

Committee for Risk Assessment
RAC

Opinion

proposing harmonised classification and labelling
at EU level of

fluopicolide (ISO); 2,6-dichloro-*N*-[3-chloro-5-(trifluoromethyl)-2-pyridylmethyl]benzamide

EC Number: -
CAS Number: 239110-15-7

CLH-O-0000006820-76-01/F

Adopted
11 June 2020

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 an opinion on the proposal for harmonised classification and labelling (CLH) of:

Chemical name: Fluopicolide (ISO)

EC Number: -

CAS Number: 239110-15-7

The proposal was submitted by **Austria** and received by RAC on **8 July 2019**.

In this opinion, all classification and labelling elements are given in accordance with the CLP Regulation.

PROCESS FOR ADOPTION OF THE OPINION

Austria has submitted 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 **12 August 2019**. Concerned parties and Member State Competent Authorities (MSCA) were invited to submit comments and contributions by **11 October 2019**.

ADOPTION OF THE OPINION OF RAC

Rapporteur, appointed by RAC: **Lea Stine Tobiassen**

Co-Rapporteur, appointed by RAC: **Peter Hammer Sørensen**

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 RAC opinion on the proposed harmonised classification and labelling was adopted on **11 June 2020** by **consensus**.

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	No current Annex VI entry										
Dossier submitters proposal	TBD	fluopicolide (ISO); 2,6-dichloro- <i>N</i> -[3-chloro-5-(trifluoromethyl)-2-pyridylmethyl]benzamide		239110-15-7							
RAC opinion	TBD	fluopicolide (ISO); 2,6-dichloro- <i>N</i> -[3-chloro-5-(trifluoromethyl)-2-pyridylmethyl]benzamide		239110-15-7	Repr. 2	H361d	GHS08 Wng	H361d			
Resulting Annex VI entry if agreed by COM	TBD	fluopicolide (ISO); 2,6-dichloro- <i>N</i> -[3-chloro-5-(trifluoromethyl)-2-pyridylmethyl]benzamide		239110-15-7	Repr. 2	H361d	GHS08 Wng	H361d			

GROUNDNS FOR ADOPTION OF THE OPINION

RAC General Comment

Fluopicolide is a fungicide used in agriculture to control oomycetes, e.g. *Phyphthora infestans*, causing potato blight.

RAC was unable to evaluate the physical and environmental hazard classes. The Committee was informed that the DS had not provided data, citing resource limitations as the reason. RAC notes that in light of it being mandatory to consider all hazard classes for the harmonised classification of pesticides and biocides (CLP regulation Art. 36(2), Art 37(1) and Annex VI Parts 1 and 2) the remaining hazard classes should therefore become the subject of a future Annex XV dossier without delay.

HUMAN HEALTH HAZARD EVALUATION

RAC evaluation of acute toxicity

Summary of the Dossier Submitter's proposal

The DS summarised three acute toxicity studies in the CLH report.

Acute oral toxicity

In an OECD TG 423 compliant oral acute toxicity study (acute toxic class method) conducted in 2000, 5 male and 5 female fasted Sprague-Dawley rats were each administered by gavage a single oral dose of 5000 mg/kg bw. There were no mortality. Clinical signs as piloerection and hunched posture were seen in all female rats and in three male rats, and abnormal gait was noted in three females. Recovery of rats, as judged by external appearance and behavior, was complete by day 3. No significant effect was reported on body weight gains throughout the study. The acute lethal oral dose of fluopicolide in rats was greater than 5000 mg/kg bw. The DS proposed not to classify for acute oral toxicity.

Acute dermal toxicity

An OECD TG 402 acute dermal limit toxicity study was conducted in Hsd Sprague-Dawley rats in 2000 with 97.7% pure fluopicolide. A single topical application of 5000 mg/kg bw of the test substance was applied under occlusion for 24 hours.

No animals died. No clinical signs or dermal response in any animal was observed throughout the study. Two females had a slightly reduced body weight on day 8. There were no macroscopic abnormalities at study termination on day 15. The fluopicolide acute lethal dermal dose (LD₅₀) to rats was greater than 5000 mg/kg bw and thus greater than the trigger value of 2000 mg/kg bw, and therefore the DS proposed not to classify fluopicolide for acute dermal toxicity.

Acute inhalation toxicity

A limit test in accordance with OECD TG 403 exposing five male and five female Sprague-Dawley rats nose only to 5.16 mg/L for four hours did not lead to any mortality.

Clinical signs during and post exposure included wet fur, hunched posture, piloerection and increased respiratory rate. Isolated occurrences of noisy respiration and red/brown staining

around the snout or eyes were also seen, but all animals recovered within a day after exposure. At autopsy, one male showed dark foci on its lungs. The LC₅₀ was thus > 5.16 mg/L.

No classification for acute inhalation toxicity was proposed by the DS.

Comments received during public consultation

No comments were received.

Assessment and comparison with the classification criteria

Limit acute toxicity tests were conducted with fluopicolide following oral, dermal and inhalation route. For the oral route, a dose of 5000 mg/kg bw was used, and no mortalities were reported. Thus, the criteria for classification (2000 mg/kg bw) for acute oral toxicity was not met, and no classification is warranted.

For the dermal route, no mortalities were reported at the limit dose of 2000 mg/kg bw, which also is the classification limit dose for classification for acute dermal toxicity. No classification for the dermal route is therefore warranted.

With respect to the inhalation route, a concentration of 5.16 mg/L for 4 hours was used. No mortalities at the doses tested were reported. As the highest limit for classification is 5 mg/L for 4h for dusts and mists, no classification for acute toxicity by inhalation is warranted.

RAC agrees with the DS that **no classification for acute toxicity through any route is warranted for fluopicolide.**

RAC evaluation of specific target organ toxicity – single exposure (STOT SE)

Summary of the Dossier Submitter's proposal

The acute studies that are relevant for the assessment of the specific target organ toxicity of fluopicolide after single exposure are assessed in the acute toxicity section. An acute neurotoxicity study is also available and is summarised below.

Table: Summary table of animal studies relevant for STOT SE

Study	Doses	Main effects	Reference
Acute oral, dermal and inhalation toxicity studies in rats	Fluopicolide as tested in the acute oral, dermal and inhalation toxicity studies	No specific target organ toxicity, also no narcotic effects, which would fall under any STOT SE criteria were noted in the acute toxicity studies.	Sec. Acute toxicity
Acute neurotoxicity Oral (gavage) US OPPTS 870.6200 (1988) GLP Rat, CD Males & Females 10/sex/group Fluopicolide (purity 95.9%) Vehicle: aqueous 1% methylcellulose	0, 10, 100 and 2000 mg/kg bw Single dose	There were no deaths or specific neurotoxicity findings. <u>2000 mg/kg bw</u> Transiently decreased body temperature in both sexes <u>100 mg/kg bw</u> No treatment-related effects <u>10 mg/kg bw</u> No treatment-related effects	Anonymous, 2002; M-208046-01-1

The studies on acute oral, dermal and inhalation toxicity demonstrated a low acute toxic potential of fluopicolide with oral and dermal LD₅₀ values of > 5000 mg/kg bw and an inhalation LC₅₀ value of > 5.16 mg/L. These values are above the classification criteria for STOT-SE classification. In addition, an acute neurotoxicity study is available in which no specific acute neurotoxicity or any narcotic effect was observed.

There is also no indication of transient effects such as respiratory tract irritation and narcotic effects after single exposure to fluopicolide. Therefore, the DS proposed no classification of fluopicolide for STOT SE.

Comments received during public consultation

No comments were received.

Assessment and comparison with the classification criteria

There was no indication of any sex-specific susceptibility in any of the acute studies. No specific, non-lethal target organ toxicity, or other significant health effects that can impair function, either reversible or irreversible, immediate and/or delayed, arising from a single exposure were seen in the acute toxicity studies. In the acute neurotoxicity study, no specific neurotoxicity effects including narcotic effects were observed. The STOT SE criteria are not fulfilled based on the results of the acute toxicity studies with fluopicolide, as shown in the following table:

Table: Summary table of acute toxicity study results

Study	Toxicological effects at LOAEL
Acute oral rat	> 5000 mg/kg bw: unspecific clinical signs
Acute dermal rat	> 5000 mg/kg bw: No clinical signs, no mortalities
Acute inhalation rat	> 5.16 mg/L/4h (highest tested dose): unspecific clinical signs, no mortalities
Acute oral neuro-toxicity rat	No effects related to specific neurotoxicity up to 2000 mg/kg bw, NOEL: 100 mg/kg bw for general toxicity

On the basis of the available studies, RAC agrees with the DS that **no classification for STOT SE is justified.**

RAC evaluation of skin corrosion/irritation

Summary of the Dossier Submitter's proposal

The DS summarised an OECD TG 404 compliant skin irritation study by semi-occluded application of 0.5 g of moistened fluopicolide for four hours using 3 New Zealand White rabbits (Anonymous, 2000). Dermal reactions were assessed 1, 24, 48 and 72 hours after removal of the dressings. No dermal irritation in any animal was elicited (mean irritation score 0.0 for erythema and oedema for all animals over 24 – 72h).

Comments received during public consultation

No comments were received.

Assessment and comparison with the classification criteria

As the scores for erythema/eschar for fluopicolide in this guideline compliant dermal irritation study in rabbits were 0 for all animals, the criteria > 2.3 for erythema/eschar in at least 2 out of 3 animals is not met. RAC thus concurs with the DS that no classification for skin corrosion/irritation is warranted.

RAC evaluation of serious eye damage/irritation

Summary of the Dossier Submitter's proposal

The eye irritating potential of fluopicolide was investigated in 2000 in an OECD TG 405 compliant study in New Zealand White rabbits, using instillation of a single dose of 100 mg of fluopicolide powder to one eye each of three rabbits. Conjunctival redness was seen in two animals with a mean score of 0.33 at the 24, 48 and 72h after exposure readings. The effect was reversed 2 days after exposure. Scores were 0 for chemosis, iritis and corneal opacity at all observation points. Thus, fluopicolide was slightly irritant to the rabbit eye but the effects were transient.

Comments received during public consultation

No comments were received.

Assessment and comparison with the classification criteria

The criteria for classification for eye irritation were not met at any observation point for any animal in the study. All parameters (corneal opacity, iritis and/or conjunctival oedema (chemosis) had mean scores for 24, 48 and 72 hours post instillation of 0.0, whilst the mean score for conjunctival redness was 0.33 in two out of three animals, thus none of the criteria for classification are fulfilled. The slight ocular reactions resolved within two days after instillation. The DS proposed no classification for serious eye damage/eye irritation. RAC concurs with the DS that **no classification of fluopicolide for eye irritation/eye damage is warranted.**

RAC evaluation of respiratory sensitisation

Summary of the Dossier Submitter's proposal

No data investigating specifically respiratory sensitisation potential of fluopicolide are available from humans nor animals. The DS evaluated the end-point of respiratory sensitisation in a weight of evidence approach including information from medical surveillance of workers in two fluopicolide production plants in which no adverse reactions were reported, and information from available animal studies that showed low systemic toxicity or local reactivity with skin and mucous membranes. Finally, the negative skin sensitisation study (Guinea pig maximization test) described below was also included in the evaluation. The DS concluded overall that fluopicolide is unlikely to induce of respiratory sensitisation.

Assessment and comparison with the classification criteria

According to the criteria for classification for respiratory sensitisation the evidence for this endpoint will normally be based on human experience. The criteria further state that (a) the size of the population exposed, and (b) the extent of exposure is taken into account. There is no positive evidence of a respiratory sensitisation potential of fluopicolide in humans. However, RAC's assessment is that due to the lack of details on e.g. exposure levels provided in the information from production sites, the negative data are insufficient to be used to conclude that the substance is not a respiratory sensitiser.

None of the alerts included in the IR&CSA guidance are present in fluopicolide.

The DS included information from acute toxicity and irritation tests to support the evaluation that fluopicolide. RAC agrees with the DS that there is a low potential for respiratory sensitisation, although the nature of tests to be included is not clear from the criteria nor the guidance on classification.

RAC notes that reference is made in the guidance to using skin sensitising properties of the substance, e.g. from non-standard versions of the LLNA in the assessment. For fluopicolide, no LLNA is available, but a negative GPMT was included by the DS in the weight of evidence evaluation leading to the conclusion that the data available were sufficient to conclude that fluopicolide is not a respiratory sensitizer.

RAC agrees with the DS that fluopicolide should not be classified for this hazard class. However, RAC was not prepared to base their conclusion solely on negative data from studies that are not designed for the assessment of respiratory sensitisation.

In conclusion, RAC considers that **fluopicolide does not warrant classification for respiratory sensitisation due to insufficient data.**

RAC evaluation of skin sensitisation

Summary of the Dossier Submitter's proposal

The skin sensitising potential of fluopicolide has been investigated in an OECD TG 406 compliant guinea-pig maximisation test (GMPT) using 10% w/v fluopicolide for intradermal induction with or without Freund's complete adjuvant, whilst the topical induction was performed 6 days later with 100% w/v fluopicolide under occlusive dressing for 48 hours. Challenge two weeks after topical induction under occlusion for 24 hours used 100% fluopicolide and 50% fluopicolide. In all cases, sterile water was used as vehicle.

Intradermal induction sites receiving Freund's Complete Adjuvant in all test and control animals showed necrosis. Slight irritation was seen in 6/20 animals on the site treated with the intradermal injections of 10% w/v fluopicolide in sterile water. No irritation was observed in controls. After topical application, slight to well-defined erythema was observed in all test animals receiving 100% w/v fluopicolide. Slight erythema was seen in one control guinea pig.

Challenge resulted in two out of 20 test animals showing slight erythema at the 24 and 48 hour readings. In the control animals, 2 out of 10 animals reacted with slight to well-defined erythema at the 48-hour reading only.

Due to the comparable reaction in the controls at a higher incidence than in the test group, and the lack of reactions in the other test animals, the DS considered the overall response to be negative. No classification for skin sensitisation is proposed.

Comments received during public consultation

No comments were received.

Assessment and comparison with the classification criteria

The incidence of slight erythema in the tested animals in 2 out of 20 animals at 24 and 48 hours after challenge is below the value relevant for classification. RAC notes that the reactions in the 2/10 controls at 48 hours after induction may be interpreted as a reduced reliability of the study. In conclusion, RAC agrees with the DS that **fluopicolide should not be classified for skin sensitisation based on the available evidence.**

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

Summary of the Dossier Submitter's proposal

Nine subchronic repeated dose study in rats, mice and dogs conducted with fluopicolide are summarised in the table below. Information from other studies using repeated exposure for specific investigations (neurotoxicity, developmental toxicity, effects on fertility or chronic/carcinogenic effects) in rats or rabbits described under the respective heading of carcinogenicity and toxicity to reproduction) are also considered in the evaluation of specific target toxicity by repeated exposure.

Table: Summary table of animal studies on STOT RE

Method, publication year, guideline, test substance purity	Species, strain, sex, no/group	Dose levels, duration of exposure	Results
Rat 28-day dietary toxicity study, OECD TG 407 (1995) GLP Fluopicolide (purity 99.0%) Year: 2000	Sprague Dawley CRL:(IGS)CDBR rats 5/dose/sex	0, 20, 200, 2000 or 20000 ppm Equivalent to: 1.78, 17.7, 179 and 1770 mg/kg bw/day (combined sexes) 28 days	<u>20 ppm (1.78 mg/kg bw/day)</u> no effects observed <u>≥ 200 ppm (17.7 mg/kg bw/day)</u> ↑ incidence of centrilobular hepatocyte hypertrophy 2/5 M (1 minimal & 1 slight) & 3/5 F (minimal) <u>≥ 2000 ppm (179 mg/kg bw/day)</u> ↓ bodyweight F (-11.3%) on day 29 ↓ body weight gain# in F (-30%) on days 0-29 ↑ water consumption in M (+18.4%) in week 3 ↑ cholesterol in M (+50%***) & F (+29%***) Pale kidneys in 3/5 M ↑ severity of phloxine tartrazine-positive granulation (hyaline droplets) in kidneys (M) ↑ incidence of centrilobular hepatocyte hypertrophy in 5/5 M (1 minimal & 4 slight) & 2/5 F (minimal) <u>20000 ppm (1770 mg/kg bw/day)</u> ↓ bodyweight (M) ↑ water consumption (F) ↓ feed intake (M/F) ↓ ALT (M) ↑ relative liver weights (M/F) ↑ absolute liver weights (M) enlarged livers (M)
Mouse 28-day dietary toxicity study,	CD-1 mice 5/dose/sex	0, 6, 64, 640 or 6400 ppm Equivalent to:	<u>6 ppm (1.07 mg/kg bw/day)</u> No effects observed <u>64 ppm (11.6 mg/kg bw/day)</u> No effects observed <u>≥ 640 ppm (115 mg/kg bw/day)</u>

Method, publication year, guideline, test substance purity	Species, strain, sex, no/group	Dose levels, duration of exposure	Results
OECD TG 407 (1995) GLP Fluopicolide (purity 99.0%) Year: 2000		1.07, 11.6, 115 and 1111 mg/kg bw/day (combined sexes) 28 days	<ul style="list-style-type: none"> ↑ ALT in M (+81%***) & F (+49%***) ↑ rel. liver weight in F (+19%***) ↑ incidence & severity of hypertrophy of centrilobular hepatocytes in 5/5 M (1 minimal, 3 slight & 1 moderate) & 4/5 F (1 minimal & 3 slight) <u>6400 ppm (1111 mg/kg bw/day)</u> ↑ AP (M) ↑ abs. & rel. liver weight (M/F) ↑ ALT in M (+148%***) & F +54%***) ↑ AP in M (+134%) ↑ rel. liver weight in M (+42%***) & F (+58%***) ↑ abs. liver weight in M (+33%***) & F (+50%***) ↑ incidence & severity of hypertrophy of centrilobular hepatocytes in 5/5 M (4 moderate & 1 slight) & 5/5 F (moderate)
Mouse 28-day dietary mechanistic toxicity study, No applicable guideline GLP Fluopicolide (purity 99.3%) Year 2004	C57BL/6 mice 15/females/dose	0 or 3200 ppm Equivalent to: 575 mg/kg bw/day 28 days	<ul style="list-style-type: none"> <u>3200 ppm (575 mg/kg bw/day)</u> ↓ body weight ↑ abs. & rel. liver weight ↑ activity of drug metabolizing enzymes in the liver ↑ incidence of perilobular to panlobular hepatocellular hypertrophy ↑ no. of mitotic cells in liver
Dog 28-day oral gavage toxicity study, OECD TG 409 (1998) GLP Fluopicolide (purity 96.9%) Year 2000	Beagle dogs 2/sex/group	0, 10, 100 and 1000 mg/kg bw/day 28 days	<ul style="list-style-type: none"> <u>10 mg/kg bw/day</u> No effects observed <u>100 mg/kg bw/day</u> No effects observed <u>1000 mg/kg bw/day</u> ↑ cholesterol in blood (M) ↑ abs. & rel. liver weight (M)
Subacute (28-days) dermal toxicity study in rats, OECD TG 410 (1981) GLP Fluopicolide (purity 97.7%) Year: 2003	Wistar rats 10/dose/sex	0, 100, 250, 500, and 1000 mg/kg bw/day semi-occlusive covering five days/ week	<ul style="list-style-type: none"> <u>100, 250, 500 and 1000 mg/kg bw/day</u> No effects observed
90-day dietary toxicity study in rats with 4-week recovery period, OECD TG 407 (1995) GLP	Sprague Dawley rats 10/dose/sex	0, 100, 1400 or 20000 ppm equivalent to: 0, 7.9, 114 or 1671 mg/kg bw/day (combined sexes)	<ul style="list-style-type: none"> <u>100 ppm (7.9 mg/kg bw/day)</u> No effects observed <u>≥ 1400 ppm (114 mg/kg bw/day)</u> ↑ cholesterol in blood (M) ↑ epithelial cells in urinary sediment (M) ↑ urine volume & ↓ specific gravity (F) ↑ rel. liver weight (M) ↓ abs. and rel. spleen weight (F) ↑ rel. kidney weight (M)

Method, publication year, guideline, test substance purity	Species, strain, sex, no/group	Dose levels, duration of exposure	Results
Fluopicolide (purity 96.9 and 97.5%) Year: 2000		13 weeks (+ 4 weeks for recovery group animals)	<p>↑ incidence hypertrophy of centrilobular hepatocytes (M) ↑ severity accumulation of hyaline droplets in the proximal kidney tubule (M) ↑ single cell death in the proximal kidney tubule epithelium (M) ↑ foci of basophilic (regenerating) tubules and granular casts (M) ↑ severity & incidence of trabecular hyperostosis of the bone joint (F)</p> <p><u>20000 ppm (1671 mg/kg bw/day)</u> ↑ hair loss & body soiling (M/F) ↓ bodyweight (M/F) ↓ feed intake (M/F) ↑ water intake (F) ↓ red blood cell parameters (M/F) ↑ APTT (M) ↑ cholesterol, protein & GGT in blood (M/F) ↑ abs. & rel. liver weight (F) ↓ abs. & rel. spleen weight (M) ↑ severity and incidence of hypertrophy of the zona glomerulosa in the adrenals (M/F) ↑ severity & incidence of trabecular hyperostosis of the bone joint (M) ↓ cellularity of the bone marrow (M/F) ↑ incidence hypertrophy of centrilobular hepatocytes (F)</p>
90-day dietary toxicity study in mice, OECD TG 408 (1998) GLP Fluopicolide (purity 97.3%) Year: 2000	CrI: CD-1 (ICR) BR mice 10/sex/ dose	0, 32, 320, 3,200 and 6400 ppm Equivalent to: 0, 5.5, 53, 545 and 1092 mg/kg bw/day (both sex combined)	<p><u>32 ppm (5.5 mg/kg bw/day)</u> No effects observed <u>≥ 320 ppm (53 mg/kg bw/day)</u> ↑ incidence hypertrophy of centrilobular hepatocytes in 9/10 M (6 minimal & 3 slight) & 2/10 F (minimal) <u>≥ 3200 ppm (545 mg/kg bw/day)</u> ↓ body weight gain (F) ↑ ALT (M/F) and AST (M) ↑ abs. & rel. liver weight (M/F) ↑ incidence of hepatocyte necrosis (F) <u>6400 ppm (1092 mg/kg bw/day)</u> ↓ body weight gain (M) ↑ AP (M) ↑ cholesterol and creatinine in blood (F) ↑ incidence of hepatocyte necrosis (M)</p>
90-day dietary toxicity study in mice, OECD TG 408 (1998) GLP Fluopicolide (purity 95.9%) Year: 2006	C57BL/6JICO mice 10/sex/ dose	0, 50, 200, 800 and 3200 ppm Equivalent to: 10.4/12.6, 37.8/52.8, 161/207, 770/965 mg/kg bw/day (M/F)	<p><u>50 ppm (10.4/12.6 mg/kg bw/day)</u> No effects observed <u>≥ 200 ppm (37.8/52.8 mg/kg bw/day)</u> ↓ cholesterol in blood in M (-26%***) & F (-21%***) <u>≥ 800 ppm (161/207 mg/kg bw/day)</u> ↓ albumin in blood (M/F) ↑ incidence centrilobular hepatocellular hypertrophy (M/F) ↑ rel. liver weight (M/F) ↑ abs. liver weight (F) <u>3200 ppm (770/965 mg/kg bw/day)</u> ↑ abs. liver weight (M) ↓ body weight gain (M/F) ↑ AP (M)</p>

Method, publication year, guideline, test substance purity	Species, strain, sex, no/group	Dose levels, duration of exposure	Results
<p>Subchronic dietary neurotoxicity study in rats</p> <p>USEPA : OPPTS 870.6200 (1998)</p> <p>GLP</p> <p>Fluopicolide (purity 97.8%)</p> <p>Year: 2002</p>	<p>CD rats</p> <p>10/sex/dose</p>	<p>0, 200, 1400 or 10000 ppm</p> <p>Equivalent to: 15.0/18.0, 107/125, 781/866 mg/kg bw/day (M/F)</p> <p>13 weeks</p>	<p><u>200 ppm (15.0/18.0 mg/kg bw/day)</u> No effects observed</p> <p><u>≥ 1400 ppm (107/125 mg/kg bw/day)</u> ↓ body weight gains (M/F) ↑ incidence of centrilobular hepatocyte hypertrophy (M) ↑ incidence and/or severity of hyaline droplets in the cortical tubules in the kidneys (M)</p> <p><u>10000 ppm (781/866 mg/kg bw/day)</u> ↓ food consumption (M/F) ↑ incidence of centrilobular hepatocyte hypertrophy (F) ↑ incidences and severities of other degenerative or regenerative changes in the kidneys including inflammation, casts and dilatation (M)</p>
<p>Dog 90-day oral gavage toxicity study,</p> <p>OECD TG 409 (1998)</p> <p>GLP</p> <p>Fluopicolide (purity 97.7%)</p> <p>Year: 2000</p>	<p>Beagle dogs</p> <p>4/sex/group</p>	<p>0, 5, 70 or 1000 mg/kg bw/day</p>	<p><u>5 mg/kg bw/day</u> No effects observed</p> <p><u>70 mg/kg bw/day</u> No effects observed</p> <p><u>1000 mg/kg bw/day</u> ↓ body weight gain (M/F) ↑ abs. & rel. liver weight (M/F)</p>
<p>52-week toxicity study by oral route (gavage) in dogs,</p> <p>OECD TG 452 (1981)</p> <p>GLP</p> <p>Fluopicolide (purity 95.9%)</p> <p>Year: 2002</p>	<p>Beagle dogs</p> <p>5/sex/group</p>	<p>0, 70, 300 or 1000 mg/kg/day</p>	<p><u>70 mg/kg bw/day</u> No effects observed</p> <p><u>≥ 300 mg/kg bw/day</u> ↑ incidence of liver enlargement (M/F)</p> <p><u>1000 mg/kg bw/day</u> ↓ bodyweight gain (M) ↑ cholesterol in blood (F)</p>
<p>Chronic toxicity and carcinogenicity study in mice,</p> <p>OECD TG 451 (1981)</p> <p>GLP</p> <p>Fluopicolide (purity 95.9%)</p> <p>Year: 2003</p>	<p>C57BL/6 mice</p> <p>60/sex/dose</p>	<p>0, 50, 400 and 3200 ppm</p> <p>Equivalent to: 7.9/11.5, 64.5/91.9, 551/772.3 mg/kg bw/day (M/F)</p> <p>78 weeks (52 week interim sacrifice)</p>	<p><u>50 ppm (7.9/11.5 mg/kg bw/day)</u> No effects observed</p> <p><u>400 ppm (64.5/91.9 mg/kg bw/day)</u> ↑ abs. & rel. liver weight week 52 and 78 (M/F) ↑ incidence of hepatocellular hypertrophy week 52 and 78 (M/F)</p> <p><u>3200 ppm (551/772.3 mg/kg bw/day)</u> ↓ body weight (M/F) ↓ feed intake (M/F) ↑ incidence of altered liver foci week 52 (F) and 78 (M/F) ↑ incidence of liver adenomas week 52 (F) and 78 (M/F)</p>

Method, publication year, guideline, test substance purity	Species, strain, sex, no/group	Dose levels, duration of exposure	Results
<p>Two-generation dietary study in rats^a,</p> <p>OECD TG 416 (1999)</p> <p>GLP</p> <p>Fluopicolide (purity 95.9%)</p> <p>Year: 2004</p>	<p>CrI: CD® (SD) IGS BR rats</p>	<p>0, 100, 500 and 2000 ppm</p> <p>Equivalent to:</p> <p><u>F0 pre-mating (Week 1-10)</u> 7.4/8.1, 36.4/41.0, 147.3/159.7 mg/kg bw/day (M/F)</p> <p><u>F0 gestation (GD 0-20)</u> 7.4, 38.1, 150.8 mg/kg bw/day</p> <p><u>F0 lactation (LD 0-14)</u> 13.5, 70.5, 281.4 mg/kg bw/day</p> <p><u>F1 pre-mating (Week 1-10)</u> 8.8/9.4, 43.7/46.9, 179.9/193.9 mg/kg bw/day (M/F)</p> <p><u>F1 gestation (GD 0-20)</u> 7.7, 39.2, 156.2 mg/kg bw/day</p> <p><u>F1 lactation (LD 0-14)</u> 15.8, 74.8, 320.4 mg/kg bw/day</p>	<p><u>100 ppm (7.4-15.8 mg/kg bw/day)</u> No effects on reproductive organs, liver & kidney observed</p> <p><u>≥ 500 ppm (36.4-74.8 mg/kg bw/day)</u> ↑ incidence of centrilobular hepatocyte hypertrophy in both generations (M/F)</p> <p><u>2000 ppm (147.3-320.4 mg/kg bw/day)</u> ↑ incidence of cortical tubular basophilia in F0 (M) and F1 (M/F) ↑ increased incidence of cortical tubules with hyaline droplets, granular casts in the medulla, hyaline tubular casts, interstitial inflammation and cortical scarring in the kidneys in both generations (M) ↑ incidence of cortical tubular dilatation and corticomedullary mineralization in kidneys of both generations (F)</p>
<p>Developmental toxicity study in rats,</p> <p>OECD TG 414 (1981)</p> <p>GLP</p> <p>Fluopicolide (purity 97.6-97.8%)</p> <p>Year: 2003</p>	<p>Hsd: Sprague Dawley</p> <p>23 mated females/group</p>	<p>0, 5, 60 or 700 mg/kg bw/day</p> <p>GD 7-20</p>	<p><u>5 mg/kg bw/day</u> No effects observed</p> <p><u>60 mg/kg bw/day</u> No effects observed</p> <p><u>700 mg/kg bw/day</u> ↓ bodyweight gain ↓ feed consumption ↓ foetal weights and crown-rump length ↑ incidence of minor skeletal defects and delayed ossification</p>
<p>Developmental toxicity study in rabbits</p> <p>OECD TG 414 (1981)</p> <p>GLP</p> <p>Fluopicolide (purity 97.8%)</p> <p>Year: 2004</p>	<p>Chbb:HM(SPF) Himalayan rabbit</p>	<p>0, 5, 20 or 60 mg/kg bw/day</p> <p>GD 6-28</p>	<p><u>5 mg/kg bw/day</u> No effects observed</p> <p><u>20 mg/kg bw/day</u> No effects observed</p> <p><u>60 mg/kg bw/day</u> ↑ mortality ↑ incidence of premature deliveries ↑ clinical signs ↓ bodyweight gain ↓ feed consumption ↓ uterus weight ↓ foetal weights and crown-rump length</p>

Method, publication year, guideline, test substance purity	Species, strain, sex, no/group	Dose levels, duration of exposure	Results
Chronic toxicity and carcinogenicity study in rats OECD TG 453 (1981) GLP Fluopicolide (purity 95.9%) Year: 2003	CrI: CD® (SD) IGS BR rats 60/sex/dose	0, 50, 200, 750 and 2500 ppm Equivalent to: 2.1/2.8, 8.4/10.8, 31.5/41.0, 109.4/142.2 mg/kg bw/day (M/F) 104 weeks (52 week interim sacrifice)	<u>50 ppm (2.1/2.8 mg/kg bw/day)</u> No effects observed <u>≥ 200 ppm (8.4/10.8 mg/kg bw/day)</u> ↑ incidence centrilobular hepatocytic hypertrophy week 104 (M) <u>≥ 750 ppm (31.5/41.0 mg/kg bw/day)</u> ↑ abs. & rel. kidney weights week 52 & 104 (M) ↑ rel. liver weights week 52 (M) ↑ incidence centrilobular hepatocyte hypertrophy week 52 (M) ↑ incidence and/or severity of foci of alteration in liver week 104 (M/F) ↑ incidence and/or severity of cortical tubular basophilia in kidneys week 52 and 104 (M) ↑ incidence and/or severity of hyperplasia of the papillary epithelium in kidney week 104 (F) ↑ incidence of cystic follicular cell hyperplasia in the thyroids week 104 (M) <u>2500 ppm (109.4/142.2 mg/kg bw/day)</u> ↑ Brown staining on the dorsal body surface (F) ↑ protein in blood up to week 52 (M/F) ↑ albumin in blood week 13 (M) ↑ creatinine in blood (M) ↑ rel. kidney weights week 52 (F) ↑ rel. liver weights week 52 (F) ↑ abs. & rel. liver weights week 104 (M) ↑ thyroid weights week 104 (M) ↑ incidence and/or severity of cystic degeneration in liver (M) ↑ incidence and/or severity of degenerative changes in kidneys week 104 (M) ↑ incidence and/or severity of cortical tubular basophilia in kidneys (F) ↑ mineralisation of the papillary/pelvic epithelium (F) ↑ increased incidence and/or severity of acinar atrophic change in pancreas week 104 (M/F) ↑ incidence of acinar atrophy with reduced colloid in prostate week 104 (M)

↑ / ↓ = increased/decreased compared with control.

M = male, F = female

* p < 0.05; ** p < 0.01; *** p < 0.001 statistically different to controls; # = no statistical analyses performed

In the rabbit developmental toxicity study, severe maternal toxicity as evidenced by mortality, marked decreases in body weight gain and food consumption were observed at the highest tested dose of 60 mg/kg bw/day. The DS considered the sensitivity of rabbits to certain chemicals and concluded that the effects in the pregnant rabbits are not relevant for classification for STOT RE.

The DS further acknowledged that severe effects (blood related parameters, bones and adrenals) observed in some repeated dose studies at high doses were above the guidance values for classification as STOT RE.

The liver was the target organ in the repeated dose toxicity studies in rats and mice. Liver effects included increases in relative weights and increased incidences of centrilobular hepatocyte

hypertrophy. Mild effects were reported in rats from around 20 mg/kg bw/day and more severe effects in mice around 100 mg/kg bw/day in 28 day-studies. The effects were also seen with longer exposure period. In addition, clinical chemical parameters (e.g. changes in blood cholesterol levels, increased plasma transaminase levels and increased protein concentration in blood) were also affected in some, but not all studies. The DS considered these liver effects to be adaptive reversible changes resulting from induction of chemical metabolising enzymes in the liver.

In males rats, the kidney was also targeted by fluopicolide with findings in several studies on kidney weights and deposition of hyaline droplets (179 mg/kg bw/day in a 28 day study, and at around 110 mg/kg bw/d in a 90 day neurotoxicity study). The droplets are a consequence of accumulation of the rat specific protein $\alpha_2\mu$ -globulin, a mechanism of low human relevance.

Effects on other organs in mice and rats occur only at doses higher than the guidance values for classification (effects on blood related parameters, bones and adrenals).

The dog was less sensitive than the rodents and generally showed no effects at dose levels relevant up to 1000 mg/kg bw/day in a 90-day study and 300 mg/kg bw/day in a one-year study.

No adverse effects were observed in a rat dermal 28-day study up to doses of 1000 mg/kg bw/day.

Comments received during public consultation

In a comment from a company, historical control data (HCD) were provided, which related to the findings of interstitial cell hyperplasia in the testes and acinar cell atrophy with associated reduced colloid in the prostate observed in mid- and high-dose males in the 2-year combined rat chronic/carcinogenicity study were provided.

Another comment from an MSCA concerned the mortality in the rabbit developmental study. The DS responded that the mortality were related to rabbit specific gastro-intestinal tract sensitivity due to a high degree of bacteria-mediated digestion in the caecum, which differs from that of e.g. rats and humans and that the mortalities in the rabbits were therefore not relevant for classification.

Assessment and comparison with the classification criteria

The criteria for classification as STOT RE refer to significant and/or severe morphological change or clear indication of functional disturbance in studies following repeated exposure. Guidance values are provided to use in a weight of evidence evaluation of the hazard class. For STOT RE category 1, the guidance value for an oral 90-day study in rats is ≤ 10 mg/kg bw/day, whilst the guidance value for STOT RE category 2 is ≤ 100 mg/kg bw/day.

Extrapolation in accordance with Haber's rule can be used to include studies of shorter or longer durations. Thus, for classification as STOT RE 1 a 28-day study the guidance value would be around 30 mg/kg bw/day - multiplying by a factor 3, whilst an exposure duration of 21 days would warrant classification as STOT RE 1 when occurring at ≤ 40 mg/kg bw/d. In a one-year study, classifiable effects would lead to classification in category 1 when occurring at ≤ 1.25 mg/kg bw/day. For classification in category 2, the corresponding guidance values are ≤ 300 mg/kg bw/day for a 28 day study, ≤ 600 mg/kg bw/day for a 14-day study and ≤ 12.5 mg/kg bw/day for effects seen in a one-year study.

Mortality:

RAC notes that mortality, when occurring after repeated exposure, is a relevant end-point for consideration under the heading of STOT RE, as described under point 3.9.2.7.3 of Annex I to the CLP Regulation.

In the rabbit developmental toxicity study (described further under "toxicity to reproduction" below), the highest dose tested was 60 mg/kg bw/day. This led to the death of 3 out of 23 dams whilst fifteen dams were killed in relation to early deliveries following exposure duration of between 16 and 22 days (GD 6-28 of gestation, mortalities occurring from day 22 of gestation). One dam of the mid-dose group was killed after early delivery. No deaths occurred in the low dose group.

In the high dose group, mean food consumption and mean body weight were decreased by up to 40% and 8.3%, respectively, compared to controls at the end of the study, but were unaffected in the low and mid-dose groups. The clinical signs seen at the highest dose level included hypoactivity, decreased hay consumption, decreased defecation, decreased body weight and/or discoloured urine for 0-4 days before death or filled stomach at autopsy in 9 out of 18 animals. In the remaining 9 animals that were killed after premature delivery, there were no clinical signs but body weight gain was reduced and filled stomach was reported.

Rabbits are known to be sensitive to antimicrobial xenobiotics interfering with the predominantly bacteria-mediated digestion. Fluopicolide is an anti-fungal agent. Therefore, RAC considers that impairment of normal gastro-intestinal tract function lead to poor condition and deaths of nine or more dams in this developmental toxicity study. In addition, pregnant rabbits are known to be sensitive to reduced food intake and decreases in body weight gain, which could be the cause of some cases of premature deliveries. In Annex I, paragraph 3.7.2.4.4 of the CLP Regulation, it is stated that "in rabbits, the body weight gain may not be useful indicators of maternal toxicity because of normal fluctuations in body weight during pregnancy".

RAC notes that no excessive mortality was reported in repeated dose studies, neurotoxicity studies or reproductive toxicity studies in rats, mice or dogs and that the rabbit is the sole species presenting a high sensitivity to oral intake of fluopicolide which supports the hypothesis that the mortality seen can be considered to be specific to the rabbit.

In conclusion, RAC considers that no classification for as STOT RE for mortality is warranted for fluopicolide.

Findings in the liver (increased weights, centrilobular hepatocyte hypertrophy, changes in blood chemistry) in studies in rats and mice of various exposure duration indicated a clear adverse effect on the liver. The severity of the effects were variable across the studies and species, but were reported to occur at relatively lower exposure levels in studies of shorter duration. The DS considered that the liver effects were the result of adaptive change to the chemical and thus not relevant for classification.

RAC notes that fluopicolide treatment resulted in the activation of CAR and weak activation of PXR in the liver, which is described below in the section on carcinogenicity. This MoA was supported by a series of associative events including the following: increased expression of genes encoding cytochrome P450s (CYPs), particularly CYP2B and (to a lesser extent) CYP3A isoforms, increased proliferation and hepatocellular hypertrophy and increased liver weight.

The kidney effects observed were increased severity of hyaline droplets and increased absolute kidney weight. The effects were only observed in male rats. Hyaline droplets have been recognized as an indicator of changes associated with the accumulation of $\alpha_2\mu$ -globulin. However, no immune-histochemical investigations are described in the CLH report and most likely these have not been performed. Hence human relevance cannot be excluded. The effects in the relevant dose range for STOT RE classification were only observed in male rats and were

characterised by increased severity of hyaline droplets in the 28-day and 90-day studies and a slightly increased absolute kidney weight. The incidences of the hyaline droplets were seen at doses above the guidance value for STOT RE Cat. 2 classification in the 13 week neurotoxicity study (107/125 mg/kg bw/day) and in the 90 day rat study (114 mg/kg bw/day). Because the effect was recorded at doses above the guidance value for STOT RE 2, RAC concludes that classification is not required. However, RAC cannot conclude that the effect is not relevant to humans, as the appropriate immune-histochemical investigations, to conclude that the effects are mediated through the rat specific protein $\alpha_2\mu$ -globulin, have not been performed. Effects on other target organs, e.g. blood related parameters, bones and adrenals, were seen in repeated dose toxicity studies at doses above the guidance values for classification.

Overall, RAC considers that the available data on repeated dose toxicity by the oral route of fluopicolide is conclusive. No classification of the substance for STOT RE via the oral route, is warranted.

No effects were observed when fluopicolide was administered dermally to rats for 28 days at doses up to 1000 mg/kg bw/day; RAC agrees with the DS that classification for STOT RE via the dermal route is not warranted.

Overall, RAC considers that, based on the available data on repeated dose toxicity, **fluopicolide should not be classified for STOT RE.**

RAC evaluation of germ cell mutagenicity

Summary of the Dossier Submitter's proposal

The genotoxic potential of fluopicolide has been investigated in nine *in vitro* studies, covering the end-points bacterial- and mammalian-cell mutation and clastogenicity, and in five *in vivo* assays (an unscheduled DNA synthesis assay in rat liver, three mouse micronucleus tests and one Comet assay in mice).

Several genotoxicity studies were performed for fluopicolide: six reverse gene mutation tests in *Salmonella typhimurium* and *Escherichia coli* strains of bacteria, one of them recently (2017), one chromosomal aberration assay in Chinese hamster V79 cells *in vitro*, one chromosomal aberration assay in human lymphocytes *in vitro* and one HPRT mutation assay in Chinese hamster V79 cells. In addition, two *in vivo* micronucleus assays were performed in mouse bone marrow cells with oral administration, one *in vivo* micronucleus assay in mouse bone marrow cells with intraperitoneal administration, one *in vivo* oral UDS assay in rats and one *in vivo* Comet assay in male mice with oral gavage administration.

2 out of 15 tests, one *in vitro* bacterial reverse mutation assay and one *in vitro* chromosome aberration assay, gave weak positive responses of doubtful biological significance. The *in vivo* mutagenicity data from the Comet assay and micronucleus tests are reliable and it is clear that no mutagenic effects were seen in animals *in vivo*. Clearly, chromosomal damage or point mutations do not occur *in vivo*. Overall, the DS concluded that fluopicolide is not genotoxic *in vivo*.

Table: Summary table of mutagenicity/genotoxicity tests in vitro

Method	Test substance	Relevant information about the study including rationale for dose selection (as applicable)	Observations/ Results	Reference
Bacterial point mutation assay (Ames test) OECD TG 471 (1997) GLP	Fluopicolide (purity 97.8%) DMSO	Test system: <i>Salmonella typhimurium</i> strains TA 1535, TA 1537, TA 98, TA 100, and <i>Escherichia coli</i> WP2uvrA The following concentrations were tested: <u>Plate incorporation</u> Experiment I: 50, 160, 500, 1600 and 5000 µg/plate (±S9) Experiment II/III (TA98 only): 50, 160, 500, 1600, 2000; 3000, 4000 and 5000 µg/plate (+S9) <u>Pre-incubation</u> Experiment I: 50, 160, 500, 1600; and 5000 µg/plate (±S9)	Positive at precipitating dose levels.	Stammberger, 2004; M-197259-02-1
Bacterial point mutation assay (Ames test) OECD TG 471 (1997) GLP	Fluopicolide Purity not reported DMSO	Test system: <i>Salmonella typhimurium</i> strains TA 1535, TA 1537, TA 98, TA 100, and <i>Escherichia coli</i> WP2uvrA The following concentrations were tested: <u>Plate incorporation</u> Experiment I: 1.6, 8, 40, 200, 1000 and 5000 µg/plate (±S9) Experiment II: 31.25, 62.5, 125, 250, 500 and 1000 µg/plate (-S9) <u>Pre-incubation</u> Experiment II: 31.25, 62.5, 125, 250, 500 and 1000 µg/plate (+S9)	Negative	Ballantyne, 2001; M-202931-01-1
Bacterial point mutation assay (Ames test) OECD TG 471 (1997) GLP	Fluopicolide (purity 95.6%) DMSO	Test system: <i>Salmonella typhimurium</i> strains TA 1535, TA 1537, TA 98, TA 100, and <i>Escherichia coli</i> WP2uvrA The following concentrations were tested: <u>Plate incorporation</u> Experiment I: 1.6, 8, 40, 200, 1000 and 5000 µg/plate (±S9) Experiment II: 31.25, 62.5, 125, 250, 500 and 1000 µg/plate (-S9) <u>Pre-incubation</u> Experiment II: 31.25, 62.5, 125, 250, 500 and 1000 µg/plate (+S9)	Negative	Ballantyne, 2001; M-202927-01-1

Method	Test substance	Relevant information about the study including rationale for dose selection (as applicable)	Observation s/ Results	Reference
Bacterial point mutation assay (Ames test) OECD TG 471 (1997) GLP	Fluopicolide (purity 95.9%) DMSO	Test system: <i>Salmonella typhimurium</i> strains TA 1535, TA 1537, TA 98, TA 100, and <i>Escherichia coli</i> WP2uvrA The following concentrations were tested: <u>Plate incorporation</u> Experiment I: 1.6, 8, 40, 200, 1000 and 5000 µg/plate (±S9) Experiment II: 31.25, 62.5, 125, 250, 500 and 1000 µg/plate (-S9) <u>Pre-incubation</u> Experiment II: 31.25, 62.5, 125, 250, 500 and 1000 µg/plate (+S9)	Negative	Ballantyne, 2001; M-202939-01-1
Bacterial point mutation assay (Ames test) OECD TG 471 (1997) GLP	Fluopicolide (purity 99.3%) DMSO	Test system: <i>Salmonella typhimurium</i> strains TA 1535, TA 1537, TA 98, TA 100, and <i>Escherichia coli</i> WP2uvrA The following concentrations were tested: <u>Plate incorporation</u> Experiment I: 1.6, 8, 40, 200, 1000 and 5000 µg/plate (±S9) Experiment II: 31.25, 62.5, 125, 250, 500 and 1000 µg/plate (-S9) <u>Pre-incubation</u> Experiment II: 31.25, 62.5, 125, 250, 500 and 1000 µg/plate (+S9)	Negative	Ballantyne, 2001; M-202935-01-1
Bacterial point mutation assay (Ames test) OECD TG 471 (1997) GLP	Fluopicolide (purity 98.2%) DMSO	Test system: <i>Salmonella typhimurium</i> strains TA 1535, TA 1537, TA 98 TA 100 and TA 102 The following concentrations were tested: <u>Plate incorporation</u> Experiment I: 3, 10, 33, 100, 333, 1000, 2500, and 5000 µg/plate (±S9) Since for the positive control of strain TA 102 with S9 mix the acceptance criteria were not met, this part of experiment I was repeated (see experiment Ia below). Experiment Ia (TA102 only): 3, 10, 33, 100, 333, 1000, 2500, and 5000 µg/plate (+S9) <u>Pre-incubation</u> Experiment II: 10, 33, 100, 333, 1000, 2500 and 5000 µg/plate (±S9)	Negative	Chang, 2017; M-595228-01-1

Method	Test substance	Relevant information about the study including rationale for dose selection (as applicable)	Observation s/ Results	Reference
In vitro chromosome aberration assay in Chinese hamster lung V79 cells OECD TG 473 (1997) GLP	Fluopicolide (purity 97.8%) DMSO	Test system: Chinese hamster lung V79 cells The following concentrations were tested: Experiment I: 25, 50, 75 and 100 µg/mL (±S9) Experiment II: 1.6, 3.2 and 6.3 µg/mL (-S9) Cytotoxicity was not measured using the parameters of relative population doubling or relative increase in cell count. Only 25-100 metaphases, instead of the currently required 300 metaphases, were analysed.	Positive at cytotoxic concentrations	Stammberger and Graeser, 2004; M-197260-02-1
<i>In vitro</i> chromosome aberration assay in human lymphocytes OECD TG 473 (1997) GLP	Fluopicolide (purity 95.9%) DMSO	Test system: Human lymphocytes The following concentrations were tested: <u>Experiment I:</u> 19.53, 78.13 and 156.25 µg/mL (-S9) 78.13, 312.5 and 625 µg/mL (+S9) <u>Experiment II:</u> 1.22, 9.77 and 19.53 µg/mL (-S9) 39.06, 156.25 and 312.5 µg/mL (+S9) Cytotoxicity was not measured using the parameters of relative population doubling or relative increase in cell count. Only 25-100 metaphases, instead of the currently required 300 metaphases, were analysed.	Negative	Allais, 2001; M-201582-01-1
<i>In vitro</i> HPRT mutation assay in Chinese hamster lung V79 cells OECD TG 476 (1997) GLP	Fluopicolide (purity 97.8%) DMSO	Test system: Chinese hamster lung V79 cells The following concentrations were tested: Experiment I: 1.2, 3.8, 12.1, 38.2, 120.8, 382, 1208 and 3820 µg/mL (±S9) Experiment II: 0.4, 0.8, 1.6, 3.2, 6.3, 12.5, 25, 50, 75, 100 and 120 µg/mL (±S9) Experiment III: 0.313, 0.625, 1.25, 2.5, 5, 10, 20, 30, 40, 50 and 60 µg/mL (±S9)	Negative	Graeser and Stammberger, 2005; M-210831-02-1

Table: Summary table of mutagenicity/genotoxicity tests in mammalian somatic or germ cells in vivo

Method	Test substance	Relevant information about the study (as applicable)	Observations/ Results	Reference
Mouse micronucleus test OECD TG 474 (1997) GLP	Fluopicolide (purity 97.8%), in 1% (w/v) methylcellulose	Test system: Mouse (HsdWin:NMRI) The following concentrations were tested: 200, 600 and 2000 mg/kg bw (oral) Target organ exposure not measured. Only 2000 instead of 4000 erythrocytes were analysed for MN. Only 200 instead of 500 cells were analysed to obtain the PCE/NCE ratio.	Negative	Anonymous, 2005; M-197261-02-1
Mouse micronucleus test OECD TG 474 (1997) GLP	Fluopicolide (purity 96.1%), in 1% (w/v) methylcellulose	Test system: Mouse (CrI:CD1) The following concentration was tested: 2000 mg/kg bw (oral). Target organ exposure not measured. Only 2000, not 4000 erythrocytes, were analysed for MN. Only 200, not 500 cells were analysed to obtain the PCE/NCE ratio.	Negative	Anonymous, 2003; M-219364-01-1
Mouse micronucleus test (i.p.) OECD TG 474 (1997) GLP	Fluopicolide (purity 99.4%), in 0.5% cremophor	Test system: Mouse (CrI:CD1) The following concentrations were tested: 150, 300 and 600 mg/kg bw (i.p.) Altered NCE/PCE ratio. Only 2000, not 4000 erythrocytes, were analysed for MN.	Negative	Anonymous, 2003; M-223119-01-1
Rat UDS assay OECD TG 486 (1997) GLP	Fluopicolide (purity 97.7%), in 1% (w/v) methylcellulose	Test system: Rat (Hsd/Ola SD) The following concentrations were tested: 600 and 2000 mg/kg bw (oral)	Negative	Anonymous, 2000; M-197230-02-1
Comet assay in mice OECD TG 489 (2016) GLP	Fluopicolide (purity 98.2%), in 1% (w/v) methylcellulose	Test system: Mouse (Hsd:ICR (CD-1)) The following concentrations were tested: 500, 1000 and 2000 mg/kg bw (oral, gavage)	Negative	Anonymous, 2018; M-635020-01-1

Based on the available studies, no classification for germ cell mutagenicity was proposed by the DS.

Comments received during public consultation

One MSCA agreed with the DS that classification for germ cell mutagenicity is not justified.

Assessment and comparison with the classification criteria

One of the earlier five bacterial reverse mutation assay showed a very slight increase in the number of revertant colonies in only one strain (TA 98) and only with metabolic activation at the highest concentration of 5000 µg/plate where precipitation was observed. Therefore, this result was considered of doubtful biological significance and four additional assays were conducted. No evidence of mutagenic activity of fluopicolide was observed in the four additional bacterial reverse

mutation assays performed with five *Salmonella typhimurium* strains and one *Escherichia Coli* strain. In addition, a recently conducted bacterial reverse mutation assay (2017) was negative. Furthermore, a Comet assay was also recently (2018) performed to confirm the negative profile for the endpoint gene mutation *in vivo*.

The chromosomal aberration assay performed in Chinese hamster V79 showed a positive response. However, the increase of aberrant cells occurred at cytotoxic concentrations where mitotic indices were clearly below the limit of 50% indicating the doubtful biological significance of these data. This chromosome aberration assay was therefore repeated in human lymphocytes and gave a clear negative response. Moreover, two *in vivo* micronucleus assays were performed in mice by the oral route up to the limit dose of 2000 mg/kg bw. Both of these assays were negative. However, one was of questionable biological significance due to the slight increase of micronucleated polychromatic erythrocytes in bone marrow of some animals given 2000 mg/kg bw as well as in one control animal. As the ratio of PCE/NCE erythrocytes was not significantly affected and no clinical signs were observed in both assays, a third assay was performed in mice by the intraperitoneal route especially to check for bone marrow exposure. This assay gave a clear negative result for clastogenicity *in vivo* at dose levels showing clear cytotoxicity of the bone marrow. The PCE/NCE ratio is strongly and significantly altered in this *in vivo* micronucleus test and therefore bone marrow exposure is considered to be demonstrated.

The HPRT mutation assay in Chinese hamster V79 cells was negative. Moreover, the *in vivo* rat hepatocyte UDS assay clearly showed that fluopicolide does not induce damage to DNA.

Based on the available data, RAC agrees with the DS that **classification of fluopicolide for mutagenicity is not warranted.**

RAC evaluation of carcinogenicity

Summary of the Dossier Submitter's proposal

The DS concluded that data for carcinogenicity are conclusive and that fluopicolide does not warrant a carcinogenicity classification.

A summary of submitted studies with carcinogenicity endpoints are presented below:

Method	Test substance, dose levels duration of exposure	Results	Reference
Combined carcinogenicity and toxicity study by dietary administration to CD rats for 104 weeks.	Fluopicolide (purity 95.9%)	<u>50 ppm</u> No effects observed	Anonymous, 2003; M-225616-01-2
CrI:CD® (SD)IGS BR rat (60/dose/sex carcinogenic phase, 20/dose/sex chronic tox. phase, 10/dose/sex treated for 52-weeks, followed by a 13-week period without treatment)	0, 50, 200, 750 or 2500 ppm (equivalent to 0, 2.1, 8.4, 31.5, 109.4 / 0, 2.8, 10.8, 41.0, 142.2 mg/kg bw/day in M/F)	<u>≥ 200 ppm</u> No adverse effects	Anonymous, 2005; M-263575-01-1
OECD TG 453 (04/1981)		<u>≥ 750 ppm</u> ↓ bodyweight gain week 1 (M/F) ↑ total protein concentration and ↓ A/G ratio in blood (M/F) ↑ cholesterol in blood (M) ↑ K ⁺ and/or Ca ²⁺ in blood (M/F) ↑ liver and kidney weights (M/F) ↓ incidence of mammary masses (F)	
GLP		↑ increased incidence and/or severity of centrilobular hepatocyte hypertrophy (M/F), incidence and/or severity of cystic degeneration and foci of alteration (M)	

Method	Test substance, dose levels duration of exposure	Results	Reference
Coagulating gland, Harderian gland, vagina and bone marrow were not sampled, fixed or examined histopathologically		<p>and ↑ increased incidence of eosinophilic foci of alteration (F) in the liver</p> <p>↑ incidence of cortical tubular basophilia and hyperplasia of the papillary epithelium (M/F)</p> <p>↑ incidence of cystic follicular cell hyperplasia in the thyroids week 104 (M)</p> <p><u>2500 ppm</u></p> <p>↓ bodyweight gain (M/F)</p> <p>↓ RBC parameters (M/F)</p> <p>↑ albumin in blood (M)</p> <p>Most of these changes reversible after a 13-week off-dose period.</p> <p>No evidence of a carcinogenic potential.</p>	
<p>Carcinogenicity study in mice via diet</p> <p>C57BL/6 mice (50/dose/sex)</p> <p>OECD TG 451 (04/1981)</p> <p>GLP</p> <p>Coagulating glands were not sampled, fixed or examined histopathologically</p>	<p>Fluopicolide (purity 95.9%)</p> <p>0, 50, 400, or 3200 ppm for 78 weeks (equivalent to 0, 7.9, 64.5, 551.0 / 0, 11.5, 91.9, 772.3 mg/kg bw/day in M/F)</p>	<p><u>50 ppm (7.9/11.5 mg/kg bw/day)</u></p> <p><u>No effects observed</u></p> <p><u>400 ppm (64.5/91.9 mg/kg bw/day)</u></p> <p><u>↑ abs. & rel. liver weight week (M/F)</u></p> <p><u>↑ incidence of hepatocellular hypertrophy (M/F)</u></p> <p><u>3200 ppm (551/772.3 mg/kg bw/day)</u></p> <p><u>↓ body weight (M/F)</u></p> <p><u>↓ feed intake (M/F)</u></p> <p><u>↑ incidence of altered liver foci week 52 (F) and 78 (M/F)</u></p> <p><u>↑ incidence of liver adenomas week 52 (F) and 78 (M/F)</u></p>	<p>Anonymous,2003; M-225595-01-1</p> <p>Anonymous, 2005; M-263591-01-1</p>

In the combined carcinogenicity and toxicity study in rats, fluopicolide was administered at concentrations of 0, 50, 200, 750 or 2500 ppm for 2 years. After 1-year treatment period, 20 animals/sex/group were sacrificed for assessment of chronic toxicity. In addition, the recovery of any effects seen during the 52-week toxicity phase was assessed in a subsequent 13-week recovery period. After the 2-year treatment period, the carcinogenicity was evaluated from all oncogenicity phase animals (60 animals/sex/group). When compared with the controls there was an effect on body weight gain. The target organs for toxicity were the liver and the kidneys with increased liver and kidney weights at 750 and 2,500 ppm. At the same dose levels, there were histopathological findings in liver comprising an increased incidence of centrilobular hepatocyte hypertrophy, an increased incidence and/or severity of cystic degeneration and foci of alteration in males, and an increased incidence of eosinophilic foci of alteration in females after 104 weeks. Secondary to the increased metabolic activity of the liver was an increased incidence of cystic follicular cell hyperplasia in the thyroids of males. In the kidneys, at 2500 ppm, there were degenerative and proliferative changes, comprising cortical tubular basophilia, at an increased severity relative to controls in both sexes. The males, in particular, had increased incidences of hyaline droplets in the cortical tubules, cortical tubular dilatation and tubular casts. Hyperplasia of the papillary epithelium at 2500 and 750 ppm was present at an increased incidence and severity in females and this was associated with mineralisation of the pelvic epithelium. No treatment-related adverse changes were observed at dose levels of ≤ 200 ppm. The overall incidence of tumour-bearing animals, the time of occurrence and the pattern of neoplastic findings did not indicate a carcinogenic effect of fluopicolide.

In a mouse oncogenicity study, fluopicolide was administered to mice in the diet at concentrations of 50, 400 and 3200 ppm for 78 weeks. After 52-weeks, 10 animals/sex/group were killed for assessment of chronic toxicity. After the 78-week treatment period, the carcinogenicity was evaluated from all oncogenicity phase animals (50 animals/sex/group).

Fluopicolide administered daily for 78 weeks produced severe reduction of the body weight gain (-45% in males and -35% in females) at 3200 ppm indicating that the maximum tolerated dose (MTD) was reached. The target organ identified was the liver. Higher liver weights, enlarged liver, increased number of masses and nodules in the liver were observed at 400 and 3200 ppm. These changes were associated with hepatocellular hypertrophy, and higher incidence of altered cell foci at 3200 ppm.

Significantly increased incidence of hepatocellular adenoma was observed at 3200 ppm at 78 weeks in both males (22% in treated animals vs. 10% in controls) and females (32% in treated animals vs. 2% in control) and at 52 weeks in females (30% vs. 0% in controls). In addition, the time of onset of the hepatocellular neoplasm was shorter in the treated females when compared with controls.

The incidence of these lesions was close to the range of the laboratory HCD (males up to 14% and females up to 22%). Moreover, no increased incidence of hepatocellular carcinoma were observed in any of the groups after the 78-week treatment period. An overview of relevant liver findings after 78-weeks is given in the following table (based on the Table in Section 10.9.2 of the CLH report).

Parameter	Control data		Low dose 50 ppm		Mid dose 400 ppm		High dose 3200 ppm	
	m	f	m	f	m	f	m	f
Final bodyweight (g) and (% control)	38.58	32.61	40.62 (+5%)	33.77 (+4%)	39.09 (+1%)	33.73 (+3%)	31.15** (-19%)	27.17** (-17%)
Liver: organ weight, relative (g/100g) and absolute (g)	1.62 4.26	1.66 5.18	1.85 4.65	1.64 4.94	1.91** 4.90**	2.20** 6.62	2.37** 7.62**	2.59** 9.37**
Non-neoplastic changes:								
Hepatocellular hypertrophy	0/50	0/49	0/50	0/50	20/50	41/50	49/50	41/50
Altered cell foci	1/50	1/49	8/50	3/50	5/50	4/50	18/50	25/50
Coagulative hepatocyte necrosis	6/50	2/49	3/50	5/50	1/50	4/50	2/50	3/50
Microfoci of necrosis	2/50	2/49	5/50	5/50	8/50	1/50	4/50	2/50
Chronic inflammation	0/50	0/49	1/50	0/50	0/50	0/50	0/50	0/50
Neoplastic changes:								
Hepatocellular adenoma (%)	5/50 (10)	1/50 (2)	0/50 (0)	2/50 (4)	5/50 (10)	0/50 (0)	11/50* (22)	16/50** (32)
Hepatocellular carcinoma (%)	3/50 (6)	0/50 (0)	1/50 (2)	0/50 (0)	0/50 (0)	2/50 (4)	2/50 (4)	0/50 (0)

* p<0.05, ** p<0.01

m: males; f: females

Findings considered related to treatment with fluopicolide are written in **bold**.

The increased number of benign liver tumours occurred only at the highest dose reaching the MTD (severe body weight gain reduction in high dose animals) suggesting a threshold mechanism. In addition, no tumours were observed with increased incidences in other mouse tissues and the liver adenoma did not progress into malignant neoplasia during the lifespan of these animals. Altogether, these findings clearly indicate that the slightly increased incidence of hepatocellular adenoma in mice is a weak carcinogenic response. The mode of action (MoA) for the increased incidence of liver adenomas was found to be CAR-mediated and thus secondary to liver enzyme induction like that of phenobarbital. This MoA is considered of no relevance in humans and therefore the DS concluded that fluopicolide does not warrant classification for carcinogenicity.

Comments received during public consultation

One MSCA supported the proposal for no carcinogenicity based on the argument that the hepatic neoplasia was limited to both sexes in one species, the lack of progression to malignancy and the plausible but incomplete mechanistic information that adenoma likely resulted from a rodent specific mechanism.

Assessment and comparison with the classification criteria

The main findings related to assessment of carcinogenicity of fluopicolide were limited to hepatocellular adenoma which were only observed in mice at the highest dose tested in both males and females. A summary of relevant findings from the 78-week mouse study is presented below:

Parameter	Control data		Low dose 50 ppm		Mid dose 400 ppm		High dose 3200 ppm	
	m	f	m	f	m	f	m	f
Neoplastic changes:								
Hepatocellular adenoma (%)	5/50 (10)	1/50 (2)	0/50 (0)	2/50 (4)	5/50 (10)	0/50 (0)	11/50* (22)	16/50** (32)
Hepatocellular carcinoma (%)	3/50 (6)	0/50 (0)	1/50 (2)	0/50 (0)	0/50 (0)	2/50 (4)	2/50 (4)	0/50 (0)

* p<0.05, ** p<0.01; m: males; f: females

Findings considered related to treatment with fluopicolide are written in **bold** text.

The DS concluded that a possible CAR/PXR mechanism could be responsible for the observed effects in the liver of the mouse. There are four additional mechanistic studies addressing the observed mouse liver oncogenicity. The first is a 28-day *in vivo* study in C57BL/6 mice focused on hepatic cellular proliferation as well as morphological changes of the liver and hepatic cytochrome P-450 isoenzyme activity. In addition, three *in vitro* studies in different hepatocyte cultures (mouse wild type, mouse CAR/PXR KO and human) were performed.

In the 28-day *in vivo* study in mice, fluopicolide was administered continuously via the diet to a group of 15 females for at least 28 days at concentrations of 0 and the dose at which liver tumours occurred in the carcinogenicity study, i.e. 3200 ppm (equivalent to 575 mg/kg bw/day), with satellite subgroups of 20 female mice per group for interim sacrifice after 7 days of treatment. Bromodeoxyuridine (BrdU) was administered in drinking water for 7 days before scheduled sacrifice for cell proliferation assessment. At both the interim and final sacrifice times, liver was weighed and sampled. Hepatic cellular proliferation was assessed as well as morphological changes of the liver. In addition, at interim sacrifice, hepatic cytochrome P450 isoenzymes were assessed.

At 3200 ppm, there were no mortalities or clinical signs during the course of the study.

At interim sacrifice, mean absolute and relative liver weights were increased by 27 to 37% compared to controls, 9/20 livers appeared to be dark and 1/20 livers was enlarged. There was a diffuse, perilobular to panlobular hepatocellular hypertrophy in all treated animals and a marked loss of diffuse, mainly centrilobular hepatocellular vacuolation in 3/20 treated animals when compared to controls. An increased number of mitotic cells and some foci of single cell necrosis/apoptosis were seen in 5/20 treated animals. The mean BrdU labelling index was approx. 6.5-fold higher in treated animals, when compared to controls, indicative of hepatocellular proliferation in the liver.

At final sacrifice, mean absolute and relative liver weights were increased by 48 to 56% compared to controls, 11/15 livers appeared to be dark and 3/15 livers were enlarged. There was a diffuse,

perilobular to panlobular hepatocellular hypertrophy in all treated animals together with a marked loss of diffuse, mainly centrilobular hepatocellular vacuolation in 3/15 treated animals, when compared to controls. Minimal single cell necrosis/apoptosis were seen in only 1/15 treated animals and an increased number of mitotic cells in 2/15 treated animals. There was no increased hepatocellular proliferation based on the results of the BrdU assay.

Fluopicolide also induced an increase in total cytochrome P450 content (+97%) as well as in benzyloxyresorufin O-dealkylation (BROD, +1785%) and pentoxyresorufin O-dealkylation (PROD, +1143%) activities. Ethoxyresorufin O-deethylation (EROD) activity was only slightly induced and lauric acid hydroxylation decreased compared to control mean as shown in the following table.

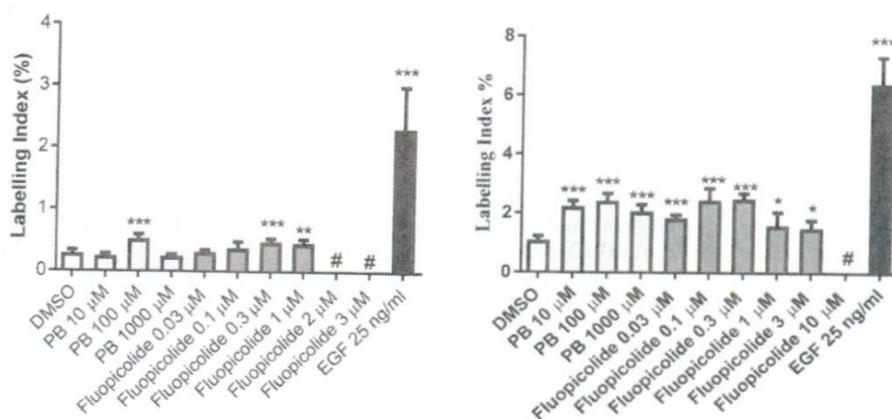
Parameter	Fluopicolide at 3200 ppm % change compared to control mean
P450	+ 97 %
BROD	+ 1785 %
EROD	+ 79 %
PROD	+ 1143 %
Lauric acid	- 67 %

In conclusion, fluopicolide at 3200 ppm in the diet induced a transient and marked hepatocellular proliferation in C57BL/6 mice after 7 days of treatment, which returned to control levels after 28 days of treatment. Fluopicolide is an inducer of BROD and PROD activities.

In the mechanistic studies hepatocytes from wildtype mice and CAR/PXR knockout (KO) mice were used *in vitro*. In these studies, cytotoxicity was evaluated by adenosine 5'-triphosphate depletion and phenobarbital (PB) was tested in parallel as an assay control to confirm hepatocytes responded to the reference compound in the expected manner (induction of CYP2B and CYP3A activities and increased cell proliferation). In addition, epidermal growth factor (EGF, 25 ng/mL) was included as a positive control for hepatocyte proliferation.

In wildtype mouse hepatocytes fluopicolide was administrated to C57BL/6 male and female mouse hepatocytes in culture induced replicative DNA synthesis in a dose-dependent manner with maximal induction at 0.3 μ M (1.7-fold in male hepatocytes and 2.3-fold in female hepatocytes). Phenobarbital induced replicative DNA synthesis to a maximum of 1.8-fold and 2.2-fold in the male and female hepatocytes, respectively; the proliferative capability of these cells in culture was confirmed using EGF.

Effects of fluopicolide, PB or EGF on DNA synthesis (S-phase) in male / female mouse hepatocytes:



PROD, BROD and 7-benzyloxyquinoline O-debenzylase (BQ) rates are indicative of CYP2B and CYP3A induction. In male mouse hepatocytes, fluopicolide caused a dose dependent increase in

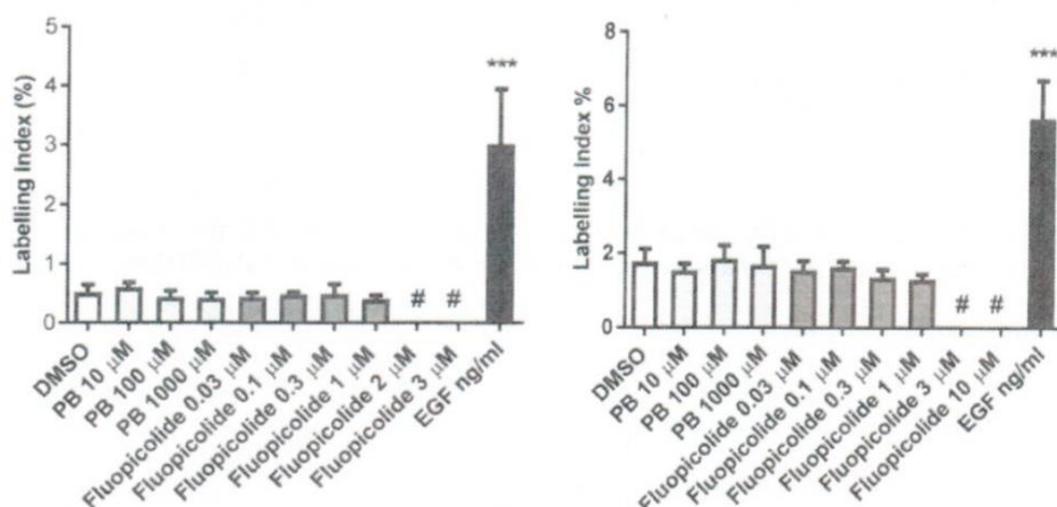
PROD and BROD (up to 3- and 2.6-fold of control respectively). BQ was also slightly increased in these cells following administration of fluopicolide at 1 and 2 μM (1.4- and 1.5-fold respectively). In female mouse hepatocytes, fluopicolide induced a dose dependent increase in PROD (up to 1.7-fold), but not BROD or BQ activities.

PB (1 mM) caused significant increases in PROD, BROD and BQ activities, which were 6.5-, 4.9- and 7.7-fold, respectively, in male mouse hepatocytes and 2.6-, 1.6- and 4.4-fold, respectively, in female mouse hepatocytes.

In conclusion, fluopicolide induced both hepatocellular S-phase replicative DNA synthesis and CYP2B enzyme activity in both male and female mouse primary hepatocyte cultures.

Treatment of **CAR/PXR KO mouse hepatocytes** with fluopicolide or PB did not induce replicative DNA synthesis in males or females at any concentration but the proliferative capability of these cells in culture was confirmed using EGF.

Effects of fluopicolide, PB or EGF on replicative DNA synthesis (S-phase) (male / female):

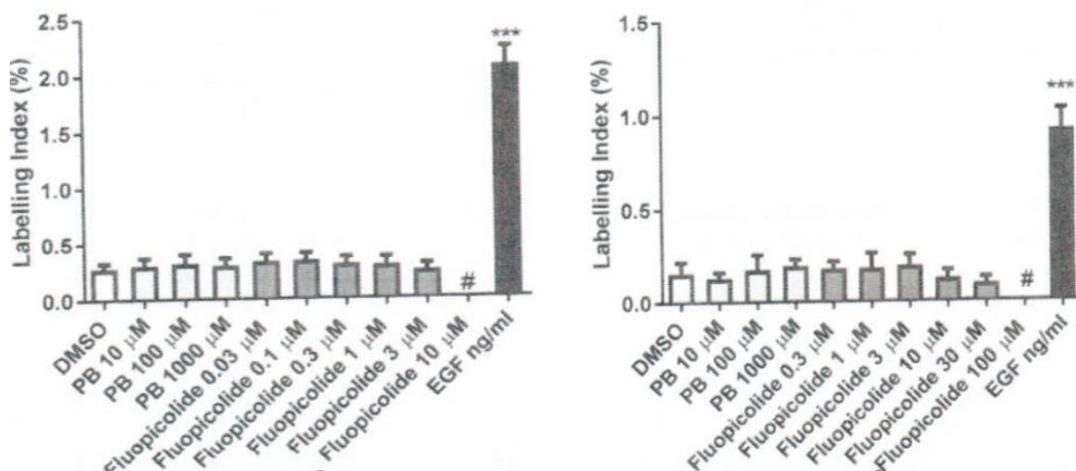


Similarly, fluopicolide did not cause any increases in PROD, BROD or BQ in male or female CAR/PXR KO mouse hepatocytes. PB administration (1000 μM) to male mouse hepatocytes slightly induced PROD, BROD and BQ to 3.5-, 1.5- and 1.7-fold respectively. 1000 μM PB also caused induction in female mouse hepatocytes in PROD and BROD 1.6- and 1.8-fold respectively, with no induction observed in BQ.

In conclusion, fluopicolide did not induce either hepatocellular S-phase replicative DNA synthesis, CYP2B or CYP3A enzyme activity in male or female CAR/PXR KO mouse primary hepatocyte cultures. These data suggest that fluopicolide requires the presence of the nuclear hormone receptors CAR and/or PXR to induce replicative DNA synthesis and enzyme activity in male and female mouse hepatocytes.

In human hepatocytes from three individual male/female donors, fluopicolide or PB administration did not induce replicative DNA synthesis. The proliferative capability of these cells was confirmed using EGF.

Effects of fluopicolide, PB or EGF on replicative DNA synthesis (S-phase) in male/females human hepatocytes:



In male human hepatocytes, fluopicolide caused slight increases in BROD and BQ activities to a maximum of 1.5 - and 2.6-fold respectively. All levels of PB resulted in statistically significant increases in BROD and BQ activities in the male human hepatocytes, with maximum increases observed at 1 mM (2.0- and 5.3-fold respectively). PROD activity could not be analysed as levels were below the level of quantification, therefore, no results are presented for this assay in the male.

In females, treatment with fluopicolide resulted in dose-dependent increases in BQ activity in hepatocytes from both donors to a maximum of 1.7- and 2.8-fold induction compared to controls. Fluopicolide caused no relevant increase in PROD or BROD activity in female hepatocytes, however, slight, but significant, decreases in BROD activity were observed at the top concentrations. In female human hepatocytes, PB consistently induced BROD and BQ activities in both donors, however, only one donor responded in a dose-dependent manner after treatment with PB.

In summary, treatment of cultured male or female human hepatocytes with fluopicolide resulted in weak induction of CYP3A enzyme activity (BROD (male only) and BQ activities (male and females)). There was no evidence of fluopicolide or PB-stimulated proliferation in cultured male or female human hepatocytes.

These data suggest that fluopicolide is a weak activator of human PXR (as shown by the effects on CYP3A enzyme activity levels).

In addition to the CAR/PXR KO mouse hepatocyte study, this study in human hepatocytes supports human non-relevance of this CAR/PXR-mediated liver tumour MoA.

The following table summarises the overall results regarding CAR/PXR activation and liver cell proliferation in the wildtype mouse, CAR/PXR KO Mouse and in human hepatocytes:

Key events	Wildtype mouse hepatocytes	CAR/PXR KO mouse hepatocytes	Human hepatocytes
CAR activation	+	-	-
PXR activation	+	-	Weak activator
Liver cell proliferation	+	-	-

Fluopicolide treatment resulted in the activation of CAR and weak activation of the PXR in the liver. This resulted in the altered expression of CAR-responsive genes that promoted a pro-proliferative and anti-apoptotic environment in the liver and an early, transient, increase in hepatocellular proliferation. Increased hepatocellular foci because of clonal expansion of spontaneously mutated cells in the mouse resulted in slight increases in liver adenomas incidence

compared to concurrent controls. This MoA was supported by a series of associative events including: increased expression of genes encoding CYPs, particularly CYP2B and (to a lesser extent) CYP3A isoforms, increased proliferation and hepatocellular hypertrophy and increased liver weight. The MoA hypothesis as postulated by the DS is presented below, with the causal key events and associative events identified:

Proposed MoA for liver adenomas in mice:

Events	Description
Key events (KE)	
KE 1	Activation of CAR/PXR nuclear receptor
KE 2	Altered gene expression secondary to CAR/PXR activation
KE 3	Increased hepatocellular proliferation
KE 4	Increased clonal expansion, leading to altered foci
KE 5	Increased incidence of hepatocellular tumours
Associative events (AE)	
AE 1	Increased CYP2B, CYP3A enzyme activity and/or protein
AE 2	Hepatocellular hypertrophy
AE 3	Increased liver weight

RAC agrees with the dossier submitter that the available data provide enough evidence to support the postulated MoA (CAR activation) to be the underlying MoA of liver adenomas observed in mice. Similar to phenobarbital (a known CAR inducer), fluopicolide did not induce DNA replication (prerequisite for tumour formation) in human hepatocytes nor in CAR/PXR KO mouse hepatocytes following induction of human CAR, in contrast to rats. Due to this qualitative difference, the liver adenomas because of CAR-activation by fluopicolide were considered to be of little relevance to humans.

AhR enzyme induction can be excluded as a potential mode of action. Fluopicolide did not produce a large increase in CYP1A EROD activity in the 28-day mechanistic study; only a slight increase of 79% was noted (compared with 1785% and 1143% increases in BROD and PROD, respectively). This increase followed a similar pattern to that observed with PB in a separate control study, in which PB induced an 83% increase in EROD activity (compared with a 6326% and 1920% increase in BROD and PROD respectively).

RAC notes that no information regarding the PPAR receptor and the gene expression of CYP4A activities was measured in the MoA studies.

The occurrence of hepatic neoplasia is limited to both sexes of one species. Furthermore, RAC considered the lack of progression to malignancy and plausible mechanistic information that the adenomas likely resulted from a rodent specific mechanism (CAR/PXR). Therefore RAC agrees with the DS that **classification for carcinogenicity is not warranted**.

RAC evaluation of reproductive toxicity

Summary of the Dossier Submitter's proposal

Effects on sexual function and fertility

The DS presented information from a preliminary dose range finding and a main 2-generation study in rats conducted in 2002 and 2003, respectively. A supplementary histopathological examination was performed in 2004 on the results of the 2-generation study.

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

Method, year, guideline, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results
<p>Preliminary reproductive performance study,</p> <p>CD rats 8 male and 8 female rats/group</p> <p>GLP</p> <p>Selected offspring were retained and treated until their sexual maturation. Year: 2002</p>	<p>Fluopicolide (purity 95.9%) dietary administration 0, 50, 200, 750 or 2500 ppm (equivalent to 0, 4.25/4.25, 16.5/18, 64.5/66.5, 197/204.25 mg/kg bw/day for M/F pre-mating) mg/kg bw/day for M/F pre-mating) Treatment from 15 days prior to mating and until termination after weaning of the resulting litters.</p>	<p><u>2500 ppm:</u> <i>Parental</i> ↓ bodyweight gain, males (days 0-49: 22%) females (GD 0-13: 19%) <i>Offspring:</i> Vaginal opening delayed compared to concurrent controls.</p> <p><u>≥ 750 ppm:</u> <i>parental</i> ↓ bodyweight gain (non-significant) F ↓ food consumption <i>Offspring:</i> ↓ bodyweight gain <u>50 and 200 ppm:</u> <i>Parental and offspring:</i> No effects.</p>
<p>Two-generation reproduction study in rats,</p> <p>CrI: CD® (SD) IGS BR rats 28 male and 28 female rats/group F1 offspring groups were culled after weaning to 24 animals/sex/group maintained for the F2 generation.</p> <p>OECD TG 416, Draft (1999)</p> <p>GLP</p> <p>Year: 2003</p> <p>Additional microscopic examination, 2004.</p>	<p>Fluopicolide (purity 95.9%) Dietary administration of 0, 100, 500 or 2000 ppm (equivalent to 0, 7.35/8.09, 36.35/41.01, 144.62/159.71 mg/kg bw/d in M/F pre-mating) throughout the two generations Treatment from 10 weeks prior to mating and until termination after weaning of the resulting litters.</p>	<p><u>2000 ppm</u> <i>Parental:</i> ↓ body weight gain (M: 6-7%/F: 14-17%) ↓ food consumption ↑ liver and kidney weights (M/F) ↓ spleen weights (F) ↑ incidence of centrilobular hepatocyte hypertrophy ↑ incidence of degenerative and regenerative changes in kidneys (M/F) <i>Offspring:</i> ↓ body weight from day 14 PND.</p> <p><u>100 and 500 ppm</u> No effects on parental or offspring and no histopathological findings.</p>

In the preliminary reproductive toxicity study, reduction in body weight gain was seen at the highest dose of 2500 ppm in males and females at different periods of pre-mating, gestation and lactation in the F0 and F1 generation. A transient effect was seen on body weight gain of females at 750 ppm during gestation. Reproductive parameters as mating performance, sperm parameter and fertility were not affected at any dose. Gestation length was slightly longer for the females treated at 2500 ppm (22.7 vs 22.3 days in controls). Gestation index was 88% in the high dose group compared to 100% in controls and other test group. The reduction was explained by parturition problems in one of the 8 dams.

Vaginal opening in females treated at 2500 ppm was delayed 3 days (36 ± 2.9) when compared to the concurrent controls (33 ± 2.0). However, the DS deemed the effect a chance finding, as the controls in the full 2-generation study showed a mean of 35 ± 3.0 days to vaginal opening.

Litter parameters incorporating litter size and offspring survival were slightly lower in the high dose group. Overall, the DS considered that fluopicolide did not affect reproduction or foetus development in this range finding study.

In the main 2-generation study, food consumption was reduced in the high dose group (2000 ppm) throughout the study, and body weight gain was reduced in both parental generations, except in females after parturition. In this dose group, kidney and liver weights were elevated, and the histopathological examination performed the following year showed centrilobular hepatocyte hypertrophy and degenerative and regenerative changes in kidneys. At 500 ppm, slight liver weight increase and centrilobular hepatocyte hypertrophy were also found, which were regarded by the DS as adaptive changes.

No effects on reproductive parameters, e.g. mating performance, fertility and fecundity, gestation length, parturition process or sperm parameters, were observed, and there was no effect on the oestrous cycling. In F1 and F2 offspring, reduced bodyweight gain was seen at 2000 ppm in both sexes from PND 14 through to weaning. The DS considered the effect linked to the direct feeding on the test diet, due to low palatability of the diet and/or systemic toxicity. Litter size, sex ratios, neonatal toxicity at birth of the F1 and F2 progeny were unaffected by treatment, as were survival to weaning. Sexual maturation (preputial separation, vaginal opening) were unaffected at any dose in this 2-generation study.

The DS concluded that fluopicolide is not toxic to sexual function or fertility.

Developmental toxicity

The CLH report includes two developmental studies in rats and two in rabbits. For each species, a dose range finding developmental toxicity study (from 2000) and a full developmental toxicity study (from 2004) were available.

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

Method, year, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels, duration of exposure	Results
Range finding developmental toxicity study in rats Sprague Dawley rats 4 female rats/group OECD TG 414 (1981) GLP Year: 2000	Fluopicolide (purity 97.6%) Administration by oral gavage 500 or 1000 mg/kg bw/d on GD 7-20 Sacrifice day 21 NB: no control animals	<u>Maternal toxicity:</u> 1000 mg/kg bw/day ↓ food consumption (~5% lower than at low dose group) ↓ bodyweight gain (-34% compared to low dose group) ↑ Incidence of post-implantation loss (31.7% compared to 0% in low dose group) 500 mg/kg bw/day ↓ food consumption <u>Developmental toxicity:</u> 1000 mg/kg bw/day ↓ foetal weight (2.8g) compared to low dose group ↓crown-rump length (32.6 mm) compared to low dose group 500 mg/kg bw/day lower foetal weight (3.1 g) and lower crown-rump length (34.1 mm) than controls of main study below (3.7 g, resp. 36.2 mm)
Developmental toxicity study in rats Sprague Dawley rats 23 dams/group OECD TG 414 (1981) with deviations: high intervals between dose levels. Parameters in newest guideline version not included: thyroid-related parameters anogenital distance not assessed. GLP Year: 2004	Fluopicolide (purity 97.6%, 97.8%) oral dosing by gavage 0, 5, 60 or 700 mg/kg bw/d on GD 7-20	<u>Maternal toxicity:</u> 700 mg/kg bw/day : ↓ body weight gain (-12%) and food consumption (-3%) No clinical signs reported. No significant effects on maternal toxicity at 5 and 60 mg/kg bw/d. <u>Developmental toxicity:</u> 700 mg/kg bw/day: One foetus with microphthalmia ↓ mean foetal weight 3.4 g (-8%)and crown-rump length 34.8 mm (-4%) ↓ ossification ↑ incidence of defects thoracic vertebral sternebrae arches (4/142, 0 in controls), centres 10/142*, 0 in controls) and ribs: fused ribs or only 9 ribs (6/142, 0 in controls) as well as wavy and/or thickened ribs (5/142, 1/148 in controls) extra rib at the 7 th cervical vertebra (5/142, 3/148 in controls) 60 mg/kg bw/day:

Method, year, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels, duration of exposure	Results
		One foetus with multiple malformations at the vertebral column and pelvis. ↑ incidence of defects thoracic vertebral sternebrae and fused and wavy ribs, non-significant (1/153 vs 0/148 in controls) 5 mg/kg bw/day: Single occurrences of wavy and/or thickened ribs (1/148, 1/150).
Range finding developmental toxicity study in rabbits Himalayan rabbits – annex states Chbb:HM(SPF) Kleinrusse 4 dams/group OECD TG 414 (1981) GLP Year: 2000	Fluopicolide (purity 97.6%-97.8%) Administration by oral gavage 25, 50, 100, 250, 500 or 1000 mg/kg bw/d Exposure on GD 6-28	<u>Maternal toxicity:</u> ≥ 100 mg/kg bw/day ↑ mortality/moribundity (4/4 dams by day 23), clinical signs and necropsy findings ↓ body weight gains and food consumption ≥ 50 mg/kg bw/day ↓ body weight gains and food consumption ↑ incidence of abortions (1/4) <u>Developmental toxicity:</u> 25 and 50 mg/kg bw/day No effects on foetuses of surviving dams (
Prenatal oral gavage developmental toxicity study in rabbits Himalayan rabbits 23 dams/dose) OECD TG 414 (1981) – NB The high dose resulted in a mortality rate > 10%. Therefore, the number of females with implantation sites that were available at necropsy was < 16 animals GLP Year: 2004	Fluopicolide (purity 97.8%) 0, 5, 20 or 60 mg/kg bw/day on exposure days GD 6-28	<u>Maternal toxicity:</u> 60 mg/kg bw/day ↑ mortality/sacrifice (18/23) ↑ clinical signs ↓ feed consumption ↓ body weight gain (-8.3%) ↑ incidence of premature deliveries (15/23) <u>Developmental toxicity:</u> 60 mg/kg bw/day ↓ mean foetal weight and crown-rump length Dead foetuses in most premature deliveries

*p < 0.05

In the rat range finding development toxicity study, the key maternal findings were a reduction in food consumption on days 7-10 and an important reduction in body weight gain throughout the period of treatment at 1000 mg/kg bw/day compared to the 500 mg/kg bw/day group. No compound-related effects were observed at necropsy of the animals. At the high-dose, post-implantation loss was elevated and included one total resorption in one out of 4 dams. Mean foetal weight and crown-rump length were reduced at 1000 and at 500 mg/kg bw/day, in comparison with the control group of the main study.

In the main developmental toxicity study in rats, dose levels of 0, 5, 60 or 700 mg/kg bw/day were used. The DS noted that the distance between the doses were higher than the recommended 2-4 fold. In the high dose group, body weights and body weight gains were decreased in the dams, with the overall body weight gain (days 1-21) being 12% lower than the concurrent control value when corrected for gravid uterine weight. The effect on body weight gain was up to -24% compared to controls in the first days of the dosing period. Food consumption was slightly decreased after the beginning of treatment at this dose level.

Mean foetal body weights, crown-rump lengths and placental weights were statistically significantly decreased (8, 4% and 9%, respectively) at this dose-level. No effects were recorded on resorption, on the number of live and dead fetuses or on sex ratios. Single litters had a small number of fetuses, but litter size was generally not affected.

One foetus of the high dose group showed microphthalmia. Visceral examination showed blood in the abdominal cavity and in the liver, and dilatation of the kidney pelvis, but the incidences were not statistically significantly different from controls.

Skeletal defects /delayed ossification were seen in the skull, vertebrae, ribs and paws. Missing or delayed ossification of thoracic vertebral arches, thoracic vertebral centres, aplastic or displaced thoracic vertebral sternbrae were reported in the high dose group, and with single incidences for some effects in the mid-dose group. Missing ossification points in caudal vertebral centra and missing ossification of forepaw metacarpal 5 were statistically significantly different from controls in all treated groups. Other skeletal effects including fused and wavy ribs and missing ossification in the hindpaw bone were recorded, but the incidences did not reach statistical significance.

One foetus of the mid-dose group had multiple skeletal effects on the vertebral column and pelvis.

The DS considered the foetal effects on body weights and crown-rump lengths and defects at the thoracic vertebrae, sternbrae and ribs to be secondary to maternal toxicity. The single incidence of microphthalmia in the high dose group was considered by the DS to be an incidental finding.

In the rabbit range finding study, dose levels of 100, 250, 500 or 1000 mg/kg bw/day group were lethal to all dams. One animal given 50 mg/kg bw/day aborted on day 29 and had decreased body weight gain and food consumption. No significant effects were observed at 25 mg/kg bw/day. No effects were seen in the fetuses of surviving dams.

The main rabbit developmental toxicity study used 60 mg/kg bw/day as the highest dose. At this dose level, 3 out of 23 animals died and 15 were sacrificed after premature delivery. Clinical signs in most of these animals included reduced hay consumption, hypoactivity, bristling coat, decreased defecation, soft stools, and discoloured urine. In addition, there was a marked decrease in body weight gains and food consumption at this dose level. At necropsy, filled stomach, red liquid in the urinary bladder and uterus as well as yellowish discoloration of the liver were observed in single animals from the high dose group.

Gravid uterus weights were slightly lower in the animals at the high dose.

One animal from the intermediate dose group was killed after premature delivery on GD 28. This animal showed decreased defecation and reduced hay consumption.

Mortality of fetuses was observed in most premature deliveries. Mean foetal body weights, crown-rump lengths and placental weights were decreased in the animals from the high dose group. There was no effect on litter size, number of live fetuses or sex ratios or on early or late resorptions. No morphological changes in the fetuses were reported at any dose.

Table: Mean maternal feed consumption in g/rabbit/day during gestation (% of control)

Parameter	Dose Group (mg/kg bw/day)				
	0	5	20	60	
				Died or aborted during gestation	Survived until termination
Number of dams	21	20	21	18	5
Day 6-19	92.4	87.2 (94 %)	87.5 (95 %)	64.0 (69 %)	79.9 (86 %)
Day 19 - sacrifice	98.5	96.7 (98 %)	70.0 (71 %)	9.1 (9 %)	58.9 (60 %)

Parameter	Dose Group (mg/kg bw/day)				
	0	5	20	60	
				Died or aborted during gestation	Survived until termination

The DS referred to several literature studies where abortions, decreased foetal and placental weight, reduced foetal ossification and pre-, peri- and postnatal death can be seen following feed restriction during gestation on developmental parameters in rabbits (Matsuzawa *et al.* 1981; Petrere *et al.* 1993; Cappon *et al.* 2005; Menchetti *et al.* 2015). In addition, the DS referred to historical control data (Viertel & Trieb, 2002) which included spontaneous abortions in this strain of rabbit up to 20%.

The mortalities of the dams were considered to be caused by specific rabbit sensitivity of the GI tract to reduced food consumption. The adverse effects on the foetuses in the rabbit developmental toxicity study are considered secondary to severe maternal toxicity, as described above.

In the 2 generation study in rats, lower body weights were seen in the offspring of the 2000 ppm group from PND 14. However, the DS considered that this effect was not a developmental effect but due to low palatability, and/or systemic toxicity resulting from direct consumption of the diet, as also seen in the parental animals.

Overall, the DS concluded that classification of fluopicolide for developmental toxicity is not warranted.

Effects on or via lactation

In the two-generation reproduction toxicity study in rats, reduced body weight in offspring were reported from around PND 14 and through to weaning, but not in the first part of lactation. The effect is thus linked to the time when the offspring started to eat the diet in addition to being nursed, suggesting a palatability effect and/or systemic toxicity of the diet rather than an effect on or via lactation. Effects of food consumption and body weights occurring in adult animals in the first days of treatment in the repeated dose studies and reproductive toxicity studies confirm this evaluation.

Data on the concentration of fluopicolide in lactating cows showed that less than 0.2% of radioactivity was excreted in the milk) with no evidence of any accumulation.

Information from the toxicokinetic studies on fluopicolide showed that fluopicolide is extensively metabolised to more polar, water soluble molecules with primarily biliary excretion.

Based on this information, no classification for lactation effects was proposed by the DS.

Comments received during public consultation

Historical control data (HCD) on the time to vaginal opening were provided by Industry, including a total of 6 range-finding and full 2-generation studies in the period from 1999 to 2002. The HCD range was from 29 days to 41 days, with a mean of 34 days and a standard deviation of 2.7. According the commenter, the data support the dismissal of the argument that the 3 day delay in vaginal opening (36 days) at the high-dose in the range-finding study was treatment related.

The data are included in the Background Document under "Additional key elements"

Assessment and comparison with the classification criteria

Sexual function and fertility

Classification in category 1A is not appropriate, as there are no data on humans on the sexual function or fertility of fluopicolide.

Classification in category 1B requires clear evidence from animal data of an adverse effect on sexual function or fertility. This is also not the case from the animal data, consisting of a two-generation reproductive toxicity study and a dose range finding study in the rat.

Category 2 would be attributed in the case of some evidence, albeit not sufficiently convincing to place the substance in category 1. The criteria further requires that the effects should be non-secondary to other toxic effects.

The available studies on fertility showed no effects on reproductive parameters such as fertility, oestrous cycling, spermatogenic function and capacity, mating, gestation or parturition, or on the F1 and F2 progeny at birth (litter size, sex ratios, neonatal toxicity), and their survival to weaning were unaffected by treatment.

The delay in vaginal opening seen in offspring of the high dose in the range-finding study can be explained by a low concurrent control value compared to that of the main study and of the HCD. Therefore, this effect is not considered sufficient to warrant classification. The reduced body weights seen in offspring from PND 14 appears to be a palatability issue, as it's onset occurs when the animals begin to feed directly, even though toxicity to the developing animals cannot be fully excluded. The effect is however considered insufficient to warrant classification, and RAC concludes that **classification for sexual function and fertility for fluopicolide is not warranted.**

Developmental toxicity

No human information on the possible developmental toxicity of fluopicolide are available, and classification in category 1A is therefore not relevant.

The available animal studies include a developmental study in the rabbit causing marked toxicity in the dams, including high mortality (18/23) in pregnant rabbits at the highest doses of 60 mg/kg bw/day. RAC notes that the lower foetal weights and reduced crown-rump lengths in the litter of the surviving 5 animals were regarded to be secondary to the severe maternal toxicity by the DS. However, RAC considers that the rabbit developmental toxicity study has limited relevance for the evaluation of effects on development of fluopicolide due to the excessive mortality seen in the dams.

In the rat developmental study, RAC notes the large distance between the dose levels in this study which makes the evaluation of possible dose-response relationship difficult leaving a concern for possible effects at doses between e.g. the mid dose of 60 mg/kg bw/day and high dose of 700 mg/kg bw/day.

At this dose, there was some maternal toxicity, that included reduced body weight gains, and food consumption, whilst no maternal toxicity was seen at 5 or 60 mg/kg bw/day.

Foetal effects on body weights and size occur at the highest dose. No significant effect on foetal size was seen at the lower dose of 60 mg/kg bw/day. RAC considers that the effects on foetal weight and size can partially be a consequence of maternal toxicity. The effects are not considered to warrant classification.

Skeletal examination of the rat foetuses showed effects of concern, as they are classified as abnormalities/malformation by ECETOC (2002). These effects include aplasia/ dysplasia of

centras and arches of vertebrae, of sternebrae and ribs and missing ossification of metacarpus (forepaw). Except for the latter, these effects occur at incidences below statistical significance. However, the effects are not common, and their incidences at the high dose exceeded the HCD ranges. Scrutiny of individual data revealed that the increased incidences of malformation at the high dose occurred in litters from a few dams with more marked reduction of body weight gain compared to the mean value. In the mid dose group, the effects on missing vertebral centra, arch, rib and vertebrae, classified as malformations, occurred in one single foetus, which diminishes the concern.

Other skeletal effects in all dose groups included decreased ossification of ribs, sternebrae and single fore- and hindpaw phalanges. Of these, statistically significant reduction in the ossification of the caudal vertebrae and of the sternebrae were seen. However, only in the high dose group were these incidences outside the historical controls. These effects are considered to be due to delayed development.

Overall, the severity of the effects on the foetuses observed in one species at a dose causing some maternal toxicity is not sufficient to warrant classification of fluopicolide for developmental toxicity in category 1B.

RAC concludes that **classification as Repr. 2 for development is warranted for fluopicolide.**

Effect on or via lactation

The criteria for classification for effects on or via lactation refers to three elements:

- a) Human evidence indicating a hazard to babies during the lactation period
- b) Results of one or two generation studies in animals which provide clear evidence of adverse effect in the offspring due to transfer in the milk or adverse effect on the quality of the milk, and/or
- c) Absorption, metabolism, distribution and excretion studies that indicate the likelihood that the substance is present in potentially toxic levels in breast milk

No information on fluopicolide absorption or interference with lactation in humans is available. Effects of fluopicolide on body weight gains of offspring in the last part of lactation in a 2-generation study in rats were reported. However, the effect is considered not to be related to lactation, but rather to direct uptake of fluopicolide, as there is no effect at the beginning of lactation, and there is evidence from adults that fluopicolide has a low palatability, resulting in low food intake and reduced body weight gain. Thus, no clear evidence of an adverse effect on the transfer to or the quality of the milk.

In addition, toxicokinetics information on fluopicolide and residue studies in lactating cows do not indicate that fluopicolide would be present in milk.

Therefore, RAC agrees with the DS that **fluopicolide should not be classified for effects on or via lactation.**

Additional references

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Viertel B, Trieb G. The Himalayan rabbit (*Oryctolagus cunuculus L.*): Spontaneous incidences of endpoints from prenatal developmental toxicity studies, Laboratory animals. 2003; 27: 19-36.

ECETOC, 2002: Monograph No. 31, Guidance on Evaluation of Reproductive Toxicity Data

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; the evaluation performed by RAC is contained in 'RAC boxes'.
- Annex 2 Comments received on the CLH report, response to comments provided by the Dossier Submitter and RAC (excluding confidential information).