Committee for Risk Assessment

RAC

Opinion

proposing harmonised classification and labelling
at EU level of

clopyralid (ISO); 3,6-dichloropyridine-2-carboxylic acid

EC Number: 216-935-4
CAS Number: 1702-17-6

CLH-O-0000007365-71-01/F

Adopted

14 September 2023

14 September 2023

CLH-O-0000007365-71-01/F

OPINION OF THE COMMITTEE FOR RISK ASSESSMENT ON A DOSSIER PROPOSING HARMONISED CLASSIFICATION AND LABELLING AT EU LEVEL

In accordance with Article 37 (4) of Regulation (EC) No 1272/2008, the Classification, Labelling and Packaging (CLP) Regulation, the Committee for Risk Assessment (RAC) has adopted on **14 September 2023** by **consensus** an opinion on the proposal for harmonised classification and labelling (CLH) of:

Chemical name: **clopyralid (ISO); 3,6-dichloropyridine-2-carboxylic acid**

EC Number: **216-935-4**

CAS Number: **1702-17-6**

Rapporteurs, appointed by RAC: **Normunds Kadiķis**

Co-Rapporteur, appointed by RAC: **Anja Menard Srpčič**

Administrative information on the opinion

Finland has submitted on **31 August 2022** a CLH dossier containing a proposal together with the justification and background information documented in a CLH report.

The CLH report was made publicly available in accordance with the requirements of the CLP Regulation at ***http://echa.europa.eu/harmonised-classification-and-labelling-consultation/*** on **17 October 2022**.

Concerned parties and Member State Competent Authorities (MSCA) were invited to submit comments and contributions by **16 December 2022**.

The opinion takes into account the comments provided by MSCAs and concerned parties in accordance with Article 37(4) of the CLP Regulation and the comments received are compiled in Annex 2.

The following table provides a summary of the Current Annex VI entry, Dossier submitter proposal, RAC opinion and potential Annex VI entry if agreed by the Commission.

Classification and labelling in accordance with the CLP Regulation (Regulation (EC) 1272/2008)

	Index No	Chemical name	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors and ATE	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	GHS05 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	
RAC opinion	607-231-00-1	clopyralid (ISO); 3,6-dichloropyridine-2-carboxylic acid	216-935-4	1702-17-6	**Add** Aquatic Chronic 1	**Add** H410	**Add** GHS09	**Add** H410	EUH066	M = 10	
Resulting Annex VI entry if agreed by COM	607-231-00-1	clopyralid (ISO); 3,6-dichloropyridine-2-carboxylic acid	216-935-4	1702-17-6	Eye Dam. 1 Aquatic Chronic 1	H318 H410	GHS05 GHS09 Dgr	H318 H410	EUH066	M = 10	

GROUNDS FOR ADOPTION OF THE OPINION

RAC general comment

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

HUMAN HEALTH HAZARD EVALUATION

RAC evaluation of skin corrosion/irritation

Summary of the Dossier Submitter's proposal

According to the data available, the dossier submitter (DS) concluded that clopyralid does not require classification as skin irritant according to the CLP Regulation. Instead, they proposed to apply the EUH066 statement - 'Repeated exposure may cause skin dryness or cracking'.

Comments received during consultation

No comments were received.

Assessment and comparison with the classification criteria

The DS provided two animal studies on skin corrosion/irritation performed with New Zealand White (NZW) rabbits (Anonymous, 1987; Anonymous, 1990).

In the first study (Anonymous, 1987), three animals per sex were tested. The test material (0.5 g of 95.4 % clopyralid) 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 h exposure period. After wiping off the test substance, the application site of each rabbit was graded according to 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 and grades for erythema and oedema were all 0.

In second study (Anonymous, 1990), five animals per sex were tested by dermal application of 0, 100, 500 or 1000 mg/kg bw/d of 95.78 % clopyralid in the powder form for 6 h/d. All rabbits were acclimated to an elastic jacket used to hold the test material dressing in dermal contact. An area approximately 10 × 15 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 the test material in dermal contact. The jacket and dressing were removed approximately six hours after application and the test site was wiped with a water-damped towel to remove any residual test material. Animals were treated 15 times during the 21-day period. The control animals were treated in the same way 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 assessed according to Draize scale. In addition, a complete histologic evaluation of treated and untreated skin was made on all control and test groups.

In the 21 d dermal study slight erythema (score 1) was observed on day 10 in two males belonging to the intermediate and high dose groups, respectively, and one female on day 3, belonging to the intermediate dose group. The histopathologic evaluation detected epidermal

hyperplasia in 1/5 males and 2/5 females treated with 100 mg/kg bw/d, 3/5 males and 1/5 females treated with 500 mg/kg bw/d, and 5/5 males and 5/5 females treated with 1000 mg/kg bw/d. The hyperplasia was considered exposure related. No significant gross lesions were found at necropsy. RAC remarks that occasional histopathologic lesions were observed in the untreated skin adjacent to the dermal test site as well. The findings consisted of degeneration of muscle fibres 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.

In addition prior to the 21 d dermal study, a probe study was conducted applying 500 and 1000 mg/kg bw/d of clopyralid for 6 h/d in one animal per sex for 4 days. No dermal irritation was noted in either the male or female rabbit.

Both the main and the probe studies were carried out in compliance with the GLP standards and the US EPA guideline No. 82-2 as well as predominantly according to OECD TG 410 (1981).

According to the CLP Regulation, a substance shall be classified as Skin Irrit. 2 when it produces reversible damage to the skin following its application for up to 4 hours. *The major criterion for the irritation category is that at least 2 of 3 tested animals have a mean score of ≥ 2.3 and ≤ 4.0.*

RAC notes that none of the criteria are fulfilled and agrees with the DS that clopyralid shall **not be classified as skin irritant**.

In addition, RAC concludes that the **EUH066 statement 'Repeated exposure may cause skin dryness or cracking' is warranted** due to epidermal hyperplasia observed after repeated exposure.

RAC evaluation of specific target organ toxicity – repeated exposure (STOT RE)

Summary of the Dossier Submitter's proposal

Clopyralid was generally well tolerated in dietary studies in several species. 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), reduced weight and blood (dogs) – reduced red blood cells. Lethality in both rabbits and rats 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/d) than the highest dose level of the oral acute toxicity study in rat (LD_{50} > 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/d after 2-5 doses (2-5 days exposure) in rats and at 250, 500 and 750 mg/kg bw/d after 5-13 doses (5-13 days of exposure) in rabbits. 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 ≤ 1000 mg/kg bw/d, they occurred within relevant guidance value for classification.

The DS proposed classification as STOT RE 2 ("H373: May cause damage to organs through prolonged or repeated oral exposure") based on lethality.

Comments received during consultation

Two comments were received during the consultation from a Member State Competent Authority (MSCA) and from an Industry representative (IND). Both rejected the justification given by the DS for the proposed classification and would prefer classifying based on the local irritating effects causing erosions and ulcers in the stomach mucosa. In addition, IND stated that "death/lethality has not been generally considered as a trigger for STOT RE" except "due to bioaccumulation of the substance or its metabolites, and/or due to the overwhelming of the detoxification process by repeated exposure to the substance or its metabolites which is not the case for clopyralid". RAC notes that "morbidity or death" is listed in the CLP Regulation as cause for STOT RE in Annex I, 3.9.2.7.3. (a).

Assessment and comparison with the classification criteria

The DS mentioned 13 oral dietary studies on different animals as well as four oral gavage studies and a dermal study, of which eight oral dietary studies, one oral gavage study and the single dermal study were considered to be acceptable (see summary in the supplemental information section below).

According to CLP, classification for STOT RE Cat. 1 is applicable for substances that have produced significant toxicity in humans or that, on the basis of evidence from studies in experimental animals, can be presumed to have the potential to produce significant toxicity in humans following repeated exposure at generally low exposure concentrations (C ≤ 10 mg/kg bw/d for oral 90 d exposure). There are no human data available on specific target organ toxicity of clopyralid. Taking into account the available data, RAC concludes that clopyralid does not fulfil the criteria for classification as STOT RE Cat. 1.

Substances shall be classified in STOT RE Cat. 2, if it can be presumed that they have potential to be harmful to human health following repeated exposure at generally moderate exposure concentrations in experimental animals (10 mg/kg bw/d < C ≤ 100 mg/kg bw/d for oral 90 d exposure). For a 28 d study, this value should be modified to C ≤ 300 mg/kg bw/d, for a 21 d study to C ≤ ~428 mg/kg bw/d and for a 13 d study to ~692 mg/kg bw/d.

It should be remarked that the numerical guidance values for experimental animals are not intended as strict demarcation values but are intended for guidance purposes.

According to the CLP guidance (Version 5.0 July 2017), paragraph 3.9.2.3.2 "Evaluation of non-human data", "*all available animal data which are of acceptable quality should be used in a weight of evidence approach based on a comparison with the classification criteria described […]. If there are differences in effects at the guidance values between studies with different duration then more weight is usually given to studies of a longer duration (28 days or more). This is because animals may not have fully adapted to the exposure in studies of shorter durations and also because longer duration studies tend to include more thorough and extensive investigations (e.g. in terms of detailed pathology and haematological effects, etc.) which can generally give more substantial information compared to shorter duration studies.*" In addition, "*any information pertaining to the relevance of findings in animals to humans must be taken into account and may be used to modify the classification from how it would be if based on the available animal data. For instance, it may be shown that the findings in animals are not relevant for humans, for example if the toxicity in animals is mediated by a mode of action or in this case by mode of administration of the substance that does not occur in humans under normal circumstances. This would potentially provide a supporting case for no classification.*"

The 28 d, 90 d or longer dietary studies in rats, mice and dogs justify no classification for STOT RE (see supplemental information in the Appendix to the opinion). Only the single study

characterised as "acceptable" by the DS and conducted for developmental toxicity assessment (clopyralid was administered by oral gavage in corn oil for gestation days (GD) 7-19, so the duration of test was ~13 days) showed 21 % mortality (i.e. 6 animals died out of 29 animals totally tested) in the 250 mg/kg bw/d group between GD13 and GD21, within the GV for STOT RE classification.

Overall, RAC concludes that **clopyralid does not fulfil the criteria for classification as STOT RE** based on a weight of evidence approach from all acceptable studies available.

RAC evaluation of reproductive toxicity

Summary of the Dossier Submitter's proposal

Fertility

Adverse effects on sexual function and fertility were investigated in a two-generation reproduction study (dRAR B.6.6.1, 1983) with supplementary histopathology the following year (1984). Clopyralid was administered in the diet of Fischer 344 rats (30 rats/sex/dose for both generations) at doses of 0, 150, 500, or 1500 mg/kg bw/d. They were reduced by 1/6 to 1/3 during mating, gestation, and lactation for F1b mating. During F2 lactation periods, dietary concentrations were reduced by 1/2 and 1/3, starting on lactation day (LD) 7 and LD 14, respectively, until weaning. According to the DAR applicant, the dietary corrected dose levels were 82.5, 275, 825 mg/kg bw/d. At the high dose, decreased body weights and food consumption in both sexes and increased liver weight in female rats in the parental (P1) and F1 rats were detected. The NOAEL 275 mg/kg bw/d was obtained based on reduction in organ weights and reduced terminal body weight. For reproductive performance, the NOAEL 825 mg/kg bw/d was derived as no effects were indicated.

No treatment-related histopathological effects in reproductive organs and accessory sex glands in randomly selected adult P1 and F1 rats at 1500 mg/kg bw/d or in major organs of randomly selected F2 rats at 1500 mg/kg bw/d were reported.

The study has several deficiencies that hamper the interpretation of the results as the study was not carried out according to OECD TG 416 (2001). Sperm counts, oestrus cycle, sexual maturation were not measured. The mating schedule design did not allow each female to be mated with one male long enough to reveal the fertility of each male. Additionally, no histopathological investigation was presented on males failing to induce pregnancy during the five-day mating period allowed for each male, and the duration of pregnancy was not recorded. The unsystematically reduced dietary levels during the mating, gestation and/or lactation periods may indicate that the NOAEL/LOAEL values based on the premating dose levels were too high and should rather be based on the lowest doses given during lactation. In addition, because the food consumption was not measured during the lactation periods, the dose levels cannot be calculated. The DS mentioned that the original DAR conclusion was that the reproduction toxicity cannot be evaluated without a doubt based on this two-generation study. Additionally, this study was discussed in the Addendum 1 (2004) to the DAR, where it was stated that, based on the intended highest dose (1500 mg/kg bw/d), the actual doses were higher than 700 mg/kg bw/d. Overall, the results do not suggest any harm in fertility or in the offspring and that a specific reproductive risk is most unlikely.

The DS proposed no classification for sexual function and fertility, due to the inconclusive results from this limited 2-generation study.

Developmental toxicity

Two studies on developmental toxicity performed according to OECD TG 414 (2001) were provided based on NZW rabbits (dRAR B.6.6.2., 1990) and Fischer-344 rats (dRAR B.6.6.2., 1981). In these studies, major malformations like polydactyly, hemivertebra, hydrocephaly, forelimb flexure, microphthalmia and anophthalmia were observed, however, with relatively low incidences. In addition, delayed ossification was observed on rabbit. These effects were partly observed in the presence of maternal toxicity or were not repeated in the second phase of the study. However, the DS considered that the overall picture was of sufficient concern and proposed to classify clopyralid as Repr. 2 for developmental effects.

Comments received during consultation

Fertility

Regarding fertility and sexual function, no comments were received.

Developmental toxicity

Two comments have been received during consultation from a MSCA and IND.

IND opposed the general statement made by the DS in support for classification for developmental toxicity: "*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*." Therefore, they considered no classification more appropriate.

The MSCA generally supported the Repr. 2, H361d classification but argued the presence of elements pro and against classification.

Additional key elements

Table: *Two-generation reproduction study (dRAR B.6.6.1, 1983)*

Doses (mg/kg bw/d)	**0**	**150**	**500**	**1500**
P1 generation (F1a 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 d survival index (%) (% liveborn pups survived for 1 days)	99 (280/281)	99 (281/282)	100 (275/275)	100 (289/289)
28 d survival index (%) (% liveborn pups survived until day 28 of lactation)	92 (198/214)	86 (179/209)	95 (192/203d)	98 (217/222)
Sex ratio on Day 28 (%male : %female)	51:49	50:50	47:53	52:48
Anophthalmiae (% affected (number affected)) pups litters	 0 0	 0.5(1) 3.6(1)	 0.5(1) 3.3(1)	 0 0

Total pups examined Total litters examined	281 28	284 28	277 30	291 28
P1 generation (F1b 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 d survival index (%) (% liveborn pups survived for 1 days)	99 (277/278)	100 (247/247)	98 (247/252)	100 (269/269)
28 d 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
F1 generation (F2a 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 (234/237)	98 (236/241)	100 (206/206)	99 (235/236)
Number of live pups/litter on Day 1	10 ± 3	10 ± 3	9 ± 3	10 ± 3
1 d survival index (%) (% liveborn pups survived for 1 days)	99 (233/234)	92 (218/236)	100 (205/206)	98 (235/236)
28 d 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 litters Runt pups litters Smaller size pups litters	 0.6(1) 4.5(1) 0 0 1.3(3) 13.0(3)	 0 0 0.4(1) 4.3(1) 0.8(2) 8.7(2)	 0.6(1) 4.5(1) 0 0 1.0(2) 9.1(2)	 1.7(3) 4.2(1) 0.4(1) 4.2(1) 3.4(8) 20.8(5)
F1 generation (F2b 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 d survival index (%) (% liveborn pups survived for 1 days)	98 (245/249)	100 (211/212)	98 (254/258)	99 (254/267)
28 d 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 litters	 0 0	 0 0	 0.5(1) 4.2(1)	 0 0
Anophthalmiae (% affected (number affected)				

pups litters	0.6(1) 4.2(1)	0.6(1) 4.0(1)	0 0	0 0
Imperforate Anal/urogenital opening (% affected (number affected) pups litters	 0 0	 0 0	 0.4(1) 4.0(1)	 0 0
Runt (% affected (number affected) pups litters	 0 0	 0.5(1) 3.8(1)	 0.4(1) 4.0(1)	 0 0
Smaller size (% affected (number affected) pups litters	 2.0(5) 11.5(3)	 0.5(1) 3.8(1)	 1.2(3) 12.0(3)	 1.5(4) 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: *Main observations in teratology study in rabbits (dRAR B.6.6.2., 1990)*

Parameters	**0 mg/kg bw/d**	**50 mg/kg bw/d**	**110 mg/kg bw/d**	**250 mg/kg bw/d**
No. of test animals	28	26	26	34
No. excluded due to aspiration of test material	0	5	1	5
No. of test animals after exclusion	28	21	25	29
% pregnant	75 % (21/28)	76.9 % (20/26)	73.1 % (19/26)	73.5 % (25/34)
Mean litter size	6.8	7.7	7.2	6.3
Number of dead foetuses	0	0	0	1
Foetal body weight (g) % of control	39.64 100 %	34.84* 87.9 %	36.19 91.3 %	34.40* 86.8 %
% of foetuses with hydrocephaly	0 %	0 %	0 %	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 %)

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

Assessment and comparison with the classification criteria

Fertility

The DS provided a single two-generation reproduction study (dRAR B.6.6.1, 1983) with supplementary histopathology study (1984) on Fischer 344 rats, see above.

According to the CLP Regulation (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 must be considered. 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.

Overall, RAC considers **no classification for reproductive toxicity for fertility is justified** as the available 2-generation study did not show any effects related to fertility (it should be emphasised that the study has major deviations decreasing its reliability).

Developmental toxicity

The DS provided two studies on developmental toxicity performed according to OECD TG 414 (2001) on NZW rabbits (dRAR B.6.6.2., 1990) and Fischer-344 rats (dRAR B.6.6.2., 1981). With respect to the rat study, a remark has been added*: "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 clopyralid (Commission Implementing Regulation (EU) No 844/2012)".*

Additional information on study design as well as summary of identified outcomes can be found under supplemental information in the Appendix to the opinion.

In addition, RAC assessed one supportive study provided by the DS on NZW rabbits (dRAR B.6.6.2., 1974) conducted partially in compliance with Directive 87/302/EE but not considered reliable for hazard assessment. The rabbits (15 females per dose plus 25 animals in control group) were treated with clopyralid in corn oil by oral gavage. Only two doses (110, 250 mg/kg bw/d) were administered daily on gestation days (GD) 6-18. On GD29, the foetuses were removed from dams by caesarean section and examined for external, skeletal and visceral malformations. Some signs of maternal toxicity were observed at the highest dose (slightly increased liver weight), but there were no mortalities or adverse clinical signs. Malformations like omphalocoele and anencephaly were similar in all groups, including control group.

In the 1990 rabbit study, body weights were recorded on days 0, then daily during the dosing period and on days 20 and 28 of gestation. All animals were observed daily for signs of toxicity. On GD28, the foetuses were removed from dams by caesarean section and examined for external, visceral and skeletal malformations. Sections of maternal liver, kidneys and stomach were preserved and histologic examination was performed on the stomachs. Several rabbits were found dead or were killed moribund (1, 5, 1, 11, from control to high dose, respectively). From the necropsy findings, it was concluded that 0, 5, 1 and 5 dams (control to high dose, respectively, not considered further in the discussion) failed to reach term because of aspiration of test material, i.e. due to intubation error. No clinical effects were observed at 50 or 110 mg/kg bw/d. At the high dose (250 mg/kg bw/d) signs of severe maternal toxicity, including significantly depressed body weight and body weight gain, stomach erosion (all animals) and excessive mortality (21 % or 6/29 dams) were reported. Laboured breathing was observed in approximately one-third (11/29) of the rabbits.

On GD0-28 maternal body weight gain is dose-dependently reduced, the difference to control being > 25 % in all dose levels. Consequently, on GD7-20 maternal body weight gain is negative in all dose levels, namely, the body weight loss is recorded:

	0 mg/kg bw/d	**50 mg/kg bw/d**	**110 mg/kg bw/d**	**250 mg/kg bw/d**
Mean weight gains (g) on GD0-28	470.8	351.4	298.2	283.3

Mean weight gains (g) on GD7-20	38.2	-91.1	-50.2	-254.3

Based on these observations, the maternal NOAEL was determined to be < 50 mg/kg bw/d and the maternal LOAEL 50 mg/kg bw/d. It should be remarked that for rabbits according to the CLP Regulation (Annex I, Section 3.7.2.4.4) "*the body weight gain may not be useful indicator of maternal toxicity because of normal fluctuations in body weight during pregnancy*". Food consumption was not measured. Nevertheless, analysis of the individual test animals weight changes during GD7-20 could support maternal toxicity from the lowest dose tested. RAC in depth analysis (see the relevant chapter below) shows clear dose dependent increase in proportion of rabbits with pronounced negative weight gain (weight loss) more than 100 g. In addition, the proportion of all rabbits with individually registered negative weight gain is approximately two times higher at the test doses compared to control group, however, no dose – response relationship is shown. RAC remarks that food consumption was not measured. The foetal body weight was decreased in all dose groups (9-13 % with respect to control), statistical significantly in the low and high doses. In low and mid dose a statistically significant delay in ossification (skull, sternebrae) was observed, which probably was related to the lower pups body weight. Based on these observations, the developmental NOAEL was estimated at < 50 mg/kg bw/d and the developmental LOAEL at 50 mg/kg bw/d. To note, these effects were observed at doses where maternal toxicity was present, however rabbit body weight gain changes might not be of biological significance according to the CLP Regulation. The historical control data (HCD) 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 Regulation 283/2013. In addition, hydrocephaly and forelimb flexure were seen in the high dose group. Detailed information is given in the section "additional key elements" below. At the highest dose, foetuses with hydrocephaly were associated with dams which lost an average of 524 g during the treatment period (GD7-20), which was substantially greater than the whole group mean weight loss (254.3 g). Despite the suggestion of a correlation between weight loss and hydrocephaly, RAC notes that at this dose the mortality was above 10 %, which is considered excessive by the CLP Regulation (Annex I, 3.7.2.4.4), thus "*the data for that dose level shall not normally be considered for further evaluation*". No other CNS malformation effects were observed in this dose group.

In the study with Fischer-344 rats (dRAR B.6.6.2., 1981), animals were observed daily for indications of toxicity. Body weights were recorded daily during dosing period and on GD16 and GD21. Food and water consumption were recorded on a three-day interval from GD6. On GD21 the foetuses were removed from dams, and examined for external, visceral and skeletal malformations. At the highest dose (250 mg/kg bw/d), the three rats died on GD10-11 for unknown reasons. Significant reductions in dams body weights and body weight gains were reported, however, the corrected body weight was not significantly lower. At this dose, dams consumed significantly less food than controls throughout most of the gestation. There was a significant decrease in absolute liver weight in rats receiving 75 and 250 mg/kg bw/d compared to controls. At 250 mg/kg bw/d, 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. In the HCD, for study conducted between 1981 and 1987, polydactyly was recorded with an incidence of 0.0 %, while the combined incidence of polydactyly in this study high dose foetuses was 0.7 %. Embryo lethality was not observed. NOAEL for decreased maternal liver weight was 15 mg/kg bw/d and for embryonic/foetal development was 250 mg/kg bw/d.

Overall, RAC considers **no classification for reproductive toxicity for development is justified** due to lack of clear evidence of adverse effects on development in the absence of other toxic effects (no effects were reported in the study in rats; in the 1990 study in rabbits,

developmental effects were observed at the doses where maternal toxicity was excessive, and no effects were reported in the supportive study in rabbits (1974)).

ENVIRONMENTAL HAZARD EVALUATION

RAC evaluation of aquatic hazards (acute and chronic)

Summary of the Dossier Submitter's proposal

Clopyralid is currently not listed in Annex VI of Regulation (EC) No 1272/2008 (CLP) with a classification for environmental hazards.

The Dossier Submitter (DS) proposed:

- **No classification for acute aquatic hazard** as all acute endpoints were above 1 mg/L with the most sensitive species, the Eurasian watermilfoil *Myriophyllum spicatum* (14 d EC_{50} > 3 mg/L), and
- **Aquatic Chronic 1 (H410) with an M-factor of 10** based on a lack of rapid degradation and a 14 d NOEC of 0.0089 mg/L for the *M. spicatum.*

Degradation

A hydrolysis study according to OECD TG 111 was run in sterile buffer solutions at pH 4, 7, 9 and in natural water under laboratory conditions for 120 hours (5 days). Degradation of clopyralid < 10 % was observed at 50 °C across pH 4, 7, 9, and natural water samples. Therefore, clopyralid can be considered hydrolytically stable. There was no need to perform a definitive test at 20 °C for 30 days, as no degradation was observed at 50 °C.

The results from two photolytic degradation in water studies indicated that clopyralid is photolytically stable in water. In the first study performed according to 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 derived SFO DT_{50} value was 271 days. In the second study, performed according to OECD TG 316, no significant degradation products were observed. The SFO DT_{50} values of 431 days and DT_{50} (as function of latitude (40° N)) of 38.933 days were derived.

There was one ready biodegradability test available for clopyralid following OECD TG 301B. It resulted in cumulative CO_2 production of 10 % and 5 % of theoretical maximum levels after 27 days in the vessels containing 10 and 20 mg/L clopyralid, respectively. Therefore, the substance was considered not readily biodegradable.

A 60 day aerobic freshwater simulation test (OECD TG 309) indicated that the substance is stable in aqueous environment (> 93.8 % of AR after 60 days) and undergoes minimal mineralisation (1.0 % at high dose and 0.1 % at low dose by day 60). The derived degradation half-live (DT_{50}) was > 1100 days for high dose. No significant degradation products were observed.

A water/sediment study carried out according to guidelines presented in Part IV of Section 5.1 of BBA (1990) and Part 1 of Section 8.2 of SETAC-Europe (1995) and OECD TG 308 was conducted using two water/sediment systems of EU origin for 100 days. Mineralisation to CO_2 was between 2.3 % and 5.3 % AR after 100 days. 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} values of 128-167 days were obtained for the dissipation from the water phase to the sediment in two water/sediment systems within 100 days. The whole system DT_{50} value for clopyralid was not calculated due to the low rate of degradation observed.

Based on the available data, the DS concluded that clopyralid is considered as not rapidly degradable for classification purposes.

Bioaccumulation

A fish bioconcentration study (US EPA OPP 165-4 US EPA OPP 72- 6) was available for clopyralid. Bluegill sunfish (*Lepomis macrochirus*) were exposed to nominal concentrations (0.1 mg/L and 1.0 mg/L) of radiolabelled clopyralid for 28 days, followed by a 14 day depuration period. The whole fish BCF values were < 1.0 for both treatment groups.

The measured octanol-water partition coefficient (log K_{ow}) at 20 ºC was -1.83 to -2.63 in pH 5-9, respectively (OECD TG 107). A higher log K_{ow} value of 1.06 is available in the EPISUITE v4.10 experimental database. KOWWIN v1.67 prediction of log K_{ow} for clopyralid is 1.63.

Based on available data, the DS concluded that clopyralid has a low potential for bioaccumulation in the aquatic environment.

Aquatic Toxicity

Reliable aquatic toxicity data are available for all three trophic levels, and a summary of the relevant information is provided in the following table (the key endpoints used in hazard classification are highlighted in bold). All study results are expressed in terms of mean measured concentrations, unless stated otherwise.

Table: Summary of relevant information on aquatic toxicity of clopyralid

Method/Test substance	Species	Endpoint	Toxicity value (mg/L)	Reference/Remarks
Short-term toxicity				
OECD TG 203, EU Method C.1., US EPA FIFRA 540/9-85-006, 72-1	Rainbow trout *(Oncorhynchus mykiss)*	96 h LC_{50}	99.9	2000 dRAR B.9.2.1/01
OECD TG 203, EU C.1., US EPA FIFRA 540/9-85-006, 72-1.	Bluegill sunfish (*Lepomis macrochirus*)	96 h LC_{50}	> 102	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*)	96 h EC_{50}	700 n (rainbow trout) 802 n (bull trout)	Fairchild *et al.* (2008)
OECD TG 202 (part 1), EU C.2, US EPA FIFRA 540/9-85-005, 72-2	Water flea (*Daphnia magna*)	48 h EC_{50}	> 99.0	2000 dRAR B.9.2.2.1/01
OECD TG 201 (1984), US EPA OPP 123-2, ECC C.3 (1992)	Freshwater green algae (*Raphidocelis subcapitata*)	72 h E_bC_{50} 72 h E_rC_{50} 96 h E_bC_{50} 96 h E_rC_{50}	30.9 30.0 32.7 33.1	2000 dRAR B.9.2.2/01 Supportive information as validity criteria not fulfilled.
OECD TG 201 (2004)	Freshwater blue algae (*Anabaena flos-aquae*)	72 h E_rC_{50}	22	2006 dRAR B.9.2.2/02 Supportive information as validity criteria partially fulfilled.
US EPA FIFRA, Subdivision J, 123-2	Freshwater blue algae (*Anabaena flos-aquae*)	120 h E_bC_{50} 120 h EC_{50}	127 37.1	2000 B.9.2.2/03 Validity of the study could not be assessed.

Method/Test substance	Species	Endpoint	Toxicity value (mg/L)	Reference/Remarks
OECD TG 201, U.S. EPA OCSPP 850.4500	Freshwater diatom (*Navicula pelliculosa*)	72 h E_rC_{50} 96 h E_rC_{50}	31.3 30.5	2014 dRAR B.9.2.2/03
US EPA OPP 122-2	Duckweed (*Lemna gibba*)	14 d EC_{50}	89	1990 dRAR B.9.2.3/01
OECD TG 239, US EPA OCSPP.SUPP	European watermilfoil (*Myriophyllium spicatum*)	14 d E_rC_{50}	**> 3** n	2015 dRAR B.9.2.3/02
Long-term toxicity				
OECD TG 210, US EPA FIFRA EPA-540/86-138, ASTM Standard E 1241-92	Fathead minnow (*Pimephales promelas*)	34 d NOEC	10.8	2000 dRAR B.9.2.1.2
OECD TG 202 (part 2, 1984)	Water flea (*Daphnia magna*)	21 d NOEC	17	1992 dRAR B.9.2.2.2
OECD TG 201 (1984), US EPA OPP 123-2, ECC C.3 (1992)	Freshwater green algae (*Raphidocelis subcapitata*)	72 h NOEC 96 h NOEC	< 3.45 24.8	2000 dRAR B.9.2.2/01 Supportive information as validity criteria not fulfilled.
OECD TG 201 (2004)	Freshwater blue algae (*Anabaena flos-aquae*)	72 h NOE_rC 96 h NOE_rC 72 h NOE_yC 96 h E_yC_{10}	13 24 13 24	2016 dRAR B.9.2.2/02 Supportive information as validity criteria partially fulfilled.
US EPA FIFRA, Subdivision J, 123-2.	Freshwater blue algae (*Anabaena flos-aquae*)	120 h NOEC	24.2	2000 B.9.2.2/03 Validity of the study could not be assessed.
OECD TG 201, U.S. EPA OCSPP 850.4500	Freshwater diatom (*Navicula pelliculosa*)	72 h E_bC_{10} 72 h E_rC_{10} 72 h E_yC_{10} 96 h E_bC_{10} 96 h E_rC_{10} 72/96 h NOEC	25.8 23.6 25.3 23 24.4 1.60	2014 dRAR B.9.2.2/04
US EPA OPP 122-2	Duckweed (*Lemna gibba*)	14 d NOEC	7.2	1990 dRAR B.9.2.3/01
OECD TG 239, US EPA OCSPP.SUP P	European watermilfoil (*Myriophyllium spicatum*)	14 d NOEC (total shoot length, fresh weight and dry weight)	**0.0089** n	2015 dRAR B.9.2.3/02
BBA 1995	Midge (*Chironomus riparius*)	28 d NOEC (emergence) 28 d NOEC (development)	50 97	2001 dRAR B.9.2.2.3.

Note: n – nominal concentration

Acute aquatic toxicity

Acute toxicity for fish, invertebrates, algae and aquatic plants were reported. The most conservative endpoints for each trophic level were:

- 96 h LC_{50} > 99.9 mg/L mean measured concentration for *Oncorhynchus mykiss*,
- 48 h EC_{50} > 99.9 mg/L mean measured concentration for *Daphnia magna*,
- 72 h E_rC_{50} = 31.3 mg/L mean measured concentration for *Navicula pelliculosa*,

and

- 14 d E_rC_{50} > 3 mg/L nominal concentration for *M. spicatum*.

As all acute endpoints for classification purposes were above 1 mg/L, the DS proposed not to classify clopyralid for acute aquatic hazard in the aquatic environment, based on the lowest 14 d E_rC_{50} > 3 mg/L for the aquatic macrophyte *M. spicatum*.

Chronic aquatic toxicity

Chronic toxicity for fish, invertebrates (and other aquatic organisms), algae and aquatic plants were reported. The most conservative endpoints for each trophic level were:

- 34 d NOEC = 10.8 mg/L mean measured concentration for *Pimephales promelas*,
- 21 d NOEC = 17 mg/L mean measured concentration for *D. magna*,
- 72/96 h NOEC = 1.60 mg/L mean measured concentration for *N. pelliculosa*, and
- 14 d NOEC = 0.0089 mg/L nominal concentration for *M. spicatum*.

Based on the 14 d NOEC of 0.0089 mg/L for *M. spicatum*, the DS proposed that clopyralid should be classified as Aquatic Chronic 1 (H410), with an M-factor of 10, for a not rapidly degradable substance.

Comments received during consultation on the ECHA website

Two Member States (MS), one National Authority, and a Company-Manufacturer provided comments.

Both MSs agreed with the proposed classification for environmental hazards. One MS pointed out the mistakes in degradation part of the CLH report.

The National Authority asked if preferred 7 day endpoints from the *Lemna gibba* study are available or could be calculated. They noted that 7 day study controls should also be considered against relevant test method criteria. The DS responded that 7 day endpoints were not available and calculated endpoints for fronds and plants on day 6 and 9 to get approximations of the 7 day toxicity (see table in RCOM document). The DS indicated that 7 day toxicity endpoints would be somewhere between the 6 day and 9 day endpoints. Taking into account that the validity criteria for OECD TG 221 was fulfilled (doubling time for frond number in the controls was < 2.5 for days 0-6 and 0-9) and that the study is not the most critical for the classification, the DS considered that presented endpoints are sufficient to show that *L. gibba* is not the most sensitive species for clopyralid. In addition, the DS noted that 14 d EC_{10} of 17 mg/L (fronds) and 18 mg/L (plants) were not reported in the CLH report.

Assessment and comparison with the classification criteria

Degradation

- RAC agrees with the DS's proposal to consider clopyralid as **not rapidly degradable**:
- The substance is hydrolytically stable at environmentally relevant pHs (pH 4-9).
- In the available ready biodegradability test (OECD TG 301B) the cumulative CO_2 production was 10 % (low dose) and 5 % (high dose) of theoretical maximum values after 27 days, indicating that the substance is not readily biodegradable.
- In the available surface water simulation test, the mineralisation was low (1.0 % (high dose) and 0.1 % (low dose)) by day 60. The derived DT_{50} was > 1100 days. No significant degradation products were observed.
- Minimal degradation and no major metabolites were observed in water/sediment system study. Low mineralisation was observed (max. 5.3 %).

Bioaccumulation

RAC agrees with the DS that clopyralid has a low potential for bioaccumulation in aquatic environment. This is based on the measured BCF value of less than 1 being below the CLP criterion of 500. This is supported by measured and estimated log K_{ow} values ranging from -2.63 to 1.63, which are below the CLP criterion of 4.

Aquatic toxicity

RAC is of the opinion that adequate acute and chronic toxicity data are available for fish, invertebrates, algae and aquatic plants.

For both, acute and chronic toxicity, aquatic plants are the most sensitive group, with the most sensitive species being *M. spicatum*. RAC notes that water-sediment *M. spicatum* toxicity test was performed according to OECD TG 239 which includes a sediment phase. All the validity criteria for OECD TG 239 guidelines were fulfilled. RAC considers that the *Myriophyllum* test is adequate for classification of clopyralid as application of the test substance is done via the water column (spiked water) and the mean measured recoveries from day 0 and 14 ranged from 98 to 104 % of the nominal concentrations of clopyralid, indicating that applied clopyralid remained in the water phase throughout the test and did not dissipate into sediment. The latter is supported by the results of the available OECD TG 308 test, where DT_{50} of clopyralid for dissipation from water to sediment was 128-167 days. Therefore, RAC considers the E_rC_{50} and NOEC values reliable and should be used for classification of clopyralid.

The lowest acute toxicity value is a 14 d E_rC_{50} value of > 3 mg/L determined for *M. spicatum*. RAC notes that all acute toxicity endpoints (L(E)C_{50}s) for fish, invertebrates, algae and aquatic plants (see table) are above the threshold value of 1 mg/L. RAC agrees with the DS that 7 d endpoint values for *L. gibba* as proposed by the National Authority would not change the classification outcome. Consequently, RAC concludes that clopyralid does not warrant classification for acute aquatic toxicity.

The lowest chronic value is a 14 d NOEC value of 0.0089 mg/L determined for *M. spicatum*. As this value is < 0.1 mg/L and the substance is considered not rapidly degradable, RAC concludes that clopyralid warrants classification as Aquatic Chronic 1 (H410) with an M-factor of 10 (0.001 < NOEC ≤ 0.01 mg/L).

In summary, on the basis of the available data, RAC considers that **clopyralid warrants classification** (in agreement with the DS's proposal) as:

Aquatic Chronic 1 (H410), M-factor = 10

ANNEXES:

Annex 1 The Background Document (BD) gives the detailed scientific grounds for the opinion. The BD is based on the CLH report prepared by the Dossier Submitter and additional information (if applicable).

Annex 2 Comments received on the CLH report, response to comments provided by the Dossier Submitter and RAC (excluding confidential information).

Appendix

Supplemental information - In depth analyses by RAC

RAC evaluation of specific target organ toxicity – repeated exposure (STOT RE)

***Table**: Summary of acceptable studies for STOT RE assessment*

Study description	Test group	Doses and duration	Description of outcomes	Reference
28 d oral dietary study Mainly according to OECD TG 407 (2008) with some deviations	CD rats 10/sex/dose	0, 150, 500, 1500 mg/kg bw/d Continuously for 28 days	No mortality or treatment-related clinical signs occurred. Males receiving 1500 mg/kg bw/d 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/d (20 % and 33 %, respectively). Absolute and relative kidney weights were increased at all dose levels in females and at 1500 mg/kg bw/d in males. 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/d) or in control animals. RAC conclusion: **no effects fulfilling the criteria for classification**	dRAR B.6.3.1., 1986
90 d oral dietary study Mainly according to OECD TG 408 (1998) with some deviations	Fischer-344 rats 15/sex/dose	0, 300, 1500, 2500 mg/kg bw/d Continuously for 98-99 days	No mortality occurred. Mean body weights and body weight gain were decreased in both sexes in 2500 mg/kg bw/d dose group (ca. 9 % and 16 %, respectively) and in females of 1500 mg/kg bw/d group (ca. 7 %). Food consumption in females was reduced at 2500 mg/kg bw/d. Mean relative liver and kidney weights were significantly increased in males at all doses and in females at 2500 mg/kg bw/d. 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/d 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 mg/kg bw/d (lesions in the stomach). RAC conclusion: **no effects fulfilling the criteria for classification**	dRAR B.6.3.2., 1983

13-week oral dietary study Mainly according to OECD TG 408 (1998) with some deviations	B6C3F1 mice 10/sex/dose	0, 200, 750, 2000, 5000 mg/kg bw/d Continuously for 95-96 days	A slight decrease in body weight was evident over the study in both sexes at 5000 mg/kg bw/d. 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/d. RAC conclusion: **no effects fulfilling the criteria for classification**	dRAR/DAR B.6.3.2.2.1., 1983
12-month oral study in dog Mainly according to OECD TG 409 (1998) with some deviations	Beagle dogs 6/sex/dose	The average daily doses calculated over the entire study period: 0, 99, 301, 983 mg/kg bw/d (males) 0, 99, 319, 977 mg/kg bw/d (females) 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 haematological changes including reductions in red blood cell counts (74 %-89 % of control), total haemoglobin concentration (79 %-85 % of control) and haematocrit (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/d (haematological effects). RAC conclusion: **no effects fulfilling the criteria for classification**	dRAR/DAR B.6.3.2.3.3., 1984
2-year chronic toxicity and oncogenicity study (oral dietary study) Mainly according to OECD TG 453 (2009) with some deviations	Fischer-344 rats 70/sex/dose	0, 15, 150, 1500 mg/kg bw/d 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/d. The effect was more frequently recorded in animals treated at 1500 mg/kg bw/d 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. RAC conclusion: **no effects fulfilling the criteria for classification**	dRAR/DAR 6.5.1.4., 1985, 1986
2-year oral dietary chronic toxicity-oncogenicity study Mainly according to OECD TG 453	B6C3F1 mice Number of animals not given	0, 100, 500, 2000 mg/kg bw/d	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. RAC conclusion: **no effects fulfilling the criteria for classification**	dRAR B.6.5/DAR B.6.5.2.2., 1984, 1986

Developmental toxicity study (oral gavage study) Mainly according to OECD TG 414 (2001)	NZW rabbits 26-34 females/dose	In corn oil Probe study: 0, 110, 250, 350 mg/kg bw/d Main study: 0, 50, 110, 250 mg/kg bw/d For gestation days 7-19	Probe study: The daily dose of 350 mg/kg bw/d resulted in weight loss, stomach erosion and significant mortality (60 %). No clinical effects were observed at 110 or 250 mg/kg bw/d. Necropsy revealed stomach erosions in all rabbits given 250 mg/kg bw/d. 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/d 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/d 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. RAC conclusion: **corresponds to classification** criteria **for STOT RE 2**	dRAR/DAR B.6.6.2.2.2., 1990
21 d dermal study Mainly according to OECD TG 410 (1981) and U.S. EPA Guideline No. 82-2 for pesticides	NZW rabbit 5/sex/dose	0, 100, 500, 1000 mg/kg bw/6 hours/day Topical application for 15 days	No treatment related systemic toxic effects. 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/d, 3/5 males and 1/5 females treated with 500 mg/kg bw/d, and 5/5 males and 5/5 females treated with 1000 mg/kg bw/d. In addition, inflammation of dermis and necrosis on epidermis (1 male, 100 mg/kg bw/d) were observed. RAC conclusion: **only local effects**	dRAR B.6.3.3./ DAR B.6.3.3.1.1., 1990

RAC evaluation of reproductive toxicity

Developmental toxicity

***Table**: Analysis on maternal body weight gain of individual rabbits on GD7-20 (dRAR B.6.6.2., 1990)*

Dose, mg/kg bw/d	Proportion of rabbits with individual negative weight gain	Proportion of rabbits with individual negative weight gain > 100 g	Maternal mortality % excluding intubation errors
0	32.1 % (9/28)	25 % (7/28)	4 % (1/28)
50	69.6 % (16/23)	34.8 % (8/23)	0 % (0/21)
110	72 % (18/25)	48 % (12/25)	0 % (0/21)
250	70.8 % (17/24)	58.3 % (14/24)	21 % (6/21)