CLH report

Proposal for Harmonised Classification and Labelling

Based on Regulation (EC) No 1272/2008 (CLP Regulation),
Annex VI, Part 2

International Chemical Identification:

clopyralid (ISO);

3,6-dichloropyridine-2-carboxylic acid

EC Number: 216-935-4

CAS Number: 1702-17-6

Index Number: 607-231-00-1

Contact details for dossier submitter: Finnish Competent Authority
Finnish Safety and Chemicals Agency (Tukes), Finland

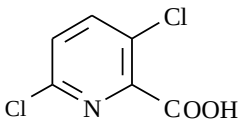
Version number: 3 **Date: 30.8.2022**

CONTENTS

1 IDENTITY OF THE SUBSTANCE

1.1 Name and other identifiers of the substance

Table 1: Substance identity and information related to molecular and structural formula of the substance

Name(s) in the IUPAC nomenclature or other international chemical name(s)	3,6-dichloropyridine-2-carboxylic acid
Other names (usual name, trade name, abbreviation)	
ISO common name (if available and appropriate)	clopyralid
EC number (if available and appropriate)	216-935-4
EC name (if available and appropriate)	
CAS number (if available)	1702-17-6
Other identity code (if available)	CIPAC number: 455
Molecular formula	$C_6H_3Cl_2NO_2$
Structural formula	
SMILES notation (if available)	
Molecular weight or molecular weight range	191.96 g/mol
Information on optical activity and typical ratio of (stereo) isomers (if applicable and appropriate)	
Description of the manufacturing process and identity of the source (for UVCB substances only)	
Degree of purity (%) (if relevant for the entry in Annex VI)	

1.2 Composition of the substance

Table 2: Constituents (non-confidential information)

Constituent (Name and numerical identifier)	Concentration range (% w/w minimum and maximum in multi-constituent substances)	Current CLH in Annex VI Table 3.1 (CLP)	Current self-classification and labelling (CLP)
clopyralid, CAS 1702-17-6	**min. 95%**	**Eye Dam. 1; H318**	**Eye Dam. 1; H318** **Eye Irrit. 2; H319** **Skin Irrit. 2; H315** **STOT SE 3; H335** **Aquatic Chronic 1; H410** **Aquatic Chronic 1; H411**

Table 3: Impurities (non-confidential information) if relevant for the classification of the substance

Impurity (Name and numerical identifier)	Concentration range (% w/w minimum and maximum)	Current CLH in Annex VI Table 3.1 (CLP)	Current self-classification and labelling (CLP)	The impurity contributes to the classification and labelling
Impurities are confidential information				It can not be excluded that impurities would contribute to classification and labelling[a]

[a] For more information see Final renewal report of the active substance clopyralid

Table 4: Additives (non-confidential information) if relevant for the classification of the substance

Additive (Name and numerical identifier)	Function	Concentration range (% w/w minimum and maximum)	Current CLH in Annex VI Table 3.1 (CLP)	Current self-classification and labelling (CLP)	The additive contributes to the classification and labelling
No additives					

Table 5: Test substances (non-confidential information) (this table is optional)

Identification of test substance	Purity	Impurities and additives (identity, %, classification if available)	Other information	The study(ies) in which the test substance is used

2 PROPOSED HARMONISED CLASSIFICATION AND LABELLING

2.1 Proposed harmonised classification and labelling according to the CLP criteria

Table 6:

	Index No	International Chemical Identification	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors	Notes
					Hazard Class and Category Code(s)	Hazard statement Code(s)	Pictogram, Signal Word Code(s)	Hazard statement Code(s)	Suppl. Hazard statement Code(s)		
Current Annex VI entry	607-231-00-1	clopyralid (ISO) 3,6-dichloropyridine-2-carboxylic acid	216-935-4	1702-17-6	Eye Dam. 1	H318	GHS 05 Dgr	H318			
Dossier submitters proposal	607-231-00-1	clopyralid (ISO) 3,6-dichloropyridine-2-carboxylic acid	216-935-4	1702-17-6	**Add** Repr. 2 STOT RE 2 Aquatic Chronic 1	**Add** H361d H373 H410	**Add** GHS08 GHS09	**Add** H361d H373 H410	EUH066	M=10	
Resulting Annex VI entry if agreed by RAC and COM	607-231-00-1	clopyralid (ISO) 3,6-dichloropyridine-2-carboxylic acid	216-935-4	1702-17-6	Repr. 2 STOT RE 2 Eye Dam. 1 Aquatic Chronic 1	H361d H373 H318 H410	GHS08 GHS05 GHS09 Dgr	H361d H373 H318 H410	EUH066	M=10	

Table 7: Reason for not proposing harmonised classification and status under public consultation

Hazard class	Reason for no classification	Within the scope of public consultation
Explosives	hazard class not assessed in this dossier	No
Flammable gases (including chemically unstable gases)	hazard class not assessed in this dossier	No
Oxidising gases	hazard class not assessed in this dossier	No
Gases under pressure	hazard class not assessed in this dossier	No
Flammable liquids	hazard class not assessed in this dossier	No
Flammable solids	hazard class not assessed in this dossier	No
Self-reactive substances	hazard class not assessed in this dossier	No
Pyrophoric liquids	hazard class not assessed in this dossier	No
Pyrophoric solids	hazard class not assessed in this dossier	No
Self-heating substances	hazard class not assessed in this dossier	No
Substances which in contact with water emit flammable gases	hazard class not assessed in this dossier	No
Oxidising liquids	hazard class not assessed in this dossier	No
Oxidising solids	hazard class not assessed in this dossier	No
Organic peroxides	hazard class not assessed in this dossier	No
Corrosive to metals	hazard class not assessed in this dossier	No
Acute toxicity via oral route	hazard class not assessed in this dossier	No
Acute toxicity via dermal route	hazard class not assessed in this dossier	No
Acute toxicity via inhalation route	hazard class not assessed in this dossier	No
Skin corrosion/irritation	data conclusive but not sufficient for classification	Yes
Serious eye damage/eye irritation	hazard class not assessed in this dossier	No
Respiratory sensitisation	hazard class not assessed in this dossier	No
Skin sensitisation	hazard class not assessed in this dossier	No
Germ cell mutagenicity	hazard class not assessed in this dossier	No
Carcinogenicity	hazard class not assessed in this dossier	No
Reproductive toxicity	harmonised classification proposed	Yes
Specific target organ toxicity-single exposure	hazard class not assessed in this dossier	No
Specific target organ toxicity-repeated exposure	harmonised classification proposed	Yes
Aspiration hazard	hazard class not assessed in this dossier	No
Hazardous to the aquatic environment	harmonised classification proposed	Yes
Hazardous to the ozone layer	hazard class not assessed in this dossier	No

3 HISTORY OF THE PREVIOUS CLASSIFICATION AND LABELLING

Clopyralid was first introduced to Annex 1 of the Directive 67/548/EEC in the Commission Directive 96/54/EC of 30 July 1996 (22nd ATP) with Xi; R41 and N, R 51-53 classifications. The classification was reviewed by the Meeting of Technical Committee C&L on the Classification and Labelling of Dangerous substances in relation to the pesticide review programme in 2005. The classification translated to the CLP Regulation was Eye Dam. 1, H318.

4 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

[A.] There is no requirement for justification that action is needed at Community level.

5 IDENTIFIED USES

Clopyralid is a contact acting and selective auxin type herbicide. It is used to control a range of broad leaf weeds in cereals and grassland.

6 DATA SOURCES

The Draft Renewal Assessment Report (2018) under Regulation (EC) 1107/2009 was used as the main data source for drafting the CLH report of clopyralid. However, the CLH report is an independent hazard assessment of clopyralid and therefore in some cases the conclusions in the CLH report may differ from those in dRAR.

7 PHYSICOCHEMICAL PROPERTIES

Table 8: Summary of physicochemical properties

Property	Value	Reference	Comment (e.g. measured or estimated)
Physical state at 20°C and 101,3 kPa	Pure: crystalline solid (99.8%) As manufactured: powdery solid (95.3%)	dRAR B.2.3/01 dRAR B.2.3/01	
Melting/freezing point	Melting Point: 149.6 ± 0.2 °C (99.8%)	dRAR B.2.1/01	
Boiling point	Clopyralid was determined to decompose at 164 ± 2 °C by Differential Scanning Calorimeter (DSC) analysis. A thermal effect due to boiling was not observed.	dRAR B.2.1/02	
Relative density	1.5747 at 20 ± 1 ^{o}C	dRAR B.2.14/01	
Vapour pressure	$1.02x10^{-5}$ mmHg or $1.36x10^{-6}$ kPa at 25°C	dRAR B.2.2/01	
Surface tension	The surface tension of a 1 g/L aqueous solution of pure (99.9%) clopyralid	dRAR B.2.12/01	

Property	Value	Reference	Comment (e.g. measured or estimated)
	was found to be 71.5 mN/m at 20°C		
Water solubility	at 20°C: Unbuffered water (pH 1.7): 0.785 g/100 mL pH 5.0: 11.8 g/100 mL pH 7.0: 14.3 g/100 mL pH 9.0: 15.7 g/100 mL	dRAR B.2.5/01	The study was not acceptable according to Regulation (EU) No 283/2013, due to lack of validation data presented in the study report. However, as such data is not required under Regulation (EC) No 1272/2008, the DS considers the study valid to be used for classification purposes.
Partition coefficient n-octanol/water	pH 5 buffer: $logK_{ow}$ = -1.81 at 20°C pH 7 buffer: $logK_{ow}$ = -2.63 at 20°C pH 9 buffer: $logK_{ow}$ = -2.55 at 20°C	dRAR B.2.7/01	The study was not acceptable according to Regulation (EU) No 283/2013 due to lack of validation data presented in the study report. However, as such data is not required under Regulation (EC) No 1272/2008, the DS considers the study valid to be used for classification purposes.
Flash point	not required as material is a solid	dRAR B.2.10/01	
Flammability	Not flammable	dRAR B.2.9/01	
Explosive properties	No sign of ignition or explosion	dRAR B.2.11/01	
Self-ignition temperature	No self-ignition temperature prior to melting at ~151°C	dRAR B.2.9/02	
Oxidising properties	Non-oxidising	dRAR B.2.13/01	
Granulometry			
Stability in organic solvents and identity of relevant degradation products			
Dissociation constant	pKa = 2.01 +/- 0.11 at 25°C	dRAR B.2.8/01	
Viscosity			

8 EVALUATION OF PHYSICAL HAZARDS

Not assessed in this dossier

9 TOXICOKINETICS (ABSORPTION, METABOLISM, DISTRIBUTION AND ELIMINATION)

Table 9: Summary table of toxicokinetic studies

Method, guideline, deviations if any	Species, strain, sex, no/group, materials and method	Results	Reference
Single dose oral study in rats No official OECD TG Non-GLP The study was performed 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 OECD TG 417 requires use of more animals, investigation of single dose administration at low and high doses, and repeat administration at the low dose.	The absorption, distribution, excretion and metabolism of clopyralid were investigated in male and female Sprague-Dawley rats following oral administration of a single dose of clopyralid (>99% radiochemical purity, dissolved in 0.7M 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 so that blood samples could be obtained at intervals up to 12 hours after 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 so that urine could be collected at eight-hour intervals up to 16 hours after administration. 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 CO2. Five days after administration, the concentration of radioactivity in the liver, kidney, muscle, perirenal fat, skin, carcass, urine and faeces were determined.	Average recovery of radioactivity was 100.55 ± 3.74% of administered clopyralid was rapidly and virtually completely absorbed 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, 96.5% 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 360 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 clopyralid at 120 hours were extremely low. 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. Following oral administration to Sprague-Dawley rat, clopyralid is rapidly and completely absorbed, and is excreted quantitatively, unchanged, in	RAR B.6.1.1. (Anonymous 1975)

Method, guideline, deviations if any	Species, strain, sex, no/group, materials and method	Results	Reference
		the urine.	
Metabolism of 14C-3,6-dichloropicolinic acid (> 99% radiochemical purity), oral and intravenous administration OECD TG 417 GLP The study does not contain plasma time-curve data. The test substance was administered in corn oil (oral dose) or saline (intravenous dose).	The Fischer 344 rats were 6 to 10 weeks old and weighed approx. 148 to 216 g (males) and 126 to 151 g (females). In a preliminary study, 2 rats/sex were dosed orally at 5 mg/kg bw 14C clopyralid and assessed for radioactivity in expired air. In the main study, the treated animals were divided into four groups. For repeat administration, rats received 14 days non-radiolabelled clopyralid (> 96% pure) treatments followed by a single dose of radiolabelled clopyralid. Urine and faeces were collected at intervals up to three days after administration and blood at the end of the collection period. The samples were analysed to follow the time course of radioactivity. Three days after administration, the concentration of radioactivity in tissues, organs and carcass was determined. Tissues analysed were: bone (femur), brain, fat (reproductive), ovaries, testes, heart, liver, kidneys, lungs, muscle (thigh), spleen, stomach, uterus, and residual carcass with skin.	Radioactivity in the urine (including cage wash) ranged from 74.1% to 97.6% of the applied dose for males and females in the four treated groups. 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 mg/kg except in the stomach where up to 0.237 and 0.189 mg/kg was found in males and females in the high dose group. **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. 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.	RAR B.6.1.1. (Anonymous 1991)
Single dose (10 mL/kg bw) toxicokinetics study and bone marrow determination of clopyralid spiked impurities through oral gavage. OECD TG 417	CD-1 mice Non-radiolabelled clopyralid spiked impurities (purity 95.4 wt%) in vehicle (1 % w/v aqueous carboxy methyl cellulose (CMC)) was mixed with 14C-radiolabeled clopyralid spiked impurities (radiochemical purity 99.0 %; specific radioactivity 35 mCi/mmol) and administered orally by gavage in dose of 10 mL/kg	No mortality and morbidity was observed during the study. All mice were normal during the study. The Cmax of the test substance was 0.3159 % at Tmax of 0.5 h. The AUClast was 0.573725 with 11.204618 h half-life. In phase 1, the Tmax was 0.5 h at which time mean concentration of clopyralid spiked impurities (Cmax) was 0.3159 % TRR under the conditions and	RAR B.6.1.1. (Anonymous 2018)

Method, guideline, deviations if any	Species, strain, sex, no/group, materials and method	Results	Reference
GLP	bw (≈ 8 µCi radioactivity/animal). The study was performed in two phases. In phase 1 (6 female mice), toxicokinetics (TK) profiling (Cmax (maximum observed/peak concentration), Tmax (time of maximum observed/peak concentration), AUC (the area under the concentration time curve), T1/2 (terminal half-life), MRT (mean residence time), Cl (clearance), Vd (volume of distribution)) of the test substance was performed on whole blood samples which were collected at 0.5, 1, 2, 4, 6, 8, 12 and 24 h post dosing. Known quantity of matrix sample was combusted in the sample oxidizer and analysed in LSA for recovered radioactivity. Based on the recovered radioactivity in each sample, TK parameters were evaluated using computer software. In phase 2 (5 female mice), whole blood samples were collected at Tmax (determined in phase 1). After blood collection, mice were sacrificed and bone marrow was collected. Known quantity of matrix sample was combusted in the sample oxidizer and analysed in LSA for recovered radioactivity.	procedures followed in this study. In phase 2, mean concentration of clopyralid spiked impurities at Tmax was 0.2207 and 0.0064 % TRR in blood and bone marrow respectively, under the conditions and procedures followed in this study. The results of this study show that clopyralid spiked impurities reaches the bone marrow after oral administration in an extreme low quantity, the peak concentration is reached soon after administration and the concentration in bone marrow will be reduced quickly. Hence, bone marrow seems not to be a suitable model in studying potential clastogenic and aneugenic effects of clopyralid spiked impurities in vivo.	
In vitro Comparative metabolism Using Liver Microsomes No guideline GLP	*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. 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	The measured concentration of the 14C-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 14C-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.	RAR B.6.1.2. (Anonymous 2016)

Method, guideline, deviations if any	Species, strain, sex, no/group, materials and method	Results	Reference
	test system. 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. Three types of negative controls were generated; vehicle control incubations (1 replicate/species), incubations lacking cofactors (1 replicate/species) and 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) ≥ 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	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, 14C-clopyralid, UDPGA and NADPH were radiochemically profiled for both species. The average limit of detection was determined to be 2.385% of the total incubation radioactivity. 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.	

Method, guideline, deviations if any	Species, strain, sex, no/group, materials and method	Results	Reference
	(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.		

9.1 Short summary and overall relevance of the provided toxicokinetic information on the proposed classification(s)

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). The majority of radioactivity (≥ 71.4 % of applied dose) was eliminated as unchanged clopyralid via urine within 24 hours. 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. Based on the studies by Anonymous (1975) and Anonymous (1991) some toxicokinetic parameters (e.g. AUC, C_{max}, T_{max} and half-lives) are left open. The toxicokinetic study in mice (Anonymous, 2018) concludes on the following parameters:
C_{max}= 0.3159 %
$T_{max=}$ 0.5 h
AUC_{last}= 0.573725
Half-life= 11.204618 h

10 EVALUATION OF HEALTH HAZARDS

Acute toxicity

10.1 Acute toxicity - oral route

Not assessed in this dossier

10.2 Acute toxicity - dermal route

Not assessed in this dosser.

10.3 Acute toxicity - inhalation route

Not assessed in this dosser.

10.4 Skin corrosion/irritation

Table 10: Summary table of animal studies on skin corrosion/irritation

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance,	Dose levels duration of exposure	Results -Observations and time point of onset -Mean scores/animal -Reversibility	Reference
Primary dermal irritation study The study was performed according to the method (USEPA 81-5 (1982)) complied with OECD guideline 404 (2015) GLP	New Zealand white rabbit 3/sex	0.5 g aliquot of clopyralid (95.4%)	The test material was applied to the back of each animal under a 2.5 cm2 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 off 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. No evidence of dermal irritation was observed in animals after treatment and grades for erythema and oedema were all 0.	dRAR B.6.2.4. (Anonymous 1987)

Table 11: Summary table of human data on skin corrosion/irritation

No data

Table 12: Summary table of other studies relevant for skin corrosion/irritation

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance,	Dose levels duration of exposure	Results -Observations and time point of onset -Mean scores/animal -Reversibility	Reference
Dermal probe study and 21-day dermal study The study was performed according to the Guideline No. 82-2 and predominantly according to OECD guideline	New Zealand White rabbit Probe study: 1/sex 21-day dermal study: 5/sex	Clopyralid (95.78 ±0.25%)	Probe study: A dermal application of 500 and 1000 mg/kg/day clopyralid, respectively, 6 hours/day for 4 days. 21-day dermal study: 0, 100, 500, 1000 mg/kg bw/day Animals were dosed a total of 15 times during the 21-day	Neither dermal irritation nor evidence of systemic toxicity was observed in the probe study. In the 21-Day Dermal Study slight erythema (score 1) was observed in test day 10 in two male rabbits belonging to the intermediate and high dose groups, respectively, and one female rabbit in test	Anonymous 1990

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance,	Dose levels duration of exposure	Results -Observations and time point of onset -Mean scores/animal -Reversibility	Reference
410 (1981). GLP			dosing period	day 3, belonging to the intermediate dose group. On the histopathologic skin lesions epidermal hyperplasia was noted in 1/5 males and 2/5 females treated with 100 mg/kg/day, 3/5 males and 1/5 females treated with 500 mg/ kg/day, and 5/5 males and 5/5 females treated with 1000 mg/kg/day.	

10.4.1 Short summary and overall relevance of the provided information on skin corrosion/irritation

The primary dermal irritation study

The study Anonymous 1987 was conducted in compliance with the GLP standards and the guideline No. 81-5. It is mainly performed in compliance with OECD guideline 404 (2015). However, 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. Also response was examined at 30 minutes instead of 60 minutes given in the guideline.

Three male and three female New Zealand white rabbits were treated with a 0.5 g aliquot of 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 (Table 13). No evidence of dermal irritation was observed in animals after treatment.

Table 13. 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

A supportive probe/21-day dermal study

The supportive probe/21-day dermal study (Anonymous 1990) 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. 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.

The test compound was applied in its powder form. All rabbits were acclimated to an elastic jacket used to hold the test material dressing in dermal contact. An area approximately 10x15 cm on the back of each rabbit was clipped free of fur prior to study initiation and as necessary thereafter. A dressing consisting of a water-moistened absorbent gauze and non-absorbent cotton was used to hold test material in dermal contact. The jacket and dressing were removed approximately six hours after application and test site was wiped with a water-damped towel to remove any residual test material.

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. 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).

A complete histologic evaluation of treated and untreated skin 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.

In the probe study there was no dermal irritation noted in either the male or female rabbit administered 500 and 1000 mg/kg/day clopyralid for 4 days, respectively. In addition, there were no observations noted during daily observations indicative of a treatment-related response.

In the 21-Day Dermal Study slight erythema (score 1) was observed in test day 10 in two male rabbits belonging to the intermediate and high dose groups, respectively, and one female rabbit in test day 3, belonging to the intermediate dose group (Table 14) . Histopathologic skin lesions at the dermal test site were present in some rabbits from the controls and all the dose level groups. A minimal degree of epidermal hyperplasia, multifocal in occurance, was also observed in several control and treated animals. The dermal microscopic changes noted is considered to be caused by the friction caused by the wrapping and unwrapping the jackets used to hold the test material in place. A treatment-related epidermal hyperplasia of greater extent than observed in control rabbits, was noted in 1/5 males and 2/5 females treated with 100 mg/kg/day, 3/5 males and 1/5 females treated with 500 mg/ kg/day, and 5/5 males and 5/5 females treated with 1000 mg/kg/day (Table 15). It can be assumed that repeated exposure has caused this hyperplasia. No significant gross lesions were found at necropsy.

Also occasional histopathologic lesions were observed in the untreated skin adjacent to the dermal test site. The findings consisted of degeneration of muscle fibers in the cutaneous trunci muscle, epidermal hyperplasia and dermal inflammation. All observations in the untreated skin were interpreted to be caused by repeated contact or compression from the caudal (posterior) margin of the elastic jacket.

Table 14 Erythema observed in rabbits (5 rabbits examined)

	Males*				Females*			
Test day	**3**	**10**	**17**	**21**	**3**	**10**	**17**	**21**
Dose mg/kg bw/day								
Control	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0
100	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0
500	0/0/0/0	**1/0/0/0**	0/0/0/0	0/0/0/0	**1/0/0/0**	0/0/0/0	0/0/0/0	0/0/0/0
1000	0/0/0/0	**1/0/0/0**	**1/0/0/0**	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0

*Results refer to: very slight erythema/well defined erythema/moderate-severe erythema/severe erythema to slight eschar formation

Table 15. Selected histopathological findings

	Males				Females			
Dose mg/kg bw/day	**Control**	**100**	**500**	**1000**	**Control**	**100**	**500**	**1000**
Skin (test site)								
Diffuse epidermal hyperplasia								
-very slight	0	0	3	5	0	2	1	3
-slight	0	1	0	0	0	0	0	2
Inflammation of dermis (multifocal)								
-very slight	0	0	4	1	0	1	1	1
-slight	0	1	1	2	0	0	0	2
-severe	0	1	0	0	0	0	0	0
Necrosis on epidermis	0	1	0	0	0	0	0	0

10.4.2 Comparison with the CLP criteria

Under the conditions of the study Anonympous 1987, the test material was considered not irritating to the skin of rabbit. Average score (24-72 h) for each rabbit was 0 for erythema and 0 for oedema. Also in the probe study (Anonymous 1990) there was no dermal irritation noted.

In the 21-day dermal study (Anonymous 1990) slight erythema (score 1) was observed in test day 3 in one female rabbit belonging to the intermediate dose group, in test day 10 in one male rabbit belonging to the intermediate dose group, and in test days 10 and 17 in one male rabbit belonging to the high dose group. At the end of study the histopathological skin lesions showed mild epidermal hyperplasia.

The CLP Regulation 1272/2008 defines criteria for classification as Skin Irrit. 2 when a substance produces reversible damage to the skin following its application for up to 4 hours. The major critera for the irritation category is that at least 2 of 3 tested animals have a mean score of $\geq 2{,}3$ and $\leq 4{,}0$. Skin Irrit. category 2 shuld be considered, if inflammation persists to the end of the observation period normally 14 days in at least 2 animals, particularly taking into account alopecia (limited area), hyperkeratosis, hyperplasia, and scaling. Based on the animal results none of the criteria is fulfilled. Also findings from the skin sensitisation tests (RAR B.6.2.6 Skin sensitisation) support the conclusion. However based on the 21-day dermal study the EUH066 statement - 'Repeated exposure may cause skin dryness or cracking' should be considered, as slight erythema (score 1) and epidermal hyperplasia was observed in repeated exposure.

10.4.3 Conclusion on classification and labelling for skin corrosion/irritation

The data available indicates that clopyralid does not require classification as skin irritant according to the CLP regulation (EC) No 1272/2008. Instead the EUH066 statement - 'Repeated exposure may cause skin dryness or cracking' should be considered.

10.5 Serious eye damage/eye irritation

Not assessed in this dosser.

10.6 Respiratory sensitisation

Not assessed in this dossier

10.7 Skin sensitisation

Not assessed in this dossier

10.8 Germ cell mutagenicity

Not assessed in this dossier

10.9 Carcinogenicity

Not assessed in this dossier

10.10 Reproductive toxicity

10.10.1 Adverse effects on sexual function and fertility

Table 16 Summary table of animal studies on adverse effects on sexual function and fertility

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
Two-generation study, Rat, Fischer 344, 30 animals/ group, non-OECD guideline, GLP status unclear	0, 150, 500, 1500 mg/kg bw/day Dietary Corrected dose levels: 82.5, 275, 825 mg/kg bw/day 96.7% test substance	P: Decreased body weights and food consumption in P1 rats of both sexes and increased liver weights in P1 female rats at 1500 mg/kg bw/day. F: NOAEL 275 mg/kg bw/day (reduction in organ weights, reduced terminal body weight), decreased body weights and food consumption in F1 rats of both sexes and increased liver weights in F1 female rats at 1500 mg/kg bw/day. Reproductive performance: NOAEL 825 mg/kg bw/day (no effects) 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. Decreased body weight and increased liver weight in F1a and F1b pups during lactation at 1500 mg/kg bw/day.	DRAR B.6.6.1 1983 1984 (supplementary histopathological examination)

In a two generation reproduction study, the test substance (96.7% pure) was administered in the diet to male and female Fischer 344 rats (30 rats/sex/concentration for both the P1 and F1 generations). Concentrations

were 0, 150, 500, and 1500 mg of the test substance/kg bw/day. Concentrations were reduced by 1/6 to 1/3 during mating, gestation, and lactation for F1b mating. During lactation periods for F2, dietary concentrations were reduced by 1/2 or 1/3, starting on Day 7 and day 14 of lactation, respectively, until weaning. The P1 rats were bred within their treatment groups to produce F1a and F1b litters after 101 days on test. Selected F1b rats were bred within their respective treatment groups to produce F2a and F2b litters after at least 120 days on test. Parameters evaluated included body weight gain, food consumption (part of the study period), clinical signs, clinical pathology, reproductive indices, litter and pup parameters, organ weights, and gross and microscopic pathology.

Supplemental histopathology

Reproductive organs (testicle, epididymis, seminal vesicle, coagulating gland, prostate, ovary, oviduct, uterus, cervix and vagina) from 10 randomly selected adults per sex in control and high dose groups from P1 and F1 generations were examined for histopathological alterations. Major organs from 10 randomly selected F2b weanlings per sex in control and top dose groups were also examined microscopically. Altogether 44 tissues were examined, including testicle, epididymis, seminal vesicle, coagulating gland, prostate, ovary, oviduct, uterus, cervix and mammary gland. Tissues from animals administered 150 or 500 mg/kg/day of the test substance were not examined microscopically since no target organs were identified in animals from the top dose group.

P1 AND F1 PARENTAL RATS

1. General observations

No clinical signs were observed in any P1 or F1 adults during the treatment period. In P1 generation a female rat at mid dose group died (drowned with litter) and another female from the mid dose group was sacrificed after weaning of F1b due to a tumour. Two F1 females (1 from control and 1 from high dose group) died spontaneously during parturition of the F2b litters. The female from high dose group died prior to giving birth on study day 218.

A slight but statistically significantly different decrease in body weights of P1 males administered 1500 mg of the test substance/kg bw/day was observed beginning with 69 days on test, and was generally consistent throughout the remainder of the study. The change to control was however less than 10%. Body weights of P1 female rats administered 1500 mg of the test substance/kg bw/day also exhibited a statistically significant decrease during most of the premating treatment period. Mean body weights of P1 females from the 500 mg/kg bw/day dose group were also statistically depressed during some of this time interval (days 13-41). In both cases the change to control was less than 10%.

Body weights of the 500 and 1500 mg/kg bw/day P1 dams were statistically significantly decreased in comparison to control dams during the F1a lactation period. Occasionally statistically significant decreases were also observed in dams of 150 mg/kg bw/day group. The changes were however within 10% compared to control.

Body weights of the 1500 mg/kg bw/day P1 dams were occasionally statistically significantly decreased in comparison to control dams (<10% to control) during the F1b lactation period; however, this was not observed in F1 females during the lactation period of the F2b litters. The administration of 1500 mg of the test substance/kg bw/day also resulted in a statistically significant decrease in overall body weight gain of P1 male and female adults.

Statistically significant decreases in body weight were observed in F1 adult males at the 1500 mg/kg bw/day dose level, and were consistent throughout most of the exposure period. Sporadic reductions in body weight were also observed in F1 adult males at the 150 and 500 mg/kg bw/day dose levels. All these changes were still < 10% compared to control. Statistically significant decreases in mean body weights were observed in adult F1 female rats at all dose levels during the last half of the premating exposure period (days -1 to 118). The observed changes were < 10% to control.

Food consumption for the P1 male rats in all three treatment groups tended to be slightly decreased in comparison to controls, particularly during the last 60 days of the premating exposure period (data only for premating period provided; study days -5 to 98). Statistically significant decreases were observed for the P1

males in the 150 and 1500 mg of the test substance/kg bw/day groups (for both dose groups change <10% compared to control), whereas the food consumption of males from the 500 mg of the test substance/kg bw/day treatment group was only sporadically decreased in comparison to controls. No clear dose-response in depression of food consumption was observed, however, the frequency and magnitude of decreases appeared to be greater in the highest dose level (1500 mg/kg bw/day) than in other treatment groups during part of the observation period. There is no data available for food consumption for later study periods.

Mean food consumption of P1 female rats from study day -5 to day 98 (premating) was sporadically decreased in all treatment groups when compared to controls, but were determined not to be of toxicological significance. All observed decreases were <10% compared to control. There is no data available for food consumption for later study periods.

No adverse effects were noted on food consumption (reported for days 0-119 for both F1 adult males and females) in adult F1 rats at any dose. There were some statistically significant decreases compared to control in all dose groups on males and on mid dose females but all changes were <10% to control. There is no data available for food consumption for later study periods.

Reproductive parameters

There were no effects on fertility indices, gestation days, gestation indices, gestation survival indices, total number of live pups per litter on Day 1 of lactation, pup survival indices on Days 1, 4, 7, 14, 21, and 28 of lactation, or sex ratio of pups at 28 days of age when compared to concurrent control group. Overall, the fertility index in P1 generation for F1a and F1b was >90%. In F1 generation fertility index was in general <90% for F2a and F2b. The gestation length in P1 generation for F1a and F1b was 26-28 days for treated groups. The gestation length in F1 generation for F2a and F2b was 25 days for treated groups. In F2a and F2b litters there were runts and smaller size observed.

Gross pathology, organ weights, and histopathology

Terminal fasted body weights and absolute and relative organ weights of the P1 males from all treatment groups were comparable to those of controls, however, a trend towards a decrease in the terminal fasted body weights of males fed 1500 mg/kg bw/day was observed. A statistically significant decrease in terminal fasted body weights was observed in P1 females in the 1500 mg/kg bw/day group (<10% to control). No adverse effects were observed in the terminal fasted body weights of P1 females in the 150 or 500 mg/kg bw/day groups.

No adverse effects on absolute or relative organ weights in P1 and F1 rats of either sex were observed.

No adverse gross pathological observations were made in any male or female P1 rats from any treatment groups, except slight hyperkeratotic changes in the nonglandular mucosa of the stomach from 1/30 male at high dose group. Also, very slight inflammation in stomach (chronic, wall, focal) was observed in 1/30 P1 male at high dose group. A single observation of slight atrophy in testes (generalized, unilateral, diffuse) was seen in P1 high dose male. One P1 female of low dose group was observed with ulcer in stomach (perforated, nonglandular wall, focal).

Small lesions were observed in the forestomach of two out of thirty adult F1 males in the 1500 mg/kg bw/day test group. Light microscopic evaluation of these nodules determined that the lesions were present in the squamous epithelium at the base of the cranial face of the limiting ridge, consisting of mucosal invaginations into the gastric wall which communicated freely with the gastric lumen. These diverticulum-like structures were lined by a keratinising squamous epithelium and their centres were filled with concentrically laminated accumulations of keratin. The stomach from one animal contained one of these nodules, while the stomach from the other contained at least two. The limiting ridge in both stomachs was covered by an irregularly undulating mucosa which was hyperkeratotic and thickened. These lesions were similar in nature to those observed in a previous dietary toxicity study following administration of 2500 mg of the test substance/kg bw/day to Fischer-344 rats. One F1 adult female (high dose) was observed with very slight haemorrhage in the stomach in gross pathological examination.

In a supplemental histopathological examination, reproductive organs from ten randomly selected adults/sex in the control and high dose groups from the P1 and F1 generations, in addition to major organs from ten

randomly selected F2b weanlings/sex in the control and high dose groups, were examined microscopically. No histopathologic changes were noted in P1 and Fl adults or F2b weanlings that were attributed to treatment with 1500 mg of the test substance/kg bw/day.

Table 17 Two-generation reproduction study: Reproductive indices for P_1 and F_1 generations

	0 mg/kg bw/day	**150 mg/kg bw/day**	**500 mg/kg bw/day**	**1500 mg/kg bw/day**
P_1 generation (F_{1a} litters):				
No. females	30	30	30	30
Fertility index (%)[a]	93 (28/30)	93 (28/30)	100 (30/30)	93 (28/30)
Gestation length (mean days ± standard deviation)	28 ± 6	27 ± 5	28 ± 6	27 ± 5
Gestation index (%)[b]	100 (28/28)	100 (28/28)	100 (30/30)	100 (28/28)
Gestation survival index (%)[c]	100 (281/281)	99 (282/284)	99 (275/277)	99 (289/291)
Number of live pups/litter on Day 1 (mean ± standard deviation)	10 ± 3	10 ± 3	9 ± 3	10 ± 2
1-Day survival index (%) (% liveborn pups survived for 1 days)	99 (280/281)	99 (281/282)	100 (275/275)	100 (289/289)
28-Day survival index (%) (% liveborn pups survived until day 28 of lactation)	92 (198/214)	86 (179/209)	95 (192/203[d])	98 (217/222)
Sex ratio on Day 28 (%male : %female)	51:49	50:50	47:53	52:48
Anophthalmia[e] (% affected (number affected))				
pups	0	0.5(1)	0.5(1)	0
litters	0	3.6(1)	3.3(1)	0
Total pups examined	281	284	277	291
Total litters examined	28	28	30	28
P_1 generation (F_{1b} litters):				
No. females	30	30	29	30
Fertility index (%)[a]	97 (29/30)	80 (24/30)	97 (28/29)	97 (29/30)
Gestation length (mean days ± standard deviation)	27 ± 5	27 ± 5	26 ± 5	27 ± 5
Gestation index (%)[b]	100 (29/29)	100 (24/24)	100 (28/28)	100 (29/29)
Gestation survival index (%)[c]	100 (278/278)	99 (247/249)	98 (252/257)	99 (269/271)
Number of live pups/litter on Day 1 (mean ± standard deviation)	10 ± 3	10 ± 2	9 ± 4	9 ± 3
1-Day survival index (%) (% liveborn pups survived for 1 days)	99 (277/278)	100 (247/247)	98 (247/252)	100 (269/269)
28-Day survival index (%) (% liveborn pups survived until day 28 of lactation)	99 (206/208)	99 (183/185)	99 (189/190)	99 (207/208)
Sex ratio on Day 28 (%male : %female)	47:53	52:48	51:49	51:49
F_1 generation (F_{2a} litters):				
No. females	30	30	30	30
Fertility index (%)[a]	77 (23/30)	77 (23/30)	73 (22/30)	80 (24/30)
Gestation length (mean days ± standard deviation)	25 ± 2	25 ± 2	25 ± 2	25 ± 2
Gestation index (%)[b]	100 (23/23)	100 (23/23)	100 (22/22)	100 (24/24)
Gestation survival index (%)[c]	99	98	100	99

	(234/237)	(236/241)	(206/206)	(235/236)
Number of live pups/litter on Day 1	10 ± 3	10 ± 3	9 ± 3	10 ± 3
1-Day survival index (%) (% liveborn pups survived for 1 days)	99 (233/234)	92 (218/236)	100 (205/206)	98 (235/236)
28-Day survival index (%) (% liveborn pups survived until day 28 of lactation)	98 (166/169)	99 (163/165)	99 (153/155)	96 (174/181)
Sex ratio on Day 28 (%male : %female)	50:50	48:52	52:48	50:50
External alterations (% affected (number affected)) Microphthalmia				
pups	0.6(1)	0	0.6(1)	1.7(3)
litters	4.5(1)	0	4.5(1)	4.2(1)
Runt				
pups	0	0.4(1)	0	0.4(1)
litters	0	4.3(1)	0	4.2(1)
Smaller size				
pups	1.3(3)	0.8(2)	1.0(2)	3.4(8)
litters	13.0(3)	8.7(2)	9.1(2)	20.8(5)
F_1 generation (F_{2b} litters):				
No. females	30	30	30	30
Fertility index (%)[a]	87 (26/30)	87 (26/30)	83 (25/30)	93 (28/39)
Gestation length (mean days ± standard deviation)	26 ± 2	25 ± 2	25 ± 2	25 ± 2
Gestation index (%)[b]	96 (25/26)	96 (25/26)	100 (25/25)	100 (27/27)
Gestation survival index (%)[c]	95 (249/262)	98 (212/216)	100 (258/259)	99 (number non-readable)
Number of live pups/litter on Day 1	10 ± 4	8 ± 3	10 ± 3	10 ± 3
1-Day survival index (%) (% liveborn pups survived for 1 days)	98 (245/249)	100 (211/212)	98 (254/258)	99 (254/267)
28-Day survival index (%) (% liveborn pups survived until day 28 of lactation)	100 (174/174)	100 (174/174)	100 (189/189)	100 (204/205)
Sex ratio on Day 28 (%male : %female)	52:48	48:52	48:52	46:54
Microphthalmia (% affected (number affected)				
pups	0	0	0.5(1)	0
litters	0	0	4.2(1)	0
Anophthalmia[e] (% affected (number affected)				
pups	0.6(1)	0.6(1)	0	0
litters	4.2(1)	4.0(1)	0	0
Imperforate Anal/urogenital opening (% affected (number affected)				
pups	0	0	0.4(1)	0
litters	0	0	4.0(1)	0
Runt (% affected (number affected)				
pups	0	0.5(1)	0.4(1)	0
litters	0	3.8(1)	4.0(1)	0
Smaller size (% affected (number affected)				
pups	2.0(5)	0.5(1)	1.2(3)	1.5(4)
litters	11.5(3)	3.8(1)	12.0(3)	14.8(4)

[a]Fertility index= no. females delivering a litter as a percentage of the total number of females placed with a male.
[b]Gestation index= No. females delivering a live litter expressed as percentage of the total number of females delivering litters.

[c] % of newborn pups that were alive at birth
[d] number 203 due to accidental death of 11 pups on days 15 and 20 of lactation by drowning
[e] % of pups and litters affected calculated using number of pups and litters available for examination after culling.

Table 18 Two-generation reproduction study: Incidences of microscopic effects, P_1 parental rats

	0 mg/kg bw/day	**1500 mg/kg bw/day**
Number of animals/tissues examined/group:	**10**	**10**
Males:		
Testicle		
Within normal limits	8[a]	10
Mineralisation, tubule(s), focal	2	0
Epididymis		
Within normal limits	10	10
Seminal vesicle		
Within normal limits	10	10
Coagulating gland		
Within normal limits	10	10
Prostate		
Within normal limits	7	6
Hyperplasia, epithelial, focal	2	3
Inflammation, suppurative, slight	2	2
Females:		
Ovary		
Within normal limits	9	9
Cyst, Corpus Luteum	1	1
Oviduct		
Within normal limits	10	10
Uterus		
Within normal limits	2	1
Dilatation, Lumen (Normal cyclic change)	2	2
Pigment-Golden Brown (Hematogenous), mesometrium (Site of previous implantation)	7	9
Vagina		
Within normal limits	10	10
Cervix		
Number of tissues examined	9	8
Within normal limits	9	8

[a] Number of animals with the specified observation for which the specified tissue was examined.

Table 19 Two-generation reproduction study: Incidences of microscopic effects, F_1 parental rats

	0 mg/kg bw/day	**1500 mg/kg bw/day**
Number of animals/tissues examined/group:	**10**	**10**
Males:		
Testicle		
Within normal limits	8[a]	10
Atrophy, tubule(s), focal	1	0
Mineralisation, tubule(s), focal	2	0
Epididymis		
Within normal limits	10	10
Seminal vesicle		
Within normal limits	10	10
Coagulating gland		
Within normal limits	10	10
Prostate		
Within normal limits	4	5
Hyperplasia, epithelial, focal	2	3
Inflammation, suppurative, slight	6	4
Females:		
Ovary		
Within normal limits	10	10
Oviduct		
Within normal limits	10	10
Uterus		
Within normal limits	2	3
Pigment-Golden Brown (Hematogenous), mesometrium (Site of previous implantation)	8	7
Endometrial Stromal Polyp, Benign, primary	0	1
Vagina		
Within normal limits	10	10
Cervix		
Number of tissues examined	9	10
Within normal limits	9	10

[a] Number of animals with the specified observation for which the specified tissue was examined.

Table 20 Two-generation reproduction study: Incidences of microscopic effects, F_{2b} weanlings

	0 mg/kg bw/day	**1500 mg/kg bw/day**
Number of animals/tissues examined/group:	**10**	**10**
Males:		
Testicle		
Within normal limits	10[a]	10
Epididymis		
Within normal limits	10	10
Seminal vesicle		
Within normal limits	9[b]	10
Coagulating gland		

Within normal limits	9[b]	9[b]
Prostate		
Within normal limits	10	10
Kidney		
Within normal limits	10	10
Lungs		
Within normal limits	2	0
Aggregates of mononuclear (predominantly lymphoid) cells, subpleural	2	3
Aspirated blood (secondary to decapitation)	6	10
Mediastinal tissue		
Within normal limits	10	9
Hemorraghe (secondary to decapitation)	0	1
Mammary gland		
No of tissues examined	8	9
Within normal limits	8	9
Females:		
Ovary		
Within normal limits	8[b]	10
Cyst, unilateral	1	0
Oviduct		
Within normal limits	9[b]	10
Uterus		
Within normal limits	10	10
Cervix		
Within normal limits	9[b]	10
Kidney		
Within normal limits	9	9
Mineralization, corticomedullary junction, focal (very slight)	0	1
Atrophy, tubules, focal	1	0
Lungs		
Within normal limits	0	0
Aggregates of mononuclear (predominantly lymphoid) cells, subpleural	5	2
Aspirated blood (secondary to decapitation)	10	10
Mammary gland		
Within normal limits	10	10

[a] Number of animals with the specified observation for which the specified tissue was examined..

[b] Tissues from 9 animals were examined, not 10.

F_1 AND F_2 LITTER AND PUP DATA

General observations

The body weights of F_{1a} pups from the 1500 mg/kg bw/day group were comparable to controls through Day 21 postpartum. However, by Day 28 a statistically significant decrease was observed in mean body weights of pups in the 1500 mg/kg bw/day group.

Mean body weights of the combined male and female F_{1b} male and female pups were comparable to controls at all three treatment levels throughout the entire 28-day lactation period. However, when separated by sex on Day 28 postpartum, the male pups in the 1500 mg/kg bw/day group had statistically lower body weights in comparison to control pups of the same sex.

Litter size and pup survival

No adverse effects were observed for mean litter size at birth and throughout lactation as well as pup survival.

Gross pathology, organ weights, histopathology

No test substance-related gross pathological observations were made in F1a, F2a, or F2b weanlings at any treatment level. In F1b females at high dose there was a single observation in liver (hernia, left middle lobe, focal , slight) and in eye (cloudy, cornea, bilateral, focal, very slight). In F1b males at high dose there were single observations in lymph nodes (moderate congestion, renal, unilateral, diffuse and moderate increased size, renal, unilateral diffuse).

Statistically significant increases in relative liver weights were noted in both male and female F1a weanlings and male F1b weanlings from the 1500 mg/kg bw/day group. No test substance-related adverse effects were noted in absolute or relative organ weights of F2a or F2b weanlings, except an incidental finding of statistically significantly decreased absolute heart weight on low dose F2a males.

Table 21 Two-generation reproduction study: F_1 generation litter weights

Parameter	0 mg/kg bw/day	150 mg/kg bw/day	500 mg/kg bw/day	1500 mg/kg bw/day
F_{1a} (n = 28-30 litters/dose)				
Postpartum Day 1 (g)	5.6 ± 0.5[a]	5.4 ± 0.4	5.6 ± 0.6	5.4 ± 0.4
Postpartum Day 4 (g)	7.6 ± 1.0	7.2 ± 1.1 (95%)	7.7 ± 1.3	7.7 ± 0.9
Postpartum Day 7 (g)	10.3 ± 1.7	9.7 ± 1.8 (94%)	10.8 ± 1.5	10.7 ± 1.4
Postpartum Day 14 (g)	19.2 ± 3.5	17.6 ± 3.7 (92%)	19.4 ± 2.1	19.5 ± 2.9
Postpartum Day 21 (g)	30.3 ± 5.0	27.8 ± 5.2 (92%)	30.1 ± 3.3	28.4 ± 4.9 (94%)
Postpartum Day 28 (g)	55.8 ± 6.7	52.0 ± 9.7	55.7 ± 5.2	50.7 ± 5.5[b] (91%)
Males—Postpartum Day 28 (g)	57.8 ± 8.4	54.8 ± 6.7	58.0 ± 5.2	52.2 ± 6.3[b] (90%)
Females—Postpartum Day 28 (g)	53.6 ± 6.7	51.3 ± 9.4	53.5 ± 5.5	49.1 ± 4.5 (92%)
F_{1b} (n = 24-29 litters/dose)				
Postpartum Day 1 (g)	5.4 ± 0.5	5.3 ± 0.3	5.5 ± 0.5	5.6 ± 0.4
Postpartum Day 4 (g)	8.2 ± 1.0	8.2 ± 0.7	8.2 ± 1.3	8.5 ± 0.9
Postpartum Day 7 (g)	11.8 ± 1.4	12.0 ± 0.9	11.8 ± 1.9	12.0 ± 1.2
Postpartum Day 14 (g)	22.1 ± 2.8	22.5 ± 1.4	21.6 ± 3.2	21.8 ± 2.2 (99%)
Postpartum Day 21 (g)	33.2 ± 3.8	33.5 ± 2.1	32.2 ± 4.9	32.3 ± 3.1 (97%)
Postpartum Day 28 (g)	58.0 ± 6.9	59.0 ± 3.0	55.8 ± 9.3	55.0 ± 6.7 (95%)
Males—Postpartum Day 28 (g)	62.0 ± 4.2	61.8 ± 3.6	59.6 ± 7.0	56.8 ± 7.4[b] (92%)
Females—Postpartum Day 28 (g)	56.0 ± 7.0	56.1 ± 3.2	53.4 ± 8.7	53.2 ± 6.4 (95%)

[a] Values are means ± standard deviation weight of pups/litter.

[b] Significantly different from control by the appropriate statistical test.

Values in parentheses are % of control values.

Table 22 Two-generation reproduction study: F_2 generation litter weights

Parameter	0 mg/kg bw/day	150 mg/kg bw/day	500 mg/kg bw/day	1500 mg/kg bw/day
F_{2a} (n = 22-24 litters/dose)				
Postpartum Day 1 (g)	5.2 ± 0.4[a]	5.2 ± 0.4	5.4 ± 0.4	5.3 ± 0.6
Postpartum Day 4 (g)	7.5 ± 0.7	7.7 ± 0.6	7.8 ± 0.6	7.7 ± 0.8
Postpartum Day 7 (g)	10.6 ± 1.0	10.8 ± 0.9	11.1 ± 0.6	10.8 ± 1.2
Postpartum Day 14 (g)	19.4 ± 1.8	19.4 ± 1.5	19.7 ± 1.3	19.1 ± 2.7 (98%)
Postpartum Day 21 (g)	29.7 ± 2.3	29.6 ± 2.3	30.1 ± 1.4	28.8 ± 3.6 (97%)
Postpartum Day 28 (g)	51.9 ± 4.7	50.5 ± 5.4	52.1 ± 3.0	49.2 ± 7.6 (95%)
Males—Postpartum Day 28 (g)	53.3 ± 5.6	51.5 ± 6.5	53.4 ± 4.2	50.8 ± 9.1 (95%)
Females—Postpartum Day 28 (g)	50.4 ± 4.7	50.1 ± 4.8	50.2 ± 3.0	48.5 ± 6.4 (96%)
F_{2b} (n = 25-27 litters/dose)				
Postpartum Day 1 (g)	5.5 ± 0.6	5.9 ± 0.7	5.6 ± 0.5	5.7 ± 0.5
Postpartum Day 4 (g)	8.2 ± 1.0	8.7 ± 1.3	8.1 ± 0.7	8.5 ± 1.0
Postpartum Day 7 (g)	11.8 ± 1.5	12.5 ± 1.8	11.8 ± 1.0	12.0 ± 1.4
Postpartum Day 14 (g)	22.2 ± 2.1	21.8 ± 2.8	21.7 ± 1.4	22.6 ± 1.8
Postpartum Day 21 (g)	34.2 ± 3.6	34.0 ± 4.3	33.1 ± 2.8	33.9 ± 3.1 (99%)
Postpartum Day 28 (g)	61.4 ± 6.2	61.1 ± 7.0	59.1 ± 5.5 (96%)	59.8 ± 5.4 (97%)
Males—Postpartum Day 28 (g)	63.8 ± 7.2	64.3 ± 8.2	61.7 ± 6.1 (97%)	62.4 ± 6.1 (98%)
Females—Postpartum Day 28 (g)	58.8 ± 5.8	58.4 ± 6.7	56.8 ± 5.1 (97%)	57.3 ± 4.8 (97%)

[a] Values are means ± standard deviation weight of pups/litter.

Values in parentheses are % of control values.

Table 23 Two-generation reproduction study: Organ weights in F_1 generation weanlings (n = 10)

Parameter	0 mg/kg bw/day	150 mg/kg bw/day	500 mg/kg bw/day	1500 mg/kg bw/day
F_{1a} males				
Absolute liver weight (g)	6.117 ± 0.832[a]	5.940 ± 0.840	5.610 ± 1.443	6.310 ± 0.964
Relative liver weight (g/100)	4.365 ± 0.242	4.329 ± 0.188	4.375 ± 0.157	4.778 ± 0.219[b] (109%)
Absolute testes weight (g)	1.687 ± 0.427	1.793 ± 0.275	1.458 ± 0.593 (86%)	1.592 ± 0.301 (94%)
Relative testes weight (g/100)	1.187 ± 0.184	1.307 ± 0.122 (110%)	1.104 ± 0.242	1.199 ± 0.102
F_{1a} females				
Absolute liver weight (g)	4.846 ± 0.648	4.769 ± 0.663	4.892 ± 0.507	4.736 ± 0.754
Relative liver weight (g/100)	4.234 ± 0.259	4.160 ± 0.207	4.249 ± 0.259	4.543 ± 0.135[b] (107%)
F_{1b} males				
Absolute liver weight (g)	6.047 ± 1.350	6.007 ± 1.292	6.127 ± 1.288[c]	5.670 ± 1.185
Relative liver weight (g/100)	4.474 ± 0.100	4.621 ± 1.223	4.527 ± 0.415	4.728 ± 0.131[b] (106%)
Terminal body weight (g)	135.1 ± 29.9	133.3 ± 26.3	136.2 ± 30.3	119.9 ± 24.7 (89%)
Absolute heart weight (g)	0.463 ± 0.084	0.454 ± 0.083	0.465 ± 0.074	0.414 ± 0.066 (89%)
Absolute kidney weight (g)	1.234 ± 0.237	1.177 ± 0.214	1.236 ± 0.181	1.098 ± 0.197 (89%)
Absolute testes weight (g)	1.460 ± 0.525	1.501 ± 0.448	1.608 ± 0.528 (110%)	1.356 ± 0.527
Relative testes weight (g/100)	1.045 ± 0.207	1.143 ± 0.327	1.158 ± 0.230 (111%)	1.094 ± 0.260
F_{1b} females				
Absolute liver weight (g)	4.872 ± 0.804	4.643 ± 0.591	4.611 ± 0.646[d] (95%)	4.711 ± 0.818
Relative liver weight (g/100)	4.369 ± 0.222	4.201 ± 0.519	4.413 ± 0.414	4.399 ± 0.248
Absolute ovaries weight (g)	0.067 ±0.009	0.063 ± 0.012	0.064 ± 0.013	0.058 ± 0.013 (87%)
Relative ovaries weight (g/100)	0.061 ± 0.007	0.057 ± 0.010	0.061 ± 0.008	0.054 ± 0.006 (89%)

[a] Values are means ± standard deviation weight of pups/litter.

[b] Significantly different from control by the appropriate statistical test.

[c] n = 9

[d] n = 11

CONCLUSION

The NOAELs in the two-generation reproduction study in rats were as follows:

Parental toxicity:	500 mg/kg bw/day, based on decreased body weights and food consumption in P_1 and F_1 rats of both sexes and increased liver weights in P_1 and F_1 female rats at 1500 mg/kg bw/day.
Reproduction and fertility:	Greater than 1500 mg/kg bw/day, based on the absence of any adverse effects on the ability of P_1 or F_1 animals to mate, reproduce, or nurse their offspring at 1500 mg/kg bw/day, the highest dose level tested.
Pup growth and development:	500 mg/kg bw/day, based on decreased body weight and in increased liver weight in F_{1a} and F_{1b} pups during lactation at 1500 mg/kg bw/day.

10.10.2 Short summary and overall relevance of the provided information on adverse effects on sexual function and fertility

The reproduction toxicity of clopyralid was investigated in one two-generation reproduction study, one supplementary histopathology study, and in three prenatal toxicity studies.

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 as described in OECD 416. 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. 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 this study, ED related parameters e.g. sperm counts, oestrus cycle, sexual maturation as according to OECD 416 (2001) were not measured. It should be noted, that microphthalmia and anophthalmia were observed in this study.

10.10.3 Comparison with the CLP criteria

According to the CLP Regulation (1272/2008):

3.7.1.3. Adverse effects on sexual function and fertility

Any effect of substances that has the potential to interfere with sexual function and fertility. This includes, but is not limited to, alterations to the female and male reproductive system, adverse effects on onset of puberty, gamete production and transport, reproductive cycle normality, sexual behaviour, fertility, parturition, pregnancy outcomes, premature reproductive senescence, or modifications in other functions that are dependent on the integrity of the reproductive systems.

Table 3.7.1(a)

CATEGORY 1

Known or presumed human reproductive toxicant Substances are classified in Category 1 for reproductive toxicity when they are known to have produced an adverse effect on sexual function and fertility, or on development in humans or when there is evidence from animal studies, possibly supplemented with other information, to provide a strong presumption that the substance has the capacity to interfere with reproduction in humans. The classification of a substance is further distinguished on the basis of whether the evidence for classification is primarily from human data (Category 1A) or from animal data (Category 1B).

Category 1A

"Known human reproductive toxicant The classification of a substance in Category 1A is largely based on evidence from humans."

No classification for fertility is proposed.

Category 1B

"Presumed human reproductive toxicant The classification of a substance in Category 1B is largely based on data from animal studies. Such data shall provide clear evidence of an adverse effect on sexual function and fertility or on development in the absence of other toxic effects, or if occurring together with other toxic effects the adverse effect on reproduction is considered not to be a secondary non-specific consequence of other toxic effects. However, when there is mechanistic information that raises doubt about the relevance of the effect for humans, classification in Category 2 may be more appropriate."

No classification for fertility is proposed.

Category 2

"*Substances are classified in Category 2 for reproductive toxicity when there is some evidence from humans or experimental animals, possibly supplemented with other information, of an adverse effect on sexual function and fertility, or on development, and where the evidence is not sufficiently convincing to place the substance in Category 1. If deficiencies in the study make the quality of evidence less convincing, Category 2 could be the more appropriate classification. Such effects shall have been observed in the absence of other toxic effects, or if occurring together with other toxic effects the adverse effect on reproduction is considered not to be a secondary non-specific consequence of the other toxic effects.*"

The classification regarding reproductive effects could not be reliably concluded because of inconclusive data and the deviations in the 2-generation study.

No classification for fertility is proposed.

Developmental toxicity

Table 24 Summary table of animal studies on adverse effects on development

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference**
Predominantly in compliance with OECD 414	0, 15, 75 and 250 mg/kg	At 250 mg/kg three deaths, significant reductions in body weight, 75 and 250 mg/kg/d reduced liver weight	dRAR B.6.6.2., 1981

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
(2001), GLP status unknown.[a]	bw/day, F-344 rat	At 250 mg/kg bw/day one litter had three foetuses with polydactyly and another litter had one foetus with a hemivertebra	
The study was performed predominantly according to OECD guideline 414 (2001), GLP.	0, 50, 110 or 250 mg/kg bw/day, NZW rabbit	250 mg/kg bw/day: signs of severe maternal toxicity, maternal body weight and body weight gain was significantly depressed (on average 525 g), multifocal erosions and/or ulcers in the gastric mucosa were observed in dams. Lower foetal weights and hydrocephaly were observed at 250 mg/kg bw/day	dRAR B.6.6.2., 1990
The study partially complies with Directive 87/302/EEC ”teratogenicity study – rodent - nonrodent”, pre-GLP.	0, 110 or 250 mg/kg bw/day, NZW rabbit	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	dRAR B.6.6.2., 1974

[a] Since no validated analytical methods were reported for the batch used in this study, the reliability of the study has been questioned in the Renewal assessment of clopyralide (Commission Implementing Regulation (EU) No 844/2012).

Developmental toxicity study in New Zealand White rabbits (dRAR B.6.6.2., 1981)

Range-finding studies

In a range-finding study, groups of 3 adult female New Zealand White rabbits were given doses of 0, 350 or 750 mg/kg/ day for 13 consecutive days in order to more dearly establish a maternally toxic dose (Hanley et al., 1990a). Dose levels of 500 and 750mg/kg/day produced excessive toxicity resulting in severe weight loss and 100% mortality or morbidity in all rabbits within the first 9 days of dosing. Necropsy of these animals revealed erosions in the stomach. Rabbits given 350 mg/kg/day survived the dosing regimen with some degree of weight loss, and one animal had stomach erosions at necropsy.

In another teratology probe study, groups of 6 - 7 inseminated female New Zealand White rabbits were administered clopyralid in corn oil at dose levels of 0, 110, 250 or 750 mg/kg/day on Days 7 -19 of gestation (Hanley et al., 1990b). Administration of 350 mg/kg/day produced stomach erosions, decreased body weights and significant mortality (60%). No clinical effects were detected at dose levels of 110or 250 mg/kg/ day, however, necropsy revealed treatment-related stomach erosions in all rabbits given 250 mg/kg/day. There were no adverse effects observed at a dose level of 110 mg/kg/day.

Test guideline and GLP

The study is predominantly in compliance with OECD 414 (2001) but the GLP status is unknown.

Material and Methods

Initially, groups of 29 - 30 (treated groups) or 35 (control group) mated female Fischer-344 rats received daily oral doses of the test substance (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 (Phase I). Due to the observation of a low number of malformations at 250 mg/kg bw/day in Phase I, an additional study groups consisting of 25 females receiving 250 mg/kg bw/day or vehicle were included (Phase II). 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. Histopathologic examination of these tissues was not considered necessary. 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. The uteri of apparently non-pregnant animals were stained and examined for evidence of implantation sites; to determine the incidence of pregnancy only. The following data on the foetuses were recorded: position and number of foetuses in utero, number of live and dead foetuses, number of resorptions, number of corpora lutea, the sex of each fetus, individual fetal weight, crown-rump length and gross external alterations.

Results

At 250 mg/kg bw/day three dams died on days 10-11 of gestations for unknown reasons. In phase I, single rat died in the high dose group on day 11 of gestation. This animal exhibited moistening of the hair in the perineal region, a slight decrease in the size of the thymus and a gastrointestinal tract devoid of feed or fecal material. A definitive diagnosis as to the cause of death could not be determined upon gross pathologic examination. In phase II, two animals at high dose group died on day 10 of gestation. Both animals had lost substantial amount of weight and exhibited exudative material from the nares. A definitive diagnosis as to the cause of death in these animals could not be ascertained upon gross pathologic examination. More detailed individual data for these three animals could not be obtained from the study report.

The dose level of 250 mg/kg bw/day caused significant reductions in body weights and body weight gains of the dams. However, when corrected by the mean foetal weight x mean number of foetuses/litter subtracted from mean maternal body weight gain (days 6-20), the dam weight was not significantly lower. 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 25 Incidence of fetal alterations among litters of rats

Parameter	**Control** (phaseI/ phaseII)	**15 mg/kg bw/day** (phaseI)	**75 mg/kg bw/day** (phaseI)	**250 mg/kg bw/day** (phaseI/ phaseII)
Number foetuses (number litters) examined				
External examination	268(29)/ 199(20)	202(22)	215(25)	243(26)/ 188(21)
Soft tissue examination	144(29)/ 107(20)	108(22)	115(25)	130(26)/ 101(21)
Skeletal examination	267(29)[1]/ 199(20)	202(22)	215(25)	243(26)/ 188(21)

Bones of the skull	123(26)[2]/ 92(19)	94(22)	100(22)[2]	113(23)[2]/ 87(19)
Total major malformations (%/No affected)				
-fetuses	0/1(1)[4]	0	0	2(4)/0
-litters	0/5(1)	0	0	8(2)/0
External alterations (%/No affected)				
Dome-shaped head				
-fetuses	0.4(1)[3]/-	0.5(1)	0	0/-
-litters	3(1) /-	5(1)	0	0/-
Edema-generalized				
-fetuses	0.4(1)[3]/-	0	0	0/-
-litters	3(1) /-	0	0	0/-
Exencephaly[b]				
-fetuses	-/1(1)	-	-	-/0
-litters	-/5(1)			-/0
Soft tissue alterations (%/No affected)				
Congenital bile duct diverticulum				
-fetuses	0/-	1(1)	0	0/-
-litters	0/-	5(1)	0	0/-
Delayed development of semilunar valve				
-fetuses	0/1(1)	0	1(1)	0/0
-litters	0/5(1)	0	4(1)	0/0
Slight cerebral hemorraghe(s)				
-fetuses	17(25)/13(14)	19(20)	9(10)*	25(32)/7(7)
-litters	55(16)/50(10)	41(9)	32(8)	58(15)/29(6)
Slightly dilated third ventricle of brain				
-fetuses	0/-	0	0	1(1) /-
-litters	0/-	0	0	4(1) /-
Focal necrosis-caudate process of liver				
-fetuses	-/17(18)	-	-	-/14(14)
-litters	-/60(12)			-/38(8)

Slightly dilated ureter-bilateral -fetuses -litters	-/1(1) -/5(1)	-	-	-/0 -/0
Hemorrhage or hematoma-liver -fetuses -litters	-/1(1) -/5(1)	-	-	-/1(1) -/5(1)
Slightly dilated lateral cerebral ventricles -fetuses -litters	-/2(2) -/10(2)	-	-	-/0 -/0
Unilateral anophthalmia[b] -fetuses -litters	-/1(1) -/5(1)	-	-	-/0 -/0
Skeletal alterations (%/No affected)				
Vertebrae-delayed ossification cervical centra -fetuses -litters	28(75)/1(1) 93(27)/100(20)	29(58) 96(21)	12(25)* 56(14)	16(37)*/0 81(21)/100(21)
Unfused thoracic centra -fetuses -litters	1(2) /- 7(2) /-	1(1) 5(1)	1(2) 8(2)	2(4) /- 15(4) /-
Bilobed thoracic centra -fetuses -litters	5(13)/7(14) 38(11)/50(10)	9(18)* 59(13)	7(15) 44(11)	4(10)/4(8) 35(9)/29(6)
Lumbar spur(s) -fetuses -litters	1(2) /- 7(2) /-	1(2) 9(2)	1(2) 8(2)	1(3) /- 8(2) /-
Hemi-vertebra[b] -fetuses -litters	0/- 0/-	0 0	0 0	0.4(1) /- 4(1) /-
Sternebrae-delayed ossification -fetuses -litters	33(87)/32(63) 93(27)/95(19)	31(63) 100(22)	32(69) 88(22)	24(58)/29(55) 89(23)/86(18)

Unfused				
-fetuses	0.4(1) /-	0	1(2)	1(2) /-
-litters	3(1) /-	0	8(2)	8(2) /-
Fused #1 and #2				
-fetuses	0/-	1(1)	0	0.4(1) /-
-litters	0/-	5(1)	0	4(1) /-
Foramen in #4 sternebra				
-fetuses	0/-	0	0	0.4(1) /-
-litters	0/-	0	0	4(1) /-
Asymmetric cartilage				
-fetuses	0/-	0	0.5(1)	0/-
-litters	0/-	0	4(1)	0/-
Unfused sternebrae				
-fetuses	-/1(1)	-	-	-/2(4)
-litters	-/5(1)			-/19(4)
Other (%/No affected)				
Polydactyly[b]				
-fetuses	0/-	0	0	1(3) /-
-litters	0/-	0	0	4(1) /-

[1]One fetus destroyed during processing, [2]Three litters at the 0, 75 and 250 mg/kg bw/day dose levels consisted of three or fewer foetuses. The heads of these foetuses were removed during the examination for soft-tissue alterations and therefore no examination could be conducted for boes of the skull. [3]Edematons body and domed head observed in a single fetus which was dead at C-section. [4]Both major malformations (exencephaly, unilateral anophthalmia) occurred in the same fetus. *Significantly different from the control value, [b]considered to be a major malformation.

Conclusions

The NOAEL for maternal toxicity was 15 mg/kg bw/day based on the decreased corrected maternal body weight gain. For developmental effects NOAEL was 75 mg/kg bw/day, based on hemivertebrae and polydactyly observed at high dose. The litter incidence of unfused thoracic centra (phase I) and unfused sternebrae (phase II) were slightly increased at the high dose group.

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 the test substance 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.

The observed malformations were discussed in the Pesticides Peer Review Meeting 175. It concluded to set the developmental NOAEL based on the malformations (hemivertebrae and polydactyly) observed at the high dose. The Rapporteur agrees with this conclusion.

Historical control data

Since no developmental studies were conducted in F344 rats before 1981, historical control data (HCD) were collected for polydactyly for studies conducted between 1981 to 1987 in F344 rats via oral treatment at the laboratory

Although polydactyly in the high dose group was observed in Phase I, the effect was not observed in the confirmatory Phase II experiment at the same dose. If a comparison is to be made with the HCD, the combined incidence in Phase I and Phase II should be used. The combined incidence of polydactyly in high dose fetuses was 0.7 percent which is very close to the HCD of 0.5 percent; and the combined incidence in high dose litters was 2.1 percent which is lower than the HCD of 4 percent. In summary, the lack of polydactyly in the Phase II study and the incidences in combined phases are close or lower than the HCD, indicating that the effect is spontaneous in origin and unrelated to exposure to clopyralid.

When comparing the provided data to the requirements for historical control data in Regulation 283/3013 it is noted that only information regarding diet was provided for the description of general conditions under which animals were maintained. In addition, four studies from years 1986-1987 did not meet the requirement of five-year period (centered as closely as possible on the date of the index study) as the index study is from year 1981. For two HCD studies (Jan 26, 1981 and May 13, 1982) incidences for polydactyly were presented, but it was also mentioned that the incidence was only observed in the low dose, and not in control, mid and high-dose groups. The Rapporteur considers this incidence cannot be used as HCD as it was not the incidence for control group and it is interpreted that the incidence for control group of these HCD studies has been 0. The HCD Range therefore should be

Fetus: percent affected (number affected)= 0

Litter: percent affected (number affected)= 0

The Reporting Member State was of the opinion that considers the incidence of polydactyly should be calculated for Phase I and Phase II dose groups separately, not as combined incidence as in the table above. In this case the incidence is Phase I: fetus: 3/243= 1.2% and litter: 1/26= 3.8% (Phase II: 0% for fetus and litter). Even though the incidences are higher than for HCD it is not possible to explicitly conclude that this would be a treatment related effect because polydactyly was seen only in one litter and in Phase II it was not observed at all.

The **Developmental toxicity study in New Zealand White rabbits (dRAR B.6.6.2., 1990)**

The study was performed predominantly according to OECD guideline 414 (2001) and under GLP.

Groups of 26-34 artificially inseminated female New Zealand White rabbits were administered daily oral doses of the test substance (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 26. 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 bw/day produced signs of severe maternal toxicity. Laboured breathing was observed in approximately one-third (11/29) of the rabbits in this group. Five rabbits in the 250 mg/kg bw/day dose group were found moribund and were submitted for necropsy; two of these animals died while awaiting necropsy. Six other rabbits in this dose group also died prior to scheduled C-section. A total of 23 rabbits were submitted for gross examination prior to the scheduled necropsy; 2 control rabbits, 6 animals at 50 mg/kg bw/day, 2 from the 110 mg/kg bw/day dose group and 13 in the 250 mg/kg bw/day dose group. Of these 23 animals, fifteen were found dead or died awaiting necropsy, five aborted or delivered early and the

remaining were euthanized moribund. As indicated above there was a low number of litters in the first group of rabbits which can be indicative of reproductive toxicity.

Maternal body weight and body weight gain was significantly depressed in the 250 mg/kg bw/day dose group as well. Multifocal erosions and/or ulcers in the gastric mucosa were observed in dams at 250 mg/kg bw/day. Lower foetal weights and hydrocephaly were observed at 250 mg/kg bw/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. However, in rabbits, the body weight gain may not be useful indicators of maternal toxicity because of normal fluctuations in body weight during pregnancy. When comparing the number of foetuses (litters) with hydrocephaly with the number of foetuses (litters) with total CNS malformation (Table 25 in the 250 mg/kg bw/day dose group it can be seen that in addition to hydrocephaly no other CNS effects were observed in this group.

Table 26: Main observations in teratology study in rabbits

Parameters	0 mg/kg bw/day	50 mg/kg bw/day	110 mg/kg bw/day	250 mg/kg bw/day
Maternal antemortem observations				
No. on test	28	26	26	34
No. excluded due to aspiration of test material	0	5	1	5
No. summarized	28	21	25	29
Number of dams with antimortem observations	2	4	4	15
Aborted	0	1	1	1
Delivered early	1	0	0	1
Moribund	0	0	0	2
Found dead	1	0	0	4
Labored breathing/rales/shallow respiration/coughing	0	3	0	11
No. aspiration of test material related	0	5	1	5
Moribund	-	0	0	3
Found dead	-	5	1	2
Labored breathing/rales/coughing	-	2	0	4
Maternal mortality % (moribund+found dead)	4 (1/28)	19 (5/26)	4 (1/26)	32 (11/34)
Total Animals with aspiration of test material excluded	4 (1/28)	0 (0/21)	0 (0/25)	21 (6/29)

Mean weight gains (g)				
- On gestation days 0-7	224.2	195.2	133.4*	179.4
- On gestation days 7-10	-49.0	-96.4	-120.3	-141.0
- On gestation days 10-13	40.2	10.0	78.6	-17.0
- On gestation days 13-16	28.9	-29.4	30.4	-4.2
- On gestation days 16-20	18.0	18.3	-39.0	-126.6
- On gestation days 20-28	169.3	222.8	188.6	274.9
- 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
% of control		74.6%	63.3%	60.2%
- Corrected days 0-28[1]	55.66	-60.02	-121.44	-80
Mean gravid uterine weight (g)	415.14	411.42	419.64	363.06
Erosions, blood and/or ulcers of the stomach	0/28	0/26	1/26	10/33
(grosspathologic)				
-ulcers, multifocal, locally extensive mucosal thickening	0	0	0	1
-erosion, multifocal, tip of mucosal fold	0	0	0	1
-blood in lumen	0	0	0	1
-erosions/ulcers, multifocal, stomach mucosa	0	0	1	7
% pregnant	75(21/28)	76.9(20/26)	73.1(19/26)	73.5(25/34)
Number of corpora lutea/dam	10.5	10.7	11.0	10.4
Number of implantations/dam	7.9	8.5	7.8	8.1
% preimplantation loss	23.00	19.90	24.53	24.15
Mean litter size	6.8	7.7	7.2	6.3
Litters with full term fetuses	19	15	18	15
Number of dead fetuses	0	0	0	1
Sex ratio (M:F), %	50:50	54:46	52:48	46:54
Foetal body weight (g)	39.64	34.84*	36.19	34.40*
% of control		87.9%	91.3%	86.8%
Resorptions				
- % of implantations	13.9	10.2	8.5	22.1
- % of litters	57.9	40.0	38.9	73.3
- number/litter	1.1	0.9	0.7	1.8
- /litters with resorptions	1.9	2.2	1.7	2.5

Malformations				
- forelimb flexure	1(1)[a]			4(3)
- hydrocephaly				8(3)
- total CNS malformations				
- ventricular septal defect	1(1)		1(1)	8(3)
- missing apical lung lobe		2(2)		1(1)
- skull, delayed ossification				
- skull, foramen	1(1)	1(1)	1(1)	2(1)
- hyoid, delayed ossification	1(1)	7(5)*	7(5)*	5(2)
- hyoid, crooked				
- vertebrae, dentoid process, delayed ossification		2(2)	1(1)	1(1)
	26(10)	24(9)	33(13)	34(11)
- atlas, fused	4(3)	2(2)	4(3)	4(4)
- atlas, missing				
- centra, delayed ossification				
- ribs, fuced				2(2)
- ribs, forked			1(1)	2(2)
- spurs, lumbar				
- sternebrae, delayed ossification		1(1)		
- sternebrae, fused	1(1)	3(2)		1(1)
- pubis, delayed ossification		2(2)		1(1)
- brachydactyly, cleft palate				
- acaudia		1(1)		
- anonychia				
- club foot, omphalocele, arthrogryposis	34(13)	22(9)	33(13)	20(9)
- cryptorchid testes	40(15)	48(13)	76(16)*	52(13)
		1(1)	2(2)	
		1(1)	2(2)	6(3)
		1(1)		
		1(1)		1(1)
	1(1)	1(1)		
				1(1)
		1(1)		
% of foetuses with hydrocephaly				8.4% (8/95)
% of foetuses skull, delayed ossification	0.8% (1/130)	6.1% (7/115)	5.4% (7/129)	5.3% (5/95)
% of foetuses sternebrae, delayed ossification	30.8% (40/130)	41.7% (48/115)	58.9%(76/129)	54.7% (52/95)
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 Number of litters with malformations	4/130 (3.1%) 4/19 (21.0%)	4/115 (3.5%) 4/15 (26.7%)	3/129 (2.3%) 3/18 (16.7%)	13/95 (13.7%) 7/15 (46.7%)

[a]number of affected foetuses(litters)

[b]including malformations

*p<0.05, Chi-square

[1]Correction: the mean gravid uterine weight subtracted from mean maternal body weight gain (days 0-28).

Conclusions

The daily doses of 250 mg/kg bw/day 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.

On days 7-20 maternal body weight gain is negative in all dose levels. On days 0-28 maternal body weight gain is dose-dependently reduced, the difference to control being > 25% in all dose levels. Also, the corrected maternal body weight gain is negative in all dose levels. Based on these observations maternal NOAEL is < 50 mg/kg bw/day and maternal LOAEL 50 mg/kg bw/day. However, as the CLP Regulation states "*In rabbits, the body weight gain may not be useful indicators of maternal toxicity because of normal fluctuations in body weight during pregnancy.*"

The fetal body weight is decreased in all dose groups, statistical significance is observed in low and high dose groups. Difference to control is 9-13% in all dose levels. The effect observed in low and mid dose is supported by statistically significant delays in ossification (skull, sternebrae) observed in low and mid dose groups. Based on these observations developmental NOAEL is < 50 mg/kg bw/day and developmental LOAEL 50 mg/kg bw/day. Hydrocephaly and forelimb flexure are seen in the high dose group.

The historical control data from the study report in considered insufficient. The studies are old (1974-1988) and the data does not cover the five-year period requested in Reg. 283/2013. Therefore, it is not possible to reliably evaluate observed fetal alterations relative to historical control data. The study is acceptable.

Developmental toxicity in rabbit (dRAR B.6.6.2., 1974)

The study partially complies with Directive 87/302/EEC "teratogenicity study – rodent - nonrodent". 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. 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 the test substance (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 6.6-10). Incidence of resorptions or malformations was similar in all groups.

Table 27 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 bw/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.

10.10.4 Adverse effects on development

Table 28: Summary table of animal studies on adverse effects on development

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
OECD 414, rat	250 mg/kg	polydactyly and hemivertebra	dRAR B.6.6.2. 1981
OECD 414, rabbit	50 mg/kg, 250 mg/kg	delayed ossification, hydrocephaly, forelimb flexure	dRAR B.6.6.2. 1990
OECD 416, rat	1500 mg/kg	increased liver weight in in F_{1a} and F_{1b}	dRAR B.6.6.1. 1983

10.10.5 Short summary and overall relevance of the provided information on adverse effects on development

In teratogenicity study on rats, there was no dose-relationship in foetal effects in F344 rats (Hayes et al., 1981). Three maternal deaths were observed at high dose group, 250 mg/kg bw/day, on days 10-11 of gestation for unknown reasons. Observed effects were moistening of the hair in the perineal region, a slight decrease in the size of the thymus and a gastrointestinal tract devoid of feed or fecal material. Two animals had lost substantial amount of weight and exhibited exudative material from the nares. A definitive diagnosis as to the cause of death in these animals could not be ascertained upon gross pathologic examination.

The number of malformed foetuses increased (statistically nonsignificant) at maternotoxic dose level of 250 mg/kg bw/day in phase I of the study. The incidence was higher than in historical control data, but it was not possible to conclude if these malformations (polydactyly and hemivertebra), were treatment related effects, because no major malformations were observed in an additional high dose group of animals, dosed 250 mg/kg bw/day (phase II of the study). The number of resorptions did not increase at the high dose level. The NOAEL for developmental effects is 75 mg/kg bw/day (based on hemivertebra and polydactyly). The

maternal NOAEL was 15 mg/kg bw/day based on the observed decrease in corrected maternal body weight gain.

In rabbits (Hanly et al., 1990), increased incidence of resorptions, malformations (hydrocephaly, total CNS malformations, forelimb flexure) and alterations were seen at the maternotoxic dose level of 250 mg/kg bw/day. The observed maternal toxicity; morbidity, clinical signs, gastric lesions, reductions in body weight and body weight gain may have had an effect on the observed malformations and resorptions, however, RMS is not aware of a specific mechanism behind. However, mortality and abortions were observed already at the lowest dose level. The maternal NOAEL from this study is < 50 mg/kg bw/day, based on negative and reduced body weight gain. The developmental NOAEL is < 50 mg/kg bw/day, based on decreased foetal body weight, supported by delays in ossification (skull, sternebrae).

In the developmental studies on rats and rabbits, major malformations, like polydactyly, hemivertebra, hydrocephaly, forelimb flexure, microphthalmia and anophthalmia were observed. However, these were observed in relatively low incidences. In addition, delayed ossification was observed on rabbit. These all effects were partly observed in maternotoxic dose levels or were not repeated in the second phase of the study. However, the overall picture shows there are elements that would support the classification as Repr. 2 for developmental effects (criteria according to CLP Regulation 1272/2008 presented in the below tables).

Classification as **Repr. 2; H361d** is proposed.

10.10.6 Comparison with the CLP criteria

According to the CLP Regulation (1272/2008):

3.7.1.4. Adverse effects on development of the offspring

Developmental toxicity includes, in its widest sense, any effect which interferes with normal development of the conceptus, either before or after birth, and resulting from exposure of either parent prior to conception, or exposure of the developing offspring during prenatal development, or postnatally, to the time of sexual maturation. However, it is considered that classification under the heading of developmental toxicity is primarily intended to provide a hazard warning for pregnant women, and for men and women of reproductive capacity. Therefore, for pragmatic purposes of classification, developmental toxicity essentially means adverse effects induced during pregnancy, or as a result of parental exposure. These effects can be manifested at any point in the life span of the organism. The major manifestations of developmental toxicity include (1) death of the developing organism, (2) structural abnormality, (3) altered growth, and (4) functional deficiency.

Table 3.7.1(a)

CATEGORY 1

Known or presumed human reproductive toxicant Substances are classified in Category 1 for reproductive toxicity when they are known to have produced an adverse effect on sexual function and fertility, or on development in humans or when there is evidence from animal studies, possibly supplemented with other information, to provide a strong presumption that the substance has the capacity to interfere with reproduction in humans. The classification of a substance is further distinguished on the basis of whether the evidence for classification is primarily from human data (Category 1A) or from animal data (Category 1B).

Category 1A

"Known human reproductive toxicant The classification of a substance in Category 1A is largely based on evidence from humans."

No classification for development of the offspring is proposed.

Category 1B

"Presumed human reproductive toxicant The classification of a substance in Category 1B is largely based on data from animal studies. Such data shall provide clear evidence of an adverse effect on sexual function and fertility or on development in the absence of other toxic effects, or if occurring together with other toxic effects the adverse effect on reproduction is considered not to be a secondary non-specific consequence of other toxic effects. However, when there is mechanistic information that raises doubt about the relevance of the effect for humans, classification in Category 2 may be more appropriate."

No classification for development of the offspring is proposed.

Category 2

"*Substances are classified in Category 2 for reproductive toxicity when there is some evidence from humans or experimental animals, possibly supplemented with other information, of an adverse effect on sexual function and fertility, or on development, and where the evidence is not sufficiently convincing to place the substance in Category 1. If deficiencies in the study make the quality of evidence less convincing, Category 2 could be the more appropriate classification. Such effects shall have been observed in the absence of other toxic effects, or if occurring together with other toxic effects the adverse effect on reproduction is considered not to be a secondary non-specific consequence of the other toxic effects.*"

Classification **Repr. 2; H361d** (Suspected of damaging the unborn child) is proposed.

10.11 Adverse effects on or via lactation

No adverse effects via lactation have been reported.

10.12 Specific target organ toxicity-single exposure

Not assessed in this dossier.

10.13 Specific target organ toxicity-repeated exposure

Table 29: Summary table of animal studies on STOT RE

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, route of exposure, dose levels, duration of exposure	Results	Reference
28-day oral study Mainly according to OECD TG 407 (2008) Deviations: Sensory reactivity to stimuli and certain haemotological and clinical parameters were not examined, epididymis, prostate, seminal vesicles, coagulating gland and thymus were not weighed and only a few tissues were examined histologically. Only a few ED endpoints were assessed. GLP Rat, CD 10/sex/dose Acceptable	Clopyralid (Lontrel T), purity 95% oral, via diet 0, 150, 500, 1500 mg/kg bw/day continuously for 28 days	No mortality or treatment-related clinical signs occurred. Males receiving 1500 mg/kg bw/day had a slight reduction in body weight gain (9%). A dose-related increase in urea nitrogen levels was observed in females receiving 500 and 1500 mg/kg bw/day (20 and 33 %, respectively). Abosolute and relative (/bw) kidney weights were increased at all dose levels in females and at 1500 mg/kg bw/day in males. In a few control and high dose animals slight histolopathological changes in kidney were reported. Lesions of the stomach epithelium including thickening of the forestomach limiting ridge, and haemorrhagic depressions of stomach were observed in both sexes in mid and high dose groups. The histopathological examination revealed minimal acanthosis of non-glandular epithelium and minimal folding of non-glandular epithelium of the limiting ridge in stomach in high dose males (10/10) and females (9/10) and in mid dose animals (5/10 in both sexes). No changes were observed in low dose (150 mg/kg bw/day) or in control animals.	dRAR B.6.3.1., 1986

2-week oral (dietary) study Mainly in accordance with OECD TG 407 GLP unclear Mouse, $B_6C_3F_1$ 5/sex/group Deviations: no individual data, clinical observations, sensory reactivity to stimuli, grip strength, motor activity, several haemotological and biochemical parameters, weigths and histopathology of several organs. Supportive only	Clopyralid (Lontrel T), purity 95% oral via diet 0, 0.2%, 1%, 2.5%, 5%, 10% in diet corresponding to approximately 0, 500, 2300, 5500, 9600 and 19200 mg/kg bw/day continuously for 2 weeks	All mice at the 10% dietary 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. Food intake was considerably reduced at 2300 mg/kg bw/day and higher dose levels in both sexes. At 9600 mg/kg bw/day this was accompanied by reduced body weight gain. Liver weights (absolute and relative) were increased in males at all dose levels and at 9600 mg/kg bw/day in females. There were no toxicologically significant treatment related gross pathological effects or remarkable adverse effects at doses that would trigger STOT RE classification.	dRAR/DAR B.6.3.1.2.1., 1982

13-day oral study No guideline GLP Rabbit, New Zealand White rabbit 3 females/group Supportive only	clopyralid, purity 96.4% in corn oil oral, by gavage 0, 350, 500 and 750 mg/kg bw/day for 13 days	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. 350 mg/kg bw/day caused no overall effect on body weight gain. 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. LOAEL: 350 mg/kg bw/day (erosions and/or ulcers in the stomach).	dRAR/DAR B.6.3.1.3.1., 1990
90-day oral study Mainly according to OECD TG 408 (1998) GLP unclear Rat, Fischer-344 15/sex/group Deviations: The dose levels did not fall into two to four time intervals and the highest dose seems too low. A number of haemotological, biochemical and neurological determinations were not made. Acceptable	clopyralid (Dowco 290), purity 96.4% oral, via diet 0, 300, 1500, 2500 mg/kg bw/day continuously for 98-99 days	No mortality. Mean body weights and body weight gain were decreased in both sexes in 2500 mg/kg bw/day dose group (ca. 9% and 16%, respectively) and in females of 1500 mg/kg bw/day group (ca. 7%). Food consumption in females was reduced at 2500 mg/kg bw/day. Mean relative liver and kidney weights were significantly increased in males at all doses and in females at 2500 mg/kg bw/day. No corresponding gross or microscopic lesions were observed in liver or kidney. 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. 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. LOAEL: 2500 (lesions in the stomach)	dRAR B.6.3.2., 1983
90-day oral study No guideline, no GLP Rat, Sprague-Dawley 15/sex/group Supportive only	clopyralid (Dowco 290) purity: 96.3% oral, via diet 0, 5, 15, 50, 150 mg/kg bw/day continuously for 90 days	One male (50 mg/kg bw/day) died on day 48 for unknown reason. There were no toxicologically significant effects. Alkaline phosphatase values of males were significantly decreased form control (24%-34%) at all dose levels but the toxicological significance of this finding remained questionable. No remarkable adverse effects at doses that would trigger STOT RE classification.	dRAR/DAR B.6.3.2.1.2., 1973

13-week oral study Mainly according to OECD TG 408 (1998) GLP unclear Mouse, $B_6C_3F_1$ 10/sex/group Deviations: Some neurological, hematological and clinical biochemistry assessments were not made. Adrenals, epididymides, uterus, ovaries, thymus, and spleen were not weigthed. Acceptable	clopyralid (Dowco 290), purity: 97% oral via diet 0, 200, 750, 2000, 5000 mg/kg bw/day countinuous for 95-96 days	A slight decrease in body weight was evident over the study in both sexes at 5000 mg/kg bw/day. There was no significant reduction in food consumption at any dose level. Relative liver weights were increased significantly in high dose males and females (by 10 and 7% in males and females, respectively). Microscopy revealed slight morphologic alteration of centrilobular hepatocytes including increased cell size and altered tinctorial properties in all high dose animals. The microscopic change was also present in most female mice at 2000 mg/kg bw/day. No remarkable adverse effects at doses that would trigger STOT RE classification.	dRAR/DAR B.6.3.2.2.1., 1983
12-month oral study in dog Mainly according to OECD TG 409 (1998) GLP unclear Dog, Beagle 6/sex/group Deviations: Blood glotting potential and the weights of gall bladder, epididymides, uterus, thyroid, parathyroid, thymus and spleen were not measured. Acceptable	clopyralid (purity 95.8% oral via diet 0, 100, 320, 1000 mg/kg bw/day, 99, 301 and 983 mg/kg bw/day and 99, 319 and 977 mg/kg bw/day, males and females respectively. continuously for 52 weeks	No treatment related changes were observed in appearance or behaviour of the dogs. The body weight of high dose females was reduced approximately 10-15% compared to controls from week 11-12 onwards. Significant dose-related haemotalogical changes including reductions in red blood cell counts (74%-89% of control), total haemoglobin concentration (79%-85% of cotrol) and hematocrit (81%-84% of control), were observed in both sexes in intermediate and high dose groups after 14, 27 and 52 weeks of treatment. LOAEL: 320 mg/kg bw/day (haemotological effects)	dRAR/DAR B.6.3.2.3.3., 1984
6-month oral study No guideline No GLP Beagle dog 4/sex/group Not acceptable	clopyralid (purity not stated) oral via diet 0, 15, 50 and 150 mg/kg bw/day continuously for 6 months	Males: No treatment related toxicological effects. Females: Increase in relative liver weight at 150 mg/kg bw/day (122 %).	dRAR/DAR B.6.3.2.3., 1976

180-day oral study No guideline No GLP Beagle dog 4/sex/group Not acceptable	clopyralid (purity not stated) oral via diet 0, 15, 50, 150 mg/kg bw/day continuously for 180 days	No treatment related toxicological effects.	dRAR/DAR B.6.3.2.3.2., 1975
21-day dermal study U.S. EPA Guideline No. 82-2 for pesticides, mainly according to OECD TG 410 (1981) GLP New Zealand White rabbit Acceptable	clopyralid (Lontrel T), purity: 95.8 % 0, 100, 500, 1000 mg/kg bw/6 hours/day topical application for 15 days	Systemic: No treatment related systemic toxic effects. Local (skin): Skin lesions at the dermal test site including mild and diffuse treatment related epidermal hyperplasia 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. In addition, inflammation of dermis and necrosis on epidermis (1 male, 100 mg/kg bw/day) were observed. See 10.2. and 10.4. for details and the study summary	dRAR B.6.3.3./ DAR B.6.3.3.1.1., 1990
Two-generation study Partly according to OECD TG 416 GLP unclear Rat, Fischer 344 30/sex/group Due to a variety of deviations from the guideline the study is considered only potentially adequate for risk assessment (see 10.10. and dRAR for details)	clopyralid (DOWCO 290), purity: 96.7% oral, via diet 0, 150, 500, 1500 mg/kg bw/day Corrected dose levels: 0, 82.5, 275, 825 mg/kg/day	No signs of systemic toxicity, altered behaviour or increased mortality in parental generations throughout the study. Gross necropsy revealed test substance-related stomach lesions in two of the thirty male F1 males in the 825 mg/kg bw/day group. See 10.10. and dRAR for details and study summary	dRAR/DAR B.6.6.1.1., 1983, 1984

Developmental toxicity study Mainly according to OECD TG 414 (2001) GLP Rat, Fischer 344 Phase I: 35 control females and 29 – 30/females/ group Phase II: 25 control females and 25 high dose (250 mg/kg bw/day) females control group Probe study: 9-10 females/group Deviations: Maternal body weights were only reported for gestation days 6, 10, 16 and 21. Gravid uteri including the cervix were not weighed. Acceptable[a]	clopyralid, (DOWCO 290), purity 97% Oral, gavage in cottonseed oil Probe study: 0, 50, 100, 250 or 500 mg/kg bw/day Main study: 0, 15, 75, 250 mg/kg bw/day gestation days 6-15	Probe study: 500 mg/kg bw/day caused maternal toxicity including significant decreases in body weight and body weight gain during gestation and food and water consumption over 9-12 days of gestation. Thymus weight was decreased. One maternal death occurred at the 500 mg/kg bw/day dose level on day 7 of gestation. Moreover, a significant increase in percent implantations resorbed was observed in this group. Main study: 250 mg/kg bw/day caused significant reduction in body weight gains (ca. 35% reduction in corrected maternal body weight gain over days 6-20) decreased food and water consumption and decreased absolute liver weight. Three dams died on days 10-11 of gestations for unknown reasons. One of these animals had moistening of the hair in the perineal region, a slight decrease in the size of the thymus and a gastrointestinal tract devoid of feed or fecal material. Two deceased animals had lost substantial amount of weight and exhibited exudative material from the nares. A definitive diagnosis as to the cause of death in these animals could not be ascertained upon gross pathologic examination. Gastrointestinal tract was not examined histopathologically in this study. See 10.10. and dRAR for details and study summary	dRAR/DAR B.6.6.2.1.1., 1981
Developmental toxicity study Mainly according to OECD TG 414 (2001) GLP Rabbit, New Zealand White 26-34 females/group Acceptable	clopyralid (DOWCO 290), purity 96.7% Oral, gavage in cottonseed oil Probe study: 0, 110, 250 and 350 mg/kg bw/day Main study: 0, 50, 110, 250 mg/kg bw/day gestation days 7-19	[b]Probe study: The daily dose of 350 mg/kg bw/day resulted in weight loss, stomach erosion and significant mortality (60%). No clinical effects were observed at 110 or 250 mg/kg bw/day. Necropsy revealed stomach erosions in all rabbits given 250 mg/kg bw/day. Main study:The dose level of 250 mg/kg/day produced signs of severe maternal toxicity. Laboured breathing was observed in ca. one-third (11/29) of the rabbits in this group. Maternal body weight gain was dose-dependently reduced at all doses (in 250 mg/kg/day dose group 60 % of the controls over days 0-28) and the corrected maternal body weight gain was negative in all doses. Several animals were found dead or were killed moribund in the study. Based on the necropsy findings in the control, 50, 110 and 250 mg/kg bw/day groups, 0, 5, 1 and 5 animals, respectively, failed to reach term due to intubation error. In addition to these, there were 1 maternal death in the control group and 6 maternal deaths in the 250 mg/kg bw/day group on gestation days 13-21 (i.e 6/29, 21% mortality). Necropsy revealed decreased ingesta and stomach lesions in all these animals. Multifocal erosions and/or ulcers in the gastric mucosa were observed in dams at 250 mg/kg/day. See 10.10. and dRAR for details and the study summary	dRAR/DAR B.6.6.2.2.2., 1990

Developmental toxicity study partially complies with Directive 87/302/EEC ”teratogenicity study rodent - nonrodent”. No GLP Rabbit, New Zealand White 25 females in control group, 15 females/dose groups Deviations: see dRAR Supportive only/additional information	Clopyralid (DOWCO 290), purity 96% 0, 110, 250, mg/kg bw/day Oral, gavage in corn oil gestation days 6-18	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. Incidence of resorptions or malformations was similar in all groups. See 10.10. and dRAR for details and study summary	dRAR/DAR B.6.6.2.2.1., 1974

2-year chronic toxicity and oncogenicity study Mainly OECD TG 453 (2009) GLP unclear Rat, Fischer 344 70/sex/treatment See study summary for deviations Acceptable	clopyralid (DOWCO 290) purity: 96.7% oral, via diet 0, 15, 150, 1500 mg/kg bw/day continuously for two years	Hyperplasia and thickening of the epithelium of the anterior surface of the gastric limiting ridge at 150 and 1500 mg/kg bw/day. 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, slightly decreased food consumption, increased relative liver and kidney weight and a grossly visible increase in the size of the gastric limiting ridge. The lesions were concluded to characterize the irritant nature of clopyralid. There was no evidence that clopyralid caused increased incidence of malignant or non-malignant tumours in the rat. see dRAR/DAR for details	dRAR/DAR 6.5.1.4., 1985, 1986
2-year combined toxicity and carcinogenicity No guideline, no GLP Rat, Sprague Dawley 40/sex/treated group, 79-80/sex/control group Not acceptable, supplementary	clopyralid (DOWCO 290) purity 92.8% oral, via diet 0, 5, 15, 50, 150 mg/kg bw/day	No toxicologically significant effects. A trend toward decrease in the mean body weight of females at 150 mg/kg bw/day was reported. See dRAR/DAR for details	dRAR /DAR 6.5.1.1., 1977, 1978, 1985
2-year dietary chronic toxicity-oncogenicity study (diet) Mainly according to OECD TG 453 GLP unclear Mouse, B6C3F1 Acceptable	clopyralid (DOWCO 290) purity 96.7% oral, via diet 0, 100, 500, 2000 mg/kg bw/day	Reduced body weight in high dose males and reduced food consumption in high dose females. Dose-related reduction in alkaline phosphatase both in males and females at 24 month examination. There was no evidence of increased tumour incidences in mouse treated with clopyralid.	dRAR B.6.5/DAR B.6.5.2.2., 1984, 1986
18-month carcinogenicity (dietary) No guideline, no GLP Mouse, CR 30 females/group, 15 males/group, 50 to 60 offspring/group Supplementary only	clopyralid (Dowco 290), purity not stated oral, via diet 35, 100, 350 ppm exposure periods 13 weeks prior to mating and 18 months for the resulting offspring	No toxicologically significant effects. See dRAR/DAR for details	dRAR/DAR 6.5.2.1., 1976

[a] Since no validated analytical methods were reported for the batch used in this study, the reliability of the study has been questioned in the Renewal assesment of clopyralide (Commission Implementing Regulation (EU) No 844/2012).

[b] The full study report of the range-finding study is not available.

Specific target organ toxicity following repeated exposure to clopyralid has been studied in several subacute and subchronic studies using oral route in the rat, mice, rabbit and dog. In addition, one 21-day study via dermal route in a rabbit is available. Other relevant studies include a rat two-generation study, rat and rabbit developmental toxicity studies and chronic toxicity and/or carcinogenicity studies in the rat and mice.

Several of these studies do not comply with OECD test guidelines and GLP and are considered only supportive or not acceptable. The studies are only briefly described below. Further details are given in dRAR, DAR and in the sections 10.4. and 10.10. of this CLH report.

28-day oral study (dRAR B.6.3.1., 1986)

The study was conducted generally according to the OECD TG 407 (2008) but has several deviations summariezed in the Table 65 and in the dRAR. Groups of 10 male and 10 female CD rats/dose level were exposed with Lontrel T (95% clopyralid) via the diet at nominal dose levels of 0, 150, 500 and 1500 mg/kg bw/day for four weeks. 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. Body weights and food consumption were checked once per week. The following organs were weighed at the end of the study: adrenals, brain, heart, kidneys, liver, ovaries, spleen and testes. Histopathological examination was restricted to heart, liver, kidneys, adrenals, stomach and any macroscopically abnormal tissue.

The mean achieved intakes of test material over the treatment period were comparable to the nominal doses (100-107 % of the nominal level). No unscheduled deaths or treatment-related clinical signs in animals occurred. Food and water consumption were comparable to that of controls. Body weight gain of high dose (1500 mg/kg bw/day) males was significantly reduced (9 %) compared to controls over the treatment period. The bodyweight gain of other treated groups was similar to that of controls.

A dose-related increase in urea nitrogen levels was observed in females receiving 500 or 1500 mg/kg bw/day (20 and 33 % compared to controls at 500 and 1500 mg/kg bw/day, respectively). There were also some statistically significant changes in other clinical biochemistry parameters in both males and females receiving 500 and 1500 mg/kg bw/day (see dRAR). These changes were slight and were not dose-related. The relative kidney weights (/body weight) of males receiving 1500 mg/kg bw/day and all treated female groups were significantly higher than those of controls (10%, 20%, 15% and 13%, increase in high dose males, low, mid and high dose females, respectively). Histopathological findings in the kidney included a few minor changes in control animals, mid dose males and high dose animals of both sexes (dRAR).

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. In males (1/10) receiving 1500 mg/kg bw/day and in females (1/10) receiving 150 mg/kg bw/day haemorrhagic depressions of stomach were also observed. The histopathological assessment revealed minimal acanthosis of non-glandular epithelium and minimal folding of non-glandular epithelium of the limiting ridge in high dose males (10/10) and females (9/10), 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. These findings were considered to indicate an irritant effect of clopyralid on the stomach.

2-week dietary probe study in mice (dRAR/DAR B.6.3.1.2.1., 1982)

The study was conducted partly according to OECD TG 407 but there were several deviations (Table 29 and dRAR). The study was designed to identify a maximum tolerated dose for Lontrel T and the primary target organ in mice in order to range dose levels for chronic toxicity study. 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) corresponding to 0, 500, 2300, 5500, 9600 or 19200 mg/kg bw/day for two weeks. Data were collected and analysed statistically on mortality, clinical appearance and behaviour, clinical chemistry, haematology, organ weights (brain, liver, kidneys, heart, thymus and testes), gross pathology and histopathological examination of liver and kidneys.

All mice at the 19200 mg/kg bw/day (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 Both sexes fed the 9600 mg/kg bw/day (5.0%) diets showed slight weight loss on day 4, concomitantly with the considerably decreased food intake (by 52 % and 40 % in males and females, respectively). The mice fed with 9600 mg/kg bw/day had only little or no body weight gain over the 2-week period and they showed decreased activity and reduced faeces during the first week. Both sexes fed the 5500 and 2300 mg/kg bw/day (2.5% and 1.0%) diets had decreased food intake for the initial 4 or 5 days. In clinical chemistry or

haematology studies, there were no treatment-related effects in 9600 mg/kg bw/day diet group. In females SGPT, AP and glucose were reduced compared to controls (19%, 17% and 15% respectively). Absolute and relative liver weights were increased compared to controls in all treated male groups and in females receiving the 9600 mg/kg bw/day. There were no toxicologically significant treatment related gross pathological effects.

13-day oral study in rabbit (dRAR/DAR B.6.3.1.3.1., 1990)

The study did not follow any guideline but complied with GLP and is considered supportive only. The study was designed to identify a maximum tolerated dose level for repeated oral exposure to clopyralid in rabbits. Groups of 3 non-pregnant female New Zealand White rabbits were administered clopyralid (96.4%) in corn oil by gavage for up to 13 consecutive 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. Only a few parameters were recorded (e.g. food consumption, hematological and clinical biochemistry determinations were missing). 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 were weighed, killed and submitted to complete necropsy. Only liver and kidney weights were recorded. Histological examination was not performed.

The 500 and 750 mg/kg bw/day doses caused excessive toxicity, including moribund state, laboured respiration and lethargy. Two rabbits treated with 750 mg/kg bw/day died by day 5 and two rabbits treated with 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 in the study. The rabbits treated with 500 and 750 mg/kg bw/day showed substantial body weight loss (Table 30). Signs of anorexia were observed in all groups, occasionally also in all animals of the control group. In the animals treated with 500 and 750 mg/kg bw/day, anorexia was observed prior to death or moribund condition. There was no overall effect of treatment at 350 mg/kg bw/day on body weight gain compared to untreated control animals. Laboured respiration and nasal exudate 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 animal treated with 750 mg/kg/day appeared to suffer from laryngeal trauma (intubation error) contributing to death on day 2. 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. There were no remarkable differences in kidney or liver weights between the groups.

Table 30: Summary of individual animal data of the 13-day oral study in rabbit

Dose (mg/kg bw/day)	Body weight (g) over the days 1-14					
	1	4	7	10	14	observations
0	4343.8	4522.3	4461.0	4169.9	4148.0	occasional anorexia
	4673.1	4514.4	4634.8	4533.3	4546.2	occasional anorexia
	4649.1	4736.4	4517.6	4221.1	4038.6	occasional anorexia
350	3346.8	3216.7	3039.8	3013.8	3101.7	occasional anorexia, multifocal stomach erosions and/or ulcers
	3484.7	3262.5	3207.8	3527.3	3422.7	occasional anorexia
	3930.9	4046.6	3691.9	3754.4	3817.4	occasional anorexia, laboured respiration and nasal exudate on days 6-13
500	4226.5	3831.2	3530.9	-	-	anorexia, found dead on day 8, perineal soiling (feces), multifocal stomach erosions and/or ulcers

	4690.4	4464.5	4314.7	-	-	anorexia, perineal soiling (urine), moribund on day 9, multifocal stomach erosions and/or ulcers
	4642.0	4517.2	3914.8	-	-	anorexia, laboured respiration, found dead on day 8, multifocal stomach erosions and/or ulcers
750	4677.9	4245.9	-	-	-	anorexia, moribund on day 6, inflammation of the cecum wall, dark urine in bladder, severe multifocal stomach erosions and/or ulcers
	4623.1	4286.8	-	-	-	anorexia, perineal soiling (feces), found dead on day 5, focal stomach erosions and/or ulcers
	4300.5	-	-	-	-	possibly aspirated test material (laryngeal trauma) contributing to death, found dead on day 2, focal hemorrage in stomach (cardia).

A 90-day study in rat (dRAR B.6.3.2., 1983)

The study is broadly in accordance with OECD guideline 408 (1998), but the GLP status is unclear (Table 65, dRAR). Groups of 15 male and 15 female Fischer-344 rats/dose level were exposed with clopyralid via diet at concentrations of 0, 300, 1500 and 2500 mg/kg bw/day for three months (98-99 days). The rats were observed at least once a day for changes in appearance and behaviour. The following organs were weighted at the end of the study: brain, heart, kidneys, liver, ovaries, testes and thymus. Liver, kidney and stomach tissues were evaluated for histopathological alterations from all animals and the samples of other tissues were evaluated only from animals of control and high dose level groups.

The achieved mean concentrations of clopyralid in the test diet were within ±14% of the targeted. There were no unscheduled deaths and impairment of general health status was found only in one animal. Mean body weights and body weight gain were decreased in the male and female high dose groups (2500 mg/kg bw/day, decreases in body weight gains 8.7 and 16.6 %, respectively) and in the female midd dose level group (1500 mg/kg bw/day, 7% decrease in body weight gain). Food consumption of females was significantly reduced compared to control following 2500 mg/kg bw/day throughout most of the study (ca. 2-8% reduction).

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. There were no treatment-related effects in urinalysis or clinical chemistry or haematological parameters. There was statistically significant decrease in alanine transaminase (ALT) values in high dose males and females and middle dose males (12-17%). The alkaline phosphase (AP) values were statistically significantly lower than control in all groups but high dose level females (12-31%). The serum urea nitrogen levels were significantly lower than control in midd and high dose males (13% decrease compared to controls in both groups).

No gross or microscopic lesions were found that corresponded with the increased relative liver or kidney weights (dRAR). 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.

90-day oral study in rats,. (dRAR/DAR B.6.3.2.1.2., 1973)

The study does not comply with any official guideline or with GLP. The study is considered supportive. 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 in all rats and the weights of heart, brain, liver, kidney and testes were determined. Specimens from different tissues were preserved and examined microscopically.

All rats survived the experimental period, except one male (50 mg/kg bw/day) that died day 48, and appeared normal throughout the study. There were no significant treatment-related differences in food intake, bodyweights, body weight gain or relative organ weights between the dose groups and controls.. Gross pathologic or microscopic examinations revealed no remarkable lesions related to treatment. No remarkable changes were observed in haematological parameters. The alkaline phosphatase values in males receiving doses of 150, 15 and 5 mg/kg bw/day were depressed with statistically significantly (by 34-24%, dRAR). However, the alkaline phosphatase values for the control males were unusually high i.e. significantly higher than those of control females, as well as, other control males used in previous and current studies in this laboratory. Therefore the significance of this finding remains questionable.

13-week dietary toxicity study in B6C3F1 mice (dRAR/ DAR B.6.3.2.2.1., 1983)

The study is broadly in accordance with OECD guideline 408 (1998) but the GLP status is unclear. 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). The mice were examined twice daily for signs of toxicity. Body weight and food consumption data were recorded weekly and blood samples were taken prior to sacrifice. 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 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.

The concentration of test substance in the determined dose levels in the diet ranged from 97 to 105% of nominal. There were no remarkable clinical signs of toxicity. A slight decrease in body weight was recorded throughout the study in both male and female mice at the 5000 mg/kg bw/day. There was no significant reduction in food consumption at any dose level. Relative liver weights were increased significantly in male and female mice at 5000 mg/kg bw/day. These increases were accompanied by morphologic alteration detected in all high dose mice: 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 in both males and females.

A 12-month oral toxicity study of 3,6-dichloropicolinic acid in beagle dogs, (dRAR/DAR B.6.3.2.3.3., 1984)

The study was conducted mainly in accordance with OECD guideline 409 (1998), with the administration period extended to 12 months. It remains unclear whether GLP was followed. Groups of 6 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. The dogs were observed twice daily for mortality and/or clinical signs. The adrenals, brain, heart, liver, kidneys, ovaries and testes of all animals were weighed. 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.

The dietary concentrations of clopyralid ranged from 91.8 to 114% of the targeted dose. The average daily doses 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 differences were observed in appearance or behaviour of the dogs. Most of the clinical findings observed are commonly observed in beagle dogs. Focal swelling of the palate and/or tonsils, redness or pallor of the buccal mucosa are however considered as not commonly observed in dogs. It is possible that irritancy of the substance has caused the observed swelling and redness.

On week 11-12 onwards the body weights of high dose females were reduced compared to controls approximately by 10-15% resulting the mean body weight of the group about 85% of that of the control group on week 53. Occasional decreases in food consumption were observed in treated females. There were no major differences in food consumption or in body weight in males.

The red blood cell count was reduced at dose levels 320 and 1000 mg/kg bw/day both on males and females with a dose-response (10-25% from control). At 100 mg/kg bw/day the red blood cell count was lowered but was not significantly different from that of the control group. Statistically sigificantly higher MCH values compared to control were seen in males on weeks 14, 27 and 52 (100.4%-110.2% of control), at week 52 with a dose-response. MCHC values were statistically higher in 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 (MCHC max. 103.3% of control).

Table 31: Haetological effecs in 12-month oral dog study

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** (89.2%)	5.54** (83.7%)	6.97	6.81	6.19* (88.8%)	5.47** (78.5%)
	27 weeks	7.44	6.85	6.45** (86.7%)	5.78** (77.7%)	7.42	7.22	6.12** (82.5%)	5.51** (74.3%)
	52 weeks	7.29	7.23	6.59 (90.4%)	6.10** (83.7%)	7.26	6.78	6.30* (86.8%)	5.55** (76.4%)
Total haemoglobin concentration (g/100 ml)	14 weeks	15.2	15.8	14.2	13.8	16.4	16.3	14.8	13.7* (83.5%)
	27 weeks	17.2	16.4	15.3*	14.3**	17.3	17.2	14.7* (85.0%)	13.6** (78.6%)
	52 weeks	16.8	17.5	16.2	15.7	17.4	16.7	15.9	14.1** (81.0%)
Hematocrit (%)	14 weeks	42.5	43.5	40.0	38.8	45.7	44.8	41.5	38.5* (84.2%)
	27 weeks	48.0	45.3	43.3*	40.3**	48.2	47.5	40.8*	39.2** (81.3%)
	52 weeks	46.7	47.2	44.0	42.7	47.2	45.3	43.5	39.5* (83.6%)
MCV	14 weeks	64.5	67.2* (104.2%)	67.7 (105.0%)	70.2** (108.8%)	65.7	66.2 (100.8%)	67.2 (102.3%)	70.5** (107.3%)
	27 weeks	64.5	66.3 (102.8%)	67.2 (104.2%)	70.0** (108.5%)	64.8	65.7 (101.4%)	66.8 (103.1%)	71.2** (109.9%)
	52 weeks	63.8	65.3 (102.4%)	66.7* (104.5%)	69.8** (109.4%)	65.0	67.0 (103.1%)	69.2** (106.5%)	71.5** (110%)
MCH	14 weeks	23.0	24.3** (105.7%)	24.0	25.0** (108.7%)	23.5	23.8	23.7	25.2** (107.2%)
	27 weeks	23.9	24.0* (100.4%)	23.8	25.0** (104.6%)	23.2	23.8	24.0	24.8** (106.9%)
	52 weeks	23.6	24.3* (103.0%)	24.7* (104.7%)	26.0** (110.2%)	24.2	24.5	25.2* (104.1%)	25.7** (106.2%)
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** (103.3%)	36.8	37.0* (102.8%)	37.0	37.0	36.2	35.7* (96.5%)

Total protein, serum albumin and/or serum globulin were significantly reduced in high dose males and females after 14 weeks of treatment (83%-89% compared to controls, dRAR). These parameters were significantly reduced in high dose males (total protein, serum albumin) and high and intermediate dose females (total protein, serum albumin) still 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 (86% compared to control). 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. Serum urea nitrogen (BUN) was statistically significantly reduced on weeks 14, 27 and 52 in high dose females (65-70% compared to controls, RAR).

Relative liver weights of high dose males and females were significantly increased (42% and 47% in males and females, respectively). The relative kidney weights were increased in high dose males (27%) and the relative heart weights in high dose females (24%).

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. 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.

A 6-month dietary feeding study in beagle dogs, (**dRAR/DAR B.6.3.2.3., 1976)**

The study does not comply with any guideline, nor was it conducted under GLP. Groups of 4 beagle dogs/sex/dose level received diets containing clopyralid (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 a 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. Gross pathologic examinations were conducted on all dogs and the brain, heart, liver, kidneys and testes were weighted and examined histopathologically.

No treatment related changes were observed in appearance or behaviour of the dogs. There were no differences in body weight gain or food consumption of the treated groups and controls. No treatment related effects were found in haematologic or clinical chemistry determinations or urinalysis. Relative liver weight of the female dogs at 150 mg/kg bw/day was increased (122 % of control).

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. Pathologic alterations (microscopic) of the genitourinary system in male beagle dogs were described as urethritis, prostatitis, focal chronic interstitial nephritis, cystitis, myodegeneration of the bladder wall and fibrinoid vascular necrosis of muscular arteries in the urinary bladder. These changes may have been caused by catheterisation since these changes occurred in only some treated males and not in any of the treated females or controls. However, the etiology of these changes is uncertain.

180-day subacute toxicity study in dogs (dRAR/DAR B.6.3.2.3.2., 1975)

The study does not comply with any guideline, nor was it conducted under GLP. Groups of 4 beagle dogs/sex/dose level received diets containing clopyralid, (Dowco 290, purity not stated) at concentrations calculated to provide doses of 0, 15, 50 and 150 mg/kg bw/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 were preserved. Tissues from the control and high dose male and female dogs were examined histopathologically.

There were no treatment related effects in body weights, food consumption, haematology, blood chemistry, ophthalmology, urinalysis or organ weights. There were no findings in necropsy.

Carcinogenicity studies in rat and mice (dRAR/DAR B.6.5.)

Altogether four dietary chronic toxicity and oncogenicity studies with clopyralid are available in rat and mice. The acceptable study in rat (dRAR/DAR B.6.5.1.4, 1985 and 1986) followed broadly OECD TG 453 (2009). It remains unclear whether GLP was followed. Groups of Fischer 344 rats, 70/sex/treatment, received clopyralid (DOWCO 290, purity 96.7%) via diet 0, 15, 150 or 1500 mg/kg bw/day for two years. Mortality, clinical appearance and behaviour were checked daily. Food consumption and body weight was determined weekly for the first 12 weeks and every four weeks thereafter. Data were collected also on haematology, clinical chemistry, urinalysis, organ weights, gross pathology and histopathology. The deviations from the guideline included the following. Blood samples for hematology and clinical chemistry and urinalysis were not taken at 3 month. MCV, MCH, MCHC, prothrombin time and activated partial thromboplastin time were not measured. Creatinine, total protein, albumin, calcium, total cholesterol were not determined. Epididymides, thyroid and uterus were not weighed.

There were no clinical observations attributable to treatment. No remarkable differences were observed in serum chemistry, urinalysis and haemotology. High dose (1500 mg/kg bw/day) and mid dose (150 mg/kg bw/day) animals had 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, slightly 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 of increased tumour incidences in rats treated with clopyralid.

The other dietary rat two-year study is not considered acceptable (dRAR/DAR B.6.5.1.1., 1977). The study did not follow any guideline and was not conducted according to GLP. There were numerous deviations from OECD TG 453 including e.g. insufficient number of animals per group, insufficient haematological, clinical chemistry, organ weight and histopathology assessments. Groups of Sprague Dawley rats (40/sex/group) received doses of 5, 15, 50 or 150 mg/kg bw/day of clopyralid (DOWCO 290, purity 92.8%) for two years via the diet. Additional groups of 80 males and 79 females were used as untreated controls. No toxicologically significant effects were reported. The only finding which may be related to treatment was a reduction in the mean body weight of female rats at the high dose level, 150 mg/kg bw/day.

In the acceptable mice two-year study, groups of B6C3F1 mice, 70/sex/treatment, received doses of clopyralid (DOWCO 290, purity 96.7%) 0, 100, 500 or 2000 mg/kg bw/day for two years (dRAR/DAR B.6.5.2.2, 1984, 1986). The study is broadly in accordance with OECD TG 453 but its GLP status is unclear (dRAR). Deviations from the guideline include the following: ophthalmological examination was not conducted, blood samples for hematology and clinical chemistry were not taken at 3 month, urinalysis was not performed. The weights of adrenals, epididymides, ovaries, spleen, thyroid and uterus were not determined. 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. Dietary administration of clopyralid at 2000 mg/kg bw/day led to a reduction in body weight in males. The values of alkaline phosphatase were clearly decreased both in males and females at 24 month examination, compared to control and in females there was a clear dose response. Also the food consumption was clearly reduced in high dose group females. There was no evidence of increased tumour incidences in mouse treated with clopyralid.

The other chronic toxicity/oncogenicity study with mice, 18-month study, is considered supplementary only (dRAR/DAR B.6.5.2.1., 1976). The study did not follow any guideline and it was not conducted under GLP. Deviations from current guidelines included e.g. 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. 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. 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. There were no toxicologically significant treatment related effects up to the highest concentration of clopyralid tested (350 ppm).

See sections 10.2., 10.4. for the 21-day dermal toxicity study (dRAR B.6.3.3/DAR B.6.3.3.1.1, 1990) and section 10.10. for the two-generation study (dRAR B.6.6.1., 1983 and 1984) and developmental toxicity studies (dRAR B. 6.6.).

10.13.1 Short summary and overall relevance of the provided information on specific target organ toxicity – repeated exposure

Clopyralid was generally well tolerated in dietary studies in rat and mice. In short-term oral studies, the main critical effects were attributed to the irritant properties of the substance such as stomach lesions (rats and rabbits). Other target organs included the liver (mice and dogs), kidneys (rats) and blood (dog).

Increased liver weights were observed in the rat, mice and dog following exposure to clopyralid doses 300-5000 mg/kg bw/day. In the mice liver weights were increased at doses 500 mg/kg bw/day and higher and were accompanied by slight morphologic alteration of centrilobular hepatocytes following exposure to high clopyralid doses (2000 and 5000 mg/kg bw/day). No correlating histopathological alterations in the liver were observed in other species. Increased kidney weights occurred in the rat at doses from 150 mg/kg bw/day without histopathological correlates. In the dog, significant dose-related haemotological changes including reductions in red blood cell counts (74%-89% of control), total haemoglobin concentration (79%-85% of control) and hematocrit (81%-84% of control), were observed in both sexes after 14, 27 and 52 weeks of dietary treatment with 320 mg/kg bw/day and 1000 mg/kg bw/day (Table 31). All the above mentioned findigs occurred at doses in excess of the guidance values for classification (STOT RE category 2 90-day: ≤ 100 mg/kg bw/day, 28-day: ≤ 300 mg/kg bw/d).

In many of the oral repeated dose studies lesions, erosion and ulcers in stomach/forestomach epithelium were observed. These included e.g. thickening of the forestomach limiting ridge, reddened area in mucosa, acanthosis and folding of non-glandular epithelium, multifocal erosion of the mucosa, fibrosis, necrosis, severe focal ulcer of the mucosa, focal cronic inflammation and haemorrhage. These effects in stomach/forestomach are most likely caused by irritatative nature of the substance rather than being target organ toxicity (mucosa, stomach/forestomach). Clopyralid is already classified as Eye Dam.1; H318. Moreover, in the 21-day toxicity study, repeated dermal administration of clopyralid for 15 days in rabbit, resulted dose-related skin lesions at the dermal test site (dRAR B.6.3.3./DAR B.6.3.3.1.1, 1990). These included mild and diffuse epidermal hyperplasia, inflammation of dermis and necrosis in epidermis. Based on these observations local LOAEL to rabbit is 100 mg/kg bw/day.

Lethality

Treatment-related lethality was reported in the 13-day toxicity study in rabbit and in both rat and rabbit developmental toxicity studies (Table 33). In all these studies clopyralid was administrated orally via gavage dissolved in corn oil or in cottonseed oil.

In the 13-day toxicity study in rabbit (dRAR/DAR B.6.3.1.3.1., 1990) 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 (Table 30). Two rabbits treated with 750 mg/kg bw/day died by day 5 and two rabbits treated with 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. 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.

In the rabbit developmental toxicity study (dRAR B.6.6, 1990) several animals were found dead or were killed moribund in the study. 0, 5, 1 and 5 deaths in control, 50, 110 and 250 mg/kg bw/day groups, respectively, were judged to be due to intubation error i.e. of aspiration of test material. After exclusion of these animals there were 1 maternal death in the control group and 6 maternal deaths in the 250 mg/kg bw/day group on gestation days 13-21. Necropsy revealed decreased ingesta and stomach lesions in all these animals. Signs of severe maternal toxicity including laboured breathing in approximately one-third (11/29) of the does and negative body weight gain was observed in this group. The study rappourter considered these effects primary stress related nonspecific effects, which has been implicated in gastric mucosal, but the possibility of direct irritation cannot be ruled out. Moreover, according to the study report of the main study administration of 350 mg/kg bw/day of clopyralid in the range-finding study (conducted prior to the main study) resulted stomach erosions, decreased body weights and significant mortality (60%). No clinical effects were detected at dose levels of 110 or 250 mg/kg bw/day. However, necropsy revealed treatment-related stomach erosions in all rabbits given 250 mg/kg bw/day. The full study report of the range-finding study is not available.

Table 32: Histopathological findings in stomach of rabbits in developmental toxicity study

Parameters	**0 mg/kg bw/day**	**50 mg/kg bw/day**	**110 mg/kg bw/day**	**250 mg/kg bw/day**
No. of tissues examined	28	26	26	33
Erosion, mucosa, multifocal				
-very slight	0	0	0	3
-slight	0	0	0	3
-moderate	0	0	0	1
Fibrosis, lamina propria, multifocal, slight	0	0	0	1
Fibrosis, submucosa, multifocal, moderate	0	0	0	1
Ulcer, mucosa, focal, severe	0	0	0	1
Necrosis with accompanying inflammation, mucosa, multifocal				
-slight	0	0	0	2
-moderate	0	0	0	1

In the rat developmental study (dRAR B. 6.6, 1981) three maternal deaths occurred in 250 mg/kg bw/day group on days 10-11 of gestation. One of these dams had moistening of the hair in the perineal region, a slight decrease in the size of the thymus and a gastrointestinal tract devoid of feed or fecal material. Two animals had lost substantial amount of weight and exhibited exudative material from the nares. A definitive diagnosis as to the cause of death in these animals could not be ascertained upon gross pathologic examination. The reporting of the study is very limited. Moreover, in the range-finding study conducted prior to the main study one maternal death occurred in 500 mg/kg bw/day group on gestation day 7. Maternal toxicity, as evidenced by statistically significant decreases in body weight, body weight gain, in the thymus weight, and food and water consumption was observed among rats in the 500 mg/kg bw/day group. In addition, a statistically significant increase in implantations resorbed was observed in this group.

In addition, in the 2-week dietary mice study all mice at the 10% dietary clopyralid dose level (19200 mg/kg bw/day) died or were moribund on day 5 because of initiation. This was judged to be due to unpalatability of the test diet and thus not related to clopyralid toxicity per se.

Table 33: Summary of treatment-related lethalities in repeated dose toxicity studies

Study	Effects	Comparison of extrapolated effective dose (lethality) with guidance values
13-day oral gavage study in rabbit dRAR/DAR B.6.3.1.3.1., 1990 0, 350, 500 and 750 mg/kg bw/day for 13 days Supportive	350 mg/kg bw/day: No effect on body weight gain. 1/3 animals had labored respiration and 1/3 had multifocal erosions and/or ulcers of the stomach. 500 mg/kg bw/day: All had laboured respiration, substantial body weight loss and lethargy. 2/3 animals died on day 8 and 1 was sacrificed in moribund. All had multifocal erosions and/or ulcers of the mucosa in the stomach. 750 mg/kg bw/day: Two rabbits that survived at least 4 days had laboured respiration, substantial body weight loss and lethargy. 2/3 animals died by day 5 (one of these apparently aspirated test material and was found dead on day 2), 1 animal was sacrificed in moribund on day 6. 1/3 animals had focal erosions and/or ulcers of the mucosa 1/3 animals had severe multifocal erosions and/or ulcers of the mucosa 1/3 animals had focal haemorrhage in the stomach. The moribund rabbit sacrificed on day 6 had also inflammation of the caecal wall and dark urine.	Lethalities at doses 500 and 750 mg/kg bw/day occurred after shorter than 9 days exposure (5 – 8 of exposure). As stated in the guidance for the application of CLP criteria the effect should be compared to a guidance value of 100 mg/kg bw/d ≤ STOT RE 2 ≤ 1 000 mg/kg bw/d 1000 mg/kg bw/day.
Oral gavage developmental toxicity study in rabbit dRAR B.6.6, 1990 0, 50, 110 and 250 mg/kg bw/day over gestation days 7-19 Acceptable	In the control, 50, 110 and 250 mg/kg bw/day groups, 0, 5, 1 and 5 dams, respectively, failed to reach term due to intubation error. In addition, 1 control and 6 high dose dams died or were sacrifized in moribund. 50 mg/kg bw/day On days 0-28 maternal body weight gain was reduced, the difference to control being > 25% and the corrected maternal body weight gain was negative. 110 mg/kg bw/day: On days 0-28 maternal body weight gain was reduced, the difference to control being > 25% and the corrected maternal body weight gain was negative. Grosspathological: Multifocal erosions/ulcers of the stomach mucosa (1/26) 250 mg/kg bw/day: Severe maternal toxicity; significantly	Maternal lethalities at 250 mg/kg bw/day, (those due to intubation errors excluded), occurred on gestation days 13-21 i.e. after 6 – 13 days of exposure. Taking into account Haber's rule, all these deaths were observed at relevant dose levels for classification as STOT RE 2 (according to Haber´s rule ≤ 690 mg/kg bw/days for 13 days exposure). The lethal dose is also within guidance value for 28-day study (30 mg/kg bw/day ≤ STOT RE 2 ≤ 300 mg/kg bw/day)

	reduced body weight gain (60% of the controls over GD 0-28), laboured breathing/rales/coughing in 11/29 of the rabbits. 6 dams were found dead or sacrifized in moribund (those due to intubation error excluded). One dam aborted and one delivered early. Grosspathological: Erosion, ulcers and/or blood in the stomach 10/33 animals, decreased ingesta Histopathological: Multifocal erosions and/or ulcers of the gastric mucosa (7), fibrosis, severe focal ulcer of the mucosa (1), multifocal necrosis with inflammation (3) In the range-finding study conducted prior to the main study administration of 350 mg/kg bw/day produced stomach erosions, decreased body weights and significant mortality (60%). No clinical effects were observed at dose level of 250 mg/kg bw/day, but necropsy revealed stomach erosions in all rabbits given 250 mg/kg bw/day.[b]	
Oral gavage developmental toxicity study in rat dRAR B.6.6, 1990, 1981 0, 15, 75, 250 mg/kg bw/day over gestation days 6-15 Acceptable[a]	<u>75 mg/kg bw/day:</u> Slight reductions in body weight gains of the dams. A slight decrease in absolute liver weight (94% of controls) <u>250 mg/kg bw/day</u>: Significantly reduced body weight gain (30%/44% of the controls over gestation days 6-10) and food consumption (84-94% of the controls over GD 6-21). A significant decrease in absolute liver weight (92-93% of the controls). Altogether 3 dams died on days 10-11 of gestation. The dam that died on day 11 of gestation (phase I) had moistening of the hair in the perineal region, a slight decrease in the size of the thymus and a gastrointestinal tract devoid of feed or fecal material. The 2 dams that died on day 10 of gestation (phase II) had lost substantial amount of weight and exhibited exudative material from the nares. A definitive diagnosis as to the cause of death in these animals could not be ascertained upon gross pathologic examination. Gastrointestinal tract was not examined grosspathologically or histopathologically in this study. In the range-finding study conducted prior to the main study one maternal death occurred in 500 mg/kg bw/day group on	Mathernal lethalities at 250 mg/kg bw/day occurred after shorter than 9 days exposure (after 4–5 of exposure). As stated in the guidance for the application of CLP criteria the effect should be compared to a guidance value of 100 mg/kg bw/d ≤ STOT RE 2 ≤ 1 000 mg/kg bw/day. The lethal dose is also within guidance value for 28-day study (30 mg/kg bw/day ≤ STOT RE 2 ≤ 300 mg/kg bw/day)

	gestation day 7. Maternal toxicity, as evidenced by reduced body weight gain, reduced thymus weight and significant decreases in food and water consumption were observed in this group.	

[a] Since no validated analytical methods were reported for the batch used in this study, the study reliability has been questioned in the Renewal assesment of clopyralide (Commission Implementing Regulation (EU) No 844/2012).

[b] This information is from the full study report of the main study. The full study report of the range-finding study has not been submitted to Tukes.

In rabbit, lethalities were reported in two studies in both gravid and nongravid females and in the range finding study at dose levels relevant for STOT RE 2 classification. The database also includes one rabbit teratology gavage study, where no lethality was reported (dRAR/DAR B.6.6.2.2.1., 1974, supplementary only). Laboured respiration, substantial body weight loss, stomach lesions and ulcers, reduced food consumption, gastrointestinal tract devoid of food and faeces were reported in the deceased animals. Rabbits are known to be very sensitive to gastrointestinal imbalances by e.g. poorly soluble and irritative substances which may result in death of rabbits. These effects are considered species-specific and therefore not relevant for humans and should not be used for regulatory purposes (Reuter et al., 2018). Clopyralid is classified as Eye Dam.1; H318 and repeated dermal administration at doses ≥ 100 mg/kg bw/day resulted dose-related skin lesions at test site in rabbit (dRAR B.6.3.3./DAR B.6.3.3.1.1, 1990). Based on this and the findings in the decedents, rabbit lethalities are most likely caused by stress, irritatative nature of clopyralid and consecutive local damage of stomach mucosa.

In rat, lethalities in gravid females in the developmental toxicity study and in the range finding study were probably related to bolus admimistration by gavage, since clopyralid caused no severe toxicity or lethality in rat dietary studies up to doses as high as 2500 mg/kg bw/day in the 90-day study, 825 mg/kg bw/day in the two-generation study and 1500 mg/kg bw/day in the two year carcinogenicity study (Table 29). However, dose-related lesions in the stomach (gastric limiting ridge, LOAEL 500 mg/kg bw/day), although less severe than in rabbit, were reported in these dietary rat studies (dRAR B.6.3.2., 1983; dRAR B.6.6.1., 1983, 1984; dRAR/DAR B.6.5.1.4, 1985, 1986). The findings in the deceased rats, e.g. body weight loss, empty gastrointestinal tract, reduced food consumption and thymus atrophy, point toward local damage of the stomach/forestomach mucosa as a cause of death. It is possible that repeated bolus administration of clopyralid in oily vehicle aggravates lesions of the stomach/forestomach mucosa resulting lethality at doses ≥ 250 mg/kg bw/day. However, the reporting of the study is very poor: no necropsy procedures or findings are reported, it is only stated that cause of death could not be ascertained upon gross pathologic examination. Therefore the cause of death of these animals can not be firmly concluded.

10.13.2 Comparison with the CLP criteria

According to Annex I of CLP regulation (EC) No 1272/2008 specific target organ toxicity (repeat exposure) means specific, target organ toxicity arising from a repeated exposure to a substance or mixture. All significant health effects that can impair function, both reversible and irreversible, immediate and/or delayed are included.

Substances are classified in Category 1 for target organ toxicity (repeat exposure) on the basis of: reliable and good quality evidence from human cases or epidemiological studies; or observations from appropriate studies in experimental animals in which significant and/or severe toxic effects, of relevance to human health, were produced at generally low exposure concentrations. Substances are classified in Category 2 for target organ toxicity (repeat exposure) on the basis of observations from appropriate studies in experimental animals in which significant toxic effects, of relevance to human health, were produced at generally moderate exposure concentrations.

For classification based on the results obtained from studies conducted in experimental animals guidance values are given to discriminate low and moderate exposure doses/concentrations. There are no human data available on specific target organ toxicity of clopyralid.

In rabbit and rat, oral repeated dosing of clopyralid resulted lesions, erosion and ulcers in stomach/forestomach epithelium and lethality at relevant dose levels for classification (STOT RE category 2 28-day: ≤ 300 mg/kg bw/d, Table 33). Clopyralid also caused dose-related skin lesions in the dermal rabbit study at similar doses (local LOAEL 100 mg/kg bw/day) and is already classified as Eye Dam.1; H318. Thus, effects in stomach/forestomach are most likely caused by irritative nature of the substance rather than being target organ toxicity to mucosa of stomach/forestomach.

Lethality in both rabbit and rat was reported following repeated oral administration of clopyralid dissolved in corn oil or in cottonseed oil via gavage at markedly lower dose levels (250 - 750 mg/kg bw/day) than the highest dose level of the oral acute toxicity study in rat (LD50 > 5000 mg/kg bw, dRAR B.6.2.1., 1987). The deaths occurred in a dose related manner at dose levels 250 and 500 mg/kg bw/day after 2-5 doses (2-5 days exposure) in rat and at 250, 500 and 750 mg/kg bw/day after 5-13 doses (5-13 days of exposure) in rabbit. Since the deaths that occurred after shorter than 9 days exposure should be compared to a guidance value of 100 mg/kg bw/d ≤ STOT RE 2 ≤ 1 000 mg/kg, these deaths occurred within relevant dose levels for classification for STOT RE 2. The deaths that occurred after ≥9 days exposure were also observed at relevant dose levels for classification as STOT RE 2 (according to Haber´s rule ≤ 690 mg/kg bw/days for 13 days exposure) and when compared to the guidance value for 28-day study (30 mg/kg bw/day ≤ STOT RE 2 ≤ 300 mg/kg bw/day). Clopyralid has low potential for accumulation in rat (dRAR B.6.1.1.).

The database shows that clopyralid generally has low systemic toxicity (Table 29). The findings in the deceased rabbits including erosions and ulcers in the stomach mucosa lead to a conclusion that rabbit lethalities are due to irritative nature of the substance. The lethality in rat seems to be related to repeated bolus administration of clopyralid in oily vehicle via gavage which could aggravate lesions of the stomach/forestomach mucosa. Thus, in both species lethality could be attributed to irritation i.e., acute toxicity, and not reflecting true repeated dose toxicity to warrant classification.

We consider this as a borderline case between no classification and classification. Since it is not possible to firmly conclude on the cause of lethality in rat, classification of clopyralid for STOT RE 2 (“H373: May cause damage to organs through prolonged or repeated oral exposure”) is proposed. Since only one study via dermal route and no inhalation studies are available, it is not proposed to specify a route of exposure.

10.13.3 Conclusion on classification and labelling for STOT RE

Based on lethality in rat, at doses within the range of guidance values for category 2, classification of clopyralid for STOT RE 2 (“H373: May cause damage to organs through prolonged or repeated oral exposure”) is proposed. It is not proposed to specify a route of exposure, specific concentration limits or target organ for STOT RE.

10.14 Aspiration hazard

Not assessed in this dossier

11 EVALUATION OF ENVIRONMENTAL HAZARDS

11.1 Rapid degradability of organic substances

Summary of relevant studies from the draft Renewal Assessment Report (dRAR) on degradation are reported briefly below (dRAR is annexed to this CLH proposal). Only relevant and valid studies for the proposal classification of clopyralid have been included from the dRAR.

Table 34: Summary of relevant information on rapid degradability

Method	Results	Remarks	Reference
Ready biodegradability			
OECD TG 301 B (1981): CO_2 evolution test (Modified Sturm Test) Technical grade clopyralid (Lontrel T 95% w/w, purity 96.4% w/w) GLP compliant	**After 27 days**, cumulative CO2 production in vessels containing clopyralid at concentrations of 10 and 20 mg/l was **10 and 5% of the theoretical maximum levels**, respectively.	The initial DOC level of the solutions was 92% of the theoretical levels, and declined by 9% and 3% by the end of the test in the solutions containing 10 and 20 mg/l of clopyralid, respectively, further confirming the results	1991 dRAR B.8.4.2.1/01
Hydrolysis			
EC Directive 92/69 Method C.7 and OECD TG 111 Test substance clopyralid (RCP (Radiochemical Purity) > 99%) GLP compliant	Clopyralid is hydrolytically stable (< 10% degradation) in sterile aqueous buffer between pH 4 and pH 9 and in natural waters at temperatures up to 50°C over a period of 5 days.	Only the preliminary test at 50 °C for 5 days was performed, and as no degradation was observed, the definite test at 20 °C for 30 days was not carried out, as recommended in the guideline. This is acceptable, as clopyralid appears to be hydrolytically stable at higher temperature than naturally occurs at European conditions. The study fulfilled the validity criteria for OECD TG 111.	2000 dRAR B.8.4.1.1/01
Other convincing scientific evidence			
Aerobic mineralisation in water			
OECD TG 309: Aerobic Mineralisation in Surface Water – Simulation Biodegradation Test [2,6-pyridinyl-^{14}C]-Clopyralid (RCP = 98.0%, specific radioactivity = 32.8 mCi/mmol) GLP compliant	DT50 (high dose): **>1100 days** Total radioactive recovery: - High dose: 94 – 100% - Low dose: 95.3 – 104.6% - Sterile: 95.1 – 99.9% Mean mineralisation to CO_2 after 60 d: - High dose: 1.0% - Low dose: 0.1% There were no significant degradation products observed in	Aerobic conditions Temperature: 20±2°C Mean pH: 6.80, 6.43 and 6.76 for high dose, low dose and sterile samples. The study fulfilled the validity criteria for OECD TG 309.	2015 dRAR B.8.4.2.2/01

Method	Results	Remarks	Reference
	the aqueous samples, high concentration, or sterile samples throughout the study period.		
Aerobic degradation in water/sediment system			
BBA, Part IV, section 5.1 (1990) SETAC-Europe, Part 1, Section 8.2 (1995) [2,6-pyridinyl-^{14}C]-Clopyralid (RCP 99.2%, specific activity 30.9 mCi/mmol) GLP compliant	DT50 (SFO, **dissipation from water to sediment phase**): - Loamy sand: **167 days** - Sandy silt loam: **128 days** Mean radioactive recovery: 97.5% and 102.4% Mineralisation to CO_2 after 100d: 2.3 and 5.3% NER: - After 30d: 7.3 and 6.2% - After 100d: 2.0 and 5.9% The minor metabolites observed consisted of at least three unidentified components, all more polar than clopyralid and comprised of a combined maximum amount of 5.4% AR.	Aerobic conditions Temperature: 20°C Two European water/sediment systems pH: - Associative water: 6.5 and 8.1	2002 dRAR B.8.4.2.3/01
Degradation in soil			
BBA Part IV, 4-1 (1991) SETAC-Europe Guideline, Procedures for Assessing the Environmental Fate and Ecotoxicity of Pesticides, Part 1, Section 1.1 (1995) (apart from minor deviations) [2,6-pyridinyl-14C]-Clopyralid (RCP ~ 99%, specific activity 26.44 mCi/mmole) GLP compliant	Results of the main test: DT50 (SFO): **16.2 – 64.6 days** Mean recovery of radioactivity: 99.3% Mineralisation to CO_2 after 374d: between 72.9 and 83.3% NER after 92d: 11.2 - 35.1% AR No degradation products besides CO_2 were observed. NER was associated mostly with the humin fraction and was deeply incorporated into the soil structure.	Conditions in the main test: Aerobic conditions Temperature: 20°C MHC: 40% Five European soils Study results recalculated as recommended by FOCUS Guidance (2006, version 2.0).	1991 dRAR B.8.1.1.1/01 2015 dRAR B.8.1.1.4/01
US EPA Pesticide Assessment Guidelines, Subdivision N,	DT50 (SFO): **8.6 days**	Aerobic conditions Temperature: 25°C	1995

Method	Results	Remarks	Reference
Paragraph 162-1 (1982). SETAC-Europe Guideline, Procedures for Assessing the Environmental Fate and Ecotoxicity of Pesticides, Part 1, Section 1.1 (1995) (apart from minor deviations) [2,6-pyridinyl-14C]-Clopyralid (RCP = 99.2%, specific activity 364 220 DPM/µg) GLP compliant	Mean recovery of radioactivity: 99.6%. Mineralisation to CO_2 after 78d: 73.6% NER after 78d (plateaued after 28d): 19% and 21% AR HPLC detected a rise in formation of several minor regions of unidentified radioactive components. None of the regions detected amounted to levels greater than 10% AR on any occasion. The most significant was region 2 (aromatic ring still intact and some form of exchangeable hydrogen attached to the ring) which comprised a maximum of 7.7% AR after 14 days and declined to <2% of applied by 78 days but could not be fully identified. The majority of NER was associated with the humin soil fraction	MHC: 75% One USA soil (silt loam)	dRAR B.8.1.1.1/02 2015 dRAR B.8.1.1.4/01
OECD TG 307 (2002) [2,6-pyridinyl-14C]-Clopyralid (RCP = 98.7%, specific activity 161.98 µCi/mg GLP compliant	DT50 (SFO): **9.8 - 16.5 days** Mean recovery of radioactivity: 91.18 - 105.77 Mineralisation to CO_2: - Sandy loam: 70.27% (after 90d) - Clay loam: 68.55% (after 90d) - Clay loam: 74.31% (after 60d) - Loam: 68.21% (after 60d) NER after 90d: 22.34 - 28.14% Majority of clopyralid was rapidly mineralised to CO_2. One minor polar metabolite was observed in the soil	Aerobic conditions Temperature: 20 ± 2°C MHC: 45% Four European soils Study fulfilled OECD TG 307 validity criteria.	2009 dRAR B.8.1.1.1/03 2015 dRAR B.8.1.1.4/01

Method	Results	Remarks	Reference
	extracts. The metabolite did not exceed *ca* 3% in any soil type and was observed at a maximum of two sampling intervals (not consecutive) in two soils.		
Photodegradation in water			
US EPA Pesticide Assessment Guidelines, Subdivision N, Paragraph 161-2 (1982). Apart from minor deviations, the study also meets the requirements of the SETAC-Europe guideline, Procedures for Assessing the Environmental Fate and Ecotoxicity of Pesticides, Part 1, Section 10 (1995). [2,6-pyridinyl-^{14}C]-clopyralid (RCP = 98.1%, specific activity 31.5 mCi/mmole) GLP compliant	DT50 (SFO): **271 days** Total radioactive recovery: 98.8 - 103.8% Low levels of minor degradation products were observed which comprised of a combined total of 4.1% AR after 21 days and consisted of several components each of which were < 2% AR.	Natural sunlight in Richmond, California, US Temperature: 25°C pH: 7	1994 dRAR B.8.4.1.2./01
OECD TG 316 - Phototransformation of Chemicals in Water – Direct Photolysis [2,6-pyridinyl-^{14}C]-clopyralid (RCP = 98.0%, specific activity 32.8 mCi/mmol) GLP compliant	DT50 (SFO): **431 days** DT50 as a function of latitude (40°N): **38,933 days** Total radiocarbon recovery: 100.7 ± 4.5% No significant degradation products observed in the aqueous samples for light exposed or dark control samples throughout the study period	Xenon lamp, average light intensity: 489 W m^{-2} Temperature: 25 ± 2°C pH: 7 The study fulfilled the quality criteria for OECD TG 316	2014 dRAR B.8.4.1.2/02
Photodegradation in soil and air			
US EPA Pesticide Assessment Guidelines, Subdivision N, Paragraph 161-3 (1982) SETAC-Europe Guideline, Procedures for Assessing the Environmental Fate and Ecotoxicity of Pesticides, Part 1, Section 2 (1995) (apart from minor deviations) [2,6-pyridinyl-^{14}C]-clopyralid (Radiochemical purity: 97.6%, specific activity: 31.5 mCi/mmole)	DT50 (PFO) > **12 years (estimate)** Total radiocarbon recovery: 93.9 - 104.8% NER formation maximum: 5%. Several small bands of unidentified radioactivity were also seen in the HPLC profile, but these reached up to only *ca* 3% AR in total, with no single component	Natural summer conditions, latitude 37.45°N One USA soil Temperature: *ca* 25°C pH: 6	1994 dRAR B.8.1.1.3/01

Method	Results	Remarks	Reference
GLP compliant	being greater than 1.7% AR.		
OECD Draft Document (2002) Phototransformation of Chemicals on Soil Surfaces US EPA OCSPP 835.2410, OPPTS 835.2410 [2,6-pyridinyl-^{14}C]-clopyralid (RCP: 98.0%, specific activity: 32.8 mCi/mmol) GLP compliant	DT50 (SFO): **553 days** Total radiocarbon recovery: 100.9 ± 2.8% Mineralisation to CO_2 after 16d: 2.1% NER formation after 16d: 4.3%	Xenon lamp with light intensity of 374 W m-2 and spectral distribution comparable to natural sunlight at 37.45°N 20 ± 2 °C pH: 5.4 One USA soil	2014 dRAR B.8.1.1.3/02
US EPA, AOPWIN v. 1.90 calculation GLP compliant	Assuming a hydroxyl radical concentration of 1.5 x 10-6 OH / cm3 in air and a 12-hour day, a half-life of 19.513 days		2002 B.8.7.2/01

11.1.1 Ready biodegradability

One ready biodegradation study was reported in the dRAR (**B.8.4.2.1/01**). The ready biodegradability of technical grade clopyralid (reported purity 96.4%) was investigated using OECD TG 301 B (Modified Strum Test). The study was performed in accordance with GLP. The test was conducted using clopyralid at nominal concentrations of 10 and 20 mg/l, to vessels containing mineral salt medium inoculated with an extract of unfertilized soil. The vessels were aerated for 28 days with CO_2 free air. The produced CO_2 was trapped in a series of Drechsel bottles containing barium hydroxide. DOC analysis was also performed by determining the concentrations of DOC of control, reference and test mixtures at the start of the test and after 27 days. The sampling took place after 2, 3, 4, 5, 7, 10, 16, 25, 27, and 28 days.

Preceding the definitive test, TOC, DOC, COD was determined, and a five-day bacterial inhibition assay was performed to mineral salt inoculated with an extract prepared from unfertilized soil, with and without sodium benzoate (OECD TG 301 D, Closed Bottle Test). Presence of 2 or 10 mg/l of clopyralid did not have significant effect on the degradation of the reference substance. COD was determined to be 103% of the calculated theoretical oxygen demand (0.71 mg O_2/mg), indicating that under the conditions of the test clopyralid was completely oxidised. In addition, the TOC and DOC of clopyralid in distilled water were determined to be 93 and 96% of the theoretical organic carbon content, indicating that clopyralid was fully oxidised under the conditions of the analysis and that all the carbon measured by TOC was in solution.

In the definitive test, observed cumulative CO_2 production was 10% and 5% of theoretical maximum levels after 27 days in the vessels containing 10 and 20 mg/l clopyralid, respectively. The DOC levels of these solutions declined by 9% in the 10 mg/l solution and 3% in the 20 mg/l solution by the end of the test, which independently confirmed the levels of CO_2 production observed. The reference substance reached the pass level of 60 % of TCO_2 in day 7 of the test.

The study partly fulfilled the validity criteria set out in OECD TG 301. Only one replicate was used for inoculum control, reference substance, low and high concentration, and therefore the difference in extremes could not be assessed. The inorganic carbon content of the test suspension in the mineral medium at the beginning of the test was not reported.

Substances are considered to be readily degradable if CO_2 production is equal to or greater than 60% of the theoretical value after 28 days in this test. Despite the deficiencies, the results clearly indicate that clopyralid cannot be considered readily biodegradable. The study can be used in the classification of clopyralid.

11.1.2 BOD_5/COD

Not available.

11.1.3 Hydrolysis

The rate of hydrolytic degradation of [2,6-pyridinyl]-^{14}C]-clopyralid (RCP >99%, specific activity 30.9 mCi/mmole) was investigated with OECD TG 111 (**B.8.4.1.1/01**). Aquatic sterile buffer solutions at pH values of 4, 7 and 9, and in natural water were treated with nominal concentration of 0.50 mg/l clopyralid under laboratory conditions. The sample of natural water was collected from Indianapolis (Indiana, USA). The incubation time was 120 hours (5 days) at five sampling intervals of 0, 2, 4, 24 and 120 hours. The test was performed at 50°C in the dark, with no headspace, in amber sterile vials.

Individual sub-samples of the buffer solutions and natural water, sufficient to allow duplicate sampling at intervals of 0, 2, 4, 24 and 120 hours (5 days), were treated with clopyralid at a nominal level of 0.50 mg/l (this is well below the clopyralid aqueous solubility of ca 1000 mg/l). The sub-samples were incubated at 50°C in the dark, with no headspace, in amber sterile vials.

The recovery of radioactivity was determined to be between 95.4% to 106.3% AR (mean 102% AR) for the buffer solutions and between 96.1% and 109.8% AR (mean 104% AR) for the natural water samples indicating a complete mass balance. Microbial analysis showed that the sterility of the buffer solutions was maintained throughout the test.

As a result of the test, < 10% degradation of clopyralid was observed at 50°C across pH 4, 7 and 9 and natural water samples. Therefore, clopyralid can be considered hydrolytically stable. There was no need to perform definitive test at 20°C for 30 days, as no degradation was observed at 50°C. Hydrolysis is not expected to be a significant route of dissipation of clopyralid in the environment. The study fulfilled the quality criteria for OECD TG 111 (2004) and can be taken account in the classification of clopyralid.

11.1.4 Other convincing scientific evidence

11.1.4.1 Field investigations and monitoring data (if relevant for C&L)

Not relevant for the classification purposes of clopyralid.

11.1.4.2 Inherent and enhanced ready biodegradability tests

Not available.

11.1.4.3 Water, water-sediment and soil degradation data (including simulation studies)

Aerobic biodegradation studies were available on water, water/sediment, and soil compartments. According to the Guidance on the Application of the CLP Criteria[1], aquatic compartment is preferred when comparing the endpoints to the CLP criteria, and therefore sediment and soil studies are summarized briefly and used only as a supportive information.

Degradation in water

Aerobic degradation of radiolabelled Clopyralid-2,6-^{14}C (RCP 98%, specific radioactivity of 32.8 mCi/mmol) in surface water was investigated with OECD TG 309 test following GLP (**B.8.4.2.2/01**). The natural surface water (from Lake Tuckahoe) was used as a test media with clopyralid in a measured concentration of 10 µg/l (low dose) and 80 µg/l (high dose). The sampling interval was 0, 3, 7, 14, 26, 38, 48 and 60 DAT, and included sterility check and measurement of pH. Oxygen concentration and temperature

[1] https://echa.europa.eu/documents/10162/2324906/clp_en.pdf/58b5dc6d-ac2a-4910-9702-e9e1f5051cc5 (*accessed 19.8.2021*)

was monitored during the test. The biological activity of the test media was confirmed with radiolabelled benzoic acid, which was > 90% degraded after incubation time of 14 days. Volatile degradation products were captured using polyurethane foam plug and a set of three 10% aq. NaOH traps connected in series.

Oxygen concentrations measured in the surface water of treated samples confirmed aerobic conditions during the incubation period. The pH values (mean 6.80, 6.43 and 6.76 for high dose, low dose and sterile samples, respectively) during incubation showed stability of the system. Similar values were measured in the control samples, indicating that the test item had no significant effects on the physico-chemical parameters of the test system. The mean temperature during incubation was 20 ± 2 °C for all systems.

Total radiocarbon recovery ranged from 94.2 to 100.0% of the applied amount in the high dose samples, 95.3 to 104.6% of the applied amount in the low dose samples, and 95.1 to 99.9% of the applied amount in the sterile samples. At the time of test termination (60 days), greater than 93.8% of the applied ^{14}C was associated with the parent compound, indicating the stability of clopyralid in aqueous environments. At high dose, the major transformation product was CO_2, where an average of 98.2% of the degraded parent clopyralid was present after 60 days. No significant difference in the degradation was observed between the high dose, low dose, and sterile samples. The mean mineralisation to CO_2 after 60 days was 1.0 and 0.1% in high and low dose.

The rate of degradation for clopyralid in surface water was calculated using single first-order (SFO) kinetics. The derived DT50 was > 1100 days, indicating that Clopyralid degrades very slowly in surface water and therefore cannot be considered rapidly biodegradable in aquatic systems. No significant degradation products were observed in the low and high concentration or sterile samples throughout the study period. The study fulfilled the quality criteria of the OECD TG 309 and can be used for classification purposes of clopyralid. Surface water simulation studies are among the preferred types of test data in the assessment of rapid degradability.

Degradation in water/sediment system

One water/sediment biodegradation simulation test is available in the dRAR (**B.8.4.2.3/01**). The study was performed in accordance with GLP and followed guidelines presented in Part IV of Section 5.1 of BBA (1990) and Part 1 of Section 8.2 of SETAC-Europe (1995). The study design was also mostly in line with OECD TG 308 and fulfilled its validity criteria. The route and rate of aquatic degradation of [2,6-pyridinyl-^{14}C]-clopyralid (RCP 99.2%, specific activity 30.9 mCi/mmol) was investigated in two representative water/sediment systems (of EU origin) under aerobic laboratory conditions over a duration of 100 days. Application was made to the surface water to mimic introduction into aquatic systems via spray-drift.

Clopyralid dissipated slowly from the water phase to the sediment phase, which comprised of < 1% AR initially to maximum levels between 28.6% and 36.4% AR over the period 60 to 100 days. The amount of radioactivity in the water phase steadily declined from 98.7% and 100.1% AR at 0 days following application to between 56.0% and 67.2% AR after 100 days. The amount of volatile radioactivity evolved was minimal throughout the study, the amount of CO2 detected slowly increased to between 2.3 % and 5.3 % AR after 100 days. The level of NER observed increased slowly to maximum levels between 6.2% and 7.3% AR after 30 days incubation and then declined to between 2.0% and 5.9% AR. Minimal degradation was observed and no major metabolites >10% of AR were formed in 100 days of incubation. The water/sediment phase SFO DT_{50} and DT_{90} values of 128 - 167 days and 425 - 556 days were obtained for the dissipation from the water phase to the sediment in two water/sediment systems within 100 days. The whole system DT50 value for clopyralid was not calculated due to the low rate of degradation observed. The results indicate that clopyralid is very slowly degradable in water/sediment systems**.** The study can be used as a supporting information in classification of clopyralid.

Degradation in soil

The dRAR includes three studies that addresses the aerobic degradation of clopyralid in soil. Which are presented briefly below. The first and second study followed BBA Part IV, 4-1 (1991) and US EPA Pesticide Assessment Guidelines, Subdivision N, Paragraph 162-1 (1982), respectively. Apart from minor

deviations, these studies also met the requirements of the SETAC-Europe Guideline, Procedures for Assessing the Environmental Fate and Ecotoxicity of Pesticides, Part 1, Section 1.1 (1995). The third study followed OECD TG 307 (2002). All studies were conducted in accordance with GLP.

In the first study (**B.8.1.1.1/02**), the route of aerobic degradation of [2,6-pyridinyl-^{14}C]-clopyralid as monoethanolamine salt was investigated in a total of five European soils (one silty loam, two sandy loams one sand, and one loamy sand) under laboratory conditions at 20 ºC and 40% moisture holding capacity (MHC) for up to 12 months. The test substance was added to the soil at a concentration of 0.3 mg/l. Additionally, a degradation rate of clopyralid in a lower temperature (10 ºC), three different moisture levels (10%, 40% and 60% of MHC) as well as in three different concentrations (0.05, 0.3 and 1 mg/kg) were investigated in three of the soils. The soils were incubated in biometer flasks in the dark. Evolved CO_2 was captured with NaOH traps. The sampling interval for the main test was 0, 1, 3, 7, 14 and 28 days and 3, 6, 9 and 12 months after application. In other parts of the test the three soils were sampled at 0, 7, 14, 28, 92 and 184 days of incubation. Mass balances were between 74.88 and 113.24% of the applied radioactivity (AR) throughout the sampling periods. In the main test (5 soils, 20°C, 40% of MHC) the mean recovery of radioactivity was 99.3% of AR, whereas the mean recoveries were 99.0% of AR for testing the effect of temperature, 102.2% of AR for testing the effect of soil moisture and 103.4% of AR for testing the use rate. All recoveries indicate a complete mass balance. The derived DT_{50} and DT_{90} values for the main study were between 16 – 64 (mean 38) days and 54 – 217 (mean 127) days. The level of NER increased to between 11.2 and 35.1% AR after 92 days and subsequently plateaued in the Marcham sandy loam soil and slowly declined in the four remaining soils. The level of volatile radioactivity recovered in the side-arm trap steadily increased throughout the study to between 11.1 and 42.0% AR by 28 days, 47.5 and 65.5% AR after 92 days and to between 72.9 and 83.3% AR after 374 days.

At lower temperature, the degradation rate was significantly slower. Mean DT_{50} and DT_{90} values were 78 – 198 days and 244 – 657 days. The moist holding capacity of the soil also affected the degradation rate. At 10%, no degradation of clopyralid was observed over the whole one-year period, whereas at 60% in one soil, the degradation rate increased significantly (DT_{50} = 7 days). At lower temperature, the degradation rate declined remarkably, with the mean DT_{50}(lab) of 124 days in three soils. For the effect of application rate, the degradation was faster in the low concentration (0.05 mg/kg; DT_{50} = 18 – 23 days) compared to the high concentration (1.0 mg/kg; DT50 = 57 – 215 days). CO_2 was the only metabolite of clopyralid observed.

In the second study (**B.8.1.1.1/03**), The route of aerobic degradation of [2,6-pyridinyl-14C]-clopyralid was investigated in a silt loam soil from USA (Marshall county) under laboratory conditions at 25 ºC for 78 days. Soil samples (50 g dry weight equivalent) were added to biometer type flasks. The side-arms to the flasks were charged with potassium hydroxide solution to collect evolved CO_2. The moisture content of the soil samples was adjusted to 75% of the MHC at 1/3 bar suction by addition of distilled water. The test substance was added to the soil at a rate of 0.28 mg/kg dry soil. The mean recovery of radioactivity was 99.6% of AR, indicating that a complete mass balance was achieved. The level of radioactivity extractable from the soil declined from 100.1% AR initially to 46.5% AR after 14 days and declined further to 8.0% AR after 78 days. The level of NER increased to 19.1% AR after 28 days and subsequently plateaued at a level between 19% and 21% AR. The level of volatile radioactivity recovered in the side-arm trap steadily increased throughout the study to 73.6% AR after 78 days. Good reproducibility was observed between the replicate samples. By day 78, 73.6% of AR evolved to CO_2. The DT_{50} and DT_{90} values were 9 and 29 days, respectively.

In the third study (**B.8.1.1.1/04**), The degradation of [2,6-pyridinyl-14C]-Clopyralid was studied in four agricultural soils (one sandy loam, two clay loam and one loam) in the dark under aerobic conditions at 20 ± 2°C for 90 days. The moisture content of the soils was determined and adjusted to *ca* 42-43% of maximum water holding capacity. Clopyralid was applied to the soil at a nominal rate of 0.27 µg/g dry weight soil). The application adjusted the soils to ca 45% of maximum water holding capacity. The soils (50 g oven dry weight equivalent) were incubated in Erlenmeyer flasks connected to a series of individual traps containing ethanediol and sodium hydroxide, for the collection of non-specific ^{14}C-volatile organic compounds and $^{14}CO_2$, respectively. Material balance, calculated as the percent of applied radioactivity (% AR), was maintained from Day 0 - Day 90. Based on the results of this study, it is concluded that [^{14}C]-Clopyralid is rapidly mineralised to $^{14}CO_2$ as most of the radioactivity was recovered as $^{14}CO_2$. One minor polar metabolite was observed in the soil extracts. The metabolite did not exceed ca 3% in any soil type and was observed at a

maximum of two sampling intervals (not consecutive) in two soils. The maximum% AR recovered as $^{14}CO_2$ in soil groups A (sandy loam), B (clay loam), C (clay loam) and D (loam) was 70.27%, 68.55%, 74.31% and 68.21%, respectively. NERs accounted for 22.34 - 28.14% AR in soils by Day 90. Highest NER recovered over the duration of the study was 32.86% AR for the group C soil at Day 21. The calculated DT50 and DT90 values varied from 10.0 – 23.1 and 16.3 – 76.7, respectively. The study was performed in accordance with the quality criteria set in OECD 307.

Further evaluation of kinetic endpoints for clopyralid from laboratory soil degradation studies presented above was performed according to FOCUS Degradation Kinetics Reports (2006) (**B.8.1.1.1/01**). The DT_{50} and DT_{90} values were derived on ten soils with an adequate variety of properties. The data was tested against SFO (Single-First order) and FOMC (First Order Multiple Compartments), out of which SFO yielded robust and visually acceptable fits for each soil. Therefore, SFO was selected as the appropriate model for deriving modelling endpoints. The estimated DT_{50} values ranged from 4.9 to 64.6 days. Based on the worst-case scenario, clopyralid does not degrade rapidly in soil under aerobic conditions. Figure 1 shows a proposed degradation pathway for clopyralid, presented in the dRAR.

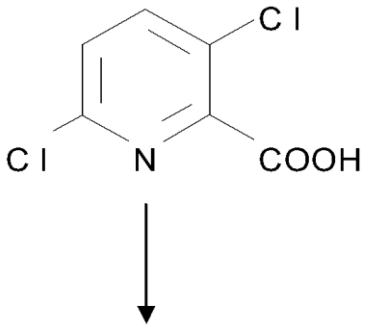


Figure 1. Proposed pathway scheme for the degradation of clopyralid in soil.

11.1.4.4 Photochemical degradation

Photolytic degradation in water

Two studies are available to address photolysis of radiolabelled clopyralid in water. In the first one (**B.8.4.1.2./01**), photodegradation of radiolabelled clopyralid at a nominal concentration of 2 mg/l was investigated in sterile aqueous buffer solutions at pH 7 under natural sunlight at 25°C. The treated solutions were exposed to natural sunlight for a period of 30 days. The study followed, apart from minor deviations, SETAC-Europe guideline, Procedures for Assessing the Environmental Fate and Ecotoxicity of Pesticides, Part 1, Section 10 (1995). Minimal degradation was observed and clopyralid was the major component detected in all samples. The level of clopyralid detected in the exposed samples declined from 99.4% AR initially to 92.7% AR after 30 days. Low levels of minor degradation products were observed which comprised of a combined total of 4.1% AR after 21 days and consisted of several components each of which were < 2% AR. The rate of observed photodegradation of clopyralid was slow and did not correlate well to first-order kinetics. The derived DT50 and DT90 values were 271 and 901 days, respectively.

The second study (**B.8.4.1.2./02**) in dRAR followed OECD TG 316. Radiolabelled clopyralid (RCP = 98.0%) in a nominal concentration of 1.80 mg/l in pH 7 buffer solution was exposed to an artificial light source at a light intensity of 489 W per m^{-2}. The study design included dark controls. The photodegradation rate of the clopyralid was calculated using according to FOCUS Kinetics Guidance (2006). Single First-Order (SFO) kinetic model was fitted to the degradation data. Further, the degradation rate was corrected to sunlight by calculating it as a function of latitude and season. The expected half-life value for clopyralid photolysis at 40° N latitude in the summer sun was 38,933 days. No significant degradation products were observed throughout the study period. The total radiocarbon recovery was 100.7 ± 4.5% and 101.4 ± 1.1% of the applied amount in the dark and in the irradiated samples, respectively.

Both presented studies were performed according to GLP. The results indicate that clopyralid is photolytically stable in water.

Photolytic degradation in soil and air

Two studies to assess photolytic degradation in soil was available in the dRAR. The first study followed US EPA Pesticide Assessment Guidelines, Subdivision N, Paragraph 161-3 (1982) which, apart from minor deviations, met the requirements of a SETAC-Europe Guideline, Procedures for Assessing the Environmental Fate and Ecotoxicity of Pesticides, Part 1, Section 2 (1995). The study was conducted in accordance with GLP. Under exposed conditions (Natural summer conditions, latitude 37.45°N at *ca* 25°C), the half-life of clopyralid was calculated to be > 12 years using pseudo first-order kinetics (R2 = 0.006). The lack of degradation seen in both the light exposed and dark control samples is reflected in the long half-life and the poor correlation co-efficient for the data set, and therefore this half-life must only be considered as an estimate.

In the second study, following OECD Draft Document (2002): Phototransformation of Chemicals on Soil Surfaces and GLP, the photodegradation of ^{14}C-clopyralid was studied in one sandy loam soil under continuous radiation of a Xenon lamp with light intensity of 374 W m^{-2} and spectral distribution comparable to natural sunlight at 37.45°N latitude for 16 days, at the temperature of 20 ± 2 °C. No significant photodegradation was observed in a sandy loam soil exposed to light using Xenon lamp as a light source. The expected DT_{50} and the DT_{90} values for clopyralid soil photolysis at 40° N latitude in the summer sun were 553 and 2040 days, respectively.

QSAR estimation of photochemical oxidation in air was performed with AOPWIN v. 1.90 (Atmospheric Oxidation Program, provided by US EPA). The hydroxyl radical rate constant was calculated to be 0.5481 x 10-12 cm3 / molecule-sec. Assuming a hydroxyl radical concentration of 1.5 x 10-6 OH / cm3 in air and a 12-hour day, a half-life of 19.513 days was obtained, indicating that photochemical oxidation of clopyralid in air is slow.

11.1.5 Conclusion on rapid degradability

The ready biodegradation study (OECD TG 301B – Modified Strum Test) pass level (>70 % mineralisation after 10 days) was not exceeded, as the CO_2 production in vessels containing 10 and 20 mg/L clopyralid was 10 and 5% of the theoretical maximum levels, respectively. Therefore, clopyralid cannot be considered readily biodegradable. According to one hydrolysis study (OECD TG 111) and two photolysis studies (OECD TG 316 and US EPA Pesticide Assessment Guidelines, Subdivision N, Paragraph 161-3 (1982)), clopyralid is also shown to be hydrolytically and photolytically stable in water.

Based on the results of simulation test in water (OECD TG 309), clopyralid is not rapidly degradable, as the determined DT_{50} of >1100 days is significantly higher than 16 days. This conclusion is further supported with the results of the presented water/sediment study (OECD TG 308), where DT_{50} of clopyralid applied to the water phase could not be determined due to low biodegradation. The DT_{50} was calculated for the dissipation from water to sediment phase instead.

According to kinetic evaluation made in accordance with FOCUS guidance from three presented soil degradation studies**,** the derived maximum DT_{50} value was 64.6 days. The photodegradation in soil is negligible (estimated DT_{50} > 12 years).

11.2 Environmental transformation of metals or inorganic metals compounds

Not relevant.

11.2.1 Summary of data/information on environmental transformation

Not relevant

11.3 Environmental fate and other relevant information

Table 35: Summary of relevant information on rapid environmental transformation

Method	Results	Remarks	Reference
Adsorption			
OECD TG 106 (1981) Adsorption/Desorption of Clopyralid in Soil: Corrections to Final Report of Study DW 2/92 from August 1993 [2,6-^{14}C]Clopyralid (purity 98.5%) GLP compliant	The soil adsorption parameters for clopyralid in four European soils: **Soil Type** / **K_f** / **K_{oc}** / **1/n** Silt loam / 0.0057 / **5.06** / 0.5577 Loamy sand / 0.0267 / **4.76** / 0.8602 Clay loam / 0.0054 / **7.34** / 0.3881 Loamy sand / 0.0125 / **3.43** / 0.7830	The sorption data indicated that clopyralid would be classified as very mobile in soil according to most classification schemes.	2002 dRAR B.8.2.1/01
OECD TG 106: [^{14}C]-Clopyralid: Adsorption to and Desorption from Five Soils Clopyralid-2,6-^{14}C (RCP 99.0%) Unlabelled Clopyralid (purity 99.9%) GLP compliant	The soil adsorption parameters for clopyralid in five European soils: **Soil Type** / **K_f** / **K_{Foc}*** / **1/n** Sandy loam / 0.01 / **0.5** / 0.489 Sandy loam / 0.08 / **2.5** / 1.047 Loamy sand / 0.03 / **4.1** / 0.889 Loam / 0.16 / **3.9** / 0.804 Clay loam / 0.14 / **2.1** / 0.829 *Coefficient adsorption per organic carbon (KF x 100/% organic carbon)	The determined KFOC values indicated that [14C]-clopyralid can be classified as being very mobile in soil	2015 dRAR B.8.2.1/02
Volatilisation			
German BBA Guidelines [2,6-pyridinyl-^{14}C]-clopyralid (RCP 97.6%, 31.5	losses due to evaporation accounted for **< 2% and ≤ 4%** AR from soil and plant surfaces respectively after 24 hours.	Clopyralid is not expected to be present in air in significant quantities for longer periods.	1994 dRAR B.8.7.1/01

Method	Results	Remarks	Reference
mCi/mmol) GLP compliant			

11.3.1 Summary of data/information on environmental fate and other relevant information

According to adsorption/desorption studies (**B.8.2.1/01** & **B.8.2.1/02**), clopyralid is very mobile substance in soil. According to evaporation study (**B.8.7.1/01**), significant volatilisation is not expected from soil or plant surface. The negligible volatilisation is further supported by low vapour pressure (1.02 x 10-5 mm Hg at 25°C) and low Henry's law constant (3.28 x 10-10 Pa m3 /mol).

11.4 Bioaccumulation

Table 36: Summary of relevant information on bioaccumulation

Method	Results	Remarks	Reference
US EPA OPP 165-4 US EPA OPP 72-6 ^{14}C labelled clopyralid (radiochemical purity >99%) non-GLP	A BCF of < 1.0 for whole fish was derived from a laboratory study in which bluegill sunfish were exposed to measured concentrations of clopyralid of 0.092 and 1.015 mg/L, under flow-through conditions for 28 days.	The study was not GLP, but the method was acceptable and sufficiently described. According to the results, clopyralid is not bioconcentrating in fish, which is further supported by its low log Kow value.	1982 dRAR B.9.2.1.4/01

11.4.1 Estimated bioaccumulation

KOWWIN v1.67 prediction of log Kow for clopyralid is 1.63.

11.4.2 Measured partition coefficient and bioaccumulation test data

According to the dRAR, the log Kow value at 20°C was -1.83 to -2.63 in pH 5-9, respectively. The values were obtained from a test following OECD TG 107. The DS notes, that the study was not acceptable in accordance with Regulation (EU) No 283/2013, due to lack of validation data presented in the study report. However, as such additional validation data is not required under CLP, the DS considers the results valid to use for classification purposes.

A higher log Kow value of 1.06 is available in the EPISUITE v4.10 experimental database.

Taken together, as the cut-off value for a substance to be considered potentially bioaccumulative for log Kow is ≥ 4, it is unlikely that clopyralid is bioaccumulative.

One bioconcentration study in fish is reported in the dRAR (**B.9.2.1.4/01**). Bluegill sunfish (*Lepomis macrochirus*; 2.5 – 3.0 in length) was exposed to nominal clopyralid concentrations of 0.1 and 1.0 mg/l for 28 days, followed by 14-day depuration in dilutant water. Concentrations of clopyralid in the test media were 92% and 101% of the nominal 0.1 and 1.0 mg/l target concentrations. No breakdown of clopyralid was observed during the exposure phase. Nine fish died during the study, which was attributed to netting and transferring the fish.

Radioactivity (dpm/g fish) in whole fish was measured. The corresponding whole fish BCF values were < 1 in both treatment groups.

The bioconcentration factor of <1 obtained in this study confirmed that clopyralid has low potential to accumulate in fish. The quality of the study was considered adequate, and the performance was sufficiently described. However, the RMS noted that there were some deficiencies in the study report, e.g., some corrections were made by hand. Indeed, DS confirmed that the protocol was corrected in some places. For example, the test duration was corrected to be 28 days instead of 30 days, and there were changes in sampling times. These corrections were signed and dated according to correct GLP procedures.

11.5 Acute aquatic hazard

Summary of relevant studies from the draft Renewal Assessment Report (dRAR) on acute aquatic hazard are reported briefly below (dRAR is annexed to this CLH proposal). Only relevant and valid studies for the proposal classification of clopyralid have been included from the dRAR. Some studies in the dRAR were conducted using Lontrel 100, which is a commercial soluble concentrate preparation of clopyralid monoethanolamine in water at a nominal concentration of 100 g clopyralid/L. These studies are not considered relevant as there are valid studies available which have been performed with the active substance.

Table 37: Summary of relevant information on acute aquatic toxicity

Method	Species	Test material	Results	Remarks	Reference
Fish					
OECD TG 203 – Fish, acute toxicity test (year not reported) EU Method C.1. US EPA FIFRA 540/9-85-006, 72-1 GLP compliant	Rainbow trout *(Oncorhynchus mykiss)*	Clopyralid (Lontrel T technical, purity 96.9%)	96h LC_{50} > 99.9 mg/l (measured concentration)	The mean measured concentration of clopyralid in the dosed vessels was 99.8 mg/l at the start and 100 mg/l at the end of the test. No mortality or sublethal effects were observed in the control or 100 mg clopyralid/L treatments. The OECD TG 203 validity criteria was fulfilled. Dissolved oxygen averaged 84%.	2000 dRAR B.9.2.1/01
OECD TG 203 – Fish, acute toxicity test EU C.1., US EPA FIFRA 540/9-85-006, 72-1. GLP	Bluegill sunfish (*Lepomis macrochirus*)	Clopyralid (Lontrel T technical, purity 96.9%)	96h LC_{50} > 102 mg/l (measured concentration)	The mean measured concentration of clopyralid in the dosed vessels was 103 mg/l at the start and 100 mg/l at the end of the test. No mortality or sublethal effects were observed in the control or 100 mg clopyralid/L treatments. The OECD TG 203 validity criteria was fulfilled. Dissolved oxygen averaged 85%.	2002 dRAR B.9.2.1/02
American Society for Toxicity and Materials (ASTM), E729-96, ASTM Standards, 2004, Vol 11-6, pp 79-100	Rainbow trout (*Onchorhyncus mykiss*), bull trout (Salvelinus confluentus)	Clopyralid salt (purity 95% a.i free acid)	All values are based on nominal concentrations. 96h EC50 = 700 mg/L (rainbow trout)	Dissolved oxygen >60% No mortality was observed in the control groups of both species. No verification of exposure concentrations No information on the specification and	Fairchild *et al.* (2008)

GLP not discussed			96h EC50 = 802 mg/L (bull trout)	impurity profile of the test substance Reliability score 1 (Klimisch) RMS Reliability score 2 (Klimisch) DS	
Aquatic invertebrates					
OECD TG 202 (part 1) - Daphnia sp. Acute Immobilisation Test EU C.2 US EPA FIFRA 540/9-85-005, 72-2 GLP	Water flea (*Daphnia magna*)	Clopyralid (Lontrel T technical, purity 96.9%)	48h EC_{50} > 99.0 mg/l (measured concentration)	The mean measured concentration of clopyralid in the dosed medium was 102 mg/l at the start and 96.0 mg/l at the end of the test. No immobilisation of daphnids was observed in the control or nominal 100 mg clopyralid/L treatments. The results are presented in. The OECD TG 202 validity criteria was fulfilled. Dissolved oxygen averaged 94%.	2000 dRAR B.9.2.2.1/01
Algae					
OECD TG 201 - Freshwater Alga and Cyanobacteria, Growth Inhibition Test (1984) US EPA OPP 123-2 ECC C.3 (1992) GLP compliant	Freshwater green algae (*Raphidocelis subcapitata*)	Clopyralid (Lontrel T technical, purity 96.9%)	All values are based on measured concentrations. 72h E_bC_{50} = 30.9 mg/l 72h E_rC_{50} = 30.0 mg/l 96h E_bC_{50} = 32.7 mg/l 96h E_rC_{50} = 33.1 mg/l	Measured concentrations of clopyralid were 94.3 to 113% of nominal at the start of the test and 97.6 to 117% at the end. OECD TG 201 validity criteria was not fulfilled.	2000 dRAR B.9.2.2/01
OECD TG 201(2004) GLP compliant	Freshwater blue algae (*Anabaena flos-aquae*)	Clopyralid (Lontrel T technical, purity 95.9%)	E_rC_{50} = 22 mg/l (measured concentration)	OECD TG 201 validity criteria was only partially fulfilled.	2006 dRAR B.9.2.2/02
US EPA FIFRA, Subdivision J, 123-2.	Freshwater blue algae (*Anabaena flos-aquae*)	Clopyralid (Lontrel T technical, purity 96.9%)	All values are based on measured concentrations. 120h E_bC_{50} 127 mg/L 120h EC_{50} 37.1 mg/L	Insufficient study reporting prevented the evaluation of validity.	2000 B.9.2.2/03
OECD TG 201	Freshwater diatom	Clopyralid (Lontrel T	E_rC_{50} = 31.3 mg/l (measured	The measured concentrations were 97 –	2014

U.S. EPA OCSPP 850.4500 GLP compliant	(*Navicula pelliculosa*)	technical, purity 95.9%)	concentration)	108% of the nominal concentrations throughout the study. OECD TG 201 validity criteria was fulfilled.	dRAR B.9.2.2/03
Aquatic macrophytes					
US EPA OPP 122-2 GLP compliant	Duckweed (*Lemna gibba*)	Clopyralid (Lontrel T technical, purity 96.9%)	14d EC_{50} = 89 mg/l (measured concentration)	Measured concentrations were similar to nominal levels throughout the study.	1990 dRAR B.9.2.3/01
OECD TG 239 - Water-Sediment *Myriophyllum Spicatum* Toxicity Test, US EPA OCSPP.SUPP GLP compliant	European watermilfoil (*Myriophyllium spicatum*)	Clopyralid (Lontrel T techinacal, purity 96.9%)	14d E_rC_{50} > 3 mg/l (nominal concentration)	Mean measured concentrations from day 0 to day 14 ranged from 98 to 104% of the nominal concentrations. OECD TG 239 validity criteria was met.	2015 dRAR B.9.2.3/02

11.5.1 Acute (short-term) toxicity to fish

Acute toxicity data on fish was available on two species: rainbow trout (*Oncorhynchus mykiss*) (**B.9.2.1/01**) and bluegill sunfish (*Lepomis macrochirus*) (**B.9.2.1/02**). Both studies followed same guidelines (OECD TG 203) and were conducted in accordance with GLP. OECD TG 203 validity criteria was fulfilled in both studies.

In the dRAR, both studies were considered reliable according to the RMS. No toxicity was observed in range finding tests with nominal concentrations of 0, 10 and 100 mg/L in either of species, and therefore definitive tests were performed as a limit tests consisting of control and 100 mg/L treatment.

The test substance concentrations were sufficiently maintained in both studies, and the mean measured concentrations were 99.9 and 102 mg/L for *O. mykiss* and for *L. macrochirus*, respectively. No mortality was observed in the 100 mg/L treatments, and therefore the derived 96h LC_{50} values for rainbow trout and bluegill sunfish were >99 and >102 mg/L, respectively. Based on the available studies clopyralid is not acutely toxic to aquatic fish up to the maximum concentration tested. The present data can be used in classification purposes of clopyralid.

One additional study was available in the dRAR for acute toxicity in fish. A 96h static acute test with clopyralid conducted by Fairchild *et al.* (2008) was performed with rainbow trout (*Oncorhynchus mykiss*) and bull trout (*Salvelinus confluentus*). This study was a peer reviewed scientific article published in a scientific journal. The study and the laboratory did not have a GLP status.

Juvenile individuals were exposed to clopyralid in nominal concentrations of 210, 420, 840, 1680 and 3360 mg/l. After 96 hours, mortality was assessed and used for calculation of acutely lethal concentrations (ALC, here on written as LC50). LC50 values were 700 mg/l and 802 mg/l for rainbow trout and bull trout, respectively. RMS in dRAR noted that due to editing restrictions of the journal, several issues were not reported in adequate detail to allow a comprehensive validity evaluation. Indeed, there was no information on e.g., whether the exposure concentrations were measured after start of the test or on the specification of the test substance. The RMS stated that Klimisch score for the study is likely 1, as the study was peer reviewed. However, DS considers that Klimisch 2 would be more suitable for this study, as the laboratory did not have GLP status, and there were deviations from the OECD TG 203 test guideline (e.g., test was conducted in 8°C)

The study can be used as a supporting information on classification of clopyralid. The results are in line with above valid studies, and indicate that clopyralid 96h LC50 for fish is > 100 mg/L.

11.5.2 Acute (short-term) toxicity to aquatic invertebrates

One study (**B.9.2.2.1/01**) with *Daphnia magna* was available to evaluate acute toxicity of clopyralid (Lontrel T technical, purity 96.9%) to aquatic invertebrates. A 48h static test was performed in compliance with GLP, following OECD TG 202 (part 1), EU C.2 and US EPA FIFRA 540/9-85-005, 72-2. Like in the fish studies, the preliminary range finding test was performed with 0, 10 and 100 mg/L nominal concentrations. No effects were observed, and the definitive test was performed as a limit test with a control and a treatment of 99.0 mg/L (mean measured).

The test substance concentration varied from 102 to 96 mg/L during the definitive test. No immobilisation of daphnids was observed in nominal 100 mg/L treatments, and consequently 48h EC_{50} was determined to be >99 mg/L. The results indicate that clopyralid is of low toxicity to aquatic

11.5.3 Acute (short-term) toxicity to algae or other aquatic plants

Four studies with freshwater green algae (*Raphidocelis subcapitata*[2]) (**B.9.2.2/01**), freshwater blue algae (*Anabaena flos-aquae*) (**B.9.2.2/02; B.9.2.2/03**) and freshwater diatom (*Navicula pelliculosa*) (**B.9.2.2/04**) were available in the dRAR to assess acute toxicity of clopyralid to algae.

Study 1 Freshwater green algae (*Raphidocelis subcapitata*)

The study followed OECD TG 201, US EPA OPP 123-2 and ECC C.3 guidelines, where an inhibitory effect of clopyralid (Lontrel T technical, purity 96.9%) on a growth is assessed over a 96h exposure period. The study was conducted in accordance with GLP and included daily measurement of light intensity, measurement of pH at the start and end of the test and continuous temperature monitoring, as well as measurement of concentrations using HPLC. A range finding test preceded the definitive test, with four nominal concentrations of clopyralid: 0.0216, 0.216, 2.16 and 21.6 mg/L. The test resulted in a 96h EC_{50} and NOEC value of > 21.6 mg/L. For the definitive test, the test system included four replicate test vessels per treatment, three of which were inoculated with 10,000 cells/ml. The nominal concentrations of clopyralid were: 0, 3.00, 6.22, 12.3, 24.2, 48.4 and 97.9 mg/L.

Measured concentrations of clopyralid were 94.3 to 113% of nominal at the start of the test and 97.6 to 117% at the end, indicating that the concentrations were well maintained during the study period. Effects on algal growth, after 72-hours exposure, ranged from a 29.2% increase in growth at 24.8 mg/L to a 98.2% inhibition of growth at 97.5 mg/L. After 96-hours, effects were 22.7% stimulation at 6.36 mg clopyralid/L to 99.1% inhibition at 97.5 mg/L. Based on mean measured concentrations, the 72-hour E_bC_{50} and E_rC_{50} values of clopyralid to *R. subcapitata* were 30.9 and **30.0 mg/L**, respectively. The 96-hour E_bC_{50} and E_rC_{50} were 32.7 and **33.1 mg/L**, respectively.

Increasements of biomass and coefficients of variation were calculated by the DS, as they were not available in the study report. The validity criteria for the OECD TG 201 test were not fulfilled: The mean increasement of biomass in the controls was <16-fold (14.2-fold, specific growth rate 0.886 day^{-1}) in the first 72 hours. The coefficient of variation of average specific growth rates in replicate controls during the whole test was 10.4% exceeding the validity criterion (< 7%). The mean coefficient of variation for section-by-section specific growth rates (days 0-1, 1-2, 2-3, 3-4) was 71.4% exceeding the criterion (< 35%).

The study can be used as a supporting information when determining the classification for clopyralid.

Study 2 & 3 Freshwater blue algae (*Anabaena flos-aquae*)

Two studies were available for *A. flos-aquae.* In the first study, effects of clopyralid (Lontrel T technical, purity 95.9%) on growth were investigated following OECD TG 201 (2004). The study was performed in accordance with GLP. The algae were exposed to nominal treatment levels of 0 (control), 3.0, 6.2, 13, 24, 47 and 99 mg a.i./L clopyralid ran in three replicates. Control treatments included six replicates. The test flasks were illuminated during the test with a light intensity range of 1900 – 2600 lux. Temperature was 23±1°C

[2] Formerly known as *Selenastrum capricornutum* and *Pseudokirchneriella subcapitata*

and pH varied from 3.1 to 7 (test initiation) and 3.0 to 6.7 (test termination). The test concentrations were confirmed with HPLC/UV and were found to be similar to nominal concentrations.

All flasks were conditioned prior to use by rinsing with the appropriate exposure or control solution. One hundred millilitres of the appropriate test solution were then placed in each replicate flask. Test duration was 5 days (120 hours), in which observations of the health of the algal cells were made at each 24-hour interval. At each interval, duplicate cell counts were conducted on each replicate vessel of the treatment levels and the control using a haemocytometer (Neubauer Improved) and a compound microscope. Two independent samples were removed from each flask for counting. One or more haemocytometer fields, each 0.10 x 0.10 cm in surface area and 0.010 cm deep and containing 0.00010 ml of culture, were examined for each sample until at least 400 algal cells or four fields were counted.

Average specific growth rate (μ_{ave}) for each replicate flask was calculated for the period from test initiation to each observation time. Yield was calculated as biomass (cell density) at each interval of the test minus the initial biomass at the start of the test. Percent inhibition of the treatment data was calculated relative to the control data. The results of the study are reported in Table 38 below

Table 38. Effects of clopyralid on algal growth based on mean measured concentrations

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

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

The validity criteria of OECD TG 201 are only partially fulfilled: The initial cell density in controls was 1 * 10^4 cells/ml. The mean control cell density at the end of the test was 54.6 * 10^4 cells/ml. Cell density grew thus 54.6 x during the test (>16x). The coefficient of variation of average specific growth rates in replicate controls during the whole test was 120 % exceeding the validity criterion (< 7 %). The mean coefficient of variation for section-by-section specific growth rates was 19.2 % fulfilling the validity criterion (< 35 %).

The study can be used as a supporting information when determining classification for clopyralid.

The second study with *A. flos-aquae* followed US EPA FIFRA, Subdivision J, 123-2 Guideline and was performed in accordance with GLP. There were four replicate test vessels per treatment, three of which were inoculated with 10,000 cells/mL. The nominal concentrations of clopyralid were: 0 (control containing algal assay medium), 0.80, 1.60, 3.20, 6.40, 12.8, 24.0 and 50.2 mg/L. The test medium was not renewed during the study.

Temperature was monitored continuously, and pH was measured at the start and end of the test. Over the 96-hour exposure period the temperature averaged 25.5 ± 0.1°C. The pH values ranged from 4.1 to 8.0 in media without algae and 4.2 to 8.6 with algae. Measured concentrations of clopyralid were 90.3 to 100% of nominal at the start of the test and 102 to 108% at the end. Effects on algal growth, after 72-hours exposure, ranged from 17.7% increase in growth at 1.60 mg/L to 97.4% inhibition of growth at 52.3 mg/L. The results of the test are reported in the Table 39. E_rC_{50} based on growth rate was not available in the study report.

Table 39. Summary of results with *A. flos-aquae*

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

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

Dossier submitter evaluated the validity of the test. Algal cell count was reported only for day 0 and day 5, which meant that the increasement of cells or coefficients of variations for growth rates could not be calculated. Therefore, the validity of the study results could not be thoroughly assessed. It is concluded that the results can be used as a supporting information in classification of clopyralid. According to the Guidance on Application of CLP criteria, "in circumstances where the basis of the EC_{50} is not specified and no E_rC_{50} is recorded, classification shall be based on the lowest EC_{50} available". Therefore, the lowest EC_{50} (37.1 mg/L) should be used.

Study 4 Freshwater diatom (*Navicula pelliculosa*)

The effects of clopyralid (Lontrel T technical, purity 95.9%) on freshwater diatom (*Navicula pelliculosa*) were investigated in a GLP compliant study that followed OECD TG 201 guideline. Before the test initiation, 6 control replicates and 4 test substance replicates containing 100 mg a.i./L parent solutions were inoculated with 1 mg a.i./L algae concentrate, containing approximately 1.0 x 106 cells/ml, resulting in a final density of approximately 1.0 x 104 cells/ml for each flask. After 30 minutes of inoculation, the test substance replicates (6 control and 4 test concentration replicates) were exposed to clopyralid in a nominal concentration of 0 (control), 1.5, 3.0, 6.0, 12, 24, and 48 mg a.i./L. The sampling took a place at 24, 48, 72, and 96 hours (±1 hour), in which the cell densities in each replicate was measured using hemocytometer.

The area under the growth curve in each treatment was calculated from 0-hour to 24, 48, 72, and 96 hours. The I_bC_{50}, I_rC_{50}, and I_yC_{50} estimates were calculated using a logistic (sigmoid-shaped) model fit to the data with percent inhibition as the dependent variable and concentration as the independent variable. The model used to describe the response to increasing test substance concentrations was the four-parameter logistic model with two parameters fixed; the minimum percent inhibition at 0%, and the maximum percent inhibition at 100%. The model was fit only in instances where the mean percent inhibition at the highest test substance treatment was greater than 45%. In instances where there was no test substance treatment inhibition that was greater than 45% any values generated from this model should be considered as estimated values. A nonlinear modeling procedure was used to estimate the slope and IC values. The distribution of xˆ method was used to estimate the 95% confidence limits. Concentrations of clopyralid were well maintained in test solutions during the test (Table 40).

Table 40. Measured concentrations of clopyralid during the test.

Nominal concentration mg/L (clopyralid)	hours					
	0	72	96	% of nominal	arithmetic mean	geometric mean
0	<MQL	<MQL	<MQL			
1.5	1.58	1.61	1.6	105 - 107	1.60	1.60
3	3.01	3.17	3.12	100 - 106	3.10	3.10
6	5.82	6.2	6.11	97 - 103	6.04	6.04
12	12.1	12.4	12.9	101 - 108	12.47	12.46

24	26.5	26.5	23.8	99 - 110	25.60	25.57
48	49.1	51.6	51.6	108 - 102	50.77	50.75

The results of the study are presented in Table 41. 72h and 96h EC_{50} based on growth was derived to be **31.3 and 30.5 mg a.i./L**.

Table 41. Algal growth inhibition results with diatom based on 72-hour arithmetic mean concentrations

Hour	IC Type	IC Value (mg a.i./L)	95% Confidence Limits (mg a.i./L)	NOEC (mg a.i./L)
72 Hours	I_bC_{05}	23.9	22.9 and 25.0	1.60
	I_bC_{10}	25.8	24.6 and 26.9	
	I_bC_{20}	27.9	26.7 and 29.2	
	I_bC_{50}	32.1	30.7 and 33.5	
	I_rC_{05}	21.5	6.29 and 36.7	1.60
	I_rC_{10}	23.6	10.9 and 36.4	
	I_rC_{20}	26.2	15.4 and 37.0	
	I_rC_{50}	**31.3**	16.5 and 46.0	
	I_yC_{05}	23.5	22.2 and 24.8	1.60
	I_yC_{10}	25.3	23.9 and 26.7	
	I_yC_{20}	27.4	25.9 and 28.9	
	I_yC_{50}	31.5	29.8 and 33.2	
96 Hours	I_bC_{05}	21.4	21.1 and 21.6	1.60
	I_bC_{10}	23.0	22.7 and 23.3	
	I_bC_{20}	25.0	24.7 and 25.2	
	I_bC_{50}	28.7	28.3 and 29.0	
	I_rC_{05}	22.7	13.8 and 31.6	1.60
	I_rC_{10}	24.4	14.8 and 34.0	
	I_rC_{20}	26.5	16.1 and 36.9	
	I_rC_{50}	**30.5**	18.5 and 42.4	
	I_yC_{05}	20.2	20.0 and 20.3	1.60

The validity criteria of OECD TG 201 were met for this study. The number of algal cells in the control was greater than 16 times the number initially inoculated after the initial 72 hours of testing and greater than 30 times the number initially inoculated after 96 hours, verifying logarithmic phase growth. The coefficient of variation for daily growth rates in the control replicates during the test did not exceed 35% during the first 72 hours of testing. The coefficient of variation for average specific growth rates in the control replicates did not exceed 10% during the initial 72 hours of testing. The coefficients of variation for mean control yield and average specific growth rates in control replicates at termination did not exceed 15%. The pH in the control did not increase more than 1.5 units during the initial 72 hours of testing. This study can be used for classification purposes of clopyralid.

Effects on aquatic macrophytes

Study 1 duckweed (*Lemna gibba*)

The inhibitory effect of clopyralid (96.4 %) on the growth of the duckweed *Lemna gibba*, by means of assessing phytotoxicity, has been assessed over a 14-day exposure period, based on US EPA OPP 122-2 guideline. The study was conducted in accordance with GLP. In preceding range-finding test, two cultures each containing 15 fronds, were treated either with clopyralid (nominal concentrations of 1, 10, 100 and 1000 mg/L) or were cultured in growth medium only for a 14-day period. The results of the range-finding test indicated that the 14-day EC_{50} would be less than 100 mg/L.

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

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

Table 42: Measured concentrations of clopyralid in test medium during a growth inhibition study with *Lemna gibba*

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

As a result, the determined 14-day EC_{50} of clopyralid to the duckweed *L. gibba* was **89 mg/L** based on mean measured concentrations under static test conditions. The study was well performed and reported and can be used in classification of clopyralid.

Study 2 Eurasian watermilfoil (*Myriophyllum spicatum*)

Eurasian watermilfoil (*Myriophyllum spicatum*) shoots were exposed to clopyralid for 14 days under static conditions, following a 7-day acclimation period. The study followed OECD TG 239 (includes sediment) and was performed in accordance with GLP. Shoots within a replicate were planted in sediment within a 300 mL borosilicate glass crystallization dish housed in a 2 L glass beaker. Parameters measured included growth rate and yield (NOEC, LOEC and EC_{50}) of total shoot lengths, total plant wet weight and total plant

dry weight. The water phase was spiked with clopyralid in nominal concentrations of 8.9, 28.6, 91.6, 293, 938 and 3000 µg a.i./L.

Mean measured recoveries from the water phase at day 0 and 14 ranged from 98 to 104% of the nominal concentrations, indicating that applied clopyralid stayed in the water phase throughout the test, and did not dissipate into the sediment. This observation is supported by the results of the OECD 308 test presented in the section 11.4.3, where DT50 of clopyralid for dissipation from water to sediment was 128 – 167 days.

The toxicity values were calculated based on nominal concentrations in units of µg a.i./L. According to ECHA Endpoint Specific Guidance R.7b, nominal concentrations can be used if measured concentrations were within 20% of the nominal concentrations. No abnormal shoot or root development was observed in any treatment level as compared to the control group throughout the study. The lowest E_yC_{50} for yield in the 14-day exposure to clopyralid was obtained for total shoot length. The statistical NOE_rC, LOE_rC and E_rC_{50} for this endpoint were 8.9, 28.6 and 1225 µg a.i./L, respectively.

The E_rC_{50} for growth rate in the 14-day exposure of the rooted aquatic macrophyte *Myriophyllum spicatum* to clopyralid was **>3 mg a.i./L for all three endpoints** (total shoot length, fresh weight, and dry weight).

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

The study can be used in classification of clopyralid. *M. spicatum* is the most sensitive species to clopyralid, and therefore the acute toxicity classification is based on this study.

11.5.4 Acute (short-term) toxicity to other aquatic organisms

Not available

11.6 Long-term aquatic hazard

Summary of relevant studies from the draft Renewal Assessment Report (dRAR) on chronic aquatic hazard are reported briefly below (dRAR is annexed to this CLH proposal).

Table 43: Summary of relevant information on chronic aquatic toxicity

Method	Species	Test material	Results	Remarks	Reference
Fish					
OECD TG 210 US EPA FIFRA EPA-540/86-138 ASTM Standard E 1241-92 GLP compliant	Fathead minnow (*Pimephales promelas*)	Clopyralid (Lontrel T technical, purity 96.9%) purity 96.9%	34d NOEC = **10.8** mg/l (measured)	Concentrations of clopyralid in the test media averaged 104 to 114% of nominal. No statistically significant ($p< 0.05$) effects, for any endpoint, compared to controls were observed up to the highest test mean measured concentration of 10.8 mg clopyralid/L.	2000 dRAR B.9.2.1.2

Aquatic invertebrates					
OECD TG 202 (part 2, 1984) GLP	Water flea (*Daphnia magna*)	Clopyralid (Lontrel T, purity 95.7%)	21d NOEC (reproduction) = **17 mg/L(**measured concentration)	Measured concentrations remained within the range of 79 – 111% of nominal throughout the study with a mean value of 99% and the results are based on mean measured concentrations. The validation criteria were met, and the study is acceptable.	1992 dRAR B.9.2.2.2
Algae					
OECD TG 201 - Freshwater Alga and Cyanobacteria, Growth Inhibition Test (1984) US EPA OPP 123-2 ECC C.3 (1992) GLP compliant	Freshwater green algae (*Raphidocelis subcapitata*)	Clopyralid (Lontrel T technical, purity 96.9%)	Results are derived from measured concentrations. **72h NOEC < 3.45 mg/l** **96h NOEC 24.8 mg/l.**	Measured concentrations of clopyralid were 94.3 to 113% of nominal at the start of the test and 97.6 to 117% at the end. OECD TG 201 validity criteria was not fulfilled.	2000 dRAR B.9.2.2/01
OECD TG 201(2004) GLP compliant	Freshwater blue algae (*Anabaena flos-aquae*)	Clopyralid (Lontrel T technical, purity 95.9%)	Results are derived from measured concentrations. **72h NOEC (growth) = 13 mg/l** **96h NOEC (growth) = 24 mg/l** 72h NOEC (yield) = 13 mg/l 96h E_yC_{10} 24 mg/l	OECD TG 201 validity criteria was only partially fulfilled.	2016 dRAR B.9.2.2/02
US EPA FIFRA, Subdivision J, 123-2.	Freshwater blue algae (*Anabaena flos-aquae*)	Clopyralid (Lontrel T technical, purity 96.9%)	120h NOEC = 24.2 mg/L (measured)	Insufficient study reporting prevented the evaluation of validity.	2000 B.9.2.2/03
OECD TG 201 U.S. EPA OCSPP 850.4500 GLP compliant	Freshwater diatom (*Navicula pelliculosa*)	Clopyralid (Lontrel T technical, purity 95.9%)	Results are derived from measured concentrations 72h E_bC_{10} = 25.8 mg/l **72h E_rC_{10} = 23.6 mg/l** 72h E_yC_{10} = 25.3 mg/l	The measured concentrations were 97 – 108% of the nominal concentrations throughout the study. OECD TG 201 validity criteria was fulfilled.	2014 dRAR B.9.2.2/04

			96h E_bC_{10} = 23 mg/l **96h E_rC_{10} = 24.4 mg/l**		
Aquatic macrophytes					
US EPA OPP 122-2 GLP compliant	Duckweed (*Lemna gibba*)	Clopyralid (Lontrel T technical, purity 96.9%)	Results are derived from measured concentrations **14d NOEC = 7.2 mg/l**	Measured concentrations were similar to nominal levels throughout the study	1990 dRAR B.9.2.3/01
OECD TG 239 - Water-Sediment *Myriophyllum Spicatum* Toxicity Test US EPA OCSPP.SUPP GLP compliant	European watermilfoil (*Myriophyllium spicatum*)	Clopyralid (Lontrel T techinacal, purity 96.9%)	Results are derived from nominal concentrations **14d NOEC (growth) = 8.9 µg/l** 14d NOEC (yield) = 8.9 µg/l	Mean measured concentratons from day 0 to day 14 ranged from 98 to 104% of the nominal concentrations. OECD TG 239 validity criteria was met.	2015 dRAR B.9.2.3/02
Other species					
BBA 1995 GLP	Midge (*Chironomus riparius*)	Clopyralid technical (purity 95.8%) [2,6-pyridinyl-^{14}C]-Clopyralid (RCP > 97.0%, specific radioactivity = 30.9 mCi/mmol)	28d NOEC (emergence) = **50 mg/l** 28d NOEC (development) ≥ **97 mg/l**	Concentrations in the overlying water were 95 to 100% of the applied dose levels at the start of the test. These levels fell to 85 to 92% on Day 28, indicating that clopyralid did not partition to the sediment phase in significant quantities. Pore water contained only 1.5 to 1.8% of the total clopyralid added after 28 days. Samples of sediment contained < 4% of applied clopyralid.	2001 dRAR B.9.2.2.3.

11.6.1 Chronic toxicity to fish

One long-term fish toxicity study with Fathead minnow (Pimephales promelas) was available in the dRAR (B.9.2.1.2). The study followed OECD 210 and was performed in accordance with GLP. Approximately 25 to 45 hours old embryos were exposed to the following nominal concentrations of clopyralid (purity 96.9%): 0 (control), solvent control (acetone), 0.78, 1.30, 2.16, 3.60, 6.00 and 10.0 mg/L. For each exposure concentration and the control (laboratory dilution water) there were four replicate aquaria each containing 850 mL of test medium and 25 embryos per replicate. Clopyralid was prepared in stock solutions with acetone before dilution and mixing with laboratory dilution water and entry to the test aquaria. During the test, the diluter system provided an average of 4.1 volume changes per 24-hour period. Embryos were incubated in circular cups suspended in a cylindrical glass incubation chamber supported by glass beads within the aquarium. Flow from the delivery tubes was directed in and around the incubation cups.

Dissolved oxygen, pH and temperature were recorded on test days 0, 7, 14, 21, 28 and 34 in each aquarium containing surviving fish. The test conditions were as follows: Temperature 24.3 to 25.7°C, pH 6.1 to 8.0, and dissolved oxygen 50 to 115%. Concentrations of clopyralid in the test media averaged 104 to 114% of nominal.

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

On day 33 of exposure, a portion of the laboratory water was diverted resulting in a decline in dissolved oxygen from a range of 75 to 115% to 50 to 81%. This caused larval mortalities which were sporadic and not treatment related. However, by this time the exposure duration was 30 days after mean hatch and the control fish survival was > 70% thereby maintaining compliance with the OECD TG 210 validity criteria. Following a 34-day exposure to clopyralid, under flow-through conditions, the NOEC for the early life-stages of Pimephales promelas was 10.8 mg/L, the highest concentration tested, based on mean measured concentrations. The LOEC was > 10.8 mg/L.

The study fulfilled OECD TG 210 validity criteria and can be used in classification of clopyralid.

11.6.2 Chronic toxicity to aquatic invertebrates

The dRAR included one OECD TG 202 (part 2) reproduction test with water flea (Daphnia magna) (B.9.2.2.2).Effects of clopyralid (purity 95.7%) on the survival and reproduction were assessed over a 21-day exposure period under semi-static conditions. The nominal exposure concentrations were 0, 5.6, 18, 56, 180 and 560 mg/L. For each exposure concentration and the control treatment (diluent water only, dechlorinated, aged laboratory tap water, hardness 350 mg/L as CaCO3), 40 daphnids were placed in 4 replicate glass flasks (10 per flask) containing 400 mL of test medium. Clopyralid was prepared in the diluent water by direct addition. Cultures were fed daily with a mixture of fry fish food and mixed algae. The test media were renewed 3 times per week on days 2, 5, 7, 9, 12, 14, 16 and 19.

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

Measured concentrations as a percent of nominal were 86 to 111% for freshly prepared and 81 to 108% for expired media. The results of the study were based on the following mean measured concentrations of clopyralid: 5.3, 17, 59, 190 and 590 mg/L. After 21 days exposure, survival of parental daphnids ranged from 100% in the control and 5.3 mg clopyralid/L treatments to 0% at 190 and 590 mg/L.

Lethal effects on the parents were most pronounced during the first 4 days of exposure with mortality occurring throughout exposure at the highest treatment level. The number of live young per female was 45 in the control and 5.3 mg/L treatments, 42 at 17 mg /L, 29 at 56 mg/L and 0 at the two highest treatment levels of 190 and 590 mg/L. The results of the study are presented in Table 44.

Table 44: Summary of results in reproduction test with D. magna

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

mean number of juveniles per surviving female at the end of the test	
EC_{10}	**EC_{20}**
23.499 (95% CL: 17.434- 28.251)	36.328 (95% CL: 30.803- 40.6)

The study fulfilled OECD TG 201 validity criteria and can be used in classification purposes for clopyralid.

11.6.3 Chronic toxicity to algae or other aquatic plants

Four studies are available for algae (species *R. subcapitata, A. flos-aquea, N. pelliculosa*) and two studies are available for the aquatic macrophytes (species *L. gibba* and *M. spicatum*). All the studies presented below are already summarised in section **11.5.3**.

Study 1 Freshwater green algae (*Raphidocelis subcapitata*)

This study followed OECD TG 201, US EPA OPP 123-2 and ECC C.3 guidelines, where an inhibitory effect of clopyralid (96.9%) on a growth is assessed over a 96h exposure period. As a result, the statistically derived 72-hour NOEC was **< 3.45 mg/L**, although at 6.38, 11.8 and 24.8 mg clopyralid/L, there was no significance difference from the controls. The 96-hour NOEC was 24.8 mg clopyralid/L.

The validity criteria for the OECD TG 201 test were not fulfilled: The mean increasement of biomass in the controls were < 16-fold (14.2-fold, specific growth rate 0.886 day^{-1}) in the first 72 hours. The coefficient of variation of average specific growth rates in replicate controls during the whole test was 10.4% exceeding the validity criterion (< 7%). The mean coefficient of variation for section-by-section specific growth rates (days 0-1, 1-2, 2-3, 3-4) was 71.4% exceeding the criterion (< 35%).

This study can be used as a supportive information.

Study 2 & 3 Freshwater blue-green algae (*Anabaena flos-aquae*)

In the first study, the effects of clopyralid (purity 95.9 %) on growth of A. flos-aquae were investigated with OECD TG 201 (2004). The study was performed in accordance with GLP. The 72-hour NOEC for growth rate was determined to be **13 mg/L**.

The validity criteria of OECD TG 201 are only partially fulfilled: The initial cell density in controls was 1 * 10 4 cells/ml. The mean control cell density at the end of the test was 54.6 * 10 4 cells/ml. Cell density grew thus 54.6-fold during the test (>16-fold). The coefficient of variation of average specific growth rates in replicate controls during the whole test was 120 % exceeding the validity criterion (< 7 %). The mean coefficient of variation for section-by-section specific growth rates was 19.2 % fulfilling the validity criterion (< 35 %).

The second study followed US EPA FIFRA, Subdivision J, 123-2 Guideline and was performed in accordance with GLP. *A. flos-aquae* was exposed to clopyralid (purity 96.9%) at a nominal concentration of 0 (control containing algal assay medium), 0.80, 1.60, 3.20, 6.40, 12.8, 24.0 and 50.2 mg/L. The derived 120-hour NOEC from the study was 24.2 mg/L.

Due to insufficient reporting, the validity of the study could not be assessed.

Studies 2 and 3 can be used as a supportive information in classification of clopyralid.

Study 4 Freshwater diatom (*Navicula Pelliculosa*)

The effects of clopyralid (purity 95.9%) on freshwater diatom (*Navicula pelliculosa*) was investigated in a study that followed OECD TG 201 and U.S. EPA OCSPP 850.4500 guidelines and was in compliance with GLP. The lowest chronic endpoint was 72h and 96h NOEC of 1.60 mg/L.

The validity criteria of OECD TG 201 were met for this study. The number of algal cells in the control was greater than 16 times the number initially inoculated after the initial 72 hours of testing and greater than 30 times the number initially inoculated after 96 hours, verifying logarithmic phase growth. The coefficient of variation for daily growth rates in the control replicates during the test did not exceed 35% during the first 72 hours of testing. The coefficient of variation for average specific growth rates in the control replicates did not exceed 10% during the initial 72 hours of testing. The coefficients of variation for mean control yield and average specific growth rates in control replicates at termination did not exceed 15%. The pH in the control did not increase more than 1.5 units during the initial 72 hours of testing.

The study can be used in classification of clopyralid.

Aquatic macrophytes

Study 1 *Lemna gibba*

The inhibitory effect of clopyralid (96.4 %) on the growth of the duckweed *Lemna gibba*, by means of assessing phytotoxicity, has been assessed over a 14-day exposure period, based on US EPA OPP 122-2 guideline. The derived NOEC was **7.2 mg/L**, measured, based on significant reductions in fronds and plants at 12.0 mg/L and above.

The study was valid and can be used in classification of clopyralid.

Study 2 *Myriophyllium spicatum*

The statistical NOE_rC and LOE_rC for all three endpoints (total shoot length, fresh weight and dry weight) were **8.9 and 28.6 µg a.i./L, respectively**.

Based on the OECD TG 239, two validity criteria are established for this test design. Both of these criteria were met during the exposure:

1) The mean total shoot length and shoot fresh weight in control plants must at least double during the exposure phase of the test and control plants must not show any visual symptoms of chlorosis.

During the exposure all control plants appeared normal, throughout the duration of the test, with no visual symptoms of chlorosis. Active growth of the control plants was demonstrated by a total shoot length yield of 37.0 cm and a wet weight yield of 1.5888 grams. This represents a 5.5x and 5.9x increase in yields for these endpoints, respectively, and thus the first validity criterion is fulfilled.

2) The mean coefficient of variation of yield based on measurements of shoot fresh weight in the control cultures must not exceed 35%. In this study the percent coefficient of variation for shoot fresh weight yield between the ten control replicates was 15.1%, thus fulfilling the second validity criteria.

M. spicatum is the most sensitive species to clopyralid, and therefore the classification is based on this study.

11.6.4 Chronic toxicity to other aquatic organisms

One development and emergence toxicity test to the sediment dwelling phase of the midge (*Chironomus riparius*) was available in the dRAR. The study followed BBA 1995 guideline and was performed in

accordance with GLP. Effects of clopyralid (purity 95.8 %) on the sediment dwelling phase of the midge (*Chironomus riparius*) were assessed under laboratory test conditions. Based on the outcome of a range-finding test, larvae of *C. riparius* were exposed to the following nominal concentrations of clopyralid applied to the aqueous phase of a water: sediment test system: 6, 13, 25, 50 and 100 mg/L. The aqueous test media were prepared with radiolabelled clopyralid and non-radiolabelled clopyralid and diluted with water ('Elga').

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

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

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

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

The study can be used as a supporting information in classification of clopyralid. However, as there are quality studies available for relevant endpoints, the relevance of this study in classification of clopyralid is low.

11.7 Comparison with the CLP criteria

11.7.1 Acute aquatic hazard

Adequate data for acute aquatic toxicity of clopyralid was available on three trophic levels: fish, aquatic invertebrates, algae/aquatic macrophytes. All acute endpoints were above 1 mg/L. The most sensitive species was aquatic macrophyte *Myriophyllum spicatum*, with a 14d-EC_{50} value of > 3 mg a.i./L (total shoot length, fresh weight and dry weight). Based on the available data it is concluded that clopyralid **does not fulfil the criteria for classification as Aquatic Acute Category 1 (≤ 1 mg/L) according to the CLP.**

11.7.2 Long-term aquatic hazard (including bioaccumulation potential and degradation)

Bioaccumulation

According to one BCF study (**B.9.2.1.4/01**) conducted on Bluegill sunfish (*Lepomis macrochirus*), the BCF of clopyralid is < 1, and therefore clopyralid has a low potential to bioaccumulate in aquatic environment. This conclusion is further supported by low experimental and estimated Log K_{ow} values that are < 4.

Biodegradation

The ready biodegradation study (OECD TG 301B – Modified Strum Test) pass level (>70 % mineralisation after 10 days) was not exceeded, as the CO_2 production in vessels containing 10 and 20 mg/L clopyralid was 10 and 5% of the theoretical maximum levels, respectively. Therefore, clopyralid cannot be considered rapidly biodegradable. According to one hydrolysis study (OECD TG 111) and two photolysis studies

(OECD TG 316 and US EPA Pesticide Assessment Guidelines, Subdivision N, Paragraph 161-3 (1982)), clopyralid is also shown to be hydrolytically and photolytically stable in water.

Non-rapid degradation is further supported with the results of simulation test in water (OECD TG 309), as the determined DT_{50} of >1100 days is significantly higher than 16 days. The results are also in line with the results from presented water/sediment study (OECD TG 308), where DT_{50} of clopyralid applied to the water phase could not be determined due to low biodegradation. The DT_{50} was calculated for the dissipation from water to sediment phase instead.

Toxicity

Sufficient long-term toxicity data was available on three tropic levels (fish, aquatic invertebrates and algae/aquatic macrophytes). The lowest endpoints for each tropic levels were as follows: 34d NOEC = 10.8 mg/L for early life-stages of fish (*Pimephales promelas*), 21d NOEC = 17 mg/L for reproduction of aquatic invertebrate (*Daphnia magna*), 72h and 96h NOEC = 1.60 mg/L for growth in algae (*Navicula pelliculosa*) and nominal NOEC = 8.9 µg/L for three endpoints (total shoot length, fresh weight and dry weight) in aquatic macrophyte (*Myriophyllum spicatum*). Based on the results, *M. spicatum* is the most sensitive species for herbicide clopyralid.

Since clopyralid is non-rapidly degradable and adequate chronic toxicity data are available for all trophic levels, clopyralid can be classified according to the criteria set out in CLP in Table 4.1.0(b)(i). In this case classification of Aquatic Chronic 1 is applicable for clopyralid based on the lowest **NOEC** value of **8.9 µg/l** for *Myriophyllum spicatum* (≤ 0.1 mg/l) with a chronic M-factor of 10 (0.001 < NOEC ≤ 0.01 mg/l).

11.8 CONCLUSION ON CLASSIFICATION AND LABELLING FOR ENVIRONMENTAL HAZARDS

Table 45 Conclusions on classification and labelling for environmental hazards of clopyralid.

Hazard Class and Category code(s)	M factor	Hazard Statement
Aquatic Chronic Category 1, H410	10	Very toxic to aquatic life with long lasting effects

12 EVALUATION OF ADDITIONAL HAZARDS

12.1 Hazardous to the ozone layer

Not assessed in this dossier.

13 ADDITIONAL LABELLING

14 REFERENCES

Fairchild et al. (2018): An ecological risk assessment of the acute and chronic toxicity of the herbicide picloram to the threatened bull trout (Salvelinus confluentus) and the rainbow trout (Onchorhyncus mykiss). Archives of environmental contamination and toxicology, 56(4), 761-769.

Final Renewal report for the active substance clopyralid (2021). Available online: https://ec.europa.eu/food/plant/pesticides/eu-pesticides-database/active-substances/?event=as.details&as_id=566

Finland, 2017. Draft Renewal Assessment Report (RAR) on clopyralid prepared by the rapporteur Member State Finland in the framework of Commission Implementing Regulation (EU) No 844/2012, 2017. Available online: https://www.efsa.europa.eu/en/consultations/call/170823

Peer review of the pesticide risk assessment of the active substance clopyralid. EFSA Journal 2018;16(8):5389, available online: https://doi.org/10.2903/j.efsa.2018.5389

Reuter et al., 2018, 46th Annual Meeting of the European Teratology Society 10.-13.09.2018, Berlin, Germany.

15 ANNEXES

Finland, 2017. Draft Renewal Assessment Report (RAR) on clopyralid prepared by the rapporteur Member State Finland in the framework of Commission Implementing Regulation (EU) No 844/2012, 2017. Available online: https://www.efsa.europa.eu/en/consultations/call/170823