.9	6.5
5000	15.9	21.2	31.1	39.1	5.3	9.9	8.0

Table 9.1-8. Food consumption of Bobwhite quail fed clopyralid in the diet for 5 days

Nominal concentration of clopyralid in the diet (ppm)	**Group mean food consumption (g/bird/day)**							
	Day –3 to –1	**Day 1**	**Day 2**	**Day 3**	**Day 4**	**Day 5**	**Day 1 to 5**	**Day 6 to 8**
Control	5.8	10.1	6.1	3.8	4.5	4.5	5.8	6.5
Control	4.1	6.2	4.3	4.1	4.1	4.8	4.7	7.7
156	4.2	6.4	3.5	3.7	3.0	4.3	4.2	5.5
313	4.2	5.8	4.7	4.7	4.8	5.4	5.1	6.2
625	4.1	6.1	4.1	3.9	4.1	4.1	4.5	6.3
1250	4.4	7.5	4.4	3.9	4.8	3.7	4.9	5.8
2500	4.5	7.1	4.6	3.8	3.9	3.7	4.6	5.7
5000	4.3	6.4	5.3	5.2	4.9	5.3	5.4	6.9

Table 9.1-9. Summary of results with bobwhite quail

Endpoint	**Clopyralid (nominal) ppm**
LC_{50} (95% C.L.)	> 5000
NOEC	5000

Conclusions:

The short-term dietary LC_{50} of clopyralid to Bobwhite quail was greater than 5000 ppm. The NOEC was 5000 ppm, since at this concentration level there were no mortalities, symptoms of toxicity or effects on body weight or food consumption.

Comments

The study was well performed and reported and in compliance with GLP. The actual concentrations in the diet were verified by measurements. The study is acceptable. The dietary toxicity of clopyralid to bobwhite quail is low.

RMS comments and evaluation:

According to the Commission Regulation 283/2013 the dietary toxicity is only required if the mode of action or results from mammalian studies indicate a potential for the dietary LD_{50} measured by the short- term dietary toxicity study to be lower than the LD_{50} based on an acute oral study. The justification of the Notifier for not providing any data for this endpoint is agreed and acceptable and no further studies are required to support the renewal for the approval of clopyralid.

B.9.1.1.3. Sub-chronic toxicity and reproduction to birds

Data to address this point were presented in the dossier submitted in 30 April 2002 for the Approval of clopyralid and were deemed acceptable following evaluation and peer review at EU level, and

therefore the study details are presented here only briefly. These data are still valid for decision making and are used in the avian long-term risk assessment of clopyralid.

CA 8.1.1.3/1 - Lontrel Herbicide: A One-Generation Reproduction Study with the Mallard (*Anas platyrhynchos*) - Final Report.

Report	[IIA 8.1.3/01] [redacted] 1985
Report title	Lontrel Herbicide: A One-Generation Reproduction Study with the Mallard (*Anas platyrhynchos*) - Final Report.
DAS Study number	103-235; Ref. J31
Guidelines	US EPA OPP 71-4 OECD 206
GLP	Yes

Methodology:

Clopyralid (termed 'Lontrel T' in the report), Batch No. AGR 192532, Purity 96.7%. The effects of clopyralid on the reproduction of the Mallard duck, *Anas platyrhynchos*, was assessed over a 5 month period.

Three treatment groups, each comprising 16 pairs of 24-week old birds (one pair per pen), weighing 1031 to 1241 g (group means) were fed diets *ad libitum*, containing clopyralid, at nominal concentrations of 100, 330 or 1000 mg/kg diet for 20 weeks. The control group were fed diet containing corn oil equivalent to the amount in the clopyralid treated diets (0.03%). Hatchlings were fed untreated diet for 14 days.

The photoperiod was 8 hours (129 lux) for the first 3 weeks of acclimation and 8 weeks of pre-photostimulation and was thereafter increased to 17 hours light for pre-egg-laying for 3 weeks and egg laying for 8 weeks. Adults were maintained at an average temperature of 17°C and 84% relative humidity, hatchlings were maintained at an average temperature of approximately 38°C until 5 days old then were held at ambient room temperature. Eggs were incubated at 37.5°C and 56% relative humidity.

The following parameters were assessed: adult toxicity and body weight, adult food consumption, eggs laid, cracked eggs laid, viable embryos (day 14 and 24), hatchling number, surviving hatchlings, hatchling body weight and egg shell thickness. Birds found dead during the study and all adults were necropsied at the end of the study. Samples of the control and dosed diets were taken for analysis by HPLC, immediately after mixing and from feeders at the end of 7-day feeding periods in weeks 1, 9 and 18. Samples were frozen immediately after collection before shipping for analysis (report Ref. J08).

Findings:

Feed samples collected immediately after preparation contained 89 to 107% of nominal concentrations of test substance and samples collected after 7 days in the feeder contained 69 to 91% of nominal concentrations.

Two mortalities were found in the 100 mg clopyralid/kg diet group; one hen was found dead during week 2 and one in week 11. Upon gross necropsy both hens had feather loss and external lesions as a result of battering by pen mates. No other mortalities occurred during the study. There were no treatment-related symptoms of toxicity observed during the study. Upon gross necropsy, all overt lesions appeared unrelated to treatment.

There were no statistically significant, treatment-related effects on adult body weight or food consumption (Tables 9.1-10 and 9.1-11). There were no statistically significant differences in reproductive parameters, egg shell thickness and hatchling and 14-day surviving chick body weights between the control and clopyralid-treatment groups (Table 9.1-12). The results are summarised in Table 9.1-13.

Table 9.1-10. Adult Mallard duck body weights following dietary exposure to clopyralid over a 20 week period

Nominal clopyralid concentration (mg/kg diet)		**Mean body weight (g)**						
		Week 0	**Week 2**	**Week 4**	**Week 6**	**Week 8**	**Term**	**Total mean change**
Control	M[a]	1241	1209	1235	1217	1235	1074	-168
	F	1089	1057	1069	1068	1111	1055	-34
100	M	1222	1191	1203	1187	1207	1060	-162
	F	1031	997	1023	1032	1071	1031	-4
330	M	1201	1168	1199	1177	1193	1068	-134
	F	1062	1033	1053	1041	1086	996	-66
1000	M	1238	1214	1228	1203	1209	1042	-196
	F	1052	1019	1024	1036	1069	1047	-4

[a] M: males, F: females.

Table 9.1-11. Adult Mallard duck food consumption following dietary exposure to clopyralid over a 20 week period

Week	**Food consumption (g/bird/day)**			
	Control	**100 mg/kg diet**	**500 mg/kg diet**	**1000 mg/kg diet**
1	80	84	84	84
2	89	85	85	88
3	99	96	106	101
4	89	92	103	94
5	91	94	95	93
6	83	90	96*	86
7	110	116	119	113
8	106	109	115	106
9	111	129	130*	128
10	101	111	110	109
11	119	126	129	117
12	163	172	166	160
13	160	170	172	159
14	167	177	170	163
15	163	171	164	181
16	184	180	167	182
17	180	175	173	178
18	168	178	162	183
19	173	174	174	171
20	131	133	124	130

* statistically different from the control at $p<0.05$

Table 9.1-12. Reproduction and offspring growth parameters of Mallard duck following dietary exposure of adults to clopyralid over a 20 week period

Parameter	**Reproduction data and offspring growth parameters**			
	Control	**100 mg/kg**	**500 mg/kg**	**1000 mg/kg**
Eggs laid	692	543	634	624
Eggs cracked	23	13	10	18
Eggs set	606	471	559	546
Viable embryos	546	377	502	474
Live 3-week embryos	523	362	482	456
Hatchlings	411	242	327	328
14-day survivors	407	240	324	320
Eggs laid/hen	43	39	40	39
Eggs laid/hen/day[a]	0.8	0.7	0.7	0.7
14-day survivors/hen	25	17	20	20
Mean egg thickness (mm)	0.417	0.411	0.419	0.408
Hatchling body weight (g)	37	37	37	37
14-day survivor body weight (g)	230	214	216	220

[a] Based on 56 days of egg production.

Table 9.1-13. Summary of results with mallard duck

Result	**Clopyralid (nominal) mg/kg diet**
NOEC	1000

Conclusions:

Dietary concentrations of clopyralid, up to 1000 mg/kg diet, did not cause any treatment-related mortality, overt symptoms of toxicity or effects on body weight or food consumption in adult Mallard ducks during a 20-week period. There were no apparent effects on reproduction or on the body weight of hatchlings at any test concentration. The nominal NOEC for clopyralid was 1000 mg/kg diet, the highest concentration tested.

Comments

The study was well performed and reported. The GLP statement was not signed by study director, but the quality assurance audits were reported satisfactorily. The actual concentrations of the diet were verified by analysis immediately after preparation and after storage of one week, and the results are acceptable. Therefore it is justified to base the results on the nominal concentrations in the diet. The study is acceptable.

The effects of technical clopyralid (purity of 96.7 %) on the reproduction of the Mallard Duck, *Anas platyrhynchos*, was assessed over a 5 month period. Dietary concentrations up to 1000 mg/kg diet, did not cause any treatment-related mortality, overt symptoms of toxicity or effects of body weight or food consumption in adult Mallard Ducks during a 20-week period. There were no apparent effects on reproduction or on the body weight of hatchlings at any test concentration. The nominal NOEC for clopyralid was 1000 mg/kg diet, the highest concentration tested.

RMS comments and evaluation:

Originally commented by the RMS in the DAR (2003): The study was well performed and reported. The GLP statement was not signed by study director, but the quality assurance audits were reported satisfactorily. The actual concentrations of the diet were verified by analysis immediately after preparation and after storage of one week, and the results are acceptable, however presented in a separate study report.

[redacted]

The study was considered as acceptable. As this study gave the lowest endpoint value (NOEC = 1000 mg/kg diet corresponding to 118 mg/kg bw/day), it was considered as critical for the avian long-term risk assessment. To avoid unnecessarily repeating vertebrate studies, this study is still considered as adequate, and the previous conclusion is still valid to use this endpoint in the avian risk assessment.

The data requirement is considered as fullfilled, and no further data on the sub-chronic and reproduction toxicity of clopyralid on birds is required.

B.9.1.2. Effects on terrestrial vertebrates other than birds

Data to address this point were presented in the dossier submitted in 30 April 2002 for the Approval of clopyralid and were deemed acceptable following evaluation and peer review at EU level, and therefore the study details are not presented here. These data are still valid for decision making and are used in the long-term risk assessment of clopyralid to terrestrial vertebrates other than birds.

Toxicity data on amphibians and reptiles was not available, as indicated by the Notifier. The studies mentioned in Table 9.1.14. below produced the critical endpoints used in the risk assessment on terrestrial vertebrates. A new endpoint value for the acute oral toxicity on rats of the representative formulation is proposed by the Notifier, as presented in Table 9.1.14.

Table 9.1.14. Clopyralid – Summary of effects on other terrestrial vertebrates

Data point	Test organism	Test substance	EU Agreed endpoint	Proposed new endpoint	Reference
Acute Oral Toxicity to Mammals					
CA 7.1.1-1	Rat	Clopyralid	LD_{50} > **5000** mg/kg bw[1]		[redacted], 1987; K-038252-033A
	Rat	GF-1374		LD_{50} = **3378** mg/kg bw	[redacted] 2005
Higher Tier Data on Mammals					
CA 5.5-1	Rat	Clopyralid	NOAEL = 50 mg/kg bw/day		[redacted] *et al*., 1977, [redacted] *et al*., 1978, [redacted] 1985 A2A-052
Effects on Terrestrial Vertebrate Wildlife (Birds, Mammals, Reptiles and Amphibians)					
CA 8.1.4	*Study data not presented*				
CP 10.1.3	*Study data not presented*				

[1] EFSA Scientific Report (2005) 50, 1-65
Bold values used in risk assessment

B.9.1.2.1. Acute oral toxicity to mammals

The acute toxicity to mammals is considered under point CA 5.2 and results are summarised in Table 9.1.14. above. The study details are presented and evaluated in the Mammalian toxicity section of the dRAR of clopyralid.

RMS comments and evaluation:

The acute endpoint of LD_{50} > 5000 mg/kg bw, as originally obtained in the DAR (2003) was considered as valid and still appropriate to be used in the risk assessment on terrestrial vertebrates other than birds. Additionally, because the representative formulation was changed since the first DAR of clopyralid, an acute toxicity study with the new formulation GF-1374 was submitted by the Notifier, evaluated by the RMS and considered as acceptable and valid, with an endpoint value of LD_{50} = 3378 mg/kg bw on rat. So the data is adequate and can be used in the risk assessment.

Acute toxicity of clopyralid in mammals is low, and therefore performing new studies on this endpoint is considered unwarranted in order to avoid unnecessary vertebrate testing. No further data is required.

B.9.1.2.2. Long-term and reproduction toxicity to mammals

The long term and reproductive toxicity to mammals is considered under points CA 5.5 and 5.6 and results are summarised in Table 9.1.14. above. The study details are presented and evaluated in the Mammalian toxicity section of the dRAR of clopyralid.

RMS comments and evaluation:

According to the EFSA conclusion (EFSA Scientific Report (2005) 50, 1-65), the overall NOAEL of 50 mg/kg bw/day for rat was agreed as representative for terrestrial vertebrates other than birds, as evaluated in the original DAR and peer reviewed during the first Annex I inclusion of clopyralid ([redacted] *et al*., 1977, [redacted] *et al.*, 1978, [redacted] 1985). Consultation with toxicology experts confirmed that this endpoint is still valid and should be used in the risk assessment. As the studies were originally evaluated in detail during the first Annex I inclusion, they are not considered here again.

Although the studies are old, the data is adequate for the ecotoxicological risk assessment and no further studies on vertebrates are warranted on mammals. The long term risk to terrestrial vertebrates from the use of clopyralid according to the GAP of the product GF-1374 is acceptable, and no further data are required.

B.9.1.3. Active substance bioconcentration in prey of birds and mammals

Investigation of the effects of bioconcentration in prey of birds and mammals is not required as the log Pow for active is <3. For example, clopyralid is not expected to bioaccumulate in animal tissues as indicated by a log P_{ow} of -2.63 and a fish BCF < 1.

RMS comments and evaluation:

The justification presented by the Notifier is acceptable and in line with the conclusion on clopyralid by EFSA peer review (EFSA Scientific Report (2005) 50, 1-65).

A bioconcentration study in fish ([redacted] 1982, DAS report GH-C 1577, Ref. J13) was submitted for the first Annex I inclusion of clopyralid, evaluated in the DAR (2003) and peer reviewed by the EFSA. The bioconcentration factor of <1 obtained in this study confirmed that clopyralid has no potential to accumulate in fish. Despite the deficiencies the study was considered of adequate quality. The risk of bioaccumulation of clopyralid in fish is negligible, and therefore performing a new study is considered unwarranted in order to avoid unnecessary vertebrate testing. No further data is required.

B.9.1.4. Other data on effects on terrestrial vertebrate wildlife (birds, mammals, reptiles and amphibians)

In absence of standard guidelines and validated methods for amphibian and reptiles, the assessment should be based on any existing relevant information. As such, there are not any publications or studies available in the literature pertaining to the toxicity of clopyralid on amphibians and reptiles.

RMS comments and evaluation:

The justification presented by the Notifier is acceptable and agreed on. Based on all vertebrate data available, clopyralid is not anticipated to be toxic to amphibians or reptiles, and the aquatic and terrestrial risk assessments are considered to sufficiently cover also these groups of vertebrates. As explained in the next sub-section, the potential for endocrine disruption of clopyralid is negligible, and therefore no amphibian or reptile studies were performed. No further data is required.

B.9.1.5. Potential for endocrine disruption

Clopyralid has undergone a comprehensive battery of in vivo toxicology studies to cover a broad spectrum of endocrine endpoints. This testing covered a tiered battery of acute, sub-chronic, chronic and reproductive tests. Furthermore, these studies have robust experimental designs, follow internationally accepted protocols and have a high level of replication and a long history of use in hazard identification and risk assessment. The results from these studies indicate no evidence of endocrine-mediated effects by clopyralid, as presented in Section 5 in detail.

In addition to in vivo studies, clopyralid is included in the U.S. Endocrine Disruption Screening program. In vitro study results for endocrine endpoints are summarized in the EDSP21 Dashboard (http://actor.epa.gov/edsp21/). Specifically, various in vitro assays were conducted for its estrogenic, androgenic, and thyroid activities. Clopyralid does not show any bioactivity in estrogen receptor (ER)-, androgen receptor (AR)-, or thyroid receptor (ThR)-related assays. Clopyralid (cytotoxic limit = 161.9274 µM) was negative in 14/15 ER assays (not tested in 3 assays). Although it was positive in one ER assay Tox21_ERa_LUC_BG1_Agonist with an AC50 of 46.3512 µM, this is likely a non-specific outcome, because only one ER assay was positive. Clopyralid also was negative in 8/8 AR assays (not tested in 3 assays), and negative in 3/3 TR assays (not tested in 1 assay). ToxCast Model Predictions for bioactivity for clopyralid were 0 for all measures including ER agonist and antagonist AUCs and AR agonist and antagonist AUCs (all equaled 0).

Studies specific to evaluating endocrine disruption in terrestrial vertebrate species were not conducted. However, chronic reproductive studies with the avian species, northern bobwhite quail, demonstrated no effects on the reproductive performance at any test concentrations. Since no reproductive effects were observed, it is unlikely that clopyralid is inducing an endocrine effect in the bobwhite quail. This finding is consistent with what was documented in mammalian species where clopyralid did not directly impact the endocrine system (estrogen receptor, androgen receptor, etc.) of vertebrate species.

Overall, robust and consistent in vivo and in vitro data provides no evidence of endocrine disrupting properties of clopyralid so further ecotoxicological studies with reptiles and amphibians were not conducted.

RMS comments and evaluation:

The explanations and justification presented by the Notifier are acceptable and agreed. The data available with birds and mammals indicate no evidence of endocrine-mediated effects of clopyralid. No further data is required.

B.9.1.6. Overall conclusions

Considering the worst-case shortcut values of the screening risk assessment, all TER_A values are in excess of their corresponding Annex VI trigger of 10, indicating acceptable acute risks to birds and mammals after application of GF-1374 at rates up to 120 g clopyralid/ha on grassland and 80 g clopyralid/ha on cereals. For long term exposure assessment of birds and mammals, application scenarios for grassland and cereals both resulted in TER_{LT} values greater than the Annex VI trigger of 5 indicating low long-term risk to birds and mammals. Overall acute and chronic risks are low for applications of clopyralid to both grassland and cereals at proposed use rates.

RMS comments and evaluation:

The conclusion of the Notifier is agreed, that the acute and long-term risks of clopyralid are negligible to birds and mammals if the representative formulation GF-1374 is used according to GAP. No further data is required. The risk assessment to birds and mammals is presented in the dRAR Vol. 3 Section CP_20, Chapter 9.2.

B.9.2. EFFECTS ON AQUATIC ORGANISMS

A summary of the critical endpoints derived from the aquatic toxicity studies on clopyralid is presented in Table 9.2.1. Main part of the studies was included in the original dossier for the Annex I inclusion presented by the Notifier in 2002, evaluated by the RMS and peer reviewed by the EFSA and considered still as valid, and therefore those studies are only briefly presented here again. Instead, the new studies submitted to support the renewal of approval of clopyralid, with proposed new endpoints, as shown in Table 9.2.1, are presented in detail below. In addition to studies owned by the Notifier, several studies published in the scientific literature are included, and evaluated in respective sub-chapters below.

Table 9.2.1. Clopyralid – Summary of effects on aquatic organisms

Data point	Test organism	Test substance	EU Agreed endpoint[1]	Proposed new endpoint	Reference
CA 8.2.1/1	Rainbow trout *(Oncorhynchus mykiss Walbaum)*	Clopyralid	**LC_{50} > 99.9 mg a.s./L**		[redacted] 2000; DAS report no. 001024, Ref. J49
CA 8.2.2.1/1	Fathead minnow *(Pimephales Promelas Rafinesque)*	Clopyralid	**NOEC = 10.8 mg a.s./L**		[redacted] 2000; DAS report no. 001017, Ref. J48
CA 8.2.2.3/1	Bluegill sunfish *(Lepomis macrochirus)*	Clopyralid	**BCF < 1**		[redacted] 1982; DAS report no. GH-C 1577, Ref. J13
CA 8.2.4.1/1	Daphnia *(Daphnia magna Strauss)*	Clopyralid	**EC_{50} > 99.0 mg a.s./L**		Marino, T. A., McClymont, E. L. & Staley, J. L. 2000; DAS report no. 001025, Ref. J52
CA 8.2.5.1/1	Daphnia *(Daphnia magna)*	Clopyralid	**NOEC = 17 mg/L**		Douglas, M. T., Bell, G. & Macdonald, I. A. 1992¸ DAS report no. DWC 615/911087, Ref. J35
CA 8.2.5.3/1	Midge *(Chironomus riparius)*	Clopyralid	**NOEC = 50 mg/L**		Barrett, K. 2001; DAS report no. GHE-T-1122, Ref. J66
CA 8.2.6.1/1	Freshwater green algae *(Selenastrum capricornutum)*	Clopyralid (mg a.s./L)	E_rC_{50} = 30 mg a.s./L		Kirk, H. D., Gilles, M. M., McClymont, E. L. & McFadden, L. G., 2000; DAS report no. 001040, J51
CA 8.2.6.2/1	Freshwater blue algae *(Anabaena flos-aquae)*	clopyralid technical (mg/L)		**E_rC_{50} = 22 mg a.s./L**	Hoberg, J. R., 2006; DAS report no. 060246
CA 8.2.6.2/2	Freshwater diatom *(Navicula pelliculosa)*	clopyralid technical (mg/L)		E_rC_{50} = 31.3 mg a.s./L	Aufderheide, J., 2015; DAS report no. 140515
CA 8.2.7/1	Duckweed *(Lemna gibba)*	Clopyralid (mg a.s./L)	EC_{50} = 89 mg a.s./L		Cowgill, U.M., Milazzo, D.P. & Potter, R.B. 1990; DAS report no. ES-2243, Ref. J28
CA 8.2.7/2	Aquatic macrophyte *(Myriophyllum spicatum)*	Clopyralid (mg/L)		**E_rC_{50} > 3.0 mg a.s./L**	Banman, C. S. & Moore, S., 2015; DAS report no. 140735

[1] EFSA Scientific Report (2005) 50, 1-65
Bold values used in risk assessment

B.9.2.1. Toxic effects to fish

B.9.2.1.1. Acute toxicity to fish

Data to address this point were presented in the dossier for the Approval of Clopyralid submitted in 30 April 2002, evaluated in the DAR (2003) and peer review at EU level. The data were deemed acceptable following evaluation, and are still valid for decision making. Therefore the study below was not evaluated in detail here again, but a brief summary is provided for convenience.

CA 8.2.1/1 - Clopyralid Technical Acute Toxicity To Fish

Report	[IIA 8.2.1./01], [redacted] 2000
Report title	Clopyralid: An Acute Toxicity Study with the Rainbow Trout, *Oncorhynchus mykiss* Walbaum
DAS Study number	001024. Ref. J49
Guidelines	OECD 203 EU Method C.1. US EPA FIFRA 540/9-85-006, 72-1
GLP	Yes

Methodology:

In a range finding test, five fish were exposed to nominal concentrations of 0 (control), 10 and 100 mg clopyralid/L. No mortalities or sublethal effects were observed after 96-hours exposure. For the definitive test, 10 fish (31 mm average length) were placed in each of three replicates of a control (laboratory dilution water) and 100 mg clopyralid/L treatment. The test vessels were 12 L glass beakers containing 10 L test medium. Clopyralid was added directly to the laboratory dilution water using sonication to aid dissolution. The test media were not replaced during the exposure period.

Fish were observed for mortality and sublethal effects at 24, 48, 72 and 96 hours and were not fed during the study. Dissolved oxygen, pH and temperature were recorded in each test vessel at 0, 24, 48, 72 and 96 hours. Dissolved oxygen averaged 84% (ASV), temperature 12.5 to 12.9°C and pH 5.6 to 7.4. On days 0 and 4, samples of test medium, from each test vessel, were taken for analysis of clopyralid by HPLC/UV.

Findings:

The mean measured concentration of clopyralid in the dosed vessels was 99.8 mg/L at the start and 100 mg/L at the end of the test (Table 9.2-2). No mortality or sublethal effects were observed in the control or 100 mg clopyralid/L treatments. The results are summarised in Table 9.2-3.

Table 9.2-2. Measured concentrations of clopyralid during an acute toxicity test with *O.mykiss*

Nominal concentration of clopyralid (mg/L)	**Measured concentration (mg/L)**		**Mean measured concentration (mg/L)**
	Day 0	**Day 4**	
Control	< LOQ	< LOQ	-
100	99.8	100	99.9

<LOQ: below the limit of analytical determination (9.92 mg clopyralid/L).

Table 9.2-3. Summary of results with rainbow trout

Result	Measured concentration of clopyralid (mg/L)
96-h LC_{50}	> 99.9
NOEC	≥ 99.9

Conclusions:

In a limit test, under static test conditions, the 96-hour LC_{50} of clopyralid to *Oncorhynchus mykiss* was > 99.9 mg/L, measured. The NOEC was ≥ 99.9 mg/L, measured.

Comments

The test was conducted using the limit test procedure, and therefore the exact LC_{50} could not be found. However, as the acute toxicity of clopyralid to rainbow trout appears to be so low, it is not necessary to perform a dose-response study. This study is acceptable as it is well performed and reported and in compliance with GLP. The result can be used in the risk assessment.

In a limit test, under static conditions, the 96-hour LC_{50} of clopyralid to Rainbow Trout, *Oncorhynchus mykiss*, was >99.9 mg/L, measured. The NOEC was ≥ 99.9 mg/L, measured.

RMS comments and evaluation:

Originally commented by the RMS in the DAR (2003): The test was conducted using the limit test procedure, and therefore the exact LC_{50} could not be defined. However, as the acute toxicity of clopyralid to rainbow trout appears to be so low, it is not necessary to perform a dose-response study. This study is acceptable as it is well performed and reported and in compliance with the GLP. The result can be used in the risk assessment.

The original specification of clopyralid is based on the batch used in this study. Based on the risk assessment for the impurities of clopyralid presented by the Notifier in Document J in December 2016, no impurity characterised in this batch is expected to substantially impact the ecotoxicological profile of clopyralid. The impurity profile of the test substance used in this study was within the limits of the new specification presented by the Notifier in December 2016.

The assessment above as given in the DAR (2003) is still valid and the endpoint of LC_{50} > 99.9 mg a.s./L can still be used in the risk assessment of clopyralid. No further fish studies are required.

Two more studies with another fish species were available and assessed in the DAR (2003), but not referred by the Notifier for the renewal of approval of clopyralid. The studies are presented below.

Study 2 – Bluegill sunfish – J50

Report: [redacted] (2000): Clopyralid: An acute toxicity study with the bluegill sunfish, *Lepomis macrochirus* Rafinesque Dow AgroSciences, unpublished report No. 001026. Dow Chemical Company Study ID: 001026, 1 June 2000. Ref. J50

Guidelines: OECD 203, EU C.1., US EPA FIFRA 540/9-85-006, 72-1.

GLP: Yes. Laboratory inspected by United States EPA, [redacted].

Methodology:

Clopyralid, Lot No. 910905 5P, Purity 96.9%. The acute toxicity of clopyralid to bluegill sunfish (*Lepomis macrochirus*) was assessed over a 96-hour exposure period, under static test conditions.

In a range finding test, five fish were exposed to nominal concentrations of 0 (control), 10 and 100 mg clopyralid/L. No mortalities or sublethal effects were observed after 96-hours exposure. For the definitive test, 10 fish (21 mm average length) were placed in each of three replicates of a control (laboratory dilution water) and 100 mg clopyralid/L treatment. The test vessels were 12 L glass beakers containing 10 L test medium. Clopyralid was added directly to the laboratory dilution water using sonication to aid dissolution. The test media were not replaced during the exposure period.

Fish were observed for mortality and sublethal effects at 24, 48, 72 and 96 hours and were not fed during the study. Dissolved oxygen, pH and temperature were recorded in each test vessel at 0, 24, 48, 72 and 96 hours. Dissolved oxygen averaged 85% (ASV), temperature 22.2 to 22.6°C and pH 5.7 to 7.4. On days 0 and 4, samples of test medium, from each test vessel, were taken for analysis of clopyralid by HPLC/UV.

Findings:

The mean measured concentration of clopyralid in the dosed vessels was 103 mg/L at the start and 100 mg/L at the end of the test (Table 9.2-4). No mortality or sublethal effects were observed in the control or 100 mg clopyralid/L treatments. The results are summarised in Table 9.2-5.

Table 9.2-4. Measured concentrations of clopyralid during an acute toxicity test with *L. macrochirus*

Nominal concentration of clopyralid (mg/L)	**Measured concentration (mg/L)**		**Mean measured concentration (mg/L)**
	Day 0	**Day 4**	
Control	< LOQ	< LOQ	-
100	103	100	102

<LOQ: below the limit of analytical determination (9.92 mg clopyralid/L).

Table 9.2-5. Summary of results with *L. macrochirus*

Result	**Measured concentration of clopyralid (mg/L)**
96-h LC_{50}	> 102
NOEC	≥ 102

Conclusions:

In a limit test, under static test conditions, the 96-hour LC_{50} of clopyralid to *Lepomis macrochirus* was > 102 mg/L, measured. The NOEC was ≥ 102 mg/L, measured.

Comments

Similarly to the test with rainbow trout, this test was conducted using the limit test procedure, and therefore the exact LC_{50} could not be found. However, as the acute toxicity of clopyralid to bluegill sunfish appears to be so low, it is not necessary to perform a dose-response study. This study is acceptable as it is well performed and reported and in compliance with GLP. The result can be used in the risk assessment.

Study 3 – Lontrel 100 – Rainbow trout – J17

Report: [redacted](1989): Lontrel 100: Determination of acute toxicity (LC_{50}) to rainbow trout (96h, static). Dow AgroSciences, unpublished report No. IRI 140485 & IRI 140731, 20 August 1989. J17

Guidelines: EC Method C.1, Directive 92/69 and OECD Guideline No. 203.

GLP: Yes. Laboratory certified by United Kingdom Good Laboratory Practice Monitoring Authority, [redacted]

Methodology:

The acute toxicity of Lontrel 100 (EF-255) to rainbow trout (*Oncorhynchus mykiss*) was determined in a 96h test conducted under static conditions according to OECD/EU guidelines in compliance with GLP. EF-255 is a soluble concentrate (SL) preparation of clopyralid monoethanolamine [redacted] at 100 g clopyralid/L, nominal (EF-255 differs from EF-1136 [redacted]. The test was carried out with material from Batch No. EB 880714-T1128 with an actual clopyralid content of 10.6% w/w.

Rainbow trout (*Oncorhynchus mykiss*) of mean weight 1.76 g and mean standard length of 48.4 mm was assessed under static exposure conditions over a period of 96 hours. Groups of ten fish were exposed to EF-255 at nominal concentrations of 62.5, 125, 250, 500 and 1000 mg product/L. The test vessels were 25 litre glass aquaria containing 20 litres media at a loading rate of 0.088 g body weight/L static volume. The test was conducted at nominally 14±1°C (actual range 13.3-15.2 °C) under a 16h light. 8h dark photoperiod in charcoal filtered dechlorinated tap water with a hardness of 88 mg/L as $CaCO_3$. Dissolved oxygen levels were 80 to 97% of the air saturation value during the study and pH values ranged from 8.2 to 8.5.

Exposure levels in the test media were monitored by measuring the concentrations of clopyralid in water samples collected at 0, 24 and 96 hours. The samples were analysed using reverse phased HPLC with UV detection at 280 nm. Mean measured concentrations of clopyralid remained essentially at nominal levels throughout the entire study with deviations within the range –2.5% to +1.2% of nominal at all exposure levels. Although results are expressed in terms of nominal concentrations, values based on measured values would be virtually identical.

Findings:

Mortality ranged from 50% at 500 mg product/L to 100% at 1000 mg product/L (see Table 9.2-6). Prior to death, fish at 1000 mg product/L became darkened in appearance and exhibited unusual swimming behaviour, including loss of equilibrium, within 1 hour of exposure. Fish at 500 mg product/L also appeared darker in colour and exhibited erratic and laboured swimming behaviour, including loss of equilibrium. No unusual appearance or behaviour was noted in fish exposed to 250 mg product/L and below.

Based on the incidence of mortality and sub-lethal effects at 500 mg product/L and above, the NOEC was considered to be 250 mg product/L (equivalent to 26.5 mg ae/L, based on 10.6% w/w clopyralid). Probit analysis of the mortality data gave the results presented in Table 9.2-7.

Table 9.2-6. Mortality data of rainbow trout

Nominal concentration*	**Mortality (%)**					
(mg EF-255/L)	**3 h**	**6 h**	**24 h**	**48 h**	**72 h**	**96 h**
Control	0	0	0	0	0	0
62.5	0	0	0	0	0	0
125	0	0	0	0	0	0
250	0	0	0	0	0	0
500	0	10	40	50	50	50

1000	100	100	100	100	100	100

* essentially identical to mean measured concentrations

Table 9.2-7. Summary of results with rainbow trout

Result	**mg EF-255/L (nominal)**
24h LC_{50} (95% c.l.)	532.0 (396.2-726.7)
48h LC_{50} (95% c.l.)	500.0 (369.1-677.3)
72h LC_{50} (95% c.l.)	500.0 (369.1-677.3)
96h LC_{50} (95% c.l.)	500.0 (369.1-677.3)
NOEC	250

Conclusions:

The 96h LC_{50} of EF-255 (equivalent to EF-1136) to rainbow trout (*Oncorhynchus mykiss*) under static conditions is 500 mg product/L, nominal (53 mg ae/L). The NOEC is 250 mg product/L, nominal (26.5 mg ae/L). These results indicate that the toxicity of clopyralid to fish is not substantially altered when formulated as EF-255.

Comments

The formulation studied (EF-255) is different from the lead formulation EF-1136 for Annex III studies. However, the Notifier clarifies the difference between the two formulations acceptably. The difference in the composition of the two formulations is of minor importance and therefore the result of this study can be used in the risk assessment of EF-1136. The study was well performed and reported and in compliance with GLP. The results are based on nominal concentrations of the test substance, but the concentrations were verified by analysis and therefore it is acceptable to use the nominal values.

B.9.2.1.2. Long-term and chronic toxicity to fish

Data to address this point were presented in the dossier for the Approval of Clopyralid submitted in 30 April 2002, evaluated in the DAR (2003) and peer review at EU level. The data were deemed acceptable following evaluation, and are still valid for decision making. Therefore the study below was presented only very briefly here again.

CA 8.2.2.1/1 - Clopyralid Technical Chronic Toxicity To Fish

Report	[IIA 8.2.2/01], [redacted]. 2000
Report title	Clopyralid: Toxicity to the Early Life Stages of the Fathead Minnow, *Pimephales Promelas* Rafinesque.
DAS Study number	001017, Ref. J48
Guidelines	OECD 210 US EPA FIFRA EPA-540/86-138 ASTM Standard E 1241-92
GLP	Yes

Methodology:

Clopyralid, Lot No. 910905 5P, Purity 96.9%. The effects of clopyralid on the early-life stages of the fathead minnow, *Pimephales promelas* Rafinesque were assessed over a 34-day exposure period under flow-through test conditions.

Healthy, post fertilised, non-eyed embryos, approximately 25 to 45 hours old, were exposed to the following nominal concentrations of clopyralid: 0 (control), solvent control (acetone), 0.78, 1.30, 2.16, 3.60, 6.00 and 10.0 mg/L. For each exposure concentration and the control (laboratory dilution water) there were four replicate aquaria each containing 850 mL of test medium and 25 embryos per replicate. Clopyralid was prepared in stock solutions with acetone before dilution and mixing with laboratory dilution water and entry to the test aquaria. During the test, the diluter system provided an average of 4.1 volume changes per 24-hour period. Embryos were incubated in circular cups suspended in a cylindrical glass incubation chamber supported by glass beads within the aquarium. Flow from the delivery tubes was directed in and around the incubation cups.

Dissolved oxygen, pH and temperature were recorded on test days 0, 7, 14, 21, 28 and 34 in each aquarium containing surviving fish. The test conditions were: hardness 62 to72 mg $CaCO_3$/L, pH 6.1 to 8.0, temperature 24.3 to 25.7°C and dissolved oxygen 50 to 115% (ASV).

Embryos and larvae were observed and counted daily. Dead or diseased embryos and larvae were removed at each observation. On day 2, embryos were thinned to 20 per replicate. After hatching was completed, total larvae were counted in each replicate. Larvae were observed for sub-lethal effects on days 3, 10, 17, 24 and 30 post-hatch and at test termination. Test fish, within two days of hatching, were fed brine shrimp twice daily and green algae was used to supplement the diet. At test termination, all surviving fish were sacrificed for weight and standard length measurements. Test media were sampled for analysis of clopyralid from one aquarium per treatment and samples were taken from the mixing chamber and diluter feedstocks on days 0, 7, 15, 21, 28 and 34. Samples were analysed using HPLC/UV.

Findings:

Concentrations of clopyralid in the test media averaged 104 to 114% of nominal (Table 9.2-8).

No statistically significant ($p < 0.05$) effects, for any endpoint, compared to controls were observed up to the highest test mean measured concentration of 10.8 mg clopyralid/L (Tables 9.2-9 and 9.2-10).

On day 33 of exposure, a portion of the laboratory water was diverted resulting in a decline in dissolved oxygen from a range of 75 to 115% to 50 to 81% (ASV). This caused larval mortalities which were sporadic and not treatment-related. However, by this time the exposure duration was 30 days after mean hatch and the control fish survival was > 70% thereby maintaining compliance with the OECD 210 validity criteria. The results are summarised in Table 9.2-11.

Table 9.2-8. Measured concentrations of clopyralid in test media during a 34-day early life-stage study with *P. promelas*

Nominal concentration of clopyralid (mg/L)	**Measured concentrations of clopyralid (mg/L)**						
	Day 0[a]	**Day 7**	**Day 15**	**Day 21**	**Day 28**	**Day 34[a]**	**Mean (% of nominal)**
0 (control)	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	-
0.78	0.906	0.908	0.816	0.905	0.883	0.837	0.876 (112)
1.30	1.43	1.46	1.38	1.42	1.36	1.43	1.41 (109)
2.16	2.46	2.44	2.69	2.43	2.33	2.39	2.46 (114)
3.60	3.83	3.87	3.68	3.92	3.62	3.78	3.78 (105)
6.00	6.28	6.40	6.22	6.36	6.03	6.21	6.25 (104)

10.0	10.3	10.6	13.1	10.9	10.0	10.2	10.8 (108)

[a] Mean of four replicate values.
< LOQ: lower than the lowest level quantified of 0.065 mg clopyralid/L.

Table 9.2-9. Summary of mean effects on the early life-stages of *P. promelas* after exposure to clopyralid for 34 days

Nominal conc. of clopyralid (mg/L)	**% pre-thinning survival**	**% embryos hatched**	**Day-to-mean hatch (days)**	**% normal at hatch**
0 (control)	100	100	3.5	95
0.78	100	100	3.3	98
1.30	100	100	4.0	96
2.16	100	100	3.8	98
3.60	100	100	3.5	99
6.00	100	100	3.3	96
10.0	100	100	3.8	100

No endpoint showed a statistically significant effect ($p<0.05$).

Table 9.2-10. Summary of mean effects on the early life-stages of *P. promelas* after exposure to clopyralid for 34 days (continued)

Nominal concentration of clopyralid (mg/L)	**% post thinning survival**	**% overall survival**	**Dry weight (mg)**	**Standard length (mm)**
0 (control)	75	75	2.48	9.8
0.78	78	78	2.69	10.2
1.30	71	71	2.60	9.9
2.16	83	83	2.96	10.8
3.60	89	89	3.07	10.9
6.00	73	73	3.07	10.7
10.0	85	85	2.81	10.5

No endpoint showed a statistically significant effect ($p<0.05$).

Table 9.2-11. Summary on results of the fish early life stage test

Result	**Mean measured concentration of clopyralid (mg/L)**
NOEC	10.8
LOEC	> 10.8

Conclusions:

Following a 34-day exposure to clopyralid, under flow-through conditions, the NOEC for the early life-stages of *Pimephales promelas* was 10.8 mg/L, the highest concentration tested, based on mean measured concentrations. The LOEC was > 10.8 mg/L.

Comments

The study was well performed and reported and in compliance with GLP. The study is acceptable and the result can be used in the chronic risk assessment to fish.

The effects of technical clopyralid (purity 96.9 %) on the early -life stages of the Fathead Minnow, *Pimephales promelas* Rafinesque, were assessed over a 34-day exposure period under flow-through test conditions. No statistically significant (<p 0.05) effects compared to controls were observed up to the highest concentration tested, 10.8 mg clopyralid / liter, based on mean measured concentration, during 34 days of exposure.

RMS comments and evaluation:

Originally commented by the RMS in the DAR (2003): The test was well performed and reported and in compliance with GLP. The study is acceptable and the result can be used in the chronic risk assessment to fish.

The Notifier has presented a new specification of the technical clopyralid. The original specification of clopyralid is based on the analysis of the batch used in this study. Based on the risk assessment for the impurities of clopyralid presented by the Notifier in Document J in December 2016, no impurity characterised in this batch is expected to substantially impact the ecotoxicological profile of clopyralid. The impurity profile of the test substance used in this study is within the limits of the new specification presented by the Notifier in December 2016.

The assessment above as given in the DAR (2003) is still valid and the endpoint of NOEC = 10.8 mg a.s./L can be used in the risk assessment of clopyralid. The impurity profile of the test substance used in this study was within the limits of the specification presented by the Notifier in December 2016. No further studies on fish are required.

In addition to the studies committed and owned by the Notifier, clopyralid has been subject to independent research since its first inclusion in Annex I, and consequently, following chronic fish studies were submitted for the AIR3 evaluation of clopyralid as a result of a literature search performed by the Notifier and conducted in accordance with EFSA guidance. Relevance and reliability of articles found in the search process were appraised in adherence with EFSA guidelines (EFSA Journal 2011;9(2):2092 and EFSA supporting publication 2013:EN-511). Summaries of the studies and the assessment of the Notifier are presented below in Tables 9.2.12. and 9.2.13.

Table 9.2.12. Comparison of the relative sensitivity of rainbow trout and bull trout to three herbicides

Using accelerated life testing procedures to compare the relative sensitivity of rainbow trout and the federally listed threatened bull trout to three commonly used rangeland herbicides (picloram, 2,4-D and clopyralid)	
KCA 9/3 (CA 8.2.1)	
Author(s)	Fairchild, J.F., Allert, A.L., Sappington, L.S., Nelson, K.J., Valle, J.
Year	2008
Journal	Environ Toxicol Chem Vol. 27(3), pp. 623-30
Relevance check	Relevant. Standard acute toxicity testing although following a superseded version of the old guideline. Methodology is comprehensively described.
Reliability check	Reliability score 1 (Klimisch)
Reasons for no reliability	Not applicable
Summary	A 96-hour static acute toxicity test was performed with rainbow trout and bull trout juveniles, exposed to clopyralid, 2,4-D or picloram. After 96 hours, mortality was assessed and used for calculation of acutely lethal concentrations

	(ALC) and the estimation of chronic effect concentrations. Picloram was more toxic than 2,4-D and clopyralid. Clopyralid showed similar toxicity to both species and also a strong effect of concentration. Rainbow trout showed an ALC_{50} for clopyralid of 700 mg/L and an estimated chronic lethal concentration causing 1% mortality in a population (CLC1) of 477 mg/L. For bull trout these values were 802 mg/L and 552 mg/L, respectively. When comparing these acute and chronic values, an acute:chronic ratio was obtained, which was 1.7 for rainbow trout and 1.5 for bull trout.
Reliability check: study details	
Parameter	**Information available**
Test protocol GLP, GEP, Guidelines (US EPA, OECD, …)	- American Society for Toxicity and Materials (ASTM), E729-96, ASTM Standards, 2004, Vol 11-6, pp 79-100 - GLP/GEP not discussed
Test substance Identification of test substance, source, purity, stability	- Test substance: Clopyralid salt o CAS 001702-17-6 o Purity: 95% a.i. free acid o Water solubility: 1000 mg/L at 25ºC o Source: Dow Agrosciences, Indianapolis, USA - Two other herbicides were also tested: 2,4-D and picloram salt
Test conditions Temperature, pH, oxygen concentration, water hardness, conductivity, photoperiod, light intensity, number of animals, food availability	- Temperature: 7.6 ± 0.8ºC - Dissolved oxygen: 8.40 ± 0.72 (83 ± 7% saturation at 650 mm Hg) - pH: 8.02 ± 0.15 - Conductivity: 679 ± 1 - Alkalinity 250 ± 0mg/L as $CaCO_3$ - Hardness: 289 ± 1 mg/L as $CaCO_3$ - Total ammonia: 0.18 ± 0.08 mg/L as N - Food: Fish were not fed during the 48 hour acclimation period and during the study
Controls Positive control, negative control	- Negative control: Well water only
Dosing system Exposure (dose, duration, frequency)	- Dose: 210, 420, 840, 1680 and 3360 mg clopyralid/L - Duration: 96 hours - Frequency: Static - Replicates: two, with 10 fish per replicate test chamber
Test species Body weight or length, gender, age/life stage, source	- Species: Rainbow trout (*Oncorhynchus mykiss*) and bull trout (*Salvelinus confluentus*) - Life stage: Juveniles - Source: Aquatic Biosystems, Fort Collins, USA - Weight: 0.55 ± 0.11 g for bull trout and 0.59 ± 0.15 g for rainbow trout - Length: 42 ± 3 mm for bull trout and 41 ± 4 mm for rainbow trout
Statistical analyses Sample size/replicates, statistical analysis of data (significance level, variability)	- Acutely lethal concentrations (ALC5, ALC10, ALC20 and ALC50) were calculated using Toxcalc Software (Tidepool Scientific Software, McKinleyville, USA) - Several parameters were calculated, which are outputs of the accelerated life testing procedures (ALT) in the US Environmental Protection Agency's Acute-to-Chronic Estimation (ACE) with Time-Concentration-Effect Models program, version 2.0, including the NOEC for mortality estimated as the chronic lethal concentration calculated to cause 1% mortality in a population (CLC1) and the acute-to-chronic estimation (ACE)
Biological effects Determined effect concentration, dose response observed	- Mortality was assessed at 2, 6, 24, 48, 72 and 96 hours of exposure - No mortality was observed in the control groups of both species - Rainbow trout and bull trout were similar in acute and chronic sensitivity to clopyralid exposure, regardless of the toxicity model used - Clopyralid showed a strong concentration effect for both fish species - The ALC_{50} for clopyralid was 700 mg/L for rainbow trout and 802 mg/L for bull trout. The CLC1 values for this herbicide were 477 mg/L and 552

	mg/L for rainbow trout and bull trout, respectively. The acute-to-chronic estimation (ACE) was calculated to be 1.7 for rainbow trout and 1.5 for bull trout. - The ALC_{50} data for the 3 tested herbicides are within the range of data published by other researchers - Picloram was more acutely toxic to rainbow trout than either 2,4-D or clopyralid
Overall assessment	- Clopyralid exposure resulted in significant pH effects at the two highest test item concentrations. Therefore, the results at the highest test item concentration were not used in statistical analyses - Methods, statistical analysis, results and discussion are well documented - The study is reliable

RMS comments and evaluation:

This study was a scientific article published in a scientific journal, and performed independently from the Notifier. The study and the laboratory did not have a GLP status. Although the OECD test guideline was not exactly followed, the study was well conducted and reported, and the study protocol was acceptable.

Rainbow trout appeared to be more sensitive to clopyralid than the other species bull trout. However, due to editing restrictions of the journal in question, several issues were not reported in adequate detail to allow a comprehensive validity evaluation. For instance, the source of the test substance studied and the analytical procedures were not reported in such detail as required for PPP active substance studies. No data is available on the specification of the test substance used in this study, so the impurity profile is unknown. The concentrations of test substances were obviously measured only once during the test period (at t = 0), and therefore the verification of exposure concentrations was not adequate throughout the study period.

Overall, the study was assessed as likely reliable, given that the report was peer reviewed prior to its publication in a scientific journal, but due to several deficiencies in reporting it was assessed that the outcome is not appropriate for changing the original acute endpoint for fish toxicity, as agreed in the EFSA conclusion on clopyralid. The study can be used as supporting data.

Table 9.2.13. Acute and chronic effects of clopyralid to rainbow trout

An ecological risk assessment of the acute and chronic effects of the herbicide clopyralid to rainbow trout (*Oncorhynchus mykiss*)	
KCA 9/4 (CA 8.2.2)	
Author(s)	Fairchild, J.F., Allert, A.L., Feltz, K.P., Nelson, K.J., Valle, J.A.
Year	2009
Journal	Arch Environ Contam Toxicol Vol. 57, pp. 725-31
Relevance check	Relevant. Following a guideline and methods are described in detail.
Reliability check	Reliability score 1 (Klimisch)
Reasons for no reliability	Not applicable
Summary	A 30-day flow-through chronic toxicity test was performed with rainbow trout juveniles, exposed to clopyralid. No significant mortality was observed. However, after 30 days, growth (measured as length and weight) decreased with increasing clopyralid concentrations. NOEC and LOEC were 68 and 136 mg clopyralid/L, respectively, for both growth endpoints. The maximum acceptable toxicant concentration (MATC; geometric mean of NOEC and LOEC) was 96 mg clopyralid/L and was used to determine the acute:chronic ratio (ACR), which was 7.3.

	When toxicity values were compared to expected environmental concentrations of clopyralid, the safety factor exceeded 1000, which indicates that low risk is expected from clopyralid exposure to rainbow trout.
Reliability check: study details	
Parameter	**Information available**
Test protocol GLP, GEP, Guidelines (US EPA, OECD, …)	- American Society for Toxicity and Materials (ASTM), E1241-05, ASTM Standards, 2004, Vol 11-6, pp 79-100 - GLP/GEP not discussed
Test substance Identification of test substance, source, purity, stability	- Test substance: Lontrel 100^{TM} (Commercial formulation of clopyralid) o CAS 1072-17-6 o Purity: 9.5% a.i. free acid o Source: Dow Agrosciences, Indianapolis, USA
Test conditions Temperature, pH, oxygen concentration, water hardness, conductivity, photoperiod, light intensity, number of animals, food availability	- Temperature: 8.48 ± 0.40 ºC - Dissolved oxygen: 7.55 ± 1.07 mg/L - pH: 7.97 ± 0.17 - Conductivity: 660 ± 76 µS - Alkalinity 245 ± 12 mg/L as $CaCO_3$ - Hardness: 273 ± 12 mg/L as $CaCO_3$ - Total ammonia: 0.93 ± 0.01 mg/L as N - Photoperiod: 14 h light/10 h darkness - Light intensity: natural diurnal cycle of dampened natural sunlight - Food: Were fed twice daily at swim-up during acclimation with #1 Finfish Diet (55% protein, 15% fat; Ziegler Brothers, Gardner, PA, USA).
Controls Positive control, negative control	- Negative control: Well water only
Dosing system Exposure (dose, duration, frequency)	- Dose: 16, 32, 64, 128 and 256 mg/L clopyralid (as free acid form) - Duration: 30 days - Frequency: Flow-through (diluter delivered 0.48 L test solution to each chamber every 20 min for a total volume of 35 L per day (turnover of 4.7 volume exchanges per day)). - Replicates: 4, with 10 fish per replicate test chamber
Test species Body weight or length, gender, age/life stage, source	- Species: Rainbow trout (*Oncorhynchus mykiss*) - Life stage: Juveniles - Source: Aquatic Biosystems, Fort Collins, USA (obtained as eyed eggs) - Weight: 0.68 ± 0.10 g - Length: 43.40 ± 2.09 mm
Statistical analyses Sample size/replicates, statistical analysis of data (significance level, variability)	- Shapiro-Wilk's test and Bartlett's test were used to test the length and weight data for normality and homogeneity of variance - These growth data were thereafter analysed using one-way analysis of variance (ANOVA) - The maximum acceptable toxicant concentration (MATC) was calculated as the geometric mean of NOEC and LOEC - The acute:chronic ratio (ACR) was calculated by dividing the previously published 96-h ALC_{50} value by the MATC
Biological effects Determined effect concentration, dose response observed	- Mortality was assessed daily during the exposure period. On days 15 and 30 growth was measured using length and weight as measurement endpoints - Clopyralid concentrations were analytically confirmed in samples collected weekly from the control, low, medium and high concentrations, using ion chromatography - No significant effects of clopyralid exposure on growth was observed at day 15, but after 30 days of exposure both length and weight showed a decrease with increasing clopyralid concentration - NOEC, LOEC and MATC were 68, 136 and 96 mg/L clopyralid for both growth endpoints, and the ACR was 7.3

	- When toxicity values were compared to expected environmental concentrations of clopyralid, the safety factor exceeds 1000. This indicates that low risk is expected from rainbow trout exposure to clopyralid
Overall assessment	- Methods, statistical analysis, results and discussion are well documented - The study is reliable

RMS comments and evaluation:

This study was a scientific article published in a scientific journal, and performed independently from the Notifier. The study and the laboratory did not have a GLP status. Although the OECD test guideline was not exactly followed, the study was well conducted and reported, and the study protocol was comprehensible.

Rainbow trout (*Oncorhynchus mykiss*) appeared to be more sensitive to clopyralid than the other species bull trout (*Salvelinus confluentus*). However, probably due to editing restrictions of the journal in question, several issues were not reported in adequate detail to allow a comprehensive validity evaluation. For instance, the source of the test substance studied and the analytical procedures were not reported in such detail as required for PPP active substance studies. No data is available on the specification of the test substance used in this study, so the impurity profile is unknown. Proportional flow-through diluters were used as test devices, where the concentrations were obviously measured only once weekly from a randomly selected aquaria of each test concentration during the test period (at t = 0), and therefore the verification of exposure concentrations was not adequate throughout the study period.

Overall, the study was assessed as likely reliable, given that the report was peer reviewed prior to its publication in a scientific journal, but due to several deficiencies in reporting it was assessed that the outcome is not appropriate for changing the original chronic endpoint for fish toxicity, as a lower endpoint value was originally determined in a valid GLP study during the first Annex I inclusion process, agreed in the EFSA conclusion. The data can be used as supporting data.

B.9.2.1.3. Potential for endocrine disruption

Clopyralid has undergone a comprehensive battery of in vivo toxicology cover a broad spectrum of endocrine endpoints. This testing covered a tiered battery of acute, sub-chronic, chronic and reproductive tests. Furthermore, these studies have robust experimental designs, follow internationally accepted protocols and have a high level of replication and a long history of use in hazard identification and risk assessment. The results from these studies indicate no evidence of endocrine-mediated effects by clopyralid.

In addition to in vivo studies, clopyralid is included in the U.S. Endocrine Disruption Screening program. In vitro study results for endocrine endpoints are summarized in the EDSP21 Dashboard (http://actor.epa.gov/edsp21/). Specifically, various in vitro assays were conducted for its estrogenic, androgenic, and thyroid activities. Clopyralid does not show any bioactivity in estrogen receptor (ER)-, androgen receptor (AR)-, or thyroid receptor (ThR)-related assays. Clopyralid (cytotoxic limit = 161.9274 µM) was negative in 14/15 ER assays (not tested in 3 assays). Although it was positive in one ER assay Tox21_ERa_LUC_BG1_Agonist with an AC50 of 46.3512 µM, this is likely a non-specific outcome, because only one ER assay was positive. Clopyralid also was negative in 8/8 AR assays (not tested in 3 assays), and negative in 3/3 TR assays (not tested in 1 assay). ToxCast Model Predictions for bioactivity for clopyralid were 0 for all measures including ER agonist and antagonist AUCs and AR agonist and antagonist AUCs (all equaled 0).

Studies specific to evaluating endocrine disruption in terrestrial vertebrate species were not conducted. However, chronic reproductive studies with the avian species, northern bobwhite quail, demonstrated no effects on the reproductive performance at any test concentrations. Since no reproductive effects were observed, it is unlikely that clopyralid is inducing an endocrine effect in the bobwhite quail. This finding is consistent with what was documented in mammalian species where clopyralid did not directly impact the endocrine system (estrogen receptor, androgen receptor, etc.) of vertebrate species.

Overall, robust and consistent in vivo and in vitro data provides no evidence of endocrine disrupting properties of clopyralid so further ecotoxicological studies with reptiles and amphibians were not conducted.

Concerning aquatic vertebrates, two screening tests on fish development published in scientific literature are included in the literature search conducted by the Notifier. These studies are summarised below in Tables 9.2.14. and 9.2.15.

Table 9.2.14. Zebrafish developmental screening with clopyralid.

Zebrafish developmental screening of the ToxCast™ Phase I chemical library	
KCA 9/7 (CA 8.2.2.1)	
Author(s)	Padilla, S., Corum, D., Padnos, B., Hunter, D.L., Beam, A., Houck, K.A., Sipes, N., Kleinstreuer, N., Knudsen, T., Dix, D.J., Reif, D.M.
Year	2012
Journal	Reprod Toxicol Vol. 33(2), pp. 174-87
Relevance check	Relevant. Clopyralid is tested. No guideline is stated but methodology is comprehensively described
Reliability check	Reliability score 2 (Klimisch)
Reasons for no reliability	Not applicable
Summary	Zebrafish (*Danio rerio*) embryos (6 to 8 hours post fertilization) were exposed for 5 days to a single dose (80 µM) or a concentration range (0.001 to 80000 µM) a large group of chemicals, including clopyralid and clopyralid-olamin. Hereafter, the larvae were assessed for lethality, hatching and malformations. For clopyralid and clopyralid-olamin no significant effects were observed compared to the control
Reliability check: study details	
Parameter	**Information available**
Test protocol GLP, GEP, Guidelines (US EPA, OECD, …)	- None described
Test substance Identification of test substance, source, purity, stability	- 309 chemicals, including clopyralid and clopyralid-olamin, from the EPA's Phase 1 ToxCast library - Purity: > 90% - The chemical was first diluted in DMSO - Chemical analysis of the chemicals over time showed little degradation over time
Test conditions Temperature, pH, oxygen concentration, water hardness, conductivity, photoperiod, light intensity, number of animals, food availability	- Temperature: 26 ± 0.1ºC - Photoperiod: 14:10 h light-dark cycle - Exposure solution: 250 µL Hanks' balanced salt solution (13.7 mM NaCl, 0.54 mM KCl, 25 µM Na_2HPO_4, 130 µM $CaCl_2$, 100 µM $MgSO_4$ and 420 µM $NaHCO_3$
Controls Positive control, negative control	- Negative control: DMSO only

Dosing system Exposure (dose, duration, frequency)	- Zebrafish embryos were exposed in 96-well plates (Millipore Multiscreen Nylon mesh plates (catalog number MANMN4050, Millipore Corp, Bedford, MA) - Dose: 80 µM chemical (in final concentration of DMSO during exposure of 0.4% v/v) in the single concentration study and a range (0.001, 0.004, 0.012, 0.030, 0.110, 0.320, 1.000, 2.960, 8.800, 26.600 and 80000 µM, also in DMSO) in the concentration response study - Duration: during 5 days (post fertilization) - Frequency: Daily dosing, chemical renewal very 24 h - Replicates: 4, containing 1 embryo
Test species Body weight or length, gender, age/life stage, source	- Species: Zebrafish (*Danio rerio*) embryos - Age: Embryos, 6 to 8 hours after fertilization - Source of the parents: Aquatic Research Organisms, Hampton, USA
Statistical analyses Sample size/replicates, statistical analysis of data (significance level, variability)	-
Biological effects Determined effect concentration, dose response observed	- The 5 days of exposure were followed by a wash-out for 1 day prior to the lethality, hatching and malformation assessments performed on day 6 (post fertilization) - Toxicity scores were assigned to mortality (40), non-hatching (20) and malformations (0-34). Any chemical with a mean toxicity above 2.24 was considered active - Clopyralid and clopyralid-olamine were both considered 'negative' for activity, as both chemicals showed a mean toxicity score below 2.24 (0.50 and 1.00, respectively). Hence, half-maximal activity concentrations (AC_{50}) could not be calculated (above highest test concentration) - As the hydrophobicity of a chemical increased, the likelihood that the chemical would be toxic also increased
Overall assessment	- Plates with > 12.5% of the controls showing lethality or significant malformations were rejected - Methods, statistics, results and discussion are described - Chemical analysis performed

RMS comments and evaluation:

This study was a research article published in a scientific journal, and performed independently from the Notifier. The study and the laboratory did not have a GLP status. No test guideline was referred to, but the methodology was otherwise clearly described. The study was well conducted and reported, and the study protocol was acceptable. However, several issues were not reported in adequate detail to allow a comprehensive validity evaluation. For instance, the source of the test substance studied, the analytical procedures and statistical analysis were not reported in such detail as required for PPP active substance studies. No data is available on the specification of the test substance used in this study, so the impurity profile is unknown. Clopyralid was considered as not posing developmental malformations in fish. Overall, the study was assessed as reliable with restrictions, given that the report was peer reviewed prior to its publication in a scientific journal. The data can be used as supporting information.

Table 9.2.15. Zebrafish developmental screening with clopyralid.

Evaluating the effects of forestry herbicides on fish development using rapid phenotypic screens	
KCA 9/8 (CA 8.2.2.1)	
Author(s)	Stehr, C.M., Linbo, T.L., Baldwin, D.H., Scholz, N.L., Incardona, J.P.

Year	2009
Journal	North American Journal of Fisheries Management Vol. 29(4) pp. 975-84
Relevance check	Relevant. No guideline is stated but methodology is comprehensively described
Reliability check	Reliability score 2 (Klimisch)
Reasons for no reliability	Not applicable
Summary	Zebrafish (*Danio rerio*) embryos were exposed for 5-day to purified substances (including clopyralid) and formulated products (including Transline, containing clopyralid) at nominal concentrations ranging from 3 µg/L to 10 mg/L. Clopyralid (purified) only caused a significant negative effect for touch response at the highest test concentration. Anatomical features, larval body length and touch responses were examined. There was no clear evidence of a toxic concentration-response relationship for any of the tested herbicides.
Reliability check: study details	
Parameter	**Information available**
Test protocol GLP, GEP, Guidelines (US EPA, OECD, …)	- No guideline described
Test substance Identification of test substance, source, purity, stability	- Test substances: Purified substances (clopyralid (purity: 99%), picloram, imazapyr, imazapic, glyphosate and triclopyr) and formulated products, containing the a.s. plus surfactants or other proprietary ingredients (Transline (clopyralid content: 40.9%), Tordon K, Habitat, Plateau, Garlon 3 A and Renovate, respectively) - Source: The purified active ingredients were obtained from Chem Service, Inc. (Pennsylvania, USA) and the formulations were supplied by the US Forest Service Regional Pesticide Coordinator (Utah, USA)
Test conditions Temperature, pH, oxygen concentration, water hardness, conductivity, photoperiod, light intensity, number of animals, food availability	- Temperature: 28.5ºC - Water: Artificial system water, prepared by filtering municipal water and adding Instant Ocean Sea Salts - Conductivity: 1500 µS/cm - pH: 7.0 – 7.4 - Photoperiod: Continuous darkness
Controls Positive control, negative control	- Negative control: System water only
Dosing system Exposure (dose, duration, frequency)	- Dose: 3, 10, 33, 100, 333, 1000 and 10000 µg/L (diluted in the system water) - Duration: 5 days - Frequency: Daily renewal - Replicates: 3 glass petri dishes, with 15 embryos per petri dish
Test species Body weight or length, gender, age/life stage, source	- Species: Zebrafish (*Danio rerio*) - Age/life stage at test initiation: 2 to 4 hours post fertilization
Statistical analyses Sample size/replicates, statistical analysis of data (significance level, variability)	- Fisher's exact test was used to analyse the proportion of fish with no developmental defects and normal touch responses - Nested analysis of variance (ANOVA) was used to analyse fish length differences. For herbicides showing significant effect of both exposure and replicate, a one-way ANOVA was performed using the means of the 3 replicates. Dunnett's test was used to compare each group with the control - Statistical analysis were performed using JMP version 5.1 (SAS Institute, Inc., North Carolina, USA) and R version 2.8 (R project for statistical computing)
Biological effects Determined effect concentration, dose response observed	- Anatomical features were examined daily during the exposure period. Larval body length (indicator of growth) was measured at the end of the exposure period (day 5). Touch response (escape reflex) was tested daily from day 3 to day 5

	- There was no clear evidence of a toxic concentration-response relationship for any of the tested herbicides - Clopyralid caused a significant decrease (down to 71% of control) in touch response at the highest test concentration (10 mg/L)
Overall assessment	- No clear effects are observed in the study. No toxic reference is used. The system might be insensitive. However, the authors discuss that this is unlikely, as the observed effects are similar to those shown for other teleosts - Methods, statistics, results and discussion were documented - The study is considered reliable (but bearing the comment above in mind, about sensitivity of the system)

RMS comments and evaluation:

This study was a research article published in a scientific journal, and performed independently from the Notifier. The study and the laboratory did not have a GLP status. No test guideline was referred to, but the methodology was otherwise clearly described.The study was well conducted and reported, and the study protocol was acceptable. However, several issues were not reported in adequate detail to allow a comprehensive validity evaluation. For instance, the source of the test substance studied and the analytical procedures were not reported in such detail as required for PPP active substance studies. No data is available on the specification of the test substance used in this study, so the impurity profile is unknown. Overall, the study was assessed as reliable with restrictions, given that the report was peer reviewed prior to its publication in a scientific journal. The data can be used as supporting information.

RMS overall evaluation of endocrine disrupting potential:

The data and explanations presented by the Notifier are adequate and acceptable to conclude that the endocrine disrupting properties of clopyralid are unlikely in aquatic or terrestrial organisms. Therefore no further vertebrate studies are justified on this issue.

B.9.2.1.4. Bioconcentration in fish

Data to address this point were presented in the dossier for the Approval of Clopyralid submitted in 30 April 2002, evaluated in the DAR (2003) and were deemed acceptable following the evaluation and peer review at EU level. These data are still valid for decision making, and therefore only briefly presented here again.

Investigation of the effects of bioconcentration in prey of birds and mammals is not required as the log Pow for active is <3. For example, clopyralid is not expected to bioaccumulate in animal tissues as indicated by a log P_{ow} of -2.63 and a fish BCF < 1.

CA 8.2.2.3/1 - Determination of the Bioconcentration Factor for 3,6-Dichloropicolinic Acid in Bluegill Sunfish During Continuous Aqueous Exposure.

Report	[IIA 8.2.3/01] [redacted] 1982
Report title	Determination of the Bioconcentration Factor for 3,6-Dichloropicolinic Acid in Bluegill Sunfish During Continuous Aqueous Exposure.
DAS Study number	GH-C-1577, Ref. J13
Guidelines	US EPA OPP 165-4; US EPA OPP 72-6
GLP	No

Methodology:

^{14}C-ring labelled clopyralid [2,6-pyridine-^{14}C] clopyralid, Batch No. GHD-1016-3C inventory 366A, specific activity 11.64 mCi/mM and radiochemical purity >99%. The radiolabelled clopyralid was mixed with unlabelled clopyralid, Batch No. AGR 117208 (analytical standard, purity not stated).

Bluegill sunfish, *Lepomis macrochirus*, 2.5 to 3.0 cm in length, were exposed to nominal clopyralid concentrations of 0.1 and 1.0 mg/L for 28 days under flow-through test conditions. The fish were then transferred to diluent water only for 14 days to depurate. Fish were fed twice daily and aquaria cleaned regularly. Diluent water (municipal supply, treated with ozone and filtered through activated charcoal) and stock solutions of clopyralid (buffered with 0.008M $NaHCO_3$) were metered into a mixing chamber from where the solution overflowed into the 14 L test aquaria. The diluent water had the following properties: pH 6.6, hardness 140 mg $CaCO_3$/L, 16°C and 10 ppm dissolved oxygen. The control treatment consisted of diluent water only. The test medium was exchanged three times daily in each aquaria. The test system was established on day -2 and 60 fish were added to each aquarium on day 0.

Water samples for analysis were pipetted from the central area of the aquarium and analysed by TLC (for the identity of clopyralid) and liquid scintillation counting (for total radioactivity) within one hour of sampling. Water was sampled on each day during the uptake phase. On each sampling occasion, from each dosed aquarium, five fish were netted at random, the brain pithed and the fish rinsed in distilled water, blotted dry, measured and weighed before evisceration. Fish from the control aquarium were sampled on days 3 and 28 during uptake and on day 14 of depuration. Fish and viscera were frozen until analysed. Fish samples were taken on days 0, 3, 7, 10, 14, 21 and 28 during uptake and on days 1, 3, 7, 10 and 14 of depuration.

Findings:

Concentrations of clopyralid in the test media were 92% and 101% of the nominal 0.1 and 1.0 mg/L target concentrations. This represented 166 and 1533 dpm/mL, respectively (Table 9.2-16). Analysis by TLC showed no breakdown of clopyralid during the exposure phase. Nine fish died during the study, representing 5% of the total fish. This was attributed to netting and transferring the fish (three control fish, four from the nominal 0.1 mg/L and two from the nominal 1.0 mg/L treatment). The remaining fish appeared to be in good condition for the duration of the study. Due to the low level of radioactivity in the fish, the combustion results are reported on whole fish, rather than separate edible and viscera portions. Combustion of whole fish showed only trace amounts of radioactivity (Table 9.2-17). Under these conditions, the highest bioconcentration factor that can be calculated is less than 1.0 (Table 9.2-18). The results are summarised in Table 9.2-19.

Table 9.2-16. Measured radioactivity of ^{14}C-clopyralid in test medium during the exposure phase of a bioconcentration study with bluegill sunfish

Parameter	**Nominal clopyralid concentration (mg/L)**	
	0.1[a]	**1.0**[b]
Mean (dpm/mL)	166	1533
Std. Dev.	6.1	38.8
Coef. Var. (%)	3.7	2.5
Std. Err.	1.1	7.0

[a] 180 dpm/mL = 0.1 mg clopyralid/L.
[b] 1510 dpm/mL = 1.0 mg clopyralid/L.

Table 9.2-17. Radioactivity in whole fish following a 28-day exposure to clopyralid

Sample day[a]	**Radioactivity in whole fish (dpm/g fish**[b]**)**	
	0.1 mg/L treatment	**1.0 mg/L treatment**
+3	71	172[b]
+7	42	151
+10	14	168
+14	46	260
+21	32	108
+28	39	151

[a] From 3 to 28 days after the start of exposure to ^{14}C-clopyralid.
[b] Arithmetic mean of five replicate fish.

Table 9.2-18. Bioconcentration of whole fish following a 28-day exposure to clopyralid

Sample day[a]	**Bioconcentration factor**	
	0.1 mg/L (nominal) treatment[b]	**1.0 mg/L (nominal) treatment**[c]
+3	0.42	0.11
+7	0.25	0.10
+10	0.09	0.11
+14	0.28	0.17
+21	0.20	0.07
+28	0.24	0.10

[a] From 3 to 28 days after the start of exposure to ^{14}C-clopyralid.
[b] Dpm/g fish, divided by the arithmetic mean of the radioactivity measurements in the test medium (166 dpm/mL equivalent to 0.092 mg clopyralid/L).
[c] Dpm/g fish, divided by the arithmetic mean of the radioactivity measurements in the test medium (1533 dpm/mL equivalent to 1.015 mg clopyralid/L).

Table 9.2-19. Summary on results of the bioconcentration study

Result	Exposure at 0.1 mg/L	Exposure at 1.0 mg/L
BCF	< 1.0	< 1.0

Conclusions:

A bioconcentration factor of < 1.0 for whole fish was derived from a laboratory study in which bluegill sunfish were exposed to measured concentrations of clopyralid of 0.092 and 1.015 mg/L, under flow-through conditions for 28 days. Analysis of the test media showed that clopyralid did not breakdown during the exposure phase.

Comments

Despite claimed by the notifier the study was not performed under GLP. There were some deficiencies in the study report, e.g. some corrections were made by hand. The protocol, not the report, was corrected by hand. These corrections were signed and dated according to correct GLP procedures. However, the method used was acceptable and the performance of the study was sufficiently described. According to the results clopyralid is not bioconcentrating in fish, and based to the other environmental properties of clopyralid bioconcentration is not anticipated. Therefore it is not necessary to repeat the test.

A bioconcentration factor of <1.0 for whole fish was derived from a laboratory study in which Bluegill Sunfish, *Lepomis macrochirus*, were exposed to measured concentrations of clopyralid of 0.092 and 1.015 mg/L, under flow-through conditions for 28 days. Analysis of the test media showed that clopyralid did not breakdown during the exposure phase.

RMS comments and evaluation:

Originally commented by the RMS in the DAR (2003): Despite claimed by the Notifier the study was not performed under GLP. The protocol, not the report, was corrected by hand. These corrections were signed and dated according to correct GLP procedures. However, the method used sas acceptable and the performance of the study was sufficiently described. According to the results clopyralid is not bioconcentrating in fish, and based to the other environmental properties of clopyralid bioconcentration is not anticipated. Therefore it is not necessary to repeat the test.

The study was evaluated and peer reviewed during the first Annex I evaluation of clopyralid. The purity of the radiolabeled active substance used in this study was >999 g/kg and thus greater than the new specification of the technical clopyralid used in other ecotoxicological studies, as presented by the Notifier in Document J in December 2016.

The study was old and performed before the adoption of OECD test guideline and GLP provisions, but otherwise adequately well performed and reported to conclude that the BCF in fish is < 1. This endpoint is still valid and therefore the study is not reassessed here again. Taking into account also the log Pow of -2.63 of clopyralid, it can be concluded that there is no risk of bioaccumulation of clopyralid in fish or other animal tissues. Therefore the risk of bioconcentration of clopyralid in food chain is negligible, and no further vertebrate studies are justified on this issue.

B.9.2.2. Effects to aquatic invertebrates

B.9.2.2.1. Acute toxicity to *Daphnia magna*

Data to address this point were presented in the dossier submitted in 30 April 2002 for the Active Approval, evaluated in the DAR (2003) and peer review at EU level. The study was deemed acceptable following the evaluation and EFSA peer review, and therefore not reassessed here again in detail. The outcomes are still valid and the endpoints can be used for decision making.

Low risk to aquatic fish, invertebrates, algae, and aquatic plants has been demonstrated for clopyralid at its proposed use rates on grassland and cereals. Due to this fact, further studies on other aquatic invertebrate species were deemed unnecessary and additional data has not been submitted.

CA 8.2.4.1/1- Clopyralid: An Acute Toxicity Study with the Daphnia, *Daphnia magna* Straus

Report	[IIA 8.2.4/01], Marino, T. A. ; McClymont, E. L. ; Staley, J. L., 2000
Report title	Clopyralid: An Acute Toxicity Study with the Daphnia, *Daphnia magna* Straus
DAS Study number	001025, Ref. J52
Guidelines	OECD 202 (Part I) EU C.2 US EPA FIFRA 540/9-85-005, 72-2
GLP	Yes

Methodology:

Clopyralid, Lot No. 910905 5P, Purity 96.9%. The acute toxicity of clopyralid to *Daphnia magna* was assessed over a 48-hour exposure period, under static test conditions.

In a range finding test, 10 daphnids per treatment (one replicate) were exposed to nominal concentrations of 0 (control), 10 and 100 mg clopyralid/L. No effects were observed after 48 hours exposure.

For the definitive test, 10 daphnids (each less than 24-hours old,) were placed in each of three replicates of a control (dilution water) and a 100 mg clopyralid/L treatment. The test vessels were 250 mL glass jars containing 200 mL test medium. Clopyralid was added directly to the laboratory dilution water using sonication to aid dissolution. The test media were not replaced during the exposure period.

Daphnids were observed for immobility at 24 and 48 hours. Dissolved oxygen, pH and temperature were recorded for each treatment at the start and end of the test. Dissolved oxygen averaged 94% (ASV), temperature 19.5 to 20.8°C and pH 7.2 to 7.9. At the start and end of the test samples of test medium were taken for analysis of clopyralid by HPLC/UV.

Findings:

The mean measured concentration of clopyralid in the dosed medium was 102 mg/L at the start and 96.0 mg/L at the end of the test (Table 9.2-20). No immobilisation of daphnids was observed in the control or nominal 100 mg clopyralid/L treatments. The results are presented in Table 9.2-21.

Table 9.2-20. Measured concentrations of clopyralid during an acute toxicity limit test with *D. magna*

Nominal concentration of clopyralid (mg/L)	**Measured concentration (mg/L)**		**Mean measured concentration (mg/L)**
	Day 0	**Day 4**	
Control	< LOQ	< LOQ	-
100	102	96.0	99.0

< LOQ: below the limit of analytical determination (7.2 mg clopyralid/L).

Table 9.2-21. Summary on results with *D. magna*

Result	**Mean measured concentration of clopyralid (mg/L)**
48-h EC_{50}	> 99.0

Conclusions:

In a limit test, under static test conditions, the 48-hour EC_{50} of clopyralid to *Daphnia magna* was > 99.0 mg/L, based on a mean measured concentration.

Comments

The test was performed using the limit test procedure. Therefore the exact EC_{50} of clopyralid could not be found but was above 99 mg/l, which means that clopyralid is of low toxicity to aquatic invertebrates. The study was well performed and reported and in compliance with GLP. The study is acceptable.

In a limit test procedure, under static test conditions, the 48-hour EC_{50} of technical clopyralid (purity of 96.9 %) to *Daphnia magna* was > 99.0 mg/L, based on a mean measured condition.

RMS comments and evaluation:

Originally commented by the RMS in the DAR (2003): The test was performed using the limit test procedure. Therefore the exact EC_{50} of clopyralid could not be found but was above 99 mg/L, which means that clopyralid is of low toxicity to aquatic invertebrates. The study was well performed and reported and in compliance with GLP. The study is acceptable.

The original specification of clopyralid is based on the analysis of the batch used in this study. Based on the risk assessment for the impurities of clopyralid presented by the Notifier in Document J in December 2016, no impurity characterised in this batch is expected to substantially impact the ecotoxicological profile of clopyralid. The impurity profile of the test substance used in this study was within the limits of the new specification presented by the Notifier in December 2016.

The conclusion above is still valid and the study can be considered as adequate for demonstrating the ecotoxicological effects of clopyralid to Daphnia. The study is valid for the risk assessment, as proposed by the Notifier. No further acute aquatic invertebrate studies are required.

B.9.2.2.2. Reproductive and development toxicity to *Daphnia magna*

Data to address this point were presented in the dossier submitted in 30 April 2002 for the Active Approval, evaluated in the DAR (2003) and peer review at EU level. The study was deemed acceptable following the evaluation and EFSA peer review, and therefore only briefly presented here again. The outcomes are still valid and the endpoints can be used for decision making.
Low risk to aquatic fish, invertebrates, algae, and aquatic plants has been demonstrated for clopyralid at its proposed use rates on grassland and cereals. Due to this fact, further studies on aquatic invertebrate species were deemed unnecessary and additional data has not been submitted.

CA 8.2.5.1/1- An Assessment of the Effects of Lontrel T on the Reproduction of *Daphnia magna*

Report	[IIA 8.2.5/01], Douglas, M. T., Bell, G. & Macdonald, I. A. 1992
Report title	An Assessment of the Effects of Lontrel T on the Reproduction of *Daphnia magna*
DAS Study number	DWC 615/911087, Ref. J35
Guidelines	OECD 202 (Part 2)
GLP	Yes

Methodology:

Clopyralid (termed 'Lontrel T' in the report), Batch No. RMM 1669, Purity 95.7%. The effects of clopyralid on the survival and reproduction of *Daphnia magna* were assessed over a 21-day exposure period under semi-static conditions.

Young daphnids, from gravid adults isolated 24 hours before the initiation of the test, were exposed to the following nominal concentrations of clopyralid: 0 (control), 5.6, 18, 56, 180 and 560 mg/L. For each exposure concentration and the control treatment (diluent water only, dechlorinated, aged laboratory tap water, hardness 350 mg/L as $CaCO_3$), 40 daphnids were placed in 4 replicate glass flasks (10 per flask) containing 400 mL of test medium. Clopyralid was prepared in the diluent water by direct addition. Cultures were fed daily with a mixture of fry fish food and mixed algae. The test media were renewed 3 times per week on days 2, 5, 7, 9, 12, 14, 16 and 19.

Temperature was recorded daily and dissolved oxygen, pH and temperature recorded before and after each test medium renewal. The test conditions were 21 ±1°C, 16 hours light, dissolved oxygen 8.4 to 8.8 mg/L and pH 6.9 (at 590 mg/L only) to 7.6 - 8.8 for the remaining treatments.

Live and dead parental daphnids were counted daily and the general condition of the animals was assessed. At each medium renewal, live and dead offspring, the number of parental daphnids with eggs or young in the brood pouch and the number of discarded eggs were recorded.

Samples of each test medium were taken on days 0, 5, 12 and 19 (freshly prepared media) and on days 2, 7, 14 and 21 (expired media) for analysis by HPLC.

Findings:

Measured concentrations as a percent of nominal were 86 to 111% for freshly prepared and 81 to 108% for expired media (Table 9.2-22).

The results of the study were based on the following mean measured concentrations of clopyralid: 5.3, 17, 59, 190 and 590 mg/L. After 21 days exposure, survival of parental daphnids ranged from 100% in the control and 5.3 mg clopyralid/L treatments to 0% at 190 and 590 mg /L (Table 9.2-23).

Lethal effects on the parents were most pronounced during the first 4 days of exposure with mortality occurring throughout exposure at the highest treatment level. The number of live young per female was 45 in the control and 5.3 mg/L treatments, 42 at 17 mg /L, 29 at 56 mg/L and 0 at the two highest treatment levels of 190 and 590 mg/L (Table 9.2-24).

No other adverse effects of exposure to clopyralid were observed. Numbers of unhatched eggs and dead young were low in all groups (≤ 1 per female). The results are summarised in Table 9.2-24.

Table 9.2-22. Measured concentrations of clopyralid in test media during a 21-day reproduction study with *D. magna*

Nominal concentration of clopyralid (mg/L)	**Measured concentrations as a percentage of nominals**							
	Day 0 (new)[a]	**Day 2 (old)**	**Day 5 (new)**	**Day 7 (old)**	**Day 12 (new)**	**Day 14 (old)**	**Day 19 (new)**	**Day 21 (old)**
Control	ND[b]	ND	ND	ND	ND	ND	ND	ND
5.6	86	81	92	98	88	99	104	107
18.0	89	88	90	92	79	100	105	103
56.0	106	100	104	102	111	108	110	107
180.0	108	108	-[c]	-	-	-	-	-
560.0	108	103	-	-	-	-	-	-

[a] New: freshly prepared test medium, old: expired test medium.
[b] ND: not detected at a limit of detection of 0.208 mg clopyralid/L.
[c] - represents no sample taken due to 100% mortality of parental daphnids.
Results corrected for an overall mean recovery of 93%.

Table 9.2-23. Survival of adults and reproduction of *D. magna* exposed to clopyralid over 21 days

Mean measured concentration of clopyralid (mg/L)	**Survival of adults (%)**	**Live young**		**Dead young**		**Unhatched eggs**	
		Total	**Per female[a]**	**Total**	**Per female**	**Total**	**Per female**
Control	100	1786	45	3	< 1	0	0
5.3	100	1801	45	0	0	0	0
17	95	1647	43	1	< 1	0	0
59	78	975*	29*	0	0	0	0
190	0	0	0	0	0	0	0
590	0	0	0	0	0	0	0

[a] Cumulative, based on counts at test medium renewal and termination.
* Significantly different to the control treatment at $p < 0.01$.

Table 9.2-24. Summary of results in reproduction test with *D. magna*

Result	**Mean measured concentration of clopyralid (mg/L)**
48-h EC_{50} (adults) (95% C.I.)	280 (220 - 360)
21-d EC_{50} (adults) (95% C.I.)	69 (58 - 82)
21-d NOEC (immobilisation of parents ≤ 10%)	17
21-d EC_{50} (reproduction: reduction in number of live young) (95% C.I.)	80 (67 – 95)
21-d NOEC (reproduction)	17

Conclusions

The 21-day EC_{50} of clopyralid to *D. magna* was 69 mg/L, based on mean measured concentrations, under semi-static test conditions. The 21-day NOEC (reproduction) was 17 mg/L, measured, based on a significant reduction in live young at exposure concentrations of 59 mg/L and above.

Comments

The study was well performed and reported and in compliance with GLP. Measured concentrations remained within the range of 79 – 111 % of nominal throughout the study with a mean value of 99 % and the results are based on mean measured concentrations. The validation criteria were met and the study is acceptable.

The 21-day EC_{50} of technical clopyralid (purity of 95.7 %) to *Daphnia magna* was 69 mg/L, based on mean measured concentrations, under semi-static test conditions. The 21-day NOEC (reproduction) was 17 mg/L, measured, based on a significant reduction in live young at exposure concentrations of 59 mg/L and above.

RMS comments and evaluation:

Originally commented by the RMS in the DAR (2003): The study was well performed and reported and in compliance with GLP. Measured concentrations remained within the range of 79-111 % of nominal throughout the study with mean value of 99 % and the results are based on mean measured concentrations. The validation criteria were met and the study is acceptable.

[redacted]

The conclusion above is still valid and the study can be considered as adequate for demonstrating the reproduction toxicity of clopyralid to Daphnia. The study is valid for the risk assessment, as proposed by the Notifier. No further studies on this issue are required.

B.9.2.2.3. Development and emergence in *Chironomus riparius*

Data to address this point were presented in the dossier submitted in 30 April 2002 for the Active Approval.The study was deemed acceptable following the evaluation and EFSA peer review, and therefore not reassessed here again in detail. The outcomes are still valid and the endpoints can be used for decision making.

CA 8.2.5.3/1- Clopyralid Technical Toxicity to the Sediment Dwelling Phase of the Midge *Chironomus riparius*

Report	[IIA 8.2.7/01], Barrett, K. , 2001
Report title	Clopyralid Technical Toxicity to the Sediment Dwelling Phase of the Midge *Chironomus riparius*
DAS Study number	GHE-T-1122, Ref. J66
Guidelines	BBA 1995
GLP	Yes

Methodology:

Clopyralid technical, Batch No. RMM 2373, Purity 95.8% w/w and radiolabelled clopyralid (3,6-dichloropicolinic acid-2,6-^{14}C), Specific Activity 30.9 mCi/mmol and Radiopurity > 97%. The effects of clopyralid technical on the sediment dwelling phase of the midge *Chironomus riparius* were assessed under laboratory test conditions.

Based on the outcome of a range-finding test, larvae of *C. riparius* were exposed to the following nominal concentrations of clopyralid applied to the aqueous phase of a water:sediment test system: 6, 13, 25, 50 and 100 mg/L. The aqueous test media were prepared with radiolabelled clopyralid and non-radiolabelled clopyralid and diluted with water (‘Elga’).

The definitive test was carried out with 13 cm tall-form glass beakers containing a layer of sediment (2 cm depth) and 20 cm of overlying water. The sediment was prepared in accordance with OECD test guideline 207 (1984). Four to five days after the establishment of the test units with sediment and water, when the sediment had settled, aeration was started in the test vessels. The test vessels were left for a further 10 days before the addition of the larvae, then 24 hours later the test substances were added to the overlying water. There was no renewal of the test medium during the toxicity test.

Each test vessel contained 25 first instar larvae. There were eight test vessels in the nominal 100 mg clopyralid/L treatment group and four vessels in the clopyralid treatments ranging from 6.0 to 50 mg/L. In addition, there were eight vessels for the control (water only) treatment. Additional vessels containing larvae were prepared for the destructive sampling of water, sediment and pore water for analysis of clopyralid on Days 1, 7 and 14.

Liquid scintillation counts were carried out on samples of overlying water and sediment immediately following application of the test substances and after 1, 3, 7, 14 and 28 days. Pore water was extracted from the sediment samples by centrifugation for 10 minutes at 3000 revolutions per minute. After removal of the pore water the sediment samples were air-dried and ground before combustion and analysis by radioassay.

Test vessels were monitored daily for growth and development of the chironomids. From day 14, when the first winged adults emerged, adults were sexed and removed from the test vessels. In addition, pH, dissolved oxygen concentrations and temperature in each vessel were recorded on days 0, 7, 14, 21 and 28. Additional pH readings were taken on day 1 since values were low compared to the control at the higher test concentrations. The cultures were maintained at a dissolved oxygen concentration of 6.3 to 9.3 mg/L, pH from 3.7 to 7.1 and a temperature of 19 to 20°C.

Findings:

The applied concentrations of clopyralid were 6.1, 12.8, 24.4, 49.9 and 97.0 mg/L. The results of the toxicity test are based on the measured concentrations applied to the overlying water.

Mean emergence, by the end of the test, ranged from 78.5% in the control treatment to 52.5% at 97 mg clopyralid/L. Emergence of adults was significantly reduced, compared to controls, at 97 mg clopyralid/L ($p< 0.05$) (Table 9.2-25). There were no significant differences in the numbers of males and females emerging from each treatment. There was no difference in the development rate of chironomids between treatments.

Based on equivalent concentrations of clopyralid from analyses of radioactivity, concentrations in the overlying water were 95 to 100% of the applied dose levels at the start of the test. These levels fell to 85 to 92% on Day 28, indicating that clopyralid did not partition to the sediment phase in significant quantities. Pore water contained only 1.5 to 1.8% of the total clopyralid added after 28 days. (Tables 9.2-26 and 9.2-27). Samples of sediment contained < 4% of applied clopyralid. The results are summarised in Table 9.2-28.

Table 9.2-25. Emergence of *C. riparius* adults following exposure to clopyralid

Measured concentration of clopyralid applied to the overlying water (mg/L)	**Mean % emergence**	**Mean development rate**
Control	78.5	0.0618
6.1	77	0.0625
13	59	0.0633
24	66	0.0624
50	69	0.0633
97	52.5*	0.0610

* Significantly different to the control at $p<0.05$.

Table 9.2-26. Mean measured concentration of clopyralid in the overlying water during a toxicity test with *C. riparius*

Measured concentration of clopyralid applied to the overlying water (mg/L)	**Mean measured concentration, mg/L (% of applied concentration)**					
	Day 0	**Day 1**	**Day 3**	**Day 7**	**Day 14**	**Day 28**
Control	ND	ND	ND	ND	ND	ND
6.1	5.8 (95)	5.8 (95)	5.7 (93)	5.5 (90)	5.6 (92)	5.5 (90)
13	13 (100)	12 (92)	12 (92)	12 (92)	12 (92)	11 (85)
24	24 (100)	23 (96)	23 (96)	23 (96)	23 (96)	22 (92)
50	48 (96)	48 (96)	47 (94)	46 (92)	47 (94)	45 (90)
97	93 (96)	91 (94)	89 (92)	89 (92)	90 (98)	87 (90)

ND: not detected at the limit of detection.

Table 9.2-27. Distribution of clopyralid in the test system on Day 28 at the end of a toxicity test with *C. riparius*

Measured concentration of clopyralid applied to the overlying water (mg/L)	**Mean measured concentration on Day 28 (% of total clopyralid added[a])**		
	Water (mg/L)	**Pore water (mg/L)**	**Sediment (mg/kg)**
Control	ND	ND	ND
6.1	5.5 (89)	5.3 (1.5)	1.75 (1.9)
13	11 (90)	11 (1.8)	ND
24	22 (92)	22 (1.7)	8.0(1.9)
50	45 (90)	44 (1.5)	20.5 (2.5)
97	87 (90)	85 (1.8)	13.5 (0.9)

[a] Amount of test material recovered, divided by the total amount added per test vessel.

Table 9.2-28. Summary of results with *C. riparius*

Result	**Applied, measured concentration of clopyralid to the overlying water (mg/L)**
28-day EC_{50} (emergence)	> 97
NOEC (emergence)	50
NOEC (development rate)	≥ 97

Conclusions:

The 28-day EC_{50} of clopyralid for emergence of the sediment dwelling midge *Chironomus riparius* was greater than 97 mg/L, based on the measured applied concentrations to the overlying water. The corresponding emergence NOEC was 50 mg/L. The NOEC based on development rate was greater than or equal to 97 mg/L, the highest concentration tested.

Comments

The study was well performed and reported and in compliance with GLP. The ”spiked water” test procedure mimics the exposure by spray drift, but not necessarily by runoff or by drainflow. Drainflow is a significant route of exposure of clopyralid. However, because clopyralid is readily soluble in water, also the exposure via these routes is considered to be mainly in soluted form. In the water/sediment study clopyralid partitioned mainly in aqueous phase. Additionally, aquatic invertebrates are not the most sensitive organisms to clopyralid and therefore the midges are anticipated to be sensitive neither. The study is considered acceptable and the result can be used in the risk assessment.

The 28-day EC_{50} of technical clopyralid (purity of 95.8 %) for emergence of the sediment dwelling midge *Chironomus riparius* was greater than 97 mg/L, based on the measured applied concentrations to the overflying water. The corresponding emergence NOEC was 50 mg/L. The NOEC based on development rate was greater than or equal to 97 mg/L, the highest concentration tested.

RMS comments and evaluation:

Originally commented by the RMS in the DAR (2003): The study was well performed and reported and in compliance with GLP. The "spiked water" test procedure mimics the exposure by spray drift,

but not necessarily by runoff or by drainflow. Drainflow is a significant route of exposure of clopyralid. However, because clopyralid is readily soluble in water, also the exposure via these routes is considered to be mainly in soluted form. In the water/sediment study clopyralid partitioned mainly in aqueous phase. Additionally, aquatic invertebrates are not the most sensitive organisms to clopyralid and therefore the midges are anticipated to be sensitive neither. The study is considered acceptable and the result can be used in the risk assessment.

[redacted]

The conclusion above is still valid and the study can be considered as adequate for demonstrating the effects of clopyralid to sediment dwelling organisms. The study is valid and the endpoint of emergence NOEC of 50 mg/L can be used for the risk assessment, as proposed by the Notifier. No further studies are required.

B.9.2.2. Effects on algal growth

Data to address the effects of clopyralid to algae were presented in the dossier submitted in 30 April 2002 for the Active Approval. Three algal studies were deemed acceptable following the evaluation and EFSA peer review, and therefore only briefly presented here again. The outcomes are still valid and the endpoints can be used for decision making.

Study 1

CA 8.2.6.1/1- Clopyralid: Growth Inhibition Test with the Freshwater Green Alga, *Selenastrum capricornutum* Printz

Report	[IIA 8.2.6/01], Kirk, H. D.; Gilles, M. M.; McClymont, E. L. ; McFadden, L.G., 2000
Report title	Clopyralid: Growth Inhibition Test with the Freshwater Green Alga, *Selenastrum capricornutum* Printz
DAS Study number	001040, Ref. J51
Guidelines	OECD 201 (1984) US EPA OPP 123-2 EEC C.3 (1992)
GLP	Yes

Methodology:

Clopyralid, Lot No. 910905 5P, Purity 96.9%. The inhibitory effect of clopyralid on the growth of the green alga *Selenastrum capricornutum* was assessed over a 96-hour exposure period.

A range-finding test, with four nominal concentrations of clopyralid (0.0216, 0.216, 2.16 and 21.6 mg/L), resulted in a 96-hour EC_{50} and NOEC value of > 21.6 mg clopyralid/L. For the definitive test, there were four replicate test vessels per treatment, three of which were inoculated with 10,000 cells/mL. The nominal concentrations of clopyralid were: 0 (control containing algal assay medium), 3.00, 6.22, 12.3, 24.2, 48.4 and 97.9 mg/L. The test medium was not renewed throughout the study. The test medium volume was 100 mL in 250 mL glass Erlenmeyer flasks.

Light intensity was measured daily, pH at the start and end of the test and temperature was monitored continuously. Over the 96-hour exposure period the photoperiod was 4637 (± 525) lux and temperature ranged from 24.1 to 24.5°C. The pH values ranged from 3.5 to 7.1 in media without algae and 3.5 to 7.3 with algae. Samples of each bulk test medium were taken at the start of the test (before dispensing to the test vessels) and from each vessel at the end of the test for analysis of clopyralid by HPLC/UV.

Algal cell density was measured using a Coulter Multisizer. Cell counts were made daily. The results of the test were based on mean measured concentrations of clopyralid in the test medium.

Findings:

Measured concentrations of clopyralid were 94.3 to 113% of nominal at the start of the test and 97.6 to 117% at the end (Table 9.2-29).

Effects on algal growth, after 72-hours exposure, ranged from a 29.2% increase in growth at 24.8 mg clopyralid/L to a 98.2% inhibition of growth at 97.5 mg clopyralid/L. After 96-hours, effects were 22.7% stimulation at 6.36 mg clopyralid/L to 99.1% inhibition at 97.5 mg clopyralid/L (Table 9.2-30). The results are summarised in Table 9.2-31.

Table 9.2-29. Measured concentrations of clopyralid in test medium during a growth inhibition study with *S. capricornutum*

Nominal concentration of clopyralid (mg/L)	**Measured clopyralid concentrations (mg/L)**		
	Day 0	**96-hours**	**Mean (% of nominal)**
Control	< LOQ	< LOQ	-
3.00	3.40	3.50	3.45 (115)
6.22	6.25	6.47	6.36 (102)
12.3	11.6	12.0	11.8 (96)
24.2	24.0	25.5	24.8 (102)
48.4	49.3	49.1	49.2 (102)
97.9	96.4	98.5	97.5 (100)

< LOQ: lower than the limit of analytical quantification (1.13 mg clopyralid/L).

Table 9.2-30. Inhibition of growth of *S. capricornutum* exposed to clopyralid over a 96-hour exposure period

Mean measured concentration of clopyralid (mg/L)	**Algal cells/mL**				
	0 hours	**24 hours**	**48 hours**	**72 hours (% inhibition)[a]**	**96 hours (% inhibition)**
Control	24920	21401	86436	355872	877999
3.45	18998	16498	54185	244598* (31.3)	728486 (17.0)
6.36	15834	20342	76175	444483 (-24.9)	1077253 (-22.7)

11.8	14300	21365	68899	356892 (-0.3)	794812 (9.5)
24.8	14099	19372	84954	459645 (-29.2)	1031279 (-17.5)
49.2	16044	7165	3322	13542* (96.2)	24832* (97.2)
97.5	12139	3944	1766	6523* (98.2)	7657* (99.1)

[a] Negative values indicate an increase in algal growth compared to the control treatment.
* Significantly different from the control at $p< 0.05$ (one-tailed Dunnett's test).

Table 9.2-31. Summary on results with *S. capricornutum*

Result	**Mean measured concentration of clopyralid (mg/L)**
72-hour E_rC_{50}[a]	30.0
72-hour E_bC_{50}[b] (95% C.I.)	30.9 (<3.45 - > 97.5)
72-hour NOEC	< 3.45[c]
96-hour E_rC_{50}	33.1
96-hour E_bC_{50} (95% C.I.)	32.7 (< 3.45 - > 97.5)
96-hour NOEC (95% C.I.)	24.8

[a] Areas under the growth curves.
[b] Reduction in the average specific growth rate.
[c] No effect at 6.38, 11.8 and 24.8 mg clopyralid/L.

Conclusions:

The 72-hour E_bC_{50} and E_rC_{50} values of clopyralid to *S. capricornutum* were 30.9 and 30.0 mg clopyralid/L, respectively, based on mean measured concentrations. The statistically derived 72-hour NOEC was < 3.45 mg clopyralid/L, although at 6.38, 11.8 and 24.8 mg clopyralid/L, there was no significance difference from the controls. The 96-hour E_bC_{50} and E_rC_{50} were 32.7 and 33.1 mg clopyralid/L, respectively, based on mean measured concentrations. The 96-hour NOEC was 24.8 mg clopyralid/L.

Comments

The study was well performed and reported and in compliance with GLP. The study is acceptable and the result can be used in the risk assessment to algae.

The 72-hour E_bC_{50} and E_rC_{50} values of technical clopyralid (purity of 96.9 %) to *S. capricornutum* were 30.9 and 30.0 mg clopyralid/L, respectively, based on mean measured concentrations. The statistically derived 72-hour NOEC was <3.45 mg clopyralid/L, although at 6.38, 11.8 and 24.8 mg clopyralid/L, there was no significant difference from the controls. The 96-hour E_bC_{50} and E_rC_{50} were 32.7 and 33.1 mg clopyralid/L, respectively, based on mean measured concentrations. The 96-hour NOEC was 24.8 mg clopyralid/L.

RMS comments and evaluation:

The study was originally evaluated and commented by the RMS in the DAR (2003): The study was well performed and reported and in compliance with GLP. The study is acceptable and the result can be used in the risk assessment to algae.

The original specification of clopyralid is based on the analysis of the batch used in this study. Based on the risk assessment for the impurities of clopyralid presented by the Notifier in Document J in December 2016, no impurity characterised in this batch is expected to substantially impact the ecotoxicological profile of clopyralid. The impurity profile of the test substance used in this study was within the limits of the new specification presented by the Notifier in December 2016.

The conclusion above is still valid and the study can be considered as adequate for demonstrating the effects of clopyralid to freshwater green alga *Selenastrum capricornutum*. The study is valid and the 72-hour endpoint of E_rC_{50} = 30 mg a.s./L can be used for the risk assessment, as proposed by the Notifier. No further studies are required.

Study 2 – Anabaena flos-aquae – J54

Report: Kirk, H.D., Gilles, M.M., McClymont, E.L. McFadden, L.G. (2000): Clopyralid: Growth inhibition test with the freshwater bluegreen alga, *Anabaena flos-aquae.* Dow AgroSciences, unpublished report No. 001039. Dow Chemical Company Study ID: 001039, 7 June 2000. Ref. J54

Guidelines: US EPA FIFRA, Subdivision J, 123-2.

GLP: Yes. Laboratory inspected by United States EPA, 401 M Street SW, Washington DC 20460, USA.

Methodology:

Clopyralid, Lot No. 910905 5P, Purity 96.9%. The inhibitory effect of clopyralid on the growth of the blue-green alga *Anabaena flos-aquae* assessed over a 120-hour exposure period.

A range-finding test, with four nominal concentrations of clopyralid (0.052, 0.52, 5.2 and 52 mg/L), resulted in a 120-hour EC_{50} of between 5.2 and 52 mg clopyralid/L and a NOEC of 5.2 mg clopyralid/L. For the definitive test, there were four replicate test vessels per treatment, three of which were inoculated with 10,000 cells/mL. The nominal concentrations of clopyralid were: 0 (control containing algal assay medium), 0.80, 1.60, 3.20, 6.40, 12.8, 24.0 and 50.2 mg/L. The test medium was not renewed during the study. The test medium volume was 50 mL in 250 mL glass Erlenmeyer flasks.

Light intensity was measured daily, pH at the start and end of the test and temperature was monitored continuously. Over the 96-hour exposure period the photoperiod was 2006 (± 102) lux and temperature averaged 25.5 ±0.1°C. The pH values ranged from 4.1 to 8.0 in media without algae and 4.2 to 8.6 with algae. Samples of each bulk test medium were taken at the start of the test (before dispensing to the test vessels) and from each vessel at the end of the test for analysis of clopyralid by HPLC/UV.

Algal cell density was measured using a Coulter Multisizer. Cell counts were made daily. The results of the test were based on mean measured concentrations of clopyralid in the test medium.

Findings:

Measured concentrations of clopyralid were 90.3 to 100% of nominal at the start of the test and 102 to 108% at the end (Table 9.2-32). Effects on algal growth, after 72-hours exposure, ranged from 17.7% increase in growth at 1.60 mg clopyralid/L to 97.4% inhibition of growth at 52.3 mg clopyralid/L (Table 9.2-33). The results are summarised in Table 9.2-34.

Table 9.2-32. Measured concentrations of clopyralid in test medium during a growth inhibition study with *A. flos-aquae*

Nominal concentration of clopyralid (mg/L)	**Measured clopyralid concentrations (mg/L)**		
	Day 0	**120-hours**	**Mean (% of nominal)**
Control	< LOQ	< LOQ	-
0.800	0.769	0.834	0.802 (100)
1.60	1.55	1.68	1.62 (101)
3.20	2.89	3.37	3.13 (98)
6.40	6.20	6.64	6.42 (100)
12.8	12.2	13.0	12.6 (98)
24.0	23.8	24.6	24.2 (101)
50.2	50.2	54.4	52.3 (104)

< LOQ: lower than the limit of analytical quantification (0.19 mg clopyralid/L).

Table 9.2-33. Inhibition of growth of *A. flos-aquae* exposed to clopyralid over a 120-hour exposure period

Nominal concentration of clopyralid (mg/L)	**Algal cells/mL**		**% inhibition after 120 hours**
	0 hours	**120 hours**	
Control	18324	577429	-
0.800	18659	465894	19.3
1.60	14139	679542	-17.7
3.20	12826	605271	-4.8
6.40	12892	675610	-17.0
12.8	12019	595980	-3.2
24.0	12198	632689	-9.6
50.2	9898	14773*	97.4

Significantly different from the control at $p < 0.05$ (one-tailed Dunnett's test).

Table 9.2-34. Summary on results with *A. flos-aquae*

Result	**Mean measured concentration of clopyralid (mg/L)**
120-hour E_bC_{50}[a]	127
120-hour EC_{50}[b]	37.1
120-hour NOEC	24.2

[a] Based on areas under the growth curve.
[b] Least squares linear regression of algal cell counts.

Conclusions:

The 120-hour E_bC_{50} and EC_{50} values of clopyralid to *A. flos-aquae* were 127 and 37.1 mg clopyralid/L, respectively, based on mean measured concentrations. The 120-hour NOEC was 24.2 mg clopyralid/L.

Comments

The study was well performed and reported and in compliance with GLP. The study is acceptable.

Study 3 – Lontrel 100 - S.capricornutum – J23

Report: Caley, C.Y., Cameron, B.D. & Chapleo, S. (1989): Lontrel 100: Alga, growth inhibition test (72h EC_{50}). Dow AgroSciences, unpublished report No. IRI 140490 & IRI 140731, 1 October 1989. J23

Guidelines: EC Method C.3, Directive 92/69 and OECD Guideline No. 201.

GLP: Yes. Laboratory certified by United Kingdom Good Laboratory Practice Monitoring Authority, Department of Health, Skipton House, 80 London Road, London SE1 6LW, UK.

Methodology:

The inhibitory effect of Lontrel 100 (EF-255) on the growth of the green alga *Selenastrum capricornutum* was determined in a 72h test conducted under static conditions according to OECD/EU guidelines in compliance with GLP. EF-255 is a soluble concentrate (SL) preparation of clopyralid monoethanolamine [redacted] at 100 g clopyralid/L, nominal (EF-255 differs from EF-1136 [redacted]). The test was carried out with material from Batch No. EB 880714-T1128 with an actual clopyralid content of 10.6% w/w.

Four replicated 100 ml cultures of *Selenastrum capricornutum* in OECD medium (hardness, 40 mg $CaCO_3$/L) at an initial cell density of $1x10^4$/mL, were exposed to a nominal test concentration series of 10, 33, 100, 330 and 1000 mg EF-255/L. The cultures were incubated for 72 hours at 21-24°C under continuous illumination in the spectral range 400-700 nm with constant stirring at 100 rpm. Six nutrient medium controls were also prepared. he pH of the test solutions ranged from 8.0-8.1 at the start of exposure and from 8.0-10.7 at the end of the test. The greatest increase in pH occurred in the control cultures and was therefore due to photosynthetic activity and was not test substance related.

Samples were taken daily from each flask and the cell density measured using a haemacytometer (Improved Neubauer). Exposure levels in the test media were monitored by measuring the concentrations of clopyralid in samples collected at 0 and 72 hours, using reverse phased HPLC with UV detection at 280 nm. Mean measured concentrations of clopyralid ranged from 71.2 to 90.1% of nominal and, consequently, results were adjusted on the basis of mean measured concentrations (as product equivalents). The corrected test series was 8.53, 25.6, 73.8, 264 and 842 mg EF-255/L.

Findings:

Exposure of *Selenastrum capricornutum* to EF-255 caused significant inhibition in growth at nominal concentrations of 842 mg product/L and above when compared to control cultures (Dunnett's test, $p<0.05$). The NOEC was therefore determined to be 264 mg EF-255/L, which is equivalent to a clopyralid concentration of 28 mg as/L (based on an active substance content of 10.6% w/w).

Analysis of the "area under the growth curve" and the "growth rate" data gave the E_bC_{50} and E_rC_{50} values summarised in Table 9.2-29. In terms of clopyralid concentration, the 72h E_bC_{50} was 47.6 mg as/L and the E_rC_{50} was 77.4 mg as/L (based on a clopyralid content of 10.6% w/w clopyralid).

Table 9.2-35. Inhibition of growth of *S. capricornutum*

Nominal concentration (mg EF-255/L)		**Corrected concentration* (µg as/L)**	**Area under growth curve at 72h ($x10^6$ cells/h/mL)**	**Growth rate 0-72h ($x10^{-3}$/h)**
Control	Mean	0	33.32	72.85
10	Mean	8.53	38.97	75.64
33	Mean	25.6	25.55	69.63
100	Mean	73.8	29.54	69.94
330	Mean	264	26.07	67.08
1000	Mean	842	3.20	27.04

* corrected on the basis of mean measured concentrations of clopyralid at 0 and 72h

Table 9.2-36. Summary on results with *S. capricornutum*

Result	**mg EF-255/L (corrected nominal*)**
72h E_bC_{50} (95% c.l.)	449 (197-1615)
0-72h E_rC_{50} (95% c.l.)	730 (661-813)
NOEC	264

* corrected on the basis of mean measured concentrations of clopyralid at 0 and 72h

Conclusions:

The 72h E_bC_{50} value of 449 mg product/L (equivalent to 47.6 mg as/L, as clopyralid) and the E_rC_{50} of 730 mg product/L (equivalent to 77.4 mg as/L, as clopyralid) are in agreement with the values of 30.9 mg as/L and 30.0 mg as/L, respectively, taken from the study on the technical grade material, also conducted on *Selenastrum capricornutum* (see Ref. J51). The NOEC is 264 mg product/L, (28.0 mg as/L). These results demonstrate that the toxicity of clopyralid to *Selenastrum* is not substantially altered when formulated as EF-255.

Comments

The formulation studied (EF-255) is different from the lead formulation EF-1136 for Annex III studies. However, the Notifier clarifies the difference between the two formulations acceptably. The difference in the composition of the two formulations is of minor importance and therefore the study is representative also for EF-1136. The study was well performed and reported and in compliance with GLP.

RMS comments and evaluation:

As the algal studies 2 and 3 are no longer supported by the Notifier in the context of AIR3 evaluation of clopyralid, the RMS has not re-evaluated them, but the above summaries are included as additional information.

Instead, new studies have been submitted, as presented below.

In addition to the data previously evaluated, the Notifier submitted new studies on further algal species for the AIR3 evaluation of clopyralid. These studies have not been previously evaluated in the context of EU approval of clopyralid, and the studies are therefore summarised and evaluated below.

CA 8.2.6.1/2- Clopyralid: Growth Inhibition Test with the Freshwater Blue Green Alga (*Anabaena flos-aquae)*

Report	Hoberg, J. R. 2006
Report title	Clopyralid Technical Grade – Growth Inhibition Test with Freshwater Blue-Green Alga (*Anabaena flos-aquae*)
DAS Study number	060246 Springborn Smithers Laboratories, Massachusetts, Lab Study No. 12550.6430
Guidelines	OECD 201(2004) U.S. EPA FIFRA Subdivision J Guidelines 122-2 and 123-2
GLP	Yes

Materials and methods

Test Item(s)

ISO Common name:	Clopyralid Technical Grade
Test item (chemical/other name):	Lontrel® T Technical, 3,6-Dichloropicolinic acid
Purity:	95.9%
Description (physical state):	Solid
Lot/batch no.:	910905 5P
CAS no.:	001702-17-6 (clopyralid)

Test System

Organism (Species):	freshwater blue-green alga (Anabaena flos-aquae)
Guideline:	OECD 201
Deviations:	None
Environmental conditions:	Test solution temperature (range): 24 to 25°C Temperature range: 23 ºC (±1 ºC) Light intensity (range): 1900 to 2600 lux (175 to 240 footcandles)
Dates of work:	3 May 2006 to 8 May 2006
GLP status:	yes
Observation intervals:	0, 24, 48, 72, 96, 120 days
Age of inoculum:	5 days
Acclimation period/conditions:	Acclimation period under conditions approximating those maintained during the definitive exposure
Initial cell density:	Approximately 1.0 x 10^4 cells/mL
Growth medium:	Name: Algal assay procedure pH at test initiation: 3.1 to 7.0 pH at test termination: 3.0 to 6.7 Constant stirring: 100 rpm
Method of test item added to the test medium:	The test solutions were prepared with AAP medium then a 0.669 mL inoculum was aseptically introduced into each flask.
No. of control replicates:	6

No. of test concentration replicates:	3 Method: measuring concentrations of clopyralid using HPLC/UV
Analytical verification:	Samples taken: 0 and 120 hrs Limit of detection: 0.0117 mg a.i./L Limit of quantitation: 0.100 mg a.i./L Recoveries from QC fortifications: 80 to 120 %
Reference substance:	Clopyralid Analytical Standard

Methodology

All flasks were conditioned prior to use by rinsing with the appropriate exposure or control solution. One hundred millilitres of the appropriate test solution was then placed in each replicate flask. At each subsequent 24-hour interval, duplicate cell counts were conducted on each replicate vessel of the treatment levels and the control using a hemacytometer (Neubauer Improved) and a compound microscope. Two independent samples were removed from each flask for counting. One or more hemacytometer fields, each 0.10 x 0.10 cm in surface area and 0.010-cm deep and containing 0.00010 mL of culture, were examined for each sample until at least 400 algal cells or four fields were counted.

Observations of the health of the algal cells were made at each 24-hour interval. Since *Anabaena flos-aquae* grows in filaments, the solutions were vigorously pipetted multiple times to break up the filaments and achieve a more homogeneous suspension prior to removing a sample for cell counts at the 24-, 48-, 72-, 96- and 120-hour intervals. The cell density in each test flask was calculated for each daily interval by dividing the number of cells counted by the number of fields examined for each cell count. Duplicate counts were averaged for replicate at each time interval. Means and standard deviations for cell density for each treatment and the control were calculated from replicate values.

Average specific growth rate (μ_{ave}) for each replicate flask was calculated for the period from test initiation to each observation time. Yield was calculated as biomass (cell density) at each interval of the test minus the initial biomass at the start of the test. Percent inhibition of the treatment data was calculated relative to the control data.

Based on the results of statistical analysis performed for 120-hour cell density, 72- and 96-hour total yield and average growth rate, the No-Observed-Effect Concentration (NOEC), the highest test concentration which demonstrated no statistically adverse effect ($p \leq 0.05$) when compared to the control data, was determined. The EC_{25} and EC_{50} values (concentrations of test substance which reduced cell density by 25 and 50%, respectively) and the 95% confidence limits were calculated for cell densities after 24, 48, 72, 96 and 120 hours of exposure. Additionally, EC_{50} values were calculated for 72- and 96-hour total yield (E_yC_{50}) and average growth rate (E_rC_{50}). The EC_{25} and EC_{50} values and their 95% confidence limits were determined by linear regression of response (percent reduction of cell density, yield and growth rate as compared with the control) versus the mean measured concentration. If less than the required response was observed (i.e., < 50% response), the EC value was empirically estimated to be greater than the highest concentration tested.

Results and discussion

At test termination (120 hours), cells exposed to the treatment levels tested and the control were observed to be normal. The 120-hour cell density in the control averaged 54.69 x 10^4 cells/mL. Cell density in the 3.0, 6.2, 13, 24, 47 and 99 mg a.i./L treatment levels averaged 56.25, 67.63, 72.71, 38.21, 0.75 and 0.00 x 10^4 cells/mL, respectively. A significant reduction in cell density was determined in treatment levels ≥ 47 mg a.i./L as compared to the control data.

The 0- to 72-hour yield in the 3.0, 6.2, 13, 24, 47 and 99 mg a.i./L treatment levels averaged 13.71, 13.08, 11.75, 2.21, 0.29 and -1.00 x 10^4 cells/mL, respectively. A significant reduction in yield was detected in treatment levels ≥ 24 mg a.i./L as compared to the control data.

The 0- to 96-hour yield in the control averaged 15.50 x 10^4 cells/mL. The 0- to 96-hour yield in the 3.0, 6.2, 13, 24, 47 and 99 mg a.i./L treatment levels averaged 21.33, 17.08, 11.17, 20.79, -0.46 and -1.00 x 10^4 cells/mL, respectively.

Statistical analysis (Kruskal-Wallis' Test) determined no significant reduction in any of the treatment levels tested when compared to the yield in the control. The 0- to 72-hour growth rate in the control averaged 0.81 $days^{-1}$. The 0- to 72-hour growth rate in the 3.0, 6.2, 13, 24, 47 and 99 mg a.i./L treatment levels averaged 0.89, 0.88, 0.84, 0.33, 0.13 and 0.00 $days^{-1}$, respectively. A significant reduction in growth rate was determined in treatment levels ≥ 24 mg a.i./L as compared to the control data. The 0- to 96-hour growth rate in the control averaged 0.67 $days^{-1}$. The 0- to 96-hour growth rate in the 3.0, 6.2, 13, 24, 47 and 99 mg a.i./L treatment levels averaged 0.78, 0.74, 0.41, 0.79, -0.09 and 0.00 $days^{-1}$, respectively. A significant reduction in growth rate was determined in treatment levels ≥ 47 mg a.i./L as compared to the control data.

The results are summarized in Table 9.2.37.

Table 9.2.37. Effects of clopyralid technical on algal growth based on mean measured concentrations

Hour	EC Type	EC Value [mg clopyralid technical/L]	95% Confidence Limits [mg clopyralid technical/L]	NOEC [mg clopyralid technical/L]
72	E_rC_{50}	22	19 - 33	13
	E_yC_{50}	19	15 - 22	13
96	E_rC_{50}	33	26 - 26	24
	E_yC_{50}	24	4.0 - 26	Not applicable
	E_rC_{50}	27	5.1 - 29	Not applicable
	E_yC_{50}	34	12 - 36	99[a]

[a] Based on Kruskal-Wallis' Test, the NOEC was determined to be 99 mg a.i./L. A more reasonable estimate of the NOEC is the E_yC10 (24 mg a.i./L) or E_yC20 (27 mg a.i./L) as suggested by the OECD Guideline (2004).

Conclusion

Based on mean measured concentrations, the 120-hour NOEC for cell density was determined to be 24 mg a.i./L. The 120-hour EC_{50} value was determined to be 29 mg a.i./L, with 95% confidence limits of 24 to 33 mg a.i./L. The 72-hour NOEC for yield was determined to be 13 mg a.i./L. The 72-

hour E_yC_{50} was determined to be 19 mg a.i./L, with 95% confidence intervals of 15 to 22 mg a.i./L. Based on Kruskal-Wallis' Test, the 96-hour NOEC for yield was determined to be 99 mg a.i./L. A more reasonable estimate to the NOEC is the E_yC_{10} or E_yC_{20}, 24 and 27 mg a.i./L, as suggested by the OECD Guideline (2004). The 96-hour E_yC_{50} was determined to be 34 mg a.i./L, with 95% confidence limits of 12 to 36 mg a.i./L. The 72-hour NOEC for growth rate was determined to be 13 mg a.i./L. The 72-hour E_rC_{50} was determined to be 22 mg a.i./L, with 95% confidence limits of 19 to 33 mg a.i./L. The 96-hour NOEC for growth rate was determined to be 24 mg a.i./L. The 96-hour E_rC_{50} was determined to be 33 mg a.i./L, with 95% confidence limits of 26 to 36 mg a.i./L. The endpoints are summarized in Table 9.2.38. below.

Table 9.2.38. The endpoints of algal growth study on clopyralid with *Anabaena flos-aquae*

Common name	Species	Test item	Time-scale	Endpoint	Toxicity value	Units of test item
Freshwater blue algae	*Anabaena flos-aquae*	clopyralid technical	72-hr	E_rC_{50}	22	mg/L
Freshwater blue algae	*Anabaena flos-aquae*	clopyralid technical	72-hr	E_yC_{50}	19	mg/L
Freshwater blue algae	*Anabaena flos-aquae*	clopyralid technical	96-hr	E_rC_{50}	33	mg/L
Freshwater blue algae	*Anabaena flos-aquae*	clopyralid technical	96-hr	E_yC_{10}	24	mg/L
Freshwater blue algae	*Anabaena flos-aquae*	clopyralid technical	96-hr	E_yC_{20}	27	mg/L
Freshwater blue algae	*Anabaena flos-aquae*	clopyralid technical	96-hr	E_yC_{50}	34	mg/L

The acceptance criteria of 96-hour cell growth in the control was 16.50 x 104 cells/mL, which was met, whereas the mean daily growth rate CV through 96 hours for the control was 469% (should be less than 35%), and the average overall growth rate of the control for the 0- to 96-hour period was 27% (should be less than 10%), indicating that these criteria were not met for this study. The reason these criteria were not achieved is that *Anabaena flos-aquae* grows in filaments and cell counts are traditionally more variable than single cell algae. The test solutions were vigorously pipetted multiple times to disperse clumped cells and provide a homogeneous suspension for counting.

In a secondary analysis of the data, control replicates D and F were empirically considered outliers and excluded from the data analysis to minimize the variability among control replicates (mean daily growth rate CV = 196%). The EC_{50} values for 120-hour cell density (28 mg a.i./L), 96-hour growth rate (31 mg a.i./L) and yield (32 mg a.i./L) remained essentially the same. Therefore the results of this study are reported based on the entire control data set.

RMS comments and evaluation:

The study was conducted after the first Annex I inclusion of clopyralid, and therefore not evaluated before in the context of EU approval. The study was well performed and reported, according to the test guideline and GLP, except the analysis of dilution water, which was conducted according to US EPA procedures in another laboratory. This deviation was not considered to have an impact on the results of the study. The method of analysis is acceptably validated for the determination of clopyralid in AAP medium. The LOQ is fit for the purpose.

The Notifier has presented a new specification for technical clopyralid. The original specification of clopyralid is based on the analysis of the batch used in this study. Based on the risk assessment for the impurities of clopyralid presented by the Notifier in Document J in December 2016, no

impurity characterised in this batch is expected to substantially impact the ecotoxicological profile of clopyralid. The impurity profile of the test substance used in this study is within the limits of the new specification presented by the Notifier in December 2016.

Although two of the acceptance criteria were not met in this study, the explanations given by the Author are reasonable. The dose-response observed during this study was well-defined and provided sound NOEC and EC_{50} values with reasonable 95% confidence limits. As the results are also in agreement with data from other algal studies, this study can be considered as acceptable. This study resulted to lowest endpoint value of algal tests, and therefore it can be used in the risk assessment of clopyralid despite the deficiencies discussed above. The impurity profile of the test substance used in this study was within the limits of the specification presented by the Notifier in December 2016.

CA 8.2.6.1/3- Clopyralid: Growth Inhibition Test with the Freshwater Diatom, *Navicula pelliculosa*

Report	Aufderheide, J. 2014
Report title	Clopyralid Technical: Growth Inhibition Test with the Freshwater Diatom, *Navicula pelliculosa*
DAS Study number	140515 ABC Laboratories, Inc. Lab Study No. 81018
Guidelines	OECD 201 U.S. EPA OCSPP 850.4500
GLP	Yes

Materials and methods

Study Sponsor:	Dow AgroSciences LLC	
Protocol Title:	Clopyralid Technical: Growth Inhibition Test with the Freshwater Diatom, *Navicula pelliculosa*	
Location of Study:	ABC Laboratories, Inc. 7200 E. ABC Lane Columbia, Missouri 65202	
ABC Study No.:	81018	
Dow Study No.:	140515	
Test Substance:	Clopyralid Technical (Test Substance No.: TSN100167; Lot No.: 910905 5P; Purity: 95.9%)	
Test Species:	*Navicula pelliculosa*	
Source of Test Species:	University of Texas at Austin (UTEX)	
Definitive Test Dates (in-life):	13 to 17 October 2014	
Test Duration:	96 hours	
Nominal Concentrations:	Based upon the range-finding tests data, the definitive nominal test concentrations were 0 (control), 1.5, 3.0, 6.0, 12, 24, and 48 mg a.i./L	
72-Hour Mean Measured Concentrations:	<MQL (control), 1.60, 3.09, 6.01, 12.3, 26.5, and 50.4 mg a.i./L	
96-Hour Mean Measured Concentrations:	<MQL (control), 1.60, 3.10, 6.04, 12.5, 25.6, and 50.8 mg a.i./L	
Environmental Conditions: (in biotic replicates)	Test Solution Temperature:	23.3 to 24.8°C
	Test Solution pH:	4.5 to 7.5
	Photoperiod:	continuous light
	Light Intensity (positions):	4,116 to 4,860 lux
	Light Intensity (daily):	4,650 to 4,690 lux

The exposure flasks were 250-mL Erlenmeyer flasks with foam stoppers and labeled with study number, treatment, replicate, and grid position. Prior to test initiation, the flasks were cleaned and autoclaved according to ABC standard operating procedures and were randomly assigned to treatments using a computer-generated random number table. The control treatment was replicated six times (replicates A, B, C, D, E, and F) and each test substance treatment was replicated four times (replicates A, B, C, and D). Each replicate contained 100 mL of the appropriate parent solution. An additional flask (replicate G) for the control and each test substance treatment were prepared to supply analytical samples at the 72-hour time points. Two additional replicates (replicates E and F) of the 1.5 mg a.i./L test substance treatment, containing 100 mL of the appropriate parent solution, was also prepared and used to evaluate the potential for incorporation of the test substance into the algal biomass. At test initiation, each biological replicate of the control (replicates A through F) and each test substance treatment (replicates A through D) as well as the additional analytical replicates (replicates G) were inoculated with 1.0 mL of an algal concentrate containing approximately 1.0 x 106 cells/mL, resulting in a final density of approximately 1.0 x 104 cells/mL for each flask. The replicates were inoculated with algae within 30 minutes after test solution preparation. At 24, 48, 72, and 96 hours (±1 hour), cell density was measured in all biological replicates of the control and test substance treatments by direct microscopic counting with a hemacytometer. Replicate E and F of the 1.5 mg a.i./L test substance treatment were not inoculated with algae.

During the four-day exposure period, the flasks were randomly positioned daily using a computer-generated random number table and incubated at 24 ± 2°C in a temperature controlled environmental chamber under continuous cool-white fluorescent lighting with an intensity of 4,300 ± 645 lux. A continuous recording of environmental chamber temperature was made from one uninoculated blank flask using an electronic data logger with thermistor probe. Light intensity was measured from each test vessel position at test initiation with a LI-COR Model LT-250A light meter equipped with a LI-COR photometric sensor. The mean light intensity over all test positions was 4,489 ± 177 lux and with a range of 4,116 to 4,860 lux. The measured light intensity was also measured daily from a centrally located test vessel position and ranged from 4,650 to 4,690 lux. The flasks were swirled on an orbital shaker table at 100 rpm throughout the test. Temperature and pH were measured in all parent solutions prior to distribution of the solutions to the test flasks. At 72 hours, temperature and pH were measured in replicate G of the control and each test substance treatment. At 96 hours, temperature and pH were measured in composite samples from the biological replicates of the control (replicates A, B, C, D, E, and F) and each test substance treatment (replicates A, B, C, and D). All temperature and pH measurements of the test solutions were performed with a WTW Model pH 330i meter.

At the end of the 96-hour exposure period, the blank control and those test concentrations exhibiting a maximal inhibition, i.e., those where the 96-hour cell density was less than or approximately equal to the initial 1.0 x 104 cells/mL cell density, were selected for the recovery test. Only the 48 mg a.i./L treatment had cell densities that were less than the initial cell density at test initiation. The recovery portion of the definitive test was conducted from 17 to 23 October 2014. For the blank control and the 48 mg a.i./L test treatment, a 0.5 mL aliquot was taken from each replicate test flask and diluted to 100 mL with untreated test medium and exposed in a single test flask. The dilution was sufficient to result in an exposure concentration that would not inhibit algal growth and growth rate based on visual observations during termination of the definitive test. The recovery exposure replicates were incubated under the same general conditions used during the definitive exposure with a temperature range of 24.0 to 24.5 ºC and light intensity range of 4,446 to 4,499 as measured from a mid-study position.

Recovery replicates were monitored for growth on day 6 of the recovery exposure.

Test solutions of clopyralid technical were analyzed using a high performance liquid chromatography system with ultraviolet detection (HPLC-UV).

Statistical Analysis: All statistical analyses were performed with SAS software (Version 9.3 for Windows).The NOEC values, based on area under the growth curve, growth rate, and yield, were estimated using a one-way analysis of variance (ANOVA) procedure and a one-tailed Dunnett's test ($p = 0.05$) where the alternate hypothesis was the mean for the growth parameter was reduced in comparison to the control. Prior to the Dunnett's test, a Shapiro-Wilk's test and a Levene's test were conducted to test for normality and homogeneity of variance, respectively, over treatments at each time point. If the results from the Shapiro-Wilk's and Levene's tests indicated normality and insignificant heterogeneity (i.e., $p > 0.01$), the analysis was performed on the nontransformed raw data. In instances of non-normality or heterogeneity (i.e., $p < 0.01$), a square root transformation was performed. If both the non-transformed raw data and the transformed data exhibited non-normality or inequality of variance, a non-parametric analysis of variance was performed on the ranks of the raw data values. Non-parametric analyses were performed on specific growth rate data at the 0-48, 0-72, and 0-96 hour time points. Parametric analyses were performed on specific growth rate at the 0-24-hour time point and area under the growth curve data and yield data at 24-, 48-, 72-, and 96-hours.

Percent Inhibition Calculations and IC Estimates: The area under the growth curve in each treatment was calculated from 0-hour to 24, 48, 72, and 96 hours. The IbC50, IrC50, and IyC50 estimates were calculated using a logistic (sigmoid-shaped) model fit to the data with percent inhibition as the dependent variable and concentration as the independent variable. The model used to describe the response to increasing test substance concentrations was the four-parameter logistic model with two parameters fixed; the minimum percent inhibition at 0%, and the maximum percent inhibition at 100%. The model was fit only in instances where the mean percent inhibition at the highest test substance treatment was greater than 45%. In instances where there was no test substance treatment inhibition that was greater than 45% any values generated from this model should be considered as estimated values. A nonlinear modeling procedure was used to estimate the slope and IC values. The distribution of $x\hat{}$ method was used to estimate the 95% confidence limits.

Results

The results are summarized in the following tables with the 72-hour and the 96-hour effects concentrations based upon the arithmetic mean measured concentrations to satisfy the OECD and OCSPP guidelines, respectively.

Table 9.2.39. Algal growth inhibition results with diatom based on 72-hour arithmetic mean concentrations

Hour	IC Type	IC Value (mg a.i./L)	95% Confidence Limits (mg a.i./L)	NOEC (mg a.i./L)
72 Hours	I_bC_{05}	23.9	22.9 and 25.0	1.60
	I_bC_{10}	25.8	24.6 and 26.9	
	I_bC_{20}	27.9	26.7 and 29.2	
	I_bC_{50}	32.1	30.7 and 33.5	
	I_rC_{05}	21.5	6.29 and 36.7	1.60
	I_rC_{10}	23.6	10.9 and 36.4	
	I_rC_{20}	26.2	15.4 and 37.0	
	I_rC_{50}	31.3	16.5 and 46.0	
	I_yC_{05}	23.5	22.2 and 24.8	1.60
	I_yC_{10}	25.3	23.9 and 26.7	
	I_yC_{20}	27.4	25.9 and 28.9	
	I_yC_{50}	31.5	29.8 and 33.2	

Table 9.2.40. Algal growth inhibition results with diatom based on 72-hour arithmetic mean measured concentrations

Hour	IC Type	IC Value (mg a.i./L)	95% Confidence Limits (mg a.i./L)	NOEC (mg a.i./L)
96 Hours	I_bC_{05}	21.4	21.1 and 21.6	1.60
	I_bC_{10}	23.0	22.7 and 23.3	
	I_bC_{20}	25.0	24.7 and 25.2	
	I_bC_{50}	28.7	28.3 and 29.0	
	I_rC_{05}	22.7	13.8 and 31.6	1.60
	I_rC_{10}	24.4	14.8 and 34.0	
	I_rC_{20}	26.5	16.1 and 36.9	
	I_rC_{50}	30.5	18.5 and 42.4	
	I_yC_{05}	20.2	20.0 and 20.3	1.60

The decline in average cell density observed during the 96-hour test exposure indicates that clopyralid technical may be algicidal in its effect on *N. pelliculosa*. Based on 1.78×10^4 cells/mL from the cell count after six days, the effects upon growth of *N. pelliculosa* were found to be algistatic as well at nominal clopyralid technical concentrations less than or equal to 48 mg a.i./L.

Conclusions

The test acceptability criteria were met for this study. The number of algal cells in the control was greater than 16 times the number initially inoculated after the initial 72 hours of testing and greater than 30 times the number initially inoculated after 96 hours, verifying logarithmic phase growth. The coefficient of variation for daily growth rates in the control replicates during the course of the test did not exceed 35% during the first 72 hours of testing. The coefficient of variation for average specific growth rates in the control replicates did not exceed 10% during the initial 72 hours of testing. The coefficients of variation for mean control yield and average specific growth rates in control replicates at termination did not exceed 15%. The pH in the control did not increase more than 1.5 units during the initial 72 hours of testing. This study satisfies the OECD and OCSPP guideline requirements for a growth inhibition test with *Navicula pelliculosa*.

RMS comments and evaluation:

The study was conducted after the first Annex I inclusion of clopyralid, and therefore not evaluated before in the EU approval context. The study was well performed and reported, according to the test guideline and GLP, except the water analysis which was not conducted in accordance to the stated GLP practices. This deviation was not considered to have an impact on the results of the study. The method of analysis of test solutions was successfully validated and the LOQ can be considered to be fit for the purpose of this study.

The Notifier has presented a new specification of the technical clopyralid. The original specification of clopyralid is based on the analysis of the batch used in this study. Based on the risk assessment for the impurities of clopyralid presented by the Notifier in Document J in December 2016, no impurity characterised in this batch is expected to substantially impact the ecotoxicological profile of clopyralid. The impurity profile of the test substance used in this study is within the limits of the new specification presented by the Notifier in December 2016.

The acceptance criteria of the study guideline were met in this study and the study is considered as valid. The results are in agreement with other algal studies available, and therefore the outcome of this study can be used in the risk assessment of clopyralid. Thus, in total there is data available on three algal species from different taxa, which represent similar toxicity level, and therefore the data requirement is fulfilled and no further studies are required.

In addition to studies generated by the Notifier, one more algal study was submitted as a result of literature search on published literature. This study is summarized in Table 9.2.41. below.

Table 9.2.41. Toxic effects of pesticide extracts on green algal species

Assessment of toxic effects of pesticide extracts on different green algal species by using chlorophyll a fluorescence	
KCA 9/1 (CA 8.2.6.1/4)	
Author(s)	Chalifour, A., Spear, P.A., Boily, M.H., DeBlois, C., Giroux, I., Dassylva, N., Juneau, P.
Year	2009
Journal	Toxicological & Environmental Chemistry Vol. 91(7), pp. 1315-1329
Relevance check	Relevant. Clopyralid is a PA herbicide. Guideline is not stated but methodology is described.
Reliability check	Reliability score 2 (Klimisch)
Reasons for no reliability	Not relevant
Summary	Three types of green algae *Chlorella vulgaris*, *Scenedesmus obliquus* and *Pseudokirchneriella subcapitata* were exposed to extracts from a reference site (‘Deborah Stairs’) or to extracts with 1 or 2 times the concentration of organophosphorus (OP) or phenoxyacid-type (PA; including clopyralid) pesticides found in the original water samples from sites of the Yamaska River associated with high agricultural activity (‘Riviere Noire’ and ‘Riviere a la Barbue’). After 96 hours of exposure, various fluorescence parameters were obtained. Results showed that *S. obliquus* was the most sensitive and *C. vulgaris* the least sensitive species, and operational PSII quantum yield (a proxy of photosynthetic electron capacity) and electron transport rate per reaction center were the most sensitive parameters. In addition, PA extracts were shown to be more toxic than OP extracts, and doubling the pesticide extract concentration did not increase the inhibitory effect by more than 10%.
Reliability check: study details	
Parameter	**Information available**

Test protocol GLP, GEP, Guidelines (US EPA, OECD, …)	- None discussed
Test substance Identification of test substance, source, purity, stability	- Concentrations of different OP and PA pesticides were analysed. The OP extracts contained at least atrazine and metolachlor, but also could contain desisopropyl-atrazine, deethyl-atrazine, simazine and dimethenamid. The PA extracts contained at least dicamba and 2,4-D, but could also contain clopyralid, MCPA and bentazon
Test conditions Temperature, pH, oxygen concentration, water hardness, conductivity, photoperiod, light intensity, number of animals, food availability	- Temperature: 22ºC - pH: 6.8 - Photoperiod: 16h light/8h darkness - Irradiance: 5 µmol photons $m^{-2}s^{-1}$ - Initial cell density: 5 x 10^5 (*C. vulgaris* and *S. obliquus*) and 2 x 10^5 (*P. subcapitata*)
Controls Positive control, negative control	- Extracts of samples from the reference site 'Deborah Stairs' for the Yamaska River; a pond in a forested, non-agricultural area, with all pesticide concentrations below the limit of detection
Dosing system Exposure (dose, duration, frequency)	- Duration: 96 hours - Frequency: Static/1 application - Dose: sample extracts with 1 and 2 times the concentration of organophosphorus (OP) or phenoxyacid-type (PA) found in the original water samples taken at 'Riviere Noire' and 'Riviere a la Barbue', two sites of the Yamaska River associated with high agricultural activity - Replication: 3
Test species Body weight or length, gender, age/life stage, source	- Green algae *Chlorella vulgaris*, *Scenedesmus obliquus* and *Pseudokirchneriella subcapitata*, cultivated in a semi-continuous culture - Source: Unknown
Statistical analyses Sample size/replicates, statistical analysis of data (significance level, variability)	- Tukey-Kramer HSD test ($p < 0.05$), using JMP 5.1 statistical software (SAS institute, USA)
Biological effects Determined effect concentration, dose response observed	- Chlorophyll a emission kinetic and chlorophyll a fluorescence transients were measured after 96 hours after exposure, to obtain various fluorescence parameters - The different green algae species did not have the same sensitivity to pesticide exposure, with the order of sensitivity of *C. vulgaris* < *P. subcapitata* < *S. obliquus* - Doubling the pesticide extract concentration did not increase the inhibitory effect by more than 10% - Relative non-photochemical quenching and the unquenched fluorescence tended to be higher for the treated algae, indicating an energy dissipation shift from photochemistry to non-photochemical processes - Higher level of J transient for treated algae - Operational PSII quantum yield (a proxy of photosynthetic electron capacity) and electron transport rate per reaction center were the most sensitive parameters - PA extracts were more toxic than OP extracts
Overall assessment	- Methodology, results and discussion are documented - Statistical analysis used was described - Study is reliable

RMS comments and evaluation:

The study was clearly reported, although not conducted according to any standard test guideline applicable for regulatory purposes for plant protection products. The herbicidal effect of phenoxy acids on green algae was clearly demonstrated in this study, compared to organophosphates that generally pose a higher toxicity to aquatic invertebrates and fish.

Because this study was not conducted according to any standard methodology, its results are not directly comparable to the data from standard single-compound and species studies as presented above and owned by the Notifier. As the test solutions were mixtures extracted from natural watersheds, it was not possible to assess the origin of clopyralid in more detail. No data is available on the specification of the test substance used in this study, so the impurity profile is unknown. However, taken that the article has undergone a peer review before publishing, the result can be considered as likely valid. Therefore its outcome can be used as supporting information to illustrate the potential exposure situation of freshwater algae to multiple residues in the environment.

B.9.2.3. Effects on aquatic macrophytes

CA 8.2.7/1- The Fourteen Day Toxicity of Lontrel T to *Lemna gibba* L G-3 (Duckweed)

Report	[IIA 8.2.8/01], Cowgill, U. M. ; Milazzo, D. P. ; Potter, R. B. , 1990
Report title	The Fourteen Day Toxicity of Lontrel T to *Lemna gibba* L G-3 (Duckweed)
DAS Study number	ES-DR-0197-3428-4 DAS Report No. ES-2243, Ref. J28
Guidelines	US EPA OPP 122-2
GLP	Yes

Methodology:

Clopyralid (termed 'Lontrel T' in the report), Batch No. AGR 0233257, Purity 96.4%. The inhibitory effect of clopyralid on the growth of the duckweed *Lemna gibba* G-3, by means of assessing phytotoxicity, has been assessed over a 14-day exposure period.

In a range-finding test, two cultures each containing 15 fronds, were treated either with clopyralid (nominal concentrations of 1, 10, 100 and 1000 mg/L) or were cultured in growth medium only (control treatment, Hoagland's medium without EDTA, yeast extract, sugar and bactotryptone) for a 14-day period. The results of the range-finding test indicated that the 14-day EC_{50} would be less than 100 mg/L.

For the definitive test, triplicate cultures, each containing 15 fronds, were treated with six (nominal) concentrations of clopyralid: 6.2, 10.4, 17.3, 28.8, 48.0, 80.0 and 150 mg/L plus a control (culture medium only). Additional vessels were established to measure pH and the concentration of clopyralid, in the absence of *L. gibba*. The test medium was not renewed during the study. Over the 14-day exposure period the photoperiod was continuous (5413 ± 377 lux) and temperature ranged from 24.5 to 26.1°C. The initial pH of each medium was 3.9 to 4.6. The test medium volume was 100 mL in 250 mL glass Erlenmeyer vessels. Light intensity, pH and plant growth (number of fronds and plants) was measured every three days throughout the study. Samples of each test medium, with and without *L. gibba* present, were taken on days 0, 7 and 14, with samples of each replicate taken on day 0 only. Samples were analysed by HPLC.

The measured concentrations of clopyralid in the test media were similar to nominal concentrations throughout the study duration (Table 9.2-42) thus clopyralid was stable under the test conditions. The results of the study were based on mean measured concentrations of clopyralid. Comparison of the day 0, 7 and 14 results show that clopyralid did not degrade in the presence of *L. gibba*, therefore the overall mean measured value was used in the estimation of the endpoints.

Findings:

After 14 days exposure, the mean number of fronds in the control treatment was 2090 (592 plants) compared to 19 (8 plants) at the highest mean measured concentration of 171.2 mg/L (Table 9.2-43). Other than at the highest concentration tested, the pH in the test medium increased by 1.6 to 1.9 units in the presence of plants from day 3 to day 14. Without plants present the pH remained stable. The results are summarised in Table 9.2-44.

Table 9.2-42. Measured concentrations of clopyralid in test medium during a growth inhibition study with *L. gibba*

Nominal concentration of clopyralid (mg/L)	**Measured clopyralid concentrations (mg/L)**			
	Day 0	**Day 7**	**Day 14**	**Mean**
Control	ND[a]	ND	ND	ND
6.2	6.9	7.3	7.5	7.2
10.4	11.5	12.4	12.3	12.0
17.3	20.6	20.2	19.9	20.3
28.8	31.6	33.4	32.7	32.4
48.0	53.8	55.3	54.8	54.5
80.0	91.8	92.8	90.2	90.7
150	175.8	168.4	167.3	171.2

[a] ND: not detected at 8 µg a.s./L.

Table 9.2-43. Inhibition of growth of *L. gibba* exposed to clopyralid over a 14-day exposure period

Mean measured concentration of clopyralid (mg/L)	**Number of plants**	**Number of fronds**
Control	592	2090
7.2	570	2010
12.0	520*	1777*
20.3	499*	1778*
32.4	506*	1819*
54.5	475*	1719*
90.7	277*	997*
171.2	8*	19*

* Significantly different from the control at $p < 0.05$ (one-tailed Dunnett's test).

Table 9.2-44. Results

Result	Mean measured concentration of clopyralid (mg/L)
14-day EC_{50} (plants) (95% C.L.)	89 (63 to 114)
14-day EC_{50} (fronds) (95% C.L.)	89 (56 to 122)

Conclusions:

The 14-day EC_{50} of clopyralid to the duckweed *L. gibba* G-3 was 89 mg/L based on mean measured concentrations under static test conditions. The NOEC was 7.2 mg/L, measured, based on significant reductions in fronds and plants at 12.0 mg/L and above.

Comments

The study was well performed and reported and in compliance with GLP. The result can be used in the risk assessment.

The 14-day EC_{50} of technical clopyralid (purity of 96.4 %) to the duckweed *Lemna gibba* G-3 was 89 mg/L based on mean measured concentrations under static test conditions. The NOEC was 7.2 mg/L, measured, based on significant reductions in fronds and plants at 12.0 mg/L and above.

RMS comments and evaluation:

Originally evaluated in the DAR (2003): The study was well performed and reported and in compliance with GLP. The result can be used in the risk assessment.

Based on the risk assessment for the impurities of clopyralid presented by the Notifier in Document J in December 2016, no impurity in the technical specification used in this study is expected to substantially impact the ecotoxicological profile of clopyralid. The impurity profile of the test substance used in this study was within the limits of the new specification presented by the Notifier in December 2016.

The conclusion above is still valid and the endpoint of EC_{50} = 89 mg/L from this Lemna study can be used in the risk assessment of clopyralid to aquatic macrophytes. The impurity profile of the test substance used in this study was within the limits of the specification presented by the Notifier in December 2016. No further comments.

In the first Annex I dossier of clopyralid submitted in 30 April 2002, additionally one report was included to study the effects of clopyralid to submersed aquatic macrophytes. The data were deemed as supporting information following the evaluation preseented in the DAR (2003). Nevertheless, in this field study significant effects were found on higher aquatic plants, although the study was not performed according to any standard guidelines and GLP. The original assessment is presented below.

Study 2 – submersed aquatic macrophytes, field trial

Report: Forsyth, D.J., Martin, P.A. and Shaw, G.G. (1997): Effects of herbicides on two submersed aquatic macrophytes, *Potamogeton pectinatus* L. and *Myriophyllum sibiricum* Komarov, in a prairie wetland. Environmental Pollution, vol. 95 no. 2, pp. 259 – 268, 1997.

Guidelines: researchers' own method, field experiment

GLP: no

Methodology:

Submersed macrophytes, *Potamogeton pectinatus* L. and *Myriophyllum sibiricum* Komarov, were planted in enclosures of polyethylene, 1 m square, in water 50-70 cm deep, that were built in a permanent pond. Water in the enclosures was treated with herbicides to yield concentrations of 0.01 and 0.1 mg a.i./l. In addition of clopyralid, also 2,4-D, picloram and a mixture of these two herbicides, as well as a non-treated control were studied in separate enclosures.

The growth (weight) of individual plants, signs of injury and number of floral spikes were examined after 30 and 60 days of exposure. Presence or absence of injuries was recorded as disintegrating roots, stems or both (DRS) and distorted leaves (DL). Frequency of injury occurrence was calculated as the percentage of plants in each treatment exhibiting any degree of injury in the two categories. Water samples were collected 6 hours after the herbicide treatment to confirm that target concentrations had been attained and clopyralid was analysed in water and sediment also after 24 hours and 7, 30 and 60 days.

Findings:

Clopyralid did not show any significant loss from water phase during the 60 days experiment, but was persistent at both concentrations studied. The measured concentrations were between 58 and 138 % of nominal concentrations originally applied at the beginning of the study. Clopyralid was not present in sediment in the lower treatment enclosures, but showed a gradual increase in concentration over time in the higher treatment enclosures (from a mean of 53 µg/kg one day after the application to a mean of 155 µg/kg 60 days later). The intended concentrations in water remained constant.

Clopyralid was the least damaging herbicide to both species of plant. Weight gain and flowering were unaffected relative to controls in *P. pectinatus* at both concentrations, but were stimulated 54 % and 89 %, respectively ($p < 0.05$), in *M. sibiricum* at 0.01 mg/l in 30 days, and flowering remained stimulated at 60 days. Injuries were induced only in *M. sibiricum* and consisted of deformed leaves at both concentrations. Roots and stems (DRS) of *P. pectinatus* were also significantly injured at higher treatment. Mean production of tubers by of *P. pectinatus* was 2.3 and 2.9 times greater than that of controls at the two concentrations. The results with other active substances are not referred here in detail, but 2,4-D caused most damages, and especially the mixture of picloram and 2,4-D affected most severely to all parameters at both concentrations and with both species.

Conclusions

According to the authors, stimulation of growth by low concentrations of herbicides is well documented with terrestrial plants and is apparent also with aquatic plants, and is probably attributable to a general promotion of growth typical of auxins at low concentrations. Herbicide concentrations in the water of prairie wetlands may increase or remain relatively constant for the duration of the summer and thereby increasing the degree of exposure for aquatic plants.

Comments

The study was well reported and published in a scientific paper. Study methods were developed by the authors and did not follow any guidelines, but were clearly described in the article. GLP was not applicable. The results with clopyralid are in line with data provided by the notifier in the dossier (water/sediment data, Lemna study), and therefore the data can be used as supporting data. Concentrations studied were slightly higher than the PEC_{sw} values resulting from spray drift from uses intended in Europe, but comparable to the drainflow concentrations.

RMS comments and evaluation:

Because this study was no longer supported by the Notifier in the context of AIR3 evaluation, the RMS has not re-evaluated it. This data can be considered as additional information. No further comments.

Instead, a new aquatic macrophyte study has been submitted and is evaluated below.

Specific sensitivity of aquatic macrophytes to clopyralid can be expected as it is a herbicide. Therefore the Notifier submitted a new laboratory toxicity test with *Myriophyllum spicatum* according to recent OECD guideline for the AIR3 evaluation, as presented below. This new study has not been evaluated before in the context of EU approval, and therefore it is described below in more detail.

CA 8.2.7/2 - Clopyralid: Toxicity to the Aquatic Macrophyte, *Myriophyllum spicatum*

Report	Banman, C. S., Moore, S. 2015
Report title	Clopyralid: Toxicity to the Aquatic Macrophyte, *Myriophyllum spicatum*
DAS Study number	140735 SynTech Research Laboratory Services LLC, Lab Study No. 14SRLS14C2
Guidelines	OECD Test Guideline 239 (2014) US EPA OCSPP.SUPP
GLP	Yes

Materials and methods

Test Item(s)

ISO Common name:	Not applicable
Test item (chemical/other name):	Clopyralid
Purity:	95.9%
Description (physical state):	White to tan powder
Lot/batch no.:	910905 5P
CAS no.:	1702-17-6

Test System

Organism (*Species*):	Aquatic plant, *Myriophyllum spicatum* L
Study type:	Laboratory study - water/sediment system
Study duration:	14 days
Parameters measured:	Test solution pH (range): 7.9 to 9.9 Test solution temperature (range): 19.6 to 20.2°C Oxygen saturation (range): 10.0 to 12.4 mg/L
Environmental conditions:	Photoperiod: 16 hours light / 8 hours dark Light intensity (range): 10,140 to 10,630 lux Temperature (range): 19.6 to 20.2°C
Observation intervals:	Daily
Test concentrations:	Nominal: Control, 8.9, 28.6, 91.6, 293, 938 and 3000 µg a.i./L Mean calculated concentrations: Control, 9.2, 28.5, 90.3, 287, 920 and 3120 µg a.i./L
Acclimation period/conditions:	16 hours light: 8 hours dark. 20.0 ± 5.0 °C.
Growth medium:	Name: Hard Processed Water (blended spring and R.O. water)
Method of test item added to the test medium:	Water stock prepared and stirred into treatment vessels
No. of control replicates:	10

No. of test concentration replicates:	5
No. of rooted apical shoots per vessel:	4 plants, thinned to 3 plants at the start of the exposure period
Analytical verification:	Method: measuring concentrations of clopyralid using LC-MS/MS Samples taken : 0 and 14days Limit of Detection: Not applicable Limit of Quantitation: 2.0 µg/L Recoveries from QC fortifications: 92 to 99%
Test substance renewal days:	None

Methodology

Following a seven day acclimation period, *Myriophyllum spicatum* shoots were exposed for 14 days under static conditions. Samples were analyzed for concentration of clopyralid. Shoots within a replicate were planted in sediment within a 300-mL borosilicate glass crystallization dish housed in a 2-L glass beaker. Parameters measured included growth rate and yield (NOEC, LOEC and EC_{50}) of total shoot lengths, total plant wet weight and total plant dry weight.

Results and discussion

Mean measured recoveries from day 0 and 14 ranged from 98 to 104% of the nominal concentrations. Samples were analyzed for clopyralid. The toxicity values were calculated based on nominal concentrations in units of µg active ingredient/L. No abnormal shoot or root development was observed in any treatment level as compared to the control group throughout the study. The lowest E_yC_{50} for yield in the 14-day exposure of the rooted aquatic macrophyte *Myriophyllum spicatum* to clopyralid was obtained for total shoot length. The statistical NOE_rC, LOE_rC and E_rC_{50} for this endpoint were 8.9, 28.6 and 1225 µg a.i./L, respectively.

The yields calculated as mean total shoot length (cm) are presented in Table 9.2.45., as mean total plant fresh weight (g) in Table 9.2.46., and as mean total plant dry weight (g) in Table 9.2.47. below.

Table 9.2.45. Mean total shoot length including side shoots (cm)

Nominal concentration (µg a.i./L)	Days after application		Yield (cm)	Reduction in yield (%)	Growth rate (1/day)	Reduction in growth rate (%)
	0[1]	14				
Control	8.2	45.2	37.0	NA	0.1221	NA
8.9		44.4	36.2	2.15	0.1208	1.04
28.6		33.7	25.6	30.9*	0.1012	17.1*
91.6		29.6	21.5	41.9*	0.0921	24.6*
293		28.9	20.8	43.9*	0.0902	26.2*
938		27.2	19.0	48.6*	0.0857	29.8*
3000		23.5	15.4	58.5*	0.0755	38.2*

* significantly different reduction compared to the pooled control

1) based on 15 additional plants, representative of those used in the test

Table 9.2.46. Mean total plant fresh weight (g)

Nominal concentration (µg a.i./L)	Days after application		Yield (cm)	Reduction in yield (%)	Growth rate (1/day)	Reduction in growth rate (%)
	0[1]	14				
Control	0.3234	1.9122	1.5888	NA	0.1264	NA
8.9		1.8512	1.5278	3.84	0.1246	1.46
28.6		1.5057	1.1823	25.6*	0.1098	13.1*
91.6		1.4273	1.1039	30.5*	0.1050	17.0*
293		1.4887	1.1652	26.7*	0.1085	14.2*
938		1.2976	0.9742	38.7*	0.0988	21.8*
3000		1.1103	0.7868	50.5*	0.0879	30.5*

* significantly different reduction compared to the pooled control
1) based on 15 additional plants, representative of those used in the test

Table 9.2.47. Mean total plant dry weight (g)

Nominal concentration (µg a.i./L)	Days after application		Yield (cm)	Reduction in yield (%)	Growth rate (1/day)	Reduction in growth rate (%)
	0[1]	14				
Control	0.0408	0.2030	0.1622	NA	0.1143	NA
8.9		0.1964	0.1556	4.03	0.1122	1.88
28.6		0.1582	0.1174	27.6*	0.0967	15.5*
91.6		0.1447	0.1039	35.9*	0.0897	21.5*
293		0.1394	0.0986	39.2*	0.0866	24.3*
938		0.1260	0.0852	47.5*	0.0790	30.9*
3000		0.1233	0.0825	49.1*	0.0781	31.7*

* significantly different reduction compared to the pooled control
1) based on 15 additional plants, representative of those used in the test

The calculated EC_{50} values, NOEC and LOEC based on growth rate and yield for each of the measured parameters (total shoot length, fresh weight and dry weight) are summarised below in Table 9.2.48.

Table 9.2.48. Summary of biological effects of clopyralid on *Myriophyllum spicatum* (based on nominal concentrations µg /L)

Parameter (µg/L)	Total shoot length		Total shoot length		Total shoot length	
	Growth rate	Yield	Growth rate	Yield	Growth rate	Yield
14-day EC_{50}	>3000	1225	>3000	2917	>3000	>3000
95% Conf. Limits	NA	271-2124	NA	1074-NA	NA	NA
14-day NOEC	8.9	8.9	8.9	8.9	8.9	8.9
14-day LOEC	28.6	28.6	28.6	28.6	28.6	28.6

Conclusion

The E_rC_{50} for growth rate in the 14-day exposure of the rooted aquatic macrophyte *Myriophyllum spicatum* to clopyralid was >3000 µg a.i./L for all three endpoints (total shoot length, fresh weight and dry weight). The statistical NOE_rC and LOE_rC for all three endpoints were 8.9 and 28.6 µg a.i./L, respectively.

The lowest E_yC50 for yield in the 14-day exposure of the rooted aquatic macrophyte *Myriophyllum spicatum* to clopyralid was obtained for total shoot length. The statistical NOE_yC, LOE_yC and EyC_{50} for this endpoint were 8.9, 28.6 and 1225 µg a.i./L, respectively.

Table 9.2.49. Summary of the toxicity of clopyralid to *Myriophyllum spicatum*

Common name	Species	Test item	Time-scale	Endpoint	Toxicity value	Units of test item
Aquatic macrophyte	*Myriophyllum spicatum*	Clopyralid	14 day	E_rC_{50}	>3000	µg/L

RMS comments and evaluation:

The study was new, well performed and reported, and in accordance with GLP and the new OECD guideline 239. It was not evaluated before in the context of EU approval, and therefore the study details were presented by the Notifier above.

The original specification of clopyralid is based on the analysis of the batch used in this study. Based on the risk assessment for the impurities of clopyralid presented by the Notifier in Document J in December 2016, no impurity characterised in this batch is expected to substantially impact the ecotoxicological profile of clopyralid. The impurity profile of the test substance used in this study is within the limits of the new specification presented by the Notifier in December 2016.

The range of test concentrations was not adequate to produce the exact E_rC_{50}, although a range-finding test was conducted. The recovery of measured test concentrations was acceptable to express the results in nominal concentrations. The method of analysis of the test solutions was acceptably validated and the LOQ was fit for this purpose.

Based on the OECD test guideline 239, two validity criteria are established for this test design. Both of these criteria were met during the exposure:
1) The mean total shoot length and shoot fresh weight in control plants must at least double during the exposure phase of the test and control plants must not show any visual symptoms of chlorosis. During the exposure all control plants appeared normal, throughout the duration of the test, with no visual symptoms of chlorosis. Active growth of the control plants was demonstrated by a total shoot length yield of 37.0 cm and a wet weight yield of 1.5888 grams. This represents a 5.5x and 5.9x increase in yields for these endpoints, respectively, and thus the first validity criteria is fulfilled.
2) The mean coefficient of variation of yield based on measurements of shoot fresh weight in the control cultures must not exceed 35%. In this study the percent coefficient of variation for shoot fresh weight yield between the ten control replicates was 15.1%, thus fulfilling the second validity criteria.

Overall, the test can be considered as valid, and its outcome can be used in the risk assessment. This is a new endpoint and not considered in the EFSA conclusion on clopyralid (2005:50). Clopyralid being a herbicide and Myriophyllum being the most sensitive aquatic organism, the aquatic risk assessment should be based on this endpoint.

The data available is adequate for the aquatic risk assessment of clopyralid, and no further studies on aquatic organisms are required.

B.9.2.4. Further testing on aquatic organisms

Low risk to non-target aquatic organisms has been demonstrated for use of clopyralid on grassland and cereals at proposed use rates. Due to this fact, further testing on aquatic organisms was considered unnecessary and additional data has not been submitted for review.

RMS comments and evaluation:

A comprehensive data package is available to assess the potential risks to aquatic organisms from the use of plant protection products containing clopyralid as active substance. The data is adequate and fulfils the data requirements for aquatic ecotoxicity set in the Commission Regulation (EU) No 283/2013. Therefore no further data are required.

B.9.3. EFFECTS ON ARTHROPODS

B.9.3.1. Effects on bees

B.9.3.1.1. Acute oral toxicity to bees

Following study to address this point was presented in the dossier for the Approval of Clopyralid submitted in 30 April 2002, evaluated in the DAR (2003) and peer review at EU level. The data were deemed acceptable following evaluation, and are still valid for decision making. Furthermore, a more recent study with the new representative formulation of clopyralid, GF-1374, was submitted and evaluated in the dRAR Section 20 Vol 3 Part B 9.

CA 8.3.1.1.1/1 - Clopyralid Technical Acute Toxicity To Honey Bees

Report	[IIA 8.3.1.1/01], Wainwright, M. 2001
Report title	Clopyralid Technical Acute Toxicity To Honey Bees
DAS Study number	GHE-T-1091, Ref. J63
Guidelines	OECD 213 (1998); OECD 214 (1998)
GLP	Yes

Methodology:

Clopyralid technical, Batch No. RMM 2373, Purity 95.8% (w/w). The acute oral and contact toxicity of clopyralid to the honeybee *Apis mellifera* were assessed over a 48-hour exposure period.

For the oral toxicity test, worker bees were fed 100 µg clopyralid/bee in a 50% sucrose solution with acetone (200 µL per cage). The bees were starved for 100 minutes prior to exposure and the doses were consumed within one hour. For the control treatments, worker bees were fed either the 50% sucrose solution only or a solution of 50% sucrose and acetone at the same concentration used to prepare the clopyralid dosing solution. Each treatment consisted of 10 worker bees in each of six replicate cages (wire mesh cylinders 11.5 x 4.0 cm dia.).

For the contact toxicity test, bees were lightly anaesthetised with carbon dioxide and a 1 µL droplet containing 100 µg clopyralid in acetone was placed on the dorsal surface of the thorax. The control bees were dosed with an equivalent volume of acetone.

For both the oral and contact test, bees were fed a 50% sucrose solution for the remainder of the test. The bees were maintained at 25.5 to 27.0 °C and 50% to 60% relative humidity. The test was carried out in darkness except for procedures which were carried out in subdued light. The bees were observed for mortality and signs of toxicity after 4, 24 and 48 hours. An acute oral and contact toxicity test with dimethoate had been carried out within one month of the test with clopyralid using bees from the same source for reference as a positive control.

Findings:

After 48 hours, mortality of bees in the oral test with 100 µg clopyralid was 3.3% and in the contact test was 1.7% (Table 9.3-1). Mortality in the corresponding oral and contact test control treatments were 1.7% and 3.3%, respectively. The untreated control mortality was 6.7%. There were no sublethal symptoms of toxicity in any treatment. The oral and contact LD_{50} values for dimethoate were 0.16 and 0.17 µg/bee, respectively, and were within the expected range for the laboratory. The results are summarised in Table 9.3-2.

Table 9.3-1. Cumulative mean mortality of honeybees 48 hours after oral and contact exposure to clopyralid

Treatment	**Mean mortality (%)**		
	4 hours	**24 hours**	**48 hours**
Oral toxicity test			
Untreated control	0	0	6.7
Acetone/sucrose control	0	0	1.7
100 µg clopyralid/bee	0	1.7	3.3
Contact toxicity test			
Acetone control	0	3.3	3.3
100 µg clopyralid/bee	0	0	1.7

Table 9.3-2. Summary on results with honey bees

Result	**Nominal concentration of clopyralid (µg/bee)**
Oral 48-hour LD_{50}	> 100
Contact 48-hour LD_{50}	> 100

Conclusions:

The 48-hour oral and contact LD_{50} values of clopyralid to the honeybee, *A. mellifera* were greater than 100 µg/bee, based on nominal concentrations.

Comments

The study was well performed and reported and in compliance with GLP. Because the limit test procedure was used, the exact LD_{50} values could not be determined. However, as clopyralid is of low toxicity to honey bees, the dose-response test is not required. The study is acceptable.

The 48-hour oral and contact LD_{50} values of technical clopyralid (purity of 95.8 % w/w) to honeybee, *Apis mellifera,* were > 100 µg/bee, based on nominal cocentrations.

RMS comments and evaluation:

The endpoint from this acute oral study with the active substance clopyralid is referred in the DAR of clopyralid (2003) and the results were used in the risk assessment presented in the EFSA conclusion on clopyralid (EFSA Scientific Report 2005: 50).

[redacted]

No other comments, the study is acceptable and the result is still valid for the risk assessment.

B.9.3.1.2 Acute contact toxicity to bees

Following study to address this point was presented in the dossier for the Approval of Clopyralid submitted in 30 April 2002, evaluated in the DAR (2003) as presented above and peer review at EU level. The data were deemed acceptable following evaluation, and are still valid for decision making. Furthermore, a more recent study with the new representative formulation of clopyralid, GF-1374, was submitted and evaluated in the dRAR Section 20 Vol 3 CP Part B 9.

B.9.3.1.1.2/1 - Clopyralid - Acute Contact Toxicity to Honey Bee

Report	[IIA 8.3.1.1/01], Wainwright, M. 2001
Report title	Clopyralid Technical Acute Toxicity To Honey Bees
DAS Study number	GHE-T-1091, Ref. J63
Guidelines	OECD 213 (1998); OECD 214 (1998)
GLP	Yes

The 48-hour oral and contact LD_{50} values of technical clopyralid (purity of 95.8 % w/w) to honeybee, *Apis mellifera,* were > 100 µg/bee, based on nominal cocentrations.

RMS comments and evaluation:

The endpoint from this acute contact toxicity study with the active substance clopyralid is referred in the DAR of clopyralid (2003) and the results were used in the risk assessment presented in the EFSA conclusion on clopyralid (EFSA Scientific Report 2005: 50).

[redacted]

No other comments, the study is acceptable and still valid for the risk assessment.

B.9.3.1.3 Chronic toxicity to bees

Acute toxicity studies conducted on honey bees were evaluated for clopyralid and GF-1374 and the endpoints were used in the submitted risk assessments (refer to MCP document). All risk assessments with the active ingredients as well as the formulated product demonstrate low acute oral and contact toxicity to honeybees following applications of GF-1374 to pasture and amenity grassland as well as cereals.

Under the Regulation (EC) No 1107/2009, the new data requirements indicate that for the active substance clopyralid, the chronic risk to adult bees should be addressed. However, validated test methods are not yet available for chronic studies. Furthermore, the new guidance document on risk assessment of bees has been published by EFSA, but is currently under review and as yet, has not been noted by the Standing Committee, with no implementation date set. It is therefore unclear whether this guidance will change or will be accepted in full or in part. According to SANCO/10181/2013-rev. 2.1, 13 May 2013, under item 4, where test methods or guidance documents are not yet available for particular data requirements, waiving of these particular data requirement points is considered acceptable.

Commission Regulation (EU) No 283/2013 sets out the data requirements for the active substances, in accordance with Regulation (EC) No 1107/2009 of the European Parliament and of the Council concerning the placing of plant protection products on the market. The new data requirements indicate that the chronic risk of clopyralid on adult bees and the effects on other honey bee life stages should be addressed. However, for the chronic study, validated test methods are not yet available; therefore, a chronic study on adult honey bees with the active substance clopyralid has not been conducted at time of submission.

Clopyralid is an herbicide that does not have any known insecticidal action. It is applied once per season and applications are made to grassland and cereals. Both these crops are not attractive to worker and foraging bees due to lack of blooming flowers containing no pollen or nectar. Applications are made to control target in-field weeds during the weed's vegetative growth. Treated weeds may be expected to be impacted soon after application due to the rapid absorption and translocation of the active substance to the meristematic portion of the plant where hormonal disruption will occur resulting in plant symptoms such as bending and twisting of the leaves, wilting, cupping of leaves, etc. resulting in the elimination of impacted weeds from the pool of flowering plants that bees may visit and where they may be exposed to systemic residues of clopyralid. Furthermore, most herbicide applications are applied at early growth stages of weeds due to the plant's susceptibility at early life stages resulting in the most effective control of herbicides. This timing was recommended because it is important that applications are made when weeds are actively growing in the vegetative state to ensure proper translocation of the chemical to the roots for complete kill. As soon as flowering begins, translocation to the roots will not be as effective because of the change from vegetative to reproductive growth resulting in poor weed control. This is extremely important for the control of creeping thistles which is the primary use of clopyralid. Therefore, treated in-field vegetation is unlikely to present any forage value to worker honey bees at the time of application and the potential of exposure is low.

There is potential for exposure of honey bees foraging on weeds off-crop or field, assuming that flowering coincides with the application. The maximum spray drift deposition rate at 1 m from the edge of the treated crop is 2.77% of the in-field rate, i.e 120 and 80 g a.s./ha clopyralid for grassland and cereals, respectively, which results in low exposure to off-target weeds and plants. Exposure of bees from plant that reside outside the fiel margins should be negligible since spray drift is the primary route

of exposure which results in very little clopyralid reaching off field plants. Furthermore, exposure to bees from dietary consumption of contaminated pollen and nectar is considered insignificant due to the minimal spray drift rate, low nectar / pollen consumption rate of bees, and low pesticide consumption in pollen and nectar following foliar applications (RUDs). Overall, exposure of bees from plants that reside outside the field margins should be negligible since spray drift is the primary route of exposure which results in very little clopyralid reaching off field plants.

RMS comments and evaluation:

The Notifier has not submitted any studies in support of the chronic risk assessment of clopyralid.

In their letter on January 22, 2016, the Notifier claimed for not intending to submit any studies to address the chronic toxicity to honeybees, grounding their decision on the non-availability of a harmonized test guideline. The Notifier has also sent a letter on this matter to the DG SANCO on February 10, 2016.

It is agreed that no harmonized guideline is as yet available for this type of study, but recent description of this study (10-d oral feeding study on adult honeybees) in Appendix M of the EFSA GD on bees and Appendix O of the final version of the EFSA GD on bees (EFSA Journal 2013; 11(7): 3295) is available and has been used by other Notifiers in support of renewal of other AIR3 active substances.

Additionally, the Commission communication (2013/C 95/02) in the framework of the implementation of the data requirement regulation 284/2013 refers to following test method in this context:

Aupinel & al. 2007. A new larval in vitro rearing method to test effects of pesticides on honey bee brood. Redia XC: 91-94.

So the RMS does not agree that it would not be possible to reliably test the chronic toxicity of plant protection products on bees. Because the exposure of pollinators is possible from flowering weeds in areas treated with the product GF-1374, waiving of this data requirement is not appropriate and the test should be submitted similarly to what has been required from other AIR3 active substances with similar use patterns.

The explanation and justification for waiving the chronic, developmental and sublethal data based on the expected low exposure, as presented by the Notifier above, is considered as understandable, but waiving of the data should be aligned with other renewal active substances according to the data requirement regulation. Therefore a formal data gap is identified, and consequently the risk assessment is inconclusive for the time being.

B.9.3.1.4 Effects on honeybee development and other honeybee life stages

Under the Regulation (EC) No 1107/2009, the new data requirements indicate that for the active substance clopyralid, the effects on honeybee development should be evaluated. However, validated test methods are not yet available for these studies. Furthermore, the new guidance document on risk assessment of bees has been published by EFSA, but this is currently under review and as yet, has not been noted by the Standing Committee, with no implementation date set. It is therefore unclear whether this guidance will change or will be accepted in full or in part. According to SANCO/10181/2013-rev. 2.1, 13 May 2013, under item 4, where test methods or guidance documents are not yet available for

particular data requirements, waiving of these particular data requirement points is considered acceptable.

Commission Regulation (EU) No 283/2013 sets out the data requirements for the active substances, in accordance with Regulation (EC) No 1107/2009 of the European Parliament and of the Council concerning the placing of plant protection products on the market. The new data requirements indicate that for clopyralid the chronic risk to adult bees and the effects on other honey bee life stages should be addressed. However, as stated previously, validated test methods are not yet available; therefore, studies with clopyralid evaluating bee brood have not been conducted at time of submission.

Clopyralid is an herbicide that does not have any known insecticidal action. It is applied once per season and applications are made to grassland and cereals. Both these crops are not attractive to worker and foraging bees due to lack of blooming flowers that contain pollen or nectar. Applications are made to control target in-field weeds during the weed's vegetative growth. Treated weeds may be expected to be impacted soon after application due to the rapid absorption and translocation of the active substance to the meristematic portion of the plant where hormonal disruption will occur resulting in plant symptoms such as bending and twisting of the leaves, wilting, cupping of leaves, etc. resulting in the elimination of impacted weeds from the pool of flowering plants that bees may visit and where they may be exposed to systemic residues of clopyralid. Furthermore, most herbicide applications are applied at early growth stages of weeds due to the plants susceptibility at early life stages resulting in the most effective control of herbicides. This timing was recommended because it is important that applications are made when weeds are actively growing in the vegetative state to ensure proper translocation of the chemical to the roots for complete kill. As soon as flowering begins, translocation to the roots will not be as effective because of the change from vegetative to reproductive growth resulting in poor weed control. This is extremely important for the control of creeping thistles which is the primary use of clopyralid.

However, there is potential for exposure of honey bees foraging on weeds off-crop, assuming that flowering coincides with the application. However, the maximum spray drift deposition rate at 1 m from the edge of the treated crop is 2.77% of the in-field rate, resulting in low exposure potential. Exposure of bees from plants that reside outside the field margins should be negligible since spray drift is the primary route of exposure which results in very little clopyralid reaching off field plants. Furthermore, exposure to bees from dietary consumption of contaminated pollen and nectar is considered insignificant due to the minimal spray drift rate, low nectar/pollen consumption rates of bees, and low pesticide concentration in pollen and nectar following foliar applications (RUDs). Overall, low risk is anticipated for bee development as well as other life stages.

RMS comments and evaluation:

The Notifier has not submitted any studies on the effects of clopyralid on honey bee development and other honeybee life stages.

In their letter on January 22, 2016, the Notifier claimed for not intending to submit any studies to address the chronic and developmental toxicity to honeybees, grounding their decision on the non-availability of a harmonized test guideline. The Notifier has also sent a letter on this matter to the DG SANCO on February 10, 2016.

It is agreed that no harmonized guideline is as yet available for this type of study, but recent description of this study (10-d oral feeding study on adult honeybees) in Appendix M of the EFSA GD on bees and Appendix O of the final version of the EFSA GD on bees (EFSA Journal 2013; 11(7): 3295) is available and has been used by other Notifiers in support of renewal of other AIR3 active substances.

Additionally, the Commission communication (2013/C 95/02) in the framework of the implementation of the data requirement regulation 284/2013 refers to following test method in this context:

Aupinel & al. 2007. A new larval in vitro rearing method to test effects of pesticides on honey bee brood. Redia XC: 91-94.

So the RMS does not agree that it would not be possible to reliably test the chronic and developmental toxicity of plant protection products on bees. Because the exposure of pollinators is possible from flowering weeds in areas treated with the product GF-1374, waiving of this data requirement is not appropriate and the test should be submitted similarly to what has been required from other AIR3 active substances with similar use patterns.

The explanation and justification for waiving the chronic, developmental and sublethal data based on the expected low exposure, as presented by the Notifier above, is considered as understandable, but waiving of the data should be aligned with other renewal active substances according to the data requirement regulation. Therefore a formal data gap is identified, and consequently the risk assessment is inconclusive for the time being.

B.9.3.1.4 Sub-lethal effects on honey bees

Clopyralid is an herbicide with no known insecticidal action. It is applied only once per season and applications are made to grasslands and cereals which do not produce nectar rich flowers. Furthermore, the target in-field weed seedlings are either at a similar immature stage of development or are woody in nature therefore unlikely to present any forage value to worker honey bees at the time GF-1374 is applied. There is potential for exposure of honey bees foraging on weeds off-crop, assuming that flowering coincides with the application.

Currently, studies to investigate sub-lethal effects of clopyralid on honey bees are considered to be unnecessary and have not been performed. However, sublethal effects are not anticipated due to the lack of acute and chronic effects for both adult and larval stages. Furthermore, based on the acute studies, no sublethal effects were observed, even at the highest dose tested for both the active and the formulated product.

RMS comments and evaluation:

It is not agreed that flowering weeds would unlikely be present in fields to be treated with the product GF-1374. Flowering dicotyledonous weeds are important nectar and pollen sources of bees and may well be present in pasture during the spray application of GF-1374. So the exposure of pollinators is possible from flowering weeds in areas treated with the product GF-1374, although the crop itself would not be attractive for bees. However, based on the acute toxicity studies with honeybees available, no sub-lethal effects were observed in studies with any of the active substances or the product GF-1374. As new studies will anyway be required on the chronic and developmental toxicity of clopyralid to honeybees (see above), these studies should cover also the possible sub-lethal effects. If any sub-lethal effects would be anticipated, they should come out in the new studies to be required.

The explanation and justification for waiving the chronic, developmental and sublethal data based on the expected low exposure, as presented by the Notifier above, is considered as understandable, but waiving of the data should be aligned with other renewal active substances according to the data requirement regulation. Therefore a formal data gap is identified, and consequently the risk assessment is inconclusive for the time being.

B.9.3.2. Effects on non-target arthropods other than bees

B.9.3.2.1. Standard laboratory testing for non-target arthropods

Following studies on non-target arthropods were submitted by the Notifier for the Annex I inclusion of clopyralid, evaluated in the DAR (2003) as presented below, and peer reviewed at EU level. The studies were deemed acceptable following evaluation, and are still valid for decision making on the active substance clopyralid. The data requirement is fulfilled. Based on this data, the risk of clopyralid to non-target arthropods other than bees was assessed as low. Therefore the studies are not reassessed here in detail.

Furthermore, more recent studies with the new representative formulation of clopyralid, GF-1374, were submitted and evaluated in the dRAR Section 20 Vol 3 CP Part B 9.

B.8.3.2.1/1 - Clopyralid - Standard laboratory testing with non-target arthropods

Report	[IIA 8.3.2/01], Sankanu A. 2000.
Report title	A laboratory study to evaluate the effects of clopyralid (EF-1136, an SL formulation containing 100 g/L clopyralid) on the parasitic wasp *Aphidius rhopalosiphi* (Hymenoptera: Braconidae).
DAS Study number	GHE-P-8725, Ref. J61 Ecotox Limited Study ID: ER-00-HMA322
Guidelines	ESCORT 1994
GLP	Yes

Methodology:

EF-1136, a soluble concentrate formulation containing 100 g clopyralid/L (nominal) and 98.1 g clopyralid/L (measured), Batch No. EB950309. The effects of EF-1136 on the survival and reproduction of the parasitic wasp *Aphidius rhopalosiphi* were assessed under laboratory test conditions.

Wasps (less than 48 hours old) were exposed to a control treatment (deionised water), a toxic reference treatment (0.1 g dimethoate/ha) and two treatments of EF-1136: 10 and 200 g clopyralid/ha. The test substances and control water were applied to glass plates using a Potter tower at a rate equivalent to 200 L/ha. Once the residues on the glass plates had dried, the test units were assembled and five female and five male adult wasps were placed in each of four replicate test units (glass plate construction) per treatment. The adults were fed with a honey and water solution via a cotton wool wick. Assessments of mortality and behaviour were carried out after 0.5, 2, 4, 24 and 48 hours from the introduction of the wasps.

After 48 hours, 15 randomly selected females from each control and EF-1136 treatment were transferred to test units containing pots of barley seedlings infested with the aphid *Rhopalosiphum padi*. Each contained test unit held one female wasp. After 24 hours the wasps were removed and 12 days later the numbers of aphid mummies were counted.

The test units were maintained in a controlled environment chamber at 19.1 to 20.6°C, 57.3 to 79.0% relative humidity and a low light intensity for 16 hours/day.

Findings:

After 48 hours, the mean mortality in the 10 and 200 g clopyralid/ha treatments of EF-1136 was 10% and 5%, respectively, compared to 20% in the control treatment (Table 9.3.3). When corrected for control mortality both rates of clopyralid gave 0.0% mortality. In the toxic reference treatment mortality was 100% after 24 hours.

The surviving females from wasps exposed to EF-1136 at 10 and 200 g clopyralid/ha produced a mean of 2.9 and 0.5 mummies/female, compared to the control wasps, which produced 5.0 mummies/female. The reduction of mummies produced in the 200 g clopyralid/ha treatment was significant at $p<0.05$ (Table 9.3.4). Reductions in

fecundity (compared with the control) of 42% and 90% were observed for EF-1136 at 10 and 200 g clopyralid/ha respectively. The results are summarised in Table 9.3.5.

Table 9.3.3. Mean mortality of *Aphidius rhopalosiphi* exposed to EF-1136 under laboratory test conditions

Time (hours)	**Percent mortality (%)[a]**			
	Control	**EF-1136 (10 g clopyralid /ha)**	**EF-1136 (200 g clopyralid /ha)**	**Toxic reference**
0.5	0.0	0.0	0.0	0.0
2	0.0	0.0	0.0	0.0
4	0.0	0.0	0.0	0.0
24	0.0	0.0	0.0	100
48	20.0	10.0	5.0	100

[a] Mean of four replicate units, each containing 5 male and 5 female wasps.

Table 9.3.4. Fecundity of female *Aphidius rhopalosiphi* following exposure to EF-1136 under laboratory test conditions

Mean number of mummies/female after 12 days		
Control	EF-1136 (10 g clopyralid/ha)	EF-1136 (200 g clopyralid /ha)
5.0	2.9 (42%)	0.5* (90%)

* Significant at $p<0.05$.

Table 9.3.5. Summary on results with *A. rhopalosiphi*

Application rate of EF-1136 (g clopyralid/ha)	**Mortality (corrected by control)**	**IOBC/WPRS classification (mortality)**	**Effects on fecundity relative to control treatment**
10	0.0%	Harmless	Not significant
200	0.0%	Harmless	Significant at $p \leq 0.05$

Conclusions:

When applied at 10 and 200 g clopyralid/ha, EF-1136 had no effect on the mortality of *A. rhopalosiphi*. According to the classification system for mortality proposed by the IOBC/WPRS Working Group, EF-1136 is classified as "harmless". Fecundity was significantly reduced at 200 g clopyralid/ha but was unaffected at 10 g clopyralid/ha.

Comments

The study was well performed and reported and in compliance with GLP. The higher treatment that caused significant reduction in fecundity is comparable to the intended use rate on cereals or oilseed rape or to the single use rate on sugar beet, but slightly lower than the use rate on pasture (240 g/ha). Multiple applications on sugar beet are also not covered by this study. According to this study the effects of clopyralid on the reproduction of the sensitive standard species *A. rhopalosiphi* at concentrations comparable to the intended use rates cannot be excluded, though the effect on mortality are acceptable. The effect on fecundity of this sensitive standard species triggers further studies with other groups of non-target arthropods.

The effects of a soluble concentrate formulation of clopyralid (EF-1136) containing 100 g clopyralid/L on the survival and reproduction of the parasitic wasp *Aphidius rhopalosiphi* were assessed under

laboratory test conditions. When applied at 10 and 200 g clopyralid/ha, EF-1136 had no effect on the mortality of *A. rhopalosiphi*. According to the IOBC/WPRS classification, EF-1136 is harmess to wasps. Fecundity was significantly reduced at 200 g clopyralid/ha but was unaffected at 10 g clopyralid/ha.

RMS comments and evaluation:

The standard laboratory studies with non-target arthropods were originally assessed in the DAR (2003) as well performed and reported, and no effects were found with any of the tested non-target arthropod species with test concentrations equivalent to recommended field rates of clopyralid. The formulation tested is no longer the representative formulation in the EU dossier of clopyralid, but the data can be used to support the renewal of the authorisation of clopyralid.

The impurity profiles of the test substances used in these studies were not reported by the Notifier, but on the basis of the impurity profiles of batches used in other ecotoxicity studies it is not anticipated that the possible impurities would significantly alter the results of these studies.

Despite the studies are considered as still valid, the products tested were different from the new AIR3 representative formulation of clopyralid. Therefore additional formulation studies with the new representative formulation GF-1374 were submitted and evaluated in the dRAR Section 20 Vol 3 CP Part B 9.

B.8.3.2.2/1 - Clopyralid - Standard laboratory testing with non-target arthropods

Report	[IIA 8.3.2/02], Sankanu A. 2000.
Report title	A laboratory study to evaluate the effects of clopyralid (EF-1136, an SL formulation containing 100 g/L clopyralid) on *Typhlodromus pyri* (Acari: Phytoseiidae).
DAS Study number	GHE-P-8416, Ref. J53. Ecotox Limited Study ID: ER-00-HMA321
Guidelines	ESCORT 1994
GLP	Yes

Methodology:

EF-1136, a soluble concentrate formulation containing 100 g clopyralid/L (nominal) and 98.1 g clopyralid/L (measured), Batch No. EB950309. The effects of EF-1136 on the survival and reproduction of the predatory mite *Typhlodromus pyri* were assessed under laboratory test conditions.

Protonymphs were exposed to a control treatment (deionised water), a toxic reference treatment (0.03 g fenproathrin/ha) and two treatments of EF-1136: 10 and 200 g clopyralid/ha. The test substances and control water were applied to glass plates using a Potter tower at a rate equivalent to 200 L/ha. Once the residues on the glass plates had dried, the test units were assembled and twenty protonymphal mites were placed in each of five replicate test units (glass plate construction) per treatment. The mites were fed with pollen and water was available via. The test units were maintained in a controlled environment chamber at approximately 25°C, 70.% relative humidity and a low light intensity for 16 hours/day.

Assessments of mortality carried out after 1, 3 and 7 days from the introduction of the mites. After 7 days, the mites were sexed to determine the numbers of males and females and sex ratio in each test unit. Reproduction assessments were 10, 12 and 14 days after infestation.

Findings:

After 7 days, the mean mortality in the 10 and 200 g clopyralid/ha treatments of EF-1136 was 16% and 20%, respectively, compared to 15% in the control treatment (Table 9.3.6). When corrected for control mortality in the 10 and 200 g clopyralid/ha treatments of EF-1136 was only 1.2% and 5.9%, respectively. Mortality was 98% after 7 days in the toxic reference treatment.

The surviving females exposed to EF-1136 at 10 and 200 g clopyralid/ha produced a mean of 4.8 and 5.7 eggs/female, compared to the control mites, which produced 7.9 eggs/female. No significant differences in fecundity between the control and either clopyralid treatments were observed (at $p<0.05$) and neither treatment lead to a reduction of 50% or greater compared to the control (Table 9.3.7). The results are summarised in Table 9.3.8.

Table 9.3.6. Mean mortality of *Typhlodromus pyri* exposed to EF-1136 under laboratory test conditions

Time (days)	Percent mortality (%)[a]			
	Control	EF-1136 (10 g clopyralid /ha)	EF-1136 (200 g clopyralid /ha)	Toxic reference
1	2.0	4.0	10	91
3	8.0	10	13	98
7	15	16	20	98

[a] Mean of five replicate units, each containing 20 mites.

Table 9.3.7. Fecundity of Typhlodromus pyri following exposure to EF-1136 under laboratory test conditions

Mean number of eggs/female after 14 days		
Control	EF-1136 (10 g clopyralid/ha)	EF-1136 (200 g clopyralid /ha)
7.9	4.8	5.7

Table 9.3-8. Summary on results with *T. pyri*

Application rate of EF-1136 (g clopyralid/ha)	Mortality (corrected by control)	IOBC/WPRS classification (mortality)	Effects on fecundity relative to control treatment
10	1.2%	Harmless	39.2%
200	5.9%	Harmless	27.7%

Conclusions:

When applied at 10 and 200 g clopyralid/ha, EF-1136 had no effect on the mortality of *T. pyri*. According to the classification system for mortality proposed by the IOBC/WPRS Working Group, EF-1136 is classified as "harmless". The effect on fecundity is borderline.

Comments

The study was well performed and reported and in compliance with GLP. The higher treatment that caused significant reduction in fecundity is comparable to the intended use rate on cereals or oilseed rape or to the single use rate on sugar beet, but slightly lower than the use rate on pasture (240 g/ha). Multiple applications on sugar beet are also not covered by this study. With respect to the intended uses of clopyralid, *T. pyri* is not a crop-

relevant species but a sensitive standard species that needs to be tested. The study is acceptable. The effect on fecundity of this sensitive standard species triggers further studies with other groups of non-target arthropods.

The effects of a soluble concentrate formulation of clopyralid (EF-1136) containing 100 g clopyralid/L on the survival and reproduction of the predatory mite *Typhlodromus pyri* were assessed under laboratory test conditions. When applied at 10 and 200 g clopyralid/ha, EF-1136 had no effect on the mortality of *T. pyri*. According to the IOBC/WPRS classification, EF-1136 is harmess to predatory mites. The effect on fecundity was borderline.

RMS comments and evaluation:

The standard laboratory studies with non-target arthropods were originally assessed in the DAR (2003) as well performed and reported, and no effects were found with any of the tested non-target arthropod species with test concentrations equivalent to recommended field rates of clopyralid. The formulation tested is no longer the representative formulation in the EU dossier of clopyralid, but the data can be used to support the renewal of the authorisation of clopyralid.

The impurity profiles of the test substances used in these studies were not reported by the Notifier, but on the basis of the impurity profiles of batches used in other ecotoxicity studies it is not anticipated that the possible impurities would significantly alter the results of these studies.

Despite the studies are considered as still valid, the products tested were different from the new AIR3 representative formulation of clopyralid. Therefore additional formulation studies with the new representative formulation GF-1374 were submitted and evaluated in the dRAR Section 20 Vol 3 CP Part B 9.

B.8.3.2.3/1 - Clopyralid - Standard laboratory testing with non-target arthropods

Report	[IIA 8.3.2/03], Miles, M. 2002.
Report title	A laboratory study to evaluate the effects on EF-1136 (100 g/L clopyralid SL) on the lacewing *Chrysoperla carnea*.
DAS Study number	GHE-P-9505, Ref. J70. Dow AgroSciences Study ID: EA99A2A028
Guidelines	ESCORT 1994
GLP	Yes

Methodology:

EF-1136, a soluble concentrate formulation containing 100 g clopyralid/L (nominal) and 98.1 g clopyralid/L (measured), Batch No. EB950309. The effects of EF-1136 on the survival and reproduction of the lacewing *Chrysoperla carnea* were assessed under laboratory test conditions.

Glass plates (80 x 80 mm) were sprayed with the test substance and control treatments using a laboratory track sprayer calibrated to deliver 200 L water/ha. Once the plates had dried, they were laid face up and an acrylic cylinder (70 mm internal diameter, 25 mm tall) was positioned on top and secured with elastic bands. The inner walls of the cylinder were pre-treated with a aqueous suspension of Fluon over which insects cannot climb.

A single 2nd instar larvae of *Chrysoperla carnea* was confined in each unit. The larvae were provided with untreated food and reared through to adulthood. Once adult lacewings were obtained, they were assessed to see whether there had been any sub-lethal treatment effects on their fecundity. For this adults were placed in

oviposition arenas and their egg-laying activity assessed. Through the study the lacewings were held under controlled environment conditions: temperature, 23°C ± 3C°, relative humidity, 45 – 90% r.h.

Findings:

The total juvenile mortality in the 200 g clopyralid/ha treatment of EF-1136 was 6.67%, compared to 0.0% in the control treatment (Table 9.3.9). Mortality was 100% in the toxic reference treatment.

The surviving females exposed to EF-1136 at 200 g clopyralid/ha produced a mean of 23.08 eggs/female/day with 85.28% egg viability, compared to the control lacewings, which produced 21.31 eggs/female/day with 92.28% egg viability. The EF-1136 treatment clearly met the criteria of greater than 15 eggs/female/day with over 80% hatch indicating no test item effect on fecundity (Table 9.3.10). The results are summarised in Table 9.3.11.

Table 9.3.9. Percentage mortality of *Chrysoperla carnea* larvae exposed to EF-1136 under laboratory test conditions

Time (days)	**Percent mortality (%)[a]**		
	Control	**EF-1136 (200 g clopyralid /ha)**	**Toxic reference**
1	0.0	0.0	100
2	0.0	0.0	100
4	0.0	0.0	100
6	0.0	0.0	100
7	0.0	0.0	100
9	0.0	0.0	100
Total juvenile mortality	0.0	6.67	100

[a] Total of 30 larvae per treatment.

Table 9.3.10. Fecundity of *Chrysoperla carnea* following exposure to EF-1136 under laboratory test conditions

Treatment	**Mean number of eggs/female/day**	**%Egg hatch (viability)**
Control	21.31	92.76
EF-1136 (200 g clopyralid /ha)	23.08	85.28

Table 9.3.11. Summary on results with *C. carnea*

Application rate of EF-1136 (g clopyralid/ha)	**Mortality**	**IOBC/WPRS classification (mortality)**	**Effects on fecundity relative to control treatment**
200	6.67%	Harmless	None

Conclusions:

When applied at 200 g clopyralid/ha, EF-1136 had no effect on the mortality or fecundity of *C. carnea*. According to the classification system for mortality proposed by the IOBC/WPRS Working Group, EF-1136 is classified as "harmless".

Comments

The study was well performed and reported and in compliance with GLP. The treatment rate is comparable to the intended use rate on cereals or oilseed rape or to the single use rate on sugar beet, but slightly lower than the use rate on pasture (240 g/ha). Multiple applications on sugar beet are also not covered by this study. With respect to the intended uses of clopyralid, *C.carnea* is a representative foliage dwelling species that is active in many crops. The study is acceptable.

The effects of a soluble concentrate formulation of clopyralid (EF-1136) containing 100 g clopyralid/L on the survival and reproduction of the lacewing *Chrysoperla carnea* were assessed under laboratory test conditions. When applied at 200 g clopyralid/ha, EF-1136 had no effect on the mortality or fecundity of *C. carnea*. According to the IOBC/WPRS classification, EF-1136 is harmess to lacewing.

RMS comments and evaluation:

The standard laboratory studies with non-target arthropods were originally assessed in the DAR (2003) as well performed and reported, and no effects were found with any of the tested non-target arthropod species with test concentrations equivalent to recommended field rates of clopyralid. The formulation tested is no longer the representative formulation in the EU dossier of clopyralid, but the data can be used to support the renewal of the authorisation of clopyralid.

The impurity profiles of the test substances used in these studies were not reported by the Notifier, but on the basis of the impurity profiles of batches used in other ecotoxicity studies it is not anticipated that the possible impurities would significantly alter the results of these studies.

Despite the studies are considered as still valid, the products tested were different from the new AIR3 representative formulation of clopyralid. Therefore additional formulation studies with the new representative formulation GF-1374 were submitted and evaluated in the dRAR Section 20 Vol 3 CP Part B 9.

B.8.3.2.4/1 - Clopyralid - Standard laboratory testing with non-target arthropods

Report	[IIA 8.3.2/04], Miles, M. 2002.
Report title	A laboratory study to evaluate the side effects of EF-1136 (100 g/L clopyralid SL) on the carabid beetle *Poecilus cupreus*.
DAS Study number	GHE-P-7173, Ref. J71. Dow AgroSciences Study ID: EA99A2A029
Guidelines	ESCORT 1994
GLP	Yes

Methodology:

EF-1136, a soluble concentrate formulation containing 100 g clopyralid/L (nominal) and 98.1 g clopyralid/L (measured), Batch No. EB950309. The effects of EF-1136 on the survival and food consumption of the carabid beetle *Poecilus cupreus* were assessed under laboratory test conditions.

Throughout the exposure period, six week old beetles were maintained in plastic vessels filled with quartz sand which were held in ventilated cabinets at a temperature of 20 ± 3°C, a 16 hour daylength and a humidity of 45-90%r h.. Six beetles (three males and three females) were placed in each vessel (five replicate vessels per treatment) and acclimatised to the test conditions for two days during which they were not fed.

The test and reference substances were applied at an equivalent rate of 400 L/ha using a laboratory track sprayer. EF-1136 was applied at a rate equivalent to 2 L/ha (nominal 200 g clopyralid/ha) and the positive reference substance dimethoate was applied at (340 g a.s./ha) and water was applied as a control. The soil moisture was maintained at 70% of water holding capacity. The beetles were fed one fly pupa immediately before the test substances were applied. Every two to three days beetles were fed with one fly pupa per survivor and the sand watered to replace lost moisture. At each observation point (2, and 6 hours, 1, 2, 4, 7, 10 and 14 days) beetles were observed for abnormal behaviour. Remnants of fly pupae were removed and new pupa placed in the test containers on days 2, 4, 7 10 and 14.

Findings:

By the end of the 14-day exposure period there were no dead beetles in the control or in the EF-1136 treatment (Table 9.3.12). In the toxic reference treatment there was 100% mortality by the end of the test. Toxic symptoms were first observed after two hours in the toxic reference treatment.

The mean number of pupae consumed per beetle per day was 0.22 in the control treatment and 0.18 in the EF-1136 treatment (Table 9.3.13). Overall an 18.28% reduction in food consumption was observed in the EF-1136 treatment compared with the control. In the dimethoate treatment on two pupae were consumed. The results are summarised in Table 9.3.14.

Table 9.3.12. Mortality of *Poecilus cupreus* after 14 days exposure to EF-1136 under laboratory conditions

Time after application	**Average cumulative mortality (%)[a]**		
	Control	**EF 1137 (200 g clopyralid/ha)**	**Toxic reference**
2 hours	0	0	16.6
6 hours	0	0	86.67
1 day	0	0	100
2 days	0	0	100
4 days	0	0	100
7 days	0	0	100
10 days	0	0	100
14 days	0	0	100

[a] Mean of five replicates.

Table 9.3.13. Numbers of pupae eaten by *Poecilus cupreus* exposed to Lontrel 100 for 14 days under laboratory conditions

Time after application (days)	**Total number of pupae eaten per beetle[a]**	
	Control	**'Lontrel 100' (120 g clopyralid/ha)**
2	0.33	0.33
4	0.30	0.17
7	0.73	0.50
10	0.77	0.63
14	0.97	0.90
Mean No pupae/beetle/day	0.22	0.18

Table 9.3.14. Summary on results with *P. cupreus*

Application rate of EF-1136 (g clopyralid/ha)	Mortality	IOBC/WPRS classification (mortality)	Effects on food consumption relative to control treatment
200	0.0%	Harmless	18.28%

Conclusions:

No effects on survival or feeding rate were observed compared to an untreated control treatment following 14 days exposure of *P. cupreus* to EF-1136 at an application rate equivalent to 200 g clopyralid/ha. According to the classification system proposed by the IOBC/WPRS Working Group, EF-1136 is classified as "harmless".

Comments

The study was well performed and reported and in compliance with GLP. Similarly to the previous studies, the studied rate of clopyralid is comparable to the intended use rate on cereals or oilseed rape or to the single use rate on sugar beet, but slightly lower than the use rate on pasture (240 g/ha). Multiple applications on sugar beet are also not covered by this study. The study is acceptable.

The effects of a soluble concentrate formulation of clopyralid (EF-1136) containing 100 g clopyralid/L on the survival and food consumption of the carabid beetle *Poecilus cupreus* were assessed under laboratory test conditions. No effects on survival or feeding rate were observed compared to untreated control treatment following 14 days exposure of *P. cupreus* to EF-1136 at an application rate equivalent to 200 g clopyralid/ha. According to the IOBC/WPRS classification, EF-1136 is harmess to carabid beetle.

RMS comments and evaluation:

The standard laboratory studies with non-target arthropods were originally assessed in the DAR (2003) as well performed and reported, and no effects were found with any of the tested non-target arthropod species with test concentrations equivalent to recommended field rates of clopyralid. The formulation tested is no longer the representative formulation in the EU dossier of clopyralid, but the data can be used to support the renewal of the authorisation of clopyralid.

The impurity profiles of the test substances used in these studies were not reported by the Notifier, but on the basis of the impurity profiles of batches used in other ecotoxicity studies it is not anticipated that the possible impurities would significantly alter the results of these studies.

Despite the studies are considered as still valid, the products tested were different from the new AIR3 representative formulation of clopyralid. Therefore additional formulation studies with the new representative formulation GF-1374 were submitted and evaluated in the dRAR Section 20 Vol 3 CP Part B 9.

B.8.3.2.5/1 - Clopyralid - Standard laboratory testing with non-target arthropods

Report	[IIA 8.3.2/05], Römbke, J. 1991.
Report title	A study of the acute toxicity for *Poecilus cupreus* (Carabidae) of Lontrel 100 (EF 255) according to the BBA guideline for testing of chemicals developed by Dr. U. Heimbach.

DAS Study number	GHE-P-2659, REf. J29. Battelle-Institut e.V. Study ID: BE-E-84-90-03-CAK6
Guidelines	BBA 1987
GLP	Yes

Methodology:

'Lontrel 100' (EF 255) (a 100 g/L soluble concentrate of clopyralid), Batch No. DB 1049-90-38B. The effects of 'Lontrel 100' on the survival and behaviour of the carabid beetle *Poecilus cupreus* were assessed over a 14-day exposure period under laboratory conditions.

Throughout the exposure period, six week old beetles were maintained in plastic vessels filled with quartz sand which were held in ventilated cabinets at a temperature of 20 ± 2°C, a 16 hour daylength and a humidity of 85%. Six beetles (three males and three females) were placed in each vessel (five replicate vessels per treatment) and acclimatised to the test conditions for three days during which they were not fed.

The test substances (and the control treatment of water only) were applied at an equivalent rate of 400 L/ha with a hydraulic sprayer. 'Lontrel 100' was applied at a rate equivalent of 1.2 L/ha (nominal 120 g clopyralid/ha) and the positive reference substance 'Afugan' (pyrazaphos 294 g/L) was applied at 1 L/ha (294 g a.s./ha) and 'E 605' (parathion 500 g/L) at 210 mL/ha (105 g a.s./ha). The soil moisture was maintained at 70% of MWC.

The beetles were fed one fly pupa immediately before the test substances were applied. Every two to three days beetles were fed with one fly pupa per survivor and the sand watered to replace lost moisture. At each observation point (2, 4, 6 and 24 hours, 2, 4, 7, and 14 days) beetles were observed for abnormal behaviour. Remnants of fly pupae were removed and new pupa placed in the test containers on days 2, 4, 7 and 14.

Findings:

By the end of the 14-day exposure period there were no dead beetles in the control or in the 'Lontrel 100' treatment (Table 9.3.15). In both positive reference treatments there was 100% mortality by the end of the test. Toxic symptoms were first observed after two hours in both of the positive reference treatments.

The mean number of pupae consumed per beetle over the entire test duration was 2.37 in the control treatment and 2.17 in the 'Lontrel 100' which was equivalent to only a 8.4% reduction (Table 9.3.16). The results are summarised in Table 9.3.17.

Table 9.3.15. Mortality of *Poecilus cupreus* after 14 days exposure to Lontrel 100 under laboratory conditions

Time after application	**Average cumulative mortality (%)[a]**			
	Control	**'Lontrel 100' (120 g clopyralid/ha)**	**'Afugan' (294 g a.s./ha)**	**'E 605' (105 g a.s./ha)**
2 hours	0	0	0	10
4 hours	0	0	0	10
6 hours	0	0	0	63.3
24 hours	0	0	100	100
2 days	0	0	100	100
4 days	0	0	100	100
7 days	0	0	100	100
11 days	0	0	100	100
14 days	0	0	100	100

[a] Mean of five replicates.

Table 9.3.16. Numbers of pupae eaten by *Poecilus cupreus* exposed to Lontrel 100 for 14 days under laboratory conditions

Time after application (days)	**Total number of pupae eaten per vessel (6 beetles)[a]**	
	Control	**'Lontrel 100' (120 g clopyralid/ha)**
2	2.00	1.65
4	2.84	2.67
7	2.00	1.99
11	2.27	2.16
14	2.67	2.33
Total	11.82	10.82
Mean pupae/beetle	2.37	2.17

[*] Expressed as the mean of all surviving beetles per vessel.

Table 9.3.17. Results

Nominal application rate of 'Lontrel 100' (g clopyralid/ha)	**IOBC/WPRS classification**
120	Harmless

Conclusions:

No effects on survival, behaviour or feeding rate were observed compared to an untreated control treatment following 14 days exposure of *P. cupreus* to 'Lontrel 100' at an application rate equivalent to 120 g clopyralid/ha. According to the classification system proposed by the IOBC/WPRS Working Group, 'Lontrel 100' is classified as "harmless".

Comments

The study was well performed and reported and in compliance with GLP, but older than the previous study. The test rate was lower than the highest recommended single use rates: half of the rate intended for pasture (240 g a.i./ha). The formulation studied (EF 255) was different from the lead formulation EF-1136 in the EU dossier. The differences with the two formulations were not clarified like was the case with the aquatic studies. Therefore it is not clear if the formulation used in this study is really comparable to EF-1136. The previous study (J71) with higher application rate confirmed the harmlessness of clopyralid to ground dwelling predators at use rates more close to the GAP. This study can be used as additional information, but not for the risk assessment of the formulation EF-1136.

The effects of a soluble concentrate formulation of clopyralid (Lontrel 100) containing 100 g clopyralid/L on the survival and behaviour of the carabid beetle *Poecilus cupreus* were assessed over a 14-day exposure period under laboratory conditions. No effects on survival, behaviour or feeding rate were observed compared to an untreated control treatment following 14 days exposure of *P. cupreus* to Lontrel 100 at an application rate equivalent to 120 g clopyralid/ha. According to the IOBC/WPRS classification, Lontrel 100 is harmess to carabid beetle.

RMS comments and evaluation:

The standard laboratory studies with non-target arthropods were originally assessed in the DAR (2003) as well performed and reported, and no effects were found with any of the tested non-target arthropod species with test concentrations equivalent to recommended field rates of clopyralid. The formulation tested is no longer the representative formulation in the EU dossier of clopyralid, but the data can be used to support the renewal of the authorisation of clopyralid.

The impurity profiles of the test substances used in these studies were not reported by the Notifier, but on the basis of the impurity profiles of batches used in other ecotoxicity studies it is not anticipated that the possible impurities would significantly alter the results of these studies.

Despite the studies are considered as still valid, the products tested were different from the new AIR3 representative formulation of clopyralid. Therefore additional formulation studies with the new representative formulation GF-1374 were submitted and evaluated in the dRAR Section 20 Vol 3 CP Part B 9.

B.8.3.2.6/1 - Clopyralid - Standard laboratory testing with non-target arthropods

Report	[IIA 8.3.2/06], Römbke, J. and Vickus, P. 1991.
Report title	A study of the acute toxicity for *Aleochara bilineata* (Staphylinidae) of Lontrel 100 (EF 255) according to the IOBC/WPRS guideline for testing of chemicals developed by Dr. L. Samsoe-Petersen.
DAS Study number	GHE-P-2489, Ref. J30. Battelle-Institut e.V. Study ID: BE-E-84-91-05-STA1
Guidelines	BBA 1987
GLP	Yes

Methodology:

'Lontrel 100' (EF 255) (a 100 g/L soluble concentrate of clopyralid), Batch No. DB 1049-90-38B. The effects of 'Lontrel 100' on the survival, behaviour, feeding rate and reproduction of the staphylinid beetle *Aleochara bilineata* were assessed.

Throughout the exposure period, seven to ten day old beetles were maintained in glass vessels filled with quartz sand which were held in ventilated cabinets at a temperature of 22 ± 2°C and a 16 hour daylength. Humidity was 85% in the test vessels. One female beetle, after copulation had been observed, was placed in each vessel (nine replicate vessels per treatment) immediately after application of the test substance to the vessel by hydraulic sprayer at a rate of 400 L/ha. 'Lontrel 100' was applied at a rate equivalent to 1.2 L/ha (nominal 120 g clopyralid/ha) and the positive reference substance 'Afugan' (pyrazaphos) was applied at 2 L/ha (588 g a.s./ha). The control treatment consisted of water only. The sand moisture content was 10% throughout the five day test period.

Each female beetle was fed 30 fly eggs *(Delia antiqua*) immediately after placing the beetles in the test containers. Feeding was repeated on days two to four. For the five days after application of the test substances daily observations were made for mortality, feeding rate and behavioural changes. On day 5, the number of eggs laid in the sand were counted. During the following week hatching and behaviour were observed.

Findings:

After five days there were no mortalities or abnormal signs of behaviour of beetles in the control or 'Lontrel 100' treatments. All but one affected beetle were dead in the positive reference treatment by day 5. The average total feeding rate was comparable in the control and 'Lontrel 100' treatments with 109.7 and 109.8 fly pupae consumed per beetle, respectively (Table 9.3.18).

Females started egg laying on day 5 in the control and 'Lontrel 100' treatments. By the end of the test, the mean number of eggs laid per female was 54.1 in the control and 49.9 in the 'Lontrel 100' treatment. The corresponding mean number of juveniles was 49.2 and 48.2, representing a hatching rate of 90.9 and 96.6%, respectively (Table 9.3.18). The results are summarised in Table 9.3.19.

Table 9.3.18. Egg production and hatch rate of *Aleochara bilineata* following exposure to 'Lontrel 100' under laboratory conditions

Parameter	**Control**	**'Lontrel 100' (120 gclopyralid/ha)**	**'Afugan' (588 g a.s./ha)**
Mortality (%)	0	0	88.9[a]
Mean total feeding rate/beetle[b]	109.7 ±7.9	109.8 ±3.7	20.3 ±8.1
Mean no. eggs laid/beetle	54.1 ±20.7	49.9 ±11.9	-[c]
Mean no. juveniles/beetle	49.2 ±18.8	48.2 ±11.3	-
% hatch	90.9	96.6	-

[a] One remaining beetle out of nine was observed to be severely affected.
[b] Out of a total of 30 fly eggs per beetle per day on days 2, 3, 4 and 5.
[c] Insufficient beetles to continue with the reproduction phase of the test.

Table 9.3.19. Summary on results with *A. bilineata*

Nominal application rate of Lontrel 100 (g clopyralid/ha)	**IOBC/WPRS classification**
120	Harmless

Conclusions:

There were no effects on the survival, behaviour, feeding rate or reproduction, compared to an untreated control treatment, following five days exposure of *A. bilineata* adults and one week incubation of eggs and hatching of larvae, to 'Lontrel 100' at an application rate equivalent to 120 g clopyralid/ha. According to the classification system proposed by the IOBC/WPRS Working Group 'Lontrel 100' is classified as "harmless".

Comments

The study was well performed and reported and in compliance with GLP. The test rate was lower than the highest recommended single use rates: half of the rate intended for pasture (240 g a.i./ha). The formulation studied (EF 255) was different from the lead formulation EF-1136 in the dossier. The differences with the two formulations were not clarified like was the case with the aquatic studies. Therefore it is not clear if the formulation used in this study is really comparable to EF-1136. This study can be used as additional information, but not for the risk assessment of the formulation EF-1136.

The effects of a soluble concentrate formulation of clopyralid (Lontrel 100) containing 100 g clopyralid/L on the survival, behaviour, feeding rate and reproduction of the staphylinid beetle *Aleochara bilineata* were assessed under laboratory conditions. No effects on the survival, behaviour, feeding rate or reproduction, compared to an untreated control treatment following five days exposure of *A. bilineata* adults and one week incubation of eggs and hatching of larvae, to Lontrel 100 at an application rate equivalent to 120 g clopyralid/ha. According to the IOBC/WPRS classification, Lontrel 100 is harmess to staphylinid beetle.

RMS comments and evaluation:

The standard laboratory studies with non-target arthropods were originally assessed in the DAR (2003) as well performed and reported, and no effects were found with any of the tested non-target arthropod species with test concentrations equivalent to recommended field rates of clopyralid. The formulation tested is no longer the representative formulation in the EU dossier of clopyralid, but the data can be used to support the renewal of the authorisation of clopyralid.

The impurity profiles of the test substances used in these studies were not reported by the Notifier, but on the basis of the impurity profiles of batches used in other ecotoxicity studies it is not anticipated that the possible impurities would significantly alter the results of these studies.

Despite the studies are considered as still valid, the products tested were different from the new AIR3 representative formulation of clopyralid. Therefore additional formulation studies with the new representative formulation GF-1374 were submitted and evaluated in the dRAR Section 20 Vol 3 CP Part B 9.

B.8.3.2.7/1 - Clopyralid - Standard laboratory testing with non-target arthropods

Report	[IIA 8.3.2/07], Heimann, D., Hof, A. and Vickus, P. 1993.
Report title	A study of the acute toxicity for *Pardosa sp*. (Araneae) of Lontrel 100.
DAS Study number	GHE-P-3126, REf. J36. Battelle-Institut e.V. Study ID: CF0033SA
Guidelines	BBA 1987
GLP	Yes

Methodology:

'Lontrel 100' (EF-1136) (a 100 g/L soluble concentrate of clopyralid), Batch No. A 629-128. The effects of 'Lontrel 100' on the survival, behaviour and feeding rate of the lycosid spider *Pardosa sp*. were assessed.

Throughout the 14-day exposure period, adult spiders, collected from the field, were maintained in glass dishes containing quartz sand in the laboratory at a temperature of 17.2 to 21.4°C and a 16 hour daylength. Four spiders were placed into each replicate dish, with three replicates containing males and three with females per treatment.

The test substances were applied to the dishes containing the spiders by hydraulic spray equipment at a rate of 400 L/ha. 'Lontrel 100' was applied at a rate equivalent to 1.2 L/ha (nominal 120 g clopyralid/ha) and the positive reference substance 'Karate' (lambda-cyhalothrin 50 g a.s./L) was applied at 50 mL/ha (2.5 g a.s./ha). The control treatment consisted of water only. The sand moisture content was approximately 70% MWC throughout the test period. In the first week, spiders were fed with five fruit flies per surviving spider. In week two, feeding took place every two to three days. Mortality, behaviour and feeding rates were observed at feeding times.

Findings:

After 14 days there were no mortalities of spiders in the control or 'Lontrel 100' treatments. In the 'Lontrel 100' treatment, one spider in each of two replicates showed abnormal behaviour, one within two hours recovering after one day, and the other after 14 days (Table 9.3.20). In the positive reference treatment mortality was 65% by the end of the test.

The mean number of fruit flies consumed per spider was 3.9 and 4.2 for the untreated controls after day 1 to 7 and day 8 to 14, respectively (Table 9.3.21). The corresponding feeding rate for spiders in the 'Lontrel 100' treatment was 3.3 and 4.3, respectively. Spiders treated with the positive reference were observed to have a lower feeding rate, compared to the control and 'Lontrel 100' treatments, over the first seven days of 1.7 fruit flies/spider. This increased to 3.9 for day 8 to 14. The results are summarised in Table 9.3.22.

Table 9.3.20. Behaviour of *Pardosa sp.* following exposure to 'Lontrel 100' under laboratory conditions for 14 days

Treatment	No. spiders affected and symptom[a]										
	2 h	**4 h**	**6 h**	**Day 1**	**Day 2**	**Day 3**	**Day 4**	**Day 7**	**Day 9**	**Day 11**	**Day 14**
Control[b]	4N	4N	4N	4N	4N	4N	4N	4N	4N	4N	4N
'Lontrel 100':											
R1	4N	4N	4N	4N	4N	4N	4N	4N	4N	4N	4N
R2	4N	4N	4N	4N	4N	4N	4N	4N	4N	4N	4N
R3	4N	4N	4N	4N	4N	4N	4N	4N	4N	4N	4N
R4	4N	4N	4N	4N	4N	4N	4N	4N	4N	4N	4N
R5	3N,1S	3N,1S	3N,1S	3N,1K	4N	4N	4N	4N	4N	4N	4N
R6	4N	4N	4N	4N	4N	4N	4N	4N	4N	4N	3N,1S
'Karate':											
R1	2K,2S	2K,2S	2K,2S	4K	3N,1K	4N	4N	4N	4N	4N	4N
R2	1K,3S	1K,3S	1K,3S	3K	3N	3N	3N	3N	3N	3N	3N
R3	3K,1S	3K,1S	3K,1S	1K	1K	1N	1N	1N	1N	1N	1N
R4	4S	4S	4S	-	-	-	-	-	-	-	-
R5	4S	4S	4S	-	-	-	-	-	-	-	-
R6	4S	4S	4S	2K	2S	2K	2K	1K	1K	1N	1S

[a] Numbers of spiders affected and behaviour - N: normal, L: slow reaction, K: co-ordination problems and S: survivors lying without movement.
[b] All replicates included.

Table 9.3.21. Feeding rate of *Pardosa sp*. following exposure to 'Lontrel 100' under laboratory conditions

Parameter	Control	'Lontrel 100'	Karate
Mean total feeding rate/spider, day 1 to 7	3.9	3.3	1.7
Mean total feeding rate/spider, day 8 to 14	4.2	4.3	3.9
Mean for entire test duration	4.0	3.7	2.4

Table 9.3.22. Summary on results with *Pardosa sp.*

Nominal application rate of 'Lontrel 100' (g clopyralid/ha)	IOBC/WPRS classification
120	Harmless

Conclusions:

There were no effects on the survival, behaviour and feeding rate, compared to an untreated control treatment, following fourteen days exposure of *Pardosa sp.* Adults to 'Lontrel 100' at an application rate equivalent to 120 g clopyralid/ha. According to the classification system proposed by the IOBC/WPRS Working Group 'Lontrel 100' is classified as "harmless". Higher tier tests (extended laboratory or semi-field tests) are therefore not required.

Comments

The study was well performed and reported and in compliance with GLP. The test rate was lower than the highest recommended single use rates: half of the rate intended for pasture (240 g ai/ha). In this test the formulation "Lontrel 100" had the code EF-1136 that means the lead formulation of clopyralid in the EU dossier. Further data on the composition of the formulation was not given, however. The result shows that clopyralid is safe for the spiders when used up to 120 g ai/ha.

The effects of a soluble concentrate formulation of clopyralid (Lontrel 100) containing 100 g clopyralid/L on the survival, behaviour and feeding rate of the lycosid spider *Pardosa sp.* were assessed under laboratory conditions. No effects on the survival, behaviour and feeding rate, compared to an untreated control treatment, following 14 days exposure of *Pardosa* adults to Lontrel 100 at an application rate equivalent to 120 g clopyralid/ha. According to the IOBC/WPRS classification, Lontrel 100 is harmess to lycosid spiders.

RMS comments and evaluation:

The standard laboratory studies with non-target arthropods were originally assessed in the DAR (2003) as well performed and reported, and no effects were found with any of the tested non-target arthropod species with test concentrations equivalent to recommended field rates of clopyralid. The formulation tested is no longer the representative formulation in the EU dossier of clopyralid, but the data can be used to support the renewal of the authorisation of clopyralid.

The impurity profiles of the test substances used in these studies were not reported by the Notifier, but on the basis of the impurity profiles of batches used in other ecotoxicity studies it is not anticipated that the possible impurities would significantly alter the results of these studies.

Despite the studies are considered as still valid, the products tested were different from the new AIR3 representative formulation of clopyralid. Therefore additional formulation studies with the new representative formulation GF-1374 were submitted and evaluated in the dRAR Section 20 Vol 3 CP Part B 9.

B.8.3.2.8/1 - Clopyralid - Standard laboratory testing with non-target arthropods

Report	[IIA 8.3.2/08], Swaminathan, R and Isaichev, V.V. 2000.
Report title	Impact of pesticides on carabids of rotational intensive cropping systems. Entomon, volume 25 (3), pages 233-239.
DAS Study number	PJ03. Published scientific article.
Guidelines	own
GLP	No

Methodology:

Ground dwelling beetles were collected with pitfall traps from biologically managed intensive rotational cropping systems of the central non-chernozome area in Russia. Adult beetles of four dominant species, *Poecilus cupreus* L., *Pterostichus melanarius* (III.), *Pseudophonus rufipes* (DeG.) and *Harpalus affinis* (Sch.), were maintained in the laboratory for one week before the bioassay. The bioassay was performed using dry-film residue technique in petri dishes that were sprayed with 0.05, 0.1, 0.15, 0.2 and 0.25 % dilutions of the formulation Lontrel 52.5 EC. Other pesticides tested included four insecticides and a herbicide product Dialen 40 EC. Ten healthy adult beetles of each species were exposed for 30 minutes and transferred to clean observation jars. Mortalities were recorded after 24 hours.

A mini-field experiment was conducted with 1 m^2 plots that were treated with Lontrel 0.3 ml/ m^2 (3 l/ha) and beetles exposed in metallic cylinders 10 beetles in each. The application rate was mentioned to be equivalent to the recommended use rate of the formulation. Mortalities were recorded 1, 3 and 7 days after treatment.

Findings:

The only result with the herbicides in the article was that the herbicides had no toxic action on the carabids. No numerical results were presented. The insecticides studied caused a substantial toxicity to the carabids. The effects were presented in tables.

Conclusions

The clopyralid formulation Lontrel 52.5 EC was concluded to be safe to the carabid beetles when used according to the recommended rate.

Comments

The article was a published study report. The study did not follow any standard procedure, but the performance of the tests was sufficiently reported. The clopyralid formulation studied was different from the lead formulation in the EU dossier. No further details of the formulation were given. No numerical results with clopyralid were available. The study can be used as additional information but the risk assessment cannot be based on this study.

Ground dwelling beetles were collected with pitfall traps from biologically managed intensive rotational cropping systems of the central non-chernozome area of Russia. Adult beetles of four dominant species were maintained in the laboratory for one week before the bioassay. The bioassay was performed using dry-film residue technique in petri dishes that were sprayed with five concentrations of Lontrel 52.5 EC formulation. Ten healthy adult beetles of each spcies were exposed for 30 minutes and transferred to clean observation jars. Mortalities were recorded after 24 hours. Additionally, a mini-field experiment was conducted with 1 m^2 plots treated with the formulation, (3 liters/ha, equivalent to the recommended field use rate) and beetles exposed in metallic cylinders 10 beetles in each. Mortalities were recorded 1, 3 and 7 days after treatment. Clopyralid was concluded to be safe to the beetles when the product was used according to the recommended field rate.

RMS comments and evaluation:

The standard laboratory studies with non-target arthropods were originally assessed in the DAR (2003) as well performed and reported, and no effects were found with any of the tested non-target arthropod species with test concentrations equivalent to recommended field rates of clopyralid. The formulation tested is no longer the representative formulation in the EU dossier of clopyralid, but the data can be used to support the renewal of the authorisation of clopyralid.

The impurity profiles of the test substances used in these studies were not reported by the Notifier, but on the basis of the impurity profiles of batches used in other ecotoxicity studies it is not anticipated that the possible impurities would significantly alter the results of these studies.

Despite the studies are considered as still valid, the products tested were different from the new AIR3 representative formulation of clopyralid. Therefore additional formulation studies with the new representative formulation GF-1374 were submitted and evaluated in the dRAR Section 20 Vol 3 CP Part B 9.

In addition to previously evaluated studies, two other studies with the former representative formulation of clopyralid were submitted for the AIR3 evaluation, which have not been evaluated berore in the context of renewal of EU approval, and are summarised below.

CA 8.3.2.1/2 - A Laboratory Study To Evaluate The Effects of Clopyralid (EF 1136, An SL Formulation Containing 100 G/L Clopyralid) on the Parasitic Wasp *Aphidius rhopalosiphi* (Hymenoptera: Braconidae)

Report	Halsall, N. 2005
Report title	A laboratory rate response test to determine the effects of EF-1136 on the parasitic wasp, *Aphidius rhopalosiphi*
DAS Study number	DAS Report No. 050171 Insect Investigations Services Wentloog Cardiff UK, Lab Study No. GLP-05-15
Guidelines	Mead Briggs et al. 2000
GLP	Yes

Materials and methods

Test Item(s)

ISO Common name:	Not applicable
Test item (chemical/other name):	EF-1136
Active substance:	100 g a.s./L (clopyralid)
Description (physical state):	Light brown liquid
Lot/batch no.:	PD021501E-01
CAS no.:	Not applicable

Test System

Organism (*Species*):	*A. rhopalosiphi*
Study type:	Definitive
Study duration:	11 days
Parameters measured:	Mortality, fecundity
Observation intervals:	2, 24, 48 h for mortality; 11 day for fecundity
Test concentrations:	g a.s./ha
Toxic reference:	Dimethoate 40 EC
Environmental conditions:	16 h photoperiod Temperature: 17– 21°C

Methodology

Based on the results of a range finding test where rates of 30, 150, 300, and 600 g a.s./ha of EF-1136 were evaluated, a limit test was conducted using rates of 8.3, 150, and 300 g a.s./ha of EF-1136. For comparative purposes, a control group treated with water and a toxic reference of Dimethoate 40 were included in the test. Treatments were applied to glass plates using Potter laboratory spray tower. Once residues had dried, arenas were formed and ten wasps (5 male and 5 female) were confined in each replicate arena (6 replicates for the water control and 5 for each rate of the test item and toxic reference). The wasps were maintained in the arenas for 48 h period during which their mortality and behaviour were assessed 2, 24, and 48 h after their initial introduction to the arenas. At 48 h, 15 female wasps from each treatment were individually confined over barley seedlings infested with *Rhopalosiphum padi*. The wasps were removed after 24 h and the number of parasitized aphis (mummies) were counted 11 days later.

Results and discussion

The results are summarised in Table 9.3.22. below.

Table 9.3.22. Mortality and fecundity effects of clopyralid on A. *rhopalosiphi*.

	Mortality (%)	Fecundity assessments	
Treatment		Mean number of mummies/♀	[1]Reduction in fecundity relative to the control (%)
Water control	0	69.8	-
EF-1136			
8.3 g a.s./ha	0	47.1	32.5
150 g a.s./ha	2	53.9	22.8
300 g a.s./ha	6	42.4	39.3
Toxic reference (0.425 mL Dimethoate 40/ha)	100	-	-

[1]Calculated from: $(1 - (R_t/R_c))*100$, where R_t is the mean number of mummies in the test item treatment and R_c is the mean number of mummies in the control.

Conclusion

Under tier I laboratory test conditions, mortality of adult *A. rhopalosiphi* 48 h after initial exposure of residues of EF-1136 applied at rates of 8.3, 150, and 300 g a.s./ha resulted in mortality of 0 (control), 0, 2, and 6%. Therefore the LR_{50} for EF-1136 is greater than 300 g a.s./ha. In fecundity assessments, mean numbers of 69.8, 47.1, 53.9, and 42.4 mummies per female were obtained for the test rates of 0, 8.3, 150, and 300 g a.s./ha, respectively. These represented reductions, relative to the control, of 32.5, 22.8, and 39.3 mummies per female.

RMS comments and evaluation:

The study was well performed and reported, in accordance with the test guideline and GLP.The test met all validity criteria, as mortality in the control did not exceed 13% (0% actual), control fecundity exceeded a mean of 5.0 mummies per female (69.8 mummies per female actual) with no more than 2 wasps producing zero values (no zeros actual), and the mortality in the toxic reference treatment (dimethoate) was between 50 to 100% after 48 h exposure (100% actual). The results are in agreement with other studies on the effect of clopyralid to non-target arthropods.

The formulation studied is different from the new representative formulation of clopyralid, but the results can be used for the risk assessment of the active substance. The impurity profile of the test substance used in this study was not reported by the Notifier, but on the basis of the impurity profiles of batches used in other ecotocicity studies it is not anticipated that the possible impurities would significantly alter the results of this study.

The highest treatment levels studied correspond to clopyralid exposure resulting from ca 1-2 X the GAP of the current representative formulation of clopyralid GF-1374. Additional formulation studies with it were submitted and evaluated in the dRAR Section 20 Vol 3 Part B 9.

B.9.3.2.2. Extended laboratory testing, aged residues studies with non-target arthropods

For the AIR3 evaluation of clopyralid, the representative formulation of clopyralid was changed. Therefore the Notifier submitted following new extended laboratory studies on beneficial arthropods with the formulation GF-1374, which are evaluated in the dRAR CP Section 20 Vol 3 Part B 9.

Data Point/Study	Rationale
CP 10.3.2.2-1 Loose (2004a) DAS Study ID 040262	The study is a new representative formulation not previously reviewed for the active approval of clopyralid
CP 10.3.2.2-2 Loose (2005) DAS Study ID 040263R	The study is a new representative formulation not previously reviewed for the active approval of clopyralid
CP 10.3.2.2-3 Loose (2004b) DAS Study ID 040264R	The study is a new representative formulation not previously reviewed for the active approval of clopyralid

Furthermore, the Notifier submitted for evaluation one extended laboratory study with the former representative formulation Lontrel 100 (EF-1136), containing clopyralid as the only active substance. This study has not been previously evaluated in the context of EU evaluation, and is therefore reviewed below.

CA 8.3.2.1/3 EF-1136 on *Aphidius rhopalosiphi* – Extended laboratory test

Report	Riches, M.N; 2004
Report title	Lontrel 100: Effects on the parasitic wasp, *Aphidius rhopalosiphi* under extended laboratory conditions
DAS Study number	DAS Study No. GHE-P-10713 CEM Analytical Services Ltd Berkshire UK; Lab Study No. CEMS-1994
Guidelines	Mead Briggs et al. 2000
GLP	Yes

Materials and methods

Test Item(s)

ISO Common name:	Not applicable
Test item (chemical/other name):	EF-1136 Lontrel 100
Active substance:	101 g a.s./L (clopyralid)
Description (physical state):	Light brown liquid
Lot/batch no.:	PD021501E-01
CAS no.:	Not applicable

Test System

Organism (*Species*):	*A. rhopalosiphi*
Study type:	Definitive

Study duration:	11 days
Parameters measured:	Mortality, fecundity
Observation intervals:	2, 24, 48 h for mortality; 11 day for fecundity
Test concentrations:	g a.s./ha
Toxic reference:	Dimethoate 40 EC
Environmental conditions:	16 h photoperiod Temperature: 17– 21°C

Methodology

Based on the results of a range finding test, the definitive rates of 42, 202, 303g a.s./ha of EF-1136 were evaluated under extended laboratory conditions. For comparative purposes, a control group treated with water and a toxic reference of Dimethoate 40 were included in the test. Treatments were applied to glass plates using Potter laboratory spray tower. Once residues had dried, arenas were formed and ten wasps (5 male and 5 female) were confined in each replicate arena (6 replicates for the water control and 5 for each rate of the test item and toxic reference). The wasps were maintained in the arenas for 48 h period during which their mortality and behaviour were assessed 2, 24, and 48 h after their initial introduction to the arenas. At 48 h, 15 female wasps from each treatment were individually confined over barley seedlings infested with *Rhopalosiphum padi*. The wasps were removed after 24 h and the number of parasitized aphis (mummies) were counted 11 days later.

Results and discussion

The mortality data are presented in Table 9.3.23. below.

Table 9.3.23. Mortality results.

Treatment	Rate tested	% mortality at 48HAI	% mortality (Abbott corrected)
Lontrel 100	3.0 L product/ha	3.33	-7.4
Lontrel 100	2.0 L product/ha	3.33	-7.4
Lontrel 100	0.416 L product/ha	3.33	-7.4
Danadim	10 mL product/ha	90.0*	88.9
Control	Water only at 200L/ha	10.0	N/A

*Result differed statistically from the control (Dunnetts test, P = 0.05, 1-tailed test)

Table 9.3.24. Reproductive effects

Treatment	Rate tested	Mean number of mummies produced per wasp (24 hrs)	% reduction in mummy numbers compared to the control.
Lontrel 100	3.0 L product/ha	20.93	4.86
Lontrel 100	2.0 L product/ha	17.80	19.09
Lontrel 100	0.416 L product/ha	19.40	11.82
Control	Water only at 400L/ha	22.00	N/A

*Result differed statistically from the control (Dunnetts-test, P = 0.05, 2-tailed test)

Conclusion

Under extended laboratory conditions, Lontrel 100 did not exceed the 50% trigger value for mortality when tested at 42, 202, 303 g a.s./ha. Lontrel 100 had no effect on the number of mummies produced by exposed wasps even at the highest dose tested. Therefore, Lontrel 100 is considered harmless to *A. rhopalosiphi.*

RMS comments and evaluation:

The study was well performed and reported, in accordance with the test guideline and GLP.The test met all validity criteria. The results are in agreement with other studies on the effect of clopyralid to non-target arthropods.

The formulation studied is different from the new representative formulation of clopyralid, but the results can be used for the risk assessment of the active substance. The impurity profile of the test substance used in this study was not reported by the Notifier, but on the basis of the impurity profiles of batches used in other ecotoxicity studies it is not anticipated that the possible impurities would significantly alter the results of this study.

The treatment levels studied correspond to clopyralid exposure resulting from ca 0.5 - 2 X the GAP of the current representative formulation of clopyralid GF-1374. Additional formulation studies with the current representative formulation were submitted and evaluated in the dRAR Section 20 Vol 3 CP Part B 9.

Additionally, as a result of the literature search conducted by the Notifier, following study published in the scientific literature was made available for the AIR3 evaluation of clopyralid.

Table 9.3.25. Summary of the published study with *T. pyri*

Total effects of selected plant protection products applied to different natural substrates on the predatory mite *Typhlodromus pyri* Sch.
KCA 9/2 (CA 8.3.2.2)

Author(s)	Czarnecka, M., Parma, P., Kulec-Poszczyca, E.
Year	2014
Journal	IOBC/WPRS Bulletin Vol. 103, pp. 51-60
Relevance check	Relevant. Study performed in line with an appropriate guideline and methodology is comprehensively described
Reliability check	Reliability score 1 (Klimisch)
Reasons for no reliability	Not applicable
Summary	*Typhlodromus pyri* were exposed for 14 days to 9 different plant protection products (PPPs), including a PPP containing clopyralid + picloram in extended laboratory studies. This PPP was tested at a concentration of 93.1 + 22.75 g clopyralid + picloram/ha, on leaves of blackberry (*Rubus* L., Rosaceae). No effects on mortality and reproduction were observed. The PPP containing clopyralid + picloram was found to be harmless and the least toxic of all the tested PPPs
Reliability check: study details	
Parameter	**Information available**
Test protocol GLP, GEP, Guidelines (US EPA, OECD, …)	- ESCORT 1 and 2 guidance documents and guidelines developed by Joint initiative of the IOBC, BART and EPPO - GLP/GEP not discussed
Test substance Identification of test substance, source, purity, stability	- Plant protection product containing clopyralid + picloram - Seven other plant protection products, containing thiophanate-methyl, chlorothalonil, pyrimethanil, paraffin oil, ethepon, MCPA (4-chloro-2-methylphenoxy acetic acid) and amidosulfuron
Test conditions Temperature, pH, oxygen concentration, water hardness, conductivity, photoperiod, light intensity, number of animals, food availability	- Temperature: 23 - 27ºC - Relative air humidity: 58 – 98% - Photoperiod: 16h light/8h darkness - Light intensity: 623 – 894 lux - Food: pine pollen and *Tetranychus urticae*
Controls Positive control, negative control	- Positive control: Dimethoate (at 0.6 g a.i./ha) - Negative control: Distilled water only
Dosing system Exposure (dose, duration, frequency)	- Exposure system: The ‘ Island method’, with exposure on discs cut out of blackberry (*Rubus* L., Rosaceae) leaves, for clopyralid + picloram (MCPA was applied to rose leaves and the other PPPs were exposed on bean plant leaves) - Exposure dose: 93.1 + 22.75 g clopyralid + picloram/ha (equal to 0.05% clopyralid and 0.01% picloram) in spray fluids on the basis of the application volume of 200 L water/ha - Replicates: 3, containing 20 mites each
Test species Body weight or length, gender, age/life stage, source	- *Typhlodromus pyri* Sch. (Acari: Phytoseiidae) - Source: Research Institute of Horticulture, Skierniewice, Poland
Statistical analyses Sample size/replicates, statistical analysis of data (significance level, variability)	- Mortalities were corrected according to Abbott’s formula - Significant difference in mortality between treated groups and control was analysed using the χ^2 test - Degree of detected relationships was determined using the Yule correlation coefficient - Significant difference in number of eggs/female between treated groups and control was analysed using Student’s t-test - Statistical analysis performed using STATISTICA 10.0.1011.7 software
Biological effects Determined effect concentration, dose response observed	- After 7 days of exposure, the surviving mites were sexed and the sex-ratio determined. The numbers of males, females, eggs and larvae were recorded at 10, 12 and 14 days of the exposure - No significant effects of clopyralid + picloram on mortality and reproduction at the tested concentration

	- Clopyralid + picloram was found to be harmless and the least toxic of all the tested PPPs
Overall assessment	- Methods, statistical analysis, results and discussion are described - Study is reliable

RMS comments and evaluation:

The study was well conducted according to the test guideline and clearly reported, although not officially fulfilling the GLP requirements. The test substances were different from the current representative formulation GF-1374, but could be used to support the evaluation of the active substance clopyralid. The specification of the test substance used in this study was not presented and hence the impurity profile is unknown. The use rate of the clopyralid + picloram formulation was within the range of the GAP of other clopyralid formulations evaluated in the DAR and dRAR. The study is acceptable and valid. The data requirement is fulfilled.

B.9.3.2.3. Semi-field studies with non-target arthropods

No effects were observed following laboratory testing in accordance with the requirements put forth in point 8.3.2 of Part A of the Annex to Regulation (EU) no 283/2013 and in point 10.3.2 of the Annex to Regulation (EU) 284/2013. Since Annex trigger values were not breached under these scenarios, semi field testing is not required.

RMS comments and evaluation:

The justification above provided by the Notifier is acceptable and no further studies on non-target arthropods are required.

B.9.3.2.4. Field studies with non-target arthropods

No effect were observed following testing in accordance with the reqirements set out in point 8.3.2 of Part A of the Annex Regulation (EU) No 283/2013 or in accordance with points 10.3.2.2 or 10.3.2.3 of Annex Regulation (EU) 284/2013. Calculated Risk Quotients (RQs) did not indicate risk to non-target arthropods so field testing was not required.

RMS comments and evaluation:

The justification above provided by the Notifier is acceptable and no further studies on non-target arthropods are required.

B.9.3.2.5. Other routes of exposure for non-target arthropods

Since GF-1374 has demonstrated low toxicity to non-target arthropods, further testing of other possible routes of exposure was deemed unnecessary.

RMS comments and evaluation: The justification above provided by the Notifier is acceptable and no further studies on non-target arthropods are required.

B.9.4. EFFECTS ON NON-TARGET SOIL MESO- AND MACROFAUNA

B.9.4.1. Effects on Earthworms

The Notifier has provided a justification for the use of a different study to address the effects of formulated plant protection product of clopyralid on the reproduction and growth of earthworms to that originally evaluated for the first Active Approval. The new earthworm study is evaluated in the dRAR Section 20 Vol 3 CP Part B 9.

Data Point/Study	Rationale
CP 10.4.2.1/1 Davies(2005) DAS Study ID 040258	Study submitted for review since the representative formulation has changed since the previous Annex I inclusion

Furthermore, studies with clopyralid active substance were available and evaluated in the DAR (2003), and peer reviewed in the context of the first EU approval of clopyralid. Therefore the studies are only briefly presented below.

B.9.4.1.1. Sub-lethal effects on earthworms

Although acute earthworm studies are no longer a specific EU data requirement, the following acute earthworm study with technical clopyralid was available and evaluated by the RMS for the first Annex I inclusion of clopyralid in 2003.

Report:	CA 8.4.1/1, Hayward, J. C. 2001
Title:	The acute toxicity of clopyralid to the earthworm *Eisenia foetida.*
Document No:	DAS Report No.GHE-T-1130, Ref. J68 CEMAS Study CEMR-1635
Guidelines:	OECD 207 (1984)
GLP	Yes

Methodology:

Clopyralid technical, Batch No. RMM 2428, Purity 96.2% (w/w). The acute toxicity of clopyralid to the earthworm *Eisenia foetida* was assessed over a 14-day exposure period under laboratory test conditions.

Based on the results of a range-finding test, earthworms (3 to 4 months old) were exposed to the following calculated concentrations of clopyralid in artificial soil: 0 (control), 99.8, 180, 320, 561 and 1000 mg/kg dry weight soil. Clopyralid (in de-ionised water) was added directly to the artificial soil.

For each treatment there were four replicate test vessels (1000 mL glass beakers with a perforated polyethylene cover) each containing 10 pre-weighed worms. The soil moisture content was 35% (w/w, dry soil basis). Soil pH and temperature were measured on days 0, 7 and 14. The cultures were maintained at a continuous mean light intensity of 533 lux, a pH of 5.15 to 5.60 and a soil temperature of 21.1 to 22.0 °C.

After 7 days the contents of each replicate vessel were emptied and worms counted and observed for symptoms of toxicity. The worms and soil were then replaced into the test vessels and after a further seven days the procedure was repeated and the worms weighed.

Findings:

After seven days exposure to clopyralid there was one mortality in the 180 and 320 mg/kg treatments (equivalent to 2.5% of the total worms). There were no further mortalities observed in these or in any other treatment (Table 9.4-1). There were no observed sub-lethal effects in any treatment. There were no statistically significant effects ($p \leq 0.05$) of mean weight change in worms exposed to clopyralid, at any exposure concentration, compared to the control treatment (Table 9.5-2). The results are summarised in Table 9.5-3.

Table 9.4-1. Cumulative mean mortality of *Eisenia foetida* exposed to clopyralid under laboratory conditions

Calculated concentration of clopyralid (mg/kg soil dry weight)	**Mean mortality (%)**	
	Day 7	**Day 14**
0 (control)	0	0
99.8	0	0
180	2.5	2.5
320	2.5	2.5
561	0	0
1000	0	0

Table 9.4-2. Mean live weights of *Eisenia foetida* exposed to clopyralid under laboratory conditions

Calculated concentration of clopyralid (mg/kg soil dry weight)	**Mean live weight (g) on Day 0**	**Mean live weight (g) on Day 14**	**Mean percent change[a]**
0 (control)	0.371	0.275	-25.8
99.8	0.386	0.293	-24.3
180	0.359	0.262	-27.2
320	0.365	0.271	-25.6
561	0.382	0.264	-30.6
1000	0.385	0.275	-28.7

[a] Mean of percentage change calculated for four replicates.

Table 9.4-3. Summary on results with *E.foetida*

Result	**Calculated concentration of clopyralid (mg/kg soil dry weight)**
14-day LC_{50}	> 1000
14-day NOEC	≥ 1000

Conclusions:

The 14-day LC_{50} of clopyralid to the earthworm *Eisenia foetida* was greater than 1000 mg/kg soil dry weight. The 14-day NOEC was ≥1000 mg/kg soil dry weight, the highest concentration tested.

Comments

The study was well performed and reported and in compliance with GLP. The test substance was mixed with the artificial soil before the earthworms were placed into the test vessels. The validity criterion was fulfilled as there were less than 10 % mortality in the control. The study is acceptable. Clopyralid is of low toxicity to earthworms.

RMS comments and evaluation:

The study was originally evaluated in the DAR (2003) as presented above. As the acute earthworm study is no longer a data requirement, the justification provided by the Notifier is acceptable and no further acute earthworm studies are required to support the renewal of approval of clopyralid.

However, the following study with the previous representative formulation of clopyralid was submitted for the evaluation in the first Annex I inclusion, and therefore summarised below as additional data. The RMS has not re-evaluated the study, because the tested formulation is no longer the representative formulation for clopyralid renewal.

Study 2 – formulation – J33

Report: Hakin, B. & Johnson, A.J. (1991). Lontrel 100 (EF-255): Acute toxicity (LC_{50}) to the earthworm (*Eisenia foetida*). Dow AgroSciences, unpublished report No. DWC 616/911407, 16 December 1991. J33

Guidelines: OECD Guideline No. 207

GLP: Yes. Laboratory certified by United Kingdom Good Laboratory Practice Monitoring Authority, Department of Health, Skipton House, 80 London Road, London SE1 6LW, UK.

Methodology:

The objective of this study was to determine the acute effects of Lontrel 100 (EF-255), on the earthworm *Eisenia fetida* in a 14-day test conducted according to OECD/EU guidelines in compliance with GLP. EF-255 is a soluble concentrate (SL) preparation of clopyralid monoethanolamine [redacted] at 100 g clopyralid/L, nominal (EF-255 differs from EF-1136 [redacted]). The test was carried out with material from Batch No. EK 901024034 with an actual clopyralid content of 9.76% w/w.

Three test material concentrations were included in the test (250, 500, and 1000 mg product/kg dry soil), plus an untreated control. Each treatment was replicated four times. Test vessels consisted of 1000 mL glass containers, covered with perforated plastic and containing soil at a mean wet weight of 738 g/replicate. The test substrate was formulated soil containing 35% moisture on a dry weight basis, and consisting of quartz sand (70%), kaolin clay (20%), sphagnum peat moss (10%), and calcium carbonate (to pH 5.6). Test soils were prepared by mixing the test dose with a small amount of soil before thorough blending with the bulk soil. Water was then gradually mixed with the treated soil to give a moisture content equivalent to 35% of the dry weight.

Groups of 10 worms (mean weight range per replicate 400-491 mg at the start of the study) were washed in water, dried, weighed and randomly placed onto the surface of the soil in the test vessels. After 7 days, the contents of the beakers were tipped out, the worms counted, and observations made on worm behaviour and condition. Where less than 10 worms were recovered, the missing worms were recorded as dead and assumed to have decomposed. The soil was then gently replaced in the containers and the worms replaced on the surface of the soil. After a further 7 days, worms were again isolated from the soil and observations made on behaviour and condition before the worms were washed, dried and weighed in replicate groups. Mean illumination during the test was 200 lux, continuous and temperature was 21-22°C. Mean soil moisture loss over the course of the test was 0-4.0%.

Approximately six weeks prior to the test, The LC_{50} value for the reference material, chloroacetamide, was determined following a similar procedure to that adopted for EF-255.

Findings:

No worms were observed on the surface of the control or treated soils between counts or on Day 7 and 14. All worms were normal in behaviour and appearance. No mortality occurred in either the control or treatment groups. Consequently, the 7 and 14 Day LC_{50} values were shown to be >1000 mg product/kg dry soil. Since weight changes were variable with no evidence of any treatment-related effect, an NOEC of ≥1000 mg product/kg dry soil is indicated.

The chloroacetamide LC_{50} value for acute toxicity was calculated from the reference data set to be 52 ppm (95% C.L. 47-58 ppm) after 7 days and 37 ppm (95% C.L. not calculable) after 14 days. These values are within the expected range for this reference material.

Table 9.4-4. Mortality of earthworms exposed to EF-255 for 14 days (4 replicates per treatment/10 individuals per replicate)

Soil Concentration (mg product/kg)	**Number of mortalities per vessel**				**Percent Mortality**
	Replicate 1	**Replicate 2**	**Replicate 3**	**Replicate 4**	
0 (control)	0	0	0	0	0
250	0	0	0	0	0
500	0	0	0	0	0
1000	0	0	0	0	0

Table 9.4-5. Mean live weights (mg) of worms on Days 0 and 14

Soil Concentration (mg product/kg)	**Replicate ID**	**Day 0 (Initiation)**	**Day 14 (Termination)**	**Change in Live Weight (%)**
0 (control)	1	405	425	
	2	416	420	
	3	467	459	
	4	483	481	
	Mean	443	446	+0.7
250	1	491	425	
	2	449	431	
	3	440	428	
	4	400	387	
	Mean	445	418	-6.1
500	1	467	475	
	2	408	425	
	3	408	430	
	4	468	459	
	Mean	438	447	+2.1
1000	1	487	472	
	2	400	410	
	3	438	429	
	4	452	442	
	Mean	444	438	-1.4

Table 9.4.6. Summary on results with *E. foetida*

Result	mg EF-255/kg dry soil
14-day LC_{50}	>1000
NOEC	≥1000

Conclusions:

The 14-day LC_{50} of EF-255 (equivalent to EF-1136) to *Eisenia foetida* in an artificial soil is >1000 mg product/kg dry soil (>97.6 mg ae/kg). The NOEC is ≥1000 mg product/kg (≥97.6 mg ae/kg). These results indicate that the toxicity of clopyralid to *Eisenia* is not substantially altered when formulated as EF-255. These results confirm the findings from the acute toxicity study on the technical material, clopyralid, in which the active ingredient was shown to have a 14-day LC_{50} value of >1000 mg as/kg (Ref. J68). The toxicity of clopyralid is not substantially altered, therefore, when formulated as EF-255.

Comments

The study was well performed and reported and in compliance with GLP. The test substance was mixed with the artificial soil before the earthworms were placed into the test vessels. The formulation studied was different from the lead formulation EF-1136 in the EU dossier, but the Notifier has clarified the composition of EF-255 and the difference is considered to be of minor importance. Therefore the study is acceptable and the result can be used in the risk assessment for EF-1136.

The acute toxicity of technical clopyralid (purity of 96.2 % w/w) to the earthworm *Eisenia foetida* was assessed over a 14-day exposure period under laboratory test conditions. The 14-day LC50 of clopyralid to the earthworm was greater than 1000 mg/kg soil dry weight. The 14-day NOEC was $\geq$ 1000 mg/kg soil dry weight, the highest concentration tested.

RMS comments and evaluation:

From the DAR (2003): The study was well performed and reported and in compliance with GLP. The test substance was mixed with the artificial soil before the earthworms were placed into the test vessels. The validity criterion was fulfilled as there was less than 10 % mortality in the control. The study is acceptable. Clopyralid is of low toxicity to earthworms.

Data on the impurity profile of the test substance used in this study was not available, but on the basis of the impurity profiles of batches used in other ecotoxicity studies it is not anticipated that the possible impurities would significantly alter the results of this study.

The conclusion above is still valid and the data is adequate for the renewal evaluation of clopyralid.

B.9.4.1.2. Long term effects on earthworms

Data to address this point were presented in the dossier submitted in 30 April 2002 for the Active Approval and were deemed acceptable following evaluation and peer review at EU level. These data are still valid for decision making.

CA. 8.4.1. Clopyralid: - earthworm reproduction

Report:	CA 8.4.1/1, Hayward, J. C. 2001
Title:	The Effects of EF-1136 on Reproduction and Growth in the Earthworm *Eisenia fetida*
Document No:	DAS Report No. GHE-T-1135, Ref. J69 CEMAS Study CEMS-1637
Guidelines:	ISO 11268-2 (1998)
GLP	Yes

Methodology:

EF-1136, a 100 g/L soluble concentrate formulation of clopyralid, Batch No. EB 950309, Purity 98.9 g clopyralid/L. The effects of EF-1136 on the mortality, growth and reproduction of the earthworm *Eisenia foetida* were assessed under laboratory test conditions.

Adult *E. foetida* (10 to 11 months old) were placed in test vessels containing two calculated concentrations of EF-1136 in artificial soil, 3 and 15 L EF-1136/ha (equivalent to nominal concentrations of 300 and 500 g clopyralid/ha, respectively). Measured application rates were 2.944 and 14.97 L EF-1136/ha (equivalent to 296 and 480 g clopyralid/ha, respectively, equivalent to 0.395 and 1.97 mg clopyralid/kg dry soil, respectively). The test substance (in deionised water) was added directly to the artificial soil. Cow manure at a rate of 1 g/100 g dry soil, was added as a food source. For each treatment of EF-1136 and the control treatment (deionised water only) there were four replicate test vessels (1.2 L plastic boxes with a perforated plastic lid) containing 600 g dry weight of artificial soil and 10 worms.

One day after the worms were introduced to the test vessels, the cow manure was added to the soil surface and thereafter once per week up to Day 28. In addition to cow manure, 5 g deionised water was added.

After 28 days, the contents of each test vessel were tipped out and observations made on the worms. The adults were removed, counted and weighed. The soil was replaced, ensuring no cocoons were visible on the soil surface. A final application of cow manure was added.

On day 56, the vessels were placed in a water bath of 40 to 60 °C to bring juveniles to the soil surface for counting. After removal of surface worms, the soil was tipped out and examined for remaining juveniles and unhatched cocoons.

Soil pH was measured on days 0 and 56 and soil temperature was measured on days 0, 28 and 56. Soil moisture was estimated weekly and replenished when required to maintain moisture content of 45.2%. The mean (continuous) light intensity was 431 lux, soil pH range 5.62 to 6.99 and soil temperature range 20.0 to 21.9 °C.

The endpoints assessed were adult mortality after 28 days, reduction in live weight of adults over 28 days, number of juveniles per surviving worm after 56 days and the number of unhatched cocoons per surviving adult after 56 days.

Findings:

After 28 days exposure to EF-1136, there was one mortality in one replicate at 1.97 mg clopyralid/kg dry soil (equivalent to 2.5% of the total). There were no observed sub-lethal effects in any treatment and no significant differences in mean weight change over the 28-day exposure period. After 56 days there were no significant differences between treatments in the numbers of live juveniles per surviving adult worm or in the number of unhatched cocoons per surviving adult (Table 9.4-7). The results are summarised in Table 9.4-8.

Table 9.4.7. Summary of mortality, mean live weight change and reproduction of *Eisenia foetida* exposed to EF-1136

Calculated soil concentration of clopyralid (mg/kg soil dry weight)	Percent mortality after 28 days	Mean percent weight change (day 0 to day 28)	Mean juveniles per surviving adult	Mean unhatched cocoons per surviving adult
0 (control)	0	+31.7	19.6	0.1
0.395	0	+23.8	22.7	0.1
1.97	2.5	+34.1	22.7	0.2

Table 9.4.8. Summary on results of the reproduction study with *E. foetida*

Result	Application rate of EF-1136 (L/ha)	Calculated soil concentration of clopyralid (mg/kg soil dry weight)
28-day NOEC (adult mortality)	15	1.97
NOEC (reproduction)	15	1.97

Conclusions:

The adult mortality 28-day NOEC for EF-1136 was 15 L/ha (equivalent to 1.97 mg clopyralid/kg soil dry weight). The corresponding NOEC for reproduction was also 15 L/ha (equivalent to 1.97 mg clopyralid/kg soil dry weight), the highest concentration tested.

Comments

The study was well performed and reported and in compliance with GLP. The test substance was mixed with the artificial soil before the earthworms were placed into the test vessels. The validity criteria (adult mortality rate, coefficient of variance for juvenile numbers and the total number of juveniles per container in the control) were all well fulfilled and therefore the test is considered as valid. The chronic toxicity of clopyralid to earthworms is low.

The effects of EF-1136, a 100 g/L soluble concentrate formulation of clopyralid (purity of 98.9 %) on the mortality growth and reproduction of the earthworm *Eisenia foetida* were assessed under laboratory test conditions. The adult mortality 28-day NOEC for EF-1136 was 15 L/ha (equivalent to 1.97 mg clopyrlid / kg soil dry weight). The corresponding NOEC for reproduction was also 15 L/ha (equivalent to 1.97 mg clopyrlid / kg soil dry weight), the highest concentration tested.

RMS comments and evaluation:

From the DAR (2003): The study was well performed and reported and in compliance with GLP. The test substance was mixed with the artificial soil before earthworms were placed into the test vessels. The validity criteria (adult mortality rate, coefficient of variance for juvenile numbers and the tofal number of juveniles per contrainer in the control) were all well fulfilled and therefore the test is considered as valid. The chronic toxicity of clypyalid to earthworms is low.

The study is still considered acceptable and valid. Data on the impurity profile of the test substance used in this study was not available, but on the basis of the impurity profiles of batches used in other ecotoxicity studies it is not anticipated that the possible impurities would significantly alter the results

of this study. The formulation tested is different from the new reprerentative formulation of clopyralid, but its outcome can be used for the risk assessment of the active substance clopyralid.

B.9.4.1.2. Earthworms – field studies

Since GF-1374 or clopyralid did not induce any chronic effects on earthworms, field testing was deemed unnecessary.

RMS comments and evaluation:

The justification above provided by the Notifier is acceptable and no further studies on earthworms are required.

B.9.4.2. Effects on non-target soil meso- and macrofauna (other than earthworms)

B.9.4.2.1. Laboratory studies with non-target arthropods

The DT_{90f} of clopyralid has a mean value of 38 days. Furthermore, the HQs for arthropods were less than 2. Due to the lack of effects and clopyralid not being persistent in soils, further studies were deemed unnecessary and data on soil meso and macrofauna was not submitted for review.

However, following new studies with the representative formulation of clopyralid, GF-1374, were submitted to address effects on non-target soil meso- and macro-fauna and not evaluated before in the context of Active Approval. The studies are evaluated in the dRAR Section 20 Vol 3 CP Part B 9.

Data Point/Study	Rationale
CP 10.4.2.1/2 Ganßmann (2012) DAS Study ID 120256	Study submitted for review since the representative formulation has changed since the previous Annex I inclusion
CP 10.4.2.1/2 Ganßmann (2012) DAS Study ID 120257	Study submitted for review since the representative formulation has changed since the previous Annex I inclusion

RMS comments and evaluation:

The explanation above provided by the Notifier is acceptable. The new studies are evaluated in the dRAR Section 20 Vol 3 CP Part B 9. No further studies on non-target soil meso- and macrofauna are required and the data requirement is fulfilled.

B.9.4.2.2. Higher tier testing

Higher tier testing of clopyralid with non-target soil meso- and macrofauna was not deemed necessary due to the lack of long-term effects in laboratory studies.

RMS comments and evaluation:

The justification above provided by the Notifier is acceptable and no further studies on non-target soil meso- and macrofauna are required.

B.9.5. EFFECTS ON SOIL NITROGEN TRANSFORMATION

A summary on the effects of clopyralid on soil microbial functions is presented in Table 9.5.1.

Since the representative formulation was changed in the AIR3 dossier of clopyralid, the Notifier submitted a new study (Schöbinger 2013) which has not been evaluated before in the context of renewal of approval of clopyralid, and evaluated first time below.

In the original DAR (2003) one acceptable study with the previous representative formulation of clopyralid was available and assessed, with no significant effects on soil respiration and nitrogen transformation. Neverthless, the summary from the DAR (2003) is presented here again as additional information below.

Additionally, as a result of the literature search conducted by the Notifier, one independent nitrification study published in the scientific literature was available and is included in the AIR3 dossier.

Table 9.5.1. Clopyralid – Summary of effects on nitrogen transformation

Data point	Test substance	Test	Soil concentration (mg/kg dry soil)	Result	New findings	Reference
CA 8.5/1	Clopyralid				<25 % deviation to the control=209 mg/kg soil <25 % deviation to the control =209 mg/kg soil	130283, Schöbinger, U., 2013
[CA 8.5/1	EF-1136 (former representative formulation of clopyralid)	Soil respiration + N transformation	4.16 mg/kg + 20.8 mg/kg	<25 % deviation to the control in both tests and both treatment levels		Hayward & Morgan 2003]

The following justification was provided by the Notifier for the use of a different study to address effects on nitrogen transformation to that originally evaluated for the first Active Approval. The new study is evaluated below.

Data point/Study	Rationale
CA 8.5/1 Schöbinger, U. 2013 DAS Study ID 130283,	Study was updated using the active ingredient so endpoint is not based on formulation study

CA 8.5/1 - Clopyralid: Effects on the Activity of the Soil Microflora under Laboratory Conditions (Nitrogen and Carbon Transformation)

Report:	CA 8.5/1, Schöbinger, U. 2013
Title:	Clopyralid: Effects on the Activity of the Soil Microflora under Laboratory Conditions (Nitrogen and Carbon Transformation)
Document No:	DAS Report No. 130283 Eurofins Agroscience Services EcoChem GmbH Study No. S13-00615
Guidelines:	OECD 216 and 217 (2000)
GLP	Yes

Materials and methods

Test Item(s)

ISO Common name:	Not applicable
Test item (chemical/other name):	Clopyralid
Purity:	95.9 wt %
Description (physical state):	Powder
Lot/batch no.:	910905 5P
CAS no.:	Not applicable

Test System

Organism (*Species*):	Soil micro-organisms
Study type:	Laboratory study with OECD guideline natural soil, assessed for: • Nitrate formation • Microbial respiration
Study duration:	56 days
Parameters measured:	Nitrogen transformation: analysis of nitrate in extracted soil samples, via ion selective electrode and WTW inoLab pH/ION 735; limits of quantification: NO_3-N: 1.06 mg/kg soil dry weight (7 days); 5.26 mg/kg soil dry weight (0, 14, 28, 42, 56 days) soil water content pH,

	Microbial respiration: soil respiration rates after addition of glucose soil water content pH
Observation intervals:	0, 7, 14, 28, 42 and 56 days
Test concentrations:	mg/kg soil dry weight
Toxic reference:	Sodium Chloride at 20.0 g a.i./kg soil dry weight (separate study)
Method of test item application:	Incorporation into the soil
Environmental conditions:	Conducted in the dark. Temperature: 18.8 – 20.1°C pH: 7.00 – 7.53 Soil source: Standard soil according to OECD 2016 and 217 (LUFA Speyer, Germany) Moisture content of soil at start: 40.9 – 42.1 % of WHCmax (nitrogen) 40.9 – 42.0 % of WHCmax (respiration) Moisture content of soil at end: 39.0 – 41.3 % of WHCmax (nitrogen) 41.0 – 41.8 % of WHCmax (respiration) Clay (%): 9.3 Silt (%): 32.0 Sand (%): 58.7 Organic Carbon (%): 0.91 Textural classification: medium silty sand (DIN)

Methodology

Incorporation of the test item at two test item concentrations into standard soil according to OECD 216 and 217 for determination of effects on nitrogen turnover (mineralization) and carbon transformation (short-term substrate-induced respiration); Incubation of test vessels in the dark at 20 ± 2 °C; Sampling of soil at 0, 7, 14, 28, 42 and 56 days after treatment (assessments at 42 and 56 days were only performed for the nitrogen turnover) after application for nitrogen transformation and carbon mineralization; Determination of pH, dry mass, nitrogen turnover and short-term respiration; Comparison of the results with the control; The test was terminated after 28 days for carbon transformation and after 56 days for nitrogen turnover, since by this time the measured activity deviated less than 25 % from the control, respectively.

Results and discussion

For the nitrate-N formation rate for the last sampling interval (day 42 to 56 day sampling) deviations from the control were -2.88 % at 0.417 mg/kg soil dry weight and +2.88 % at 209 mg/kg soil dry weight.

For the short-term respiration, deviations from the control were -2.93 % at 0.417 mg/kg soil dry weight and -18.2 % at 209 mg/kg soil dry weight at the end of the 28-day incubation period.

Table 9.5.2. Effects of Clopyralid on the nitrate formation rate

Interval sampling days	Control	0.417 mg/kg sdw			209 mg/kg sdw		
	[mg/kg/day [1]]	[mg/kg/day [1]]	[%[2]]	[sig[3]]	[mg/kg/day [1]]	[%[2]]	[sig[3]]
0-7	-2.46	-2.44	+0.813	--	-2.36	+4.07	--

Interval sampling days	Control	0.417 mg/kg sdw			209 mg/kg sdw		
	[mg/kg/day [1]]	[mg/kg/day [1]]	[%[2]]	[sig[3]]	[mg/kg/day [1]]	[%[2]]	[sig[3]]
7-14	0.393	0.356	-9.41	--	1.42	+261	--
14-28	0.832	1.11	+33.4	--	1.12	+34.6	--
28-42	0.350	0.250	-28.6	--	-0.529	-251	--
42-56	1.04	1.01	-2.88	n.s.	1.07	+2.88	n.s.

[1] mean mg NO_3-N/kg soil dry weight per day
[2] deviation from control
[3] statistical significance : n.s. not significant

Conclusion

Based on the results of this study and in accordance with OECD Guidelines 216 and 217, the test item had no adverse effect on soil respiration after 28 days of exposure (< 25 % deviation between treatments and control) and no long-term effect on nitrogen turnover after 56 days of exposure (< 25 % deviation between test item groups and control) in a field soil tested up to and including 209 mg/kg soil dry weight.

Table 9.5.3. Conclusions of the effects of clopyralid on soil microbial functions.

Common name	Species	Test item	Time-scale	Endpoint	Toxicity value	Units of test item
Soil micro organisms	N/A	Clopyralid	56 day – nitrogen transformation	<25 % deviation to the control	209	mg/kg soil
Soil micro organisms	N/A	Clopyralid	28 day – carbon respiration	<25 % deviation to the control	209	mg/kg soil

RMS comments and evaluation:

The study was well performed according to the OECD test guidelines and GLP, well reported and fulfilled the validity criteria, as the variation between replicate control samples was less than ± 15 %. The toxic reference data originated from a separate study. The soil fulfilled the requirements for sand content, pH, organic C content and microbial biomass set in the test guideline.

The Notifier has presented a new specification of technical clopyralid. The original specification of clopyralid is based on the analysis of the batch used in this study. Based on the risk assessment for the impurities of clopyralid presented by the Notifier in Document J in December 2016, no impurity characterised in this batch is expected to substantially impact the ecotoxicological profile of clopyralid. The impurity profile of the test substance used in this study is within the limits of the new specification presented by the Notifier in December 2016.

As a conclusion, no long-term effects on soil micro-organisms were observed at treatment levels equivalent to > 6.68 times the maximum application rate of GF-1374 to cereals and pastures. Overall, an acceptable risk to soil micro-organisms is therefore expected following the use of clopyralid at rates of 120 g clopyralid/ha on grasslands and 80 g clopyralid on cereals.

The data requirement is fulfilled and no further studies on this issue are required.

2. Study originally reported and evaluated in the DAR (2003):

Report: Hayward, J.C. & Morgan, A.J. 2003. EF-1136: Effects on Soil Microflora Activity. CEMAS Study No. CEMS-2007. Dow AgroSciences Project No. 031001. 04 March 2003. Ref J72

Guidelines: OECD 216 (2000) and OECD 217 (2000)

GLP: Yes

Methodology

A single sandy soil as recommended by the test guidelines was used as test soil. The soil originated from a set-aside area of an organic arable field in UK. The main characteristics of the soil were: 70 % sand, 15 % silt and 15 % clay, organic carbon content 1.3 %, pH 6.1 – 6.4, microbial biomass 3.7 % of organic C_{tot}, water holding capacity 49.2% at 0.001 bar, cation exchange capacity 7.8 meq/100g. The soils was classified as sandy loam.

The soil was treated with clopyralid 1x and 5x field rate, equivalent to 300 g and 1500 g clopyralid/ha, or 4.16 mg and 20.8 mg EF-1136 / kg dry soil. Dinoseb applications of 4.95, 15.0 and 45 mg/kg soil were studied as toxic reference substance.

For soil respiration mesurements the soil samples amended with glucose were incubated for 28 days. Microbial biomass was determined using a substrate-induced respiration method. The soils for nitrogen transformation were amended with ground lucerne as a source of nitrogen and were incubated for 28 days. Measurements were made after 6 hours and 7, 14 and 28 days. Analysis of the data was carried out using the Dunnett's two-tailed test for determining the location of statistically significant differences between treatments and controls.

Findings

Using the reference substance dinoseb the soil respiration rates were –22 %, -35 % and –11 % from the control mean by day 28. The nitrate-nitrogen transformation rates were –26 %, +222 % and –10 %, respectively. Statistically significant (P=0.05) effect on both soil respiration and nitrate-nitrogen transformation was seen consistently over a 28-day period.

Soil microbial respiration in soil treated with clopyralid showed no deviation from the control mean greater than 7.8 % during the study, as summarised in Table 9.5.4. The total range of between-replicate variation for the controls was –0.7% to +0.8% during the study.

Table 9.5.4. Summary table of percentage variation in mean respiration rates relative to the mean control value

Sampling time (days)	1x field rate = 300 g clopyralid/ha	5x field rate = 1500 g clopyralid/ha
Day 0 (6 hours after initiation)	+1.5 %	+0.1 %
Day 7	+0.3 %	-4.8 % *
Day 14	-1.4 %	-6.3 % *
Day 28 (end of the study)	-2.0 %	-7.8 % *

* = Statistically significant differences were found for mean respiration rates (mg CO_2/kg/hour) when compared with the controls (P=0.05), tested using the two-tailled Dunnett's test.

The variations of the nitrate-nitrogen transformation rates in soil treated with clopyralid are presented in Table 9.5.5. Soil nitrate-nitrogen transformation rates showed the greatest deviation from the control mean of +16 % and +138 % between days 0 and 7. The range of percentage variation within the control damples for nitrate-nitrogen concentrations was from –3.1 % to +1.7 %.

Table 9.5.5. Summary table of percentage variation of mean nitrate-nitrogen transformation rates relative to the mean control value

Sample time (days)	1x field rate = 300 g clopyralid/ha	5x field rate = 1500 g clopyralid/ha
Day 0 (start) to 7	+16 %	+138 % *
Day 7 to 14	+1.0 %	+8.3 % *
Day 14 to 28 (end of the study)	+4.6 % *	+18 % *

* = Statistically significant differences were found for mean nitrate-nitrogen rates (mg N/kg/day) when compared with the controls (P=0.05), tested using the two-tailled Dunnett's test.

Conclusions

The required validity criteria were met for both soil respiration and nitrogen transformation according to the respective test guidelines.

By day 28 the soil nitrate-nitrogen transformation rates at the 1x and 5x field rates of clopyralid differed by +4.6 % and +18 % from the control mean, respectively. These values are below the 25 % criterion of the effect as stated in the guideline OECD 216.

By day 28 the soil respiration rates at the 1x and 5x field rates of clopyralid differed by –2.0 % and –7.8 % from the control mean, respectively. These values are below the 25 % criterion of effect as stated in the guideline OECD 217.

Comments

The study was new, well performed and reported, in compliance with GLP and acceptable. The only deviation from GLP was that histrorical details for the site of soil sampling were provided by the land manager and were not generated according to GLP principles. This study confirms the findings of the previous two studies that have been evaluated as not adequate. The results of this study can be used in the risk assessment to soil micro-organisms and the data requirement is fulfilled. No further data is required on soil micro-organisms.

RMS comments and evaluation:

Because this formulation is no longer the representative formulation for clopyralid renewal, the RMS has not re-evaluated this study for AIR3. The data can be used as additional data.

The data requirement is fulfilled and no further studies on the functions of soil micro-organisms are required to support the renewal of approval of clopyralid.

In addition to Notifier-owned studies presented above, the literature search conducted by the Notifier yielded one study published in the scientific literature, and considered as relevant. The study is reviewed in Table 9.5.6. below.

Table 9.5.6. Summary of the published study on the effect on nitrification

Effect of soil contamination with herbicides on the nitrification process	
KCA 9/5 (CA 8.5)	
Author(s)	Kucharski, J., Bacmaga, M., Wyszkowska, J.
Year	2009
Journal	Ecological Chemistry and Engineering A Vol. 16(8), pp. 947-52

Relevance check	Relevant. Study does not follow an appropriate guideline but methodology is comprehensively described.
Reliability check	Reliability score 2 (Klimisch)
Reasons for no reliability	Not applicable
Summary	Effect of four herbicides Faworyt 300 SL (clopyralid), Harpun 500 SC (isoproturon), Akord 180 OF (phenmedipham, desmedipham and ethofumesate) and Mocarz 75 WG (tritosulfuron) on nitrogen transformation was assessed over an exposure period of 70 days. On day 14, 28, 42, 56 and 70 the nitrate nitrogen contents were determined. Faworyt 300 SL enhanced the nitrification process and this stimulating effect was still observed at the end of the experiment. Harpun 500 SC and Mocarz 75 WG both inhibited the nitrogen transformation process, but this was not observed at the end of the exposure period anymore. Akord 180 OF also inhibited the process during the first weeks, but a stimulating effect was observed after that.
Reliability check: study details	
Parameter	**Information available**
Test protocol GLP, GEP, Guidelines (US EPA, OECD, …)	- None described
Test substance Identification of test substance, source, purity, stability	- Faworyt 300 SL (clopyralid (monoethanolamine salt) at 300 g/dm^3) - Three other herbicides were also tested: Harpun 500 SC (containing isoproturon), Akord 180 OF (with phenmedipham, desmedipham and ethofumesate) and Mocarz 75 WG (containing tritosulfuron)
Test conditions Temperature, pH, oxygen concentration, water hardness, conductivity, photoperiod, light intensity, number of animals, food availability	- Test system: o Soil type: Typical brown soil, with a granulometric composition of loamy sand o pH: 6.5 o Hydrolytic acidity: 8.25 mmol/kg o Total exchangeable alkaline cations: 78 mmol/kg o Organic carbon content: 6.3 g/kg - Moisture content: 60% of maximum water capacity - Temperature: 25ºC
Controls Positive control, negative control	- Negative control: No herbicide added
Dosing system Exposure (dose, duration, frequency)	- Exposure system: 50 g of air-dried soil in each 100 cm^3 test beaker, with a dose of nitrogen (aqueous solution of $(NH_4)_2SO_4$ at 0 or 300 mg N/kg soil added at same time as the herbicide - Dose: 0 (control), 1 (dose recommended by manufacturer) and doses 50, 100, 150 and 200 times higher than recommended by manufacturer - Replicates: 9 - Incubation time: 14, 28, 42, 56 and 70 days
Test species Body weight or length, gender, age/life stage, source	- Micro-organisms present in the soil
Statistical analyses Sample size/replicates, statistical analysis of data (significance level, variability)	- Quantity (%) of nitrified nitrogen was calculated based on the formula by Wyszkowska (2002) - Results were processed statistically with Duncan's multiple range test, using Statistica application
Biological effects Determined effect concentration, dose response observed	- On day 14, 28, 42, 56 and 70 the content of N-NH_4 ions were determined with the application of Nessler's reagent, and N-NO_3 levels with the use of phenoldisulfonic acid - When applied at the highest test dose (200 x recommended), Faworyt 300 SL enhanced the nitrification process by 22%. This stimulating effect was still observed at the end of the experiment (day 70)

	- Harpun 500 SC and Mocarz 75 WG both inhibited the nitrification process, but this was not a lasting effect. Akord 180 OF also inhibited the process during the first weeks, but a stimulating effect was observed at day 42 and 56
Overall assessment	- Methods, statistical analysis, results and discussion are described - Study is reliable

RMS comments and evaluation:

The study did not follow GLP requirements or any official test guideline, but the protocol was well reported and in general aligned with appropriate test guidelines on this area. The clopyralid formulation tested was different from the representative formulation for AIR3 assessment, but the results were in accordance with studies owned by the Notifier. The specification of the test substance used in this study was not presented and hence the impurity profile is unknown. As the article was peer reviewed before its publication, the results are considered as valid and the data can therefore be used as supporting data.

B.9.6. EFFECTS ON TERRESTRIAL NON-TARGET HIGHER PLANTS

B.9.6.1. Summary of screening data

Additional screening data (apart from experimental data presented under Chapter 9.6.2.) on the effects of clopyralid on terrestrial non-target plants has not been conducted and is not presented by the Notifier.

RMS comments and evaluation:

As appropriate experimental data was available, it is agreed that screening data are not necessary. The experimental test reports are assessed in the next chapter.

B.9.6.2. Testing on non-target plants

Two new standard laboratory studies were submitted by the Notifier to address the effects of clopyralid on terrestrial non-target plants (Rockliff 2013 a, b). These studies have not been evaluated before in the context of renewal of approval of the active substance. The studies are evaluated below.

Furthermore, one study originally submitted and previously evaluated in the DAR (2003) is briefly summarised below. However, as the tested formulation is no longer representative for AIR3 evaluation, the study has not been re-evaluated here again and the data can be used as supporting data.

Additionally, one independent study published in the scientific literature was submitted as a result of literature search conducted by the Notifier, and is summarised below.

CA 8.6.2/1 - EF-797 (clopyralid potassium, 750 g a.e/kg, SG) GLP Seedling Emergence and Seedling Growth Test Terrestrial Non Target Plants (based on OECD Guideline 208) – China, 2013

Report:	8.6.2/1, Rockliff, C. 2013a
Title:	EF-797 (clopyralid potassium, 750 g a.e/kg, SG) GLP Seedling Emergence and Seedling Growth Test Terrestrial Non Target Plants (based on OECD Guideline 208) – China, 2013
Document No:	DAS Report No. 130095 Stockbridge Technology Centre Ltd., Lab Study No. STC/13/E754
Guidelines:	OECD 208 according to the July 2006 revision
GLP	Yes

Materials and methods

Test Item(s)

ISO Common name:	Not applicable
Test item (chemical/other name):	EF-797
Purity:	750 g a.e/kg clopyralid potassium
Description (physical state):	Soluble granule (SG)
Lot/batch no.:	ZL21278964
CAS no.:	Not applicable

Test System

Monocotyledonous species:	Ryegrass, oats, onion
Dicotyledonous species:	Soybean, oilseed rape, lettuce, sugar beet, carrot, cucumber, tomato
Study type:	Greenhouse study assessing Seedling Emergence and Seedling Growth
Parameters measured:	Emergence counts: at 14, 21 and 28 days (harvest) after 50% emergence Number of dead plants: at 14, 21 and 28 days (harvest) after 50% emergence Shoot fresh weight: at 28 days (harvest) after 50% emergence Shoot length: at 28 days (harvest) after 50% emergence Phytotoxicity rating system: at 14, 21 and 28 days (harvest) after 50% emergence. Visual injury scale 0% = no visual injury, 1% -39% = slight visual injury, 40% - 69% = moderate visual injury, 70% - 99% = severe visual injury, 100% = all plants dead
Growth conditions:	Temperature (range): actual 13.6°C to 26.6°C and 13.6°C to 27.4°C Photoperiod: natural day-length plus supplementary lighting with 5000 lux to extend day-length to 16 hours Light intensity (range): >5000 lux Relative humidity: actual 37% to 79% and 37% to 79% Water regime and schedules: Following treatment pots placed in plastic saucers and lightly watered overhead with a hosepipe and rose. All subsequent water was applied to the saucers. Plants inspected daily and watered according to crop requirements. Final watering applied one, two or three days before harvest. Water source/type: mains tap water Pest control method /fertilisation, if used: NPK fertiliser applied as liquid feed
Growth medium:	Soil type: sandy loam

	Details of nutrient medium, if used: sand 60.7%, silt 27.8%, clay 11.6%, Organic matter 1.1% pH: 7.5
Test concentrations:	Nominal: 5, 10, 20, 40, 80, 160, and 320 g a.e/ha – soybean, lettuce, tomato and carrot 40, 80, 160, 320 and 640 g a.e/ha – ryegrass, oats, onion, oilseed rape, cucumber and sugar beet Mean calculated concentrations: g test item /ha n/a
Analytical verification:	By HLS Application 13 February 2013 Highest treatment rate (I): clopyralid = 99% recovery Application 8 March 2013 Highest treatment rate (I): clopyralid = 98% recovery
Test material application:	Method: Pre-emergence application using gas pressurised Oxford Precision Sprayer with a 2m boom fitted 4 fan tip 80° standard nozzles (ISO size:01 F80) mounted on a battery powered track sprayer Application interval: 1 Reference chemical (if used): N/A
Seeds:	Source: commercial seed lots Method of seeding: by hand Prior seed treatment/sterilisation: none Number of seeds per replicate pot: 10 except cucumber with 5
Growth stage at application:	Pre-emergence
Number of control replicates:	5
Number of test concentration replicates:	5

Methodology

Seeds were sown on the day before treatment application. Treatments were applied starting with the untreated water only control. Then the test item EF-797 was measured out for the highest rate, 640 g a.e/ha, and diluted in water. This solution was diluted 1:1 in sequence to produce the lower rates. All treatment applications were made using a track sprayer calibrated to deliver 200 L water/ha (+/- 10%).

After treatment application the pots were removed to a glasshouse and laid out in randomised blocks. All pots were placed in saucers and lightly watered overhead with a hosepipe and rose. All subsequent watering was to the saucers to avoid leaching. Treatment application was made to six species on 13th February 2013 and to four species on 8th March 2013.

Seedlings were assessed for emergence, visual injury and survival at 14, 21 and 28 days after 50% emergence of the untreated water only control for each species. Foliar fresh weight was recorded at harvest (28 days after 50% emergence of the untreated water only control for each species). The ER_{25} and ER_{50} values for all ten species were calculated using foliar fresh weight data, shoot length and plant survival expressed as a % of the untreated control and capped at 100%. Dunnett's Test to determine NOEL levels were carried out for comparison with the untreated water only control.

Results and discussion

The results are presented in the following tables 9.6.1 – 9.6.3.

Table 9.6.1. Observations of plant mortality: % Emergence, % survival, shoot length (mm), shoot fresh weight (g): Monocotyledonous species

Treatment: EF-797 g a.e/ha	Lolium perenne (Ryegrass)				Avena sativa (Oats)				Allium cepa (Onion)			
	Emergence (%)	Survival (%)	Shoot length (mm)	Shoot weight (g)	Emergence (%)	Survival (%)	Shoot length (mm)	Shoot weight (g)	Emergence (%)	Survival (%)	Shoot length (mm)	Shoot weight (g)
Untreated	94	100	127	0.64	96	100	305	8.47	100	100	102	1.43
40	96	100	134	0.63	98	100	439	17.28	100	100	109	1.43
80	100	100	133	0.74	94	100	414	14.80	100	100	110	1.44
160	100	96	138	0.71	94	100	370	11.46	100	100	107	1.42
320	96	100	141	0.73	98	100	393	14.26	98	98	107	1.45
640	98	100	131	0.59	98	100	321	8.49	86	95.3	107	1.12

Table 9.6.2. Observations of plant mortality: % Emergence, % survival, shoot length (mm), shoot fresh weight (g): Dicotyledonous species

Treatment: EF-797 g a.e/ha	Brassica napus (Oilseed rape)				Glycine max (Soybean)				Cucumis sativa (Cucumber)			
	Emergence (%)	Survival (%)	Shoot length (mm)	Shoot weight (g)	Emergence (%)	Survival (%)	Shoot length (mm)	Shoot weight (g)	Emergence (%)	Survival (%)	Shoot length (mm)	Shoot weight (g)
Untreated	100	100	197	35.88	74	100	199	29.15	84	95.2	22	3.20
5	-	-	-	-	64	100	176	18.05	-	-	-	-
10	-	-	-	-	74	100	150	21.95	-	-	-	-
20	-	-	-	-	78	89.7	187	22.78	-	-	-	-
40	100	100	192	35.40	58	72.4	165	12.24	92	95.7	25	3.49
80	100	100	200	38.01	48	50	131	5.76	88	95.5	24	2.92
160	100	100	201	38.02	28	42.9	101	2.77	96	100	27	3.79
320	96	100	195	36.08	42	4.8	27	0.30	84	90.5	25	2.61
640	98	100	199	35.88	-	-	-	-	88	90.9	32	2.91

Treatment: EF-797 g a.e/ha	Beta vulgaris (Sugar beet)				Lactuca sativa (Lettuce)				Lycopersicon esculentum (Tomato)			
	Emergence (%)	Survival (%)	Shoot length (mm)	Shoot weight (g)	Emergence (%)	Survival (%)	Shoot length (mm)	Shoot weight (g)	Emergence (%)	Survival (%)	Shoot length (mm)	Shoot weight (g)
Untreated	92	100	89	8.98	94	100	54	6.30	100	100	100	17.96
5	-	-	-	-	94	100	57	6.69	96	100	91	15.75
10	-	-	-	-	96	97.9	61	7.20	100	100	92	16.03
20	-	-	-	-	94	100	62	6.86	96	100	81	11.67
40	78	100	95	8.20	90	82.2	71	4.18	98	98	99	14.32
80	86	100	100	8.52	94	85.1	71	4.02	94	93.6	99	11.18
160	92	100	101	9.60	94	78.7	67	2.61	96	93.8	97	10.02
320	86	100	97	8.17	36	77.8	48	1.09	98	69.4	65	2.77
640	90	100	96	7.28	-	-	-	-	-	-	-	-

Treatment: EF-797 g a.e/ha	Daucus carota (Carrot)			
	Emergence (%)	Survival (%)	Shoot length (mm)	Shoot weight (g)
Untreated	100	100	99	3.28
5	100	100	104	3.14
10	98	100	105	2.90
20	98	100	96	2.48
40	100	100	94	2.46
80	94	100	95	2.19
160	86	97.7	85	1.48
320	92	87	58	1.00
640	-	-	-	-

Table 9.6.3. Reported NOEL, ER_{25} and ER_{50} values based % Emergence, % Survival, Shoot Length, Shoot fresh weight

Species	% Emergence			% Survival			Shoot length			Shoot weight		
	NOEL	ER_{25}	ER_{50}	NOEL	ER_{25}	ER_{50}	NOEL	ER_{25}	ER_{50}	NOEL	ER_{25}	ER_{50}
Ryegrass				>640	>640	>640	>640	>640	>640	>640	>640	>640
Oats				>640	>640	>640	>640	>640	>640	>640	>640	>640
Onion				320	>640	>640	>640	>640	>640	>640	>640	>640
Oilseed rape				>640	>640	>640	>640	>640	>640	>640	>640	>640
Soybean				40	19.43	48.80	40	55.51	162.03	20	15.04	21.47
Cucumber				>640	>640	>640	320	>640	>640	>640	>640	>640
Sugar beet				>640	>640	>640	>640	>640	>640	>640	>640	>640

Species	% Emergence			% Survival			Shoot length			Shoot weight		
Lettuce				160	111.82	238.12	>320	>320	>320	80	27.45	105.94
Tomato				160	264.47	>320	160	259.76	>320	40	32.17	160.26
Carrot				>320	>320	>320	160	209.64	>320	40	24.70	140.14

Soybean: Log-linear – plant survival, Linear – shoot length, Log-log – shoot weight.

Conclusion

Based on foliar fresh weight reduction:

The monocotyledon species were not sensitive to pre-emergence application of EF-797 with ER_{25} and ER_{50} values of >640 g a.e/ha. The most sensitive dicotyledon species to pre-emergence application of EF-797 was soybean with an ER_{25} value of 15.04 g a.e/ha and an ER_{50} value of 21.47 g a.e/ha.

Based on shoot length reduction:

The ER_{25} and ER_{50} values for all monocotyledon species were >640 g a.e/ha. The most sensitive dicotyledon species to pre-emergence application of EF-797 was soybean with an ER_{25} value of 55.51 g a.e/ha and an ER_{50} value of 162.03 g a.e/ha.

Based on survival:

The ER_{25} and ER_{50} values for all monocotyledon species was >640 g a.e/ha. The most sensitive dicotyledon species to pre-emergence application of EF-797 was soybean with an ER_{25} value of 19.43 g a.e/ha and an ER_{50} value of 48.80 g a.e/ha.

Table 9.6.4. The endpoints for soybean from the seedling emergence test with clopyralid.

Common name	Species	Test item	Time-scale	Endpoint	Toxicity value	Units of test item
Soybean	*Glycine max*	EF-797	28 days	Survival NOEL	40	g a.e/ha
Soybean	*Glycine max*	EF-797	28 days	Survival ER_{25}	19.43	g a.e/ha
Soybean	*Glycine max*	EF-797	28 days	Survival ER_{50}	48.80	g a.e/ha
Soybean	*Glycine max*	EF-797	28 days	Shoot length NOEL	40	g a.e/ha
Soybean	*Glycine max*	EF-797	28 days	Shoot length ER_{25}	55.51	g a.e/ha
Soybean	*Glycine max*	EF-797	28 days	Shoot length ER_{50}	162.03	g a.e/ha
Soybean	*Glycine max*	EF-797	28 days	Shoot weight NOEL	20	g a.e/ha
Soybean	*Glycine max*	EF-797	28 days	Shoot weight ER_{25}	15.04	g a.e/ha
Soybean	*Glycine max*	EF-797	28 days	Shoot weight ER_{50}	21.47	g a.e/ha

RMS comments and evaluation:

The study was well performed and reported, according to GLP provisions and the test guideline. The test substance studied was different from the current representative formulation of clopyralid, but as the product contained clopyralid as only active substance, the data can be used to support the AIR3

active substance evaluation. The specification of the test substance used in this study was not presented and hence the impurity profile is unknown. A few deviations from the test guideline were reported. Repeat application was necessary for four species due to poor emergence. Some natural radiation, maximum temperature, minimum temperature and humidity records were not recorded on several dates due to recording problems with the environmental computer. These deviations were considered not affecting the outcomes significantly.

Soybean appeared to be the most sensitive species for clopyralid in this seed emergence test. The study is acceptable and its outcomes are valid to be used in the risk assessment for terrestrial non-target plants.

CA 8.6.2/2 - EF797 (clopyralid potassium, 750 g a.e/kg, SG) GLP Vegetative Vigour Test Terrestrial Non Target Plants (based on OECD Guideline 227) – China 2013

Report:	8.6.2/2, Rockliff, C. 2013b
Title:	EF-797 (clopyralid potassium, 750 g a.e/kg, SG) GLP Vegetative Vigour Test Terrestrial Non Target Plants (based on OECD Guideline 227) – China 2013
Document No:	DAS Report No. 130094 Stockbridge Technology Centre Ltd, Lab Study No. STC/13/E753
Guidelines:	OECD Guideline 227 according to the July 2006 revision
GLP	Yes

Materials and methods

Test Item(s)

ISO Common name:	Not applicable
Test item (chemical/other name):	EF-797
Purity:	750 g a.e/kg clopyralid potassium
Description (physical state):	Soluble granule (SG)
Lot/batch no.:	ZL21278964
CAS no.:	Not applicable

Test System

Monocotyledonous species:	Ryegrass, oats, onion
Dicotyledonous species:	Soybean, oilseed rape, lettuce, sugar beet, carrot, cucumber, tomato
Study type:	Greenhouse study assessing Vegetative Vigour
Parameters measured:	Number of dead plants: at 21 days (harvest) Shoot fresh weight: at 21 days (harvest) Shoot length: at 21 days (harvest) Phytotoxicity rating system: 7, 14 and 21 days (harvest) Visual injury scale 0% = no visual injury, 1% - 39% = slight visual injury, 40%-69% = moderate visual injury, 70% - 99% = severe visual injury, 100 % = all plants dead
Growth conditions:	Temperature (range): actual 16.6°C to 26.6°C Photoperiod: natural day-length plus supplementary lighting with 5000 lux to extend the day-length to 16 hours Light intensity (range): >5000 lux

	Relative humidity: actual 49% to 79% Water regime and schedules: Following treatment pots placed in plastic saucers and water applied to the saucers. Plants inspected daily and watered according to crop requirements. Final watering was applied one day before harvest. Water source/type: mains tap water Pest control method /fertilisation, if used: NPK liquid feed applied if required
Growth medium:	Soil type: sandy loam Details of nutrient medium, if used: sand 60.7%, silt 27.8%, clay 11.6%, organic matter: 1.1% pH: 7.5
Test concentrations:	Nominal: 5, 10, 20, 40, 80, 160, and 320 g a.e/ha – soybean, lettuce, tomato and carrot 40, 80, 160, 320 and 640 g a.e/ha – ryegrass, oats, onion, oilseed rape, cucumber and sugar beet Mean calculated concentrations: N/A
Analytical verification:	By HLS: Highest Treatment (I) (640 g a.e/ha) clopyralid = 99% recovery
Test material application:	Method: gas pressurised Oxford Precision Sprayer with a 2 m boom fitted with 4 fan tip 80o standard nozzles (ISO size: 01F80) mounted on a battery powered track sprayer. Application interval: 1 Reference chemical (if used): N/A
Seeds:	Source: commercial seed lots Method of seeding: By hand Prior seed treatment/sterilisation: None Number of seeds per replicate pot: 5 plants (onion 10 and soybean 4) Growth stage at application: 2-4 true leaves
Number of control replicates:	5
Number of test concentration replicates:	5

Methodology

Seeds were sown on a range of dates to produce plants at the required growth stage at treatment application. The test item was diluted in water to give the highest treatment rate and then diluted 1:1 in sequence to produce the lower rates. All treatment applications were made using a track sprayer calibrated to deliver 200 L water/ha (+/- 10%) starting with the water only control and then the highest to lowest application rates. After treatment application the pots were removed to a glasshouse and laid out in randomised blocks. All pots were placed in saucers and water was applied directly into the saucers to avoid leaching. Plants were assessed for visual injury and plant death. Foliar fresh weights and shoot length were recorded at harvest (21 days after treatment application).

The ER_{25}, ER_{50} and NOEL values for each species were calculated using foliar fresh weight, shoot length and plant survival data expressed as the % of the untreated control and capped at 100 % using JMP statistical package.

Results and discussion

The results are summarised in the following tables 9.6.5 – 9.6.7.

Table 9.6.5. Observations of % survival, % visual injury and shoot fresh weight (g): Monocotyledonous species

Treatment EF-797 g a.e/ha	*Lolium perenne* (Ryegrass)				*Avena sativa* (Oats)				*Allium cepa* (Onion)			
	Survival	Visual injury	Shoot length	Fresh weight	Survival	Visual injury	Shoot length	Fresh weight	Survival	Visual injury	Shoot length	Fresh weight
Untreated	100	0	197	1.58	100	0	529	18.06	100	0	181	6.52
40	100	0	202	2.04	100	0	498	15.78	100	0	185	6.76
80	100	0	201	1.37	100	0	505	15.45	94	0	184	5.58
160	100	0	193	1.09	100	0	505	14.64	100	0	205	6.98
320	100	0	185	1.56	100	0	519	15.61	100	7	194	6.20
640	100	0	192	1.36	100	0	546	17.05	96	20	196	5.46

Table 9.6.6. Observations of % survival, % visual injury and shoot fresh weight (g): Dicotyledonous species

Treatment EF-797 g a.e/ha	*Brassica napus* (Oilseed rape)				*Glycine max* (Soybean)				*Cucumis sativa* (Cucumber)			
	Survival	Visual injury	Shoot length	Fresh weight	Survival	Visual injury	Shoot length	Fresh weight	Survival	Visual injury	Shoot length	Fresh weight
Untreated	100	0	153	31.07	100	0	168	20.24	100	0	97	25.11
5	-	-	-	-	100	3.4	170	17.45	-	-	-	-
10	-	-	-	-	100	15	154	16.35	-	-	-	-
20	-	-	-	-	100	28	146	15.74	-	-	-	-
40	100	0	136	26.02	100	49	108	14.27	86.7	7	109	26.25
80	100	0	152	29.96	100	75	89	9.90	100	17	106	23.61
160	100	0	151	30.59	95	81	78	7.97	93.3	29	94	19.05
320	100	0	147	28.37	35	96	54	1.98	100	34	116	23.21
640	100	0	154	27.93	-	-	-	-	93.3	41	116	21.82

Treatment EF-797 g a.e/ha	*Beta vulgaris* (Sugar beet)				*Lactuca sativa* (Lettuce)				*Lycopersicon esculentum* (Tomato)			
	Survival	Visual injury	Shoot length	Fresh weight	Survival	Visual injury	Shoot length	Fresh weight	Survival	Visual injury	Shoot length	Fresh weight
Untreated	100	0	119	23.75	100	0	107	56.13	100	0	154	25.20
5	-	-	-	-	100	0	113	57.09	100	3.8	187	24.98
10	-	-	-	-	100	0	112	49.88	100	21	170	20.26
20	-	-	-	-	100	13	108	45.08	100	42	153	20.56
40	100	0	124	21.62	96	43	106	26.53	92	72	123	14.61
80	100	0	136	25.53	20	86	40	5.91	40	92.6	54	4.70
160	100	1.2	133	21.74	4	99	16	0.26	12	98.4	27	0.41
320	100	5.8	136	23.50	0	100	0	0	56	97.2	75	2.19
640	100	15	137	24.27	-	-	-	-	-	-	-	-

Treatment EF-797 g a.e/ha	*Daucus carota* (Carrot)			
	Survival	Visual injury	Shoot length	Fresh weight
Untreated	100	0	97	2.56
5	100	1.4	122	2.60
10	100	3.6	135	2.94
20	100	11	119	2.59
40	100	25	159	4.33
80	96	42	130	2.82
160	96	60	108	1.97
320	68	86	98	0.91
640	-	-	-	-

Table 9.6.7. Reported NOEL, ER_{25} and ER_{50} values for survival, shoot length and shoot fresh g a.e/ha

Species	Shoot length			Shoot weight		
	NOEL	ER_{25}	ER_{50}	NOEL	ER_{25}	ER_{50}
Ryegrass	>640	>640	>640	>640	>640	>640
Oats	>640	>640	>640	>640	>640	>640
Onion	>640	>640	>640	>640	>640	>640
Oilseed rape	>640	>640	>640	>640	>640	>640
Soybean	20	22.47	131.61	20	24.96	131.30
Cucumber	>640	>640	>640	>640	>640	>640
Sugar beet	>640	>640	>640	>640	>640	>640
Lettuce	40	40.84	61.66	20	13.45	33.78
Tomato	40	24.46	103.67	20	13.14	37.61
Carrot	>320	>320	>320	160	127.81	272.33

Soybean: Log-linear – shoot length, Lettuce: Log-linear - shoot weight

Conclusion

Based on Foliar fresh weight reduction:

1. The monocotyledon species were not sensitive to post-emergence application of EF-797 with ER_{25} and ER_{50} values >640 g a.e/ha.

2. The most sensitive dicotyledon species to post-emergence application of EF-797 was lettuce with an ER_{25} value of 13.45 g a.e/ha and an ER_{50} value of 33.78 g a.e/ha and tomato with an ER_{25} value of 13.14 g a.e/ha and an ER_{50} value of 37.61 g a.e/ha.

Based on Shoot length reduction:

1. The monocotyledon species were not sensitive to post-emergence application of EF-797 with ER_{25} and ER_{50} values >640 g a.e/ha.

2. The most sensitive dicotyledon species to post-emergence application of EF-797 was soybean with an ER_{25} value of 22.47 g a.e/ha and an ER_{50} value of 131.61 g a.e/ha and lettuce with an ER_{25} value of 40.84 g a.e/ha and an ER_{50} value of 61.66 g a.e/ha.

The resulting endpoint values are summarized in Table 9.6.8. below.

Table 9.6.8. Summary of the endpoints from the vegetative vigour test with clopyralid.

Common name	Species	Test item	Time-scale	Endpoint	Toxicity value	Units of test item
Soybean	*Glycine max*	EF-797	21 days	% shoot length NOEL	20	g a.e/ha
Soybean	*Glycine max*	EF-797	21 days	% shoot length ER_{25}	22.47	g a.e/ha
Lettuce	*Lactuca sativa*	EF-797	21 days	% shoot length ER_{50}	61.66	g a.e/ha
Tomato	*Lycopersicon esculentum*	EF-797	21 days	% shoot weight NOEL	20	g a.e/ha
Tomato	*Lycopersicon esculentum*	EF-797	21 days	% shoot weight ER_{25}	13.14	g a.e/ha
Lettuce	*Lactuca sativa*	EF-797	21 days	% shoot weight ER_{50}	33.78	g a.e/ha

RMS comments and evaluation:

The study was well performed and reported, according to GLP provisions and the test guideline. The test substance studied was different from the current representative formulation of clopyralid, but as the product contained clopyralid as only active substance, the data can be used to support the AIR3 active substance evaluation. The specification of the test substance used in this study was not presented and hence the impurity profile is unknown. A slight deviation from the study plan was to cultivate four soybean plants instead of five as stated in the Study Plan. This deviation was considered not affecting the outcomes significantly.

In this vegetative vigour test soybean appeared to be the most sensitive species for clopyralid as measured by sooth length reduction, and tomato as measured by sooth weight reduction. The study is acceptable and its outcomes are valid to be used in the risk assessment for terrestrial non-target plants. The data requirement is fulfilled and no further studies are required on this issue.

Study 1 originally evaluated in the DAR (2003):

Non-target plants, – J99

Report: Paterson, E. (2001): Evaluation of the phytotoxicity of Clopyralid (Based on OECD guideline 208 vegetative vigour test terrestrial non target plants, Dow AgroSciences unpublished report No. GHE-P-9045, Study ID EA00A2A024, 25 January 2001. Ref J99

Guidelines: OECD guideline 208 vegetative vigour test terrestrial non target plants.

GLP: Yes (certified laboratory).

Methodology:

The objective of this study was to determine the effects of Lontrel 100 (EF-1136) on non target terrestrial plants. EC_{25} and EC_{50} values (based on g as/ha) were determined and calculated using foliar fresh weight data for Lontrel 100 on a range of monocotyledon and dicotyledon species. Lontrel 100 was formulated as EF-1136, a SL containing 100 g as/l of clopyralid. Applications were made post-emergence, in a total volume of 200 l/ha. Applications ranged from 0.937 to 120 g as/ha.

A total of 3 monocotyledon and 3 dicotyledon species from six different families were tested. The species evaluated represented members of the Gramineae, Liliaceae, Cyperaceae, Brassicaceae, Leguminosae and Chenopodiaceae families. The growth stage at application ranged from BBCH 12 - 14 depending on the species. The plant species were raised and the study was conducted under glasshouse conditions. Visual injury was assessed on a 0-100 linear scale at 7 day intervals post application. At the final assessment timing (21 days) visual injury, number of dead plants per pot and foliar fresh weight were recorded. EC_{25} and EC_{50} values were calculated using Minitab Statistical package 12.2.

Findings:

No visual injury was observed on *Avena sativa, Allium cepa, Cyperus esculentus, Brassica napus* and *Beta vulgaris*, 21 days after application of Lontrel 100. The higher tolerance of these species to Lontrel 100 meant that it was not possible to carry out regression analysis and predict EC_{25} and EC_{50} values, for foliar fresh weight reduction. EC_{25} and EC_{50} values were therefore estimated to be >120 g as/ha. The EC_{25} and EC_{50} values (g as/ha) for *Glycine max* foliar fresh weight were 7.4 and 25.4 respectively. The R-Sq value for the non-linear model used was 0.96.

Comments

The study was well performed and reported and in compliance with GLP. The study is acceptable.

RMS comments and evaluation:

The study was originally evaluated in the DAR (2003), as presented above. Because the tested formulation is no longer representative for AIR3 evaluation of clopyralid, the study has not been re-evaluated here again. The data can be used as supporting additional data.

The data requirement is fulfilled and no further studies are required on this issue.

In addition to the Notifier-owned studies, one study published in the scientific literature was submitted to support the AIR3 evaluation of clopyralid. The study is summarised in Table 9.6.9. below.

Table 9.6.9. Effects of clopyralid on the seed production of pea (*Pisum sativum*).

Pea (*Pisum sativum*) seed production as an assay for reproductive effects due to herbicides	
KCA 9/6 (CA 8.6)	
Author(s)	Olszyk, D., Pfleeger, T., Lee, E.H., Plocher, M.
Year	2009
Journal	Environ Toxicol Chem (2009) Vol. 28(9), pp. 1920-9
Relevance check	Relevant. Study follows a standard test guideline with slight adaptations. Methodology is comprehensively described
Reliability check	Reliability score 1 (Klimisch)
Reasons for no reliability	Not applicable
Summary	Garden pea (*Pisum sativum* L. 'Dakota') were treated with the herbicide Stinger Dow AgroSciences (containing clopyralid) at a vegetative stage of growth (14 days after emergence) or at flowering (20 days after emerging). The application rates were 0.001, 0.002, 0.01 and 0.1 x field application rate (FAR; 210 g clopyralid/ha). After 14 days of exposure, plant height and dry weight were measured and 35 days after emerging pea seeds were harvested and pea seed dry

	weight was measured. Clopyralid exposure did not affect plant height and dry weight. Pea seed dry weight showed a reduction with clopyralid exposure rate. Plant developmental stage (14 or 20 days after emerging) had no effect on the observed effects of clopyralid. The NOEC was > 0.01 x FAR and the $EC25_{seed\ dry\ weight}$ was 0.07 x FAR.
Reliability check: study details	
Parameter	**Information available**
Test protocol GLP, GEP, Guidelines (US EPA, OECD, …)	- US EPA (1996) OPPTS 850.4150, adapted for single plants in pots grown for a longer period of time to produce seed - GLP/GEP not discussed
Test substance Identification of test substance, source, purity, stability	- Test substance: Stinger Dow AgroSciences, containing clopyralid - Other herbicides tested: Banvel Micro Flo (containing dicamba), Beacon Syngenta Crop Protection (with primisulfuron), Oust E.I. du Pont de Nemours and Company (containing sulfometuron), Rely Bayer CropScience LP (with Glufosinate), Phomene Nufarm (containing MCPA) and Roundup Original Monsanto Company (with Glyphosate) - Chemical analysis was performed, which verified that the herbicide solutions were within the expected range
Test conditions Temperature, pH, oxygen concentration, water hardness, conductivity, photoperiod, light intensity, number of animals, food availability	- Temperature: 18.4 – 26.7 ºC - Relative humidity: 57 – 72% - Photosynthetically active radiation: 126 – 258 µmol m^{-2} s^{-1} - CO_2: 417 – 419 ppm - Test system: Substrate was a pasteurized sandy loam mineral soil with 3% organic matter (coarse-loamy, mixed, superactive, mesic Fluventic Haploxerolls) - Fertilizer: Scotts slow-release Osmocote, containing N, P and K, with 10 g mixed in the 1.6 kg (dry weight) of soil per pot at potting - Watering: Reverse osmosis water was added to saucers under the pots whenever the top of the soil was dry to a depth of 1 to 2 cm
Controls Positive control, negative control	- Negative control: a no-spray and a zero herbicide concentration
Dosing system Exposure (dose, duration, frequency)	- Dosing: a logarithmic sequence of increasing rates of 0.001, 0.002, 0.01 and 0.1 times the field application rate (FAR; of 210 g a.i./ha for clopyralid) - Duration: Plant height and leaf injury were evaluated at day 14 of exposure period, pea seeds were harvested at day 35 - Frequency: 1 - Replicates: 6 pots, with one plant per pot
Test species Body weight or length, gender, age/life stage, source	- Species: Garden pea (*Pisum sativum* L. 'Dakota') - Source: the Territorial Seed Company (PE631) - Life stage at test initiation: Just before initiation of flowering (14 to 15 days after emergence (DAE)) or at opening of the first flowers (18 to 21 DAE)
Statistical analyses Sample size/replicates, statistical analysis of data (significance level, variability)	- Statistical differences between treatments were analysed with three-way analysis of variance (ANOVA), using SAS/STAT software (SAS institute) - NOEC was calculated with Dunnett's test - Post-ANOVA testing on 25% reduction in plant response was calculated using Weibull model - Nonlinear regression calculations were performed with the nonlinear regression procedure (PROC NLIN) - All analysis were performed using SAS/STAT software (SAS institute), version 8 for Unix and in version 9.1 on a XP platform for PC
Biological effects Determined effect concentration, dose response observed	- No differences in sensitivity between plants treated at a vegetative state compared with a flowering state of development - Clopyralid had essentially no effect on stem height or dry weight, but some leaf injury and significant reductions in pea seed dry weight were observed

	- NOEC > 0.1 x FAR and EC_{25} for seed dry weight was 0.07 x FAR for clopyralid exposure
Overall assessment	- Methods, statistics, results and discussion are well described - Study is reliable

RMS comments and evaluation:

The study was well performed and reported, according to US EPA test guideline, although not fulfilling the GLP requirements. The clopyralid formulation tested was different from the representative formulation for AIR3 evaluation of clopyralid, but originating from the same company. The specification of the test substance used in this study was not presented and hence the impurity profile is unknown. The performance and the results were clearly reported. The EC_{25} for seed dry weight of 14.7 g clopyralid/ha obtained in this study corresponds to 12 - 18 % of the intended use rates for clopyralid resulting from the uses of the product GF-1374 on pasture and cereals. This is in the order of the results from the previously presented seedling emergence and vegetative vigour studies with other plant species, owned by the Notifier.

Given that the research article has undergone a peer review before publication, the study can be considered as acceptable and valid, and therefore the data can be used as supporting data for the AIR3 evaluation of clopyralid. Data requirement on the effects to terrestrial non-target plants is fulfilled and no further data are required.

B.9.7. EFFECTS ON OTHER TERRESTRIAL ORGANISMS (FLORA AND FAUNA)

No additional data on the effects of clopyralid on other terrestrial organisms has been generated due to the lack of effects on terrestrial organisms (as demonstrated in this dossier). Due to this reason, no further information is provided.

RMS comments and evaluation:

The justification provided by the Notifier is acceptable and agreed, and no further data on this issue are required.

B.9.8. EFFECTS ON BIOLOGICAL METHODS FOR SEWAGE TREATMENT

Data to address this point were presented in the dossier submitted in 30 April 2002 for the Active Approval and were deemed acceptable following evaluation and peer review at EU level. These data are still valid for decision making. As the study was originally presented and evaluated in the DAR (2003), the details are summarised only briefly here again.

CA 8.8/1- The Effects of Clopyralid Technical On Activated Sewage Sludge Respiration

Report	[IIA 8.7/01], Mallett, M. J. , 2000
Report title	The Effects of Clopyralid Technical On Activated Sewage Sludge Respiration
DAS Study number	CEMR-1322, Ref. J57 CEM Analytical Services Ltd, North Ascot, Berkshire, UK
Guidelines	OECD 209 (1984)
GLP	Yes

Methodology:

Clopyralid technical, Batch No. RMM 2373, Purity 95.8% w/w. The effect of clopyralid on the rate of respiration of activated sewage sludge was assessed after an incubation period of three hours.

In a range-finding test, three concentrations of clopyralid were prepared at 1, 10 and 100 mg/L. One vessel containing dilution water, synthetic sewage sludge and activated sewage but no clopyralid served as the control treatment. Three further vessels with 1, 10 and 100 mg clopyralid/L without activated sewage sludge were prepared to determine the extent of abiotic oxygen consumption. The activated sewage sludge was collected from Thames Water, Ascot Works, UK and maintained at 20 ± 2°C, under aeration with synthetic sewage sludge added. The test vessels were 1 L glass beakers filled with 500 mL of test medium and covered with polyethylene film. The synthetic sewage sludge was prepared in accordance with the OECD 209 guideline.

For the definitive (limit) test, there were three replicate vessels prepared with 100 g clopyralid/L, three replicate control vessels and one vessel each containing 5, 15 or 30 mg 3,5-DCP/L as a reference substance. The test vessels were maintained at 20 ± 2°C throughout the study and the test media were vigorously aerated.

The total duration of the incubation phase was three hours. After three hours, respiration rates were measured over 10 minute periods using a polarographic oxygen electrode. The respiration rate was calculated by plotting measured dissolved oxygen concentrations against time in minutes. The rates were assessed from the linear phase of the oxygen decline curves. The oxygen consumption for each treatment was compared to the control consumption rate and the percent inhibition calculated.

Temperature and pH were measured in each vessel after removal of medium to measure respiration rate. The pH of the vessels in the definitive test ranged from 7.62 to 7.84 and temperature from 20.4 to 20.5 °C.

Findings:

The results from the preliminary test indicated that there was no difference in respiration rate between the controls and the clopyralid treatments up to and including 100 mg clopyralid/L. No oxygen consumption was recorded in the clopyralid treatments without activated sewage sludge indicating that there was no abiotic oxygen consumption associated with the presence of clopyralid.

In the definitive test, the mean control respiration rate of 32.5 mg O_2/L/h was similar to that in the 100 mg/L clopyralid treatment of 32.0 mg O_2/L/h (Table 9.9-1). The small difference was not significant at $p<0.05$. Therefore, clopyralid had no effect on the respiration rate of activated sewage sludge at 100 mg/L over a three hour exposure period. The EC_{50} value for the reference substance 3,5-DCP was 7.1 mg/L, satisfying the OECD 209 test guideline validity criterion (EC_{50} of 5 to 30 mg 3,5-DCP/L). The results are summarised in Table 9.9-2.

Table 9.8-1. Inhibition of respiration of activated sewage sludge exposed to clopyralid over a three hour incubation period

Replicate	**Respiration rate (mg O_2/L/h)**		
	Control	**Clopyralid (100 mg/L)**	**3,5-DCP (concentration in brackets)**
1	32.25	31.50	19.50 (5 mg/L)
2	32.25	32.25	9.00 (15 mg/L)
3	33.00	32.25	6.00 (30 mg/L)
Mean	32.50	32.00	-

Table 9.8-2. Summary on the results of respiration inhibition of activated sludge

Result	**Nominal concentration of clopyralid (mg/L)**
EC_{50}	> 100 mg/L

Conclusions:

Clopyralid had no adverse effect on activated sewage sludge respiration at 100 mg/L. The EC_{50} value for the inhibition of respiration of activated sewage sludge was > 100 mg clopyralid/L.

Comments

The study was well performed and reported and in compliance with GLP. The validity criteria given in the test guideline were met and the study is acceptable.

The effect of technical clopyralid (purity of 95.8% w/w) on activated sewage sludge respiration was assessed after an incubation period of 3 hours. No adverse effects on activated sewage sludge was observed at 100 mg/L resulting in the 3 h EC_{50} for the inhibition of respiration of activated sludge reported as > 100 mg/L. Therefore no risk is expected following applications of 120 g clopyralid/ha to grassland and 80 g clopyralid/ha to cereals.

RMS comments and evaluation:

From the DAR (2003): The study was well performed and reported and in compliance with GLP. The validity criteria given in the test guideline were met and the study is acceptable.

The outcome of this study is still valid and can be used in the risk assessment.

[redacted]

Data requirement on the effects on biological sewage treatment is fulfilled and no further data are required.

It is unlikely that sewage treatment plants would be significantly exposed by clopyralid following the intended field uses on plant protection products containing clopyralid, as the products are used on cereals or pasture, according to GAP.

B.9.9. MONITORING DATA

No additional ecotoxicological monitoring data for the active substance has been conducted, so further data has not been submitted for review.

RMS comments and evaluation:

The justification above provided by the Notifier is acceptable. As the data requirements according to the Commission Regulation (EU) 283/2013 are fulfilled, no further data are required on this issue.

B.9.10. BIOLOGICAL ACTIVITY OF METABOLITES POTENTIALLY OCCURRING IN GROUNDWATER

B.9.10.1. Introduction

This assessment is conducted according to SANCO 221/2000 - rev 10 - final (Guidance document on the assessment of the relevant of metabolites in groundwater, 25 February 2003).

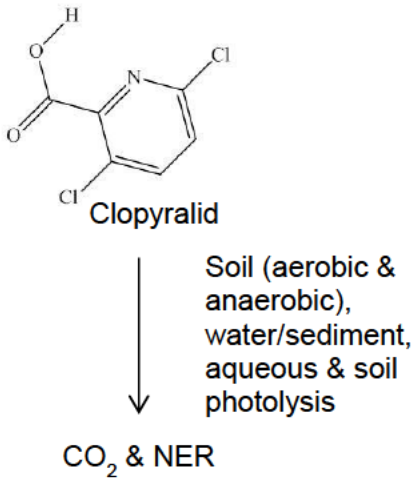
The representative formulation for active clopyralid is GF-1374, which is an emulsifiable concentrate formulation containing a nominal 144 g/L fluroxypyr-meptyl (100 g a.e./L fluroxypyr), 80 g/L clopyralid and 2.5 g/L florasulam as the active ingredients. Comparison between representative GAPs for fluroxypyr (EFSA, 2011) and florasulam (EFSA, 2015) and that for GF-1374 indicates that the relevance of metabolites in ground water for fluroxypyr and florasulam were sufficiently addressed. Therefore, no relevance assessment is presented for fluroxypyr and florasulam but a reference is made to the respective EFSA Conclusions Reports.

B.9.10.2. Fate and behaviour in the environment

B.9.10.2.1. Summary of Degradation Pathway in Soil

Clopyralid metabolism in soil was studied under various environmental conditions (dark aerobic, soil photolysis, dark anaerobic). Under neither conditions metabolites > 5% AR are formed. Clopyralid mineralizes to CO_2 under aerobic conditions whereas in anaerobic conditions very minimal degradation occurs and no transformation products are formed. CO_2 accounted for 68.21% to 74.31% at 90 days in aerobic soil study. In anaerobic soil and soil photolysis study similar behaviour was observed.

The study confirmed clopyralid mineralizes to CO_2; there was no pH correlation observed.



B.9.10.2.2. Summary of Identification of Metabolites in Soil

No metabolites were identified for clopyralid.

B.9.10.2.3. Relevance of metabolites in groundwater

Step 1: Exclusion of Degradation Products of No Concern

No metabolites are identified for clopyralid; therefore, no degradation product is of concern.

Step 2: Quantification of Potential Groundwater Contamination

No quantification required for clopyralid.

Step 3: Hazard Assessment: Identification of relevant metabolites

No further assessment required for clopyralid.

Step 3, Stage 1: Screening for biological activity

No further assessment required for clopyralid.

Step 3, Stage 2: Screening for genotoxicity

No further assessment required for clopyralid.

Step 3, Stage 3: Screening for toxicity

No further assessment required for clopyralid.

Step 4: Exposure assessment – threshold of concern approach

No further assessment required for clopyralid.

Step 5: Refined risk assessment for non-relevance of metabolites

No further assessment required for clopyralid.

Overall Conclusion

No metabolites are identified for clopyralid; therefore, it is determined that relevance of metabolite in ground water is not of concern.

RMS comments and evaluation:

The evaluation of the biological activity of metabolites in groundwater, as presented by the Notifier above, is acceptable and it is agreed that no metabolites are formed to be assessed for the ecotoxicological relevance in groundwater. The data requirement is therefore fulfilled and no further data are required.

B.9.11. REFERENCES RELIED ON

Literature search:
The results of a search for relevant data published post-submission of the Active Approval, conducted in accordance with Article 8 (5) of Regulation (EC) No. 1107/2009, was presented by the Notifier. Relevance and reliability of articles found in the search process were appraised in adherence with EFSA guidelines (EFSA Journal 2011;9(2):2092 and EFSA supporting publication 2013:EN-511). For clopyralid, its metabolites and appropriate trade names, the review of the published literature identified eight articles of relevance to the ecotoxicology part of the regulatory data package. These eight articles are summarised in this evaluation and included in this reference list below as data points KCA. The explanation given by the Notifier on how the literature search was conducted, is acceptable for the RMS evaluator. The relevant studies are evaluated under respective sections. The reliability scoring follows the system of Klimisch & al. (1997):

Klimisch, H-J., Andreae, M. & Tillmann, U. (1997) A Systematic Approach for Evaluating the Quality of Experimental Toxicological and Ecotoxicological Data. Regulatory Toxicology and Pharmacology **25** pp 1-5.

In this list of references the *studies presented in the dossier in April 2002* for the Active Approval were deemed acceptable following evaluation and peer review at EU level. These data are still valid for decision making and are listed below in *italics,* and marked for the previous evaluation in the DAR 2003. Other references listed are new tests, studies or information not previously evaluated before and required to support the active substance renewal submission. The purpose of submission is indicated in the last column.

All studies except those published in the scientific literature are owned by the Notifier Dow AgroSciences (= DAS).

Data protection claims made by the Applicant are based on the following Table.

Data owner: DAS = Dow AgroSciences.

Data Point	Author(s)	Year	Title Compagny Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner	Previous evaluation
CA 8.1.1.1/1	[redacted]	1980	Acute Oral LD50 – Mallard Duck – DOWCO 290 DAS Report No. GH-RC 164 [redacted] GLP/GEP (Y/N): No Published (Y/N): No	Yes	No	N/A	DAS	DAR 2003
CA 8.1.1.3/1	[redacted]	1985	Lontrel Herbicide: A One-Generation Reproduction Study with the Mallard (Anas platyrhynchos) - Final Report. DAS Report No. 103-235 [redacted] GLP/GEP (Y/N): Yes Published (Y/N): No	Yes	No	N/A	DAS	DAR 2003
CA 8.2.1/1	[redacted]	2000	Clopyralid: An Acute Toxicity Study with the Rainbow Trout, Oncorhynchus mykiss Walbaum DAS Report No. 001024 [redacted] GLP/GEP (Y/N): Yes Published (Y/N): No	Yes	No	N/A	DAS	DAR 2003
CA 8.2.2.1/1	[redacted]	2000	Clopyralid: Toxicity to the Early Life Stages of the Fathead Minnow, Pimephales Promelas Rafinesque. DAS Report No. 001017	Yes	No	N/A	DAS	DAR 2003

Data Point	Author(s)	Year	Title Compagny Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner	Previous evaluation
	[redacted]		[redacted] GLP/GEP (Y/N): Yes Published (Y/N): No					
KCA 9/3 (CA 8.2.1)	Fairchild, J.F., Allert, A.L., Sapping-ton, L.S., Nelson, K.J., Valle, J.A.	2008	Using accelerated life testing procedures to compare the relative sensitivity of rainbow trout and the federally listed threatened bull trout to three commonly used rangeland herbicides (picloram, 2,4-D and clopyralid). Environ. Toxicol. Chem. Vol. 27(3): 623-630. GLP/GEP (Y/N): No Published (Y/N): Yes	Yes	No	N/A	-	Submitted for the purpose of renewal
KCA 9/4 (CA 8.2.2)	Fairchild, J.F., Allert, A.L., Feltz, K.P., Nelson, K.J., Valle, J.A.	2009	An ecological risk assessment of the acute and chronic effects of the herbicide clopyralid to rainbow trout (Oncorhynchus mykiss). Arch. Environ. Contam. Toxicol. Vol. 57: 725-731. GLP/GEP (Y/N): No Published (Y/N): Yes	Yes	No	N/A	-	Submitted for the purpose of renewal
KCA 9/7 (CA 8.2.2.1)	Padilla, S., Corum, D., Padnos, B., Hunter, D.L., Beam, A.,	2012	Zebrafish developmental screening of the ToxCast™ Phase I chemical library. Reprod. Toxicol. Vol. 33(2): 174-187. GLP/GEP (Y/N): No	Yes	No	N/A	-	Submitted for the purpose of renewal

Data Point	Author(s)	Year	Title Compagny Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner	Previous evaluation
	Houck, K.A., Sipes, N., Klein-streuer, N., Knudsen, T., Dix, D.J., Reif, D.M.		Published (Y/N): Yes					
KCA 9/8 (CA 8.2.2.1)	Stehr, C.M., Linbo, T.L., Baldwin, D.H., Scholz, N.L., Incardona, J.P.	2009	Evaluating the effects of forestry herbicides on fish development using rapid phenotypic screens. North American Journal of Fisheries Management Vol. 29(4): 975-984. GLP/GEP (Y/N): No Published (Y/N): Yes	Yes	No	N/A	-	Submitted for the purpose of renewal
CA 8.2.2.3/1	[redacted]	1982	Determination of the Bioconcentration Factor for 3,6-Dichloropicolinic Acid in Bluegill Sunfish During Continuous Aqueous Exposure. DAS Report No. GH-C 1577 [redacted] GLP/GEP (Y/N): No Published (Y/N): No	Yes	No	N/A	DAS	DAR 2003
CA 8.2.4.1/1	Marino, T. A. ; McClymon	2000	Clopyralid: An Acute Toxicity Study with the Daphnia, Daphnia magna Straus DAS Report No. 001025	No	No	N/A	DAS	DAR 2003

Data Point	Author(s)	Year	Title Compagny Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner	Previous evaluation
	t, E. L. ; Staley, J. L.		Dow AgroSciences LLC, Midland, Michigan, United States GLP/GEP (Y/N): Yes Published (Y/N): No					
CA 8.2.5.1/1	Douglas, M. T. ; Bell, G. ; Macdonald , I. A.	1992	An Assessment of the Effects of Lontrel T on the Reproduction of Daphnia magna DAS Report No. DWC 615/911087 Huntingdon Research Center Ltd, Huntingdon, Cambridgeshire, United Kingdom GLP/GEP (Y/N): Yes Published (Y/N): No	No	No	N/A	DAS	DAR 2003
CA 8.2.5.3/1	Barrett, K.	2001	Clopyralid Technical Toxicity to the Sediment Dwelling Phase of the Midge Chironomus riparius DAS Report No. GHE-T-1122 Huntingdon Life Sciences Ltd, Huntingdon, Cambridgeshire, United Kingdom GLP/GEP (Y/N): Yes Published (Y/N): No	No	No	N/A	DAS	DAR 2003
CA 8.2.6.1/1	Kirk, H. D. ; Gilles, M. M. ; McClymon t, E. L. ; McFadden, L. G.	2000	Clopyralid: Growth Inhibition Test with the Freshwater Green Alga, Selenastrum capricornutum Printz DAS Report No. 001040 Dow AgroSciences LLC, Midland, Michigan, United States GLP/GEP (Y/N): Yes Published (Y/N): No	No	No	N/A	DAS	DAR 2003

Data Point	Author(s)	Year	Title Compagny Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner	Previous evaluation
CA 8.2.6.2/1	Hoberg, J. R.	2006	Clopyralid Technical Grade - Growth Inhibition Test with Freshwater Blue Green Alga (Anabaena flos-aquae) DAS Report No. 060246 Springborn Smithers Laboratories 790 Main Street Wareham, Massachusetts, United States 02571 GLP/GEP (Y/N): Yes Published (Y/N): No	No	Yes	Active substance data submitted with an application under Article 15 of the Regulation (renewal) and with the application for renewal of authorisation of the corresponding product. Applies also to data submitted with applications for authorisation for products containing AIR2 substances (Regulation (EU) No 1141/2010).	DAS	Submitted for the purpose of renewal
CA 8.2.6.2/2	Aufder-heide, J.	2015	Clopyralid Technical: Growth Inhibition Test with the Freshwater Diatom, Navicula pelliculosa DAS Report No. 140515 ABC Laboratories, Inc. 7200 E. ABC Lane Columbia, Missouri 65202 USA GLP/GEP (Y/N): Yes Published (Y/N): No	No	Yes	Active substance data submitted with an application under Article 15 of the Regulation (renewal) and with the application for renewal of	DAS	Submitted for the purpose of renewal

Data Point	Author(s)	Year	Title Compagny Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner	Previous evaluation
						authorisation of the corresponding product. Applies also to data submitted with applications for authorisation for products containing AIR2 substances (Regulation (EU) No 1141/2010).		
KCA 9/1 (CA 8.2.6.1/4)	Chalifour, A., Spear, P.A., Boily, M.H., DeBlois, C., Giroux, C., Dassylva, N., Juneau, P.	2009	Assessment of toxic effects of pesticide extracts on different green algal species by using chlorophyll a fluorescence. Toxicological & Environmental Chemistry Vol 91(7): 1315-1329	No	No	N/A	-	Submitted for the purpose of renewal
CA 8.2.7/1	Cowgill, U. M. ; Milazzo, D. P. ; Potter, R. B.	1990	The Fourteen Day Toxicity of Lontrel T to Lemna gibba L G-3 (Duckweed) - ES-DR-0197-3428-4 DAS Report No. ES-2243 Dow AgroSciences LLC, Midland, Michigan, United States GLP/GEP (Y/N): Yes Published (Y/N): No	No	No	N/A	DAS	DAR 2003

Data Point	Author(s)	Year	Title Compagny Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner	Previous evaluation
CA 8.2.7/2	Banman, C. S., Moore, S.	2015	Clopyralid: Toxicity to the Aquatic Macrophyte, Myriophyllum spicatum DAS Report No. 140735 SynTech Research Laboratory Services LLC 17745 South Metcalf Avenue Stilwell, Kansas 66085-9104 GLP/GEP (Y/N): Yes Published (Y/N): No	No	Yes	Active substance data submitted with an application under Article 15 of the Regulation (renewal) and with the application for renewal of authorisation of the corresponding product. Applies also to data submitted with applications for authorisation for products containing AIR2 substances (Regulation (EU) No 1141/2010).	DAS	Submitted for the purpose of renewal
CA 8.3.1.1.1/1	Wain-wright, M.	2001	Clopyralid Technical Acute Toxicity To Honey Bees DAS Report No. GHE T-1091 Huntingdon Life Sciences Ltd, Huntingdon, Cambridgeshire, United Kingdom GLP/GEP (Y/N): Yes Published (Y/N): No	No	No	N/A	DAS	DAR 2003

Data Point	Author(s)	Year	Title Compagny Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner	Previous evaluation
CA 8.3.1.1.2/1	Wain-wright, M.	2001	Clopyralid Technical Acute Toxicity To Honey Bees DAS Report No. GHE T-1091 Huntingdon Life Sciences Ltd, Huntingdon, Cambridgeshire, United Kingdom GLP/GEP (Y/N): Yes Published (Y/N): No	No	No	N/A	DAS	DAR 2003
CA 8.3.2.1/1	Sankanu, A.	2000a	A Laboratory Study To Evaluate The Effects of Clopyralid (EF 1136, An SL Formulation Containing 100 G/L Clopyralid) on the Parasitic Wasp Aphidius rhopalosiphi (Hymenoptera: Braconidae) DAS Report No. GHE-P-8725 Ecotox Ltd, Tavistock, Devon, United Kingdom GLP/GEP (Y/N): Yes Published (Y/N): No	No	No	N/A	DAS	DAR 2003
CA 8.3.2.1/2	Halsall, N.	2005	A laboratory rate response test to determine the effects of EF-1136 on the parasitic wasp, Aphidius rhopalosiphi DAS Report No. 050171 Insect Investigations Services Wentloog Cardiff UK GLP/GEP (Y/N): Yes Published (Y/N): No	No	Yes	Active substance data submitted with an application under Article 15 of the Regulation (renewal) and with the application for renewal of authorisation of the corresponding product. Applies also to data	DAS	Submitted for the purpose of renewal

Data Point	Author(s)	Year	Title Compagny Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner	Previous evaluation
						submitted with applications for authorisation for products containing AIR2 substances (Regulation (EU) No 1141/2010).		
CA 8.3.2.1/3	Riches, M.N.	2004	Lontrel 100: Effects on the parasitic wasp, Aphidius rhopalosiphi under extended laboratory conditions DAS Report No. GHE-P-10713 CEM Analytical Services Ltd Berkshire UK GLP/GEP (Y/N): Yes Published (Y/N): No	No	Yes	Active substance data submitted with an application under Article 15 of the Regulation (renewal) and with the application for renewal of authorisation of the corresponding product. Applies also to data submitted with applications for authorisation for products containing AIR2 substances (Regulation (EU) No 1141/2010).	DAS	Submitted for the purpose of renewal

Data Point	Author(s)	Year	Title Compagny Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner	Previous evaluation
CA 8.3.2.2/1	Sankanu, A.	2000b	A Laboratory Study To Evaluate The Effects of Clopyralid (EF-1136, An SL Formulation Containing 100 G-L Clopyralid) on Typhlodromus pyri (Acari: Phytoseiidae) DAS Report No. GHE-P-8416 Ecotox Ltd, Tavistock, Devon, United Kingdom GLP/GEP (Y/N): Yes Published (Y/N): No	No	No	N/A	DAS	DAR 2003
CA 8.3.2.3/1	Miles, M. J.	2002a	A Laboratory Study To Evaluate The Side Effects of EF-1136 (100 G/L Clopyralid Sl) on the, Lacewing Chrysoperla carnea DAS report No. GHE-P-9505 EC Directive 91/414/EEC; EC Directive 96/12/EC GLP/GEP: Yes Published (Y/N): No	No	No	N/A	DAS	DAR 2003
CA 8.3.2.3/2	Miles, M. J.	2002b	A Laboratory Study To Evaluate The Side Effects of EF-1136 (100 G/L Clopyralid Sl) on the Carabid Beetle Poecilus Cupreus DAS report No. GHE-P-7173 Directive 96/12/EC GLP/GEP: Yes Published (Y/N): No	No	No	N/A	DAS	DAR 2003
CA 8.3.2.3/3	Rombke, J	1991	The Study of the acute toxicity for Poecilus cupreus (carabidae) of Lontrel 100 (EF-255) according to the BBA Guideline for testing of chemicals developed by Dr. U. Heimback DAS report No. BE-E084-90-03-CAK6 BBA GLP/GEP: Yes	No	No	N/A	DAS	DAR 2003

Data Point	Author(s)	Year	Title Compagny Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner	Previous evaluation
			Published (Y/N): No					
CA 8.3.2.3/4	Rombke, J., Vickus, P.	1991	The Study of the acute toxicity for Aleochara bilineata (Staphylinidae) of Lontrel 100 (EF-255) according to IOBC/WPRS Guideline for Testing Chemical Developed by Dr. L. Samsoe-Petersen DAS report No. GHE-P-2489 IOBC/WPRS GLP/GEP: Yes Published (Y/N): No	No	No	N/A	DAS	DAR 2003
CA 8.3.2.3/5	Heimann, D.A., Vickus, P.	1991	A Study of the Acute Toxicity For Pardosa Sp. (Araneae) of Lontrel 100 DAS report No. GHE-P-3126 GLP/GEP: Yes Published (Y/N): No	No	No	N/A	DAS	DAR 2003
KCA 9/2 (CA 8.3.2.2)	Czar-necka, M., Parma, P., Kulec-Poszczyca, E.	2014	Total effects of selected plant protection products applied to different natural substrates on the predatory mite Typhlodromus pyri Sch. IOBC/WPRS Bulletin Vol. 103: 51-60. GLP/GEP: No Published (Y/N): Yes	No	No	N/A	-	Submitted for the purpose of renewal
[CA 8.4.1/1	Hayward, J. C.	2001	The acute toxicity of clopyralid to the Earthworm Eisenia fetida DAS Report No. GHE-T-1130 CEMAS Study CEMR-1635 GLP/GEP (Y/N): Yes	No	No	N/A	DAS	DAR 2003]

Data Point	Author(s)	Year	Title Compagny Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner	Previous evaluation
			Published (Y/N): No					
CA 8.4.1/1	Hayward, J. C.	2001	The Effects of EF-1136 on Reproduction and Growth in the Earthworm Eisenia fetida DAS Report No. CEMS-1637 CEM Analytical Services Ltd, North Ascot, Berkshire, United Kingdom GLP/GEP (Y/N): Yes Published (Y/N): No	No	No	N/A	DAS	DAR 2003
CA 8.5/1	Schö-binger, U.	2013	Clopyralid: Effects on the Activity of the Soil Microflora under Laboratory Conditions (Nitrogen and Carbon Transformation) DAS Report No. 130283 Eurofins Agroscience Services EcoChem GmbH Eutinger Str. 24 D-75223 Niefern-Öschelbronn Germany GLP/GEP (Y/N): Yes Published (Y/N): No	No	Yes	Active substance data submitted with an application under Article 15 of the Regulation (renewal) and with the application for renewal of authorisation of the corresponding product. Applies also to data submitted with applications for authorisation for products containing AIR2 substances (Regulation (EU) No 1141/2010).	DAS	Submitted for the purpose of renewal

Data Point	Author(s)	Year	Title Compagny Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner	Previous evaluation
[CA 8.5.1	Hayward & Morgan	2003	Soil respiration + N transformation GLP/GEP (Y/N): Yes Published (Y/N): No	No	No	N/A	DAS	DAR 2003]
KCA 9/5 (CA 8.5)	Kucharski, J., Bacmaga, M., Wysz-kowska, J.	2009	Effect of soil contamination with herbicides on the nitrification process. Ecological Chemistry and Engineering A Vol. 16(8): 947-952. GLP/GEP (Y/N): No Published (Y/N): Yes	No	No	N/A	-	Submitted for the purpose of renewal
CA 8.6.2/1	Rockliff, C.	2013	EF-797 (clopyralid potassium, 750 g a.e/kg, SG) GLP Seedling Emergence and Seedling Growth Test Terrestrial Non Target Plants (based on OECD Guideline 208) – China, 2013 DAS Report No. 130095 Stockbridge Technology Centre Ltd., Cawood, Selby, North Yorkshire, YO8 3TZ. UK. GLP/GEP (Y/N): Yes Published (Y/N): No	No	Yes	Active substance data submitted with an application under Article 15 of the Regulation (renewal) and with the application for renewal of authorisation of the corresponding product. Applies also to data submitted with applications for authorisation for products containing AIR2	DAS	Submitted for the purpose of renewal

Data Point	Author(s)	Year	Title Compagny Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner	Previous evaluation
						substances (Regulation (EU) No 1141/2010).		
CA 8.6.2/2	Rockliff, C.	2013	EF-797 (clopyralid potassium, 750 g a.e/kg, SG) GLP Vegetative Vigour Test Terrestrial Non Target Plants (based on OECD Guideline 227) – China 2013 DAS Report No. 130094 Stockbridge Technology Centre Ltd, Cawood, Selby, North Yorkshire, UK. GLP/GEP (Y/N): Yes Published (Y/N): No	No	Yes	Active substance data submitted with an application under Article 15 of the Regulation (renewal) and with the application for renewal of authorisation of the corresponding product. Applies also to data submitted with applications for authorisation for products containing AIR2 substances (Regulation (EU) No 1141/2010).	DAS	Submitted for the purpose of renewal
KCA 9/6 (CA 8.6)	Olszyk, D., Pfleeger, T., Lee, E.H., Plocher, M.	2009	Pea (*Pisum sativum*) seed production as an assay for reproductive effects due to herbicides. Environ Toxicol Chem (2009) Vol. 28(9), pp. 1920-9	No	No	N/A	-	Submitted for the purpose of renewal

Data Point	Author(s)	Year	Title Compagny Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner	Previous evaluation
CA 8.8/1	Mallett, M. J.	2000	The Effects of Clopyralid Technical on Activated Sludge Respiration. DAS Report No CEMS-1322 CEM Analytical Services Ltd, North Ascot, Berkshire, UK GLP/GEP (Y/N): Yes Published (Y/N): No	No	No	N/A	DAS	DAR 2003