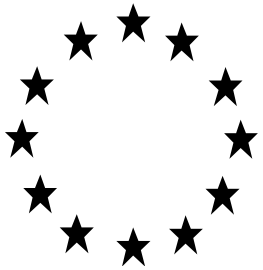
Draft Renewal Assessment Report
under Regulation (EC) 1107/2009



CLOPYRALID

Volume 3 – B.6 (AS)

RMS: Finland
Co-RMS: Poland

May 2017

Volume 1

Level 1: Statement of subject matter and purpose for which this report has been prepared and background information on the application

Level 2: Summary of active substance hazard and of product risk assessment

Level 3: Proposed decision with respect to the application
Appendix 1: Guidance documents used in this assessment
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Annex A: List of the tests, studies and information submitted

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Annex B.2 (AS): Physical and chemical properties of the active substance
Annex B.3 (AS): Data on application
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Annex C: Confidential information and, where relevant, details of any task force formed for the purpose of generating tests and studies submitted

List of Endpoints

Version History

When	What
2017/ May	DRAR- First version submitted to EFSA

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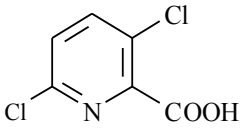
B.6. TOXICOLOGY AND METABOLISM DATA

B.6.1. ABSORPTION, DISTRIBUTION, METABOLISM AND EXCRETION IN MAMMALS

Following administration in the rat, clopyralid was rapidly absorbed and excreted quantitatively unchanged, mainly in the urine. There were no differences in distribution of radioactivity between dose rates, sex, route (oral vs. intravenous) or frequency of administration. Three days after dose administration tissue levels were negligible (< 0.01 % of the applied dose). Clopyralid was not metabolised in the rat. Clopyralid has low potential for accumulation. In the *in vitro* comparative metabolism study no unique human metabolites were formed when compared to rat metabolism and there was no observed metabolism.

Clopyralid (3,6-dichloropicolinic acid)

Structural formula:



Molecular formula:
$C_6H_3Cl_2NO_2$

Table B.6.1-1. Summary of toxicokinetic studies of clopyralid

Route **Guideline** **GLP**	**Species** **No of animals**	**Type of study**	**Reference**
Oral administration No official guideline Non- GLP	Rat (Sprague-Dawley) 5 animals (3 males, 2 females)/ dose 6 animals (3 males, 3 female)/ dose	Absorption, distribution, excretion and metabolism of clopyralid (purity not stated) following oral administration of a single dose of ^{14}C-clopyralid (>99% radiochemical purity) at 10 mg/kg bw.	[redacted] *et al.*, 1975 Supplementary
Oral and intravenous administration Guideline 85-1, mainly in accordance with the OECD guideline 417 (2010) Good Laboratory Standards	Rat (Fischer-344) Preliminary study : 4 animals (2 males, 2 females)/ dose Main study : 10 animals (5 males, 5 females)/ dose Control : 2 animals (1 male, 1 female)	Absorption, distribution, excretion and metabolism of clopyralid following oral and iv administration of ^{14}C-clopyralid (> 99% radiochemical purity). Preliminary study : oral administration of 5 mg/kg bw ^{14}C-clopyralid Main study: oral, intravenous and repeated oral administration of 5 mg/kg bw ^{14}C-clopyralid, oral administration of 150 mg/kg bw ^{14}C-clopyralid	[redacted] 1991 Acceptable
In vitro No guideline Good Laboratory Practice Standards	Rat and human liver microsomes	Comparative metabolism *in vitro* for clopyralid-2,6-^{14}C, using liver microsomes from rat and human donors.	[redacted] *et al*., 2016 Acceptable

B.6.1.1. Absorption, distribution, metabolism and excretion by oral route

Study:	The fate of 3,6-dichloropicolinic acid (DOWCO 290) following oral administration in rats ([redacted] 1975)
Previous evaluation:	This study was submitted to DAR (2003). It was not performed according to any official guideline or GLP. It was considered as supplementary information. No new

	evaluation has been performed but text has been modified. Conclusions have not changed.

Test guideline and GLP
The study was not performed using any official guideline or test method, and it was not done under GLP. The study was performed in 1975 before the existence of OECD test guidelines or GLP guidelines. The method is an early version of a metabolism study investigating single administration at one dose level. The current test guideline OECD 417 (year 2010) requires use of more animals and prefers using a minimum of two doses.

Materials and methods
The absorption, distribution, excretion and metabolism of clopyralid (purity not stated) were investigated in male and female Sprague-Dawley rats following oral administration (via feeding needle and syringe) of a single dose of ^{14}C-clopyralid (>99% radiochemical purity, dissolved in 0.7 M phosphate buffer, pH 7.4) at 10 mg/kg bw. Animals were fasted for 12 hours prior to and 45 minutes after dosing.

Five rats (three males and two females) were housed in metabolism cages and blood samples were obtained at intervals up to 12 hours after oral administration. The samples were analysed to follow the time course of radioactivity in the plasma.

An additional three male and three female rats were housed in metabolism cages that allowed collection of urine, feces and expired CO_2. Urine was collected at eight-hour intervals. The samples were analysed to follow the time course of radioactivity in the urine. Faecal samples were collected at 24 h intervals. Expired air was bubbled through 5M ethanolamine in 2-methoxyethanol to trap expired CO_2 and the solutions were collected every 24 hours. Five days after administration samples were taken (liver, kidneys, muscle, perirenal fat, skin, carcass and metabolism cage rinse water).

Results
Average recovery of radioactivity was 100.55 ± 3.74% of administered ^{14}C. Clopyralid was rapidly and virtually completely absorbed (radioactivity recovered in urine, CO_2, tissues and carcass 97.32% of administered ^{14}C) following oral administration, and was excreted unchanged in the urine, with 92.2 ± 3.55% excreted in the urine by 120 hours post dose. Of this, 96.46% was excreted during the first 32 hours following administration, with a half-life of 3.05 hours, and the remainder with a half-life of 24.7 hours. Clopyralid was the only radioactive residue detected; 94 – 99% of the radioactivity co-chromatographed with clopyralid analytical standard.

The limit of detection in plasma was approximately 0.05 µg clopyralid per g of plasma. The peak in plasma concentration was reached 18 minutes after dosing, indicating that absorption is rapid. The plasma concentration fell below the limit of detection 480 minutes after dosing. The declining portion of the plasma time-curve does not represent a log-linear process. This, together with the fact that plasma levels dropped below the level of detection after 6 hours, yet urinary excretion continued throughout the 120-hour duration of the experiment, indicates that clopyralid may be distributed into tissues before ultimately being excreted via the urine.

Tissue levels of ^{14}C at 120 hours were extremely low. Of the administered ^{14}C 5.09± 1.80% was detected in tissues, skin and carcass. With an average concentration in all the tissues examined, it was less than 0.018% per g, and in the remaining carcasses it was 0.025% per g. Radioactivity in expired air accounted for 0.03% of the administered radioactivity. Excretion by this route was considered negligible. Faecal radioactivity accounted for 2.69% of dose. The report was uncertain, whether this small amount represented excretion of absorbed material or a small proportion of unabsorbed dose.

Conclusions
Following oral administration to Sprague-Dawley rat, clopyralid is rapidly and virtually completely absorbed, and is excreted quantitatively, unchanged, in the urine. The study is supplementary.

Study:	Metabolism of 14C-3,6-Dichloropicolinic Acid in Rats. ([redacted] 1991)
Previous evaluation:	This study was submitted to DAR (2003). It was mentioned to be performed according to the Guideline 85-1. The current OECD guideline 417 was updated after the study was previously evaluated. The compliance of the study with the current guideline was evaluated and text has been modified. The study is acceptable.

Test guideline and GLP
The study was reported to be conducted in accordance with the Environmental Protection Agency Pesticide Programs Good Laboratory Standards and the guideline 85-1. The study was performed mainly in accordance with the current OECD guideline 417 (2010). Less animals was used in the control and preliminary test groups than required. No specific rationale for fasting the animals was reported. The study does not contain plasma time-curve data.

Materials and methods
The absorption, distribution, excretion and metabolism of clopyralid were investigated in male and female Fischer-344 rats following oral and iv administration of ^{14}C-clopyralid (> 99% radiochemical purity). The test substance was administered as a solution, either in corn oil (oral dose) or saline (intravenous dose). Acetone was used to dissolve the test substance. The rats were 5 to 9 weeks old at arrival and were acclimated for at least 7 days before the test. The animals weighed approximately 147 to 216 g (males) and 126 to 151 g (females) when dosed with clopyralid. The animals were housed in metabolism cages.

The oral dose was administered via gavage in a volume of 5 ml/kg and iv dose via tail vein in a volume of 1 ml/kg. Animals in oral dose groups were fasted overnight through four hours post dose.

In a preliminary study, 2 rats/sex were dosed orally at 5 mg/kg bw ^{14}C-clopyralid and expired air, urine and feces were collected in intervals of 0-12 h and 12-24 h post dose. In addition cage washes were analysed.

In the main study, the treated animals were divided into four groups (Table B.6.1-2). For repeat administration, rats received 14 days non-radiolabelled clopyralid (> 96% pure) followed by a single dose of radiolabelled clopyralid. Urine and faeces were collected at intervals up to three days after administration (urine: 0-6 h, 6-12 h, 12-24 h then daily, faeces 24 h intervals) and blood at the end of the collection period. Cage washes were taken to analyses. The samples were analysed to follow the time course of radioactivity. Tissues collected for radio analysis were: bone (femur), brain, fat (reproductive), ovaries, testes, heart, liver, kidneys, lungs, muscle (thigh), spleen, stomach, uterus, and residual carcass with skin.

The control group received vehicle only as a single oral dose. Feces and urine were collected up to 24 hours after administration (urine: 0-6 h, 6-12 h, 12-24 h, faeces 0-24 h). Cage washes were taken to analyses. Blood and same tissues as in the main study were collected for analysis after sacrifice.

Table B.6.1-2. Design of clopyralid metabolism study in the rat

Group	Dose (mg/kg bw)	No. of animals	No. of doses/route of administration	Time of sacrifice (h) after last dose
P	5	2m + 2f	1/oral	24
A	0	1m + 1f	-	24
B	5	5m + 5f	1/intravenous	72
C	5	5m + 5f	1/oral	72
D	5	5m + 5f	14 day repeated non-labelled + 1 labelled/oral	72
E	150	5m + 5f	1/oral	72

m = male; f = female

Results
One animal from group D died during the study. It was replaced by another animal. Effects observed on the animals in group C were soft feces and in animals in group D were sores on the head, swollen eye, staining by urine, thinness, hunched and chromodacryorrhea. All other animals showed no overt signs of toxicity.

In the group P the total average percent of radioactive dose in feces was 3.90 % (males) and 0.30 % (females) and in urine 102 % (males) and 61 % (females). The preliminary study indicated that there was no excretion of radioactivity in expired air, so this route was not assessed in the main study. It was mentioned in the report that from the control group A the levels of radioactivity were generally nondetectable.

For the groups B, C, D and E the mean concentration of radioactivity in the blood was below 0.04 ppm, three days after administration. Radioactivity in the urine ranged from 74.1% to 97.6% of the applied dose for males

and females in the four treated groups (Table B.6.1-3). Radioactivity in cage washings ranged from 10.47% to 21.83% of the applied dose. Radioactivity in the faeces ranged from 0.83% to 4.51% of the applied dose, in the carcass from 0.06% to 2.81%, and in the tissues it was less than 0.01%.
There were no apparent differences between treated groups or sexes; multiple applications did not change the tissue distribution or elimination pattern. In individual tissues/organs (excluding carcass), residues were generally less than 0.002 ppm except in the stomach where up to 0.237 and 0.189 ppm (mean) was found in males and females in the high dose group E.

Recovery: Total recoveries (mean) ranged from 95% to 115% for the different treatment groups.
Absorption and excretion: Following intravenous and oral administration, clopyralid was rapidly absorbed and excreted with the majority of radioactivity being excreted in the urine during the first 24 hours.
Metabolism: Only clopyralid was recovered from the urine; no metabolites were detected. Most of the radioactivity in the faeces was also unchanged clopyralid.

Table B.6.1-3. Excretion data following administration of clopyralid

		Excretion after oral administration (mean % of applied dose)							
Group		B (Low dose)		C (Low dose)		D (Repeat dose)		E (High dose)	
		Intravenous		Oral		Oral		Oral	
Sex		male	female	male	female	male	female	male	female
Urine	0 – 6 h	69.4	81.7	45.1	49.4	43.9	39.3	36.0	22.3
	6 – 12 h	6.76	7.76	25.3	11.9	33.8	21.5	31.5	45.8
	12 – 24 h	7.89	6.24	13.9	10.1	8.90	12.4	11.0	19.2
	24 – 48 h	1.01	1.50	2.21	1.86	1.68	1.44	2.29	2.33
	48 – 72 h	0.82	0.41	0.60	0.79	0.83	0.50	1.19	0.51
	Subtotal 0 – 24 h	84.05	95.7	84.3	71.4	86.6	73.2	78.5	87.3
	0 – 48 h	85.06	97.2	86.51	73.26	88.28	74.6	80.79	89.63
	0 – 72 h	85.9	97.6	87.1	74.1	89.1	75.1	82.0	90.1
Faeces	0 – 24 h	1.84	0.27	2.28	3.18	1.93	1.29	2.38	3.69
	24 – 48 h	0.12	0.44	0.79	0.57	0.71	0.49	0.57	0.50
	48 – 72 h	0.26	0.12	0.44	0.20	0.48	0.45	0.82	0.32
	Subtotal 0 – 48 h	1.96	0.71	3.07	3.75	2.64	1.78	2.95	4.19
	0 – 72 h	2.22	0.83	3.51	3.95	3.12	2.23	3.77	4.51
Carcass		0.13	0.06	0.69	2.81	0.18	0.14	0.39	0.49
Cage Wash[1]		18.08	16.27	11.92	14.28	10.47	21.83	14.17	12.19
Tissues		< 0.01	ND	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Total Recovery		106	115	103	95.0	103	99.4	100	107

[1] Cage wash + cage rinse + cage wipe.
ND not detectable

Conclusions
Following oral and intravenous administration in the rat, clopyralid was rapidly absorbed and excreted unchanged in the urine. There were no differences in distribution of radioactivity between dose rates, sex, route or frequency of administration. Clopyralid was not metabolised in the rat. The study is acceptable.

Initial estimation of absorption is done by excluding the dose in GI tract and faeces from the total recovery. Since the dose only for stomach was determined (not for the rest of the GI tract) and the percent of radioactive dose was < 0.01 % only the dose in faeces was excluded. This gives the estimates between 91.1 % (females, group C) to 114.2 % (females, group B). The dose excreted in faeces can represent the excreted dose or a portion of unabsorbed dose. Biliary excretion was not investigated.

In total, the excretion (urine and faeces) at 48 h was between 76.4 % and 97.9 %. Urinary excretion at 48 h was between 73.26 % and 97.2 %. In 24 hours 71.4 % to 95.7 % of the applied dose was excreted via urine and 0.27 % to 3.69 % via faeces.

Bioavailability could not be determined since the study does not contain plasma concentration time curve.

At study termination (72 h) the radioactivity measured in the tissues was < 0.01 % of the applied dose. Due to low levels detected no specific target organs or wideness of distribution could be determined. No information about the tissue distribution at the time of test substance peak plasma/blood concentration or peak rate urinary excretion is available in the study.

When considering the rate of urinary excretion, and the dose found in the faeces and cage wash in comparison to levels found in tissues and carcass it is considered that clopyralid has low potential for accumulation.

B.6.1.2. Absorption, distribution, metabolism and excretion by other routes

Study:	Clopyralid: *In vitro* Comparative metabolism Using Liver Microsomes from F344 Rat and Human Donors. ([redacted] *et al*., 2016)
Previous evaluation:	This is a new study submitted for AIR 3 evaluation. No guideline mentioned. The study is acceptable.

Test guideline and GLP
No guideline mentioned. The study was mentioned to be conducted in compliance with Good Laboratory Practice Standards.

Materials and methods
In vitro metabolism data for clopyralid-2,6-^{14}C (Radiochemical purity 99.0%) was generated using liver microsomes from F344/DuCrl rat and human donors. The microsomes from the rat were pooled samples from a single gender (greater than or equal to 3 animals per species per sample). The microsomes from human donors were comprised of liver microsomes from individual donors. The single gender microsomes were pooled in equal portions by species to provide a mixed gender microsome pool for both rat and human donors.

Incubations of rat and human donor liver microsomes were performed according to Table B.6.1-4. The incubations with the positive control 7-ethoxycoumarin (purity: 99.9%) were performed to assess metabolic activity of the test system. Common and appropriate co-factors for Phase I CYP-based metabolism were also added to the test system.

Table B.6.1-4. Incubation Sample Scheme

Reagent	**Final Concentration**				
	Incubations (N=3)	**Positive Control** (N=2)	**No Enzyme** (N=1)	**No Cofactor Control** (N=1)	**Vehicle Control** (N=1)
Microsomes (Rat or Human)	1 mg/mL	0.05 mg/mL	NA	1 mg/mL	1 mg/mL
^{14}C-Clopyralid	9.60 ppm (50 µM)	NA	9.60 ppm (50 µM)	9.60 ppm (50 µM)	(DMSO)
7-Ethoxycoumarin	NA	500 µM	NA	NA	NA
NADPH	1.3 mM	1.3 mM	1.3 mM	1.3 mM*	1.3 mM
UDPGA	5.0 mM	NA	5.0 mM	5.0 mM	5.0 mM
Alamethicin	10 µg/mL	NA	10 µg/mL	10 µg/mL	10 µg/mL
Magnesium Chloride	3.3 mM	NA	3.3 mM	3.3 mM	3.3 mM
Potassium Phosphate Buffer	0.1 M; pH 7.4	0.1 M; pH 7.4	0.1 M; pH 7.4	0.1 M; pH 7.4	0.1 M; pH 7.4

NA= not applicable for indicated incubation, * Added after kill solution, DMSO= dimethyl sulfoxide

Based on the water solubility of ^{14}C-clopyralid (at a pH of 8 approximately 500 mg/l), a concentration of 50 µM was selected for this study as it allowed for sufficient quantitation of the substrate concentration in the final incubation sample without saturating the microsomes. Confirmation of the test material concentration in the dose solution(s) was conducted concurrently with the study and radioactivity in the dose solutions was quantified by liquid scintillation spectrometry (LSS).

A single stock solution of the positive control test material, 7-ethoxycoumarin, was prepared at a concentration of 50 mM in DMSO to administer a dose to incubation samples equivalent to an incubation exposure concentration of 500 µM.

For the test item incubations, thawed and pooled mixed gender microsomes were aliquoted into vials and placed on wet ice with alamethicin. A final substrate concentration of 50 µM was used in a 1 mL incubation volume with a final protein concentration of 1 mg/mL. Incubations were initiated upon the addition of NADPH, UDPGA and the clopyralid dose stock solution. Incubations were placed into an oscillating water bath (30 min, 60 rotations/minute, 37°C) and the incubations were then quenched with acetonitrile containing 1% (v/v) formic acid (i.e. 'kill solution'). Samples were centrifuged at 15000 rcf for 10 minutes and the supernatant transferred to high-recovery autosampler vials and stored at -80°C until analysis.

Duplicate incubations (per species) were performed with the positive control 7-ethoxycoumarin at a final substrate concentration of 500 µM in an incubation volume of 1mL with a final protein concentration of 0.05 mg/mL. The cofactor UDPGA was not added in order to avoid possible Phase II metabolite formation. For a given1 mL incubation sample, KPBS, NADPH, and diluted microsomes were combined in ultracentrifuge tubes and incubated at 37°C for 2 minutes. The 7-ethoxycoumarin dose solution was then added to the samples and the samples were then incubated for another 20 minutes at 37°C. The incubations were then quenched with kill solution. Samples were centrifuged at 15000 rcf for 10 minutes and the supernatant transferred to high-recovery autosampler vials and stored at -80°C until analysis.

Three types of negative controls were generated in this study. Vehicle control incubations (1 replicate/species) were prepared as well as incubations lacking cofactors (1 replicate/species). The third negative control was a single incubation without microsomes (*i.e.,* no enzyme; 1 replicate/species).

The amount of radioactivity recovered in the final processed samples for all samples was measured by LSS and compared to the radioactivity recovered before and after centrifugation. Radioactive recovery values were comparable, therefore radioactive counts acquired after centrifugation were used for mass balance calculations. The mass balance of test material and/or metabolites recovered from the incubation test system was evaluated by comparison of radioactivity added to the sample to the amount of radioactivity recovered in the final supernatants.

The incubation supernatants from the ^{14}C-clopyralid incubation samples were radiochemically profiled by HPLC with radiochemical flow detection (RAM). The total radioactivity of HPLC eluent from representative incubation samples of each species was used to determine ^{14}C-activity system recovery. The limit of detection (LOD) was determined based on the signal to noise ratio (S/N) $\geq$ 3.

The authentic solvent standard, matrix standard, supernatants of representative liver microsome incubation samples of each species and the supernatants of negative control incubation samples were analyzed by HPLC with electrospray quadrupole–time-of-flight high resolution mass spectrometry (HPLC/ESI/Q-TOF MS) for the identification of the metabolite observed in the radiochemical profiles.

The metabolite of 7-ethoxycoumarin (umbelliferone) from each positive control incubation sample was measured by LC/UV. Based on the LC/UV method, the umbelliferone concentrations from all positive control incubations were determined and the resulting umbelliferone formation rates in pooled microsomes from rat and human were calculated and also compared to the vendor supplied values of the appropriate species.

Results

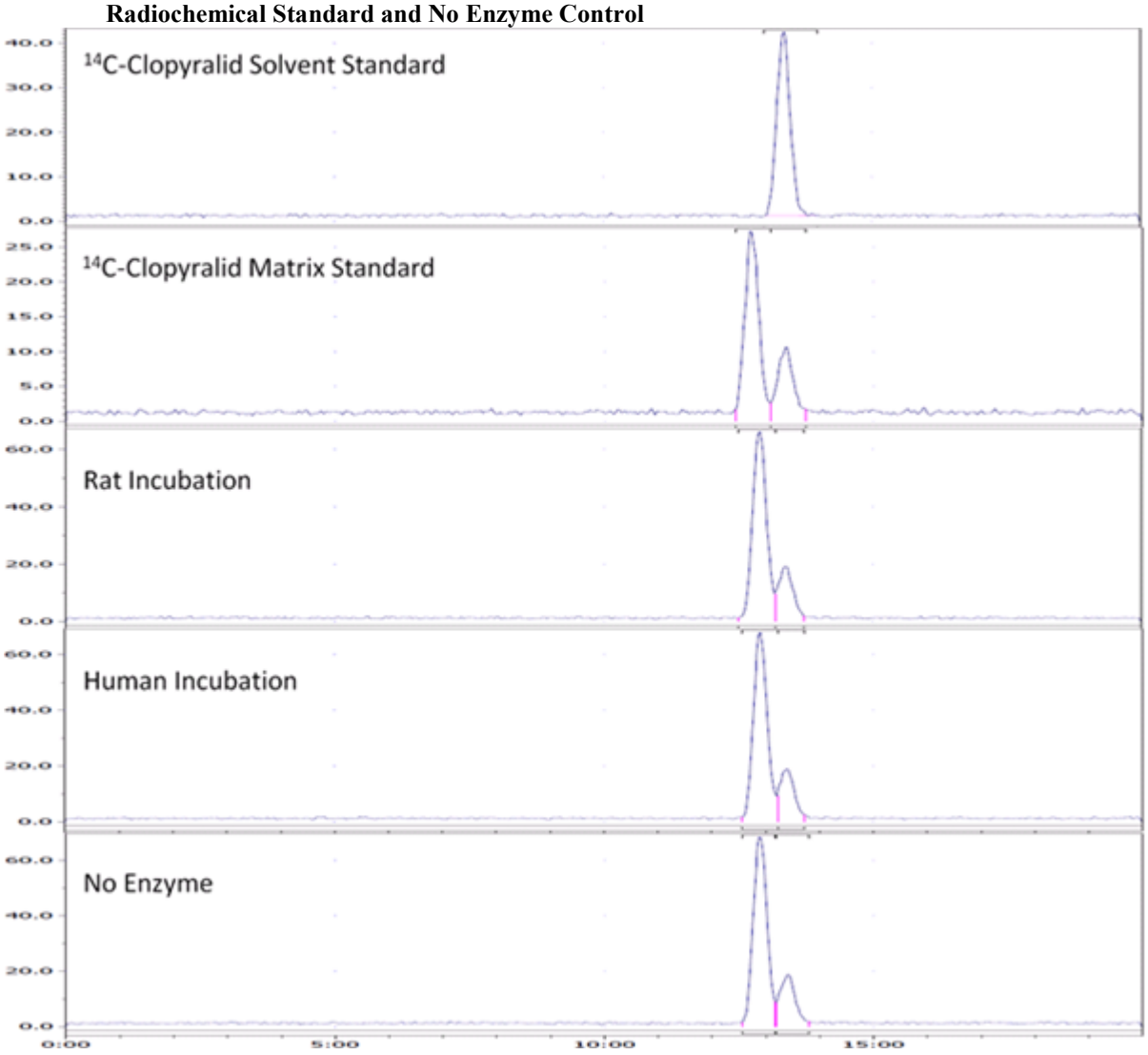
The measured concentration of the ^{14}C-clopyralid dose stock was 106 % (1016 µg/mL) of the target concentration. The dose solution was homogenous with 2.0% relative standard deviation between aliquots. The radioactive homogeneity was determined to have a relative standard deviation of 0.35%. The measured concentration of the 7-ethoxycoumarin dose stock was 94.1% (8.95 mg/mL) of the target concentration with a relative standard deviation of 0.6%.

The mass balance (incubation recovery) values for the ^{14}C-clopyralid incubation samples averaged from 101% to 100% in rat and human donors, respectively. The No Enzyme incubation had a radioactive recovery of 109% and the No Cofactor Controls had radioactive recoveries of 105% and 98.5% for rat and human donors, respectively.

The measured metabolite formation rates in pooled microsomes from rat and human donors were 498 and 286 pmol/mg protein/min, respectively. The percent target of measured metabolite formation rates for the rat and human liver microsomes were 39% and 36%, respectively. The difference between the measured metabolite formation rates was comparable and the averaged vendor values were approximately within a factor of three.

The definitive incubation supernatants from whole incubation systems containing microsomes, alamethicin, ^{14}C-clopyralid, UDPGA and NADPH were radiochemically profiled for both species. Figure 1 shows representative radiochemical chromatograms for the ^{14}C-clopyralid solvent standard, ^{14}C-clopyralid matrix standard, rat incubation, human incubation and no enzyme incubation. The ^{14}C-clopyralid solvent standard only appeared as a single peak on the LC/RAM radiochromatogram. However, ^{14}C-clopyralid in the matrix standard formed doublet peaks in the LC/RAM chromatograms. The change in peak shape for ^{14}C-clopyralid is attributed to a pH effect in the matrix. The average limit of detection was determined to be 2.385% of the total incubation radioactivity.

Figure 1. Representative Radiochemical Chromatograms from Complete Liver Microsomal Incubations of ^{14}C-Clopyralid in F344 Rat and Human Donors in the Presence of NADPH with Radiochemical Standard and No Enzyme Control



Conclusions

The study showed that under these experimental conditions no unique human metabolites were formed when compared to rat metabolism and that there was no observed metabolism. The study is acceptable.

B.6.2. ACUTE TOXICITY

The acute toxicity, skin and eye irritancy and skin sensitisation potency of clopyralid is presented in table B.6.2-1. The acute toxicity of clopyralid (Lontrel T) was studied by oral, dermal and inhalation routes using rat and rabbit as a test animal. The studies done were briefly described, but according to the study reports, they were conducted in compliance with GLP standards, the methods of USEPA and mainly according to OECD guidelines. Clopyralid was of low toxicity by all routes. The highest attainable concentration achieved in an acute inhalation study after grinding the material was only 1.0 mg/l. An earlier study only succeeded in generating 0.2 mg/l. However, there was no mortality.

Clopyralid did not induce irritation on rabbit skin. Clopyralid caused marked irritation to eyes of rabbit and symptoms were still present after 21 days. Therefore, clopyralid is to be classified according to regulation 1272/2008 as Eye Dam. 1; H318.

In sensitivity tests, clopyralid was mildly irritating and sensitising in Magnusson & Kligman test but showed no signs of erythema or oedema in Buehler test. The studies were considerd supportive. Classification was not possible based on the available data.

There were no studies submitted on phototoxicity and there was not sufficient information to draw the final conclusion.

Table B.6.2-1. Summary of acute toxicity of clopyralid

Route/method	Species	Result/Comment	Classification according to regulation 1272/2008	Reference
Oral	Rat	LD_{50} > 5000 mg/kg bw	None	[redacted], *et al*. 1987
Dermal	Rabbit	LD_{50} > 2000 mg/kg bw	None	[redacted] *et al*. 1987
Inhalation	Rat	LC_{50} > 0.2 mg/l/4 h[1)]	- (study for supportive information only)	[redacted] *et al.* 1987
Inhalation	Rat	LC_{50} > 1.0 mg/l/4 h[1)]	None	[redacted] 1991
Dermal	Rabbit	Average scores (24-72 h) for each rabbit is 0 for erythema and 0 for oedema.	None	[redacted] 1987
Eye	Rabbit	Severe irritation; effects still present after 21 days	Eye Dam. 1; H318	[redacted] 1987
Magnusson & Kligman	Guinea pig	Slightly sensitising	- (study for supportive information only)	[redacted]. 1996
Buehler test method	Guinea pig	No signs of erythema or oedema	- (study for supportive information only)	[redacted] 1987
Phototoxicity	-	No studies submitted		-

[1)] Highest attainable concentration

B.6.2.1. Oral

Study:	Lontrel T herbicidal chemical (Penta process): Acute oral toxicity study in Fischer 344 rats ([redacted] 1987)
Previous evaluation:	This study was submitted to DAR (2003). It was mentioned to be performed according to the Guideline No. 81-1. The compliance of the study with the OECD guideline 423 (limit test, 2001) was evaluated and text has been modified. Conclusions have not changed. The study is acceptable.

Test guideline and GLP

The study was reported to be conducted in compliance with GLP standards and the Guideline 81-1. The study report only briefly described the study performed, and some issues required in the OECD guideline 423 were not given, e.g. volume of dose, humidity and temperature data. Both sexes were dosed. Details of observation period were not given for the first hours/days after dosing. Stepwise dosing is mentioned in the guidance, however in

the test all animals seemed to be dosed at the same time. These deviations are considered not to have major effect on the study acceptability.

Materials and methods
Lontrel T (95.4% clopyralid) was administered as a suspension in corn oil (50 % w/v) at a dose of 5000 mg/kg bw to groups of 5 male and 5 female Fischer 344 rats. All animals were fasted the night before treatment and observed thereafter at least once each day for 14 days. Body weights and clinical observations were examined. All animals were submitted to necropsy, including examination of the eyes, after the observation period.

Results
All rats survived a single oral dose of 5000 mg/kg bw of Lontrel T. Since all rats survived the limit test, no other dose levels were tested. All rats were in good health throughout the observation period. No evidence of any treatment-related gross lesions was observed at necropsy. All animals gained weight from day 1 to day 14. On two female rats the weight had slightly decreased on day 7.

Conclusion
The acute oral toxicity of Lontrel T in rat was low. The LD_{50} was greater than 5000 mg/kg bw. The study is acceptable. No classification is required according to regulation 1272/2008.

B.6.2.2. Dermal

Study:	Lontrel T herbicidal chemical (Penta process): Acute dermal toxicity study in New Zealand white rabbits ([redacted] 1987)
Previous evaluation:	This study was submitted to DAR (2003). It was performed according to the Guideline 81-2 that in the DAR was mentioned to comply with OECD guideline 402 (limit test). No new evaluation has been performed since this guideline has not been updated since previous evaluation of the study. Text has been modified. Conclusions have not changed. The study is acceptable.

Test guideline and GLP
The study was reported to be conducted in compliance with GLP standards and the Guideline 81-2. In the DAR it was mentioned to comply with OECD guideline 402 (limit test).

Materials and methods
Five New Zealand White rabbits per sex were treated with 2000 mg/kg bw of Lontrel T (95.4% clopyralid). The test material was applied to the back of each animal and held in contact with the fur free skin with a gauze dressing and non-irritating tape. Five millilitres of distilled water was added under the plastic wrap to ensure sufficient contact. The wrappings were removed after a 24-hour exposure period and observations were recorded for any irritation at the application site. Thereafter the treated skin was washed. The observation period lasted 14 days and body weight and clinical observations were examined. After the observation period animals were submitted to necropsy that included examination of the eyes.

Results
All rabbits survived a single 24-hour dermal exposure of 2000 mg/kg bw of Lontrel T. Since all animals survived the limit test, no other dose levels were tested. No clinical evidence of any treatment-related effects was observed during the two-week observation period. Gross pathologic examination did not reveal any treatment-related effects. The few observations were considered incidental findings (pale area/heart on one female, foci-depressed kidneys on one female). One female had decreased body weight from day 1 to day 14. Two males had decreased body weight from day 7 to day 14.

Conclusion
The acute dermal toxicity of Lontrel T in rabbits was low. The LD_{50} for male and female rabbits was greater than 2000 mg/kg bw. The study is acceptable. No classification is required according to regulation 1272/2008.

B.6.2.3. Inhalation

Study:	Lontrel T herbicidal chemical (Penta process): An acute aerosol inhalation study in

	Fischer 344 rats ([redacted] 1987)
Previous evaluation:	This study was submitted to DAR (2003). It was mentioned to be performed according to the Guideline 81-3 that in the DAR was mentioned to comply with OECD guideline 403 (limit test). The study was considered as supportive information for evaluation of inhalation toxicity. No new evaluation has been performed but text has been modified.

Test guideline and GLP
The study was reported to be conducted in compliance with GLP standards and the Guideline 81-3. In the DAR it was mentioned to comply with OECD guideline 403 (limit test). In contrary to the recommendation in the OECD guideline 403 (limit test) the animals were group housed during whole-body exposure. According to the guideline animals should be housed individually during exposure to prevent them from filtering the test aerosol through the fur of their cage mates. MMAD from the study did not meet the recommended values (1 to 4 μm) from the OECD guideline.

Materials and methods
Five male and five female Fischer 344 rats were exposed (whole-body) for 4 hours to a solid aerosol of Lontrel T (95.4% clopyralid) at maximum test time-weighted average concentration of 0.2 mg/l (highest attainable concentration). The mass median aerodynamic diameter (MMAD) of the aerosol was 13.45 μm, while the geometric standard deviation (σg) of the particle size distribution was 2.11. Although a large quantity of material was initially aerosolized, only a fraction remained suspended. Most test material particles settled in the glass ductwork which led to the inhalation chamber. During exposure animals were group housed. The observation period lasted 14 days.

Observations included evaluation of the fur, eyes, mucous membranes and respiration. Also behaviour pattern, nervous system activity, tremors, convulsions, salivation, lacrimation, diarhhea, lethargy and other signs of altered central nervous system function were assessed. Two weeks post-exposure a gross pathologic examination, including examination of the eyes, was conducted.

Results
During exposure, four animals had red (porphyrin-like) stains around their nares and one salivated. Following exposure, three males and all five females had reddish stains around the nares and all animals were urine stained in the perineum. By test day six, all rats appeared normal. There was no mortality during this study and only a slight decrease in average male body weight on test day two. There were no exposure-related gross pathologic observations. Pre-exposure and pre-termination unilateral or bilateral small linear streaks were observed on the corneas of several animals. These were observed also at necropsy. After formalin fixation the streaks were not visible. This change was observed only one eye of a female rat characterized by a very slight mineralization of the corneal epithelium. This change was considered to be incidental.

Conclusion
The LC_{50} for male and female rats was greater than 0.2 mg/l/4 h. The study is supportive for evaluation of inhalation toxicity.

Study:	Lontrel T: Acute inhalation toxicity study with Fischer 344 rats ([redacted] 1991)
Previous evaluation:	This study was submitted to DAR (2003). The study was considered acceptable. It was mentioned to be performed according to FIFRA Guideline 81-3. It is mainly performed in compliance with OECD guideline 403 (2009). The OECD guideline was updated since previous evaluation and the compliance of the study with the current guideline was evaluated. Text has been modified.

Test guideline and GLP
The study was reported to be conducted in compliance with GLP standards and the FIFRA Guideline No. 81-3. It is mainly performed in accordance with OECD guideline 403 (limit test) (2009). The chamber atmosphere humidity during exposure was lower (13±2 %) than what recommended in the guideline.

Materials and methods

Five male and five female Fischer 344 rats were exposed (nose-only) for 4 hours to the highest practically attainable concentration of 1.0 mg/l of Lontrel T (95.8% clopyralid). This was obtained only by grinding the test material twice and by stirring it every 10 minutes to eliminate cavities within the reservoir of the dust generator. The mass median aerodynamic diameter (MMAD) and geometric standard deviation (σg) were 3.9 µm ±2.4. The observation period lasted 14 days. Observations included evaluation of the fur, eyes, mucous membranes and respiration. Also behaviour pattern, nervous system activity, tremors, convulsions, salivation, lacrimation, diarhhea, lethargy and other signs of altered central nervous system function were assessed. On test day 15 rats were submitted to gross necropsy examination, including examination of the eyes.

Results
All animals survived the exposure as well as the two-week post-exposure period. There were no clinical effects noted during or after the exposure period. On test day two, mean body weights for male and female animals were decreased 1-2% from pre-exposure values. Thereafter the animals achieved expected body weight gains. There were no exposure-related gross pathologic observations. Chamber relative humidity values were low (13±2 %). In the study report it was assumed that test material might have interfered with the humidity gauge.

Conclusion
The LC_{50} for male and female rats was greater than 1.0 mg/l/4 h, which was the highest attainable concentration. The study is acceptable. No classification is required according to regulation 1272/2008.

B.6.2.4. Skin irritation

Study:	Lontrel T herbicidal chemical (penta process): Primary dermal irritation study in New Zealand white rabbits ([redacted] 1987)
Previous evaluation:	This study was submitted to DAR (2003). It was mentioned to be performed according to the Guideline 81-5. The OECD guideline 404 was updated after the study was previously evaluated and the compliance of the study with the current guideline was evaluated. Text has been modified. The study is acceptable.

Test guideline and GLP
The study was reported to be conducted in compliance with the GLP standards and the guideline No. 81-5. It is mainly performed in compliance with OECD guideline 404 (2015). Not all information was reported, e.g. temperature, humidity and the age of animals. The size of the area where the test material was applied was smaller than recommended in the guideline. Response was examined at 30 minutes instead of 60 minutes given in the guideline.

Materials and methods
Three male and three female New Zealand white rabbits were treated with a 0.5 g aliquot of Lontrel T (95.4% clopyralid). The test material was applied to the back of each animal under a 2.5 cm^2 gauze patch and held in contact with the fur free skin with non-irritating tape. The gauze patch was then moistened with water and covered with bandage. The wrappings were removed after a 4-hour exposure period. After wiping of the test substance, the application site of each rabbit was graded (Draize scale) for erythema/eschar and oedema within thirty minutes and 24, 48 and 72 hours after patch removal.

Results
No evidence of dermal irritation was observed in animals after treatment. Skin irritation grades for erythema and oedema are given in the table B.6.2-2

Table B.6.2-2. Skin irritation grades for erythema and oedema

	Animal					
	1 (male)	2 (male)	3 (male)	4 (female)	5 (female)	6 (female)
Erythema						
30 min	0	0	0	0	0	0
24 h	0	0	0	0	0	0
48 h	0	0	0	0	0	0
72 h	0	0	0	0	0	0
Average score (24-72 h)	0	0	0	0	0	0

Edema						
30 min	0	0	0	0	0	0
24 h	0	0	0	0	0	0
48 h	0	0	0	0	0	0
72 h	0	0	0	0	0	0
Average score (24-72 h)	0	0	0	0	0	0

Conclusion
Under the conditions of this study, the test material was considered not irritating to the skin of rabbit. The study is acceptable. Average scores (24-72 h) for each rabbit is 0 for erythema and 0 for oedema. No classification is required according to regulation 1272/2008.

B.6.2.5. Eye irritation

Study:	Lontrel T herbicidal chemical (penta process): Primary eye irritation study in New Zealand white rabbits ([redacted] 1987)
Previous evaluation:	This study was submitted to DAR (2003). It was mentioned to be performed according to the Guideline 81-4. The OECD guideline 405 was updated after the study was previously evaluated and the compliance of the study with the current guideline was evaluated. Text has been modified. The study is acceptable.

Test guideline and GLP
The study was reported to be conducted in compliance with GLP standards and the Guideline No. 81- 4. It is mainly performed in compliance with OECD guideline 405 (2012). Not all information was reported, e.g. temperature, humidity and the age of animals. Anaesthetics or analgesics were not used. Initial test was not performed.

Materials and methods
Three male and three female New Zealand white rabbits were treated with a 0.1 g aliquot of Lontrel T (95.4% clopyralid). The test material was instilled into the conjunctival sac of the right eye of the animals. The left eye remained untreated and served as a control. The eyes of all rabbits remained unwashed. Both eyes of the rabbits were examined and graded (Draize's scale) for conjunctival redness and chemosis, discharge, corneal opacity and reddening of the iris at 1, 24, 48 and 72 hours and 7, 14 and 21 days post-installation.

Results
Exposure to Lontrel T dust revealed a severe irritating effect in the eyes of rabbits. Slight discomfort was observed in all animals immediately upon instillation of the test material. Observations of the conjunctivae post-treatment were characterised as slight to marked redness and chemosis. All animals had a marked amount of discharge from the treated eye. Reddening of the iris was observed in all animals. Corneal opacity was observed in all animals and ranged from scattered or diffuse areas of opacity to marked opacity. Signs of irritation were present in all animals 21 days post treatment. These observations were characterised as slight to moderate redness and chemosis, slight discharge and reddening of the iris. Two animals had scattered or diffuse areas of opacity and three animals had moderate opacity 21 days post-treatment. Individual animal eye irritation scores are summarised in Table B.6.2-3.

Table B.6.2-3. Acute eye irritation of Lontrel T in rabbits

	Animal					
	1 (male)	2 (male)	3 (male)	4 (female)	5 (female)	6 (female)
Conjunctivae redness						
1 h	1	1	1	1	1	1
24 h	1	3	1	2	2	2
48 h	2	3	2	3	2	2
72 h	2	3	2	3	2	3
7 d	2	3	3	2	2	2
14 d	1	2	3	2	1	1
21 d	1	2	2	2	1	1
Average score	1.67	3	1.67	2.67	2	2.33

(24-72 h)						
Conjunctivae chemosis						
1 h	3	3	3	3	3	3
24 h	4	2	4	3	4	3
48 h	4	2	4	3	3	2
72 h	3	2	3	2	3	2
7 d	3	1	2	1	2	1
14 d	2	0	2	1	1	0
21 d	2	0	2	1	1	0
Average score (24-72 h)	3.67	2	3.67	2.67	3.33	2.33
Corneal Opacity						
1 h	3	0	3	1	3	3
24 h	3	2	4	2	3	3
48 h	3	2	4	2	3	3
72 h	3	2	3	2	3	3
7 d	3	1	3	1	3	3
14 d	3	1	3	1	3	1
21 d	3	1	3	0	3	1
Average score (24-72 h)	3	2	3.67	2	3	3
Reddening of iris						
1 h	1	1	1	1	1	1
24 h	1	1	1	1	1	1
48 h	1	1	1	1	1	1
72 h	1	1	1	1	1	1
7 d	1	1	1	1	1	1
14 d	0	0	0	1	1	0
21 d	0	0	0	1	1	0
Average score (24-72 h)	1	1	1	1	1	1

Conclusion
Exposure to Lontrel T revealed a severe irritating effects in the eyes of rabbits. The effects were not reversible within 21 days post-exposure. The study is acceptable. Based on the observed effects still present on study termination the classification as Eye Dam. 1; H318 is required.

B.6.2.6. Skin sensitization

Study:	Lontrel T (clopyralid technical): Delayed contact hypersensitivity study in the guinea pig ([redacted] 1996)
Previous evaluation:	This study was submitted to DAR (2003). It was performed mainly according to OECD guideline 406. Text has been modified and conclusions have changed. The study is of supportive information.

Test guideline and GLP
The study was reported to be performed in compliance with the principles of GLP and mainly according to OECD guideline 406 (Maximisation test method) (1992).

Materials and methods
A preliminary study was conducted to establish the irritant and non-irritant concentrations of the test material. Skin reactions to Lontrel T (97.9% clopyralid) were tested on 4 Dunkin-Hartley albino guinea pigs by intradermal injections where two animals received 0.1 ml 5%, 10% and 30% w/v of Lontrel T in vehicle or in FCA or other two animals 0.1 ml 0.5%, 1%, 3% of Lontrel T in vehicle or in FCA. Two guinea pigs received by topical application 0.25 ml 5%, 10%, 30% and 50% Lontrel T in vehicle and three guinea pigs 0.03 ml 1%, 3%, 5%, 10%, Lontrel T in vehicle.

Six to eight week old (on treatment day 1) Dunkin-Hartley albino guinea pigs were acclimatized 6-16 days before placing on the test in the main study. An area of skin was clipped free of hair day before study commenced. Induction by intradermal injection (0.1 ml) was administered on day 1 and second induction by topical application (0.6 ml) on day 8. The application site was covered by occlusive dressing for 48 hours. On day 21 hair was removed by clipping and on day 22 by shaving. About an hour later the challenge dose (0.03 ml) was administered by topical application. The application site was covered by an occlusive dressing for 24 hours. The degree of reactions to challenge was scored on a four point scale (0 (= no response) – 3 (= severe erythema)) 24 and 48 hours after removal of the occlusive dressings. Positive control animals were not used, but results from two other studies (year 1995) with challenge with undiluted hexyl cinnamic aldehyde and 50% or 30% w/v hexyl cinnamic aldehyde in propylene glycol were reported.

In the main study, the test group of 10 male and 10 female Dunkin-Hartley albino guinea pigs and the control group of ten animals received the doses presented in the table 6.2-4.

Table 6.2-4. Doses administered to test and control groups.

	Test group	**Control group**
First induction (intradermal injection)	- Freund's complete adjuvant (FCA) (1:1) with purified water - 3% w/v clopyralid in propylene glycol - 3% w/v clopyralid in FCA (1:1) with purified water	- FCA (1:1) with purified water - propylene glycol - propylene glycol in FCA (1:1) with purified water
Second induction (topical)	50% w/v Lontrel T in propylene glycol	propylene glycol
Challenge (topical)	- 10% w/v Lontrel T in propylene glycol - 3% w/v Lontrel T in propylene glycol - propylene glycol	- 10% w/v Lontrel T in propylene glycol - 3% w/v Lontrel T in propylene glycol - propylene glycol

Results

Preliminary study

The dose selected for intradermal injection for the main study, 3% w/v clopyralid in propylene glycol, caused slight erythema and superficial eschar in one of the two animals. The other animal had no reactions. The higher concentrations tested (5%, 10% and 30% w/v of Lontrel T in vehicle) caused also slight to moderate erythema in both animals tested but also ulceration and pallor. Both of these animals on higher concentration group were killed for humane reason after 48 hour examination. With the dose of 3% w/v clopyralid in FCA, selected for the main study, slight erythema in both animals and superficial eschar in one animal was observed.

The dose selected for topical induction for the main study, 50% w/v Lontrel T in propylene glycol, caused slight erythema at 24 h and exfoliation at 24 h and 48 h on both animals. This was the highest concentration tested.

The doses selected for topical challenge for the main study, 10% w/v and 3% w/v Lontrel T in propylene glycol, caused no reaction in both animals. No higher concentrations were tested.

Table 6.2-5. Results of the preliminary study

Intradermal administration				
Animal	**Concentration of test material**	**Dermal responses at time after injection**		
		24 h	**48 h**	**7 days**
31M	30% w/v in vehicle[+] 10% w/v in vehicle 5% w/v in vehicle 30% w/v in FCA [+A] 10% w/v in FCA [A] 5% w/v in FCA	2 2u 2u 1 1 2p	1d 1u 1u 1 1 1p	§
37F	30% w/v in vehicle[+] 10% w/v in vehicle 5% w/v in vehicle 30% w/v in FCA [+A] 10% w/v in FCA [A]	1u 1p 1u 1 1	1u 1u 1u 1 1p	§

	5% w/v in FCA	1u	1	
32M	3% w/v in vehicle	0	0	0
	1% w/v in vehicle	2p	1d	0e*
	0.5% w/v in vehicle	2p	1p	0e*
	3% w/v in FCA	1	1	0
	1% w/v in FCA	1	1	0
	0.5% w/v in FCA	2p	1p	0e*
38F	3% w/v in vehicle	1	1	0e*
	1% w/v in vehicle	1p	1	0
	0.5% w/v in vehicle	1	1	0
	3% w/v in FCA	1	1	0e*
	1% w/v in FCA	1p	1p	1e*
	0.5% w/v in FCA	1	1p	0e*
Topical induction administration				
Animal	**Concentration of test material in vehicle**	**Dermal responses at time after removal of dressings**		
		24h	**48h**	**7 days**
33M	50% w/v	1f	0f	0
	30% w/v	1f	0	0
	10% w/v	0f	0	0
	5% w/v	0	0	0
39F	50% w/v	1f	0f	0
	30% w/v	1f	0f	0
	10% w/v	0f	0f	0
	5% w/v	0f	0	0
Topical challenge administration				
Animal	**Concentration of test material in vehicle**	**Dermal responses at time after removal of dressings**		
		24h	**48h**	
34M	10% w/v	0	0	
	5% w/v	0	0	
	3% w/v	0	0	
	1% w/v	0	0	
35M	10% w/v	0	0	
	5% w/v	0	0	
	3% w/v	0	0	
	1% w/v	0	0	
40F	10% w/v	0	0	
	5% w/v	0	0	
	3% w/v	0	0	
	1% w/v	0	0	

0 No response
1 Slight erythema
2 Moderate erythema
FCA Freunds Complete Adjuvant
(explanation for "d" not given in the study report)
+ Maximum practicable concentration
A Difficult to dose
e* Superficial eschar
p Pallor
u Ulceration
§ Animals killed for humane reasons after 48 h examination
f Exfoliation

Main study

The animals remained in overt good health and achieved anticipated overall bodyweight gains.

At 24 h reading intradermal injection of 3% w/v Lontrel T in propylene glycol caused slight erythema and discoloration and isolated cases of pallor and ulceration. However, 12 animals out of 20 had no reactions. Intradermal injection of 3% w/v Lontrel T in the adjuvant caused moderate erythema, pallor, ulceration and single case of discoloration. One animal out of 20 had no reactions. Intradermal injection of the adjuvant alone caused moderate erythema in all animals. In the control group FCA and vehicle in FCA caused moderate erythema in all animals. In addition pallor was observed in one animal dosed with vehicle in FCA. Vehicle alone caused no response.

Table 6.2-6. Results of the main study – responses after intradermal injection (test group)

Response	Number of animals showing response+					
	FCA		Test material in vehicle		Test material in vehicle in FCA	
	Male	Female	Male	Female	Male	Female
No response			5	7		1
Slight erythema			5	3		
Moderate erythema	10	10			10	9
Discolouration			2	3		1
Pallor			1		8	3
Ulceration			2		6	4

+ Ten animals in each sex-group FCA Freunds Complete Adjuvant

At 24 h reading after removal of the occlusive dressings topical induction application of 50% w/v Lontrel T in propylene glycol caused slight or moderate erythema, ulceration, exfoliation and isolated oedema, while topical induction application of propylene glycol alone (control group) caused slight erythema and exfoliation in one animal only.

Challenge application of 10% w/v Lontrel T in propylene glycol caused eschar formation in two test animals and slight erythema in three test and one control animal. Exfoliation was also seen in six test animals and one control. Challenge application of 3% w/v Lontrel T in propylene glycol or propylene glycol alone caused no dermal reaction. The responses to challenge applications are summarised in Table 6.2-7.

Table 6.2-7. Responses to challenge applications of Lontrel T

Group	Challenge treatment	No. of animals	Incidence of significant responses+		Total responders
			24 hours	48 hours	
Control	Vehicle alone	10	0	0	0
Test	Vehicle alone	20	0	0	0
Control	10% Lontrel T	10	1	0 (1 Exfoliation)	1
Test	10% Lontrel T	20	3	3 (6 Exfoliation)	3
Control	3% Lontrel T	10	0	0	0
Test	3% Lontrel T	20	0	0	0

+ Slight erythema or a more marked response (Grade 1 or above)

In the reported studies with positive control hexyl cinnamic aldehyde significant responses were seen in at least 70 % of animals.

Conclusion

Based on the results of the pre-test the dose of 3% w/v Lontrel T in propylene glycol or in adjuvant was selected for the main test. When comparing the results of the pre-test for 3% in vehicle (intradermal administration, 24&48h) with the next higher dose of 5% in vehicle the main difference is the ulceration noted in both animals in both time points at 5% dose. In addition, the 3% dose did not cause any effects on the other animal at any time point. The scores for 5%-30% doses in vehicle were comparable.

When comparing the results of the pre-test for 3% in FCA (intradermal administration, 24&48h) with 5% in FCA the scores did not differ much and ulceration was seen at only one time point in one animal at 5% in FCA dose. Superficial eschar was seen at 3% in FCA in one animal. Again, the scores for 5%-30% doses in FCA were comparable. Both animals on higher concentration group (5%-30%) were killed for humane reason after 48 hour examination but further clarification was not given in the study report.

According to OECD guideline 406 the dose for induction should cause mild-to moderate skin irritation. It should be noted that in the main test intradermal injection of 3% w/v Lontrel T in propylene glycol caused no reactions in 12 animals out of 20. In the guideline, it is said that consideration should be given to the use of FCA treated animals and in the main test intradermal injection of 3% w/v Lontrel T in the adjuvant caused moderate erythema and one animal out of 20 had no reactions.

Based on the results of the pre-test, the dose selected for topical induction for the main study was 50% w/v Lontrel T in propylene glycol. This caused slight erythema at 24 h and exfoliation at 24 h and 48 h on both animals. This is considered as faint reaction. It is noted that this was mentioned to be the maximum practicable concentration for topical administration.

Based on the pre-test, the dose of 10% w/v Lontrel T in propylene glycol was selected for topical challenge for the main study. This dose caused no reaction in animals and it was the highest dose tested. It was not clear why higher doses were not tested as for topical induction administration also doses of 30% and 50% test material in vehicle were tested. 50% w/v Lontrel T in propylene glycol was mentioned to be maximum practicable concentration for topical administration. In the pre-test topical induction with the dose of 30% test material in vehicle caused slight erythema on 24 h reading. This leaves open the results for doses between 10% -30 % of test substance in vehicle.

Based on all these reasons and considering the overall view there are uncertainties on dose selection. There is some indication of a possible sensitization potential as 15% of test group animals showed dermal responses with the selected doses. It is possible that if higher concentration was used on challenge phase it would have revealed more animals with dermal responses. It is considered that the doses should have been more properly selected as some uncertainties remain.

Under the conditions of this study, repeated applications of Lontrel T showed slight sensitising potential in the guinea pig, but because of uncertainties related to selected doses the study is considered as supportive information.

Study:	Lontrel T herbicidal chemical (penta process): Dermal sensitization potential in the Hartley albino guinea pig ([redacted] 1987)
Previous evaluation:	This study was submitted to DAR (2003). It was evaluated as supportive in the DAR. No re-evaluation has been performed and the text has not been modified.

Test guideline and GLP
The study was performed according to GLP and the method (USEPA 81-6 (1982)) complied principally with OECD guideline 406 (Buehler test method) (Directive 67/548/EEC, Annex V, Method B.6). However, in the study report, sensitivity or reliability data of the technique assessed was not provided. No justification for the choice of vehicle was included. A range finding test was done with only one animal and the dilutions used did not cause any irritation on guinea pig skin. In the main study, insufficient number of animals (10) was used in the treatment group and 3 induction applications of test substance are not nowadays always considered sufficient.

Materials and methods
A range finding test was done with one male Hartley albino guinea pig. A single application of 0.4 ml of 1% and 10% Lontrel T (95.4% clopyralid) in Dowanol DPM was topically applied to the skin of guinea pigs for six hours. No irritation occurred, so the 10% dilution of the formulation was chosen for the study.

For induction, ten male Hartley albino guinea pigs received 3 applications of 0.4 ml aliquots of 10% Lontrel T in Dowanol DPM once a week. A 10% solution of DER 331 epoxy resin in Dowanol DPM was used as a positive control and applied in a similar manner in another group of ten guinea pigs. The chambers were removed after a 6-hour exposure period and observations for erythema/oedema were recorded on the following day. The concentration of DER 331 was decreased to 5% for the third induction application and challenge, due to erythema observed after the second induction application.

The challenge phase was performed two weeks after the last induction application. The 10% Lontrel T or 5% DER 331 was applied to the guinea pigs in similar manner as throughout the induction phase for a period of 6 hours. Following washing and chemical depilation, the skin reactions were assessed 24 and 48 hours after the challenge application using a 4-score grading system for erythema.

Results
During induction or challenge, none of the guinea pigs treated with 10% Lontrel T revealed any signs of erythema or oedema. Five of ten animals challenged with 5%DER 331 revealed slight erythema.

Conclusion
Based on the results of this study, performed with too few animals, Lontrel T was not considered a potential skin sensitiser at the concentration tested. The study is supportive.

B.6.2.7. Phototoxicity

No studies were submitted.

RMS is of the opinion that the requirements for testing according to Commission Regulation (EU) No 283/2013 are fulfilled as clopyralid absorbs electromagnetic radiation between 290 - 700 nm and is expected to reach eyes or light exposed areas of skin. In addition the ultraviolet/visible molar extinction/absorption coefficient of the active substance is bigger than 10 L × mol^{-1} × cm^{-1}.

The notifier provided the following waiver request for phototoxicity study:

"In accordance with Commission Regulation (EU) No 283/2013, phototoxicity study is required for active substances in the circumstances: "*The in vitro study shall be required where the active substance absorbs electromagnetic radiation in the range 290-700 nm and is liable to reach the eyes or light-exposed areas of skin, either by direct contact or through systemic distribution*" and "*If the Ultraviolet/visible molar extinction/absorption coefficient of the active substance is less than 10 L × mol^{-1} × cm^{-1}, no toxicity testing is required*."

In the case of clopyralid, test substance solutions with concentrations of 19.4 and 58.3 µg/mL were prepared in pH <2, pH 7, and pH >10 buffer solutions. The pH <2 samples showed absorbance maxima (λmax) at 201, 226, and approximately 282 nm at the 19.4-µg/mL sample concentration and approximately 206, 223, and 282 nm at the 58.3-µg/mL sample concentration. The pH 7 samples showed absorbance maxima (λmax) at 198, 221, and 280 nm at the 19.4-µg/mL sample concentration and approximately 202, 220, and 280 nm at the 58.3-µg/mL sample concentration. The pH >10 samples showed absorbance maxima (λmax) at 199, 221, and 280 nm at the 19.4-µg/mL sample concentration and 203, 219, and 280 nm at the 58.3-µg/mL sample concentration. **No absorption maxima were observed above 290 nm in the pH <2, pH 7, or pH >10 samples** (Elliott, 2014).

Although clopyralid didn't absorb maximum light above 290 nm, the molar absorption coefficient was greater than 10 L x mol^{-1} x cm^{-1} between 290-312 nm (Table 1 below from [redacted] 2014).

If a study were conducted for phototoxicity, the wavelength would be between 290-312 nm which is a UVB range. According to only available test guideline for phototoxicity testing (OECD 432), "*generally UVB (320-290 nm) is of less relevance but is highly cytotoxic; the cytotoxicity increases 1000-fold as the wavelength goes from 313 to 280 nm".* Therefore, light at UVB range causes high level of cytotoxicity and conducting OECD 432 with clopyralid at UVA range would not be relevant.

The phototoxicity study is not warranted and was not conducted due to following reasons.

(1) Light of the UVA and visible regions (*i.e.,* 700-320 nm) is usually associated with phototoxicity reactions *in vivo* (OECD 432) at which clopyralid has absorption coefficient < 10 L x mol^{-1} x cm^{-1} .
(2) There are currently no established and accepted test guideline for UVB absorbers
(3) Clopyralid is stable following photolysis under 250-700 nm wavelengths in both aqueous and soil photolysis studies ([redacted] et al., 1994; [redacted] et al., 1994). As clopyralid toxicity is well understood, there are no unknown toxicity associated with the exposure. As a result, phototoxicity in humans from exposure to clorpyralid is not a concern

Table 1: Ultraviolet/visible molar extinction/absorption coefficient of clopyralid

Absorbance Wavelength (nm)	Absorbance (AU)	Molar Absorption Coefficient (L mol^{-1} cm^{-1})	Absorbance Wavelength (nm)	Absorbance (AU)	Molar Absorption Coefficient (L mol^{-1} cm^{-1})
250	2.27351	898.2	282	3.31663	1,310
251	2.23470	882.8	283	3.27268	1,293
252	2.22540	879.2	284	3.25512	1,286
253	2.23869	884.4	285	3.23930	1,280
254	2.28483	902.6	286	3.20882	1,268
255	2.35034	928.5	287	3.17703	1,255
256	2.43375	961.5	288	3.10566	1,227
257	2.53177	1,000	289	3.10037	1,225
258	2.65075	1,047	290	3.02565	1,195
259	2.79088	1,103	291	2.98188	1,178
260	2.93976	1,161	292	2.91487	1,152
261	3.08340	1,218	293	2.85673	1,129
262	3.22647	1,275	294	2.72068	1,075
263	3.29518	1,302	295	2.53156	1,000
264	3.33154	1,316	296	2.30656	911.2
265	3.41670	1,350	297	2.01667	796.7
266	3.41954	1,351	298	1.71636	678.1
267	3.37951	1,335	299	1.41607	559.4
268	3.44304	1,360	300	1.13426	448.1
269	3.43224	1,356	301	0.88065	347.9
270	3.52785	1,394	302	0.67013	264.7
271	3.40834	1,347	303	0.49981	197.5
272	3.32607	1,314	304	0.36692	145.0
273	3.31043	1,308	305	0.26656	105.3
274	3.34837	1,323	306	0.19145	75.63
275	3.32774	1,315	307	0.13818	54.59
276	3.31604	1,310	308	0.09995	39.49
277	3.37539	1,333	309	0.07304	28.86
278	3.36440	1,329	310	0.05391	21.30
279	3.32051	1,312	311	0.04061	16.04
280	3.28046	1,296	312	0.03142	12.41
281	3.30103	1,304	313	0.02473	9.768
Molecular Weight g/mol=	192.00		Cell Path Length=	1.000 cm	

References:

Batzer, F.R.; Concha, M.; Schelpler, K. (1994) Phocodegradatrion of [^{14}C] clopyralid in/on soil by natural sunlight. Dow AgroSciences. Study ID: GH-C 3489

Concha, M.; Shepler, K. (1994) Photodegradtion of [^{14}C] clopyralid in buffered aqueous solution at pH 7 by Natural Sunlight. Dow AgroSciences. Study ID: GH-C 3392

Elliott, T. (2014) Clopyralid: Determination of UV/Vis Absorption and Molar Absorptivity. Dow AgroSciences. Study ID: NAFST-14-91"

In the notifier's waiver request it was presented that if a study was conducted for phototoxicity the wavelength would be between 290 – 312 nm. The issue of testing wavelengths below 320 nm was discussed at Pesticide Peer review Meeting 137, and the opinion was:

the experts proposed that the phototoxicity test should not be performed if it has been demonstrated that the test material only absorbs at wavelengths lower than 313 nm and there is insufficient absorption at longer wavelengths to trigger testing in the absence of further guidance. (Technical report on the outcome of the pesticides peer review meeting on general recurring issues in mammalian toxicology. EFSA supporting publication 2016:EN-1074.).

However, there is still some uncertainty with regard to molar absorption coefficient in different pHs at 313 nm. It remains unclear what the molar absorption coefficient would be for the pH-values <2, 7 and >10 at 313 nm, and is possible that it might be considerably higher than for the example case of pH 2.66, taking into account their higher starting value at 290 nm.

RMS conclusion:

There is not sufficient information to draw the final conclusion.

B.6.3. SHORT-TERM TOXICITY

The summary of short-term toxicity of clopyralid is presented in Table B.6.3-1.

Rat: Dietary administration of clopyralid in a 4-week study at 500 mg/kg bw/day caused an irritant effect on the stomach characterised as folding of the non-glandular epithelium of the limiting ridge. In a 3-month study at higher doses, liver and kidney weight increases were recorded in males following 300 to 2500 mg/kg bw/day and in females at 2500 mg/kg bw/day. Males and females at 2500 mg/kg bw/day showed lesions of the gastric limiting ridge. The NOAEL for rat could not be established from the two acceptable studies.

Mouse: Following oral administration of clopyralid in the diet in a short-term study over 13 weeks, the liver was identified as the target organ based on liver weight increases and microscopic alterations to the centrilobular hepatocytes. Based on the acceptable study, the NOAEL for mouse is 750 mg/kg bw/day.

Dog: In a 12-month oral dog study, with a higher dose (320 mg/kg/day) there was an increase in liver weight and haematological effects were also observed. Based on the acceptable study, the NOAEL for dog is 100 mg/kg bw/day.

Rabbit: Repeated dermal administration of clopyralid up to 1000 mg/kg bw/day for 15 days in a 21-day rabbit study did not cause systemic toxicity at any dose (NOAEL 1000 mg/kg bw/day). In the supportive 13 day oral study on rabbits, multifocal erosions and/or ulcers of the stomach were seen.

The vapour pressure of clopyralid is less than 10^{-2} Pa and according to information in the dossier it is intended to be used only in field applications. Therefore the assessment of short term (28-day or 90-day) inhalation toxicity is not required. The results of the acute dermal toxicity study (B 6.2.2) and repeated dermal toxicity study (B 6.3.3) indicate that clopyralid has low toxicity by this route. A dermal 90-day toxicity study in the rat is therefore not required.

Table 6.3-1. Summary of subchronic toxicity studies of clopyralid

Study	Dose levels	NOAEL	LOAEL	Effects at LOAEL	Reference
4-week oral (dietary) rat	0, 150, 500, 1500 mg/kg bw/day	Males: 150 mg/kg bw/day Females: < 150 mg/kg bw/day	Males: 500 mg/kg bw/day Females: 150 mg/kg bw/day	Males: Changes in clinical biochemistry parameters, histopathological changes on stomach. Females: Kidney weight↑.	[redacted] *et al*., 1986 **Acceptable**
2-week oral (dietary) $B_6C_3F_1$ mouse	0, 0.2%, 1%, 2.5%, 5%, 10% dietary concentrations (about 0, 500, 2300, 5500, 9600, 19200) mg/kg bw/day	Males: <500 mg/kg bw/day Females: 500 mg/kg bw/day	Males: 500 mg/kg bw/day Females: 2300 mg/kg bw/day	Reduction in food consumption and slight histopathological alteration in the liver, increase of relative liver weight (males).	[redacted] *et al.* 1982 Supportive
13-day oral New Zealand White rabbit (Female)	0, 350, 500, 750 mg/kg bw/day	< 350 mg/kg bw/day	350 mg/kg bw/day	Multifocal erosions and/or ulcers of the stomach.	[redacted] *et al*., 1990 Supportive
3-month oral (dietary) rat	0, 300, 1500, 2500 mg/kg bw/day	Males: <300 mg/kg bw/day Females: 300 mg/kg	Males: 300 mg/kg bw/day Females: 1500 mg/kg	Males: Mean relative liver and kidney weights significantly↑, serum chemistry value (alkaline phosphase) ↓, microscopical	[redacted] *et al.*, 1983 **Acceptable**

		bw/day	bw/day	investigations on liver. Females: Mean body weight and bodyweight gain ↓, serum chemistry value (alkaline phosphase) ↓, microscopical investigations on liver.	
90-day oral (dietary) Sprague-Dawley rat	0, 5, 15, 50, 150 mg/kg bw/day	>150 mg/kg bw/day	>150 mg/kg bw/day	No toxicologically significant effects.	[redacted] *et al.,* 1973 Supportive
13-week oral (dietary) $B_6C_3F_1$ mouse	0, 200, 750, 2000, 5000 mg/kg bw/day	Males: 2000 mg/kg bw/day Females: 750 mg/kg bw/day	Males: 5000 mg/kg bw/day Females: 2000 mg/kg bw/day	Microscopical changes in the liver, mean relative liver weights ↑ (males).	[redacted] *et al*., 1983 **Acceptable**
12-month oral (dietary) Beagle dog	0, 100, 320, 1000 mg/kg bw/day	100 mg/kg bw/day	320 mg/kg bw/day	Haematological effects, liver weights ↑(males).	[redacted] *et al*., 1984 **Acceptable**
6-month oral (dietary) Beagle dog	0, 15, 50, 150 mg/kg bw/day	Males: >150 mg/kg bw/day Females: 50 mg/kg bw/day	Males: >150 mg/kg bw/day Females: 150 mg/kg bw/day	Males: No treatment related toxicological effects. Females: Increase in relative liver weight.	[redacted] *et al*., 1976 Supportive
180-day oral (dietary) Beagle dog	0, 15, 50, 150 mg/kg bw/day	>150 mg/kg bw/day	>150 mg/kg bw/day	No treatment related toxicological effects.	[redacted] 1975 Supportive
21-day dermal New Zealand White rabbit	0, 100, 500, 1000 mg/kg bw/day	Systemic NOAEL >1000 mg/kg bw/day	>1000 mg/kg bw/day	No treatment related systemic toxic effects.	[redacted] *et al.,* 1990 **Acceptable**

B.6.3.1. Oral 28-day study

Study:	Lontrel T toxicity to rats by dietary admixture for 4 weeks ([redacted] 1986)
Previous evaluation:	This study was submitted to DAR (2003). No guideline was mentioned but the study was performed mainly according to OECD guideline 407. The guideline was updated since the study was previously evaluated. Text has been modified and conclusions has changed. The study is acceptable.

Test guideline and GLP
The study was reported to be performed in compliance with the GLP standards. No guideline was mentioned but the study was performed mainly according to OECD guideline 407 (2008). Prior to assignment to the study

animals should have had veterinary examination, however as a deviation this was carried out on treatment day 5. Sensory reactivity to stimuli of different types was not studied on fourth week of the study. According to OECD guideline 407 hematological examinations should include also haematocrit and reticulocytes and clinical biochemistry determinations should include bile acids. These seemed not to be examined. The blood sample was taken at week 4 although as recommended in the guideline it should be done just prior to or as part of the procedure of euthanasia. During necropsy some organs were dissected free of fat and weighed, but epididymides, prostate + seminal vesicles with coagulating glands and thymus were not included although recommended by the guideline. Many tissues were preserved but only few were examined histopathologically (heart, liver, kidneys, adrenals, stomach and any macroscopically abnormal tissue). In the guideline mandatory endpoints recommended for the detection of endocrine disrupters (EDs) were listed. Only few of the endpoints were measured (weight of epididymides and prostate + seminal vesicles with coagulating glands not determined, histopathology of several tissues missing). Therefore there is not sufficient information in this study for screening EDs.

Materials and methods
Groups of 10 male and 10 female CD rats/dose level were exposed with Lontrel T (95% clopyralid) via the diet at concentrations of 0, 150, 500 and 1500 mg/kg bw/day for four weeks. The dose levels were chosen on the basis of previous work, however no further explanation was given. Samples of diet from week 1 and 4 were analysed for the accuracy of preparation. The animals were inspected twice per day for dead or moribund animals and clinical symptoms were registered for individual animals. Detailed individual examinations were performed once per day on every week day (examinations were not carried out at weekends), including recording of all signs of ill health, any behavioural changes or reaction to treatment. Body weights and food consumption were checked once per week. Water consumption was checked over daily periods during week 3. Because the terminal procedures took two days to complete, the treated animals continued to receive test material in the diet until the day on which they were killed. Prior to necropsy bone marrow for smears were collected. However, no further investigation was performed. The following organs were weighed at the end of the study: adrenals, brain, heart, kidneys, liver, ovaries, spleen and testes. Samples of blood were collected during week 4 for hematological investigations and clinical biochemistry on blood. Pathological study of some organs was carried out at the end of the test period (heart, liver, kidneys, adrenals, stomach and any macroscopically abnormal tissue), although many tissues were preserved. Histopathological examination was restricted to macroscopically abnormal tissues, the specified list of tissues and other tissue identified as a target organ for toxicity by other results of the study from all animals from the control group, and all animals from the high dosage level group and any macroscopically abnormal tissue in any animal. Statistical analyses were performed separately for males and females for food and water consumption, bodyweight, organ weight and clinical pathology data.

Results
During the study, no unscheduled deaths, treatment-related effects on behaviour or condition occurred. Food and water consumption was comparable to that of controls. Over the treatment period, a significantly reduced weight gain was recorded for males receiving 1500 mg/kg bw/day in comparison to controls. The bodyweight gain of other treated groups was similar to that of controls. The group mean intake of test material achieved over the treatment period was comparable to the nominal levels (100-107 % of the nominal level).

There was a slight but statistically significant increase in red blood cell count in males receiving 1500 mg/kg bw/day. Also, an increase in mean corpuscular haemoglobin concentration (MCHC) and mean corpuscular volume (MCV) following all doses was observed in males. However, the increases were not dose-related and were within expected control ranges.

In clinical biochemistry studies, a dosage-related increase in urea nitrogen levels was noted in treated females receiving 1500 or 500 mg/kg bw/day. Males were not affected. Females receiving 1500 mg/kg bw/day also exhibited reduced ALAT (alanine aminotransferase) levels. However, this change was considered to be of no toxicological significance in the study report. Significant changes in electrolyte levels were also recorded for treated animals receiving 1500 or 500 mg/kg bw/day. These changes included reduced calcium and chloride levels in males and increased sodium and potassium levels in females. Statistically significant reductions in plasma protein were recorded in males receiving 500 or 1500 mg/kg bw/day and females receiving 1500 mg/kg bw/day.

Kidney weights of all treated female groups and males receiving 1500 mg/kg bw/day were higher than those of controls. However, this finding in treated females was not dosage related in degree and in the study report it was thought to be probably unrelated to treatment. However, the increases in kidney weights were statistically

significant. Hydronephrosis in kidneys was observed in males (2/10) receiving 1500 mg/kg bw/day. Also pale cortical area was observed on one male receiving 500 mg/kg bw/day.

Table 6.3-2 Kidney weights of female rats

Group/dosage (mg/kg/day)	Kidneys (g)	% of control
Control	A 1.89 (1.91)	
150	2.26** (2.34)	119.6 (122.5)
500	2.17** (2.10)	114.8 (109.9)
1500	2.13** (2.10)	112.6 (109.9)

A organ weights adjusted for final bodyweights as covariate. Where data have been adjusted, the absolute values are given in parentheses.
Level of significance, Williams' test: ** $p<0.01$ in comparison with controls.

In kidneys, a group on basophilic cortical tubules on males (2/10) and female (1/10), pelvic dilatation (right) on males (2/10), occasional groups of basophilic cortical tubules on males (1/10), cystic medullary tubule on males (1/10) and minimal focal mineralisation at the corticomedullary junction on females (3/10) receiving 1500 mg/kg bw/day was observed. Also an area of dilated/basophilic tubules with inflammatory cells on one male receiving 500 mg/kg bw/day was observed. No histopathological determinations from the kidneys of the animals receiving 150 mg/kg bw/day or females receiving 500 mg/kg bw/day were made.

Thickening of the forestomach limiting ridge was noted in females (7/10) and males (2/10) receiving 1500 mg/kg bw/day. This was also observed in a single female treated with 500 mg/kg bw/day. On males (1/10) receiving 1500 mg/kg bw/day and on females (1/10) receiving 150 mg/kg bw/day haemorrhagic depressions of stomach were observed.

In stomach, in the histopathological assessment, minimal acanthosis of non-glandular epithelium and minimal folding of non-glandular epithelium of the limiting ridge were observed in males (10/10) and females (9/10) receiving 1500 mg/kg bw/day, and in males (5/10) and females (5/10) receiving 500 mg/kg bw/day. These changes were not seen in animals receiving 150 mg/kg bw/day or in control animals.

On the macroscopical examination also on males (1/10) receiving 1500 mg/kg bw/day also congested thymus, small testes, minimal contents on seminal vesicle and ulcerated, tip missing tail was observed. In microscopic examination also focus on myocarditis on heart of one female receiving 1500 mg/kg bw/day was observed. In addition on one male receiving 1500 mg/kg bw/day minimal congestion and haemorrhage on thymus and on testes minimal atrophic seminiferous tubules was observed.

Conclusion
The NOAEL for females could not be determined since there was a statistically significant increase in kidney weight observed in all dose levels. The NOAEL for males is 150 mg/kg bw/day based on lesions of the stomach epithelium following administration of 500 and 1500 mg/kg bw/day of Lontrel T. This indicated an irritant effect on the stomach. The NOAEL of the study could not be established. The study is acceptable.

Study:	Lontrel T (Dowco 290): results of a 2-week dietary probe study in B6C3F1 mice ([redacted] *et al.* 1982)
Previous evaluation:	This study was submitted to DAR (2003). It was evaluated as supportive in the DAR. No re-evaluation has been performed, the text is slightly modified.

Test guideline and GLP
The study was performed according to GLP. The study was not performed using any official guideline or test method, but is broadly in accordance with OECD guideline 407 (Directive 67/548/EEC, Annex V, Method B.7). The study was designed to identify a maximum tolerated dose for Lontrel T and the primary target organ in mice to aid in the selection of dose levels for subchronic and chronic toxicity-oncogenicity studies in mice.

Materials and methods
Groups of 5 male and 5 female B6C3F1 mice/dose level were exposed to clopyralid via diet containing 0, 0.2, 1.0, 2.5, 5.0 or 10% of Lontrel T (95% clopyralid) (0, 500, 2300, 5500, 9600 or 19200 mg Lontrel T/kg bw/day) for two weeks. All mice at the 10% level died or were moribund on day 5 because of inanition, judged to be due

to unpalatability of the test diets, and were excluded from further toxicity evaluation. The mice were monitored twice daily during the workweek and once daily during weekends. Body weights and food consumption was monitored twice weekly. Data were collected and analysed statistically on mortality, clinical appearance and behaviour, clinical chemistry (control and 5.0% groups only), haematology (control and 5.0% groups only), organ weights, gross pathology and histopathological examination of liver and kidneys.

Results
Consumption of the 9600 mg/kg bw/day diet was considerably reduced in both sexes for the first 5 days of the study and continued to be somewhat decreased for the remainder of the study. The mice with 9600 mg/kg bw/day diet showed decreased activity and reduced faeces during the first week but appeared to be normal thereafter. Both sexes fed the 5500 and 2300 mg/kg bw/day diets had decreased food intake for the initial 4 or 5 days, but consumption of these test diets was comparable to controls thereafter. Both sexes fed the 9600 mg/kg bw/day diets showed slight weight loss on day 4, concomitant with the observed decrease in food intake. Some recovery of body weight occurred, although there was little or no overall weight gain for the 2-week period.

In clinical chemistry or haematology studies, there were no treatment-related effects in 9600 mg/kg bw/day diet group. Absolute and relative liver weights showed increases compared to controls in all treated male groups and in females receiving the 9600 mg/kg bw/day concentration (Table 6.3-3). However, the organ weight data for males in general appeared atypical, since there were several parameters (brain, heart, kidneys) that showed isolated, non-dose related significant differences in treated groups. There were no toxicologically significant treatment related gross pathological effects.

Table 6.3-3 Liver weight effects following 2-weeks dietary administration of clopyralid to mice

Parameter	Males					Females				
Dosage (mg/kg bw/day)	0	500	2300	5500	9600	0	500	2300	5500	9600
Final Body weight (g)	21.2	21.5	21.8	20.9	19.1	20.9	21.0	20.4	20.0	18.4
Absolute liver weight (g) (% of control)	1.23	1.38* (112)	1.44* (117)	1.44* (117)	1.42* (115)	1.32	1.29	1.24	1.36	1.42
Relative liver weight (g/100g) (% of control)	5.79	6.45* (111)	6.60* (114)	6.90* (119)	7.40* (128)	6.28	6.14	6.04	6.80	7.71*
% Increase	-	11.4	14.0	19.2	27.8	-	-2.2	-3.8	8.3	22.8

* Statistically significantly different from untreated controls at $p \leq 0.05$.

Conclusion
The NOAEL for female mice was the 0.2% dietary concentration (500 mg/kg bw/day), based on a reduction in food consumption following administration of Lontrel T at a dietary concentration of 1.0% (2300 mg/kg bw/day). Unpalatability of the test diets, was evident with higher doses during the first week of the study. For males, a NOAEL-value could not be set because absolute and relative liver weights of the animals increased with all doses. The study is supportive.

Study:	Clopyralid: 13-day repeated oral gavage study in New Zealand White rabbits ([redacted] *et al*., 1990)
Previous evaluation:	This study was submitted to DAR (2003). It was evaluated as supportive in the DAR. No re-evaluation has been performed and the text has not been modified.

Test guideline and GLP
The study was performed according to GLP. The study was not performed using any official guideline or test method. The study was designed to identify a maximum tolerated dose level for repeated oral exposure to clopyralid in rabbits.

Materials and methods
Groups of 3 non-pregnant female New Zealand White rabbits were administered clopyralid (96.4%) in corn oil by gavage for up to 13 days at dose levels of 350, 500 and 750 mg/kg bw/day. A control group of 3 female rabbits was administered the corn oil vehicle for 20 days. During the course of the study, all animals were observed daily for signs of toxicity and body weights were recorded. Animals that died or appeared moribund were submitted for a complete necropsy. On the day following the final dose, all animals in the treatment groups

that survived the dosing regimen were weighed, killed and submitted to complete necropsy. Liver and kidney weights were recorded. Histological examination was not performed.

Results
Two rabbits treated at 750 mg/kg bw/day died by day 5 and two rabbits treated at 500 mg/kg bw/day died on day 8. The third rabbit in both groups was moribund and was therefore sacrificed prior to scheduled termination. There were no other mortalities. The 500 and 750 mg/kg bw/day doses caused excessive toxicity, including moribund state, laboured respiration and lethargy. These animals showed also substantial body weight loss. There was no overall effect of treatment at 350 mg/kg bw/day on body weight gain compared to untreated control animals. Laboured respiration occurred in one animal treated at 350 mg/kg bw/day.

All animals treated at 500 and 750 mg/kg bw/day had either focal or multifocal erosions and/or ulcers of the gastric mucosa or focal haemorrhage in the stomach. The moribund rabbit sacrificed on day 6 had also inflammation of the caecal wall and dark urine. One rabbit treated at 350 mg/kg bw/day had multifocal erosions and/or ulcers of the stomach but two other rabbits in the group had no gross lesions.

Conclusion
A NOAEL-value for female rabbits could not be determined due to toxic effects recorded at 350 mg/kg bw/day, the lowest dose tested. One animal in this group exhibited stomach erosions and/or ulcers at necropsy. Similar gross lesions were noted in most rabbits given higher doses. The study is supportive.

B.6.3.2. Oral 90- day study

Study:	Dowco 290 Herbicide: Results of A Three-Month Dietary Toxicity Study in Rats ([redacted] 1983)
Previous evaluation:	This study was submitted to DAR (2003). No guideline was stated but the study was performed broadly according to OECD guideline 408. Text has been modified but conclusions has not changed. The study is acceptable.

Test guideline and GLP
According to information from DAR (2003) the study was performed according to GLP. However, it seems that the only statement of GLP was in the Quality assurance statement, mentioning that in compliance with GLP regulations the study was inspected by the QA Unit. No official guideline was stated but the study is broadly in accordance with OECD guideline 408 (1998). The dose levels used in the study did not fall in the two to four fold interval recommended in the OECD guideline. According to the guideline the highest dose level should be chosen with the aim to induce toxicity but not death or severe suffering. Based on a brief description of the previous study results which were used as basis to choose the dose levels, effects were seen on dose levels higher than 2500 mg/kg bw/day, which was selected as the highest dose level. Only some of the haematological, clinical biochemistry and urinalysis determinations recommended in the guideline were investigated (ALT, AP, BUN, red and white blood cells count, haemoglobin, platelets count, packed cell volume, and from urinalysis ph, protein, glucose, ketones, bilirubin, occult blood, urobilinogen and specific gravity measured). The samples for hematology investigations were taken only on days 86-87, and not at the end of the test, from only some rats of each group. The samples for urinalysis were taken on day 84 although the guideline recommends taking it on the last week of the study. The weight of all of the tissues recommended in the guideline were not determined (adrenals, epididymides, uterus, spleen missing). Ophthalmological examination was not made. No sensory reactivity to stimuli of different types, assessment of grip strength and motor activity assessment was done. It seems that detailed clinical examinations were not made once a week during the study.

Materials and methods
Groups of 15 male and 15 female Fischer-344 rats/dose level were exposed with Dowco 290 herbicide (96.4% clopyralid) via diet at concentrations of 0, 300, 1500 and 2500 mg/kg bw/day for three months (98-99 days). The dose levels were chosen on the basis of previous work. Samples of control feed, premixes and diets were analysed for concentration, homogeneity and stability. The rats were observed at least once a day for changes in appearance and behaviour. Body weights and food consumption were recorded once per week. The following organs were weighted at the end of the study: brain, heart, kidneys, liver, ovaries, testes and thymus. Serum biochemistry (alanine transaminase, alkaline phosphatase, urea nitrogen) and haematology values (packed cell volume, red blood cells, haemoglobin, white blood cells, platelets, WBC differential), urinalysis and pathological study of organs was carried out at the end of the test period. Liver, kidney and stomach tissues were

evaluated for histopathological alterations from all animals. The samples of other tissues (45) were evaluated only from animals of control and high dose level groups. Results of the serum chemistry, haematology, urine specific gravity results, body weight and body weight gain, food consumption figures and final body and organ weight results were analysed statistically.

Results
During the study, there were no unscheduled deaths and gross impairment of general health status was found only on one animal (bloody urine, male high level dose group). Mean body weights and body weight gain were decreased in the male and female high dose groups (2500 mg/kg bw/day) and in the female middle dose level group (1500 mg/kg bw/day). There were no treatment-related effects on food consumption in males. Food consumption in females was significantly reduced compared to control following 2500 mg/kg bw/day throughout most of the study. Reduced food consumption at 1500 mg/kg bw/day was occasionally seen. The detected mean concentrations of clopyralid in the test diet were within ±14% of the targeted.

Mean relative liver and kidney weights were significantly increased in males following all doses and in females following the 2500 mg/kg bw/day dose (Table B.6.3-4). There was also increase in the mean relative brain weight, decrease in the absolute heart weight and decrease in the mean absolute thymus weight in the high dose level females.

According to the study report there were no treatment-related effects on urinalysis or clinical chemistry and haematological parameters. Regarding serum chemistry, there was statistically significant decrease in ALT (alanine transaminase) values in high dose level males and females and middle dose level males. The AP (alkaline phosphase) values were statistically significantly lower than control in all groups but high dose level females. BUN (serum urea nitrogen) values were statistically significantly lower than control in middle and high dose level males.

No gross or microscopic lesions were found that corresponded with the increased relative liver or kidney weights. In liver, in the microscopic investigation very mild to mild focal aggregations of mononuclear cells were observed on all dose groups (including control) on males and females (on 10-15/15 animal/dose). In addition, on all dose levels on females very mild to mild hepatocellular vacuolar change consistent with fatty change was observed (9-12/15 animals/ dose). In kidneys, small cortical cysts were observed on one female at low dose group. On lungs, mild peribronchiolar lymphocytic infiltration (on 11-15/15 animal/dose) and mild to moderate focal perivascular granulomatous inflammation was observed (on 5-11/15 animal/dose) in control and high dose level animals.

Table B.6.3-4 Effect of clopyralid on kidney and liver weights in rats following administration of clopyralid for three months

Parameter	Males				Females			
Dosage (mg/kg bw/day)	0	300	1500	2500	0	300	1500	2500
Final body weight (g)	298.5	294.1	297.2	283.9*	168.9	165.4	163.2	154.2*
Kidney weight (g)	2.031	2.083	2.162*	2.121	1.208	1.202	1.195	1.179
Relative kidney weight (g/100g) (% of control)	0.681	0.708* (104)	0.727* (106.8)	0.747* (109.7)	0.716	0.727	0.733	0.766*
% Increase	-	4.0	6.8	9.7	-	1.5	2.4	7.0
Liver weight (g)	7.586	7.683	7.968	7.958	4.390	4.287	4.303	4.152
Relative liver weight (g/100g) (% of control)	2.542	2.611* (102.7)	2.680* (105.4)	2.802* (110.2)	2.599	2.593	2.638	2.694*
% Increase	-	2.7	5.4	10.2	-	-	1.5	3.7

* Statistically significantly different from untreated controls at $p \leq 0.05$.

Slight irregularities and accentuation of the limiting ridge at the junction of the squamous and glandular portions of the stomach were found. This lesion was found in most (14/15 males and 10/15 females) animals fed with 2500 mg/kg bw/day diets, but in none of the males or females of the control group or lower dose-groups. Microscopically the lesion was found to consist of increased thickness of the gastric mucosa caused by irregular folds and corrugations of the stratified squamous epithelium on the anterior face of the limiting ridge.

Conclusion
The NOAEL for male rats could not be established due to statistically significant increased relative liver and

kidney weights recorded at all doses. At dose level 300 mg/kg bw/day also the AP (alkaline phosphase) values were statistically significantly lower than control. Some microscopical investigations were made on livers. The NOAEL for female rats is 300 mg/kg bw/day, based on the reduced body weight gain at 1500 mg/kg bw/day and increased relative liver and kidney weight (statistically significant) following 2500 mg/kg bw/day dose. The study is acceptable.

Study:	Dowco 290 pesticide: results of a 90-day dietary feeding study in rats ([redacted] *et al.,* 1973)
Previous evaluation:	This study was submitted to DAR (2003). It was evaluated as supportive in the DAR. No re-evaluation has been performed and the text has not been modified.

Test guideline and GLP
The study was not performed using any official guideline or test method, and it was not done under GLP. The study was performed in 1973 before the existence of OECD or GLP guidelines.

Materials and methods
Fifteen Sprague-Dawley Spartan rats/sex/group received doses of 0, 5, 15, 50 or 150 mg/kg bw/day Dowco 290 (96.3% clopyralid) for 90 days. The animals were weighed weekly. Observations for changes in appearance and behaviour were made at least weekly in addition to the times when body weights or food consumption were determined. Data were collected on haematology, clinical chemistry, urinalysis, organ weights, gross pathology and histopathology. Haematological evaluations and urinalysis were performed on 5 rats of each sex from the control and highest dose level groups at 30 days and approximately 1 week prior to termination of the study. Clinical chemistry determinations of blood urea nitrogen (BUN), serum alkaline phosphatase activity (AP) and serum alanine aminotransferase (ALAT) were made on 5 rats/sex/dose level at the termination of the study. A gross pathologic examination was conducted on all rats and the weights of heart, brain, liver, kidney and testes were determined. Specimens from different tissues (29) were preserved and examined microscopically. Statistical evaluation of the results was done.

Results
All rats survived the experimental period and appeared normal throughout the study. The food consumption and weight gain of animals receiving various doses was normal at all recording intervals. Also, no significant differences were observed between test groups and controls relative to the weights of the body, brain, heart, liver, kidney and testes weights or to the respective organ to body weight ratios. Gross pathologic or microscopic examinations revealed no visible lesions related to treatment.

Most parameters of haematology and clinical chemistry appeared normal. Only alkaline phosphatase values in males receiving doses of 150, 15 and 5 mg/kg bw/day were slightly depressed. The 50 mg/kg bw/day dose level group was comparable to control group. The apparent depression of alkaline phosphatase were likely to reflect the unusually high control values. The alkaline phosphatase values for the control males were significantly higher than those of control females, as well as, other control males used in previous and current studies in this laboratory.

Conclusion
In this study, NOAEL for male and female rats was >150 mg/kg bw/day, based on the absence of toxicologically significant effects following administration of Dowco 290 for 90 days. The study is supportive.

Study:	Dowco 290: Results of a 13-week dietary toxicity study in B6C3F1 mice ([redacted] 1983)
Previous evaluation:	This study was submitted to DAR (2003). No official guideline was stated but the study was performed broadly according to OECD guideline 408. Text has been modified but conclusions has not changed. The study is acceptable.

Test guideline and GLP
The study was performed in accordance with Good laboratory practices for non-clinical studies. No official guideline was stated but the study is broadly in accordance with OECD guideline 408 (1998). It seems that detailed clinical observations were not made once a week. Ophthalmological examination was made only during necropsy (glass slide technique). Sensory reactivity to stimuli of different types, assessment of grip strength and

motor activity assessment were not examined. All of the hematological and clinical biochemistry determinations mentioned in the guideline were not made. Part of the determinations were made only on high dose level and control group. The weight of all of the tissues recommended in the guideline were not determined (adrenals, epididymides, uterus, ovaries, thymus, spleen missing)

Materials and methods
Groups of 10 $B_6C_3F_1$ mice/sex/dose level received diets containing Dowco 290 (97% clopyralid) at concentrations calculated to provide doses of 0, 200, 750, 2000 or 5000 mg/kg bw/day for 13 weeks (95-96 days on the test). Analyses of diets were conducted three times during the study. The mice were examined twice daily for signs of toxicity and changes in behaviour. Body weight and food consumption data were recorded weekly. Blood samples were taken prior to sacrifice. Clinical biochemistry determinations of urea nitrogen concentration, glutamic pyruvic transaminase activity, glutamic oxaloacetic transaminase activity, alkaline phosphatase activity, glucose, total protein, albumin and globulin were made from serum samples from all mice at all dose levels. Haematologic evaluations of packed cell volume, haemoglobin, total erythrocytes, leukocyte count, indices (MCV, MCH, MCHC) and platelets were performed on all mice at all dose levels. Stained blood smear examination and differential leukocyte counts were conducted on mice at the control and high dose levels. After 13 weeks the mice were sacrificed and subjected to gross necropsy. Necropsy examinations included an ophthalmologic examination. Weights of the brain, liver, kidneys, heart and testes were recorded. A set of tissues (50) from all males and females were preserved. These were examined microscopically from all mice in the control and top dose group and selected tissues from mice in the intermediate dose groups. Data on body weights, food consumption, clinical chemistry data, hematology data, absolute and relative organ weights were evaluated statistically.

Results
The mice appeared normal throughout the 13-week study and the in-life clinical observations were not remarkable. One mouse died during the study (traumatic injury). A slight, but statistically different from control, decrease in body weight was evident throughout the study in both male and female mice at the 5000 mg/kg bw/day dose level. However, there was no significant reduction in food consumption at any dose level. In few cases statistical outliers were excluded from calculations of food consumption data. The concentration of test substance in the determined dose levels in the diet ranged from 97 to 105% of nominal.

Relative liver weights (Table 6.3-5) were increased significantly in male and female mice at the 5000 mg/kg bw/day. These increases were accompanied by morphologic alteration detected in all mice at the 5000 mg/kg bw/day. The centrilobular hepatocytes of the livers were increased in size and had altered tinctorial properties. The microscopic change was also present in most female mice (8/10) at the 2000 mg/kg bw/day dose level. In addition very slight aggregates of reticuloendothelial cells was observed on all dose levels on both males and females (4-6/9-10 males and 6-8/10 females). Statistically different (lower) weights in absolute liver weights (groups 200 and 750 mg/kg bw/day) were observed in males.

Table 6.3-5 Effect of clopyralid on mean liver weights in mice following administration of clopyralid for 13 weeks

Parameter	Males (mg/kg bw/day)					Females (mg/kg bw/day)				
	0	200	750	2000	5000	0	200	750	2000	5000
Liver weight (g)	1.43	1.30*	1.30*	1.35	1.43	1.34	1.31	1.30	1.32	1.41
Relative liver weight (%)	5.15	4.90	5.00	5.11	5.67*	5.55	5.49	5.50	5.58	5.92*

* Statistically significantly different from untreated controls at $p \leq 0.05$.

In males also statistically different (higher) weights in relative brain weight (group 5000 mg/kg bw/day) and in relative testes weight (groups 750 and 5000 mg/kg bw/day) were seen. Statistically different (lower) weights in absolute heart and kidney weight (both in group 5000 mg/kg bw/day) were observed.

Only occasional statistically different values from control were seen in mean hematology or clinical chemistry data.

Gross pathologic observations included alopecia of abdomen in females on all dose levels (on 5-10 /10 animals) and 1/9 males on middle dose level. Also alopecia on thorax was observed but on fewer animals.

Histopathological observatios included inflammation in gallbaldder (1/10 males and 2/10 females), pancreas (1/10 females), lungs (1/10 males and 2/10 females), aorta (1/10 females), skin (1/10 males, 3/9 females), tongue (2/10 males, 1/10 females) and nasal turbinates (1/10 females). In adrenals focus of altered cells (cortex, focal) on 1/10 males, very slight hyperplasia (spindle cell, cortex) on 8/10 males and slight hyperplasia (spindle cell, cortex) on 10/10 females was observed on high dose level. These lesions in adrenals were observed in control group approximately at the same incidence. In kidneys very slight aggregates of mononuclear (predominantly lymphoid) cells (cortex) (all dose levels, 1-2/9-10 males, 1-3/10 females), very slight degeneration of tubules (focal) (all dose levels 1-3/9-10 males and two dose levels 1-2/10 females) and very slight mineralization (1/10 females) was observed. In urinary bladder very slight aggregates of mononuclear (predominantly lymphoid) cells (submucosa) was observed on 1/10 males and 2/10 females in the high dose group. These lesions in urinary bladder were observed in control group at the same incidence. In thyroid gland hyperplasia (focal, epithelial follicles) was observed on 1/10 females in the high dose group. In tongue metaplasia (cartilage, submucosa, focal) was observed on 1/10 males in the high dose group.

Conclusion
The NOAEL for male mice was 2000 mg/kg bw/day and 750 mg/kg bw/day for females. These are based on the microscopical changes in the liver (centrilobular hepatocytes of the livers were increased in size and had altered tinctorial properties) on females at the dose level 2000 mg/kg bw/day and on males on dose level 5000 mg/kg bw/day. Also relative liver weights were increased significantly in male and female mice at dose level 5000 mg/kg bw/day. The study is acceptable.

Study:	A 12-month oral toxicity study of 3,6-dichloropicolinic acid in the beagle dogs ([redacted] 1984)
Previous evaluation:	This study was submitted to DAR (2003). No official guideline was stated but the study was performed broadly according to OECD guideline 409. Text has been modified but conclusions have not changed. The study is acceptable.

Test guideline and GLP
The study was performed in compliance with the United States FDA GLP regulations. The study was not performed using any official guideline or test method, but is predominantly in accordance with OECD guideline 409 (1998), with the administration period extended to 12 months. It seems that detailed clinical examinations were not made once a week, however, e.g. skin and eye observations were made during the clinical examinations. It is mentioned that the blood sample was taken at week 52 but it is not completely sure if this was part of the procedure of sacrifice or not. Measurement of glotting potential was not made on hematology investigations. Serum electrolytes were not investigated but it seems to be an area of consideration in the guideline. The weight of the following organs recommended in the guideline were not measured: gall bladder, epididymides, uterus, thyroid with parathyroid, thymus, spleen.

Materials and methods
Groups of 6 beagle dogs/sex/dose level received diets containing 3,6-dichloropicolinic acid (purity 95.8% clopyralid) at concentrations calculated to provide doses of 0, 100, 320 and 1000 mg/kg bw clopyralid/day for a minimum of 52 weeks. Samples of diet prepared for weeks 1, 4, 12, 26, 38 and 50 were submitted for analysis. The dogs were observed twice daily for mortality and/or clinical signs. Body weights were recorded weekly during the 4-week acclimatisation period and during treatment. Food consumption was determined daily during the pre-treatment and treatment periods up to study day 3.1 (expression from the study report, exact day unclear). Thereafter, food consumption was measured over weekly intervals. Ophthalmoscopy was conducted one week before dosing and during weeks 27 and 52 of dosage. Laboratory investigations were performed on each animal twice prior to the treatment and during treatment weeks 14, 27 and 52. Clinical biochemistry determinations of serum urea nitrogen concentration, serum glutamic pyruvic transaminase (SGPT), serum glutamic oxalactic transaminase (SGOT) and serum alkaline phosphatase (SAP) activity, glucose, total protein, albumin (A), globulin (G), A/G ratio, total cholesterol and total bilirubin was determined. Haematological measurements of haematocrit, haemoglobin, platelet count, total erythrocytes and leukocytes, differential leukocyte count and calculation of Wintrobe's constants (MCV, MCH, MCHC) were measured. From urine samples, the following analyses were conducted: colour, volume, appearance, specific gravity, pH, bilirubin, glucose, occult blood, urobilinogen, ketones, proteins, and microscopic examination of the centrifuged deposit. Faecal flotation test was performed from each dog before the treatment and after 6 and 12 months of treatment.

The adrenals, brain, heart, liver, kidneys, ovaries and testes of all animals were weighed. Representative portions of these and other tissues (40) were preserved. Tissues from all control and high dose male and female dogs were examined histopathologically. Additional histopathological evaluation was performed on the livers, kidneys, adrenals, lungs and any abnormalities in the intermediate and low dose groups. Results were evaluated statistically.

Results

The dietary concentrations of 3,6-dichloropicolinic acid ranged from 91.8 to 114% of the targeted dose. The average daily doses of 3,6-dichloropicolinic acid, calculated over the entire study period, for males and females were 99, 301 and 983 mg/kg bw/day and 99, 319 and 977 mg/kg bw/day, respectively.

No treatment related changes were observed in appearance or behaviour of the dogs. The clinical findings observed (variety of skin conditions, eye abnormalities, digestive system disturbances and less frequently also focal swelling of the palate and/or tonsils, redness or pallor of the buccal mucosa, the presence of a brown discharge in the ear canal, the presence of worms in the feces, heat on females) were reported to occur on control group about the same frequency, and were presented to be commonly observed on beagle dogs.

Animals in all treatment groups gained weight comparable to controls. However, at least on week 53 the group mean body weight of high dose females (10.82 kg) was lower than that of controls (12.78 kg), being about 85% of the weight of the controls. There were no differences in food consumption. There were no changes in the eyes, which could be ascribed to treatment. Occasionally noted, infrequent and mild changes observed included conjunctivitis, inflammation of the membrane nictitans, iritis, ciliary infection, the presence of cells in the anterior chamber of the eye and neovascularization of the cornea. Urinalysis did not reveal any differences between animals from treated or non-treated groups. In fecal flotation analysis ova from the intestinal parasites were occasionally observed.

Highly significant reductions in red blood cell counts for males and females in intermediate and high dose groups were observed after 14, 27 and 52 weeks of treatment (Table 6.3-6). The red blood cell count of the low dose groups was also lowered, but not significantly different from that of the control group. Haematological parameters which are dependent on the red blood cell count, such as total haemoglobin concentration and hematocrit, also tended to be reduced in the intermediate and the high dose groups during treatment in comparison to control animals. Not all of these differences were statistically significant.

Table 6.3-6 Effect of clopyralid on haematology parameters in dogs following administration of clopyralid for 12 months

Parameter, assessment time		Males (mg/kg bw/day)				Females (mg/kg bw/day)			
		0	100	320	1000	0	100	320	1000
Red blood cell count (x10^6)	14 weeks	6.62	6.49	5.91**	5.54**	6.97	6.81	6.19*	5.47**
	27 weeks	7.44	6.85	6.45**	5.78**	7.42	7.22	6.12**	5.51**
	52 weeks	7.29	7.23	6.59	6.10**	7.26	6.78	6.30*	5.55**
Total haemoglobin concentration (g/100 ml)	14 weeks	15.2	15.8	14.2	13.8	16.4	16.3	14.8	13.7*
	27 weeks	17.2	16.4	15.3*	14.3**	17.3	17.2	14.7*	13.6**
	52 weeks	16.8	17.5	16.2	15.7	17.4	16.7	15.9	14.1**
Hematocrit (%)	14 weeks	42.5	43.5	40.0	38.8	45.7	44.8	41.5	38.5*
	27 weeks	48.0	45.3	43.3*	40.3**	48.2	47.5	40.8*	39.2**
	52 weeks	46.7	47.2	44.0	42.7	47.2	45.3	43.5	39.5*
MCH	14 weeks	23.0	24.3**	24.0	25.0**	23.5	23.8	23.7	25.2**
	27 weeks	23.9	24.0*	23.8	25.0**	23.2	23.8	24.0	24.8**
	52 weeks	23.6	24.3*	24.7*	26.0**	24.2	24.5	25.2*	25.7**
MCHC	14 weeks	35.7	36.2	35.3	35.5	36.0	36.3	35.8	35.5*
	27 weeks	35.7	36.2	35.3	35.3	35.8	36.3	35.8	34.5
	52 weeks	36.0	37.2**	36.8	37.0*	37.0	37.0	36.2	35.7*

Statistically significantly different from untreated controls at * $p \leq 0.05$ or ** at $p \leq 0.01$.

MCV and MCH were statistically greater in the high dose males and females on weeks 14, 27 and 52 and on intermediate dose males and females on week 52. Statistically different (higher) MCH values from control were also seen on low dose males on weeks 14, 27 and 52. MCHC values were statistically higher on low and high dose males on week 52 and statistically lower on high dose females on weeks 14 and 52. Significant differences in females in white blood cell and platelet counts and in the percent segmented neutrophils and lymphocytes were seen occasionally.

Total protein, serum albumin and/or serum globulin were significantly reduced in high dose males and females after 14 weeks of treatment. These parameters were significantly reduced in high dose males and high and intermediate dose females after 27 weeks of treatment. After 52 weeks of treatment, a trend toward reduced total protein and serum albumin and/or globulin existed for high dose males and females, but these differences were statistically significant only for serum albumin in the high dose females. In addition, during both pre-treatment and treatment period there were occasional significant differences between control and treated groups in various other clinical biochemistry parameters. BUN was significantly reduced on weeks 14, 27 and 52 on high dose females.

Absolute liver weights of intermediate dose males and the absolute and relative liver weights of high dose males and females increased significantly (Table 6.3-7). Also, there was a trend toward increased relative kidney and heart weights in high dose males and females, with significant increases being observed in the relative kidney weight of high dose males and the relative heart weight of high dose females. There was also significantly reduced relative adrenals weight in low dose males but this was not observed on other dose levels or on females.

Table 6.3-7 Effect of clopyralid on liver weights in dogs following administration of clopyralid for 12 months

Parameter	Males (mg/kg bw/day)				Females (mg/kg bw/day)			
	0	100	320	1000	0	100	320	1000
Liver weight (g) (% of control)	344.39	356.76 (103.6%)	388.79** (112.9%)	450.77** (130.9%)	312.98	303.95 (97%)	348.23 (111.3%)	396.07* (126.5%)
Relative liver weight (%)	25.60	24.92	26.58	36.37**	26.02	24.24	29.91	38.11**

Statistically significantly different from untreated controls at * $p \leq 0.05$ or ** at $p \leq 0.01$.

At necropsy, the presence of areas, foci or nodules of various colours and consistencies in the lungs of test substance treated dogs, were revealed at all dose levels. Such changes were not seen in control dogs. Histologically, the lung lesions were presented as chronic bronchiolitis with or without granulomas around foreign material. The foreign material appeared to be plant material suggestive of food origin. The test material has been shown to be irritant, and the report states that such irritancy may have caused the dogs to "mouth" their food, which may have resulted in the incidental inhalation of some particles. Other findings in the lungs were interstitial pneumonia (1 male, 3 females), pleural fibrosis (1 male, 2 females), focal dilatation of alveoli (4 males, 3 females) and mononuclear cell infiltration (1 control and 1 test group male, 2 control and 2 test group females). In some control and treated animals also histiocytosis, brown pigment around bronchioles, alveolar oedema, bronchopneumonia and alveolar epithelial hyperplasia were observed.

Reddening, scab formation and alopecia on skin was observed. In histological observation inflammatory changes like acanthosis and hyperkeratosis were seen. Acute ulcerative dermatitis was observed in one control and two high dose females. In adrenals coarse vacuolation of cortical cells were observed in control and treated animals. In kidneys focal mineralization of tubules, cortical cyst and medullary fibrosis was noted. Other macroscopical and histological changes were incidental.

Table 6.3-8 Amount of animals with histopathological findings on adrenals

Parameter	Males (mg/kg bw/day)				Females (mg/kg bw/day)			
	0	100	320	1000	0	100	320	1000
Focal coarsely vacuolated enlarged cortical cells	1	2	1	3	1	2	4	4

Conclusion

At dose level 100 mg/kg bw/day the red blood cell count was lowered but was not significantly different from that of the control group. Statistically different (higher) MCH values from control were seen on males on weeks 14, 27 and 52. MCHC values were statistically higher on males on week 52. At this dose level a statistically significant effect was not seen on females on MCH or MCHC. This was considered not significant effect as the values were only slightly higher than that of controls.

There was also significantly reduced relative adrenals weight on males on this dose level and in adrenals coarse vacuolation of cortical cells was observed at all dose levels. This significant effect on the weight was not observed at higher dose levels. The weight of adrenals on females was not affected. The histopathological findings observed were seen on 1 control and 1-4 test group animals (males and females) and there was no clear dose response on males. On females the intermediate and high dose groups observations were the same (4/6 animals).

Histopathological changes in the lungs and kidneys were observed on males and females at this dose level. Only few of the changes in lungs were observed also in control animals. Effects seen only on test animals on this dose level were pleural fibrosis (1 female), chronic bronchiolitis (2 males and 1 female with clear dose response), granulomas (2 males and 1 female), bronchopneumonia (1 female). The histopathological findings in kidneys were observed at the same incidence also on control animals or only on 1-2 animals. These findings were considered incidental and the chronic bronchiolitis is possibly related with the plant material suggestive of food origin.

The NOAEL for male and female dogs was 100 mg/kg bw/day based on haemotological effects and, additionally in males, to the increase in absolute liver weights observed at dose level 320 mg/kg bw/day. The study is acceptable.

Study:	Dowco 290 herbicide (3,6-dichloropicolinic acid): results of a six-month dietary feeding study in beagle dogs ([redacted] *et al*., 1976)
Previous evaluation:	This study was submitted to DAR (2003). It was evaluated as supportive in the DAR. No re-evaluation has been performed and the text has not been modified.

Test guideline and GLP
The study was not performed using any official guideline or test method, and nor was it done under GLP. The study was performed in 1976 before the existence of OECD test guidelines or GLP guidelines.

Materials and methods
Groups of 4 beagle dogs/sex/dose level received diets containing Dowco 290 (purity not stated) at concentrations calculated to provide doses of 0, 15, 50 and 150 mg/kg bw/day for 6 months. The dogs were observed daily for changes in appearance and behaviour. Body weights were recorded twice during the first week and weekly thereafter. Food consumption was recorded twice each week. Clinical biochemistry determinations of serum urea nitrogen concentration, alanine aminotransferase (ALAT), alkaline phosphatase activity and ASAT (aspartate aminotransferase) activity were determined from the preterminal samples. Haematologic measurements of packed cell volume, haemoglobin, total erythrocytes and differential leukocyte count were measured on all dogs prior to starting the study and on day 173 of the experiment. Urine samples were collected from all dogs before and after the treatment. The following analyses were conducted: specific gravity, pH, semiquantitative determinations of sugar protein, bilirubin, ketones, occult blood and microscopic examination of the sediment. Gross pathologic examinations were conducted on all dogs and the brain, heart, liver, kidneys and testes were weighted. Representative portions of these and other tissues (32) were preserved and examined histopathologically. Results were evaluated statistically.

Results
No treatment related changes were observed in appearance or behaviour of the dogs. Animals in all treatment groups gained weight comparable to controls. Likewise, there were no differences in food consumption. In addition, no treatment related effects were found in haematologic or clinical chemistry determinations or urinalysis.

An increased relative liver weight of the group of female dogs at 150 mg/kg bw/day dose level was the only significant difference between treatment groups and controls. Urinary tract changes were noted in 1 male dog in 15 and 150 mg/kg bw/day treatment groups and in 2 male dogs at the 50 mg/kg bw/day level. As suggested in

the study report, these changes may have been caused by catheterisation rather than being related to treatment, since these changes occurred in only some treated males and not in any of the treated females or controls.

Conclusion
The NOAEL for female dogs was 50 mg/kg bw/day, based on the increase in relative liver weight following administration of Dowco 290 at 150 mg/kg bw/day. The NOAEL for male dogs was >150 mg/kg/day, based on the absence of treatment related toxicological effects. The study is supportive.

Study:	180-day subacute toxicity study in dogs ([redacted] 1975)
Previous evaluation:	This study was submitted to DAR (2003). It was evaluated as supportive in the DAR. No re-evaluation has been performed and the text has not been modified.

Test guideline and GLP
The study was not performed using any official guideline or test method, and nor was it done under GLP. The study was performed in 1975 before the existence of OECD test guidelines or GLP guidelines.

Materials and methods
Groups of 4 beagle dogs/sex/dose level received diets containing Dowco 290 (purity not stated) at concentrations calculated to provide doses of 0, 15, 50 and 150 mg/kg bw clopyralid/day for 180 days. The dogs were observed daily for changes in appearance and behaviour. Body weights and food consumption were recorded weekly. The following laboratory studies were performed on each dog prior to starting the study, after 90 days and prior to termination. Clinical biochemistry determinations of serum urea nitrogen concentration, alanine aminotransferase (ALAT) activity, alkaline phosphatase activity and ASAT (aspartate aminotransferase) activity were determined. Haematologic measurements of haematocrit, haemoglobin, total erythrocytes and leucocytes, and differential leukocyte count were measured. From urine samples, the following analyses were conducted: colour, albumin, appearance, acetone, specific gravity, pH, bilirubin, glucose, occult blood and microscopic examination of the sediment.

The brain, heart, liver, kidneys and testes of all animals were weighted. Representative portions of these and other tissues (24) were preserved. Tissues from the control and high dose male and female dogs were examined histopathologically. Results were evaluated statistically.

Results
Studies of body weight, food consumption, haematology, blood chemistry, ophthalmology, urinalysis, organ weight or findings at necropsy did not show any treatment related effects. Significant increase in relative liver weight was detected only in the high dose group females.

Conclusion
Under the conditions of this study, the NOAEL for male and female dogs was >150 mg/kg bw/day, based on the absence of treatment related toxicological effects following administration of Dowco 290. The study is supportive.

B.6.3.3. Other routes

Study:	Clopyralid: probe and 21-day dermal toxicity study in New Zealand White rabbits. ([redacted] 1990)
Previous evaluation:	This study was submitted to DAR (2003). The study was performed according to the Guideline No. 82-2 and predominantly according to OECD guideline 410 (1981). Text has been modified but conclusions have not changed. The study is acceptable.

Test guideline and GLP
The study was conducted in compliance with GLP standards and the Guideline No. 82-2 and predominantly according to OECD guideline 410 (1981). However, in the present 21-day dermal study, animals were treated for three 5-day periods, i.e. 15 days treatment in all. Also, adrenals were not weighed at necropsy. The dose levels to be used were established by the probe study. At the highest level neither dermal irritation nor evidence of systemic toxicity was observed. According to the guideline the highest dose level should result in toxic effects,

however the highest dose level used is the same as presented as minimum to be used in a limit test. It was noticed that the weight of the animals at the beginning of the test were higher than what suggested in the guideline and detailed information about the housing conditions was not given.

Materials and methods
Dermal probe study. The purpose of the probe study was to establish acceptable dose levels to be used in the 21-day dermal study. One male and one female New Zealand White rabbit received a dermal application of 500 and 1000 mg/kg bw/day clopyralid (95.78 ± 0.25%), respectively, 6 hours/day for 4 days. The test item was applied in powder form, a dressing consisting of a water-moistened absorbent gauze and non-absorbent cotton held the test item in dermal contact. In addition an elastic jacket was used to hold the test material dressing in dermal contact. Animals were weighed prior to the first application and at study termination. A thorough evaluation of skin at the application site was made on each test day after unwrapping. The rabbits were observed daily for evidence of toxicity. Neither dermal irritation nor evidence of systemic toxicity was observed in the probe study.

21-day dermal study. Groups of 5 male and 5 female New Zealand White rabbits received topical applications of 0, 100, 500 and 1000 mg/kg bw/6 hours/day of clopyralid (95.78 ±0.25%). Animals were treated a total of 15 times during the 21 day period. The control animals were treated in the same way as the treatment group animals with the exception that test material was not administered under the absorbent gauze. All animals were weighted prior to the first application and at approximately weekly intervals thereafter. Food consumption was not quantitated since the rabbit consumed their entire ration of 113 g/day. A careful clinical examination was conducted on all animals prior to the first application and at approximately weekly intervals thereafter. A cageside examination of behaviour of animals and possible evidence of toxicity was made each day of the workweek. The condition of the dermal test-site was evaluated when daily wraps were removed on the last day of a dosing week and on the afternoon prior to necropsy using a standard scoring system (Draize).

Haematological measurements of hematocrit, haemoglobin, platelet count, total erythrocyte count and total leukocyte count were measured from all animals. Also a complete blood smear examinations were conducted which included differential leukocyte counts. The following clinical biochemistry determinations were evaluated for each animal: serum urea nitrogen concentration, ALAT (alanine aminotransferase), ASAT (aspartate aminotransferase) and alkaline phosphatase activity, glucose, total protein, albumin, globulin, bilirubin, creatinine, phosphorus, calcium, sodium, potassium and chloride. All animals were examined for gross pathological changes. The necropsy included examination of the eyes. Weights of the liver, kidneys and testes were recorded. Representative portions of these and other tissues (52) were preserved. A complete histologic evaluation of treated and untreated skin, liver and kidneys was made on all control and high-dose animals. In addition, histologic evaluation of normal and treated skin from all animal in the low and intermediate dose groups was performed. Results were evaluated statistically.

Results
Application of clopyralid to rabbits elicited no clinical signs of systemic toxicity. Only slight erythema (score 1) was observed in two male rabbits belonging to the intermediate and high dose groups, respectively, and one female rabbit belonging to the intermediate dose group. No evidence of skin irritation was observed in the control rabbits. No treatment related changes were noted in haematological parameters or in relative or absolute weights of liver, kidneys or testes. However, the liver weight of males treated with 1000 mg/kg bw/day was about 112 % of that of the control group and the liver/body weight was about 114 % of that of the control group. This was not identified as statistically significant, but it seemed that p-value was calculated with regard all doses and both sexes. On females this effect was not observed.

In some clinical chemistry parameters there were minimal statistically identified differences but in the study report these were considered not treatment related as there was lack of dose response and they were not related to any identifiable tissue pathology. There was a slight but statistically significant decrease in the body weight of males in the high dose group.

No gross pathological lesions attributable to treatment were observed at necropsy. In kidneys clear cyst (cortex, unilateral, focal) and dark focus (cortex, unilateral, focal) and in skin both at dermal test site and at untreated site multifocal erythema were observed on treated animals. Histopathological skin lesions at the dermal test site were present in some rabbits from controls and all dose level groups. Mild and diffuse treatment related epidermal hyperplasia was noted in 1/5 males and 2/5 females treated with 100 mg/kg bw/day, 3/5 males and 1/5 females treated with 500 mg/kg bw/day, and 5/5 males and 5/5 females treated with 1000 mg/kg bw/day. This was

considered by the authors of the report, to be caused by the mechanical irritation of the powdered test material and the friction caused by the wrapping and unwrapping the jackets used to hold the test material in place. Also inflammation of dermis, necrosis on epidermis (1 male, 100 mg/kg bw/day) and multifocal epidermal hyperplasia were observed. Occasional histopathologic lesions (epidermal hyperplasia, degeneration of muscle fibers, inflammation of dermis) were observed in the untreated skin adjacent to the dermal test site in some control and treated rabbits. The observations from liver (aggregates of mononuclear cells) and kidneys (congestion of blood vessels, cyst, degeneration or mineralization of tubules on treated animals) were, in the study report, considered to be unassociated with exposure to clopyralid.

One male from intermediate dose group was sacrificed (day 21) due to traumatic fracture of the lumbar vertebra.

Conclusion
The systemic NOAEL for male and female rabbits was >1000 mg/kg bw/day, based on the absence of treatment related systemic toxic effects following repeated dermal administration of Lontrel T up to 1000 mg/kg bw/day. Local effects that were observed were slight erythema in the intermediate and high dose groups and mild and diffuse treatment related epidermal hyperplasia at all dose levels. The study is acceptable.

B.6.4. GENOTOXICITY

Summary of genotoxity studies
There are two Ames tests (*Salmonella* typhimurium reverse mutation assay) in the original DAR (Richold *et al*., 1981, Bruce and Gollapudi, 1987). Clopyralid did not induce gene mutations in these tests where no bacterial strain capable of detecting cross-linking mutagens was included. *In vitro* host mediated assay with *Salmonella* typhimurium strains TA 1530 and G-46 and *Saccharomyces cerevisiae* strain D-3 (Sibinovic, 1973) was performed using no official guideline or test method, and it was not done under GLP. The study is supportive only. *In vitro* mammalian cell gene mutation assay in Chinese hamster ovary cells ([redacted] and [redacted] 1987) gave a negative result.

In the *in vitro* chromosome aberration test in cultured rat lymphocytes ([redacted] *et al*., 2001) frequency of aberrant cells was increased significantly although the frequency was within the historical control range. As this highest tested concentration (697.5 µg/mL) does not fulfil the OECD Guideline 473 requirement for the dose selection and neither does the reduction in mitotic index show that the dose was high enough, no definite evidence for the positive or negative result can be indicated. Hence, the result of the study is considered equivocal. Clopyralid did not demonstrate genotoxic activity in an *in vitro* test of unscheduled DNA synthesis (UDS) performed in isolated rat hepatocytes ([redacted] and [redacted] 1985). Because of the deviations in relation to the OECD Test Guideline, the study is considered supportive only.

In an *in vivo* micronucleus test ([redacted] *et al*., 1991), the highest dose exceeded the maximum tolerated dose and was thus useless for evaluation. The remaining two doses did not fulfill the OECD Test Guideline requirements for the proper dose selection with three analysable dose levels covering a range from the maximum to little or no toxicity. Dose selection was inappropriate also in an acute and subacute *in vivo* cytogenetic non-GLP study in rats ([redacted] 1973). Because of the wrong dose selection, both the *in vivo* micronucleus test and the acute and subacute *in vivo* cytogenetic study were not acceptable.

In vivo dominant lethal mutagenesis assay (non-GLP) ([redacted] 1973) is not acceptable as test substance concentrations have been too low in the study and the procedure did not cover all phases of male germ cell maturation in rat.

There is no acceptable or unquestionable chromosome test in the whole dossier and hence, a data gap for addressing clastogenic and aneugenic end point is identified.

Table 6.4-1. Summary of *in vitro* and *in vivo* genotoxicity studies

Test	Test Object	Concentration	Result	Reference
In vitro gene mutation assays				
In vitro bacterial reverse mutation (Ames)	*Salmonella* typhimurium TA 98, TA 100, TA 1535, TA 1537, TA 1538	125, 250, 500 and 1000 µg/plate in the presence and absence of S9 mix	Negative	Richold, M. *et al*., 1981
In vitro bacterial reverse mutation (Ames)	*Salmonella* typhimurium TA 98, TA 100, TA 1535, TA 1537	50, 158, 500, 1580 and 5000 µg/plate in the presence and absence of S9 mix	Negative	Bruce, R. & Gollapudi, B., 1987
In vitro host mediated assay	*Salmonella* typhimurium TA 1530, G-46; *Saccharomyces cerevisiae* D-3	10%, 20% or 50% saturated solution of clopyralid	Negative Study supportive	Sibinovic, K., 1973
In vitro mammalian cell forward mutation	Chinese hamster ovary cells (CHO/HGPRT)	125, 250, 500, 700, 750, 1000 and 1500 µg/ml in the absence of S9 mix; 1750, 2000, 2250, 2500 and 2750 µg/ml in the presence of S9 mix	Negative	[redacted] 1987
In vitro cytogenetic assay				
In vitro chromosome aberration	Cultured rat lymphocytes	43.6 to 2790 µg/ml in the presence and absence of S9 mix	Equivocal	[redacted] *et al*., 2001
Unscheduled DNA synthesis (UDS)				
In vitro mammalian UDS	Rat hepatocytes	5×10^{-5}, 1.56×10^{-4}, 5×10^{-4}, 1.56×10^{-3}, 5×10^{-3}, 1.56×10^{-2}, 5×10^{-2} M	Negative Study supportive	[redacted] 1985
In vivo studies in somatic cells				
In vivo micronucleus test	Mouse bone marrow	500, 1667 or 5000 mg/kg bw, single administration by gavage	Study not acceptable	[redacted] *et al*., 1991
In vivo chromosome aberration	Rat bone marrow	4, 40 or 400 mg/kg bw, single administration by gavage (acute study); 4, 40 or 400 mg/kg bw/day administration by gavage once daily for five days (subacute study) to males	Study not acceptable	[redacted] 1973
In vivo studies in germ cells				
In vivo dominant lethal mutagenesis assay	Sprague-Dawley CD rats	4, 40 or 400 mg/kg bw/day administration by gavage once daily for five days to males	Study not acceptable	[redacted] 1973

B.6.4.1. In vitro studies

Study:	Ames metabolic activation test to assess the potential mutagenic effect of 3,6-dichloropicolinic acid technical ([redacted] 1981)
Previous evaluation:	This study was submitted to DAR (2003). Text has been slightly modified but conclusion has not been changed. Deviations from the current OECD Test Guideline 471 (1997): No strain detecting cross-linking mutagens was included in the study. Only 2-aminoanthracene was used to indicate the efficacy of the S9-mix. The study is acceptable.

Test guideline and GLP
The study was performed according to GLP and the method used complies predominantly with OECD Test Guideline 471 (1997). However, only four levels of active substance were used compared to five required by the current OECD guideline. No strain detecting cross-linking mutagens was included in the study. Only 2-aminoanthracene was used to indicate the efficacy of the S9-mix.

Materials and methods
The test was conducted in the presence and absence of an externally supplied metabolic activation system of mammalian microsomal enzymes derived from Aroclor-induced rat liver (S9) using *Salmonella* typhimurium strains TA 98, TA 100, TA 1535, TA 1537 and TA 1538. Based on the range-finding study, the concentrations of 3,6-dichloropicolinic acid technical (95% clopyralid; Batch No 222A) chosen to the incorporation method were 125, 250, 500 and 1000 µg/plate in the presence and absence of S9 mix. The assay used three plates per concentration. The validity of the study was confirmed by using positive control chemicals for assays with and without S9. Negative control cultures were treated with the solvent DMSO (dimethylsulfoxide) used to dissolve the test material.

Results
In a dose-range finding study, clopyralid was cytotoxic to the test strains at the 10000 µg/plate dose level.

In the main experiment, there was no substantial increase in the number of revertant colonies with any of the strains, either in the presence or absence of S9. The positive control chemicals substantially increased the mean number of revertant colonies in the presence and absence of S9 confirming the validity of the experimental conditions for detecting induced mutations.

Conclusion
Under the conditions of this study, 3,6-dichloropicolinic acid technical was negative in the Ames test; i.e. 3,6-dichloropicolinic acid technical did not show any evidence of mutagenic potential in the bacterial reverse gene mutation assay. The study is acceptable.

Study:	Lontrel T herbicidal chemical (Penta process): evaluation in the Ames *Salmonella*/ mammalian-microsome mutagenicity assay ([redacted] 1987)
Previous evaluation:	This study was submitted to DAR (2003). Text has been slightly modified but conclusion has not been changed. Deviations from the current OECD Test Guideline 471 (1997): No strain detecting cross-linking mutagens was included in the study. Only 2-anthramine was used to indicate the efficacy of the S9-mix.The study is acceptable.

Test guideline and GLP
The study was performed according to GLP and the method used complies predominantly with OECD Test Guideline 471 (1997). However, only four bacterial strains were used compared to five required by the guideline. No strain detecting cross-linking mutagens was included in the study. 2-anthramine was used as positive control in the presence of S9.

Materials and methods
The test was conducted in the presence and absence of an externally supplied metabolic activation system of mammalian microsomal enzymes derived from Aroclor-induced rat liver (S9) using *Salmonella* typhimurium strains TA 98, TA 100, TA 1535 and TA 1537. The concentrations of Lontrel T (95.4% clopyralid, Batch No AGR 233257) chosen to the preincubation method were 50, 158, 500, 1580 and 5000 µg/plate in the presence and absence of S9 mix. Pre-incubation was done at 30°C for 30 minutes. Thereafter, 2 ml of top agar was added and poured onto the plates and they were incubated two days at 37°. The assay used three plates per concentration. The validity of the study was confirmed by using positive control chemicals for assays with and without S9. Negative control cultures were treated with the solvent DMSO (dimethylsulfoxide) used to dissolve the test material.

Results

In an initial dose-range finding study, clopyralid was not cytotoxic to *S.* typhimurium TA 100 at up to 5000 µg/plate.

In the main experiment, the other three strains were also tested up to 5000 µg/plate. Clopyralid did not increase the number of histidine-independent revertants with any of the strains. The positive control chemicals gave a marked increase in the mean number of revertant colonies in the presence and absence of S9 confirming the validity of the experimental conditions for detecting induced mutations.

Conclusion
Under the conditions of this study, Lontrel T was negative in the Ames test; i.e. clopyralid did not show any evidence of mutagenic potential in the bacterial reverse gene mutation assay. The study is acceptable.

Study:	Evaluation of Lontrel*T herbicidal chemical (penta process) in the Chinese hamster ovary cell/hypoxanthine-guanine-phosphoribosyl transferase (CHO/HGPRT) forward mutation assay ([redacted] 1987)
Previous evaluation:	This study was submitted to DAR (2003). The study was performed according to the method that complies predominantly with OECD Test Guideline 476 (1997), which was applicable at the time of dossier submission. Conclusion has not been changed. Historical control data of the laboratory has been included. The study is acceptable.

Test guideline and GLP
The study was performed according to GLP and the method used complies predominantly with OECD Test Guideline 476 (1997), which was applicable at the time of dossier submission.

Materials and methods
The mutagenic potential of Lontrel T (95.4% clopyralid, Batch No AGR 233257) was evaluated in the *in vitro* Chinese hamster ovary cell (CHO-K_1-BH_4) hypoxanthine guanine phosphoribosyl transferase (CHO/HGPRT) forward gene mutation assay. The test was conducted in the presence and absence of an externally supplied metabolic activation system of mammalian microsomal enzymes derived from Aroclor-induced rat liver (S9). The concentrations of clopyralid (based on initial toxicity tests) were 250, 500, 750, 1000 and 1500 µg/ml in the absence of S9 mix (first experiment), 125, 250, 500, 700 and 1000 µg/ml in the absence of S9 mix (second experiment) and 1750, 2000, 2250, 2500 and 2750 µg/ml in the presence of S9 mix. The exposure period was four hours. Following treatment and during the phenotypic expression period, cells were subcultured every three days. At each subculture, cells within each treatment group were pooled and replated. At the end of the expression period the cultures were plated (5 dishes/treatment) on selection media and incubated for seven to nine days to allow colonies to form and mutation frequencies to be determined.

The validity of the experimental conditions for detection of induced mutation was confirmed by using positive control chemicals, ethyl methanesulphonate for assay without S9 and 20-methylcholanthrene for assay with S9. Negative control cultures were treated with the solvent (culture medium) used to dissolve the test material.

Results
In the first experiment, in the absence of S9, mutation frequencies following clopyralid at 250 and 750 µg/ml were significantly higher than with the negative control (Table 6.4-2). However, there was no dose response and mutation frequencies at all clopyralid doses were within laboratory historical negative control values in the absence of S9 mix in 22 tests over two years.

Clopyralid did not increase the number of mutation frequencies in the second experiment in the absence of S9, or in the presence of S9. The positive control chemicals induced significant increases in mutation frequencies confirming the validity of the experimental conditions for detecting mutants induced by direct and indirect acting genotoxins.

Table 6.4-2 Summary of mutation frequency in CHO cells exposed to Lontrel T

Treatment/ concentration (µg/ml)	Without S9						With S9		
	Experiment I			Experiment II			Experiment I		
	Total	Range	No/10^6 clonable cells	Total	Range	No/10^6 clonable cells	Total	Range	No/10^6 clonable cells
Negative control	0	-	0	1	0 - 1	1.5	2	0 - 1	2.4
125	-	-	-	1	0 - 1	1.2	-	-	-
250	6	0 - 3	8.9**	0	-	0	-	-	-
500	3	0 - 2	3.6	1	0 - 1	0.9	-	-	-
700	-	-	-	2	0 - 1	2.0	-	-	-
750	8	0 - 4	12.5**	-	-	-	-	-	-
1000	1	0 - 1	1.5	1	0 - 1	1.2	-	-	-
1500	0	-	0	-	-	-	-	-	-
1750	-	-	-	-	-	-	2	0 - 1	2.1
2000	-	-	-	-	-	-	0	-	0
2250	-	-	-	-	-	-	2	0 - 1	3.1
2500	-	-	-	-	-	-	4	0 - 2	6.1
2750	-	-	-	-	-	-	0	-	0
Positive control	103	16 - 25	335.5**	172	25 - 40	400.0**	108	19 - 30	199.3**

** $p \leq 0.01$ statistically significantly different from negative controls

Table 6.4-3 Historical control data of the laboratory

Table 4: Historical Negative Control Values of 6-Thioguanine Resistant (TG^r) Mutation Frequency in CHO-K_1-BH_4 Cultures

	No. TG^r Mutants Per 10^6 Clonable Cells		
Mo./Yr	-S9	+S9	Solvent*
11/85	12.9		2
11/85		2.6	1
12/85	11.2		2
12/85	7.0		2
12/85	10.1		2
12/85	4.7		2
12/85	7.0		2
12/85		3.6	1
12/85		12.2	1
12/85		12.0	1
12/85		7.5	1
12/85		6.7	1
1/86	8.2	4.4	1
1/86	3.8	13.4	1
2/86	6.6	3.0	2
3/86	10.2	5.0	1
3/86	12.2	2.4	1
3/86	0	1.3	1
5/86		4.2	1
6/86	1.5	8.1	1
6/86	1.3		1
7/86	2.8	0	1
7/86	13.4	6.8	1
11/86	4.6	4.8	1
12/86	2.3	2.1	1
1/87	4.4	2.2	1
3/87	0	2.4	4
4/87	2.7	1.2	3
4/87		2.1	1
4/87	1.5		4

*1 & 3 = DMSO; 2 = Water; 4 = Culture Medium. Five microliters of the solvent were added per ml of the treatment medium in 1 and 2. In 3, ten microliters of the solvent were added per ml of the treatment medium.

Conclusion

Under the conditions of this study, Lontrel T was negative in the CHO/HGPRT gene mutation assay. The study is acceptable.

Study:	The evaluation of Lontrel T herbicide in the rat hepatocyte unscheduled DNA synthesis assay ([redacted] 1985)
Previous evaluation:	This study was submitted to DAR (2003). Some text has been added but conclusion has not been changed. Deviations from the OECD Test Guideline 482 (1986): Only 30 cells/concentration instead of 50 required by the guideline were counted. Result was not confirmed in an independent experiment. Because of these deviations in relation to the guideline, the study is considered supportive only. OECD Test Guideline 482 was deleted on 2nd April 2014.

Test guideline and GLP
The study was performed according to GLP and the method used complies predominantly with OECD Test Guideline 482 (1986).

Materials and methods
Lontrel T (95.6% clopyralid, Batch No AGR 192532) was evaluated in the unscheduled DNA synthesis (UDS) assay. The test material was added to cultures of hepatocytes isolated from the liver of male Fischer 344 rats at concentrations of 0 (negative control), 5 x 10^{-5}, 1.56 x 10^{-4}, 5 x 10^{-4}, 1.56 x 10^{-3}, 5 x 10^{-3}, 1.56 x 10^{-2} and 5 x 10^{-2} M dissolved in Williams Media E. Additional cultures were treated with the positive control substance 2-acetylaminofluorene (2-AAF) dissolved in DMSO (dimethylsulfoxide) at concentrations of 0, 10^{-7}, 10^{-6} and 10^{-5} M in Williams Media E. DMSO served as the negative control for 2-AAF treated cultures.

The test chemical and ^{3}H-thymidine were incubated with the cultures (3 per treatment) for 18 hours. The cultures were washed with thymidine, thereafter nuclei were swelled with sodium citrate and fixed with ethanol:acetic acid (3:1). The cells on a slide were coated with a thin layer of photographic emulsion, refrigerated, developed and stained. Slides were scored blind. UDS was quantified by counting the number of grains in the nucleus and subtracting the mean number of grains in three nuclear sized areas in the cytoplasm adjacent to the nucleus. A total of 30 cells (15 cells/slide) were counted per treatment.

Results
Clopyralid was toxic to hepatocyte cultures at concentrations of 1.56 x 10^{-2} M and above. Clopyralid did not show significant UDS at any concentration tested. In contrast, 2-acetylaminofluorene led to a statistically significant increase in UDS at concentrations of 10^{-6} and 10^{-5} M compared to the negative control.

Conclusion
Under the conditions of this study, clopyralid did not demonstrate genotoxic activity in the UDS assay. Because of the deviations in relation to the OECD Test Guideline, the study is considered supportive only.

Study:	*In vitro* and subacute *in vivo* host-mediated assay for mutagenesis. Final report - compound DOWCO 290 (Sibinovic, 1973)
Previous evaluation:	This study was submitted to DAR (2003). The study was not performed according to any official test guideline and not according to GLP. No new evaluation has been performed. The study is supportive.

Test guideline and GLP
The study was not performed using any official guideline or test method, and it was not done under GLP. The study was performed in 1973 before the existence of OECD test guidelines or GLP guidelines. The *in vivo* method is a version of the Ames test, where the requirement for metabolic activation was met by injecting the test bacteria/yeast cells into the peritoneal cavity of mice previously dosed with the test material. The *in vitro* method, known as the "spot test", was developed for rapid screening of test substances, and is less sensitive than the plate incorporation test. A negative test result in the spot test does not give sufficient evidence of non-mutagenicity in *Salmonella*.

Materials and methods
The mutagenic potential of DOWCO 290 (purity not stated) was evaluated in *in vitro* and *in vivo* host-mediated assays for mutagenesis using *Salmonella* typhimurium strains TA 1530 and G-46 and *Saccharomyces cerevisiae* strain D-3.

In the *in vitro* test, test organism suspensions were placed on plates and discs containing 0.1 ml of a 10%, 20% or 50% saturated solution of DOWCO 290 in corn oil were added. The plates were incubated at 37ºC for 24 hours and examined for zones of inhibition.

In the *in vivo* test, 10 Charles River ICR male mice/group received clopyralid in corn oil at 0 (control), 4.0, 40.0 or 400.0 mg/kg bw/day by gavage, daily for five days. Half an hour after the last administration, intraperitoneal injections of the test organism suspensions were made. Four hours after administration of the test organisms, the animals were sacrificed and the peritoneal fluid was removed and plated. Plates were incubated at 30°C (time not stated) and colonies were counted. The validity of the study was confirmed by treating other plates with validated positive control chemicals. Negative control cultures were treated with the vehicle (saline).

Results
Dowco 290 did not significantly increase the number of mutant or recombinant frequencies in the *in vivo* test. The *in vitro* test was also negative. The positive control chemicals gave a marked increase in the frequency of mutant or recombinant frequencies in the *in vivo* test confirming the validity of the experimental conditions for detecting induced mutations.

Conclusion
Under the conditions of this study, Dowco 290 was non-mutagenic. The study is supportive.

Study:	Evaluation of clopyralid in an *in vitro* chromosomal aberration assay utilizing rat lymphocytes ([redacted] 2001)
Previous evaluation:	This study was submitted to DAR (2003). The study was performed according to the method that complies predominantly with OECD Test Guideline 473 (1997), which was applicable at the time of dossier submission. In the re-evaluation, text has been modified and tables as well as historical control data included in the text. After the re-evaluation, the result of the study is considered equivocal.

Test guideline and GLP
The study was performed according to GLP and the method complied principally with OECD guideline 473 (1997), which was applicable at the time of dossier submission.

Materials and methods
Clopyralid (purity 96.9%, Batch No TSN100167) was evaluated in an *in vitro* chromosomal aberration assay with rat lymphocytes. Whole blood samples were obtained from male Crl:CD BR rats by cardiac puncture and established in culture for 48 hours at 37 °C. Thereafter, in Experiment 1 cells were treated for 4 hours with concentrations of 0, 43.6, 87.2, 174.4, 348.8, 697.5, 1395 and 2790 µg/mL in the presence and absence of S9. Cells were harvested 20 hours later (24 hours after treatment initiation).

In Experiment 2, cells were treated continuously for 24 hours with concentrations of 0, 43.6, 87.2, 174.4, 348.8, 697.5, 1050, 1395, 2100 and 2790 µg/mL in the absence of S9 and with concrentrations of 174.4, 348.8, 697.5, 1395 and 2790 µg/mL for 4 hours in the presence of S9. Cultures were harvested 24 hours after treatment initiation.

Positive controls were mitomycin C in the absence of S9 and cyclophosphamide monohydrate in the presence of S9.

After slide preparation, one hundred metaphases/replicate (a total of 200 metaphases/treatment) were examined for structural abnormalities and incidence of polyploidy on coded slides. Only those metaphases containing 42 ± 2 centromeres were scored, except for cells with multiple aberrations (defined as cells having five or more aberrations). Gaps were not included in totals.

Results
In Experiment 1, based upon the mitotic indices (21 % reduction in mitotic index compared to the control at 2790 µg/mL with S9, no toxicity without S9), concentrations of 0, 697.5, 1395 and 2790 µg/mL were selected for the chromosome aberration analysis. In the absence of metabolic activation, the fequency of cells with aberrations in the negative control, as well as the clopyralid treated cultures, was 1%. In the presence of S9

activation , aberrant cell frequencies were 0.5, 1.5 and 1.5% in cultures treated with the test material at concentrations of 697.5, 1395 and 2790 µg/mL, respectively, compared to the negative control value of 0.5%. Differences between the negative control and any of the treated cultures either with or without S9 activation were not statistically significant.

Table 6.4-4 Results of the chromosomal aberration assay 24 hours after treatment in the absence of S9; Experiment 1 Replicates are designated A & B

	Neg. control			Clopyralid 697.5 µg/mL			Clopyralid 1395 µg/mL			Clopyralid 2790 µg/mL			Pos. control		
	A	B	A+B	A	B	A+B	A	B	A+B	A	B	A+B	A	B	A+B
No. of cells scored	100	100	200	100	100	200	100	100	200	100	100	200	50	50	100
Chromatid gaps	0	0	0	1	1	2	2	0	2	0	2	2	0	1	1
Chromosome gaps	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Chromatid breaks	1	0	1	1	1	2	1	0	1	0	1	1	6	8	14
Chromatid exchanges	0	0	0	0	0	0	0	0	0	2	0	2	11	16	27
Chromosome breaks	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0
Chromosome exchanges	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0
Total aberrations (excluding gaps)[a]	2	0	2 (1.0)	1	1	2 (1.0)	1	1	2 (1.0)	2	1	3 (1.5)	17	24	41 (41.0)
No. of cells with aberr. (excluding gaps)[a]	2	0	2 (1.0)	1	1	2 (1.0)	1	1	2 (1.0)	1	1	2 (1.0)	11	16	27 (27.0)[b]
Miscellaneous aberr.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cells with multiple aberr. (5 or more aberr.)	0	0	0	0	0	0	0	0	0	0	0	0	0	2	2

[a] Values in parentheses are percentages.
[b] Significantly (alpha <0.05) different from negative control.

Table 6.4-5 Results of the chromosomal aberration assay 24 hours after treatment in the presence of S9; Experiment 1 Replicates are designated A & B

	Neg. control			Clopyralid 697.5 µg/mL			Clopyralid 1395 µg/mL			Clopyralid 2790 µg/mL			Pos. control		
	A	B	A+B	A	B	A+B	A	B	A+B	A	B	A+B	A	B	A+B
No. of cells scored	100	100	200	100	100	200	100	100	200	100	100	200	50	50	100
Chromatid gaps	0	0	0	2	1	3	0	2	2	2	1	3	1	0	1
Chromosome gaps	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Chromatid breaks	1	0	1	1	0	1	1	1	2	2	1	3	11	7	18
Chromatid exchanges	0	0	0	0	0	0	0	0	0	1	0	1	16	12	28
Chromosome breaks	0	0	0	0	0	0	0	1	1	1	0	1	0	1	1
Chromosome exchanges	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Total aberrations (excluding gaps)[a]	1	0	1 (0.5)	1	0	1 (0.5)	1	2	3 (1.5)	4	1	5 (2.5)	27	20	47 (47.0)
No. of cells with aberr. (excluding gaps)[a]	1	0	1 (0.5)	1	0	1 (0.5)	1	2	3 (1.5)	2	1	3 (1.5)	20	18	38 (38.0)[b]
Miscellaneous aberr.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cells with multiple aberr. (5 or more aberr.)	0	0	0	0	0	0	0	0	0	0	0	0	4	2	6

[a] Values in parentheses are percentages.
[b] Significantly (alpha <0.05) different from negative control.

In Experiment 2, concentrations of 1050 to 2790 µg/mL were toxic without S9: compared to the negative control reductions in mitotic indices of 76.9 to 100% were observed, respectively. Compared to the negative control reductions in mitotic indices of 45.9, 27.4, 14.3, 6.5 and 5.2% were observed in the remaining concentrations of 697.5, 348.8, 174.4, 87.2 and 43.6 µg/mL, respectively. There was little to no toxicity at any treatment level in the presence of S9. Based upon these results, cultures treated with 174.4, 348.8 and 697.5 µg/mL in the absence of S9 and cultures treated with 697.5, 1395 and 2790 µg/mL in the presence of S9 were selected for chromosome aberration analysis.

In the non-activation assay of Experiment 2, the frequency of cells with aberrations in the negative control was 0% and the corresponding values at treatment levels of 174.4, 348.8 and 697.5 µg/mL were 1.5, 1.5 and 2.5%, respectively. Frequency of aberrant cells at 697.5 µg/mL was significantly different from the concurrent negative control.However, this result was within the historical (past 5 years) negative control range of the laboratory and is therefore, considered to be of no biological significance.

In the activation assay of Experiment 2, cultures treated with clopyralid at concentrations of 697.5, 1395 and 2790 µg/mL had aberrant cell frequencies of 0, 3.5 and 3.0%, respectively, when compared to the negative control value of 1.5%. Differences between negative control and any of the treated cultures with S9 were not statistically significant. The frequencies of aberrant cells observed in clopyralid treated cultures were within the laboratory historical background range.

The positive control chemicals gave a marked increase in the frequency of cells with aberrations.

Table 6.4-6 Results of the chromosomal aberration assay 24 hours after treatment in the absence of S9; Experiment 2 Replicates are designated A & B

	Neg. control			Clopyralid 174.4 µg/mL			Clopyralid 348.8 µg/mL			Clopyralid 697.5 µg/mL			Pos. control		
	A	B	A+B	A	B	A+B	A	B	A+B	A	B	A+B	A	B	A+B
No. of cells scored	100	100	200	100	100	200	100	100	200	100	100	200	50	50	100
Chromatid gaps	0	1	1	0	1	1	1	0	1	0	1	1	3	4	7
Chromosome gaps	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Chromatid breaks	0	0	0	1	1	2	1	1	2	1	4	5	18	4	22
Chromatid exchanges	0	0	0	0	0	0	0	0	0	0	0	0	9	11	20
Chromosome breaks	0	0	0	0	1	1	1	0	1	0	0	0	0	0	0
Chromosome exchanges	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Total aberrations (excluding gaps)[a]	0	0	0 (0.0)	1	2	3 (1.5)	2	1	3 (1.5)	1	4	5 (2.5)	27	15	42 (42.0)
No. of cells with aberr. (excluding gaps)[a]	0	0	0 (0.0)	1	2	3 (1.5)	2	1	3 (1.5)	1	4	5 (2.5)[b]	25	14	39 (39.0)[b]
Miscellaneous aberr.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cells with multiple aberr. (5 or more aberr.)	0	0	0	0	0	0	0	0	0	0	0	0	5	2	7

[a] Values in parentheses are percentages.
[b] Significantly (alpha <0.05) different from negative control.

Table 6.4-7 Results of the chromosomal aberration assay 24 hours after treatment in the presence of S9; Experiment 2 Replicates are designated A & B

	Neg. control			Clopyralid 697.5 µg/mL			Clopyralid 1395 µg/mL			Clopyralid 2790 µg/mL			Pos. control		
	A	B	A+B	A	B	A+B	A	B	A+B	A	B	A+B	A	B	A+B
No. of cells scored	100	100	200	100	100	200	100	100	200	100	100	200	50	75	125
Chromatid gaps	0	1	1	1	1	2	2	1	3	1	0	1	0	0	0
Chromosome gaps	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Chromatid breaks	0	3	3	0	0	0	4	0	4	3	1	4	4	9	13
Chromatid exchanges	0	0	0	0	0	0	4	0	4	0	1	1	11	16	27
Chromosome breaks	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1
Chromosome exchanges	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Total aberrations (excluding gaps)[a]	0	3	3 (1.5)	0	0	0 (0.0)	8	0	8 (4.0)	3	2	5 (2.5)	15	26	41 (32.8)
No. of cells with aberr. (excluding gaps)[a]	0	3	3 (1.5)	0	0	0 (0.0)	6	1	7 (3.5)	3	3	6 (3.0)	17	20	37 (29.6)[b]
Miscellaneous aberr.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cells with multiple aberr. (5 or more aberr.)	0	0	0	0	0	0	0	1	1	0	1	1	6	2	8

[a] Values in parentheses are percentages.
[b] Significantly (alpha <0.05) different from negative control.

Table 6.4-8 Historical background aberration frequencies

Year	S-9 (- or +)	FREQUENCIES [a] Mean	Std. Dev.	Minimum	Maximum	N [b]
1987	-	3.8	0.35	3.5	4.0	2
	+	2.8	2.36	1.0	5.5	3
1988	-	1.6	0.80	0.5	3.0	7
	+	1.7	1.33	0	4.0	6
1989	-	1.0	0.29	0.5	1.5	7
	+	1.1	0.74	0	2.0	8
1990	-	1.0	0.50	0.5	1.5	3
	+	1.5	0.50	1.0	2.0	3
1991	-	1.3	0.88	0	3.5	26
	+	1.2	1.12	0	4.0	26
1992	-	1.6	1.40	0	5.5	19
	+	1.8	1.00	0	3.5	19
1993	-	2.1	2.10	0	6.5	14
	+	1.9	1.55	0.5	5.5	14
1994	-	1.7	1.21	0	4.5	21
	+	1.6	0.87	0	3.5	20
1995	-	1.61	0.82	0	3.0	9
	+	0.78	0.51	0	1.5	9
1996	-	1.25	0.91	0	3.0	14
	+	1.36	1.47	0	5.0	14
1997	-	1.50	1.00	0.5	3.0	7
	+	2.14	1.52	0	4.5	7
1998	-	1.75	0.98	0.5	3.5	10
	+	2.10	1.94	0	6.5	10
1999	-	1.23	0.68	0	2.0	11
	+	1.30	1.53	0	5.0	10
2000	-	1.27	0.88	0	3.0	13
	+	0.77	0.48	0	1.5	13

[a] Frequencies are % cells with aberrations excluding gaps.

[b] Number of experiments

Conclusion

Despite low cytotoxicity the concentration selection mainly fulfils the study requirements demanding the highest concentration to be 10 mM which means 1920 µg/mL clopyralid. The highest concentrations in the study exceed this value except the highest evaluated concentration of 697.5 µg/mL in Experiment 2 in the absence of S9 where 45.9 % reduction in mitotic index in relation to the negative control was observed.

At this concentration of 697.5 µg/mL in Experiment 2 in the absence of S9, frequency of aberrant cells was significantly different from the negative control but within the historical control range of the past five years. As the concentration of 697.5 µg/mL does not fulfil the OECD Guideline 473 requirement for the dose selection and neither does the 45.9 % reduction in mitotic index, no definite evidence for the positive or negative result of the study can be indicated. Hence, the result of the study is considered equivocal.

B.6.4.2. In vivo studies in somatic cells

Study:	Evaluation of clopyralid in the mouse bone marrow micronucleus test ([redacted], 1991)
Previous evaluation:	This study was submitted to DAR (2003). After the new evaluation, the study is not considered acceptable anymore. Only 1000 polychromatic erythrocytes/animal were scored instead of 2000 required by the OECD Test Guideline 474 (1997) valid at the moment of the dossier preparation. According to the current OECD Test Guideline (2014) at least 4000 PCEs/animal should be scored. As 4/5 and 2/5 males and 3/5 and 2/5 females at the highest dose 5000 mg/kg bw died at 24 and 48 hours, respectively, the maximum tolerated dose (MTD) was exceeded and hence, the dose selection does not fulfil the study requirements. 72 hours sampling time is not appropriate for bone marrow. Based on the inappropriate dose selection and too few cells scored, the study is considered to be not acceptable.

Test guideline and GLP
The study was performed according to GLP but the method deviates from the OECD Test Guideline 474 (1997) (valid at the moment of dossier preparation) as only 1000 polychromatic erythrocytes/animal were scored instead of 2000 required by the test guideline. According to the current OECD Test Guideline (2014) at least 4000 PCEs/animal should be scored. The highest dose level exceeded the MTD and hence, the dose selection does not fulfil the study requirements. 72 hours sampling time used is not appropriate for bone marrow.

Materials and methods
Clopyralid (96.1% pure; Batch No AGR 233257) was evaluated in the mouse bone marrow micronucleus test. The test material was administered to CD-1 (ICR) mice as single gavage doses of 0 (negative control), 500, 1667 or 5000 mg/kg bw in corn oil. Groups of mice (5/sex/treatment) were sacrificed at 24, 48 or 72 hours after treatment. Mice treated with 120 mg/kg bw cyclophosphamide were used as positive controls and sacrificed 24 hours after treatment. Dose solutions were prepared freshly and analysed for test substance content.

A total of 1000 polychromatic erythrocytes (PCE) were examined from each animal and the number of micronucleated polychromatic erythrocytes (MN-PCE) was recorded. The ratio (%) of (PCE) to normochromatic erythrocytes (NCE) in the bone marrow was determined by examining 1000 erythrocytes.

Results
4/5 and 2/5 males and 3/5 and 2/5 females at the highest dose 5000 mg/kg bw died at 24 and 48 hours, respectively. Hence, the maximum tolerated dose (MTD) was clearly exceeded and the dose selection does not fulfil the study requirements of three analysable dose levels covering a range from the maximum to little or no toxicity.

The frequencies of MN-PCE in males at 1667 mg/kg bw at 24 hours sacrifice and in females at 500 and 1667 mg/kg bw at 48 hours were more than double as high as in the control animals. The result of the 5000 mg/kg bw females at 48 hours with 4.5 times increased frequency of MN-PCE is discarded based on the three survived animals only. The positive control mice showed significant increases in MN-PCE.

Table 6.4-9 Summary of the data on the frequencies of micronucleated polychromatic erythrocytes (MN-PCE) in the bone marrow of male mice treated with the test material or cyclophosphamide (CP)

Treatment (mg/kgBW)	24 h Sacrifice N[a]	24 h Sacrifice MN-PCE[b]	24 h Sacrifice % PCE[b]	48 h Sacrifice N[a]	48 h Sacrifice MN-PCE[b]	48 h Sacrifice % PCE[b]	72 h Sacrifice N[a]	72 h Sacrifice MN-PCE[b]	72 h Sacrifice % PCE[b]
Negative Control[c]	5	1.0± 1.2	58.8± 7.4	5	1.8± 1.1	65.1± 2.0	5	1.4± 1.7	58.6 6.7
Positive Control[d]	5	50.6± 17.6	53.0± 5.7	--[e]	--	--	--	--	--
500	5	1.2± 1.1	65.0± 5.0	5	0.8± 0.8	63.3± 3.8	5	1.2± 0.4	63.4± 5.9
1667	5	2.2± 2.3	63.7± 3.9	5	0.4± 0.9	61.7± 8.5	5	1.2± 0.4	58.1± 4.2
5000	1	1.0	66.6	3	2.0 0.0	60.8 7.6	2	2.0 1.4	60.6 6.1

[a]N = number of mice. Five mice were treated/group. The numbers shown are the mice surviving at the time of scheduled sacrifice. 1000 PCE were examined from each survivor.

[b]Data are means and standard deviations.

[c]Vehicle used to mix the test material

[d]120 mg/kgBW Cyclophosphamide

[e]Not done

Table 6.4-10 Summary of the data on the frequencies of micronucleated polychromatic erythrocytes (MN-PCE) in the bone marrow of female mice treated with the test material or cyclophosphamide (CP)

Treatment (mg/kgBW)	24 h Sacrifice N[a]	MN-PCE[b]	% PCE[b]	48 h Sacrifice N[a]	MN-PCE[b]	% PCE[b]	72 h Sacrifice N[a]	MN-PCE[b]	% PCE[b]
Negative Control[c]	5	1.0± 0.7	60.5± 3.8	5	0.6± 0.9	56.7± 21.3	5	1.6± 1.1	56.9± 9.0
Positive Control[d]	5	43.2± 16.0	54.7± 3.6	--[e]	--	--	--	--	--
500	5	0.4± 0.9	60.6± 6.8	5	1.4± 1.5	64.6± 5.6	5	1.2± 0.4	61.7± 6.9
1667	5	1.4± 0.5	65.1± 4.1	5	1.4± 1.1	70.6± 8.1	5	1.4± 1.1	67.3± 6.4
5000	2	1.5± 2.1	57.0± 20.9	3	2.7± 2.1	62.8± 12.4	4	1.3± 1.3	58.8± 20.6

[a]N = number of mice. Five mice were treated/group. The numbers shown are the mice surviving at the time of scheduled sacrifice. 1000 PCE were examined from each survivor.
[b]Data are means and standard deviations.
[c]Vehicle used to mix the test material
[d]120 mg/kgBW Cyclophosphamide
[e]Not done

Conclusion
Increased frequencies of MN-PCE were observed in males at 1667 mg/kg bw at 24 hours sacrifice and in females at 500 and 1667 mg/kg bw at 48 hours where the frequencies of MN-PCE were more than double as high as in the control animals. The result of the 5000 mg/kg bw females with 4.5 times increased frequency of MN-PCE is discarded based on the three survived animals only.

Too few cells/animal were scored and the highest dose exceeded the MTD. Hence, the study requirements were not fulfilled and performing a new study to confirm the positive result is recommended.

Based on the inappropriate dose selection and too few cells scored, the result of the study should be confirmed and overall the study is considered to be not acceptable.

Study:	Acute and subacute *in vivo* cytogenetic study in rats ([redacted] 1973)
Previous evaluation:	This study was submitted to DAR (2003). The study was not performed according to any official test guideline and not according to GLP. After the new evaluation, the study is not considered acceptable anymore. No cytotoxicity, measured as a reduction in mitotic index compared to negative control, was observed in test substance treated animals. Hence, the test substance concentrations studied can be deduced to have been too low. This is obvious also on the grounds of the dose levels used in the *in vivo* micronucleus test ([redacted] 1991). Based on the too low dose levels, the

	study is not acceptable.

Test guideline and GLP
The study was not performed using any official guideline or test method, and it was not done under GLP. The study was performed in 1973 before the existence of OECD or GLP guidelines. The method used is an early version of the *in vivo* Mammalian bone-marrow chromosome aberration test (Directive 67/548/EEC, Annex V, Method B.11), where in addition to a single-dose followed by three sacrifice intervals, groups of animals were also dosed for 5 days prior to sacrifice.

Materials and methods
DOWCO 290 (purity not stated) was evaluated in a cytogenetic study in Sprague-Dawley rats. Groups of male rats were administered DOWCO 290 by gavage at doses of 4, 40 or 400 mg/kg bw in corn oil in an acute study (single administration; 5 animals/treatment/time interval) and in a subacute study (administration once daily for five days; 5 animals/treatment). In the acute study, the negative controls were corn oil alone (administered by gavage; 5 animals/treatment/time interval) and saline alone (administered intraperitoneally; 5 animals/treatment, single time interval, as with positive control) and the positive control was triethylene melamine (administered intraperitoneally; 5 animals/treatment, single treatment). In the subacute study, the negative control was corn oil (administered by gavage; 5 animals/treatment/time interval). A positive control was not employed in the subacute study.

In the acute study, bone marrow cells were examined at metaphase from 5 animals/treatment, 6, 24 and 48 hours after administration (24 hours after treatment only for the saline negative control and the triethylene melamine positive control). In the subacute study, bone marrow cells were examined at metaphase from all animals 5 days after the final administration.

Diploid cells were scored for chromatid gaps and breaks, chromosome gaps and breaks, reunions, cells with greater than 10 aberrations, polyploidy, pulverisation and any other chromosome aberrations. Metaphase (50) spreads were scored for each animal. Mitotic indices were calculated as the number of cells in mitosis divided by the number of cells observed from a count of at least 500 cells.

Results
In the acute and subacute studies, there were no significant aberrations of chromosomes following administration of clopyralid. In contrast, the positive control led to significant aberrations.

No cytotoxicity, measured as a reduction in mitotic index compared to negative control, was observed in test substance treated animals. Hence, the test substance concentrations studied can be deduced to have been too low. This is obvious also on the grounds of the dose levels used in the *in vivo* micronucleus test ([redacted] 1991).

Conclusion
Under the conditions of this study, DOWCO 290 did not induce chromosome aberrations in rats following administration at up to 400 mg/kg bw/day. However, the test substance concentrations studied were too low. Hence, the study is not acceptable.

B.6.4.3. In vivo studies in germ cells

Study:	Dominant lethal mutagenesis assay ([redacted] 1973)
Previous evaluation:	This study was submitted to DAR (2003). The study was not performed according to any official test guideline and not according to GLP. The study is not considered acceptable anymore. No justification for dose selection was given. No signs of toxicity caused by the test substance were observed. Based on this fact and the knowledge on dose selection in *in vivo* studies in somatic cells, test substance concentrations studied can be deduced to have been too low. The procedure with 7 matings does not cover all phases of male germ cell maturation in rat. To ensure that all phases of male germ cell maturation are evaluated for dominant lethal mutation induction, for five daily dose

	administrations, there should be 10 matings in rat conducted at weekly intervals following the last treatment. If the goal is to determine whether a substance induces dominant lethal mutations *per se*, it would be necessary to expose an entire round of spermatogenesis and mate once at the end (OECD Test Guideline No. 478; adopted 28th July 2015). Because of these deviations, the study is not considered acceptable and no detailed evaluation was performed.

Test guideline and GLP
The study was not performed using any official guideline or test method, and it was not done under GLP. The study was performed in 1973 before the existence of OECD or GLP guidelines. The method used is an early version of the Dominant Lethal test (OECD guideline 478 / Directive 67/548/EEC, Annex V, Method B.22), employing smaller numbers of animals.

Materials and methods
Groups of male Sprague-Dawley CD rats (10/treatment) were administered DOWCO 290 (purity not stated) by gavage at doses of 4, 40 or 400 mg/kg bw/day in corn oil once daily for five days. Similar groups received a single intraperitoneal administration of saline only, oral corn oil only (negative controls) or intraperitoneal administration of triethylene melamine (positive control).

Following treatment, the animals were mated with two virgin females each week for seven weeks. The females were necropsied two weeks after the middle of the cohabitation week and the uterus examined for the number of *corpora lutea*, total implantations and foetal mortalities.

Results
There were occasional significant differences ($p \leq 0.05$) following clopyralid treatment compared to the negative control for fertility index at 40 mg/kg bw/day in week 2 and implantation losses at 4 and 400 mg/kg bw/day in week 5. These differences were considered incidental and not toxicologically significant, as they were not dose-related. Overall, there were no treatment-related reductions in fertility index, number of implantations, number of *corpora lutea*, pre-implantation losses or resorptions following administration of clopyralid.

The positive control significantly reduced the number of implantations ($p \leq 0.01$), and increased pre-implantation losses ($p \leq 0.01$) and resorptions ($p \leq 0.01$) compared to the negative control on three of the first four matings.

Conclusion
Under the conditions of this study, DOWCO 290 showed no detectable dominant lethal activity following administration at up to 400 mg/kg bw/day.

As the test substance concentrations studied were too low and the study protocol does not cover all phases of male germ cell maturation, the study is not acceptable.

B.6.5. LONG-TERM TOXICITY AND CARCINOGENESIS

Summary of long term toxicity and carcinogenicity
From the one acceptable study, administration of clopyralid at 150 and 1500 mg/kg bw/day to Fischer-344 rats caused hyperplasia and thickening of the epithelium of the anterior surface of the gastric limiting ridge. The effect was more frequently recorded in animals treated at 1500 mg/kg bw/day and this dose level was also associated with reduced body weight, decreased food consumption, increased relative liver and kidney weight and a grossly visible increase in the size of the gastric limiting ridge. Although the gastric limiting ridge is not present in human's stomach, the lesions detected in rats characterize the irritant nature of clopyralid rather than being species specific effect. There was no evidence that clopyralid caused increased incidence of malignant or non-malignant tumours in the rat. NOAEL-value for rat is 15 mg/kg bw/day. In the supplementary study, no toxicologically significant effects were associated with ingestion of Dowco 290 for 2 years. The only finding which was detected and may be related to the ingestion of Dowco 290 in the diet was a reduction in the mean body weight of female rats at the high dose level, 150 mg/kg bw/day.

In an acceptable 2-year study in $B_6C_3F_1$ mice, dietary administration of clopyralid at 2000 mg/kg bw/day led to a reduction in body weight in males. No other significant toxicological effects were recorded in males and no significant toxicological effects were recorded in female mice at all. There was no evidence that clopyralid caused increased incidence of malignant or non-malignant tumours in the mouse. NOAEL-value for mouse is 500 mg/kg bw/day.

Table B.6.5-1 Summary of long term toxicity and carcinogenicity of clopyralid

STUDY (ROUTE)	SPECIES/ STRAIN	DOSAGES	NOAEL	LOAEL	EFFECTS AT LOAEL	REFERENCE
2-year chronic toxicity and oncogenicity study (diet)	Rat/ Fischer-344 (male/female)	15, 150, 1500 mg/kg bw/day	15 mg/kg bw/day	150 mg/kg bw/day	Lesions of the gastric limiting ridge, slightly reduced food consumption	[redacted] *et al.*, 1985 and 1986 **Acceptable**
2-year combined toxicity and carcinogenicity (dietary)	Rat/ Sprague-Dawley (male)	5, 15, 50, 150 mg/kg bw/day	>150 mg/kg bw/day	> 150 mg/kg bw/day	ND	[redacted] *et al.*, 1977 **Supplementary**
	Rat/ Sprague-Dawley (female)	5, 15, 50, 150 mg/kg bw/day	50 mg/kg bw/day	150 mg/kg bw/day	Reduction in body weight	[redacted] *et al.*, 1978 [redacted] 1985
2-year dietary chronic toxicity-oncogenicity study (diet)	Mouse/ $B_6C_3F_1$ (male)	100, 500, 2000 mg/kg bw/day	500 mg/kg bw/day	2000 mg/kg bw/day	Reduction in body weight	[redacted] *et al.*, 1984 [redacted] et al., 1986 **Acceptable**
	Mouse/ $B_6C_3F_1$ (female)	100, 500, 2000 mg/kg bw/day	2000 mg/kg bw/day	>2000 mg/kg bw/day	ND	
18-month carcinogenicity (dietary)	Mouse/ CR strain (male/female)	35, 100, 350 ppm	>350 ppm (> 52,5 mg/kg bw/day)	> 350 ppm (> 52,5 mg/kg bw/day)	ND	[redacted] 1976 **Supportive**

ND: Not determined: no adverse effects

Study:	DOWCO 290: 2-year rat diet chronic toxicity and oncogenicity study. Final report ([redacted] 1986) DOWCO 290: 2-year rat diet chronic toxicity and oncogenicity study. 1-year interim report ([redacted] 1985)
Previous evaluation:	This study was submitted to DAR (2003). The study was performed predominantly according to OECD guideline 453 (2009). Text has been modified but conclusions have not changed. The study is acceptable.

Test guideline and GLP

According to information from DAR (2003) the study was performed according to GLP. However, it seems that the only statement of GLP was in the Quality assurance statement, mentioning that in compliance with GLP regulations the study was inspected by the QA Unit. The method used complies predominantly with OECD test guideline 453 (2009). It is not completely clear whether the observation of general health status was done in more general level than what described in the guideline. It seems that ophthalmological examination was not done but eyes were examined during necropsy. Blood samples for hematology and clinical chemistry and urinalysis were not taken at 3 month. In the hematology investigations MCV, MCH, MCHC, prothrombin time and activated partial thromboplastin time were not measured. In the clinical chemistry investigations creatinine, total protein, albumin, calcium, total cholesterol were not determined. In the urinalysis determinations investigations on appearance, volume and occult blood were negleted. The weight of epididymides, thyroid and uterus was not determined, adrenals and spleen were weighed only at 24 month necropsy. Lacrimal gland was not preserved during necropsy.

Materials and methods
The chronic toxicity and carcinogenicity of DOWCO 290 (96.7% clopyralid) to rats was evaluated over two years. Groups of Fischer-344 rats, 70/sex/treatment, received doses of 0 (control), 15, 150 or 1500 mg/kg bw/day for two years. Dietary concentrations were adjusted weekly for the first three months of the study, and thereafter monthly to give the correct dosages. Approximately 3 month intervals samples of diets were taken to verify test material concentration. Data were collected on mortality, clinical appearance and behaviour daily. Palpation examination was conducted once during prestudy period, prior to 6 month necropsy (on 10 rats/sex/group) and all rats prior to 12 month necropsy and monthly thereafter. Food consumption and body weight was determined weekly for the first 12 weeks of the study and every four weeks thereafter. Data were collected also on haematology, clinical chemistry, urinalysis, organ weights, gross pathology and histopathology. The investigations on hematology included packed cell volume, haemoglobin concentration, red and white blood cell counts, platelet counts and WBC differential counts. Serum chemistry values included glucose, alanine transaminase, aspartate transaminase, alkaline phosphatase, urea nitrogen, sodium, potassium, and chloride. In urinalysis specific gravity, pH, protein, glucose, ketones, bilirubin and urobilinogen were investigated. Carcasses and tissues of all rats found dead or killed moribund were examined. Gross necropsies included examination of the eyes. Representative portions of tissues (48) were preserved in the necropsy and liver, kidneys, brain, heart, testes, ovaries, thymus gland (6 month necropsy only), adrenal gland and spleens (24 month necropsy only) were weighed. Interim sacrifices (10/sex/group) were made at 6 and 12 months. Additional measurements of haematology, clinical chemistry and urinalysis (10/sex/group) were also made at 18 months and at termination (20/sex/group). The study design is summarised in Table B.6.5-2.

Table B.6.5-2 Design of two year chronic toxicity/carcinogenicity study of DOWCO 290 in Fischer 344 rats

Parameter	Number of rats/sex/group investigated			
	6 month	12 month	18 month	24 month
Haematology	10	10	10	20
Clinical chemistry	10	10	10	20
Urinalysis	10	10	10	20
Necropsy	10	10	0	50
Organ weights	10	10	0	<50[1]
Histopathology[2]	10	10	0	50

[1] All survivors at termination. [2] Selected tissues in all groups, all tissues in control and highest dose group.

Results
The observed test material concentration in the diet was > 82% of the targeted concentration throughout the study in all dose groups.

The cumulative mortality of the high dose level males (4 animals dead) at the end of the study was statistically different from controls (13 animals dead). Such difference was not observed in any other dose group. However, the cumulative mortality of females seemed to have a dose response. From the most probable causes of death of animals found dead or in moribund states necessitating sacrifice, pituitary neoplasms (adenoma and adenocarcinoma) and Fischer rat leukemia were the two most common ones (together more than 50 % of early deaths). Other reasons (neoplasms) observed only in the test item dose group animals were observed only in one animal, except mesothelioma observed in three males (1 animal low dose group, 2 animals middle dose group).

According to study report there were no clinical observations attributable to administration of the test material. The animals in high dose level groups (1500 mg/kg bw/day), and occasionally males in the middle dose level group, tended to eat little (<10%) less than controls. Logically, statistically significant lower mean body weights were seen in both male and female high dose groups. No differences attributable to administration of the test material occurred in serum chemistry, urinalysis or haemotological assessment. In the interim report statistically significant decrease from control were found at the 6 month evaluation for packed cell volume, haemoglobin and red blood cells of males in the high dose group and a dose response was observed. These effects were not seen in females and did not appear in any male groups in later examinations. At 6 month evaluation glucose was statistically significantly decrease from control on males at low, middle and high dose groups and there was a dose response. This effect was not seen at 12 month evaluation. The same effect was observed also on alkaline phosphatase. On females there was a statistically significantly decrease in alanine transaminase from control at 12 month evaluation at low, middle and high dose groups. Group mean serum chemistry levels of chloride for females in the high dose group were statistically significantly decreased at 18- and 24 month evaluations. The decrease was small (<3%) and no statistically significant decreased values of sodium and potassium were observed.

The final fasted body weights of animals in the high dose group at the necropsy (24 month) were lower than control group; however, the difference was not statistically significant on males. These decreases were mentioned to be attributable to administration of test material. The final fasted body weight was also lower at 6 and 12 month evaluations but was not statistically significant.

Relative (to body weight) liver and kidney weights were significantly increased in animals receiving 1500 mg/kg bw/day after 6, 12 and 24 months (males) and 6 and 12 months (females) (Table 6.5-3). The significant increases in absolute kidney weights recorded in females after 6 months were not apparent after 12 or 24 months. There was also a statistically significant decrease in the absolute and relative weights of spleen on animals at dose level 1500 mg/kg bw/day after 24 months. The relative brain and heart weight were statistically significantly increased on females at dose level 1500 mg/kg bw/day after 24 months. Absolute heart weight was statistically significantly decreased. On males the relative heart weight was statistically significant increased at 6 month at low and high dose levels. Also the relative weight of ovaries was statistically significantly increased at low and high dose level females after 24 months. Relative thymus weight was statistically significantly increased at high dose level females at 6 month evaluation.

Table 6.5-3 Effect of clopyralid on kidney, liver and spleen weights in rats following administration of clopyralid for 2 years

Parameter	Assessment time (months)	Males (mg/kg bw/day)				Females (mg/kg bw/day)			
		0	15	150	1500	0	15	150	1500
Liver weight (g)	6	8.977	8.800	8.681	9.121	4.919	4.907	5.052	5.367*
	12	9.075	8.906	8.991	9.415	5.605	5.705	5.528	5.801
	24	10.299	11.009	10.473	10.688	7.884	7.407	7.280*	7.273*
Relative liver weight (g/100 g bw)	6	2.751	2.738	2.685	2.892*	2.731	2.676	2.676	3.073*
	12	2.473	2.447	2.459	2.627*	2.479	2.489	2.468	2.764*
	24	2.699	2.822	2.698	2.904*	2.783	2.654	2.584	2.848
Kidney weight (g)	6	2.378	2.257	2.309	2.442	1.372	1.391	1.473*	1.456
	12	2.577	2.436*	2.583	2.618	1.642	1.630	1.625	1.634
	24	2.903	2.896	2.910	3.002	2.136	2.049	2.067	2.047
Relative kidney weight (g/100 g bw)	6	0.729	0.705	0.714	0.774*	0.762	0.758	0.781	0.836*
	12	0.702	0.669*	0.706	0.731*	0.727	0.711	0.725	0.780*
	24	0.763	0.743	0.751	0.815*	0.756	0.732	0.734	0.805
Spleen weight (g)	24	1.253	2.782	1.282	0.910*	0.837	1.068	0.768	0.416*
Relative spleen weight (g/100 g bw)	24	0.326	0.749	0.332	0.247*	0.297	0.411	0.275	0.164*

* Statistically significantly different from untreated controls at $p \leq 0.05$.

Increased prominence of the gastric limiting ridge was recorded in the stomach of most animals (49 out of 50 males; 48 out of 50 females) receiving 1500 mg/kg bw/day for two years. Thickening of the epithelium of the anterior surface of the limiting ridge (increased cells in the stratum spinosum) and hyperplasia (increased cellular activity in the *stratum basale*) was recorded in most males and females receiving 1500 mg/kg bw/day. Thickening of the limiting ridge was recorded in a few animals and hyperplasia in approximately half of the animals receiving 150 mg/kg bw/day. Aggregation of inflammatory cells in the gastric mucosa was discernible in animals at all dose levels, also in control. According to study report the observed exacerbation of inflammatory cells in the gastric mucosa in animals at high dose level was judged to be an effect of treatment with the test material.

In histopathological observations there were aggregates of mononuclear cells in lacrimal/harderian glands and in high dose level males this was statistically different from control. In females mammary gland galactocele and benign fibroadenoma were observed at all dose levels, however no dose response was present. In pituitary benign adenoma was observed in animals at all dose levels. The incidence was approximately the same as on controls and no clear dose response was present. Pituitary adenocarcinoma was observed only on controls. In spleen malignant alterations (leukemia, metastasis) were observed at all dose levels and for males there was a decreasing dose response present. In testes benign leydig cell tumor was observed in almost all of the males in control and test item dose groups.

The number of total animals with benign or malignant tumors or the number of total benign or primary malignant tumors shows no clear difference between control and test item dose groups (Table 6.5-4).

Table B.6.5-4 Tumor Incidence in Fischer-344 rats.

Parameter	Males (mg/kg bw/day)				Females (mg/kg bw/day)			
	0	15	150	1500	0	15	150	1500
No. Examined Microscopically	50	50	50	50	50	50	50	50
Total Animals with Primary tumors	50	50	50	50	39	40	35	43
Total Primary Tumors	105	98	99	98	66	64	58	65
Total Animals with Benign Tumors	50	50	50	50	30	36	31	36
Total Benign Tumors	91	77	79	92	51	56	46	53
Total Animals with Malignant Tumors	12	18	18	4	14	8	12	12
Total Primary Malignant Tumors	14	21	20	6	15	8	12	12

Conclusions
Under the conditions of this study, following administration of clopyralid at 15, 150 and 1500 mg/kg bw/day for two years, the NOAEL for male and female rats was 15 mg/kg bw/day, based on lesions of the gastric limiting ridge and slightly reduced food consumption. The lesions in stomach were detected also in rat in subchronic studies. Although the gastric limiting ridge is not present in human's stomach, the lesions detected in animals characterize the irritant nature of clopyralid rather than being species specific effect. Administration of clopyralid at 1500 mg/kg bw/day was also associated with reduced body weight, increased relative liver and kidney weight and macroscopically visible increase in the size of the gastric limiting ridge. There was no evidence that clopyralid caused an increased incidence of malignant or non-malignant tumours in the rat at any dose level. The study is acceptable.

Study:	DOWCO 290 (3,6-dichloropicolinic acid): results of a two-year chronic toxicity and oncogenicity study in rats by the dietary route ([redacted] *et al*., 1977)
Previous evaluation:	This study was submitted to DAR (2003). It was evaluated as supplementary in the DAR. No re-evaluation has been performed and the text has not been modified.

Test guideline and GLP
The study was not performed using any official guideline or test method, and it was not done under GLP. The study was finalized in 1977 before the existence of OECD test guidelines or GLP guidelines.

Deviations from current guideline (OECD 453/Directive 67/548/EEC, Annex V, Method B.33): The guideline requires clinical investigations on satellite groups of animals, distinct from the main groups, whereas this study did not have satellite animals. Group sizes were too small for current guidelines. Haematology was performed

only after one year (5 males/treatment at one year) and at termination (five males, five females, control and high dose only). Investigations did not assess clotting potential. Clinical chemistry suite did not assess total protein, albumin, or glucose levels, and it was performed only between 3 and 12 animals per group after one year and at termination, not at six-monthly intervals. Organs weighed and taken for histopathology were insufficient for modern guidelines: adrenals and female gonads were not weighed, and thymus, spleen, female mammary gland, skin, bone marrow and femur plus joint were not taken, though abnormal tissues were taken. Tissues were examined histopathologically from only seven rats in control and high dose groups, plus animals that died or were killed during the course of the study.

Materials and methods
Groups of Sprague-Dawley rats, 40/sex/treatment, received doses of 5, 15, 50 or 150 mg/kg bw/day of Dowco 290 (clopyralid 92.8%) for two years via the diet. Additional groups of 80 males and 79 females were used as untreated controls. Data were collected on mortality, clinical appearance and behaviour, body weights, food consumption, haematology, clinical chemistry, urinalysis, organ weights, gross pathology and histopathology. Report text states that bodyweight data was collected monthly. However, the data are presented for weekly bodyweights for the first five months. Clinical examinations were conducted less frequently than required (see above). The study design is summarised in Table B.6.5-5.

Table B.6.5-5 Number of animals studied in two year chronic toxicity/carcinogenicity study of clopyralid

Parameter	Number of rats/sex/group investigated			
	Males		Females	
	12 months	24 months	12 months	24 months
Haematology	5	10[1]	5[1]	10[1]
Clinical chemistry	0	12, 3 - 7[2]	0	12
Urinalysis	5[1]	10[1]	5[1]	10[1]
Necropsy	0	80, 40	0	79, 40
Organ weights	0	12, 3 - 7[2]	0	31, 15 - 18
Histopathology	0	80, 40[3]	0	79, 40[3]

[1] High dose and control groups only. [2] Number of rats per group examined varied as shown. Control group contained more animals than treated groups, and where numbers vary, table gives 'control, range for test groups' e.g. '12, 3-7'. [3] Histopathology included all major tissues from 7 rats/sex in control and high dose groups, plus all macroscopically abnormal tissues from any animal.

Results
No toxicologically significant effects were associated with ingestion of Dowco 290 for 2 years. The only finding which was detected and may be related to the ingestion of Dowco 290 in the diet was a trend toward decrease in the mean body weight of female rats at the high dose level, 150 mg/kg bw/day.

Conclusions
Under the conditions of this study, the NOAEL for male rats was 150 mg/kg bw/day based on the absence of significant toxicological effects at the highest dose tested. The NOAEL for female mice was 50 mg/kg bw/day based on the body weight reduction in females receiving 150 mg/kg bw/day for two years. The study is supplementary.

Study:	Supplemental report on DOWCO 290 (3,6-dichloropicolinic acid): results of a two-year chronic toxicity and oncogenicity study in rats by the dietary route ([redacted] *et al.*, 1978)
Previous evaluation:	This study was submitted to DAR (2003). No re-evaluation has been performed and the text has not been modified.

Test guideline and GLP
See above study ([redacted] *et al.*, 1977). This report gives data for additional histopathological investigations.

Materials and methods
The chronic toxicity and carcinogenicity of DOWCO 290 to rats was evaluated over two years ([redacted] *et al.*, 1977). Supplementary histopathological examinations were conducted on an additional 8 rats/sex in the high dose group (150 mg/kg bw/day) and the untreated control group.

Results
There were no toxicologically significant treatment-related histopathological effects.

The data from the additional animals were combined with the original data and assessed for toxicological and statistical significance. In four cases, the increased group size resulted in findings, which had been statistically significant in the original data (but described as being of no toxicological significance), losing their statistical significance.

Conclusions
This supplementary report does not change the conclusions of the original report ([redacted] *et al*., 1977).

Study:	DOWCO 290 (3,6-dichloropicolinic acid): results of a two-year chronic toxicity and oncogenicity study in rats by the dietary route. Supplemental report on the histopathological evaluation of thyroid gland and pituitary gland tissues from female rats in the 50, 15 and 5 mg/kg bw/day groups ([redacted] 1985)
Previous evaluation:	This study was submitted to DAR (2003). No re-evaluation has been performed and the text has not been modified.

Test guideline and GLP
See above study ([redacted] *et al*., 1977). This report gives data for additional histopathological investigations.

Materials and methods
The chronic toxicity and carcinogenicity of DOWCO 290 to rats was evaluated over two years ([redacted] *et al*., 1977). In response to a request from the US EPA, supplementary histopathological examinations were conducted to evaluate tumour incidence in the thyroid and pituitary glands from female rats treated with clopyralid at 5, 15 and 50 mg/kg bw/day for two years. Microscope slides from the original investigation were re-read along with the additional slides, to establish and apply uniform diagnostic criteria across time.

Results
There were no toxicologically significant treatment-related histopathological effects. Also, there was no indication of an oncogenic effect on the thyroid or pituitary of treated females at any dose level.

Conclusions
This supplementary report does not change the conclusions of the original report ([redacted] *et al*., 1977).

Study:	DOWCO 290: 2-year dietary chronic toxicity-oncogenicity study in mice. (Final Report) ([redacted] 1986) DOWCO 290: 2-year chronic toxicity and oncogenicity study in mice: 1-year interim report ([redacted] 1984)
Previous evaluation:	This study was submitted to DAR (2003). The study was performed predominantly according to OECD guideline 453 (2009). Text has been modified but conclusions have not changed. The study is acceptable.

Test guideline and GLP

According to information from DAR (2003) the study was performed according to GLP. However, it seems that the only statement of GLP was in the Quality assurance statement, mentioning that in compliance with GLP regulations the study was inspected by the QA Unit. The method used complies predominantly with OECD test guideline 453 (2009). It is not completely clear whether the observation of general health status was done in more general level than what described in the guideline. It seems that ophthalmological examination was not conducted on mice. Blood samples for hematology and clinical chemistry were not taken at 3 month. In the hematology investigations MCV, MCH, MCHC, prothrombin time and activated partial thromboplastin time were not measured. Only some of the parameters of clinical chemistry were determined (alanine transaminase, alkaline phosphatase, urea nitrogen, total protein, albumin and glucose, globulin was calculated). Urinalysis was not performed. The weight of adrenals, epididymides, ovaries, spleen, thyroid and uterus were not determined.

Materials and methods
Groups of $B_6C_3F_1$ mice, 70/sex/treatment, received doses of Dowco 290 (96.7% clopyralid) 0 (control), 100, 500 or 2000 mg/kg bw/day for two years. Samples were taken for analysis of the test material concentration in the feed. Data were collected on mortality, clinical appearance and behaviour daily. Palpation examination was conducted once during prestudy period, prior to 12 month necropsy and approximately monthly intervals thereafter for the duration of the study. Food consumption and body weight was determined weekly for the first three months of the study and monthly thereafter. Data were collected also on haematology, clinical chemistry, organ weights, gross pathology and histopathology. The investigations on hematology included packed cell volume, percent haemoglobin, red and white cell counts, platelet counts and WBC differential counts. Serum chemistry values included glucose, alanine transaminase, alkaline phosphatase, urea nitrogen, total protein and albumin (globulin was calculated). Mice that died or were killed moribund were necropsied. Representative portions of tissues (48) were preserved in the necropsy and liver, kidneys, brain, heart and testes were weighed. Interim sacrifices (10/sex/group) were made at 6 and 12 months. The study design is summarised in Table B.6.5-6.

Table B.6.5-6 Design of two year chronic toxicity/carcinogenicity study of Dowco 290 in $B_6C_3F_1$ mice.

Parameter	Number of mice/sex/group investigated		
	6 months	12 months	24 months
Haematology	10	10	20
Clinical chemistry	10	10	20
Necropsy	10	10	50
Organ weights	10	10	50[1]
Histopathology[2]	10	10	50

[1] All survivors. [2] Selected tissues in all groups, all tissues in control and highest dose group.

Results
The observed test material concentration in the diet was > 84 % of the targeted concentration throughout the study in all dose groups.

The cumulative mortality of the middle dose level males (16 animals dead) at the end of the study was higher than controls (7 animals dead) but not statistically different. Most of the deaths were observed during last two months of the study and there was no dose relationship. The mortality of females was comparable to controls at all dose levels. From the most probable causes of early mortality, lymphosarcoma (13 animals), hepatocellular carcinoma (9 animals) and histiocytic sarcoma (six animals) were the three most common reasons. These were observed in the controls as well.

According to study report there were no clinical observations made during the study which were considered to be related to treatment. Food consumption of male mice in all dose groups was not statistically significantly different form control during the study. Food consumption of female mice in the middle and high dose group was statistically significantly lower than controls during the second year of the study.

In male mice there was a persistent decrease in mean body weight (10 – 12%) in the top dose group (2000 mg/kg bw/day) throughout most of the two years. In females the statistically significant increase in the mean body weights at dose level 500 mg/kg bw/day was observed approximately from fourth month of the study forward.

In the hematology investigations there was a statistically significant increase in haemoglobin and red blood cell count in females at low and middle dose levels at 6 months evaluation. At this time point white blood cell count was statistically significantly decreased at the high dose group females. At 12 months evaluation platelets were statistically significantly increased at low dose males and decreased in middle dose females. There was a statistically significant decrease in platelets in the low dose females at 24 month evaluation.

In clinical chemistry investigations there was a statistically significant increase in glucose at middle and high dose males and high dose females at 6 month evaluation. At 24 month evaluation there was a statistically significant increase in glucose at middle and high dose males and decrease in alkaline phosphatase at high dose females.

There were statistically significant decreases and increases in some organ and body weights at 6 and 12 month evaluations, however no statistically significant differences were observed in the final body weights or the organ weights of males and females at any dose level when compared to controls at 24 month evaluation.

At the end of the study there were pale focus observed in the livers and lungs of males and there was a dose response. Also mass/nodule was observed in livers and lungs of many animals but approximately at the same incidence as on controls. In females cystic endometrial hyperplasia was observed in uterus at the end of the study on 33-45 animals at all dose levels and control. However, there was no dose response.

In histopathological observations at the end of the study there was spindle cell hyperplasia in adrenals of males and females at all dose levels. In males the dose response had decreasing trend and on females there was no clear dose response. There was also mineralization in brains of males and females and on testes at all dose levels and there was a dose response. However, the incidence at the high dose level was approximately the same as on controls. Degeneration axons of peripheral nerve was observed in male mice at all dose levels and there was a dose response. The incidence at high dose level was lower than at control but the difference was statistically significant.

In histopathological observations e.g. the following neoplasms were observed: lacrimal/harderian gland benign cystadenoma (5-6 males and 3-5 females all dose levels), benign hepatocellular neoplasm/adenoma (total 20-30 males and 11-17 females all dose levels), malignant hepatocellular neoplasm/carcinoma (total 9-11 males and 2-5 females all dose levels), benign primary lung neoplasm/adenoma (total 10-16 males and 2-9 females all dose levels), malignant primary lung neoplasm/adenocarcinoma (total 2-5 males all dose levels). However, no clear dose response was seen, the incidence was approximately the same as on controls and the results were not statistically significant. Also benign anterior adenoma in pituitary was observed in females at all dose levels with decreasing dose response. The tumor incidence in $B_6C_3F_1$ mice is presented in Table B.6.5-7.

Table B.6.5-7 Tumor Incidence in $B_6C_3F_1$ mice.

Parameter	Males (mg/kg bw/day)				Females (mg/kg bw/day)			
	0	100	500	2000	0	100	500	2000
No. Examined Microscopically	50	50	50	50	50	50	50	50
Total Animals with Primary tumors	41	41	46	42	37	39	39	41
Total Primary Tumors	100	77	114	105	67	67	60	76
Total Animals with Benign Tumors	34	29	38	37	27	28	23	28
Total Benign Tumors	77	56	92	91	42	44	35	48
Total Animals with Malignant Tumors	18	17	19	13	21	20	21	26
Total Primary Malignant Tumors	23	21	22	14	25	23	25	28

Conclusions
Under the conditions of this study, the NOAEL for male mice was 500 mg/kg bw/day based on the body weight reduction in males receiving 2000 mg/kg bw/day for two years. The NOAEL for female mice was >2000 mg/kg bw/day based on the absence of significant toxicological effects at the highest dose tested. There was no evidence that clopyralid caused an increased incidence of malignant or non-malignant tumours in the mouse at any dose level. The study is acceptable.

Study:	18-month mouse oncology study with DOWCO 290 ([redacted] 1976)
Previous evaluation:	This study was submitted to DAR (2003). It was evaluated as supportive in the DAR. No re-evaluation has been performed and the text has not been modified.

Test guideline and GLP
The study was not performed using any official guideline or test method, and it was not done under GLP. The study was performed in 1976 before the existence of OECD test guidelines or GLP guidelines. Deviations from current guidelines (OECD 451 / Directive 67/548/EEC, Annex V, Method B.32) are common. E.g. in the study, food consumption was not recorded, no clinical biochemistry or haematological studies were done, and at necropsy for histopathology, aorta, skin, oesophagus, female mammary gland and femur (including joint) were not sampled.

Materials and methods
Groups of Charles River strain Swiss albino mice, 30 females/treatment and 15 males/treatment, received diets containing Dowco 290 (purity not stated) at 0 (control), 35, 100 or 350 ppm for 13 weeks. After this period, the females and males from the same dose group were mated. The resulting offspring were distributed into the same dose groups (50 to 60/sex/treatment) and fed diets containing clopyralid at the same concentration for 18 months. Data were collected on mortality, clinical appearance and behaviour, body weights, gross necropsy and histopathology.

Results
There were no changes in behaviour, clinical appearance or body weight of either parents or offspring. Since food consumption was not recorded, it was not possible to calculate the intake of clopyralid in mg/kg bw/day. After termination of the study, there were no changes in any of the tissues evaluated gross pathologically or microscopically.

Conclusions
Under the conditions of this study, the NOAEL for male and female mice was >350 ppm (about 52,5 mg/kg bw/day (IPSC Environmental Health Criteria No. 70, WHO (1987)) based on the absence of toxicologically significant treatment related effects at the highest concentration of clopyralid tested. The study is supportive.

B.6.6. REPRODUCTIVE TOXICITY

The reproduction toxicity of DOWCO 290 was investigated in one two-generation reproduction study, one supplementary histopathology study, and in three prenatal toxicity studies. The two-generation study was considered supportive for evaluation of the reproduction toxicity especially concerning male reproductive health. Two of three prenatal toxicity studies were acceptable. The NOAELs and LOAELs determined from these studies are expressed in Table 6.6-1.

In the two-generation study, several defects hamper the interpretation of the results. The mating schedule design did not allow each female to be mated with one male long enough to reveal the fertility of each male. Additionally, no histopathological investigation was presented on males failing to induce pregnancy during the five-day mating period allowed for each male, and the duration of pregnancy was not recorded. The unsystemically reduced dietary test article levels during the mating, gestation and/or lactation periods may indicate that the NOAEL/LOAEL values based on the premating dose levels are too high and should rather be based on lowest doses given during lactation. In addition, because the food consumption was not measured during the lactation periods, the dose levels cannot be calculated. The NOAEL for adults was 150 mg/kg bw/day based on the reduction in parental body weight and food consumption, and induced stomach lesions. The NOAEL for offspring was higher, 500 mg/kg bw/day, based on the reduction in F1 pup weights and increased liver weight. However, because the dietary concentrations were reduced during mating, pregnancy and lactation periods, but the food consumption was not recorded, lower NOAELs may be possible. Originally, in the DAR the conclusion was that based on this two-generation study in rats, the toxicity for reproduction cannot be evaluated without a doubt. The need for a new study should be considered. Additionally, this study was discussed in the Addendum 1 (2004) to DAR. It was presented that as the intended highest dose level was as high as 1500 mg/kg bw/day, thus, the actual doses were, at least, more than 700 mg/kg bw/day. However, the conclusion was that the results do not suggest any harm in the fertility of dams or in the offspring and that a specific reproductive risk is most unlikely.

In teratogenicity studies, clopyralid did not induce specific malformations or increased the incidence of spontaneous malformations at non-maternotoxic dose level.

There was no dose-relationship in foetal effects in F344 rats. The number of malformed foetuses increased (statistically nonsignificant) at maternotoxic dose level of 250 mg/kg bw/day. These malformations (polydactyly and hemivertebra), however, were considered incidental because no major malformations were observed in an additional group of animals dosed to 250 mg/kg bw/day. The number of resorptions did not increase at the high dose level. The NOAEL for developmental effects was >250 mg/kg bw/day. The maternal NOAEL was 15 mg/kg bw/day based on decreased liver weight and food consumption.

In rabbits, increased incidence of resorptions, malformations and alterations were seen at the maternotoxic dose level of 250 mg/kg bw/day (NOAEL 110 mg/kg bw/day) in the acceptable study. The observed maternal toxicity; morbidity, clinical signs, gastric lesions, reductions in body weight and body weight gain may have caused the observed malformations and resorptions. However, mortality and abortions were observed already at the lowest dose level.

Table 6.6-1 Summary of reproduction and developmental toxicity studies conducted in rats and rabbits

Species Strain	Test material	Application dates: day of gestation	Doses tested/ Route	NOAELs/LOAELs	Reference
Rat, Fischer 344	DOWCO 290 96.7%	Two-generation study	0, 150, 500, 1500 mg/kg bw/day Dietary	**Adults:** NOAEL 150 mg/kg bw/day (females) and 500 mg/kg bw/day (males), based on decreased body weight, reduced food consumption, stomach lesions **Offspring:** NOAEL 500 mg/kg bw/day based on decreased pup weights and increased pup liver weights in F1 generations. **Reproduction:** NOAEL >1500 mg/kg bw/day	[redacted] *et al*., 1983 Supportive
				Supplementary histopathological examinations on samples collected in the above study No treatment-related histopathological effects in reproductive organs and accessory sex glands in randomly selected adult F0 and F1 rats/sex at 1500 mg/kg bw/day or in major organs of randomly selected F2B weanlings/sex at 1500 mg/kg bw/day	[redacted] 1984 **Acceptable**
Rat, Fischer 344	DOWCO 290 97%	6-15	0, 15, 75, 250 mg/kg bw/day Oral gavage	**Maternal:** NOAEL 15 mg/kg bw/day based on decreased liver weight and food consumption **Embryotoxicity/teratogenicity**: NOAEL >250 mg/kg bw/day, no LOAEL (malformed foetuses detected were considered incidental)	[redacted] *et al*., 1981 **Acceptable**
Rabbit, New Zealand White	DOWCO 290 96.4%	7-19	0, 50, 110, 250 mg/kg bw/day Oral gavage	**Maternal**: NOAEL 110 mg/kg bw/day based on decreased body weight and body weight gain, gastric lesions, clinical signs and morbidity **Embryotoxicity/teratogenicity:** NOAEL 110 mg/kg bw/day based on decreased mean foetal weight, slightly increased spontaneous malformations	[redacted] *et al.,* 1990 **Acceptable**
Rabbit, New Zealand White	DOWCO 290 96%	6-18	0, 110, 250, mg/kg bw/day Oral gavage	**Maternal**: NOAEL >250 mg/kg bw/day, no LOAEL **Embryotoxicity/teratogenicity**: NOAEL 250 mg/kg bw/day, no LOAEL	[redacted] *et al.*, 1974 Additional information

B.6.6.1. Generational studies

Study:	DOWCO 290: Two generation dietary reproduction study in Fischer-344 rats ([redacted] 1983)
Previous evaluation:	This study was submitted to DAR (2003). There were deviations from the OECD guideline 416 and the study was evaluated as supportive for evaluation of reproduction toxicity of clopyralid. This study was discussed also in the Addendum 1 (2004) and the text is included after the study summary. AIR 3 evaluation conclusion is that the study is supportive only because of deviations from OECD guideline. This differs from what is presented in Conclusion on the peer review of clopyralid (EFSA Scientific Report (2005) 50, 1–65) where the study was considered acceptable.

Test guideline and GLP
Design and conduct of the study was not in compliance with OECD test guideline 416/Directive 67/548/EEC, Annex V,"two generation reproduction test".
GLP: Self-certified

Deviations:
Dietary levels of test article were reduced in F0 dams during the mating, gestation and lactation periods of F1B litters, and in F1 dams during F2A and F2B lactation periods. The dietary levels were decreased from 1/6 to 1/3 (F0) or ½-1/3 (F1) of the concentration available in the premating test diets. This was done to avoid overdosing since a dam's food consumption increases markedly during lactation without concurrent increases in body weight. Food consumption of F0 and F1 females was not recorded after the beginning of the mating period. The mating schedule and periods (two 5-day periods/male with or without a 7-day resting period) are too short to evaluate the fertility, and the duration of pregnancy was not recorded. Pregnancy rates (pregnant females/mated females) were not recorded. Only 10 weaned offspring/sex/dose were selected for gross necropsy. The ages of weanlings necropsied ranged too much, from 29 to 50 days. No histopathological investigation was presented on males failing to induce pregnancy. No individual data was presented.

Material and methods
A two-generation reproduction study was conducted with clopyralid (DOWCO 290; purity: 96.7%) administered in the diet at dose levels of 0 (vehicle control), 150, 500, and 1 500 mg/kg bw/day to 30 male and 30 female Fischer 344 rats (50 days old) per dose level. The concentration of clopyralid in the test diets was adjusted weekly during the premating exposure periods based on group mean body weight and food consumption data in order to maintain the appropriate dose levels. F0-generation animals were bred twice within their treatment groups to produce F1 litters (F1A and F1B) after 101 days on test. Randomly selected F1B weanlings at age of 28 days from the various treatment groups (30 males and 30 females) were dosed for 120 days prior to mating and then bred to produce F2A and F2B litters. The breeding program consisted of a 5-day mating period with one male, a 7-day resting period (for F0 parents only), and a second 5-day mating period with another male. After weaning, a minimum of a 10-day rest period was allowed before the second mating.

Litters were culled by random selection to a total of eight pups (four/sex) on day 4 post-partum. Mortality, behaviour, body weight development (except during mating and pregnancy), food consumption (except during mating, pregnancy and lactation), mating performance, interval between first paring and parturition, reproduction and litter data including visible implantation sites were examined. Liver, kidneys, brain, heart, ovary and testes weights were recorded for adult rats and weanlings, and their eyes were examined. Tissues were collected for histopathological examination according to guidelines. Histopathological examination of F0 and F1 parent tissues collected at necropsy was limited to tissues in which grossly visible nodules or masses were apparent.

The pups from each first mating (F1A and F2A) were killed at the age of 29-50 days.

Results
There were no treatment-related mortalities during the study. Three females and one female with litter were lost during the study: two F0 females at 500 mg/kg bw/day died, one drowned with her litter and another was sacrificed due to a tumour (anaplastic carcinoma). One control and one high dose F1 females died at or around parturition due to difficulties with parturition.

Mean body weight was significantly reduced at 1 500 mg/kg bw/day; for F0 parents during the premating and lactation (females) periods and for F2 males during the exposure period and for F1A pups and F1B male pups after the lactation period (Table 6.6-2). At 500 mg/kg bw/day, mean body weight was significantly reduced also in F0 females during the premating and lactation periods. Over the course of the study, body weight gain was reduced in F0 animals receiving 1500 mg/kg bw/day. Food consumption was reduced in F0 parents at 1500 mg/kg bw/day. There was a significant increase in relative liver weight in male F1 parents receiving 1500 mg/kg bw/day. One F0 male had slight hyperkeratotic changes in the nonglandular mucosa of the stomach and two F1 males had small lesions in the forestomach (mucosal invaginations into the gastric wall) at 1500 mg/kg bw/day. The fertility seems not to be reduced after any mating.

Table 6.6-2 Main observations in two-generation reproduction study

Dose level (mg/kg bw/d)	150	500	1 500
F0 generation			
-Mean body weights		↓* (females)[a]	↓ *
-Body weight gain			↓ *
-Food consumption	↓ * (males)	↓* (males)	↓ *
-Stomach lesions[b]	One female		Two males
F1 generation, adults			
-Mean body weights	↓ up to *	↓ up to*	↓ *
-Food consumption	↓ up to * (males)	↓ up to *	↓ up to * (males)
-Relative liver weight			↑ * (males)
-Stomach lesions, adults[c]			One female and two males
-Relative testicular weight			↑*
F1 generation, pups			
-Mean body weights			↓*[d]
-Smaller size			↑* (F2A)
-Relative liver weights			↑ * (F1A, F1B males)
- Anophthalmia[e]	One pup	One pup	
F2 generation			
-Mean birth weights			↓ (F2A)
-Microphthalmia/ anophthalmia[f]	One pup	One pup	Three pups

[a]change observed only in indicated sex and/or generation
[b]focal hyperkeratosis or focal ulcer in the nonglandular mucosa or focal cronic inflammation
[c]very slight haemorrhage or nodule
[d]F1A and F1B females: decreased, but not statistically significantly
[e]none in control or in F1B generation
[f]incidence of microphthalmia: 1, 0, 1 and 3 (in one litter) in F2A pups at 0, 150, 500 and 1500 mg/kg bw/day, respectively; incidence of anophthalimia: 1, 1, 1 and 0 in F2B pups at 0, 150, 500 and 1500 mg/kg bw/day, respectively.
* $p < 0.01$ (statistics: parametric or nonparametric analysis of variance)

Conclusions
NOAEL for reproduction was >1500 mg/kg bw/day and NOAEL for maternal toxicity was 150 mg/kg bw/day.

The deviations from the guideline hamper the validity of the study. The results, as they are, do not suggest any harm in the fertility of dams or in the offspring. The dose levels during the mating, gestation and lactation period remain open. The mean concentrations of clopyralid in the test diet throughout the study were within ±25% of nominal. Individual litter data was not available. The study is supportive for evaluation of reproduction toxicity of clopyralid. Need for a new study should be considered.

Discussion and conclusions from Addendum 1 (2004)
The submitted 2-generation rat study is flawed by a number of deficiencies and deviations from the current guideline requirements. In the study, the NOAEL for adults was 150 mg/kg bw/day based on the reduction in parental body weight and food consumption, and induced stomach lesions. The NOAEL for offspring was higher, 500 mg/kg bw/day, based on the reduction in F1 pup weights and increased liver weight.

The variation of the mean concentration of clopyralid (±25%) in the diet and intentionally reduced concentrations of clopyralid in the diet hamper the evaluation of dose levels of clopyralid especially during the lactation period. The concentration of clopyralid in the diet was adjusted based on food consumption and body weight data. However, during mating, gestation and lactation periods, food consumption was not measured. Dietary concentrations of clopyralid were reduced by 1/6-1/3 from the premating concentrations during some mating, gestation and/or lactation periods. Thus, the dose levels during these periods remain open. Reduced dietary concentrations of clopyralid were given during the F1B mating, gestation and lactation periods and during the F2A and F2B lactation periods. Based on data provided on reduced dietary concentrations, the actual dose level during late lactation period may have been only half of the intended dose level (if the food consumption of dams is considered to be double comparing to the food consumption during premating period). However, the intended highest dose level was as high as 1500 mg/kg bw/day, thus, the actual doses were, at least, more than 700 mg/kg bw/day.

Mating schedule was different for F0 and F1 parents; F0 parents were mated with two 5-day mating periods separated with 7 days resting, and F1 parents without the resting period. Males were changed for different 5-day mating periods. No individual data for mating, gestation lengths or male fertility were provided. Based on the available data, it is difficult to draw any conclusions from "gestation length" (from paring to parturition). Typically "gestation length" was 28±6 days for F1A gestation, 27±5 days for F1B and 25±2 days for F2A and F2B gestations. The number of males failing to induce pregnancy is not clear from the data because of short mating periods with different males. According the notifier, there was no dose-related increase in males failing to induce pregnancy (F1a: 2, 2, 0, 2; F1b: 1, 6, 2, 1; F2a: 7, 7, 8, 6; F2b: 4, 4, 5, 3).

Nonetheless, the results, as they are, even with the high doses used, do not suggest any harm in the fertility of dams or in the offspring. Additionally, taking into account the whole toxicological profile of clopyralid, a specific reproductive risk is most unlikely.

Conclusion AIR 3 evaluation:
The study is considered as supportive only because of the deviations, e.g. the actual doses have been remarkably less than what intended, perhaps only 700 mg/kg bw/day at the highest dose level. However, the results do not suggest any harm in the fertility of dams or in the offspring and when taking into account the whole toxicological profile of clopyralid, a potential of reproductive toxicity is unlikely.

Study:	DOWCO 290 Herbicide: two-generation dietary reproduction study in Fischer-344 rats – Supplemental histopathology ([redacted] 1984)
Previous evaluation:	This study was submitted to DAR (2003). Text has not been modified and conclusions have not changed.

Test guideline and GLP
Guidelines: Not relevant.
Deviations: Not relevant.
GLP: Self-certified.

Material and Methods
Supplementary histopathological examinations of reproductive organs and accessory sex glands (testes, epididymides, prostate, coagulating gland, seminal vesicle/ovaries, oviduct, uterus, cervix, vagina) were conducted on 10 randomly selected F0 and F1 adults/sex in the high dose group (1500 mg/kg bw/day) and the control group. In addition, microscopic examinations were made of major organs (those listed in EC guideline 87/302/EEC) from 10 randomly selected F2B weanlings/sex in the high dose (1500 mg/kg bw/day) and the control group.

Results
There were no treatment-related histopathological effects.

Conclusions
This supplementary report does not change the conclusions of the original report. This supplemental study is acceptable.

B.6.6.2. Developmental toxicity studies

Study:	DOWCO 290 – oral teratology study in Fischer-344 rats ([redacted] 1981)
Previous evaluation:	This study was submitted to DAR (2003). The study was performed predominantly according to OECD guideline 414 (2001). Text has been modified. Conclusions have not changed from DAR but the NOAEL for maternal toxicity is different from what is at Conclusion on the peer review of clopyralid (EFSA Scientific Report (2005) 50, 1–65). The study is acceptable.

Test guideline and GLP
According to information from DAR (2003) the study was GLP (self certified), but conducted prior to the enforcement of GLP regulations. It seems that the only statement of GLP was in the Quality assurance statement, mentioning that in compliance with GLP regulations the study phases were inspected by the QA Unit. Design and conduct of the study is predominantly in compliance with OECD 414 (2001). Maternal body weights was reported to be recorded daily during dosing period and on days 16 and 21 of gestation but only weights on gestation days 6, 10, 16 and 21 were presented. Gravid uteri including the cervix were not weighed.

Material and Methods
Groups of 29 - 35 mated female Fischer-344 rats received daily oral doses of DOWCO 290 (purity: 97%) at levels of 0, 15, 75 and 250 mg/kg bw/day administered by gavage in cottonseed oil suspension from gestation day 6 through 15. Additional study groups consisting of 25 females receiving 250 mg/kg bw/day or vehicle were included. Animals were observed daily for indications of toxicity. Body weights were recorded daily during dosing period and on gestation days 16 and 21. Food and water consumption were recorded three day intervals from gestation day 6. Kidneys, liver and thymus were weighed and preserved. On gestation day 21, the foetuses were removed from dams by caesarean section, and all foetuses were examined for external and visceral malformations (autopsy, serial sectioning of fixed heads of one-half of each litter) and for skeletal malformations (staining with Alizarin Red).

Results
At 250 mg/kg bw/day three dams died on days 10-11 of gestations for unknown reasons. The dose level of 250 mg/kg bw/day caused significant reductions in body weights and body weight gains of the dams (Table 6.6-3). There was a significant decrease in absolute liver weight in dams receiving 75 and 250 mg/kg bw/day compared to controls. At 250 mg/kg bw/day dams consumed significantly less food than controls throughout most of the gestation. At 250 mg/kg bw/day, one litter had three foetuses with polydactyly and another litter had one foetus with a hemivertebra. These were considered incidental, since no malformations were seen among additional high dose group foetuses. Embryo lethality was not observed. NOAEL for decreased maternal liver weight was 15 mg/kg bw/day and for embryonic/foetal, development was 250 mg/kg bw/day.

Table 6.6-3 Main results of the rat teratogenicity study

Parameter	Control	15 mg/kg bw/day	75 mg/kg bw/day	250 mg/kg bw/day
Maternal body weights (g)				
-day 6	203/199[a]	199	199	201/199
-day 10	211/211	208	206	204*/204*
-day 16	231/234	229	226	219*/221*
-day 21	268/274	267	261	257/259*
Maternal body weight gain (g)				
-days 6-9	8/12	9	7	2*/5*
-days 10-15	20/23	21	19	15*/17*
-days 16-20	37/40	38	36	38/37*
-days 6-20	65/75	67	62	56/60*
Food consumption (g/rat/day)				
-days 6-8	13/12	14	14	12*/10*
-days 9-11	14/13	14	14	12*/11*
-days 12-14	15/14	15	14*	14*/11*
-days 15-17	17/16	17	17	16*/14*
-days 18-20	17/16	17	17	16/15*
Water consumption (g/rat/day)				
-days 6-8	20/18	20	20	19/15*
-days 9-11	24/20	22	22	23/18*
-days 12-14	26/22	26	25	26/20*
-days 15-17	31/27	31	30	31/24*
-days 18-20	29/27	29	29	29/25
Maternal liver weight (g)	10.45/9.90	10.36	9.84*	9.67*/9.19*
Foetal body weights (g)	4.36/4.27	4.38	4.52*	4.42/4.27
Number of foetuses (litters) with malformations[b]	1(1)[c]	0	0	4/2[d]
Total resorptions	0/1	0	0	0/1
Mean number of resorptions -without dams with total resorptions	0.7/1.1	1.0	0.7	0.6/1.3

*p<0.05, Dunnet´s test

[a]results of two different groups separated by a slash

[b]results of both separate control and 250 mg/kg bw/day groups combined

[c]one foetus with exencephaly and anophthalmia

[d]one foetus with lumbar hemivertebra and three foetuses in another litter had polydactyly

Conclusions

The NOAEL for maternal toxicity was 15 mg/kg bw/day (decreased maternal liver weight) and >250 mg/kg bw/day for foetal toxicity/teratogenicity. No treatment-related malformations were observed.

Placental weights were not recorded. Food consumption data for days 18-20 for additional groups (control and 250 mg/kg bw/day) were not available in result tables, but were found in individual data. In the probe study, groups of 9 or 10 female rats were administered 0, 50, 100, 250 or 500 mg/kg bw/day of DOWCO 290 as a suspension in cottonseed oil by gavage on days 6 through 15 of gestation. Maternal toxicity, as evidenced by decreases in body weight and the amount of body weight gained during gestation, was observed at 500 mg/kg bw/day. One maternal death occurred at 500 mg/kg bw/day. A statistically significant increase in percent implantations resorbed was observed in this group. The study is acceptable.

Study:	Clopyralid: oral gavage teratology study in New Zealand White rabbits [redacted] 1990)
Previous evaluation:	This study was submitted to DAR (2003). The study was performed predominantly according to OECD guideline 414 (2001). Text has been modified but conclusions have not changed. The study is acceptable.

Test guideline and GLP
The study was performed predominantly according to OECD guideline 414 (2001). Food consumption was not measured. Housing conditions were not reported in detail. Acclimation time of the second group of rabbits was not given. GLP: OECD principles of Good Laboratory Practice, Paris 1982; and USEPA (1983) and Japan GLP regulations (1984).

Material and Methods
Groups of 26-34 artificially inseminated female New Zealand White rabbits were administered daily oral doses of DOWCO 290 (purity 96.4%) in corn oil by gavage at dose levels of 0, 50, 110 or 250 mg/kg bw/day on days 7-19 of gestation (insemination day 0). The first group of rabbits consisted of 16 rabbits/treatment but due to low number of litters with pups a second group of rabbits was added to the study. Analysis of the dosing solutions was performed to verify the concentration. Body weights were recorded on days 0, daily during dosing period and on days 20 and 28 of gestation. All animals were observed daily for signs of toxicity. On gestation day 28, the foetuses were removed from dams by caesarean section and examined for external malformations. All foetuses were dissected under a microscope to examine visceral malformations, eviscerated and stained before examination for skeletal malformations. Sections of maternal liver, kidneys and stomach were preserved and histologic examination was performed on the stomachs.

Results
The concentrations of dosing solutions were > 98% of the target concentration. Several animals were found dead or were killed moribund in the study (Table 6.6-4). From the findings at necropsy, it was concluded that in the control, 50, 110 and 250 mg/kg bw/day groups, 0, 5, 1 and 5 animals, respectively, failed to reach term because of aspiration of test material, i.e. due to intubation error. Administration of the dose level of 250 mg/kg/day produced signs of severe maternal toxicity. Laboured breathing was observed in approximately one-third of the rabbits in this group. Six rabbits from this group were submitted for necropsy prior to scheduled caesarean section because of clopyralid-induced morbidity or mortality. Maternal body weight and body weight gain was significantly depressed in the 250 mg/kg/day dose group as well. Multifocal erosions and/or ulcers in the gastric mucosa were observed in dams at 250 mg/kg/day. Lower foetal weights and hydrocephaly were observed at 250 mg/kg/day. The affected foetuses were from dams that lost an average of approximately 525 g during treatment, thus the foetal effects may have been stress-related and secondary to the severe maternal toxicity. When comparing the number of foetuses (litters) with hydrocephaly with the number of foetuses (litters) with total CNS malformation (Table 6.6-5) in the 250 mg/kg/day dose group it can be seen that in addition to hydrocephaly no other CNS effects were observed in this group. The NOAEL for clopyralid for both maternal and foetal parameters was 110 mg/kg/day.

Table 6.6-4 Main observations in teratogenicity study on rabbits

Parameters	0 mg/kg bw/day	50 mg/kg bw/day	110 mg/kg bw/day	250 mg/kg bw/day
Number of dams	28	26	26	34
Number of deaths	1	5	1	6
Number of dams excluded due to aspiration	0	5	1	5
Number of moribund				5
Number of dams with antimortem observations	2	4	4	15
Number of dams with abortion	0	1	1	1
Number of litters delivered early	1	0	0	1
Mean weight gains (g)				
- On gestation days 7-20	38.2	-91.1	-50.2	-254.3*
- On gestation days 0-28	470.8	351.4	298.2	283.3
Erosions, blood and/or ulcers of the stomach	0/28	0/26	1/26	10/33
Foetal body weight (g)	39.64	34.84*	36.19	34.40*
Resorptions				
- % of implantations	13.9	10.2	8.5	22.1
- % of litters	57.9	40.0	38.9	73.3
- mean per dam	1.1	0.9	0.7	1.8
Malformations (more than one per group)				
- forelimb flexure	1(1)[a]			4(3)
- hydrocephaly				8(3)
- total CNS malformations	1(1)		1(1)	8(3)
- ventricular septal defect		2(2)		1(1)
- missing apical lung lobe	1(1)	1(1)	1(1)	2(1)
- skull, delayed ossification	1(1)	7(5)*	7(5)*	5(2)
- skull, foramen		2(2)	1(1)	1(1)
- hyoid, delayed ossification	26(10)	24(9)	33(13)	34(11)
- hyoid, crooked	4(3)	2(2)	4(3)	4(4)
- vertebrae, dentoid process, delayed ossification				2(2)
- atlas, fused			1(1)	2(2)
- centra, delayed ossification	1(1)	3(2)		1(1)
- ribs, fuced		2(2)		1(1)
- spurs, lumbar	34(13)	22(9)	33(13)	20(9)
- sternebrae, delayed ossification	40(15)	48(13)	76(16)*	52(13)
- sternebrae, fused		1(1)	2(2)	
- pubis, delayed ossification		1(1)	2(2)	6(3)
Number of foetuses with external alterations[b]	2/130 (1.5%)	2/115 (1.7%)	0/129 (0%)	6/95 (6.3%)
Number of foetuses with visceral alterations	5/130 (3.9%)	6/115 (5.2%)	4/129 (3.1%)	12/95 (12.6%)
Number of foetuses with skeletal malformations	0/130 (0%)	3/115 (2.6%)	1/129(0.7%)	2/95(2.1%)
Number of foetuses with malformations	4/130 (3.1%)	4/115 (3.5%)	3/129 (2.3%)	13/95 (13.7%)
Number of litters with malformations	4/19 (21.0%)	4/15 (26.7%)	3/18 (16.7%)	7/15 (46.7%)

[a]number of affected foetuses(litters)
[b]including malformations
*$p<0.05$, Chi-square

Conclusions
The NOAEL-levels were 110 mg/kg bw/day for dams (decreased bodyweight gain, stomach erosions) and intrauterine development (decreased foetal body weight, slightly increased resorptions and slightly increased number of foetuses with spontaneous malformations).

The doses were selected based on results in a range finding study and a teratology probe study involving doses of 0, 350, 500 and 750 mg/kg bw/day and 0, 110, 250 and 350 mg/kg bw/day, respectively. The daily doses of 350 mg/kg bw/day and above resulted in weight loss, stomach erosion and mortality. No clinical effects were observed at 110 or 250 mg/kg bw/day. At 250 mg/kg bw/day, stomach erosions were observed in all rabbits. The study is acceptable.

Study:	The effect of DOWCO® 290 (3,6-dichloropicolinic acid) on the developing embryo and foetus of pregnant rabbits ([redacted] 1974)
Previous evaluation:	This study was submitted to DAR (2003). It was evaluated as additional information in the DAR. No re-evaluation has been performed and the text has not been modified.

Test guideline and GLP
Guidelines: The study partially complies with Directive 87/302/EEC "teratogenicity study – rodent - nonrodent".

Deviations: The dosing period started and ended one day earlier than stated in the guideline. Only two dose levels were investigated. Food consumption was not measured.

GLP: Non-GLP. This study was conducted prior to the enforcement of GLP regulations.

Material and Methods
Groups of 15 artificially inseminated female New Zealand White rabbits were administered daily oral doses of DOWCO 290 (purity: 96%) at levels of 110 or 250 mg/kg bw/day by gavage in corn oil from day 6 to day 18 of gestation (insemination day 0). A group of 25 rabbits given corn oil alone served as controls. On gestation day 29, the foetuses were removed from dams by caesarean section, and examined for external and skeletal malformations. Also, one-third of each litter was dissected under a microscope to examine visceral malformations.

Results
There were no mortalities, clinical signs or body weight changes during the study. Mean foetal body weight was slightly reduced at 250 mg/kg bw/day and relative maternal liver weight was slightly increased at 250 mg/kg bw/day (Table 6.6-5). Incidence of resorptions or malformations was similar in all groups.

Table 6.6-5 Main observations in teratogenicity study on rabbits

Parameters	0 mg/kg bw/day	110 mg/kg bw/day	250 mg/kg bw/day
Number of dams with total resorption	0	1	0
Mean weight gains (g)			
- gestation days 6-18	-0.05	0	-0.01
- gestation days 6-29	0.14	0.13	0.12
Relative liver weight (g/100 g)	25.00	25.82	27.13
Number of resorptions/litters with resorptions	9/6	10/4	4/4
Foetal body weights (g)	35.62	36.01	33.79
Litters with malformations			
-omphalocoele	1	0	0
-anencephaly	0	1	0

Conclusions
The NOAEL-levels were >250 mg/kg/d for dams and intrauterine development. The number of pregnant female was rather low; 14, 12, 12 at 0, 110 and 250 mg/kg bw/day, respectively. An additional dose level of 50 mg/kg bw/day was not administered as specified in the original protocol. As no teratogenic effects were found at 250 or 110 mg/kg bw/day, the lowest or 50 mg/kg bw/day doses were omitted from the study. The study is acceptable to give additional information.

B.6.7. NEUROTOXICITY

B.6.7.1. Neurotoxicity studies in rodents

No study submitted. The results from toxicological studies on clopyralid suggest that there are no specific neurotoxic effects, even though the observations in the e.g. short term studies did not necessarily include e.g. motor activity observations.

B.6.7.2. Delayed polyneuropathy studies

Clopyralid is not an anticholinesterase and, as such, has no potential to cause delayed neurotoxicity. Clopyralid is not an organophosphate.

B.6.8. OTHER TOXICOLOGICAL STUDIES

In the Addendum 1 to DAR (2004) the Repeated insult patch test for [redacted] ([redacted] 1987) was reported. The study was evaluated as supportive information. The study was not performed using any official guideline or test method and the study was not done under GLP. In this study one hundred humans completed a repeated insult patch test in which dilutions of tridiphane, triclopyr, clorpyrifos and clopyralid were applied to the skin of the back of the subjects, all chemicals to all subjects. During an induction period of 22 days each sample was applied 9 times to the same site. The subjects were instructed to remove the patches 24 hours after application. Induction period was followed by a 2-week rest period, then a challenge application was performed to a naïve site to test for reactions indicative of contact sensitisation. It was concluded that based on the test conditions, clopyralid did not produce skin irritation or contact sensitisation on subjects.

The study is not included in this evaluation based on following points in Regulation 1107/2009:

(13) For ethical reasons, the assessment of an active substance or a plant protection product should not be based on tests or studies involving the deliberate administration of the active substance or plant protection product to humans with the purpose of determining a human 'no observed effect level' of an active substance. Similarly, toxicological studies carried out on humans should not be used to lower the safety margins for active substances or plant protection products.

Article 8, 2. The complete dossier shall contain the full text of the individual test and study reports concerning all the information referred to in points (b) and (c) of paragraph 1. It shall not contain any reports of tests or studies involving the deliberate administration of the active substance or the plant protection product to humans.

B.6.8.1. Toxicity studies on metabolites and relevant impurities

No studies submitted.

B.6.8.2. Supplementary studies on the active substance

Study:	Clopyralid: An evaluation of mammalian toxicology: Addendum: A comparison of manufacturing process and clopyralid toxicity ([redacted] 1995)
Previous evaluation:	This review was submitted to DAR (2003). The review is not re-evaluated and text or conclusions have not changed.

Guidelines and GLP
Not applicable. The paper is a review of mammalian toxicology of clopyralid.

Materials and methods
Clopyralid was first manufactured before 1980. The method of manufacture changed from the so-called 'Hydrazino' process to the so-called 'Penta' process in 1987. Prior to the change in manufacturing process, a comprehensive data base existed on the toxicity of clopyralid. The change in manufacturing process was associated with a change in the specification for the active substance and appropriate bridging studies were conducted and, at the same time, a few additional tests were completed to further update the clopyralid data package. Following this, the majority of toxicology study endpoints were addressed using the 'Penta' process. The Addendum reviews the endpoints from the studies, and makes a comparison of toxicity of technical material from the two production processes.

Results

The Addendum concludes that there were no significant differences between the two production processes, in terms of the toxicity of the technical materials. In both cases, the materials were of low acute toxicity, and low general toxicity, with no evidence of adverse effects on carcinogenicity, reproduction, teratogenicity or mutagenicity. Findings were limited to changes to the gastric epithelium in rats only, and at high doses, lowered body weights and occasionally food consumption, in all species tested. High dose levels were also associated with adaptive increases in liver and occasionally kidney weight.

Conclusion
The toxicities of the technical materials produced by the two production processes are essentially similar.

Study:	Clopyralid: Assessment of Immunotoxic Potential using the Sheep Red Blood Cell Assay after 28-Day Dietary Exposure to Male F344/DuCrl Rats ([redacted] 2010)
Previous evaluation:	This is a new study. The study was mentioned to be performed according to USEPA, OPPTS 870.7800 (1998). The study is considered acceptable.

Test guideline and GLP
The study was mentioned to be performed according to USEPA, OPPTS 870.7800 (1998). The study was conducted in compliance with GLP Standards.

Material and Methods
Clopyralid (purity 95.9 %) was administered to groups of 10 male F344/DuCrl rats in diet at dose levels of 0, 150, 500 or 1000 mg/kg bw/day for 29 days. A group of 10 male F344/DuCrl rats at dose level of 0 mg/kg bw/day served as positive immunosuppressive control group and was administered cyclophosphamide at 20 mg/kg via ip injection on study days 24-28. The dietary concentrations of test item were adjusted based on the most recent data on body weight and food consumption. Analyses of dose confirmation were made of all dose levels. Observations made were daily cage-side examinations, detailed clinical observations once per week, body weight/body weight gain, feed consumption and hematology (hematocrit, hemoglobin, red blood cell, total white blood cell, differential WBC count, platelet count, reticulocyte count, MCH, MCV, and MCHC). Rats were immunized with SRBC five days prior to sacrifice. Serum samples were analysed for anti-SRBC IgM by ELISA. Rats were sacrificed at the end of the study and subjected for gross necropsy. Spleen and thymus were weighed and from vehicle control and test material groups samples of liver, kidney, spleen, thymus, sternum, mesenteric lymph node, Peyer's patch and gross lesions were preserved.

Results
The mean concentration of clopyralid in the diet was > 91.5 % of the targeted. There was no mortality and according to study report no treatment related findings on detailed clinical and cage-side observations. There were no statistically identified differences compared to controls in the body weights and feed consumption was similar to controls. According to study report there were no treatment related changes in the hematology parameters or in organ weights. There was a statistically significant increase in absolute and relative thymus weight on males at 500 and 1000 mg/kg bw/day; however there were no corresponding histopathological effects. According to study report there were no treatment related observations in gross pathology or in histopathology (slight sinus histiocytosis in mesenteric lymph node observed in one animal at dose levels 0, 150 and 1000 mg/kg bw/day). In SRBC antibody response no statistically significant difference from control was identified at any dose level of the test item groups.

Conclusions
The NOAEL from this study is 150 mg/kg bw/day based on the increased thymus weight at dose levels 500 and 1000 mg/kg bw/day. No other treatment related effects were observed. The study is acceptable as additional information.

B.6.8.3. Studies on endocrine disruption

No studies were submitted regarding endocrine disruptive properties.
Clopyralid is not classified as Carc. 2 or Repr. 2 according to Regulation (EY) N:o 1272/2008. In the submitted short and long term studies some effects were seen. For example in the short term studies, in histopathological investigations on mice, there were histopathological changes (hyperplasia) in adrenals and in thyroid gland

([redacted]1983). On dogs reduced relative adrenals weight and in adrenals coarse vacuolation of cortical cells were observed ([redacted] 1984). In the submitted chronic toxicity - oncogenicity study, for example in histopathological investigations on mice, hyperplasia in adrenals was observed ([redacted] 1984, 1986). However, it is concluded that clopyralid does not fulfill the interim criteria for endocrine disruptive properties.

The following information is provided by the notifier. It has been slightly modified by RMS to avoid duplication of the data.

Clopyralid has undergone a comprehensive battery of in vivo toxicology that covers 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. 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).

The results for clopyralid in the endocrine specific high throughput assays from the ToxCast™ database (available at: actor.epa.gov/edsp21/) indicated no activity related to the estrogen, androgen, or thyroid receptor. Specifically, clopyralid was inactive in the 8 androgen receptor assays for which it was evaluated, 14 out of 15 estrogen receptor assays for which it was evaluated and 3 thyroid receptor assays for which it was evaluated (Table 6.8-1). The one positive ER assay Tox21_ERa_LUC_BG1_Agonist with an AC50 of 46.35 µM is likely a non-specific outcome, because only one ER assay was positive (Table 6.8-1).

Table 6.8-1 Summary of Clopyralid Results in the Endocrine Disruption Screening Program for the 21st Century

Assay	Biological Target	Endocrine Pathway	AC50 (µM)
ATG_AR_TRANS_up	AR Transcription factor Activity (human)	Androgen	Inactive
NVS_NR_cAR	AR Binding (Chimpanzee)	Androgen	Not Tested
NVS_NR_hAR	AR Binding (Human)	Androgen	Not Tested
NVS_NR_rAR	AR Binding (rat)	Androgen	Not Tested
OT_AR_ARELUC_AG_1440	Gene Expression via AR and ARE (human receptor, Chinese hamster cell line)	Androgen	Inactive
OT_AR_ARSRC1_0480	AR protein dimerization with SRC-1 (human)	Androgen	Inactive
OT_AR_ARSRC1_0960	AR protein dimerization with SRC-1 (human)	Androgen	Inactive
Tox21_AR_BLA_Agonist_ratio	Gene Expression via AR and ARE (human)	Androgen	Inactive
Tox21_AR_BLA_Antagonist_ratio	Gene Expression via AR and ARE (human)	Androgen	Inactive
Tox21_AR_LUC_MDAKB2_Agonist	Gene Expression via AR and ARE (human)	Androgen	Inactive
Tox21_AR_LUC_MDAKB2_Antagonist	Gene Expression via AR and ARE (human)	Androgen	Inactive
ACEA_T47D_80hr_positive	Proliferation of estrogen-sensitive human breast cells	Estrogen	Inactive

Assay	Biological Target	Endocrine Pathway	AC50 (µM)
ATG_ERE_CIS_up	Gene Expression via ER and ERE (human)	Estrogen	Inactive
ATG_ERa_TRANS_up	ESR1 Transcription factor Activity (human)	Estrogen	Inactive
NVS_NR_bER	ESR1 Binding (Bovine)	Estrogen	Not Tested
NVS_NR_hER	ER Binding (Human)	Estrogen	Not Tested
NVS_NR_mERa	ESR1 Binding (mouse)	Estrogen	Not Tested
OT_ER_ERaERa_0480	ESR1 protein homodimerization (human)	Estrogen	Inactive
OT_ER_ERaERa_1440	ESR1 protein homodimerization (human)	Estrogen	Inactive
OT_ER_ERaERb_0480	ESR1 and ESR2 protein heterodimerization (human)	Estrogen	Inactive
OT_ER_ERaERb_1440	ESR1 and ESR2 protein heterodimerization (human)	Estrogen	Inactive
OT_ER_ERbERb_0480	ESR2 protein homodimerization (human)	Estrogen	Inactive
OT_ER_ERbERb_1440	ESR2 protein homodimerization (human)	Estrogen	Inactive
OT_ER_EREGFP_0120	Gene Expression via ESR1 and ERE (human)	Estrogen	Inactive
OT_ER_EREGFP_0480	Gene Expression via ESR1 and ERE (human)	Estrogen	Inactive
Tox21_ERa_BLA_Agonist_ratio	Gene Expression via ESR1 and ERE (human)	Estrogen	Inactive
Tox21_ERa_BLA_Antagonist_ratio	Gene Expression via ESR1 and ERE (human)	Estrogen Receptor	Inactive
Tox21_ERa_LUC_BG1_Agonist	Gene Expression via ESR1 and ERE (human)	Estrogen	46.35 µM
Tox21_ERa_LUC_BG1_Antagonist	Gene Expression via ESR1 and ERE (human)	Estrogen	Inactive
ATG_THRa1_TRANS_up	THRA Transcription factor Activity (human)	Thyroid	Inactive
NVS_NR_hTRa	THRA binding (human)	Thyroid	Not Tested
Tox21_TR_LUC_GH3_Agonist	Regulation of catalytic activity via THRA and THRB and TRE (rat cell line, human receptor)	Thyroid	Inactive
Tox21_TR_LUC_GH3_Antagonist	Regulation of catalytic activity via THRA and THRB and TRE (rat cell line, human receptor)	Thyroid	Inactive

In summary, robust and consistent in vivo and in vitro data provides no evidence of endocrine disrupting properties of clopyralid.

B.6.9. MEDICAL DATA AND INFORMATION

B.6.9.1. Medical surveillance on manufacturing plant personnel and monitoring studies

The following information is provided by the notifier.
Clopyralid has been manufactured in the U.S. since 1975 in [redacted]. Review of medical surveillance data on the plant employees from 2002 to present disclosed no abnormalities suspected to be of

occupational aetiology. Review of medical data January 2002-June 2015 disclosed no incidents involving chemical exposure in the clopyralid plant.

No epidemiology studies focused on health effects of clopyralid have been done on these employees.

Methodology:
Employees work at a manufacturing plant in [redacted] producing Clopyralid as a batch process 12 months/year, 24 hours/day. The employees work 12-hour shifts for two continuous days followed by two days leave. This sequence continues with shifts alternating between days and nights. The process includes a [redacted] Operator intervention is required to periodically change the centrifuge filter cloths. Area sampling for airborne dust in the vicinity of a process trough was conducted in 2014. Four area samples were collected above a process trough at a location estimated to be in the range of employee breathing zones. Monitoring times ranged from 477 to 571 minutes. Results for three of the area samples were below the limit of detection (0.09 mg/m3) and the remaining sample was 0.1 mg/m3.

Employees wear Saranex hoods and suits as well as nitrile gloves to minimize skin and clothing contact, and full face air purifying respirators while performing tasks with potential Clopyralid exposure.

A health screen is provided to all employees every one (age 40 and above) or every two years (< age 40), comprising the following:

A health surveillance questionnaire
Electrocardiogram
Pulmonary function test
Audiogram
Vision tests
Complete blood count
Urinalysis
Blood chemistry parameters
Health surveillance review
Physical exam with site physician

Data from 23 past and present employees was evaluated in 2015.
Findings:
There were no abnormalities consistent with chemical exposure. No employee reported symptoms related to working in the manufacturing plant. No abnormalities were noted related to work with Clopyralid.

Conclusions:
The surveillance data indicate that workers involved in the production of Clopyralid are healthy and do not show any acute or chronic medical problems.

Study:	Worker exposure assessment to Lontrel herbicide ([redacted] 1991)
Previous evaluation:	This report was submitted to DAR (2003). The report is not re-evaluated and text or conclusions have not changed.

Guidelines and GLP
Not relevant.

Materials and methods
Employees working at a manufacturing plant in [redacted] producing clopyralid as a batch/continuous process 12 months/year and 24 hours/day, since 1985, were monitored for health effects. The employees worked 8-hour shifts, for seven continuous days followed by a two day leave.

Employees blowing the filter heel, sampling the filter, sampling a dryer and dumping (emptying) the dryer were monitored for exposure to clopyralid dust. Employees wore Tyvek suits and gloves to minimise skin and clothing contact, and respiratory protection on two of the above four tasks (not specified which). Exposure of the workers doing short-term tasks (of less than 25 minutes duration) ranged from below the limit of detection (< 0.1 mg/m^3) to 15.9 mg/m^3.

A voluntary health screen was offered to all employees every two years, comprising of the following: a health surveillance questionnaire, electrocardiogram, chest X-ray, pulmonary function test, hearing tests, vision tests, complete blood count, urinalysis, blood chemistry parameters and health surveillance review.

Data from 17 past and present employees was evaluated in 1991 and only one employee did not participate.

Results
There were no abnormalities consistent with chemical exposure. None of the employees reported work-related symptoms in the manufacturing plant. One male had developed angioedema, which was characterised by localised tongue swelling in 1989. This disappeared following treatment with antihistamine and removal from the manufacturing plant but reappeared when the individual returned to work. The effect was diagnosed as an allergic reaction to a chemical, but the causative agent could not be identified. However, after being transferred to another plant that did not produce clopyralid, the individual did not develop further episodes of angioedema and his health has been normal since then.

Conclusion
According to the surveillance data of the notifier, the workers involved in the production of clopyralid were healthy and did not show any acute or chronic medical problems. One worker developed an allergic reaction, which subsided after he was transferred to another plant. However, the reaction was not positively correlated with clopyralid.

B.6.9.2. Data collected on humans

The following information is provided by the notifier.
Since the previous submission in 2002, no new reports from studies on humans related to toxicokinetics or metabolism, or reports on specific exposure testing on humans were found in an open literature search. Animal studies reveal clopyralid administered by gavage or IV injections is excreted unaltered in the urine.

B.6.9.3. Direct observation

The following information is provided by the notifier.
No reports related to clinical poisoning cases by Clopyralid were found in refereed journals or official reports in an open literature search of PubMed in May 2015.

Dow Agrosciences collects data from reported human exposure incidents (or alleged exposure) from the general public, industry and medical facilities its Adverse Effects Reporting Committee (AERC) database. These are not related to refereed journals or official reports, and it is recognised that many of these data are somewhat subjective in terms of reliable exposure information and plausible symptomatology. Also, in the public/end user domain, usage is generally of formulated product, and symptoms may be related to the other components –rather than the active ingredient. There are often co-exposures reported, which confound further. Additionally, "SafetyCall International" (SC-I) handles acute incident reports from similar sources, mainly from North and South America, Canada and China; and are subject to the same confounders as above. Often the reports are duplicates of those documented in AERC. In most cases, follow up and outcomes cannot be tracked because of lack of available information (some reporters who are unwilling to give details) and the confidentiality constraints on medical managers.

Adverse Effects Reporting Committee (AERC) database

Since 2002, when the last submission was made, 112 reports exist in the AERC database relating to alleged, possible, or actual product exposure, and multiple product exposure. The majority of these reports (89) allude to mainly mild symptoms or no symptoms - some calls are just for general advice post-exposure. 23 were

considered moderate or major, the most significant exposure being a 10g ingestion in a suicide attempt in 2012 in China. In this case lavage was performed – no outcome available. 72 of these reports are duplicated in the Safety Call database summarised below.

"Safety Call International" (SC-I) database

The vast majority of the reports relate to mild skin irritation or eye irritation from accidental direct contact exposure or occasionally spray drift; irritation, itching, rashes, "lumps" and soreness being the commonest skin complaints. Eye irritation, pain, soreness, watering and blurred vision were the commonest complaints after eye contact. There were no reports of serious eye injury.

Other less commonly reported effects of exposure included headache, dizziness, nausea, vomiting, diarrhoea, lethargy, ill-defined aches and pains, leg weakness, high blood pressure, throat and respiratory irritation and coughing. It must be remembered that these cases are likely to have had exposure to formulated product which contains several other components, for example isopropanol, capable of causing symptoms.

In DAR (2003) it was reported that according to the notifier, since October 1991, there have been 57 reports in the U.S.A. and 3 non-U.S. reports of alleged human health effects associated with clopyralid reported to Dow AgroSciences (DAS). These allegations were received from PROSAR, a human health poison control centre, employees of DAS, customers, and other sources. The majority of the incidents involved skin exposure to individuals handling clopyralid, with symptoms of hives, welts, redness & itching, blistering rash, swelling of lips, numbness and pain, and one instance of chemical burn. Also, frequent was eye exposure, with symptoms of blurred vision, irritation, pain, conjunctivitis, twitching, and corneal erosion. There were two instances involving litigation in which a variety of symptoms was alleged, including blurred vision, headache, fatigue and coughing in an individual following 3 days of spraying. In the second litigation incident, an individual who entered a park following spray treatment alleged symptoms of headache, vomiting, cramps, sadness, lethargy, dizziness, nausea, transient hypothyroidism and Hashimoto's disease. There were a few instances of alleged inhalation exposure with symptoms of irritation, shortness of breath, respiratory distress, disorientation, dizziness, and nausea.

Searches of the open literature through Medline, Toxline, and Science Direct databases in January 2002 produced no reports of adverse health effects in humans from clopyralid other than the reports of alleged health effects listed above.

B.6.9.4. Epidemiological studies

According to the notifier, since the previous submission in 2002, no new studies have been identified which provide new end-points in this section. In DAR (2003) it was mentioned that there have been no known exposures to the general public in the commercial use of clopyralid, and no reports of health effects associated with clopyralid other than the alleged health effects listed above (B.6.9.3).

B.6.9.5. Diagnosis of poisoning (determination of active substance, metabolites), specific signs of poisoning, clinical test

The following information is provided by the notifier.
Since the last submission in 2002, no new human information was uncovered which provides new end-points in this section. Data is based primarily on animal studies covered elsewhere in this submission. Target organs are mainly liver and kidney with very high doses, and lethargy has also been reported. The mechanism of action of Clopyralid and other pyridine herbicides is through mimicking the action of auxin, a natural growth inhibiting hormone in plants. Clopyralid has very low general systemic toxicity via the usual routes of exposure.

Acute oral LD_{50} for rats 4000-5000 mg/kg
Acute dermal LD_{50}, rabbit > 2,000 mg/kg; low irritancy.
Acute inhalational LC_{50}, 4 h, dust, rat > 1.3 mg/l.
Eye contact exposure – clopyralid is capable of producing severe eye irritation and even permanent damage with direct contact.

No specific poisoning syndrome or specific tests for poisoning have been identified. Pyridine herbicides can be detected in the urine of exposed persons, but do not correlate with any clinical effect.

Repeated Dose Toxicity:-
Based on available data, repeated exposures are not anticipated to cause additional significant adverse effects.

Chronic Toxicity and Carcinogenicity:-
Did not cause cancer in laboratory animals. Not classified as a carcinogen.

Developmental Toxicity:-
Clopyralid caused birth defects in test animals, but only at greatly exaggerated doses that were severely toxic to the mothers. No birth defects were observed in animals given clopyralid at doses several times greater than those expected during normal exposure.

Reproductive Toxicity:-
In animal studies, did not interfere with reproduction.

Genetic Toxicology:-
In vitro genetic toxicity studies were negative. Animal genetic toxicity studies were negative

With regard to expected effects of poisoning, since the last submission in 2002, no new information was discovered which provides new end-points in this section. Clopyralid is considered to be of low overall systemic toxicity; no specific acute toxic syndrome has been identified after ingestions, and long term effects are not expected following exposures related to normal handling and usage. Significant and permanent eye damage can occur following significant direct contact exposure to the eyes.

B.6.9.6. Proposed treatment: first aid measures, antidotes, medical treatment

The following treatments are proposed by the notifier:
Since the last submission in 2002, no new information was discovered which provides new end-points in this section. Clopyralid is considered to be of low overall systemic toxicity, and there is no specific antidote. Mild exposures or small ingestions are unlikely to cause significant systemic toxicity, and first aid measures are, for the most part, generic to such products and related to supportive care relative to any symptoms.

Description of first aid measures

*General advice**:*** First Aid responders should pay attention to self-protection and use the recommended protective clothing (chemical resistant gloves, splash protection).

Inhalation: Move person to fresh air. If person is not breathing, call an emergency responder or ambulance, then give artificial respiration; if by mouth to mouth use rescuer protection (pocket mask for example). Call a poison control centre or doctor for treatment advice.

*Skin Contact***:** Take off contaminated clothing. Rinse skin immediately with plenty of water for 15-20 minutes. Call a poison control centre or doctor for treatment advice.

Eye Contact: Wash immediately and continuously with flowing water for at least 30 minutes. Remove contact lenses after the first 5 minutes and continue washing. Chemical eye burns may require extended irrigation. Obtain prompt medical consultation, preferably from an ophthalmologist. Suitable emergency eye wash facility should be immediately available.

Ingestion: No emergency medical treatment should be necessary for small accidental ingestions – however, where larger amounts have been swallowed call a poison control centre or doctor immediately for treatment advice. Have person sip a glass of water if able to swallow. Do not induce vomiting unless told to do so by the poison control centre or doctor. Never give anything by mouth to an unconscious person

No specific antidote. Treatment of exposure should be directed at the control of symptoms and the clinical condition of the patient.

B.6.9.7. Literature data

The applicant provided a description and results of the open literature search. The RMS considers the literature search provided as acceptable.

Databases:
STN Toxicology Database Cluster, includes CAPLUS, RTECS and TOXCENTER.
Dialog databases, includes AGRICOLA, AGRIS International, Aqualine, ASFA, BIOSIS, BIOTECHNO, CAB Abstracts, CEABA®, Ecology Abstracts, Embase, Environmental Engineering Abstracts, ESBIOBASE, Foodline®: SCIENCE, FSTA®, GEOBASE, MEDLINE, Meteorological and Geoastrophysical Abstracts, PASCAL, Pollution Abstracts, SCISEARCH, ToxFile, TOXLINE and Water Resources Abstracts
(Patents and conference papers were excluded from the search)

Time window: 2005 to January 2015 and January 2015 to July 2015.

Input parameters: CAS number, CAS name´, IUPAC name, common name, different chemical names, company experimental names, product names and development codes.

Results: A total of 501 summary records were retrieved after removing duplicates from all database searches. After a rapid assessment for relevance based on title/abstract total of 477 summary records were excluded. This resulted for 24 summary records for further assessment. After detailed assessment for relevance, based on abstract/fulltext, 16 studies were excluded. The 8 studies considered relevant and reliable related to the ecotoxicology data points. There were no articles with relevance for toxicology section.

The main categories of relevance for which the open literature was searched included e.g. toxicological and metabolism studies on the active substance (KCA Section 5) or on the plant protection product (KCP Section 7) (Regulations 283/2013 and 284/2013) and other data requirements for which information may have a direct or indirect effect on overall risk assessment. The overview of specific relevance criteria considered, relating to toxicology data requirements:
- Well-defined test material
- *In vivo* tests in relevant test species
- *In vitro* tests
- PBPK modelling (ADME studies)
- Relevant route of exposure
- Specific endpoint can be clearly related to the data requirement
- Epidemiological studies
- Poisonings, clinical cases
- Field studies and calculations (exposure)

The reliability of the relevant studies was mentioned to be carried out according to Klimisch *et al.* (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), with following categories: reliable without restriction, reliable with restriction, not reliable, not assignable.

B.6.10. REFERENCES RELIED ON

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
B.6.1.1	[redacted]	1975	The Fate Of 3,6-Dichloropicolinic Acid (DOWCO 290) Following Oral Administration in Rats. DAS Study No. NBK 102 GLP/GEP (Y/N): No Published (Y/N): No	Y	N		Dow AgroSciences	DAR (2003)
B.6.1.1	[redacted]	1991	Metabolism of 14C-3,6-Dichloropicolinic Acid in Rats. HWI 6148-115 DAS Study No. HWI 6148-115 [redacted] GLP/GEP (Y/N): Yes Published (Y/N): No	Y	N		Dow AgroSciences	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
B.6.1.2	[redacted]	2016	Clopyralid: *In Vitro* Comparative Metabolism Using Liver Microsomes from F344 Rat and Human Donors DAS study no. 160839 GLP/GEP (Y/N): Yes Published (Y/N): No	N	Y	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).	Dow AgroSciences	Submitted for the purpose of renewal
B.6.2.1	[redacted]	1987	Lontrel T Herbicidal Chemical (Penta Process): Acute Oral Toxicity Study in Fischer 344 Rats. DAS Study No. K-038252-033A [redacted] GLP/GEP (Y/N): Yes Published (Y/N): No	Y	N		Dow AgroSciences	DAR (2003)

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B.6.2.2	[redacted]	1987	Lontrel T Herbicidal Chemical (Penta Process): Acute Dermal Toxicity Study in New Zealand White Rabbits. DAS Study No. K-038252-033D [redacted] GLP/GEP (Y/N): Yes Published (Y/N): No	Y	N		Dow AgroSciences	DAR (2003)
B.6.2.3	[redacted]	1987	Lontrel T Herbicidal Chemical (Penta Process): An Acute Aerosol Inhalation Study in Fischer 344 Rats. DAS Study No. K-038252-034 [redacted] GLP/GEP (Y/N): Yes Published (Y/N): No	Y	N		Dow AgroSciences	DAR (2003)
B.6.2.3	[redacted]	1991	Lontrel T: Acute Inhalation Toxicity Study With Fischer 344 Rats. DAS Study No. K-038252-045 [redacted] GLP/GEP (Y/N): Yes Published (Y/N): No	Y	N		Dow AgroSciences	DAR (2003)

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B.6.2.4	[redacted]	1987	Lontrel T Herbicidal Chemical (Penta Process): Primary Dermal Irritation Study in New Zealand White Rabbits. DAS Study No. K-038252-033B [redacted] GLP/GEP (Y/N): Yes Published (Y/N): No	Y	N		Dow AgroSciences	DAR (2003)
B.6.2.5	[redacted]	1987	Lontrel T Herbicidal Chemical (Penta Process): Primary Eye Irritation Study in New Zealand White Rabbits. DAS Study No. K-038252-033C [redacted] GLP/GEP (Y/N): Yes Published (Y/N): No	Y	N		Dow AgroSciences	DAR (2003)
B.6.2.6	[redacted]	1996	Lontrel T (Clopyralid Technical): Delayed Contact Hypersensitivity Study in the Guinea Pig DAS Study No. DES/363 DAS Report No. GHE-T-1150 [redacted] GLP/GEP (Y/N): Yes Published (Y/N): No	Y	N		Dow AgroSciences	DAR (2003)

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B.6.2.6	[redacted]	1987	LONTREL* T Herbicidal Chemical (Penta Process): Dermal Sensitization Potential in the Hartley Albino Guinea Pig [redacted] DAS Report No.: K-038252-033E GLP/GEP (Y/N): Y Published (Y/N): N	Y	N		Dow AgroSciences	DAR (2003)
B.6.3.1	[redacted]	1986	Lontrel T - Toxicity To Rats By Dietary Admixture For 4 Weeks (Final Report) DAS Study No. DWC 463/86108 [redacted] GLP/GEP (Y/N): Yes Published (Y/N): No	Y	N		Dow AgroSciences	DAR (2003)
B.6.3.1	[redacted]	1982	LONTREL* T (DOWCO* 290): Results of a 2-week Dietary Probe Study in $B_6C_3F_1$ Mice [redacted] DAS Report No.: HET K-038252-(16) GLP/GEP (Y/N): Y Published (Y/N): N	Y	N		Dow AgroSciences	DAR (2003)

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B.6.3.1	[redacted]	1990	Clopyralid: 13-day Repeated Oral Gavage Study in New Zealand White Rabbits [redacted] DAS Report No.: K-038252-040 GLP/GEP (Y/N): Y Published (Y/N): N	Y	N		Dow AgroSciences	DAR (2003)
B.6.3.2	[redacted]	1983	Dowco 290 Herbicide: Results of A Three-Month Dietary Toxicity Study in Rats. DAS Study No. A2A-054 [redacted] GLP/GEP (Y/N): Yes Published (Y/N): No	Y	N		Dow AgroSciences	DAR (2003)
B.6.3.2	[redacted]	1973	DOWCO 290 - pesticide: results of a 90 day dietary feeding study in rats. DAS Study No. A2A-030 [redacted] GLP/GEP (Y/N): No Published (Y/N): No	Y	N		Dow AgroSciences	DAR (2003)

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B.6.3.2	[redacted]	1983	Dowco 290: Results of A 13-Week Dietary Toxicity Study in B6C3F1 Mice. DAS Study No. A2A-029 [redacted] GLP/GEP (Y/N): Yes Published (Y/N): No	Y	N		Dow AgroSciences	DAR (2003)
B.6.3.2	[redacted]	1984	A 12-Month Oral Toxicity Study of 3,6-Dichloropicolinic Acid in the Beagle Dog. DAS Study No. 81347 [redacted] GLP/GEP (Y/N): Yes Published (Y/N): No	Y	N		Dow AgroSciences	DAR (2003)
B.6.3.2	[redacted]	1976	DOWCO* 290 Herbicide (3,6-Dichloropicolinic Acid): Results of a Six-month Dietary Feeding Study in Beagle Dogs [redacted] DAS Report No.: HET K-38252-(5) GLP/GEP (Y/N): N Published (Y/N): N	Y	N		Dow AgroSciences	DAR (2003)

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B.6.3.2	[redacted]	1975	180-Day Subacute Toxicity Study in Dogs - DOWCO 290 [redacted] DAS Report No.: GH-RC-21 GLP/GEP (Y/N): N Published (Y/N): N	Y	N		Dow AgroSciences	DAR (2003)
B.6.3.3	[redacted]	1990	Clopyralid: probe and 21 day dermal toxicity study in New Zealand White rabbits DAS Study No. K-038252-044 [redacted] GLP/GEP (Y/N): Yes Published (Y/N): No	Y	N		Dow AgroSciences	DAR (2003)
B.6.4.1	Richold, M.; Jones, E.; Fleming, P.M.	1982	Ames Metabolic Activation Test To Assess The Potential Mutagenic Effect of 3,6-Dichloropicolinic Acid Technical DAS Study No. DWC 339/81801 DAS Report No. GHE-T-041 Huntingdon Research Center Ltd, Huntingdon, Cambridgeshire, United Kingdom GLP/GEP (Y/N): Yes Published (Y/N): No	N	N		Dow AgroSciences	DAR (2003)
B.6.4.1	Bruce, R. J.; Bhaskar Gollapudi, B.	1987	Lontrel T Herbicidal Chemical (Penta Process): Evaluation in the Ames Salmonella/Mammalian-Microsome Mutagenicity Assay. DAS Study No. K-038252-036 Dow Chemical Company, Freeport, Texas, United States GLP/GEP (Y/N): Yes Published (Y/N): No	N	N		Dow AgroSciences	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
B.6.4.1	[redacted]	2001	Evaluation of Clopyralid in an *In Vitro* Chromosomal Aberration Assay Utilizing Rat Lymphocytes *DAS Study No. 001155* [redacted] *GLP/GEP (Y/N): Yes* *Published (Y/N): No*	N	N		Dow AgroSciences	DAR (2003)
B.6.4.1	[redacted]	1987	Evaluation of Lontrel T Herbicidal Chemical (Penta Process) in the Chinese Hamster Ovary Cell/Hypoxanthine-Guanine-Phosphoribosyl Transferase (CHO/HGPRT) Forward Mutation Assay DAS Study No. K-038252-037 [redacted] GLP/GEP (Y/N): Yes Published (Y/N): No	N	N		Dow AgroSciences	DAR (2003)
B.6.4.1	[redacted]	1985	The evaluation of Lontrel T herbicide in the rat hepatocyte unscheduled DNA synthesis assay DAS Study No. HET K 038252 -031 [redacted] GLP/GEP (Y/N): Yes Published (Y/N): No	N	N		Dow AgroSciences	DAR (2003)

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B.6.4.1	[redacted]	1973	In Vitro and Subacute In Vivo Host-mediated Assay for Mutagenesis: Final Report - Compound DOWCO 290 [redacted] DAS Report No.: GHE-T-1151 GLP/GEP (Y/N): N Published (Y/N): N	Y	N		Dow AgroSciences	DAR (2003)
B.6.4.2	[redacted]	1991	Evaluation of Clopyralid in the Mouse Bone Marrow Micronucleus Test. DAS Study No. K-038252-042 [redacted] GLP/GEP (Y/N): Yes Published (Y/N): No	Y	N		Dow AgroSciences	DAR (2003)
B.6.4.2	[redacted]	1973	Acute and subacute in vivo cytogenetic study in rats. Final report - compound DOWCO 290. DAS Study No. GHE-T-1152 [redacted] GLP/GEP (Y/N): No Published (Y/N): No	Y	N		Dow AgroSciences	DAR (2003)
B.6.4.3	[redacted]	1973	Dominant Lethal Assay for Mutagenesis Final Report - Compound DOWCO 290. DAS Study No. 2421 [redacted] GLP/GEP (Y/N): No Published (Y/N): No	Y	N		Dow AgroSciences	DAR (2003)

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B.6.5	[redacted]	1985	Dowco 290: 2-Year Rat Diet Chronic Toxicity and Oncogenicity Study 1-Year Interim Report. DAS Study No. K-038252-25, part 1 [redacted] GLP/GEP (Y/N): Yes Published (Y/N): No	Y	N		Dow AgroSciences	DAR (2003)
B.6.5	[redacted]	1986	Dowco 290: 2-Year Rat Diet Chronic Toxicity and Oncogenicity Study Final Report. DAS Study No. K-038252-25 (2), part 2 [redacted] GLP/GEP (Y/N): Yes Published (Y/N): No	Y	N		Dow AgroSciences	DAR (2003)
B.6.5	[redacted]	1977	DOWCO 290 (3,6 dichloropicolinic acid): results of a two-year chronic toxicity and oncogenicity study in rats by the dietary route DAS Study No. A2A-052 [redacted] GLP/GEP (Y/N): No Published (Y/N): No	Y	N		Dow AgroSciences	DAR (2003)

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B.6.5	[redacted]	1978	Supplemental report on DOWCO 290 (3,6 dichloropicolinic acid): results of a two-year chronic toxicity and oncogenicity study in rats by the dietary route DAS Study No. HET K-038252 (4) [redacted] GLP/GEP (Y/N): No Published (Y/N): No	Y	N		Dow AgroSciences	DAR (2003)
B.6.5	[redacted]	1985	DOWCO 290 (3,6 dichloropicolinic acid): results of a two-year chronic toxicity and oncogenicity study in rats by the dietary route. Supplemental report on the histopathological evaluation of thyroid gland and pituitary gland tissues from female rats in the 50, 15 and 5 mg/kg bw/day groups DAS Study No. HET K-038252 (4) [redacted] GLP/GEP (Y/N): No Published (Y/N): No	Y	N		Dow AgroSciences	DAR (2003)
B.6.5	[redacted]	1984	Dowco 290: 2-Year Chronic Toxicity and Oncogenicity Study in Mice: 1-year Interim Report DAS Study No. K-038252-24 part 1 [redacted] GLP/GEP (Y/N): Yes Published (Y/N): No	Y	N		Dow AgroSciences	DAR (2003)

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B.6.5	[redacted]	1986	Dowco 290: 2-Year Dietary Chronic Toxicity-Oncogenicity Study in Mice (Final report) DAS Study No. K-038252-24 part 2 [redacted] GLP/GEP (Y/N): Yes Published (Y/N): No	Y	N		Dow AgroSciences	DAR (2003)
B.6.5	[redacted]	1976	18-month Mouse Oncology Study with DOWCO 290 (Powder) [redacted] DAS Report No.: GHR-C-93 GLP/GEP (Y/N): N Published (Y/N): N	Y	N		Dow AgroSciences	DAR (2003)
B.6.6.1	[redacted]	1983	Dowco 290: Two Generation Dietary Reproduction Study in Fischer-344 Rats DAS Study No. K-038252-021 [redacted] GLP/GEP (Y/N): Yes Published (Y/N): No	Y	N		Dow AgroSciences	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
B.6.6.1	[redacted]	1984	DOWCO 290 herbicide: two generation dietary reproduction study in Fischer 344 rats - supplemental histopathology DAS Study No. K-038252-(21) PT3 [redacted] GLP/GEP (Y/N): Yes Published (Y/N): No	Y	N		Dow AgroSciences	DAR (2003)
B.6.6.2	[redacted]	1981	DOWCO 290 - Oral Teratology Study in Fischer 344 Rats. DAS Study No. HET K-38252-(10) [redacted] GLP/GEP (Y/N): Yes Published (Y/N): No	Y	N		Dow AgroSciences	DAR (2003)
B.6.6.2	[redacted]	1990	Clopyralid: Oral Gavage Teratology Study in New Zealand White Rabbits. DAS Study No. K-038252-039 [redacted] GLP/GEP (Y/N): Yes Published (Y/N): No	Y	N		Dow AgroSciences	DAR (2003)

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B.6.6.2	[redacted]	1974	The Effect of DOWCO® 290 (3,6-dichloropicolinic Acid) on the Developing Embryo and Foetus of Pregnant Rabbits [redacted] DAS Report No.: HET K-038252-(7) GLP/GEP (Y/N): N Published (Y/N): N	Y	N		Dow AgroSciences	DAR (2003)
B.6.8.2	[redacted]	1995	Clopyralid An Evaluation of Mammalian Toxicology [redacted] DAS Report No.: None GLP/GEP (Y/N): No Published (Y/N): No	No	No		Dow AgroSciences	DAR (2003)

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B.6.8.2	[redacted]	2010	Clopyralid: Assessment of Immunotoxic Potential using the Sheep Red Blood Cell Assay after 28-Day Dietary Exposure to Male F344/DuCrl Rats DAS Study No. 101062, K-038252-132 [redacted] GLP/GEP (Y/N): Yes Published (Y/N): No	Y	Y	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).	Dow AgroSciences	Submitted for the purpose of renewal
B.6.9.1	[redacted]	1991	Worker Exposure Assessment to LONTREL Herbicide (Letter Report) [redacted] DAS Report No.: None GLP/GEP (Y/N): N Published (Y/N): N	N	N		Dow AgroSciences	DAR (2003)