CLH report

Proposal for Harmonised Classification and Labelling

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

International Chemical Identification:

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

EC Number: **not allocated**

CAS Number: **239110-15-7**

Index Number: **not allocated**

Contact details for dossier submitter:

Version number: 02 **Date: June 3, 2019**

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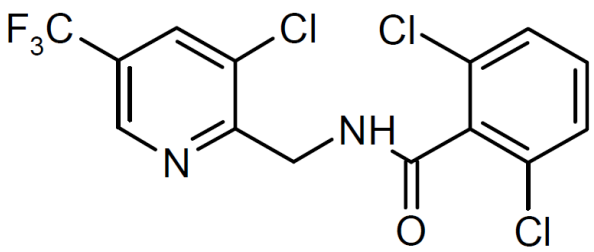
1 IDENTITY OF THE SUBSTANCE

1.1 Name and other identifiers of the substance

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

Name(s) in the IUPAC nomenclature or other international chemical name(s)	IUPAC name: 2,6-dichloro-N-{[3-chloro-5-(trifluoromethyl)-pyridin-2-yl]methyl}benzamide CAS name: Benzamide, 2,6-dichloro-*N*-[[3-chloro-5-(trifluoromethyl)-2-pyridinyl]methyl]-
Other names (usual name, trade name, abbreviation)	Fluopicolide
ISO common name (if available and appropriate)	Fluopicolide
EC number (if available and appropriate)	Not allocated
EC name (if available and appropriate)	Not allocated
CAS number (if available)	239110-15-7
Other identity code (if available)	CIPAC: 787
Molecular formula	$C_{14}H_8Cl_3F_3N_2O$
Structural formula	
SMILES notation (if available)	FC(F)(F)c1cnc(CNC(=O)c2c(Cl)cccc2Cl)c(Cl)c1
Molecular weight or molecular weight range	383.59 g/mol
Information on optical activity and typical ratio of (stereo) isomers (if applicable and appropriate)	Not applicable
Description of the manufacturing process and identity of the source (for UVCB substances only)	Not applicable, fluopicolide is not an UVCB.
Degree of purity (%) (if relevant for the entry in Annex VI)	min. 97.0% w/w

1.2 Composition of the substance

Table 1-2: Constituents (non-confidential information)

Constituent (Name and numerical identifier)	Concentration range (% w/w minimum and maximum in multi-constituent substances)	Current CLH in Annex VI Table 3 (CLP)	Current self-classification and labelling (CLP)
Fluopicolide	Min. 97.0% w/w	No entry in Annex VI	

Table 1-3: Impurities (non-confidential information) if relevant for the classification of the substance

Impurity (Name and numerical identifier)	Concentration range (% w/w minimum and maximum)	Current CLH in Annex VI Table 3 (CLP)	Current self-classification and labelling (CLP)	The impurity contributes to the classification and labelling
Toluene CAS: benzene, methyl [108-88-3]	Max. 0.3% w/w	Flam. Liq. 2 H225 Skin Irrit. 2 H315 Asp. Tox. 1 H304 STOT SE 3 H336 STOT RE 2 H373 Repr. 2 H361d		No

Table 1-4: Additives (non-confidential information) if relevant for the classification of the substance

Additive (Name and numerical identifier)	Function	Concentration range (% w/w minimum and maximum)	Current CLH in Annex VI Table 3 (CLP)	Current self-classification and labelling (CLP)	The additive contributes to the classification and labelling
Not relevant					

2 PROPOSED HARMONISED CLASSIFICATION AND LABELLING

2.1 Proposed harmonised classification and labelling according to the CLP criteria[#]

Table 2-1:

	Index No	Chemical name	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors and ATEs	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	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
Dossier submitters proposal	n.a.	Fluopicolide (ISO); 2-6-dichloro-N-[3-chloro-5-(trifluoromethyl)-2-pyridylmethyl]benzamide	n.a.	239110-15-7	none	none	none	none	none	none	n.a.
Resulting Annex VI entry if agreed by RAC and COM	n.a.	Fluopicolide (ISO); 2-6-dichloro-N-[3-chloro-5-(trifluoromethyl)-2-pyridylmethyl]benzamide	n.a.	239110-15-7	none	none	none	none	none	none	n.a.

[#] only health hazards are assessed
n.a.: not applicable

Table 2-2: Reason for not proposing harmonised classification and status under public consultation

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

3 HISTORY OF THE PREVIOUS CLASSIFICATION AND LABELLING

Fluopicolide is an active substance in the scope of the Regulation (EC) No 1107/2009 (repealing Council Directive 91/414/EEC). There is no harmonised classification and labelling in Annex VI of regulation 1272/2008 (CLP) and there have been no previous classification and labelling discussions of this active substance. The active substance is therefore subject to the harmonized classification and labelling process in accordance with Article 36(2) of CLP and no further justification is required. However, **only health hazards** are assessed in this dossier. The member state Austria proposed to ECHA on Novmeber 28, 2017, to limit the CLH report to human health hazards only, owing to a lack of resources. Furthermore, clarity is required on human health classification (particularly with regard to reproductive toxicity) prior to the EU approval renewal of the active substance under regulation (EC) 1107/2009, because a reproductive classification would directly impact on the data required for this renewal. This proposal was initially accepted by ECHA on December 22, 2017.

The peer review of the pesticide risk assessment of fluopicolide (EFSA Scientific Report (2009) 299, 1-158) concluded that fluopicolide was of low acute toxicity (via the oral, dermal and inhalation routes), was not a skin irritatant and that only slight eye irritation (insufficient for classification purposes) was observed. The review concluded that fluopicolide was unlikely to be genotoxic and that no classification for carcinogenicity, fertility or development was warranted. There were no effects on specific target organs at doses relevant for classification into STOT SE or STOT RE. All of these health hazards have been addressed in this CLH report.

Overall, following the peer review of fluopicolide, no classification was proposed with regard to the physical and chemical data or the toxicological data; however, based on the fate and behaviour data R53 was proposed for fluopicolide and based on the ecotoxicological data N, R50, R53, S60 and S61 were proposed (see https://www.efsa.europa.eu/en/efsajournal/pub/rn-299 for further details).

4 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

There is no requirement for justification that action is needed at Community level; fluopicolide is an active substance in the scope of Regulation (EC) No 1107/2009 (repealing Council Directive 91/414/EEC).

5 IDENTIFIED USES

Fluopicolide containing plant protection products are developed for foliar and seed treatment application to control diseases caused by pathogen fungi from the Oomycete (Phycomycete) class including downy mildews and late blight in crops like vines and potatoes.

6 DATA SOURCES

For human health, the data used in this CLH report consist of studies which have been submitted for Annex I inclusion under Council Directive 91/414/EEC and studies which will be additionally submitted for EU approval renewal under Regulation (EC) No 1107/2009 in November 2020.

7 PHYSIOCHEMICAL PROPERTIES

Physical hazards are not assessed in this dossier. Only health hazards are assessed.

8 EVALUATION OF PHYSICAL HAZARDS

Physical hazards are not assessed in this dossier. Only health hazards are assessed.

8.1 Explosives

Please refer to Section 8.

8.1.1 Short summary and overall relevance of the information provided on explosive properties

Please refer to Section 8.

8.1.2 Comparison with the CLP criteria

Please refer to Section 8.

8.1.3 Conclusion on classification and labelling for explosive properties

Please refer to Section 8.

8.2 Flammable gases (including chemically unstable gases)

Please refer to Section 8.

8.2.1 Short summary and overall relevance of the provided information on flammable gases (including chemically unstable gases)

Please refer to Section 8.

8.2.2 Comparison with the CLP criteria

Please refer to Section 8.

8.2.3 Conclusion on classification and labelling for flammable gases

Please refer to Section 8.

8.3 Oxidising gases

Please refer to Section 8.

8.3.1 Short summary and overall relevance of the provided information on oxidising gases

Please refer to Section 8.

8.3.2 Comparison with the CLP criteria

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8.3.3 Conclusion on classification and labelling for oxidising gases

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8.4 Gases under pressure

Please refer to Section 8.

8.4.1 Short summary and overall relevance of the provided information on gases under pressure

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Please refer to Section 8.

8.4.3 Conclusion on classification and labelling for gases under pressure

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8.5.1 Short summary and overall relevance of the provided information on flammable liquids

Please refer to Section 8.

8.5.2 Comparison with the CLP criteria

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8.5.3 Conclusion on classification and labelling for flammable liquids

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Please refer to Section 8.

8.6.1 Short summary and overall relevance of the provided information on flammable solids

Please refer to Section 8.

8.6.2 Comparison with the CLP criteria

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8.6.3 Conclusion on classification and labelling for flammable solids

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8.7 Self-reactive substances

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8.7.1 Short summary and overall relevance of the provided information on self-reactive substances

Please refer to Section 8.

8.7.2 Comparison with the CLP criteria

Please refer to Section 8.

8.7.3 Conclusion on classification and labelling for self-reactive substances

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8.8 Pyrophoric liquids

Please refer to Section 8.

8.8.1 Short summary and overall relevance of the provided information on pyrophoric liquids

Please refer to Section 8.

8.8.2 Comparison with the CLP criteria

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8.8.3 Conclusion on classification and labelling for pyrophoric liquids

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8.9 Pyrophoric solids

Please refer to Section 8.

8.9.1 Short summary and overall relevance of the provided information on pyrophoric solids

Please refer to Section 8.

8.9.2 Comparison with the CLP criteria

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8.9.3 Conclusion on classification and labelling for pyrophoric solids

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8.10 Self-heating substances

Please refer to Section 8.

8.10.1 Short summary and overall relevance of the provided information on self-heating substances

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8.10.2 Comparison with the CLP criteria

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8.10.3 Conclusion on classification and labelling for self-heating substances

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8.11 Substances which in contact with water emit flammable gases

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8.11.1 Short summary and overall relevance of the provided information on substances which in contact with water emit flammable gases

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8.11.3 Conclusion on classification and labelling for substances which in contact with water emit flammable gases

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8.12.1 Short summary and overall relevance of the provided information on oxidising liquids

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8.14 Organic peroxides

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8.14.3 Conclusion on classification and labelling for organic peroxides

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8.15 Corrosive to metals

Please refer to Section 8.

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8.15.2 Comparison with the CLP criteria

Please refer to Section 8.

8.15.3 Conclusion on classification and labelling for corrosive to metals

Please refer to Section 8.

9 TOXICOKINETICS (ABSORPTION, METABOLISM, DISTRIBUTION AND ELIMINATION)

Toxicokinetics are not assessed in this dossier, only health hazards are assessed; however, as it is relevant to the human health evaluation, a short summary of the available toxicokinetic data is provided below.

Table 9.1: Summary table of toxicokinetic studies

Method	Results	Remarks	Reference
[Phenyl-U-^{14}C]-AE C638206: Single high & low dose rat A.D.E. study US EPA OPPTS 870.7485 JMAF 59 NohSan No 4200 GLP	The major elimination route was via faeces (82 to 88% of dose), while urine contained 5 to 13% dose. Almost complete excretion occurred within 48 hours for the low dose group and 24 hours for the high dose group. At 168 hours post dose tissues were low (0.75 to 1.25% of the dose). Liver and kidneys contained highest residues, and also skin & fur for females of the high dose group.	ADE report. 2 separate metabolism reports (see below). [Phenyl-U-^{14}C]-Fluopicolide: ADE: 4 male & 4 female rats at 10 mg/kg bw; 4 male & 4 female rats at 100 mg/kg bw.	Anonymous; 2001; M-204781-01-1
[Phenyl-U-^{14}C]-AE C638206: Rat metabolism following administration of a single oral low dose - (including Amendment No. 1) US EPA OPPTS 870.7485 JMAF 59 NohSan No 4200 GLP	[Phenyl-U-^{14}C]-fluopicolide was very extensively metabolised in low dose rats (10 mg/kg bw/day) with up to 55 metabolites in urine (9 to 13% dose in urine) and 52 in faecal extracts (81 to 82% dose eliminated in faeces). Biotransformations observed included aromatic ring hydroxylation, hydrolysis, dealkylation, acetylation, oxidative N-dealkylation and conjugation with glucuronic acid, sulphate and glutathione. Glutathione conjugates were further metabolised by loss of glycine and glutamic acid to leave cysteine conjugates. The cysteine conjugates were further metabolised by acetylation to form the mercapturic acids or to dealkylated and S-methylated to form S-methyl metabolites. The S-methyl metabolites were oxidised to both sulphones and sulphoxides. The formation of an acetylated version of AE C653711 (M-01, BAM), indicated that fluopicolide could be cleaved which is consistent with results from the other radiolabelled metabolism study with [Pyridyl-2,6 - ^{14}C]-fluopicolide.	[Phenyl-U-^{14}C]-Fluopicolide: Metabolism: 4 male & 4 female rats at 10 mg/kg bw (see above).	Anonymous; 2004; M-227026-02-1

Method	Results	Remarks	Reference
[Phenyl-U-^{14}C]-AE C638206: Rat metabolism following administration of a single oral high dose - (including Amendment No. 1) US EPA OPPTS 870.7485 JMAF 59 NohSan No 4200 GLP	[Phenyl-U-^{14}C]- fluopicolide was also extensively metabolised in high dose animals (100 mg/kg bw/day) with 46 metabolites detected in urine (4 to 6% dose in urine) and 14 in faecal extracts (86 to 87% dose eliminated in faeces). The same routes of metabolism as seen in the low dose group were observed in high dose animals.	[Phenyl-U-^{14}C]-Fluopicolide: Metabolism: 4 male & 4 female rats at 100 mg/kg bw (see above).	Anonymous; 2004; M-227025-02-1
[Pyridyl-2,6 - ^{14}C]-AE C638206 - Single oral low dose rat A.D.E. study US EPA OPPTS 870.7485 JMAF 59 NohSan No 4200 GLP	The major elimination route was via faeces (69 to 72% of dose), while urine contained 21 to 27% dose. Almost complete excretion occurred within 48 hours. At 168 hours post dose tissues contained between 0.7 to 0.5% of the dose. Liver, kidneys and blood consistently contained highest residues.	Separate ADE and Metabolism reports (see below). [Pyridyl-2,6-^{14}C]-Fluopicolide: ADE: 4 male & 4 female rats at 10 mg/kg bw.	Anonymous; 2001; M-202609-02-1
[Pyridyl-2,6-14C]-AE C638206: Rat metabolism following administration of a single oral low dose US EPA OPPTS 870.7485 JMAF 59 NohSan No 4200 GLP	[Pyridyl-2,6-^{14}C]- fluopicolide was also very extensively metabolised in the rat with up to 28 metabolites in urine (17 to 21% dose in urine) and 31 in faecal extracts (63% dose eliminated in faeces). The same biotransformations as seen in rats dosed with [phenyl-U-^{14}C]-fluopicolide were observed in rats dosed with [pyridyl-2,6-^{14}C]-fluopicolide. The formation of AE C657188 (M-02, PCA), indicated that fluopicolide could be cleaved in the rat by oxidative N-alkylation of the carboxamide amine portion of the molecule.	[Pyridyl-2,6-^{14}C]-Fluopicolide: ADE: 4 male & 4 female rats at 10 mg/kg bw (see above).	Anonymous; 2004; M-227023-01-1

Method	Results	Remarks	Reference
[Phenyl-U-14C]-AE C638206: Repeat oral low dose A.D.M.E. study in the rat - (including amendment No. 1) US EPA OPPTS 870.7485 JMAF 59 NohSan No 4200 GLP	No evidence of accumulation was observed. Following 14 daily oral administrations of [phenyl-U-^{14}C]-fluopicolide the major route of elimination was via faeces (73 to 79% dose). Repeated dosing enhanced elimination via urine compared with the single oral dose (15 to 22% dose). Tissue levels were consistently low (mean 0.38%). Liver, kidneys (organs of excretion and metabolism) and blood contained the highest concentrations of radioactivity in both sexes. [Phenyl-U-^{14}C]- fluopicolide was also extensively metabolised in repeat dose animals with 46 metabolites detected in urine and 14 in faecal extracts. A large number of metabolites were observed in the excreta (up to 57 in the urine and 45 in the faeces). The observed routes of metabolism included glutathione conjugation and its subsequent biotransformation products, hydroxylation, conjugation with glucuronic acid, conjugation with sulphate and oxidative N-dealkylation.	[Phenyl-U-^{14}C]-Fluopicolide: Metabolism: 5 male & 5 female rats at 10 mg/kg bw	Anonymous; 2004; M-227027-02-1
[Phenyl-U-^{14}C]-AE C638206: Rat bile excretion study US EPA OPPTS 870.7485 JMAF 59 NohSan No 4200 GLP	Biliary elimination was a major route in low dose animals (10 mg/kg bw/day). 77% of the low dose for males and 83% for the females (mean 80%) was detected in the bile of cannulated rats dosed with [phenyl-U-^{14}C]-fluopicolide. At the high dose level, the values were 34% for the males and 41% for the females (mean 37%), demonstrating absorption had been saturated by this dose level.	[Phenyl-U-^{14}C]-Fluopicolide: 4 male & 4 female bile-duct cannulated rats at 10 mg/kg bw; 4 male & 4 female bile-duct cannulated rats at 100 mg/kg bw.	Anonymous; 2002; M-212243-01-1
[Pyridyl-2,6-^{14}C]-AE C638206: Single oral low dose rat bile excretion study US EPA OPPTS 870.7485 JMAF 59 NohSan No 4200 GLP	When dosed with [pyridyl-U-^{14}C]-fluopicolide 59% of the low dose for males and 64% for the females (mean 62%) was detected in the bile of cannulated rats. The difference between the two radiolabels likely indicates a portion of the fluopicolide dose is metabolised to form single ring metabolites AE C653711 (M-01, BAM) and AE C657188 (M-02, PCA), which behave differently in the rat.	[Pyridyl-U-^{14}C]-Fluopicolide: 4 male & 4 female bile-duct cannulated rats at 10 mg/kg bw;	Anonymous; 2003; M-230976-01-1

Method	Results	Remarks	Reference
[Phenyl-U-^{14}C]-AE C638206 and [pyridyl-2,6-^{14}C]-AE C638206: Rat blood and plasma kinetics study US EPA OPPTS 870.7485 JMAF 59 NohSan No 4200 GLP	The general pharmacokinetic profiles were similar between radiolabels and sexes. Fluopicolide was absorbed relatively rapidly with maximal concentrations achieved between 7 and 10 hours post dose at 10 mg/kg bw.	[Phenyl-U-^{14}C]-Fluopicolide: 4 male & 4 female rats at 10 mg/kg bw; 4 male & 4 female rats at 100 mg/kg bw. [Pyridyl-U-^{14}C]-Fluopicolide: 4 male & 4 female rats at 10 mg/kg bw; 4 male & 4 female rats at 100 mg/kg bw.	Anonymous; 2002; M-221902-01-1
[Phenyl-U-^{14}C]-AE C638206 rat tissue kinetic study	Fluopicolide was rapidly and widely distributed into the tissues. No significant sex difference was found. In rats dosed with [phenyl-U-^{14}C]-fluopicolide, highest tissue concentrations were in the intestine and contents with next highest concentrations observed in liver, kidneys and adrenals, which decreased with time. The compound was extensively metabolised with 13 metabolites detected in liver by 8 hours post dose, of which AE C653711 (M-01, BAM), AE 0717559, AE C643890 (M-06) and AE 0717560 were identified.	[Phenyl-U-^{14}C]-Fluopicolide: 16 male & 16 female rats at 10 mg/kg bw; 16 male & 16 female rats at 100 mg/kg bw. Sacrificed at 8, 24 or 30, 36 or 48, 72 (males) & 120 hours (females).	Anonymous; 2003; M-221892-01-1
[2,6-Pyridyl-^{14}C]-AE C638206: Rat tissue kinetic study	In rats dosed with [pyridyl-U-^{14}C]-fluopicolide, the compound was similarly distributed into tissues, followed by a significant and rapid decrease in tissue concentrations. Again, no significant sex difference was found. Highest radioactivity concentrations were in the intestine and contents presumably as a result of biliary excretion of radioactivity. The next highest concentrations were in the liver, kidneys, adrenals and cardiac blood which declined with time post dose.	[Pyridyl-U-^{14}C]-Fluopicolide: 16 male & 16 female rats at 10 mg/kg bw. Sacrificed at 6 or 7, 24, 36, 48 (males) & 120 hours (females).	Anonymous, 2003; M-221885-01-1

Method	Results	Remarks	Reference
(^{14}C)-AE C638206: Preliminary toxicokinetic studies in the rat Non guideline preliminary study GLP	The findings aer consistent with later studies. Fluopicolide was relatively rapid adsorped with blood C_{max} between 8 to 12 hours. At low dose residues were below 0.10 µg/g in all tissues except the liver, kidney and blood 168 h after dosing. The major metabolic reactions identified were aromatic hydroxylation of the phenyl ring, glucuronidation of the phase I hydroxyl products and sequential metabolism through the mercapturic acid pathway.	Combined ADME & kinetics preliminary study (25 & 500 mg/kg bw) [Phenyl-U-^{14}C]-Fluopicolide: ADME: 2 male & 2 female rats at 25 mg/kg bw; 2 male & 2 female rats at 500 mg/kg bw. Blood kinetic: 2 male & 2 female rats at 25 mg/kg bw; 2 male & 2 female rats at 500 mg/kg bw. [Pyridyl-2,6-^{14}C]-Fluopicolide: ADME: 2 male & 2 female rats at 25 mg/kg bw. Blood kinetic: 2 male & 2 female rats at 25 mg/kg bw.	Anonymous; 2000; M-197858-01-1
Interspecies comparison of in vitro metabolism of [phenyl-UL-^{l4}C] fluopicolide using mouse, rat, dog and human liver microsomes Non guideline preliminary study GLP	[Phenyl-U-^{14}C]-Fluopicolide was significantly metabolised by liver microsomes from mice, rat, dog and humans, with a total of 8 metabolites detected. No human-specific metabolites were observed.	[Phenyl-U-^{14}C]-Fluopicolide: *In vitro* (1 and 10 µM) with liver microsomes from mice, rat, dog and humans.	Anonymous; 2019; M-653630-02-1

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

In vivo studies

Toxicokinetic studies on the absorption, distribution, metabolism and excretion of fluopicolide, were conducted in the rat. Studies were performed using two different radiolabels; [phenyl-U-^{14}C]-fluopicolide or [pyridyl-2,6-^{14}C]-fluopicolide.

The major route of elimination of fluopicolide was via the faeces for both the 10 and 100 mg/kg bw oral dose and for both pyridyl (69 to 72% of the administered dose) and phenyl (82 to 88%) ring radiolabels. No significant sex difference was observed. There was a tendency towards a higher urinary excretion level with the pyridyl radiolabel (19% in males and 22% in females for the 10 mg/kg bw dose) compared to the phenyl radiolabel (10% in males and 13% in females for the 10 mg/kg bw dose). This suggests that a proportion of the metabolites that were formed differed between the two radiolabels and were presumably linked to the formation of AE C657188 (M-02, PCA) from the pyridyl ring moiety and AE C653711 (M-01, BAM) from the phenyl ring.

Following repeated (14x) daily oral administrations of [phenyl-U-^{14}C]-fluopicolide the total recovery of radioactivity was approx. 96% of the administered dose; with the faeces, again, being found to be the major route of elimination representing 79% for the males and 72% for the females. The urine was found to represent 15% of the administered dose for the males and 21% for the females. It appeared that repeated dosing enhanced elimination via urine compared with the single oral dose.

Tissue radioactivity levels were consistently low and ranged between 0.46 to 1.25% of the administered dose for the single dose studies and a mean of 0.38% for the repeat dose study.

Investigations in bile-cannulated rats over 48 hours showed a large proportion of the radioactivity found in the faeces had been absorbed and then eliminated via the bile. The extent of oral absorption based on the biliary excretion study only for the 10 mg/kg bw oral dose, was 80% of the administered dose for the phenyl radiolabel and 62% for the pyridyl radiolabel. However, blood and plasma pharmacokinetic data show the systemic exposure was similar between both the radiolabels and the sexes. The bioavailability of fluopicolide, taking into account the material undergoing entero-hepatic recirculation, was calculated to be 75 to 88% of the administered dose.

Fluopicolide was well distributed into organs and tissues (blood T_{max} 5.5 to 7.5 hours and plasma T_{max} 6.5 to 8 hours for 10 mg/kg bw) followed by a moderately rapid elimination such that the majority was eliminated by 48 hours post dose followed by a slower terminal elimination phase with a mean half-life of approx. 99 hours for blood. A lower mean half-life of 16 hours was observed for plasma due to the difference in limits of quantification.

The highest tissue residues were found in the liver and kidney and (to a lesser extent) in the spleen and blood.

In tissue kinetic studies the highest tissue concentrations were observed in the intestine and contents, reflecting a combination of unabsorbed material and biliary excretion. The next highest concentrations were consistently observed in the liver, kidneys and adrenals albeit that the concentrations were decreasing with time post dosing. AE C653711 (M-01, BAM), AE 0717559, AE C643890 (M-06) and AE 0717560 were identified in the liver 8 hour post dosing with [phenyl-U-^{14}C]-fluopicolide.

Fluopicolide was extensively metabolised in the rat. The formation of the metabolites AE C653711 (M-01, BAM) and AE C657188 (M-02, PCA) was confirmed during the course of the biotransformation investigations and indicated that fluopicolide could be cleaved in the rat by oxidative N-alkylation of the carboxamide amine portion of the molecule. Generally, the biotransformations observed included aromatic ring hydroxylation, hydrolysis, dealkylation, acetylation, oxidative N-dealkylation and conjugation with glucuronic acid, sulphate and glutathione. The glutathione conjugates were seen to be further metabolised by loss of glycine and glutamic acid to leave cysteine conjugates. The cysteine conjugates were seen to be further metabolised by acetylation to form the mercapturic acids or to be dealkylated and S-methylated to form S-methyl metabolites. The S-methyl metabolites were seen to be oxidised to both sulphones and sulphoxides.

In vitro study

The comparative *in-vitro* metabolism of fluopicolide was studied with liver microsomes from CD-1 mouse, Wistar rat, Beagle dog and human. Incubations were performed with [phenyl-U-^{14}C]-fluopicolide at two concentrations (1 and 10 µM) at 1, 60 and 120 minutes.

[Phenyl-U-^{14}C]-Fluopicolide was significantly metabolised by liver microsomes from all four species. Conversion of fluopicolide was 98% in dog, 82% in mouse, 68% in human and 54% in rat microsomes after 120 minutes of incubation. A total of 8 metabolites were detected, named Metabolite 1 to 8 based on their HPLC retention time. Metabolites accounting for ≥ 5% were considered as main metabolites. Overall, five main metabolites were detected: Metabolite 1 (mouse, rat and human) and Metabolite 2 (mouse and rat), Metabolite 3 and Metabolite 5 (mouse, dog and human) and Metabolite 6 which was detected as a main metabolite in the four species. Metabolite 2 was detected in the mouse and rat microsome incubations only. No human-specific fluopicolide metabolites were detected.

10 EVALUATION OF HEALTH HAZARDS

10.1 Acute toxicity - oral route

The acute oral toxicity of fluopicolide has been investigated in rats.

Table 10-1: Summary table of animal studies on acute oral toxicity

Method, guideline, deviations if any	Species, strain, sex, no/ group	Test substance	Dose levels, duration of exposure	Value LD_{50}	Reference
Acute oral toxicity OECD 423(1996) GLP	Rat, Hsd: Sprague-Dawley (CD) male & female 5/sex/ dose level	Fluopicolide (purity 97.7%)	Single oral gavage at doses of 5000 mg/kg bw, in 1% w/v aqueous methylcellulose	> 5000 mg/kg bw	Anonymous.; 2000; M-197224-01-1

Table 10-2: Summary table of human data on acute oral toxicity

Type of data/report	Test substance	Relevant information about the study (as applicable)	Observations	Reference
No human data				

Table 10-3: Summary table of other studies relevant for acute oral toxicity

Type of study/data	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
No other studies				

10.1.1 Short summary and overall relevance of the provided information on acute oral toxicity

In an acute toxicity study by the acute toxic class method, 5 male and 5 female fasted Sprague-Dawley rats were each administered by gavage a single oral dose of 5000 mg/kg bw.

No mortality was observed. Clinical signs of reaction to treatment were confined to piloerection and hunched posture, seen in all female rats and in three male rats with abnormal gait notable in three females. Recovery of rats, as judged by external appearance and behavior, was complete by Day 3. All animals were considered to have achieved satisfactory body weight gains throughout the study.

The acute lethal oral dose in rats of fluopicolide (AE C638206) was greater than 5000 mg/kg bw.

10.1.2 Comparison with the CLP criteria

The guidance on the application of the CLP criteria (Regulation (EC) No 1272/2008) gives a cut-off LD_{50} value of 2000 mg/kg bw for the classification of acute toxicity via the oral route. Under the conditions of this study the LD_{50} value of fluopicolide for oral toxicity was found to be > 5000 mg/kg bw. Therefore **no classification** for acute oral toxicity is proposed.

10.1.3 Conclusion on classification and labelling for acute oral toxicity

The data on the acute toxicity potential of fluopicolide (AE C638206) are conclusive. Based on the oral LD_{50} of > 5000 mg/kg bw after acute oral administration to rats, an acute toxicity classification is **not warranted** according to Regulation (EC) No 1272/2008 (CLP).

10.2 Acute toxicity - dermal route

The acute dermal toxicity of fluopicolide has been investigated in rats.

Table 10-4: Summary table of animal studies on acute dermal toxicity

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance	Dose levels, duration of exposure	Value LD_{50}	Reference
Acute dermal toxicity OECD 402 (1987) GLP	Rat, Hsd: Sprague-Dawley (CD) male & female 5/sex/ dose level	Fluopicolide (purity 97.7%)	Single dermal dose of 5000 mg/kg bw, in 1% w/v aqueous methylcellulose	> 5000 mg/kg bw	Anonymous; 2000; M-197225-01-1

Table 10-5: Summary table of human data on acute dermal toxicity

Type of data/report	Test substance	Relevant information about the study (as applicable)	Observations	Reference
No human data				

Table 10-6: Summary table of other studies relevant for acute dermal toxicity

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations	Reference
No other data				

10.2.1 Short summary and overall relevance of the provided information on acute dermal toxicity

A group of ten rats (five males and five females) received a single topical application of the test substance, administered as supplied at a dose level of 5000 mg/kg bw. The application site was occluded for 24 hours. All animals were observed daily for 14 days and body weights were recorded at weekly intervals post dosing.

No mortality was observed. There were no clinical signs of reaction to treatment observed in any animal throughout the study. There was no evidence of a dermal response to treatment observed in any animal throughout the study. A slightly reduced body weight was evident in 2/5 females on Day 8. All other animals were considered to have achieved satisfactory bodyweight gains throughout the study.

No macroscopic abnormalities were observed for animals killed at study termination on Day 15. The acute lethal dermal dose (LD_{50}) to rats of fluopicolide (AE C638206) was greater than 5000 mg/kg bw and thus greater than the trigger value of 2000 mg/kg bw.

10.2.2 Comparison with the CLP criteria

The application on the guidance of the CLP criteria (Regulation (EC) No 1272/2008) gives a cut off LD50 value of 2000 mg/kg bw for acute dermal toxicity classification. Under the conditions of this study fluopicolide (AE C638206) had an LD_{50} of > 2000 mg/kg bw and as such **no classification** for acute dermal toxicity is proposed.

10.2.3 Conclusion on classification and labelling for acute dermal toxicity

The data on the acute dermal toxicity potential of fluopicolide (AE C638206) are conclusive. Based on the LD_{50} value of > 5000 mg/kg bw after acute dermal administration to rats, according to the CLP guidance (Guidance to Regulation (EC) No 1272/2008), an acute dermal toxicity classification of fluopicolide (AE C638206) is **not warranted**.

10.3 Acute toxicity - inhalation route

The acute toxicity of fluopicolide via the inhalation route has been investigated in rats.

Table 10-7: Summary table of animal studies on acute inhalation toxicity

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance, form and particle size (MMAD)	Dose levels, duration of exposure	Value LC_{50}	Reference
Acute inhalation toxicity OECD 403 (1981) GLP	Rat, Sprague-Dawley Crl:CD®BR male & female 5/sex/dose level	Fluopicolide (purity 98.3%) Dust MMAD ± GSD = 3.37 ± 2.09 µm	Mean concentration of 5.16 mg/L (9.09 mg/L nominal) was administered by 4 hour nose-only exposure	>5.16 mg/L (4 hours)	Anonymous; 2000; M-197229-01-1

Table 10-8: Summary table of human data on acute inhalation toxicity

Type of data/report	Test substance	Relevant information about the study (as applicable)	Observations	Reference
No human data				

Table 10-9: Summary table of other studies relevant for acute inhalation toxicity

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations	Reference
No other data				

10.3.1 Short summary and overall relevance of the provided information on acute inhalation toxicity

The acute inhalation toxicity of fluopicolide (AE C638206) was investigated by exposing a group of five male and five female Sprague-Dawley (CD) rats to a dust atmosphere of the limit concentration of test substance of 5.16 mg/L. The test group was subjected to a single four-hour, continuous, snout only exposure. Signs of reaction to treatment were recorded during a subsequent 14-day observation period. The animals were sacrificed at the end of the observation period and were subjected to detailed necropsy.

No mortality was recorded. Common observations noted both 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. Animals recovered quickly to appear normal on the first day after exposure. Normal bodyweight gain was noted during the study.

No macroscopic abnormalities were noted for 9/10 animals. One male showed dark foci on its lungs. The LC_{50} was therefore > 5.16 mg/L.

10.3.2 Comparison with the CLP criteria

The guidance of the application of the CLP criteria (Regulation (EC) No 1272/2008) gives a 4-hour LC_{50} cut-off value of 5 mg/L to trigger classification for acute inhalation toxicity. Under the conditions of this study the 4-hour LC_{50} of fluopicolide was > 5.16 mg/L. **No classification** for acute inhalation toxicity is proposed.

10.3.3 Conclusion on classification and labelling for acute inhalation toxicity

The data on the acute inhalation toxicity potential of fluopicolide (AE C638206) are conclusive. Based on the LC_{50} value of > 5.16 mg/L after acute inhalative administration to rats according to the CLP guidance (Guidance to Regulation (EC) No 1272/2008)) an acute inhalation toxicity classification of fluopicolide (AE C638206) is **not warranted**.

10.4 Skin corrosion/irritation

The potential of fluopicolide to induce acute skin corrosion or irritation has been investigated in rabbits.

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

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance	Dose levels, duration of exposure	Results - Observations and time point of onset - Mean scores/ animal - Reversibility	Reference
Acute skin irritation study OECD 404 (1992) GLP	Rabbit, New Zealand White albino Females 3/group	Fluopicolide (purity 97.7%)	0.5 g (powder moistened with water prior applying to skin) 4 hours, semi-occlusive	None of the three rabbits showed any substance-related lesions at the examination time-points 1, 24, 48 and 72 hours after patch removal. The mean irritation score over 24 – 72 h was 0.0 for erythema and oedema respectively.	Anonymous; 2000; M-197226-01-1

Table 10-11: Summary table of human data on skin corrosion/irritation

Type of data/report	Test substance	Relevant information about the study (as applicable)	Observations	Reference
No human data				

Table 10-12: Summary table of other studies relevant for skin corrosion/irritation

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations	Reference
No other data				

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

The potential of fluopicolide (AE C638206) to cause inflammatory or corrosive changes upon first contact with skin was assessed by semi-occluded application of 0.5 g of the test material to the closely-clipped dorsa of three New Zealand White rabbits for four hours. Dermal reactions were assessed 1, 24, 48 and 72 hours after removal of the dressings.

The single semi-occlusive application of fluopicolide (AE C638206) to intact rabbit skin for four hours elicited no dermal irritation in any animal during the study. The mean irritation score over 24 – 72 h was 0.0 for erythema and oedemafor all animals.

10.4.2 Comparison with the CLP criteria

The guidance on the application of the CLP criteria (Regulation (EC) No 1272/2008) requires that mean irritation score are > 2.3 for erythema/eschar in at least 2 out of 3 animals, before classification as a skin irritant is triggered. The scores for erythema/eschar for fluopicolide in this study were 0 for all animals. Fluopicolide was found to be not irritating to the skin of the rabbit under the conditions of this study, therefore **no classification** for skin corrosion/irritation is proposed.

10.4.3 Conclusion on classification and labelling for skin corrosion/irritation

Since this valid skin corrosion/irritation study with fluopicolide (AE C638206) in rabbits did not show signs of skin irritation, the results are conclusive, but do **not warrant** classification as skin irritant according to the CLP guidance (Guidance to Regulation (EC) No 1272/2008)).

10.5 Serious eye damage/eye irritation

The potential of fluopicolide to induce serious eye damage / eye irritation has been investigated in rabbits.

Table 10-13: Summary table of animal studies on serious eye damage/eye irritation

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance	Dose levels duration of exposure	Results - Observations and time point of onset - Mean scores/animal - Reversibility	Reference
Acute eye irritation study OECD 405 (1987) GLP	Rabbit, New Zealand White albino Females 3/group	Fluopicolide (purity: 97.7%)	100 mL (93 mg)	Eyes were examined and irritation was assessed at 1, 24, 48 and 72 hours after administration; mean scores were calculated from 24-72 h values The mean eye irritation score (24/48/72 hours) for conjunctival redness was 0.33 in 2 animals and 0 in 1 animal and 0 in all 3 animals for all other endpoints scored. Reactions had resolved in all instances two days after instillation. Thus, fluopicolide was transiently slightly irritant to the rabbit eye.	Anonymous; 2000; M-197227-01-1

Table 10-14: Summary table of human data on serious eye damage/eye irritation

Type of data/report	Test substance	Relevant information about the study (as applicable)	Observations	Reference
No human data				

Table 10-15: Summary table of other studies relevant for serious eye damage/eye irritation

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations	Reference
No other data				

10.5.1 Short summary and overall relevance of the provided information on serious eye damage/eye irritation

The eye irritating potential of fluopicolide was investigated in rabbits. A single dose of 100 mL of fluopicolide powder was administered to one eye each of three Himalayan rabbits. Eye irritation was assessed at 1, 24, 48 and 72 hours.

A single instillation of fluopicolide into the unrinsed eye of the rabbit elicited slight conjunctival irritation in all animals (in one animal minimal (grade 1) conjunctival redness only at the 1 hour observation). The ocular reactions resolved in all instances within two days after instillation. There were no observations of corneal opacity, iritis or chemosis. Thus, fluopicolide was transiently slightly irritant to the rabbit eye.

10.5.2 Comparison with the CLP criteria

According to the grading criteria as described in Regulation (EC) No 1272/2008, substances that produce in at least 2 of 3 tested animals, a positive response of: (a) corneal opacity ≥ 1 and/or (b) iritis ≥ 1, and/or (c) conjunctival redness ≥ 2 and/or (d) conjunctival oedema (chemosis) ≥ 2 calculated as the mean scores following grading at 24, 48 and 72 hours after installation of the test material, and which fully reverses within an observation period of 21 days shall be classified as category 2 eye irritant.

These criteria were not met at any observation point for any animal in the study. Mean scores for 24, 48 and 72 hours post instillation were 0.0 for all parameters, with the exception of conjunctival redness, which had a score of 0.33 in two out of three animals. The ocular reactions resolved in all instances within two days after instillation.Therefore **no classification** for serious eye damage/eye irritation is proposed.

10.5.3 Conclusion on classification and labelling for serious eye damage/eye irritation

The results of the eye irritation study with fluopicolide are conclusive, but they do **not warrant** an eye irritation classification according to the CLP guidance (Guidance to Regulation (EC) No 1272/2008).

10.6 Respiratory sensitisation

No data on respiratory sensitisation available. Fluopicolide was of low toxicity in an acute inhalation study and was negative in a skin sensitisation study (see below); therefore, it is unlikely that it would induce respiratory sensitisation.

10.6.1 Short summary and overall relevance of the provided information on respiratory sensitisation

Medical surveillance data on manufacturing personnel was obtained during the pilot-scale production of fluopicolide (2004-2005 in Lyon, France, and Dormagen, Germany). No incidences of adverse reactions were reported during the pilot-scale manufacture/formulation of fluopicolide (fluopicolide DAR 2005). No formally recognised and validated animal tests currently exist for respiratory sensitisation. However data from some animal studies may be indicative of the potential of a substance to cause respiratory sensitisation in humans and may provide supportive evidence in case human evidence is available. This information may also be combined with information on structural alerts for respiratory sensitisation and information on the skin sensitising properties of a substance and should be used in a weight of evidence assessment.

In the animal studies conducted with fluopicolide, no evidence of respiratory tract irritation (local cytotoxic effects) was obvious; also the acute rat inhalation data did not provide evidence for functional impairment of the respiratory system. Moreover, fluopicolide has no skin sensitizing potential. According to the CLP criteria, a substance which is negative in sensitization assays, most probably also lacks the potential for respiratory allergy so that based on this no evidence of a respiratory sensitization potential of fluopicolide exists. Since in addition the skin and eye irritation studies in rabbits, and the rat acute and repeated dose dermal toxicity studies did not indicate an irritating potential on skin and mucous membranes, a respiratory irritation potential of fluopicolide is not likely.

10.6.2 Comparison with the CLP criteria

According to the CLP criteria for respiratory sensitization, evidence that a substance can lead to specific hypersensitivity will normally be based on human experience. In this context, hypersensitivity is normally seen as asthma, but other hypersensitivity reactions such as rhinitis/conjunctivitis and alveolitis are also considered. The condition will have the clinical character of an allergic reaction.

In the animal studies with fluopicolide, no evidence of respiratory tract irritation (local cytotoxic effects) was obvious, also the acute rat inhalation data do not provide evidence for functional impairment of the respiratory system. According to the CLP criteria, substances which are negative in sensitization studies, most likely have no potential for respiratory allergy. Therefore, based on this no evidence of a respiratory sensitization potential of fluopicolide exists. Also the skin and eye irritation studies in rabbits, and the rat acute and repeated dose dermal toxicity studies did not indicate a severe irritating or corrosive potential on skin and mucous membranes, so that a respiratory irritation potential of fluopicolide (AE C638206) is not likely.

10.6.3 Conclusion on classification and labelling for respiratory sensitisation

Since in the animal studies with fluopicolide (AE C638206) no evidence of respiratory sensitisation was obvious (no evidence of local irritation, respiratory tract impairment or skin sensitisation) and since limited human data (up to 2005) shows no evidence of adverse respiratory effects, no classification for respiratory sensitisation is proposed.

10.7 Skin sensitisation

The skin sensitising potential of fluopicolide has been investigated in a guinea-pig maximisation test (GMPT).

Table 10-16: Summary table of animal studies on skin sensitisation

Method, guide-line, deviations if any	Species, strain, sex, no/group	Test substance	Dose levels duration of exposure	Results	Reference
Guinea-pig maximisation test (GPMT according to Magnusson and Kligman) OECD 406 (1992) GLP	Guinea pigs, Dunkin-Hartley guinea-pigs Females 20 animals for the test item group and 10 control animals	Fluopicolide (purity: 97.7%)	Intradermal induction 10% w/v fluopicolide in sterile water or in a 50:50 mixture of Freund's complete adjuvant in sterile water Topical induction 6 days later 100% w/v fluopicolide in sterile water, occlusive dressing for 48 hours. Challenge Two weeks after the topical induction, all animals were challenged by occluded application of 100% fluopicolide in sterile water to the anterior site on the flank and 50% fluopicolide in sterile water to the posterior site on the flank.	**Negative** Slight erythema was observed in 2/20 test animals (10%) at the 24 and 48 hour reading compared to slight to well-defined erythema for 2/10 control animals (20%) at the 48 hour reading only. As the reactions observed were of similar severity and the incidence was greater in control animals (20% of controls compared with 10% of tested animals) and no reactions were observed for any of the remaining animals the reactions in the test animals are not considered relevant.	Anonymous; 2000; M-197228-01-1

Table 10-17: Summary table of human data on skin sensitisation

Type of data/report	Test substance	Relevant information about the study (as applicable)	Observations	Reference
No human data				

Table 10-18: Summary table of other studies relevant for skin sensitisation

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations	Reference
No other data				

10.7.1 Short summary and overall relevance of the provided information on skin sensitisation

The potential of fluopicolide to cause delayed contact hypersensitivity in guinea pigs was investigated in a GPMT according to Magnusson-Kligman. Based on a preliminary study, the closely-clipped backs of twenty female Dunkin-Hartley guinea-pigs were subject to intradermal injections of Freund's Complete Adjuvant, 10% w/v fluopicolide (AE C638206) in sterile water and 10% w/v fluopicolide (AE C638206) in a 50:50 mixture of Freund's complete adjuvant in sterile water on Day 1. Six days later, the same area of skin was treated by topical application of 100% w/v fluopicolide in sterile water and the test site was covered by an occlusive dressing for 48 hours. The same induction procedures were carried out on 10 control animals, except that the test material was replaced by vehicle in all doses. Two weeks after the topical induction, all animals were challenged by occluded application of 100% fluopicolide in sterile water to the anterior site on the flank and 50% fluopicolide in sterile water to the posterior site on the flank. The occlusive dressings were removed on the following day and the condition of the test sites was assessed approx. 24 and 48 hours later.

There were no deaths or signs of ill health or toxicity. Body weight changes were similar between control and treated animals. Necrosis was observed at sites receiving Freund's Complete Adjuvant in all test and control animals following intradermal injections. Slight irritation was seen in 6/20 animals on the site treated with 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.

The challenge application produced no dermal reactions indicative of skin sensitization in any of the animals. Slight erythema was observed in two test animals at the 24 and 48 hour reading compared with slight to well-defined erythema for two control animals at the 48 hour reading only. The reactions observed were noted to be of similar incidence and severity and as no reactions were observed for any of the remaining test or control animals, the overall response was considered negative.

Fluopicolide was not a skin sensitizer in this GPMT.

10.7.2 Comparison with the CLP criteria

In a GPMT with fluopicolide a similar incidence and severity of reactions were observed (10% of animals of the test group and 20% of the control responded); Therefore, the overall response is considered negative. Moreover, the incidence of 10% in the test group is below the guidance value of responses in ≥ 30% of animals in an adjuvant test that would lead to classification. Therefore **no classification** for skin sensitisation is proposed.

10.7.3 Conclusion on classification and labelling for skin sensitisation

Since for fluopicolide no evidence of a sensitizing potential from a Maximization study in guinea-pigs exists, the data are conclusive that they do **not warrant** a skin sensitization classification of fluopicolide according to the CLP guidance (Guidance to Regulation (EC) No 1272/2008).

10.8 Germ cell mutagenicity

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).

Table 10-19: Summary table of mutagenicity/genotoxicity tests *in vitro*

Method, guideline, deviations if any	Test substance	Relevant information about the study including rationale for dose selection (as applicable)	Observations/ Results	Reference
Bacterial point mutation assay (Ames test) OECD 471 (1997) GLP	Fluopicolide (purity 97.8%) DMSO	Test system: *Salmonella typhimurium* strains TA 1535, TA 1537, TA 98, TA 100, and *Escherichia coli 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 precipi-tating dose levels.	Anonymous; 2004; M-197259-02-1
Bacterial point mutation assay (Ames test) OECD 471 (1997) GLP	Fluopicolide Purity not reported DMSO	Test system: *Salmonella typhimurium* strains TA 1535, TA 1537, TA 98, TA 100, and *Escherichia coli 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**	Anonymous; 2001; M-202931-01-1

Method, guideline, deviations if any	Test substance	Relevant information about the study including rationale for dose selection (as applicable)	Observations/ Results	Reference
Bacterial point mutation assay (Ames test) OECD 471 (1997) GLP	Fluopicolide (purity 95.6%) DMSO	Test system: *Salmonella typhimurium* strains TA 1535, TA 1537, TA 98, TA 100, and *Escherichia coli WP2uvrA* The following concentrations were tested: Plate incorporation 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) Pre-incubation Experiment II: 31.25; 62.5; 125; 250; 500 and 1000 µg/plate (+S9)	**Negative**	Anonymous; 2001; M-202927-01-1
Bacterial point mutation assay (Ames test) OECD 471 (1997) GLP	Fluopicolide (purity 95.9%) DMSO	Test system: *Salmonella typhimurium* strains TA 1535, TA 1537, TA 98, TA 100, and *Escherichia coli WP2uvrA* The following concentrations were tested: Plate incorporation 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) Pre-incubation Experiment II: 31.25; 62.5; 125; 250; 500 and 1000 µg/plate (+S9)	**Negative**	Anonymous; 2001; M-202939-01-1

Method, guideline, deviations if any	Test substance	Relevant information about the study including rationale for dose selection (as applicable)	Observations/ Results	Reference
Bacterial point mutation assay (Ames test) OECD 471 (1997) GLP	Fluopicolide (purity 99.3%) DMSO	Test system: *Salmonella typhimurium* strains TA 1535, TA 1537, TA 98, TA 100, and *Escherichia coli WP2uvrA* The following concentrations were tested: Plate incorporation 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) Pre-incubation Experiment II: 31.25; 62.5; 125; 250; 500 and 1000 µg/plate (+S9)	**Negative**	Anonymous; 2001; M-202935-01-1
Bacterial point mutation assay (Ames test) OECD 471 (1997) GLP	Fluopicolide (purity 98.2%) DMSO	Test system: *Salmonella typhimurium* strains TA 1535, TA 1537, TA 98 TA 100 and TA 102 The following concentrations were tested: Plate incorporation 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). Experiment Ia (TA102 only): 3; 10; 33; 100; 333; 1000; 2500; and 5000 µg/plate (+S9) Pre-incubation Experiment II: 10; 33; 100; 333; 1000; 2500 and 5000 µg/plate (±S9)	**Negative**	Anonymous; 2017; M-595228-01-1

Method, guideline, deviations if any	Test substance	Relevant information about the study including rationale for dose selection (as applicable)	Observations/ Results	Reference
In vitro chromosome aberration assay in Chinese hamster lung V79 cells OECD 473 (1997) GLP Deviations: Cytotoxicity was not measured using the parameters of relative population doubling (RPD) or relative increase in cell count (RICC). Only 25-100 metaphases, instead of the currently required 300 metaphases, were analysed.	Fluopicolide (purity 97.8%) DMSO	Test system: Chinese hamster lung V79 cells The following concentrations were tested: <u>Experiment I:</u> 25; 50; 75 and 100 µg/mL (±S9) <u>Experiment II:</u> 1.6; 3.2 and 6.3 µg/mL (-S9)	**Positive** at cytotoxic concentrations	Anonymous; 2004; M-197260-02-1
In vitro chromosome aberration assay in human lymphocytes OECD 473 (1997) GLP Deviations: Cytotoxicity was not measured using the parameters of relative population doubling (RPD) or relative increase in cell count (RICC). Only 25-100 metaphases, instead of the currently required 300 metaphases, were analysed.	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)	**Negative**	Anonymous; 2001; M-201582-01-1

Method, guideline, deviations if any	Test substance	Relevant information about the study including rationale for dose selection (as applicable)	Observations/ Results	Reference
In vitro HPRT mutation assay in Chinese hamster lung V79 cells OECD 476 (1997) GLP	Fluopicolide (purity 97.8%) DMSO	Test system: Chinese hamster lung V79 cells The following concentrations were tested: <u>Experiment I:</u> 1.2; 3.8; 12.1; 38.2; 120.8; 382; 1208 and 3820 µg/mL (±S9) <u>Experiment II:</u> 0.4; 0.8; 1.6; 3.2; 6.3; 12.5; 25; 50; 75; 100 and 120 µg/mL (±S9) <u>Experiment III:</u> 0.313; 0.625; 1.25; 2.5; 5; 10; 20; 30; 40; 50 and 60 µg/mL (±S9)	**Negative**	Anonymous; 2005; M-210831-02-1

Table 10-20: Summary table of mutagenicity/genotoxicity tests in mammalian somatic or germ cells *in vivo*

Method, guideline, deviations if any	Test substance	Relevant information about the study (as applicable)	Observations/ Results	Reference
Mouse micronucleus test OECD 474 (1997) GLP Deviations: 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.	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)	**Negative**	Anonymous; 2005; M-197261-02-1

Method, guideline, deviations if any	Test substance	Relevant information about the study (as applicable)	Observations/ Results	Reference
Mouse micronucleus test OECD 474 (1997) GLP Deviations: 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.	Fluopicolide (purity 96.1%), in 1% (w/v) methylcellulose	Test system: Mouse (Crl:CD1) The following concentration was tested: 2000 mg/kg bw (oral)	**Negative**	Anonymous; 2003; M-219364-01-1
Mouse micronucleus test (i.p.) OECD 474 (1997) GLP Deviations: Altered NCE/PCE ratio. Only 2000, not 4000 erythrocytes, were analysed for MN.	Fluopicolide (purity 99.4%), in 0.5% cremophor	Test system: Mouse (Crl:CD1) The following concentrations were tested: 150, 300 and 600 mg/kg bw (i.p.)	**Negative**	Anonymous; 2003; M-223119-01-1
Rat UDS assay OECD 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

Method, guideline, deviations if any	Test substance	Relevant information about the study (as applicable)	Observations/ Results	Reference
Comet assay in mice OECD 489 (2016) GLP	Fluopicolide (purity 98.2%), in 1% (w/v) methylcellulose	Test system: Mouse (Hsd:ICR (CD-1)), male, 6/dose, liver and kidney cells examined The following concentrations were tested: 500, 1000 and 2000 mg/kg bw (oral, gavage, dose volume 10 mL/kg) Positive control: methyl methanesulfonate (MMS) 40 mg/kg bw Two doses were administered approx. 21 h apart (positive control dose was administered once on day 2) Animals were euthanized 3-4 h following last treatment 150 cells/animal examined	**Negative** No dose increase observed in % tail DNA Positive control gave the expected results The % tail DNA in the negative control was within the laboratory historical control data	Anonymous; 2018; M-635020-01-1

Table 10-21: Summary table of human data relevant for germ cell mutagenicity

Type of data/report	Test substance	Relevant information about the study (as applicable)	Observations	Reference
No human data				

10.8.1 Short summary and overall relevance of the provided information on germ cell mutagenicity

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*, one HPRT mutation assay in Chinese hamster V79 cells, two *in vivo* micronucleus assays in mouse bone marrow cells with oral administration, one *in vivo* micronucleus assay in mouse bone marrow cells with intraperitoneal administration, one *in vivo* UDS assay in rat hepatocytes by oral route and one *in vivo* Comet assay in male mice with oral gavage administration. Several deviations were reported from the current OECD test guidelines for a number of these studies, specifically, two *in vitro* chromosome abberation tests (OECD 473) and three *in vivo* micronucleus tests (OECD 474); however, the dossier submitter considers that these deviations do not affect the validity or results of the studies.

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 typhymurium* strains and one *Escherichia coli* strain. In addition a recently conducted bacterial reverse mutation assay (2017) was also negative and also confirmed the overall negative outcome in this study type. Furthermore, a Comet assay was also recently (2018) performed to confirm the negative profile for the endpoint gene mutation *in vivo*. In this *in vivo* Comet assay, no statistically significant or dose related increases in % tail DNA were observed in liver or kidney cells of treated male mice (6/dose) up to doses of 2000 mg/kg bw/day. The positive

control gave the expected response and the increase in % tail DNA observed in the vehicle control group was within the range of the laboratory historical control data, thus confirming the validity of the study (a detailed summary of this comet assay is provided in Annex I of this CLH report).

The chromosomal aberration assay performed in Chinese hamster V79 showed a positive response. However, the increase of aberrant cells occured at cytotoxic concentrations where mitotic indices were clearly below the limit of 50% indicating the doubtful biological signifiance 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 polychromatic to normochromatic 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 to increase the likelihood of bone marrow exposure. This assay gave a clear negative result for clastogencity *in vivo* at dose levels showing clear cytotoxicity of the bone marrow.

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.

10.8.2 Comparison with the CLP criteria

In summary, 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 Comet assay and micronucleus tests are reliable and it is clear that no mutagenic effects were seen in whole animals. Clearly, chromosomal damage or point mutations do not occur *in vivo*. Overall, therefore, it is concluded that fluopicolide is not genotoxic *in vivo*. Furthermore, in the carcinogenicity studies (see section 10.9) fluopicolide caused an increase in hepatocellular adenomas in male and female mice by a mechanism considered not relevant to humans, and increased neoplasms were observed only at or above the maximum tolerated dose (MTD).

No information is available on the genotoxicity of fluopicolide in humans. Therefore, it clearly does not meet the criteria for classification in category 1A. Since fluopicolide was negative in *in vivo* tests in mammals and there is no information on its mutagenicity in germ cells, classification in category 1B is not appropriate.

The lack of histopathological findings in the male and reproductive organs would suggest (in the absence of relevant toxicokinetic data) that the potential for fluopicolide or its metabolites to reach and interact with the genetic material of the germ cells is low. Classification for germ cell mutagenicity category 2 may be considered on the basis of positive somatic cell mutagenicity tests *in vivo*, in mammals; or other positive *in vivo* somatic cell genotoxicity tests that are supported by positive results from *in vitro* mutagenicity assays; or positive *in vitro* mammalian mutagenicity assays for substances that also show chemical structure activity relationship to known germ cell mutagens. Since none of these conditions was met, classification in category 2 is not appropriate.

No classification for germ cell mutagenicity is proposed.

10.8.3 Conclusion on classification and labelling for germ cell mutagenicity

Since the results of the conducted guideline genotoxicity studies with fluopicolide did not reveal a genotoxic potential, the criteria for genotoxicity classification are not met. Thus, the data for fluopicolide are conclusive, but they do **not warrant** genotoxicity classification according to the CLP guidance (Guidance to Regulation (EC) No 1272/2008).

10.9 Carcinogenicity

The chronic toxicity and carcinogenic potential of fluopicolide has been investigated in one two-year long-term toxicity/carcinogenicity study in rats and an 18-month carcinogenicity study in mice.

Table 10-22: Summary table of animal studies on carcinogenicity

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference**
Combined carcinogenicity and toxicity study by dietary administration to CD rats for 104 weeks. Crl: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) OECD 453 (04/1981) GLP Coagulating gland, Harderian gland, vagina and bone marrow were not sampled, fixed or examined histopathologically	Fluopicolide (purity 95.9%) 0, 50, 200, 750 or 2,500 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>50 ppm</u> No effects observed <u>≥ 200 ppm</u> No adverse effects <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) ↑ increased incidence and/or severity of centrilobular hepatocyte hypertrophy (M/F), incidence and/or severity of cystic degeneration and foci of alteration (M) and ↑ increased incidence of eosinophilic foci of alteration (F) in the liver ↑ incidence of cortical tubular basophilia and hyperplasia of the papillary epithelium (M/F) ↑ incidence of cystic follicular cell hyperplasia in the thyroids week 104 (M) <u>2,500 ppm</u> ↓ bodyweight gain (M/F) ↓ RBC parameters (M/F) ↑ albumin in blood (M) Most of these changes reversible after a 13-week off-dose period. No evidence of a carcinogenic potential.	Anonymous; 2003; M-225616-01-2 Anonymous; 2005; M-263575-01-1

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
Carcinogenicity study in mice via diet C57BL/6 mice (50/dose/sex) OECD 451 (04/1981) GLP Coagulating glands were not sampled, fixed or examined histopathologically	Fluopicolide (purity 95.9%) 0, 50, 400, or 3,200 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)	50 ppm (7.9/11.5 mg/kg bw/day) No effects observed 400 ppm (64.5/91.9 mg/kg bw/day) ↑ abs. & rel. liver weight week (M/F) ↑ incidence of hepatocellular hypertrophy (M/F) 3,200 ppm (551/772.3 mg/kg bw/day) ↓ 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)	Anonymous; 2003; M-225595-01-1 Anonymous; 2005; M-263591-01-1

M = male F = female

Table 10-23: Summary table of human data on carcinogenicity

Type of data/report	Test substance	Relevant information about the study (as applicable)	Observations	Reference
No human data				

Table 10-24: Summary table of other studies relevant for carcinogenicity

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
28-day explanatory dietary toxicity study in C57Bl/6 female mice to investigate liver cell proliferation and cytochrome P450 induction	Fluopicolide (purity 99.3%) 15 mice for at least 28 days at concentrations of 0 (control) and 3,200 ppm (equivalent to 575 mg/kg bw/day) Satellite subgroups of 20 mice for interim sacrifice after 7 days of treatment	↑ hepatocellular proliferation in C57BL/6 mice after 7 days of treatment, which returned to control levels after 28 days of treatment. ↑ liver weight ↑ increased incidence of hepatocellular hypertrophy ↓ diffuse, mainly centrilobular hepatocellular vacuolation ↑ total cytochrome P-450 and BROD and PROD activities As confirmed by a separate positive control study (Langrand-Lerche, C.; 2004; M-232813-01-1), fluopicolide induced hepatic changes, both histopathological and in terms of enzyme induction activities with a phenobarbital-like profile.	Anonymous; 2004; M-229594-01-1

<table>
<tr><th>Method, guideline, deviations if any, species, strain, sex, no/group</th><th>Test substance, dose levels duration of exposure</th><th>Results</th><th>Reference</th></tr>
<tr><td>In vitro tests with fluopicolide in cultured male and female C57BL/6 Mouse hepatocytes, CarKO/PxrKO Mouse hepatocytes and human hepatocytes</td><td>Fluopicolide (purity 98.2%)

0, 0.03, 0.1, 0.3, 1, 2, 3 and 10 µM</td><td>Overview of key results relevant for the liver tumor MOA:
<table>
<tr><th>Key results</th><th>Wildtype mouse hepatocytes</th><th>CarKO/PxrKO mouse hepatocytes</th><th>Human hepatocytes</th></tr>
<tr><td>CAR activation</td><td>+</td><td>-</td><td>-</td></tr>
<tr><td>PXR activation</td><td>+</td><td>-</td><td>(+)</td></tr>
<tr><td>Liver cell proliferation</td><td>+</td><td>-</td><td>-</td></tr>
</table>
The in vitro studies confirmed a rodent specific CAR/PXR MOA since they demonstrated that significant CAR and PXR activation and hepatocyte proliferation was induced in WT hepatocytes but not in CarKO/PxrKO or human hepatocytes.</td><td>Anonymous; 2017; M-600904-01-1

Anonymous; 2017; M-603455-01-1

Anonymous; 2017; M-600908-01-1

Anonymous; 2017; M-604080-01-1

Anonymous; 2017; M-600911-01-1

Anonymous; 2017; M-604094-01-1</td></tr>
</table>

10.9.1 Chronic/carcinogenicity study in rats

Fluopicolide was administered to Sprague Dawley rats in the diet at concentrations of 0, 50, 200, 750 or 2,500 ppm for 2 years. After 1-year treatment period, 20 animals/sex/group were killed 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 a marked effect on body weight gain in the first week of treatment in animals receiving 2,500 ppm and in females receiving 200 or 750 ppm. Subsequent weight gain by animals receiving 2,500 ppm tended to be lower than that of the controls, though the difference was less than was seen during the first week of treatment. For males and females given 2,500 ppm, the overall weight gain was 5 and 25% lower than controls, respectively, at the end of Week 52 in the Toxicity phase, and 11 and 17% lower than controls at the end of the Carcinogenicity phase. The subsequent body weight gain of females receiving 200 or 750 ppm was similar to that of the controls. 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 hepatocytic hypertrophy and 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 2,500 ppm there were degenerative and proliferative changes, comprising cortical tubular basophilia at an increased severity 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 2,500 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 200 ppm; at this dose a slight increase in hepatocellular hypertrophy in males was the only non-neoplastic finding (an adaptive response secondary to

liver enzyme induction), whilst the only neoplastic finding was a decrease in mammary and/or adrenal masses in females (which is not toxicologically relevant). There were no findings at 50 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.

Therefore, the NOAEL for toxicity was 200 ppm in both males and females, (equivalent to 8.4 and 10.8 mg/kg bw/day, in males and females, respectively). Furthermore, there was no evidence of carcinogenicity with fluopicolide up to and including the dose level of 2,500 ppm (equivalent to 109.4 and 142.2 mg/kg bw/day, in males and females, respectively).

10.9.2 Chronic/carcinogenicity study in mice

In a mouse oncogencity study, fluopicolide was administered to C57BL/6 mice in the diet at concentrations of 0 (control), 50, 400 and 3,200 ppm for 78 weeks. After 52-week treatment period, 10 animals/sex/group were killed for assessment of chronic toxicity. After 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 3,200 ppm indicating that the Maximal 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 3,200 ppm at 52 and 78 weeks. These changes were associated with hepatocellular hypertrophy at 52 and 78 weeks, and higher incidence of altered cell foci at 3,200 ppm at 78 weeks.

Generally, the number of animals with neoplasms, the number of animals with more than one primary neoplasm and the number of animals with benign and malignant tumors were comparatively similar in all groups. A significant increased incidence of hepatocellular adenoma was observed at 3,200 ppm at 78 weeks in both males (22% vs. 10% in control)) and females (32% vs. 2% in control) and at 52 weeks in females (30% vs. 0% in control). In addition, the time of onset of the hepatocellular neoplasm was shorter in the treated females when compared with controls. However, no increased incidence of hepatocellular carcinoma were observed in any of the groups after the 78-week treatment period. An overview about relevant liver findings is given in the following tables.

Liver findings in the mouse oncogenicity study at 52 weeks

Parameter	Control data		Low dose 50 ppm		Mid dose 400 ppm		High dose 3,200 ppm	
	m	**f**	**m**	**f**	**m**	**f**	**m**	**f**
Final bodyweight (g) and (% control)	37.66	36.23	37.90 (+1%)	34.14 (-6%)	42.56 (+13%)	34.03 (-6%)	**31.25* (-17%)**	**26.58** (-27%)**
Liver: organ weight, relative (g/100g) and absolute (g)	1.59 4.21	1.51 4.21	1.71 4.53	1.44 4.20	**2.06**** **4.84****	1.57 4.61	**2.15**** **6.85****	**2.26**** **8.39****
Non-neoplastic changes:								
Hepatocellular hypertrophy	0/10	0/10	0/10	0/10	**5/10**	**6/10**	**10/10**	**9/10**
Altered cell foci	0/10	0/10	0/10	0/10	0/10	0/10	0/10	**2/10**
Coagulative hepatocyte necrosis	1/10	1/10	0/10	0/10	1/10	0/10	0/10	0/10
Microfoci of necrosis	4/10	3/10	1/10	2/10	2/10	1/10	1/10	1/10
Chronic inflammation	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10
Neoplastic changes:								
Hepatocellular adenoma	0/10 (0)	0/10 (0)	0/10 (0)	0/10 (0)	0/10 (0)	1/10 (10)	0/10 (0)	**3/10 (30)**
Hepatocellular carcinoma	0/10 (0)	0/10 (0)	0/10 (0)	0/10 (0)	0/10 (0)	0/10 (0)	0/10 (0)	0/10 (0)

* p<0.05, ** p<0.01
m: males; f: females
Findings considered related to treatment with fluopicolide are written in **bold**.

Liver findings in the mouse oncogenicity study at 78 weeks

Parameter	Control data		Low dose 50 ppm		Mid dose 400 ppm		High dose 3,200 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**.

Therefore, an increased number of benign liver tumours occured only at the highest dose reaching the MTD (severe body weight gain reduction in high dose animals) suggesting a threshold mechanism (see also Annex II). 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 for the increased incidence of liver adenomas was found to be CAR-mediated and thus secondary to liver enzyme induction like that of phenobarbital (see below). This MoA is considered of no relevance in humans.

Therefore, the NOAELs of the study are 50 ppm for toxicity (equivalent to 7.9 and 11.5 mg/kg bw/day, in males and females, respectively) and 400 ppm for carcinogenicity (equivalent to 64.5 and 91.9 mg/kg bw/day, in males and females, respectively).

10.9.3 Other studies relevant for carcinogenicity

There are four additional mechanistic studies addressing the observed mouse liver oncogenicity. The first one 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 isoenzymes activity. Furthermore three *in vitro* studies in different hepatocyte cultures (mouse wild type, mouse CarKO/PxrKO and human) were performed.

10.9.3.1 28-day mechanistic study in mice

In an *in vivo* study in mice, fluopicolide (AE C638206) was administered continuously via the diet to a group of 15 female C57BL/6 mice for at least 28 days at concentrations of 0 (control) and the dose at which liver tumors occurred in the carcinogencity study, i.e. 3,200 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 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 P-450 isoenzymes were assessed.

At 3,200 ppm, there were no mortalities or clinical signs during the course of the study. There was a body weight loss (-2.1 g) between Days 1-7. The mean body weight gain was thereafter transiently higher and then again lower than controls between Days 15-28 resulting in a mean body weight reduction throughout treatment (-6 to -9%). Mean food consumption was also lower than controls between Days 1-7 (-25%).

At interim sacrifice, mean terminal body weight was statistically significantly lower (-7%) when compared to controls. 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 labeling index was approx. 6.5-fold higher in treated animals, when compared to controls, indicative of a marked hepatocellular proliferation in the liver.

At final sacrifice, mean terminal body weight was not affected. 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 a marked increase in total cytochrome P-450 content (+97%) as well as in BROD (+1785%) and PROD (+1143%) activities. EROD activity was only slightly induced and lauric acid hydroxylation decreased compared to control mean as shown in the following table.

Total cytochrome P-450 content and enzymatic activities at interim sacrifice

Parameter	**Fluopicolide at 3,200 ppm** % change compared to control mean
P450	+ 97 %
BROD	+ 1785 %
EROD	+ 79 %
PROD	+ 1143 %
Lauric acid	- 67 %

In conclusion, fluopicolide at 3,200 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. In addition, fluopicolide is a marked total cytochrome P-450 and BROD and PROD activities inducer. As confirmed by a separate positive control study (Anonymous.; 2004; M-232813-01-1), fluopicolide induced hepatic changes, both histopathological and in terms of enzyme induction activities with a phenobarbital-like profile.

10.9.3.2 Mechanistic studies in hepatocytes

This MoA is further supported by *in vitro* studies in hepatocytes from wildtype mice and CAR/PXR-knockout mice, since under the assumption of a CAR/PXR-mediated liver tumor MoA, a proliferative effect in mice which are lacking CAR/PXR receptors should not occur.

The goal of these *in vitro* studies was to investigate the potential of fluopicolide to stimulate cell proliferation (measured as the change in replicative DNA synthesis during S-phase of the cell cycle) and modulate cytochrome P450 (Cyp) enzyme activities in isolated male and female C57BL/6 mouse hepatocyte cultures in comparison to isolated male and female constitutive androstane receptor knockout/pregnane x receptor knockout (CarKO/PxrKO) mouse hepatocyte cultures and to cryopreserved male and female human hepatocyte cultures from three independent donors (Anonymous.; 2017; M-603455-01-1 / Anonymous.; 2017; M-604080-01-1 / Anonymous.; 2017; M-604094-01-1).

In these studies, cytotoxicity was evaluated by adenosine 5'-triphosphate (ATP) 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.

Mechanistic study in wildtype C57BL/6 male and female mouse hepatocytes

Fluopicolide administration 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 µM (1.7-fold in male hepatocytes and 2.3-fold in female hepatocytes). PB 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 (25 ng/mL).

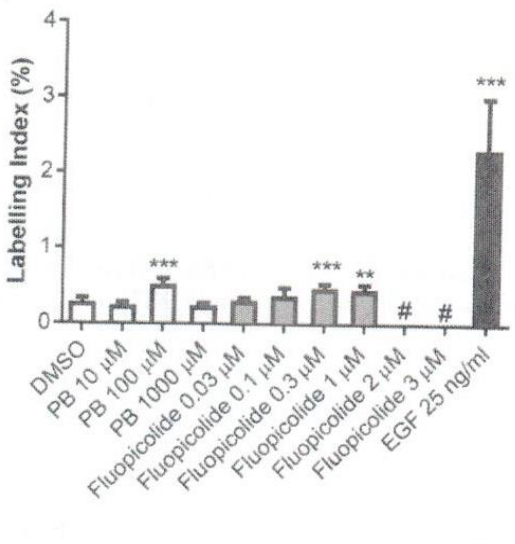

Figure 10- 1: Effect of fluopicolide, PB or EGF on replicative DNA synthesis (S-phase) in male C57BL/6 mouse primary hepatocytes

Effect of Fluopicolide, PB or EGF on replicative DNA synthesis (S-phase) in female C57BL/6 mouse primary hepatocytes

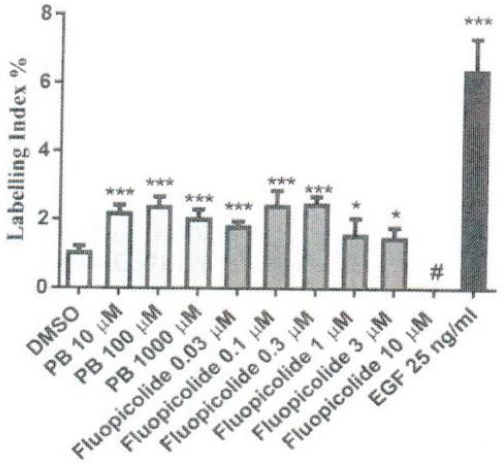

Figure 10- 2: Figure 10- 1: Effect of fluopicolide, PB or EGF on replicative DNA synthesis (S-phase) in female C57BL/6 mouse primary hepatocytes

Hepatic pentoxyresorufin-O-dependentylation (PROD), benzyloxyresorufin O-debenzylase (BROD) and benzoxyquinoline-O-debenzylation (BQ) rates are indicative of Cyp2b and Cyp3a induction. In male C57BL/6 mouse hepatocytes, fluopicolide caused a dose dependant 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 C57BL/6 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 (6.5-fold), BROD (4.9-fold) and BQ (7.7-fold) activities in the male mouse hepatocytes. PB (1 mM) also caused significant increases in PROD, BROD and BQ activities in the female mouse hepatocytes, increasing activities by 2.6-, 1.6- and 4.4-fold, respectively. Therefore, treatment with the positive control items PB and EGF gave the expected set of responses, indicating the suitability of the test system.

In conclusion, fluopicolide induced both hepatocellular S-phase replicative DNA synthesis and Cyp2b enzyme activity in both male and female C57BL/6 mouse primary hepatocyte cultures. These data suggest that fluopicolide activated the nuclear hormone receptor constitutive androstane receptor (CAR) in male and female C57BL/6 mouse hepatocytes.

Mechanistic study in male and female CarKO/PxrKO Mouse hepatocytes

Treatment with fluopicolide or PB did not induce replicative DNA synthesis in male or female CarKO/PxrKO hepatocytes at any concentration but the proliferative capability of these cells in culture was confirmed using EGF (25 ng/mL).

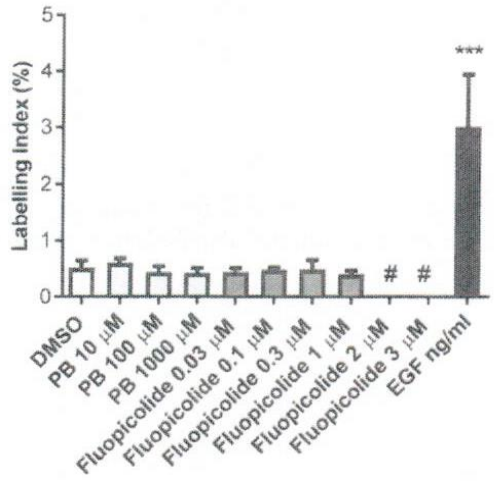

Figure 10- 3: Effect of fluopicolide, PB or EGF on replicative DNA synthesis (S-phase) in male CarKO/PxrKO mouse primary hepatocytes

Effect of Fluopicolide, PB or EGF on replicative DNA synthesis (S-phase) in female *Car*KO/*Pxr*KO mouse primary hepatocytes

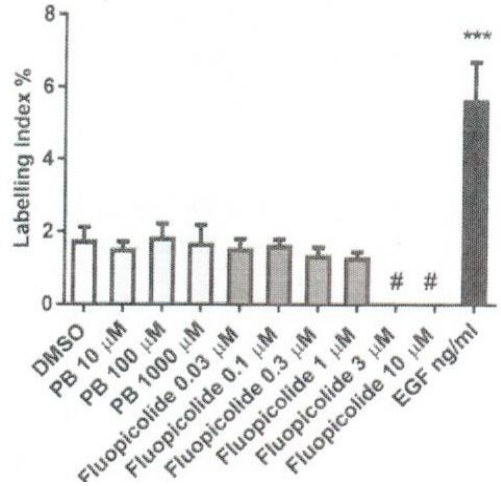

Figure 10- 4: Effect of fluopicolide, PB or EGF on replicative DNA synthesis (S-phase) in female CarKO/PxrKO mouse primary hepatocytes

Similarily, fluopicolide did not cause any increases in PROD, BROD or BQ in male or female CarKO/PxrKO mouse hepatocytes. PB administration (1000 µM only) 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 CarKO/PxrKO 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.

Mechanistic study in human hepatocytes from three Individual Donors

Neither administration with fluopicolide nor PB induced replicative DNA synthesis in cultured male or female human hepatocytes. However, the proliferative capability of these cells in culture was confirmed using EGF (25 ng/mL).

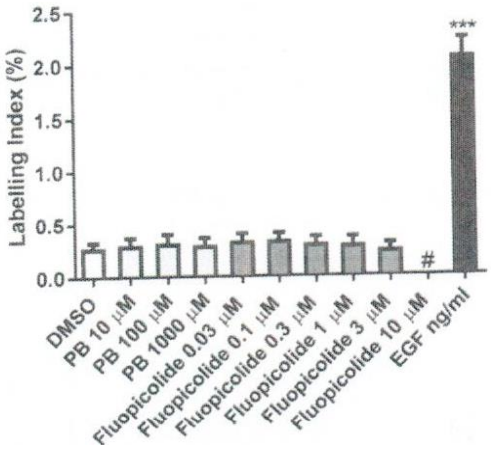

Figure 10- 5: Effect of fluopicolide, PB or EGF on replicative DNA synthesis (S-Phase) in male human hepatocytes, donor 8210

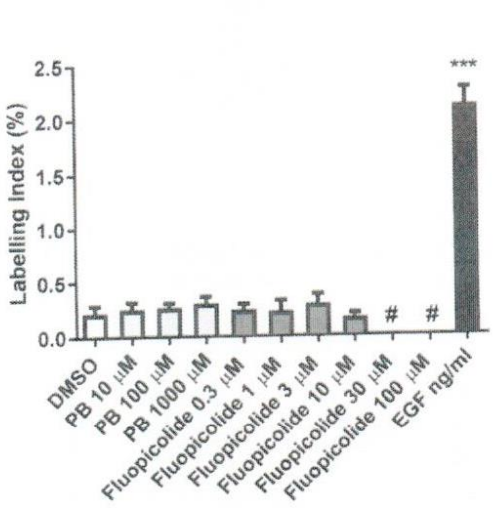

Figure 10- 6: Effect of fluopicolide, PB or EGF on replicative DNA synthesis (S-Phase) in female human hepatocytes, donor 8239

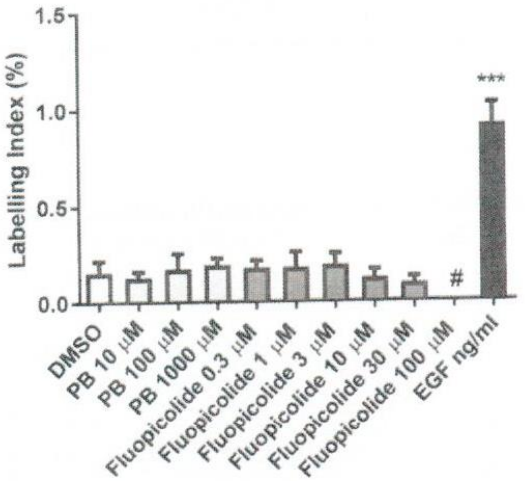

Figure 10- 7: Effect of fluopicolide, PB or EGF on replicative DNA synthesis (S-Phase) in female human hepatocytes, donor 1765

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 Donor 8210.

Treatment with fluopicolide resulted in dose-dependent increases in BQ activity in female hepatocytes from both donors to a maximum of 1.7- and. 2.8-fold induction compared to control. 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 activites in both donors, however, only donor 1765 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) which in humans is normally involved in xenobiotic metabolism with no effect on DNA-synthesis in male or female human hepatocytes.

Most importantly, in addition to the CarKO/PxrKO Mouse hepatocyte study, this study in human hepatocytes strongly supports human non-relevance of this CAR/PXR-mediated liver tumor MoA.

The following table summarizes the overall results regarding CAR/PCR activation and liver cell proliferation in wildtype mouse, CarKO/PxrKO Mouse and human hepatocytes:

Overview of key effects relevant for the liver tumor MOA

Key events	Wildtype mouse hepatocytes	CarKO/PxrKO mouse hepatocytes	Human hepatocytes
CAR activation	+	-	-
PXR activation	+	-	(+)
Liver cell proliferation	+	-	-

This overview makes clear that the key event of CAR/PXR activation which lead to liver cell proliferation and eventually to liver tumors in rodents, is not relevant to humans in which the important key events, CAR induction and liver cell proliferation do not occur after exposure to fluopicolide.

10.9.3.3 Proposed mode of action (MOA) overview

An evaluation of the mode of action (MOA) of the liver tumor induction according to the IPCS/human Framework method is provided in Annex II to this document. The main conclusions are summarized in the following.The weight of evidence indicates a hypothesized MOA via the constitutive androstane receptor and/or pregnane X receptor (CAR/PXR) activation leading to the observed liver tumours in mice. The key events involved are summarized in the following table.

Listing of key events and associative events for a CAR-mediated liver tumour MOA

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

(From Elcombe et al, Crit Rev Toxicol. 2014;44(1): 64-82)

MOA studies demonstrated these key events for fluopicolide. Fluopicolide induced BROD and PROD activities in the conducted mechanistic 28-day in vivo mouse study (Langrand-Lerche, C.; 2004; M-229594-01-1) which indicates that CAR/PXR receptors were activated by fluopicolide with subsequently increased protein expression/enzyme activity of CYP2B and CYP3A (KE 1, 2 and AE 1). Moreover, a marked transient hepatocellular proliferation (KE 3) were demonstrated at 7-day interim sacrifice. At terminal sacrifice significantly increased absolute and relative weights and a diffuse, perilobular to panlobular hepatocellular hypertrophy was seen in all treated animals (AE 2). Moreover, the PCNA assessment on liver tissue from animals at 3200 ppm in the subchronic mouse study (Wason, S. M.; 2006; M-205579-02-1) showed that fluopicolide did not produce hepatocellular proliferation on Day 90. This is completely consistent with the lack of cell proliferation observed on Day 28 with the BrdU assessment. These findings emphasize that a transient liver cell proliferation followed by a return to control levels is a prerequisite for the development of hepatocellular adenoma following a long term exposure period to fluopicolide.

Hepatocellular hypertrophy (AE 2), increased liver weights (AE 3), elevated incidences of altered foci (KE 4) and eventually liver adenomas (KE 5) were seen in the standard subchronic and chronic mouse studies with fluopicolide (Anonymous.; 2006; M-205579-02-1, Anonymous.; 2000; M-197623-01-1, Anonymous.; 2003; M-225595-01-1).Therefore, all key and associative events for a CAR-mediated liver tumour MOA were observed after fluopicolide treatment *in vivo*.

These rodent-specific findings are very similar to that demonstrated for phenobarbital which causes liver tumours in rodents. In a 28-day study in the same strain of mice (Anonymous.; 2004; M-232813-01-1) phenobarbital at 80 mg/kg/day induced a marked hepatocellular proliferation in male and female C57BL/6 mice after 7 days of treatment, which remained statistically significant but slight in males and returned to control levels in females after 28 days of treatment. In addition, phenobarbital was found to be a strong inducer of hepatocellular hypertrophy and of total cytochrome P-450 and BROD and PROD activities. Clofibric acid at 300 mg/kg/day induced a marked hepatocellular proliferation in male and female C57BL/6 mice after 7 days of treatment, which returned to control levels after 28 days of treatment. In addition, clofibric acid was found to be a strong inducer of hepatocellular hypertrophy and of lauric acid hydroxylation activities. That this transient effect on hepatocellular cell proliferation is the key event for non-genotoxic induction of hepatocellular carcinogenesis by xenobiotic compounds is supported by widely published work (e.g. Schulte-Hermann, R. (1974)[1]; Schulte-Hermann, R. (1979)[2]; Hildebrand, B. et al. (1991)[3]). However, despite the long history of phenobarbital use in human medicine no evidence of an increased carcinogenic risk for humans was seen. Therefore, also for fluopicolide (AE C638206) which caused similar tumors via CAR and PXR human relevance can be excluded.

In addition to the *in vivo* MOA study also *in vitro* hepatocyte studies were conducted with exposure of wild-type (WT) mice (Chatham, L.; 2017; M-603455-01-1), CAR/PXR-knockout (CarKO/PxrKO) mice (Chatham, L.; 2017; M-604080-01-1) and human (Chatham, L.; 2017; M-604094-01-1) hepatocyte cultures to fluopicolide. These studies confirmed the CAR/PXR MOA since they demonstrated that hepatocyte proliferation was induced in WT mice hepatocytes but not in CarKO/PxrKO mice hepatocytes. Human hepatocytes did not show a proliferation or significant CAR(PXR activation which clearly confirms that human hepatocytes are not sensitive to this liver tumour MOA and thus that this MOA is not relevant to humans.

[1] Schulte-Hermann R. Induction of liver growth by xenobiotic compounds and other stimuli. CRC Crit Rev Toxicol. 1974 Sep;3(1):97-158.

[2] Schulte-Hermann R. Adaptive liver growth induced by xenobiotic compounds: its nature and mechanism. Arch Toxicol Suppl. 1979;(2):113-24. Review.

[3] Hildebrand B, Grasso P, Ashby J, Chamberlain M, Jung R, van Kolfschoten A, Loeser E, Smith E, Bontinck WJ. Validity of considering that early changes may act as indicators for non-genotoxic carcinogenesis. Mutat Res. 1991 Jun;248(2):217-20. Review

Other MOAs can be excluded based on the available results. Thus, a genotoxic MOA can be excluded based on the results of the genotoxicity studies which did not indicate a genotoxic potential. Furthermore, the results of the 28-day mechanistic study in mice (Anonymous.; 2004; M-232813-01-1) show that Arylhydrocarbon receptor- or PPARα-mediated effects and thus such MOAs can be excluded since no relevant effects on EROD or Lauric acid, respectively were noted.

Also oxidative stress and severe liver cytotoxicity as a mode of action for the liver tumours can be excluded, since in the repated dose toxicity studies with fluopicolide even at the highest doses, no signs of severe cytotoxicity in the liver, like inflammatory signs, broad hepatic necrosis, hepatocellular death, fibrosis, cirrhosis or severely increased transaminase activities were observed.

Thus, it can be summarized that the investigations demonstrated a non-genotoxic rodent-specific CAR/PXR mediated MOA for the liver tumours after fluopicolide treatment which has a clear threshold for the underlying key events and the final liver tumour induction (see also Annex II: Evaluation of the proposed MOA of fluopicolide according to the IPCS/human Framework method). This MOA is the same that has been demonstrated for phenobarbital and many other non-genotoxic compounds which caused liver tumours in rodents. This MOA has no relevance to humans which was shown in the mechanistic *in vitro* hepatocyte studies and supported by the fact that despite the long history of phenobarbital use in human medicine no evidence of an increased carcinogenic risk for humans was seen.

10.9.4 Comparison with the CLP criteria

According to the CLP guidance (Guidance to Regulation (EC) No 1272/2008 on classification, labelling and packaging (CLP) of substances and mixtures Version 5.0 July 2017), category 1A (known to have carcinogenic potential for humans) classification is largely based on human evidence, or Category 1B (presumed to have carcinogenic potential for humans) classification is largely based on animal evidence. The classification in Category 1A and 1B is based on strength of evidence together with additional considerations. Such evidence may be derived from human studies that establish a causal relationship between human exposure to a substance and the development of cancer (known human carcinogen); or animal experiments for which there is sufficient evidence to demonstrate animal carcinogenicity (presumed human carcinogen). In addition, on a case-by-case basis, scientific judgement may warrant a decision of presumed human carcinogenicity derived from studies showing limited evidence of carcinogenicity in humans together with limited evidence of carcinogenicity in experimental animals.

The placing of a substance in Category 2 is done on the basis of evidence obtained from human and/or animal studies, but which is not sufficiently convincing to place the substance in Category 1A or 1B, based on strength of evidence together with additional considerations. Such evidence may be derived either from limited evidence of carcinogenicity in human studies or from limited evidence of carcinogenicity in animal studies.

No human studies or epidemiological publications regarding effects on humans are known, which could trigger classification of fluopicolide.

With regard to animals studies, it can be summarized that the dietary administration of fluopicolide produced higher incidence of hepatocellular adenoma only at very high doses reaching MTD in male and female mice following chronic treatment (Anonymous.; 2003; M-225595-01-1). Given that these hepatocellular adenoma were not observed at lower dose levels in mice, not observed in rats following a 2-year treatment period and taken into account the lack of genotoxicity potential of fluopicolide, the higher incidence of hepatocellular adenoma was thus considered to be subsequent to a threshold mechanism with a Phenobarbital-like mechanism of action (hepatocellular hypertrophy and transient cell proliferation) which is a well-known mechanism of action specific to the mouse and of no relevance to humans. This is also confirmed by *in vivo* and *in vitro* mode of action studies. Other MOAs can be excluded based on the available study and literature results.

Based on the MOA of a CAR/PXR-mediated effect together with the high dose reaching the MTD at which liver adenomas were seen, the liver adenomas in mice are not regarded as relevant to humans.

10.9.5 Conclusion on classification and labelling for carcinogenicity

No carcinogenic potential in humans is known from epidemiological literature due to an absence of any publications in this regard. Also no carcinogenic potential of fluopicolide was evident from the rat carcinogenicity study.

The liver adenomas at the highest dose in the mouse oncogencity study were caused by a rodent-specific liver enzyme induction via a CAR/PXR receptor-mediated MOA, like that of phenobarbital which is known to be non-relevant to humans.

Therefore, the data are conclusive but do **not warrant** a carcinogenicity classification according to the CLP guidance (Guidance to Regulation (EC) No 1272/2008 on classification, labelling and packaging (CLP) of substances and mixtures Version 5.0 July 2017).

10.10 Reproductive toxicity

10.10.1Adverse effects on sexual function and fertility

Table 10-25: Summary table of animal studies on adverse effects on sexual function and fertility

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
Preliminary study on reproductive per-formance in by dietary administration to CD rats 8 male and 8 female rats/group OECD 451 (1981) GLP	Fluopicolide (purity 95.9%) Oral administration via diet at 0, 50, 200, 750 or 2,500 ppm (equivalent to 0, 5.2/6.4, 25.5/32.9 and 103.4/127.3 mg/kg bw/day for M/F premating) Treatment started 15 days prior to pairing and continued uninterrupted until termination after weaning of the resulting litters.	<u>50 and 200 ppm</u> No effects <u>≥ 750 ppm</u> ↓ bodyweight gain and food consumption in parental females <u>2,500 ppm</u> ↓ bodyweight gain parental males and offspring Reproductive parameters were considered to be unaffected by treatment with fluopicolide up to and including the highest tested dose of 2,500 ppm.	Anonymous.; 2002; M-215068-01-1

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
Two generation reproduction study in rats. Crl: CD® (SD) IGS BR rats 28 male and 28 female rats/group OECD 416, Draft (1999) GLP	Fluopicolide (purity 95.9%) Oral administration via diet at concentrations of 0, 100, 500 or 2,000 ppm throughout the two generations Equivalent to: F0: 0, 5.2, 25.5 & 103.4 mg/kg bw/day in males and 0, 6.4, 32.9 & 127.3 mg/kg bw/day in females during premating F1: 0, 5.7, 28.3 & 117.1 mg/kg bw/day in males and 0, 6.8, 34.6 & 141.6 mg/kg bw/day in females during premating	<u>100 and 500 ppm</u> No effects <u>2,000 ppm</u> *Parental:* ↓ bodyweight gain and food consumption (M/F) ↑ liver and kidney weights (M/F) ↓ spleen weights (F) *Offspring:* ↓ bodyweight gain Reproductive parameters and sexual maturation were considered to be unaffected by treatment with fluopicolide up to and including the highest tested dose of 2000 ppm.	Anonymous; 2003; M-232532-01-1
Additional microscopic examination to the 2-generation reproduct-ion study in rats	Fluopicolide (purity 95.9%) Oral administration via diet at concentrations of 0, 100, 500 or 2,000 ppm throughout the two generations.	<u>100 and 500 ppm</u> No adverse effects <u>2,000 ppm</u> *Parental:* ↑ incidence of centrilobular hepatocyte hypertrophy ↑ incidence of degenerative and regenerative changes in kidneys (M/F)	Anonymous; 2004; M-247289-01-1
Supplementary information: External expert statement regarding fluopicolides reproductive and developmental toxicity potentials	-	This document contains a summary and review of the two-generation and the developmental toxicity studies with rats and rabbits by an external expert for reproductive toxicity concluding that on the basis of the available studies, fluopicolide should not be classified as a reproductive toxicant.	Anonymous; 2018; M-638869-01-1

M = male F = female

Table 10-26: Summary table of human data on adverse effects on sexual function and fertility

Type of data/report	Test substance	Relevant information about the study (as applicable)	Observations	Reference
No human data				

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

The data of a preliminary dose range finding and main 2-generation study in rats are available.

In a **preliminary study** treatment levels for a two-generation study in **rats** were examined. Fluopicolide was administered orally, via the diet, at concentration levels of 0, 50, 200, 750 or 2,500 ppm to groups of 8 males and 8 females, 15 days prior to pairing until termination after weaning of the resulting litters. Selected offspring (constituting an F1 generation) continued to receive the diets from about the time of weaning until termination following attainment of sexual maturation.

This preliminary study showed that dietary concentrations of 2,500 ppm of fluopicolide induced general toxicity observed as bodyweight gain reductions in F0 males during the premating period, and in F0 females during the gestation and lactation period. A transient effect in F0 females was also observed during the gestation period at 750 ppm. F1 offspring body weight gains were also reduced at 2,500 ppm. Reproductive parameters were considered to be unaffected by treatment with fluopicolide up to and including the highest tested dose of 2,500 ppm. Therefore, a dose level of maximum up to 2,500 ppm was regarded as suitable as the high concentration in a main study of reproductive performance.

In the main **2-generation study in rats** the influence of fluopicolide on the fertility and reproductive performance of two successive generations was assessed in male and female rats of the Crl: CD® (SD) IGS BR strain. Fluopicolide was administered continuously in the diet at concentrations of 0, 100, 500 or 2,000 ppm to groups of rats throughout the two generations. The F0 generation, which comprised 28 males and 28 females in each group, received the treated diet for 10 weeks before pairing and throughout mating, gestation, littering and lactation. Offspring survival and growth to weaning were evaluated at which point 24 male and 24 female offspring per group were selected to form the F1 generation.

Parental toxicity

Body weight gain and food consumption were low for adult animals treated at 2,000 ppm throughout the study, with the exception of the low body weight gain which was not apparent in the females following parturition.

At 2,000 ppm, kidney and liver weights were high for parental males and females in both generations, when compared with the controls and a retrospective histopathological examination showed treatment-related findings in both organs (centrilobular hepatocyte hypertrophy and degenerative and regenerative changes in kidneys) at this dose level. Group mean body weight-relative liver weights were also slightly higher for females treated at 500 ppm, when compared with the controls and centrilobular hepatocyte hypertrophy was also present in in males at 500 ppm from both generations. Since these findings are common in the livers of rodents which have been administered xenobiotics, they are considered to be an adaptive change and not a toxic effect of treatment at this dose level.

Reproductive parameters

Oestrous cycles, mating performance, fertility and fecundity were similar in all groups. Gestation length, parturition process and sperm parameters were unaffected by treatment. The return of females to oestrous cycling following lactation was not influenced by treatment in either generation.

Offspring toxicity

Litter parameters at birth of the F1 and F2 progeny and their survival to weaning showed no detrimental effects of treatment. Although initial group mean body weight values were similar in all groups, both male and female offspring at 2,000 ppm displayed a similar pattern of significantly reduced body weight from day 14 through to weaning, coinciding with the time when the offspring start to eat the diet, suggesting a palatability effect and/or systemic toxicity due to direct consumption of the test diet and not a lactational effect. Sexual maturation, as assessed by the age and bodyweight at the time of attainment of vaginal opening or balano-preputial separation, was also not affected by treatment with fluopicolide at doses up to and including 2,000 ppm.

In conclusion, a dietary concentration of fluopicolide at 500 ppm should be considered as the No Observed Adverse Effect Level (NOAEL). The minimum mean achieved dosages for the F0 animals at this NOAEL (500 ppm) are 25.5 mg/kg bw/day for the males and 32.9 mg/kg bw/day for the females. The No Observed Effect Level (NOEL) for developing offspring is considered to be 500 ppm, equivalent to a minimum mean achieved dosage of 35.8 mg/kg bw/day for F0 females during gestation and lactation based on the decreased bodyweight gain at 2,000 ppm. Since reproductive parameters were considered to be unaffected the NOEL for reproductive parameters is considered to be the highest tested dose of 2000 ppm, equivalent to a mean achieved dosage of at least 103.4 mg/kg bw/day for F0 males and at least 127.3 mg/kg bw/day for F0 females before pairing. Reproductive parameters and sexual maturation were considered to be unaffected.

10.10.3 Comparison with the CLP criteria

According to the CLP criteria, reproductive toxicity includes adverse effects on sexual function and fertility in adult males and females, as well as developmental toxicity in the offspring. The definitions presented in the CLP criteria are adapted from those agreed as working definitions in IPCS/EHC Document N°225, Principles for Evaluating Health Risks to Reproduction Associated with Exposure to Chemicals. In this classification system, reproductive toxicity is subdivided under two main headings:

- Adverse effects on sexual function and fertility;

- Adverse effects on development of the offspring.

According to the CLP criteria, a ‘Known or presumed human reproductive toxicant’ is a substance known to have produced an adverse effect on sexual function and fertility, or on development in humans or when there is evidence from animal studies, possibly supplemented with other information, to provide a strong presumption that the substance has the capacity to interfere with reproduction in humans. Such a substance is classified as Category 1A (evidence for classification is primarily from human data) or Category 1B (evidence for classification is primarily from animal data).

According to the CLP criteria, a ‘Suspected human reproductive toxicant’ or reprotoxicant Category 2 is a substance for which is some evidence from humans or experimental animals, possibly supplemented with other information, of an adverse effect on sexual function and fertility, or on development, and where the evidence is not sufficiently convincing to place the substance in Category 1. If deficiencies in the study make the quality of evidence less convincing, Category 2 could be the more appropriate classification. Such effects shall have been observed in the absence of other toxic effects, or if occurring together with other toxic effects the adverse effect on reproduction is considered not to be a secondary non-specific consequence of the other toxic effects.

As described before, the rat dietary multigeneration reproductive toxicity study, including the dose range finding study with oral administration of fluopicolide (AE C638206) via diet at concentrations of up to 2,000 and 2,500 ppm did not reveal any effects on reproductive parameters, like fertility, oestrous cycling, spermatogenic function and capacity, mating, gestation or partiurition. Moreover, litter parameters at birth of the F1 and F2 progeny (litter size, sex ratios, neonatal toxicity), their survival to weaning and their sexual maturation (balano-preputial separation, vaginal opening) showed no adverse effects of treatment. The only offspring effect was a reduced bodyweight gain in both male and female offspring at 2,000 and 2,500 ppm from day 14 through to weaning which is considered due to a palatability effect and/or systemic toxicity due to direct consumption of the test diet.

With reference to the CLP criteria (Regulation (EC) No 1272/2008 on classification, labelling and packaging (CLP) of substances and mixtures) there are no results from this study of reproduction and fertility in the rat that determine a need for classification i.e. there are no adverse effects on sexual function and fertility and no adverse effect on prenatal or postnatal development of the offspring, in the presence of parental toxicity. Lower body weight from postnatal day 14, in the F1 offspring at 2000 ppm, was not a developmental effect but a palatability effect and/or systemic toxicity resulting from direct consumption of the diet. Fluopicolide is not a reproductive toxicant.

10.10.4 Adverse effects on development

Table 10-27: Summary table of animal studies on adverse effects on development

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels, duration of exposure**	**Results (% difference refers to difference from concurrent control unless otherwise stated)**	**Reference**
Rat oral gavage range finding develop-mental toxicity study Sprague Dawley rats 4 inseminated female rats/group OECD 414 (1981) GLP	Fluopicolide (purity 97.6%) 500 or 1,000 mg/kg bw from Day 7-20 of pregnancy. No statistical analysis was performed in this range-finding study	<u>Maternal toxicity:</u> <u>There were no deaths at any dose</u> ***1000 mg/kg bw/day*** Clinical signs: pultaceous faeces in 2 females (Days 10-13 of gestation) ↓ food consumption on Days 7-10 (-16% compared with Days 4-7) & lower than at 500 mg/kg bw/day throughout. ↓ bodyweight gain on Days 0-21 (-30% compared with 500 mg/kg bw/day dose-group) ***500 mg/kg bw/day*** ↓ food consumption on Days 7-10 (-7% compared with Days 4-7) <u>Developmental toxicity</u> ***1000 mg/kg bw/day*** ↑ Incidence of post-implantation loss 31.7% (total litter loss in 1 female and 75% resorptions in another) ↓ fetal weight (-24% compared with main study control) ↓ crown-rump length (-10% compared with main study control) ***500 mg/kg bw/day*** ↓ fetal weight (-16% compared with main study control) ↓ crown-rump length (-6% compared with main study control) Based on the results of this study, the high dose in the main study should be between 500 and 1,000 mg/kg bw/day.	Anonymous; 2000; M-198488-01-1

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels, duration of exposure**	**Results (% difference refers to difference from concurrent control unless otherwise stated)**	**Reference**
Rat oral gavage developmental toxicity study Sprague Dawley rats 23 dams/group OECD 414 (1981) GLP dose levels were not set in two- to four-fold intervals thyroid-related parameters were not assessed & anogenital distance of all live rodent foetuses was not measured as required in the newest guideline version	Fluopicolide (purity 97.6%, 97.8%) 0, 5, 60 or 700 mg/kg bw from Day 7-20 of pregnancy	Maternal toxicity: There were no deaths or clinical signs of toxicity ***700 mg/kg bw/day*** ↓ body weight gain (-12%* on Days 1-21; maximum -24%* on Days 7-10) ↓ food consumption (-3% on Days 1-21) ***60 mg/kg bw/day*** No effects ***5 mg/kg bw/d*** No effects Developmental toxicity: ***700 mg/kg bw/day*** ↓ mean foetal weight (-8%*) ↓ crown-rump length (-4%*) ↓ ossification (foetus/litter incidence %): caudal vertebrae (82.4/100*), sternebrae (71.8/100*) & forepaw (71.8/100*), hindpaw metatarsal (7/28.6[1]) & hindpaw toe (5.6/23.8[1]) ↑ incidence of minor defects (foetus/litter %): thoratic vertebrae centra (7/28.6*), thoratic vertebral arches (2.8/14.3[1]), sternebrae (2.1/14.3[1]) , various minor rib defects (4.2/14.3[1], 3.5/9.5[1] and 3.5/14.3[1]) ***60 mg/kg bw/day*** ↓ ossification (foetus/litter incidence %): caudal vertebrae (24.2/72.7*), sternebrae (20.9/63.6*) & forepaw (19/63.6*) ***5 mg/kg bw/day*** ↓ ossification (foetus/litter incidence %): caudal vertebrae (34/81*), sternebrae (20.7/47.6*) & forepaw (27.3/66.7*) There was no evidence of treatment-related teratogenic effects at any dose level. NOEL: 60 mg/kg bw/day for maternal toxicity and for developmental toxicity	Anonymous; 2004; M-202155-02-1

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels, duration of exposure**	**Results (% difference refers to difference from concurrent control unless otherwise stated)**	**Reference**
Rabbit oral gavage range finding developmental toxicity study. Himalayan rabbits 4 dams/group OECD 414 (1981) GLP	Fluopicolide (purity 97.6%-97.8%) 25, 50, 100, 250, 500 or 1,000 mg/kg bw from Day 6-28 of pregnancy	<u>Maternal toxicity:</u> ***1000 mg/kg bw/day*** ↑ mortality/moribundity: 4/4 dead (Days 13-15) Clinical signs in all animals: impairment of mobility/consciousness, respiratory sounds, coat bristling, ↓ defication, hyper/hypoactivity, discoloured urine and tray, ↓ hay consumption Necropsy findings: beige discoloured heart, liver & kidneys & petechial bleeding in stomach (all animals) ↓ body weight: (10% lower than 25 mg/kg bw/day dose-group at 100% mortality on Day 13) ↓ food consumption throughout study (-97% on Days 10-13 compared with pre-treatment values) ***500 mg/kg bw/day*** ↑ mortality/moribundity: 4/4 dead (Days 15-16) Clinical signs in all animals: impairment of mobility/consciousness, respiratory sounds, coat bristling, ↓ defication, hyper/hypoactivity, discoloured urine and tray, ↓ hay consumption Necropsy findings: beige discoloured heart, liver & kidneys & petechial bleeding in stomach (all animals) Necropsy findings: beige discoloured heart, liver & kidneys & petechial bleeding in stomach (all animals) ↓ body weight: (9% lower than 25 mg/kg bw/day dose-group at 100% mortality on Day 16) ↓ food consumption throughout study (-98% on Days 13-16 compared with pre-treatment values) ***250 mg/kg bw/day*** ↑ mortality/moribundity: 4/4 dead (Days 17-23) Clinical signs in all animals: impairment of mobility/consciousness, respiratory sounds, coat bristling, ↓ defication, hyper/hypoactivity, discoloured urine and tray, ↓ hay consumption Necropsy findings: beige discoloured heart, liver & kidneys & petechial bleeding in stomach (all animals) ↓ body weight: (17% lower than 25 mg/kg bw/day dose-group at 100% mortality on Day 19) ↓ food consumption throughout study (-92% on Days 16-19 compared with pre-treatment values) ***100 mg/kg bw/day*** ↑ mortality/moribundity: 4/4 dead (Days 16-22) Clinical signs in all animals: impairment of mobility/consciousness, respiratory sounds, coat bristling, ↓ defication, hyper/hypoactivity, discoloured urine and tray & ↓ hay consumption Necropsy findings: beige discoloured heart, liver & kidneys & petechial bleeding in stomach (all animals)	Anonymous; 2000; M-211192-01-1

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels, duration of exposure	Results (% difference refers to difference from concurrent control unless otherwise stated)	Reference
		1 dam aborted (Day 22) ↓ body weight: (16% lower than 25 mg/kg bw/day dose-group at 100% mortality on Day 19) ↓ food consumption throughout study (-98% on Days 16-19 compared with pre-treatment values) ***50 mg/kg bw/day*** There were no deaths Clinical signs (1 animal): ↓ defication & discoloured tray 1 dam aborted (Day 29) ↓ body-weight gain: (57% lower than 25 mg/kg bw/day dose-group throughout study) ↓ total food consumption (-20% on Days 0-29 compared with 25 mg/kg bw/day dose-group) ***25 mg/kg/bw/day*** No findings <u>Developmental toxicity:</u> Gravid uterus and fetal weights were normal and embryofoetal development was unaffected at 25 and 50 mg/kg bw/day. Based on the results of this study, a dose level in the region of 50 mg/kg bw/day was considered to be a suitable high dose for the main study.	

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels, duration of exposure**	**Results (% difference refers to difference from concurrent control unless otherwise stated)**	**Reference**
Prenatal oral gavage developmental toxicity study in rabbits. Himalayan rabbits 23 dams/dose) OECD 414 (1981) GLP 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	Fluopicolide (purity 97.8%) 0, 5, 20 or 60 mg/kg bw on Days 6-28 of gestation	<u>Maternal toxicity:</u> ***60 mg/kg bw/day*** ↑ mortality: 18/23 dams dead (3 found dead on Days 24, 25 & 29 and 15 killed after premature delivery) Clinical signs: ↓ defecation, hypoactivity, bristling coat, pultaceous faeces & discoloured urine & ↓ hay consumption ↓ body weight gain (-86% Days 6-29 & -57% Days 0-29) ↓gravid uterine weight (-29%) ↓feed consumption (-43%* Days 23-26 & -54%* Days 26-29) Necropsy findings: tautly filled stomach (6/23), red fluid in urinary bladder (2/23), red fluid in uterus (2/23) & yellow discoloured liver (1/23) ↑ incidence of abortions (12/23 dams aborted litter) ↑ incidence of premature deliveries in 3/23 dams (0/7, 1/5 and 7/10 foetuses born dead in these litters) ***20 mg/kg bw/day*** 1 dam killed after aborting on Day 28 ↓ defecation (dam killed on Day 29) ↓ food consumption (-19% on Day 26 in dam killed on Day 29) ***5 mg/kg bw/day*** No findings <u>Developmental toxicity:</u> ***60 mg/kg bw/day*** ↓ mean foetal weight (-14%) ↓ crown-rump length (-6%) ***20 mg/kg bw/day*** No findings ***5 mg/kg bw/day*** No findings No teratogenic effects were observed in the fetuses at any dose level. NOAEL: 20 mg/kg bw/day for maternal toxicity and developmental toxicity	Anonymous; 2004; M-202513-02-1

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels, duration of exposure	Results (% difference refers to difference from concurrent control unless otherwise stated)	Reference
Supplementary information: External expert statement regarding fluopicolides reproductive and developmental toxicity potentials	-	This document contains a summary and review of the two-generation and the developmental toxicity studies with rats and rabbits by an external expert for reproductive toxicity concluding that on the basis of the available studies, fluopicolide should not be classified as a reproductive toxicant.	Anonymous; 2018; M-638869-01-1

% difference refers to concurrent control unless otherwise stated
* Statistically different from control
[1] not statistically significant but outside historical data (based on 21 studies in Sprague Dawley rats)

Table 10-28: Summary table of human data on adverse effects on development

Type of data/report	Test substance	Relevant information about the study (as applicable)	Observations	Reference
No human data				

Table 10-29: Summary table of other studies relevant for developmental toxicity

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations	Reference
No other data				

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

10.10.5.1 Rat developmental toxicity studies

A rat range finding study was conducted to select suitable doses of fluopicolide for a subsequent developmental toxicity (teratogenicity) study in Sprague Dawley rats. Groups of 4 mated female Sprague Dawley rats received technical fluopicolide in aqueous methylcellulose (1% w/v) by oral gavage once daily at the dose levels of 500 or 1,000 mg/kg bw from Day 7-20 of pregnancy and were sacrificed on Day 21 of pregnancy.

The key maternal findings in this study included a reduced gain in body weight throughout the period of treatment at 1,000 mg/kg bw/day (-34% compared to 500 mg/kg bw/day and -27% when corrected for gravid uterine weight). Food consumption showed a marked initial (Days 7-10) decrease at this dose level and a slight reduction at 500 mg/kg bw/day. No compound-related effects were observed at necropsy of the animals. Post-implantation loss was elevated at 1,000 mg/kg bw/day and included one total resorption. Mean fetal weight and crown-rump length were reduced at 1000 and 500 mg/kg bw/day. Therefore, the highest dose selected for the definitive study of developmental toxicity was 700 mg/kg bw/day.

In the main developmental toxicity study in rats, groups of 23 mated female Sprague Dawley rats received fluopicolide by oral gavage in 1% methylcellulose once daily at the dose levels of 0, 5, 60 or 700 mg/kg bw/day from Day 7-20 of pregnancy (Day 0: day of mating, Day 1: day of sperm detection). They were sacrificed on Day 21 of pregnancy.

Body weights and weight gains were decreased in the animals from the high dose group, especially at the beginning of the treatment period during gestational Days 7-10 (-24% when compared with the control value), a gestational phase which is considered to be highly susceptible to influences regarding skeletal development. These animals showed also a slight initial decrease in food consumption after beginning of treatment. Overall body weight gain (Days 1-21) was 9% lower than the concurrent control value and 12% lower when corrected for gravid uterine weight.

Mean foetal body weights, crown-rump lengths and placental weights were slightly, but statistically significantly decreased in the high dose group. However, litter size, number of live and dead foetuses as well as sex ratios were unaffected by the administration of the test substance. Incidences of early and late conceptuses undergoing resorption were also not affected by the administration of the test compound up to and including the highest tested dose level of 700 mg/kg bw/day.

Morphological examination of the foetuses revealed one foetus with multiple malformations at the vertebral column and pelvis in the intermediate dose group and one foetus with microphthalmia in the high dose group. These findings are considered to be incidental due to their isolated occurrence.

Foetuses from the high dose group showed increased incidences of minor skeletal defects at the thoracic vertebrae, sternebrae and ribs. However, only a small number of foetuses in single litters was affected and these findings are not considered to have adverse consequences for the foetuses in postnatal life. The observations represent mostly a perturbation of ossification, transient in nature, being resolved as ossification progresses. In addition a delayed ossification was detected at 700 mg/kg bw/day which indicated together with the decreased foetal weight and length a generally retarded foetal development at this maternally toxic dose level.

In conclusion, oral administration of fluopicolide to the pregnant rat at the dose of 700 mg/kg bw/day caused maternal toxicity as evidenced by decreased body weight gains and slightly decreased food consumption. Mean foetal body weights and crown-rump lengths were also slightly decreased at700 mg/kg bw/day. In addition, minor defects at the thoracic vertebrae, sternebrae and ribs as well as delayed ossification were observed more frequently at this dose level and are considered secondary to the above described maternal toxicity. Fluopicolide was not teratogenic in this developmental toxicity study in rats.

Fluopicolide did not cause any maternal toxicity or embryotoxicity at the dose of 60 mg/kg bw/day or below. Therefore, with regard to the present study the No Observed Effect Level (NOEL) is 60 mg/kg bw/day for maternal toxicity and for developmental toxicity.

10.10.5.2 Rabbit developmental toxicity studies

A rabbit range finding study with dose levels of 25, 50, 100, 250, 500 or 1,000 mg/kg bw/day was conducted in rabbits in order to select a suitable high dose level of fluopicolide for a subsequent developmental toxicity study in Himalayan rabbits. All animals from the 100, 250, 500 or 1,000 mg/kg bw/day group were found dead, killed moribund or killed after abortion up to Day 23 of the study. At the dose of 50 mg/kg bw/day one animal aborted on Day 29 and decreased bodyweight gain and food consumption was recorded. No significant effects were observed at 25 mg/kg bw/day. Based on the results of this study, a dose level in the region of 50 mg/kg bw/day was considered to be a suitable high dose for the main study.

In the main rabbit developmental toxicity study, groups of 23 mated female Himalayan rabbits received fluopicolide by oral gavage in 1% methylcellulose once daily at the dose levels of 0, 5, 20 or 60 mg/kg bw/day from Day 6-28 of gestation and were sacrificed on Day 29 of gestation.

The study report author stated that three animals of the high dose group were found dead and 15 animals of this group were killed after premature delivery from Day 22-29 of gestation; however, further examination of the raw data has revealed that 12 dams aborted whole litters (with no live pups), whilst 2 dams delivered prematurely with partial live litters (dam 178 delivered 1 dead and 4 live pups and dam 180 delivered 7 dead and 3 live pups). In addition a further dam prematurely delivered 7/7 live pups. These dams showed decreased defecation, reduced hay consumption, hypoactivity, bristling coat, pultaceous feces, and discoloured urine. One animal from the intermediate dose group (20 mg/kg bw/day) was killed after aborting on Day 28 of

gestation. This animal showed decreased defecation and reduced hay consumption. The dossier submitter considers this to be a spuorious finding and not related to treatment with fluopicolide, owing to its isolated occurrence in this dose group, supported by the the dose-range finding study, in which no abortions were observed at a similar dose (25 mg/kg bw/day) and only one dam aborted at a much higher dose (50 mg/kg bw/day). Furthermore, according to published historical data, up to 20% abortions have been reported for this strain of rabbit (Viertel & Trieb 2002[4]). Therefore, this isolated single abortion at this dose is considered incidental and not treatment-related.

Body weight gains and food consumption were markedly decreased in the animals from the high dose group (-57% for Days 0-29 and -54% for Days 26-29 for body-weight gain and food consumption respectively when compared with the control group). Gravid uterus weights were 29% lower than controls in the animals from the high-dose group; nonetheless, when adjusted for gravid uterine weight, the dams in the high-dose group showed mean body-weight losses (for Days 0-29) of 148 g, compared with a mean loss of 15 g in the control group.

At necropsy, tautly filled stomach, red liquid in urinary bladder and uterus as well as yellowish discoloration of the liver were observed in single animals from the high dose group. No compound-related effects were observed in the low and intermediate dose group.

Twelve dams aborted their litters whilst two dams prematurely delivered litters that contained 1/5 and 7/10 dead foetuses respectively; a further dam prematurely delivered a litter conaining only live foetuses (7/7 live foetuses). It is more likely that the observed abortions are secondary to the maternal toxicity observed at this dose, and not a consequence of malformations in the aborted foetuses; no malformations were detected in these foetuses or in foetuses at the lower doses. Furthermore, variations which could potentially progress into malformations (with an increase in dose) were not detected at 50 or 20 mg/kg bw/day. Mean fetal body weights, crown-rump lengths and placental weights were decreased in the animals from the high dose group. Of the remaining surviving litters, litter size, number of live and dead foetuses as well as sex ratios remained unaffected by the administration of the test compound (litters from dams found dead or killed after abortion/premature delivery were excluded from subsequent calculations). Likewise, incidences of early and late conceptuses undergoing resorption were not affected by the administration of the test substance. Morphological examination of the fetuses did not reveal any compound-related effects up to and including the highest tested dose level of 60 mg/kg bw/day.

There are several publications describing the effects of feed restriction during gestation on developmental parameters in rabbits (Matsuzawa *et al.* 1981[5]; Petrere *et al.* 1993[6]; Cappon *et al.* 2005[7]; Menchetti *et al.* 2015[8]). In most of them feed was restricted during the phase of organogenesis between approx. GD 6-19 resulting in abortions, decreased foetal and placental weight, reduced foetal ossification and also higher rate of pre-, peri- and postnatal death. A feed restriction by 87.7-90% resulted in all three publications with feed restriction during organogenesis in an increased rate of abortions whereas pregnancy was not affected at the next higher tested feed level (feed restriction to 23.3%, 40% or 50% of control, respectively).

Reduced fetal weight and delayed ossification was already detected by Cappon *et al.*[7] (2005) starting at a feed restriction of 50% of control. In addition, Menchetti *et al.*[8] (2015) showed that the late gestational phase from

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

5 Matsuzawa T, Nakata M, Goto I, Tsushima M. Dietary deprivation induces fetal loss and abortion in rabbits. Toxicology. 1981;22(3):255-9.

6 Petrere JA, Rohn WR, Grantham LE 2nd, Anderson JA. Food restriction during organogenesis in rabbits: effects on reproduction and the offspring. Fundam Appl Toxicol. 1993 Nov;21(4):517-22.

7 Cappon GD, Fleeman TL, Chapin RE, Hurtt ME. Effects of feed restriction during organogenesis on embryo-fetal development in rabbit. Birth Defects Res B Dev Reprod Toxicol. 2005 Oct;74(5):424-30.

8 Menchetti L, Brecchia G, Canali C, Cardinali R, Polisca A, Zerani M, Boiti C. Food restriction during pregnancy in rabbits: effects on hormones and metabolites involved in energy homeostasis and metabolic programming. Res Vet Sci. 2015 Feb;98:7-12

GD 19-28 is even more sensitive regarding secondary effects of decreased feed consumption. A feed restriction of 30% during this gestational period already lead to a minimal increase of abortions and slightly decreased number of live born pups whereas this was not observed if feed was restricted to the same proportion from GD 0-9 or GD 9-18.

Therefore, the increased incidence of abortions (12/23 dams) and premature deliveries with some dead foetuses (2/23 dams), along with the reduced fetal weights and crown-rump lengths in the highest dose group in the present study are considered as secondary consequences of severe maternal toxicity as evidenced by mortality (3/23 dams found dead) and decreases in body weight gain due to markedly reduced food consumption. As shown in the table below the food consumption was drastically reduced to 9% of control in the high dose dams that died or had abortions from gestation day 19 till death or sacrifice whereas the high dose dams that survived till termination showed a food consumption reduction to 60% of control which is in good agreement with the published results in rabbits.

Mean maternal feed consumption in g/animal/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[a]	98.5	96.7 *(98 %)*	70.0 *(71 %)*	9.1 *(9 %)*	58.9 *(60 %)*

[a] death in case of 3 animals in the high dose group

In conclusion, oral administration of fluopicolide to pregnant rabbits at the dose of 60 mg/kg bw/day caused severe maternal toxicity as evidenced by mortality, clinical signs, decreases in body weight gain and drastically reduced food consumption. Only secondary to this severe maternal toxicity were an increased incidence of abortion (12/23 dams), reduced foetal body weights and reduced crown lump lengths observed. There was no evidence of treatment-related teratogenic effects at any dose level.

At doses up to and including 20 mg/kg bw/day, fluopicolide did not cause any maternal toxicity or embryotoxicity. Therefore, the No Observed Adverse Effect Level (NOAEL) is 20 mg/kg bw/day for maternal toxicity and for developmental toxicity in the present study.

10.10.6 Comparison with the CLP criteria

There is no data on humans to inform on the developmental toxicity of fluopicolide, and thus classification in category 1A is not appropriate.

Classification in category 1B for developmental toxicity is not appropriate as there is no clear evidence of an adverse effect on development in experimental animals.

Substances are classified in category 2 when there is some evidence from humans or experimental animals of an adverse effect on development, and where the evidence is not sufficiently convincing to place the substance in category 1. Furthermore, the effects shall have been observed in the absence of other toxic effects, or if occurring together with other toxic effects the adverse effect on reproduction is considered not to be a secondary non-specific consequence of the other toxic effects.

In the 2-generation study, there were no effects on embryo or fetal lethality or on pup survival during lactation and weaning. It is concluded that slightly reduced bodyweight development of pups during lactation was likely a palatability effect and/or systemic toxicity due to direct consumption of the test diet and not a specific toxic effect of fluopicolide on postnatal development.

In the rat developmental toxicity study, dam body weights and bodyweight gains were decreased in the high dose group during the treatment phase, especially at the beginning of the treatment period during gestational Days 7-10, a gestational phase which is considered to be highly susceptible to influences regarding skeletal development. Fluopicolide was found not to be teratogenic in the rat; the incidence of major malformations was low and clearly incidental to the administration of fluopicolide. Foetuses from the high dose group showed treatment-related increased incidences of minor skeletal defects at the thoracic vertebrae, sternebrae and ribs. However, only a small number of foetuses in single litters was affected and these findings are not considered to have adverse consequences for the foetuses in postnatal life. The observations represent mostly a perturbation of ossification, transient in nature, being resolved as ossification progresses. For this reason, it is considered that the anomalies should be considered non-adverse such that classification of fluopicolide as a developmental toxicant is not warranted with reference to the CLP guidance statement that 'classification is not necessarily the outcome in the case of minor developmental changes, when there is only a small reduction in fetal/pup body weight or retardation of ossification when seen in association with maternal toxicity'. In addition a delayed ossification was detected at 700 mg/kg bw/day which indicated together with the decreased foetal weight and length a generally retarded foetal development at this maternally toxic dose level.

In the rabbit developmental toxicity study, 15 animals of the 60 mg/kg bw/day group were killed after abortion or premature delivery on Days 22-29 of gestation. Twelve dams aborted their litters, whilst three dams delivered prematurely (two of these dams produced litters with 1/5 and 7/10 dead foetuses respectively and the remaining dam delivered a live litter). Mean fetal body weights, crown-rump lengths and placental weights were decreased in the animals from this dose group. However, all these findings are considered secondary to severe maternal toxicity as evidenced by mortality (3 animals) and decreases in body weight gain due to drastically reduced feed consumption at the hightest tested dose. There was no evidence of treatment-related teratogenic effects at any dose level.

It can be summarized that in both species developmental effects only occurred at high doses which caused maternal toxicity and even mortality in rabbits. Therefore, the developmental findings are considered to be secondary, non-specific consequences of maternal toxicity in the conducted developmental toxicity studies in rats and rabbits.

In conclusion, the data regarding adverse effects on development are conclusive but do **not warrant** a developmental toxicity classification.

10.10.7 Adverse effects on or via lactation

Table 10-30: Summary table of animal studies on effects on or via lactation

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
No special studies on lactation available			
Preliminary study on reproductive performance by dietary administration to CD rats 8 male and 8 female rats/group OECD 451 (1981) GLP	Fluopicolide (purity 95.9%) Oral administration via diet at 0, 50, 200, 750 or 2,500 ppm (equivalent to 0, 5.2/6.4, 25.5/32.9 and 103.4/127.3 mg/kg bw/day for M/F premating) Treatment started 15 days prior to pairing and continued uninterrupted until termination after weaning of the resulting litters.	Dietary concentrations of 2500 ppm induced toxicity observed as body weight gain reductions in F0 males during the premating period, and in F0 females during the gestation and lactation period. Thus, F1 offspring body weight reductions from approx. day 14 of age at the same dose level suggest a systemic toxicity due to direct consumption of the test diet and /or a palatability effect and no direct lacational effect.	Anonymous; 2002; M-215068-01-1
Two generation reproduction study in rats. Crl: CD® (SD) IGS BR rats 28 male and 28 female rats/group OECD 416, Draft (1999) GLP	Fluopicolide (purity 95.9%) Oral administration via diet at concentrations of 0, 100, 500 or 2,000 ppm throughout the two generations	Litter parameters at birth (F1 and F2) and survival to weaning not affected and sexual maturation was not affected. Body weight at birth were also comparable with control. Reduced body weight apparent from approx. day 14 of age on in both generations is considered to be due to a palatability effect and/or systemic toxicity due to direct consumption of the test substance and not a direct lactational effect.	Anonymous; 2003; M-232532-01-1
Supplementary information: External expert statement regarding fluopicolides reproductive and developmental toxicity potentials	-	This document contains a summary and review of the two-generation and the developmental toxicity studies with rats and rabbits by an external expert for reproductive toxicity concluding that on the basis of the available studies, fluopicolide should not be classified as a reproductive toxicant.	Anonymous; 2018; M-638869-01-1

Table 10-31: Summary table of human data on effects on or via lactation

Type of data/report	Test substance	Relevant information about the study (as applicable)	Observations	Reference
No human data				

Table 10-32: Summary table of other studies relevant for effects on or via lactation

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations	Reference
ADME study following repeated oral administration to the lactating cow	Fluopicolide (purity 99.3%) (^{14}C)-Fluopicolide	The absorption, distribution, metabolism and excretion of (^{14}C)-fluopicolide were investigated after twice daily oral administrations for 7 consecutive days in two lactating cows. Excretion balance and tissue distribution investigations were performed at two dose levels, nominally at 1 ppm (equivalent to 0.024 mg/kg bw/day) and 10 ppm (0.28 mg/kg bw/day) in the diet.	In the milk, the level of radioactive residues reached a steady state with a maximum value of 18.8 ng equivalents/g (19 ppb) 5 days after the first dose at the high dose. Therefore, the residues in milk were below the concentration requiring extensive analysis (<50 ppb). The major compound identified was unchanged fluopicolide, which accounted for 29% of the TRR in the milk.	Anonymous; 2008; M-218626-02-1
Distribution and Metabolism of [^{14}C]-fluopicolide in the lactating cow	Fluopicolide (purity 99.3%) (^{14}C)-Fluopicolide	Two lactating cows were orally dosed by gelatine capsules twice daily with [^{14}C]- fluopicolide for 7 consecutive days, by gelatin capsule at a daily dose level of 1 ppm (0.03 mg/kg bw/day) and 10 ppm (0.35 mg/kg bw/day). Milk was collected from each animal twice daily, immediately prior to the morning and afternoon dosing.	The concentrations of total radioactivity in milk were low for both dose levels, with a maximum concentration of 10 ng equivalents/g reached at 32 h post dose at the 10 ppm dose level. For the 1 ppm dose level, levels did not rise above 1 ng equivalents/g.	Anonymous; 2009; M-233391-02-1
Residues and major metabolites in milk and edible cattle tissues following 28 days dosing of fluopicolide to lactating cows	Fluopicolide (purity 96.1%)	3 lactating cows/group were dosed with gelatine capsules at 0, 0.5, 1.5 and 5 mg/kg bw/day fluopicolide daily for 28-days. Cows were milked twice daily and residues of fluopiclode and ist major metabolites were quantified.	Apart from two values above 0.01 mg/kg, all milk samples analysed gave results below the LOQ of 0.01 mg/kg for fluopicolide and its main metabolites. The two values above the LOQ were from Day 4 and Day 28 in one animal from the top dose group.	Anonymous; 2004; M-219457-01-1

10.10.8 Short summary and overall relevance of the provided information on effects on or via lactation

The potential of fluopicolide to elicit adverse effects on or via lactation has been investigated in a preliminary dose range finding and in a two-generation reproduction toxicity study in rats (see Section 10.10.1). No data from humans are available.

Reduced body weight apparent in offspring from approx. day 14 through to weaning, coinciding with the time when the offspring started to eat the diet suggesting a palatability effect and/or systemic toxicity due to direct consumption of the test diet and not a lactational effect. This is supported by the fact that reduced food consumption and body weight development was observed in all repeated dose rat studies during the initial treatment phase and also in the parental animals of the present reproductive studies. Therefore, in the preliminary and main rat dietary multigeneration reproductive toxicity study, no effects on the lactation or any negative subsequent effects on the offspring which could be mediated via lactation are considered.

In conclusion, it can be summarized that based on these results there is **no clear evidence** of an adverse effect due to transfer in the milk, or an adverse effect on the quality of the milk.

In addition, three studies are available conducted in lactating cows designed to investigate the concentration of fluopicolide and its major metabolites in milk (see Table 10-32) showing a low to very low transfer into milk (less than 0.2% of radioactivity was excreted in the milk) with no evidence of any accumulation.

10.10.9 Comparison with the CLP criteria

Under CLP, substances that are absorbed by women and have been shown to interfere with lactation shall be classified and labelled to indicate this property hazardous to breastfed babies. Effects in the mother can adversely impact the breast milk (either in terms of the quantity produced or the quality produced). However, if a substance causes overt toxicity in the mother, this may indirectly impair milk production or impair maternal care as a non-specific secondary effect and should not lead to classification.

a) Human evidence indicating a hazard to babies during the lactation period

No data from humans are available.

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

Reduced body weight apparent in offspring from approx. day 14 through to weaning, coinciding with the time when the offspring started to eat the diet suggest a palatability effect and/or systemic toxicity due to direct consumption of the test diet and not a lactational effect. This is supported by the fact that reduced food consumption and body weight development was observed in all repeated dose rat studies during the initial treatment phase and also in the parental animals of the present reproductive studies. In conclusion, in the preliminary and main rat dietary multigeneration reproductive toxicity study there is no clear evidence of an adverse effect due to transfer in the milk, or an adverse effect on the quality of the milk.

c) Absorption, metabolism, distribution and excretion studies that indicate the likelihood that the substance is present in potentially toxic levels in breast milk

The available toxicokinetic data suggest that fluopicolide is extensively metabolised to more polar, water soluble molecules followed by a moderately rapid elimination primarily via bile. On this basis, it is unlikely that fluopicolide or its metabolites would be transferred in significant amounts into the milk. This is supported by studies conducted in lactating cows, which show low to very low transfer of fluopicolide to the milk with no evidence of any accumulation.

Based on the above assessment and comparison with the classification criteria, fluopicolide **does not meet** the criteria for classification for effects on or via lactation.

10.10.10 Conclusion on classification and labelling for reproductive toxicity

On the basis of the results of the two-generation reproduction study in the rat and prenatal developmental toxicity studies in rats and rabbits, there is **no justification for classification** of fluopicolide as a reproductive toxicant based on the CLP criteria (Regulation (EC) No 1272/2008 on classification, labelling and packaging (CLP) of substances and mixtures). This is supported by an external expert review of the experimental study results in relation to the criteria for classification (Anonymous; 2018; M-638869-01-1).

10.11 Specific target organ toxicity-single exposure

The acute studies that are relevant for the assessment of the specific target organ toxicity of fluopicolide after single exposure are reported in Sections 10.1 to 10.3. An acute neurotoxicity study is also available and is summarised below.

Table 10-33: Summary table of animal studies relevant for STOT SE

Study, species, test substance, purity	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.	See Sections 10.1 to 10.3
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 2,000 mg/kg bw Single dose	There were no deaths or specific neurotoxicity findings. 2,000 mg/kg bw Transiently decreased body temperature in both sexes 100 mg/kg bw No treatment-related effects 10 mg/kg bw No treatment-related effects	Anonymous; 2002; M-208046-01-1

Table 10-34: Summary table of human data on STOT SE

Type of data/report	Test substance	Relevant information about the study (as applicable)	Observations	Reference
No human data				

Table 10-35: Summary table of other studies relevant for STOT SE

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations	Reference
No other data				

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

Studies which can be used to evaluate potential STOT SE effects were already summarized in Sections 10.1 to 10.3. These studies on acute oral, dermal and inhalation toxicity demonstrated a low acute toxic potential of fluopicolide (AE C638206) with oral LD_{50} values of > 5,000 mg/kg bw after oral and dermal administration and an inhalation LC_{50} 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 was no indication of any sex-specific susceptibility in any of the acute studies. Since no specific, non-lethal target organ toxicity, or other significant health effects that can impair function, both reversible and irreversible, immediate and/or delayed, arising from a single exposure were seen in the studies on acute toxicity, according to the ECHA Guidance a classification in STOT SE Category 1 and also in 2 is not applicable. The STOT SE criteria are not fulfilled based on the results of the acute toxicity studies with fluopicolide, as shown in the following table:

Summary of acute toxicity study results

Study	Toxicological effects at LOAEL
Acute oral rat	> 5,000 mg/kg bw: unspecific clinical signs
Acute dermal rat	> 5,000 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 2,000 mg/kg bw, NOEL: 100 mg/kg bw for general toxicity

Moreover, there were no acute effects observed in repeated dose toxicity studies with fluopicolide.

Furthermore, the ECHA Guidance specifies criteria that trigger a classification for STOT SE Category 3. These criteria are generally independent from the aforementioned guidance values and include transient target organ effects, focusing on overt narcotic effects and respiratory tract irritation (respiratory tract irritation covers two different effects: ‘sensory irritation’ and ‘local cytotoxic effects’). Specifically, the following examples for findings from single and repeated inhalation toxicity studies are mentioned as possible triggers for a STOT SE Category 3 classification: clinical signs of toxicity (dyspnoea, rhinitis etc.) and histopathology (e.g. hyperemia, edema, minimal inflammation, thickened mucous layer) which are reversible.

According to the CLP criteria, also physicochemical properties, such as pH, physical form, solubility, vapour pressure, particle size, which can be important parameters in evaluating toxicity studies and in determining the most appropriate classification especially with respect to inhalation where physical form and particle size can have a significant impact on toxicity. For fluopicolide none of these parameters indicate a potential to fall under STOT SE Category 3.

10.11.2 Comparison with the CLP criteria

According to the CLP criteria, a classification for STOT SE needs to be considered if the substance causes non-lethal target organ toxicity after a single exposure (i.e. significant health effects that can impair function, both reversible and irreversible, immediate and/or delayed and not covered by acute toxicity, skin corrosion / irritation, eye damage / irritation, respiratory or skin sensitisation, genotoxicity, carcinogenicity and reproductive toxicity should be taken into consideration).

Based on the results after acute exposure to fluopicolide (AE C638206) in toxicological studies with single and repeated dosing, no significant toxic effects on specific target organs were observed at non-lethal dose levels at or below reference values assigned in the guidance on the application of the CLP criteria. Thus, classification of fluopicolide for STOT SE Category 1 or 2 is not warranted.

There is also no indication of transient effects like respiratory tract irritation (RTI) and narcotic effects (NE) after single exposure to fluopicolide. Therefore, classification of fluopicolide for STOT SE Category 3 is therefore also **not warranted**.

10.11.3 Conclusion on classification and labelling for STOT SE

A comparison of the toxicological effects in acute oral, dermal and inhalation toxicity studies, furthermore of the lack of effects in other studies, like in an acute neurotoxicity rat study, with the aforementioned classification criteria reveals that the results are conclusive and that a STOT SE Category 1, 2 and 3 classification of fluopicolide (AE C638206) according to the CLP guidance (Guidance to Regulation (EC) No 1272/2008 on classification, labelling and packaging (CLP) of substances and mixtures Version 5.0 July 2017) is **not warranted**.

10.12 Specific target organ toxicity-repeated exposure

The following tables provide a detailed overview of potentially classification-relevant toxicological findings of fluopicolide (AE C638206) with the respective applicable CLP criteria (following the Guidance to Regulation (EC) No 1272/2008 on classification, labelling and packaging (CLP) of substances and mixtures, Version 4.1, July 2017) for specific target organ toxicity after repeated exposure (STOT RE). The specific target-organ toxicity of fluopicolide upon repeated exposure has been investigated in nine short-term oral studies in rats, mice and dogs ranging from 28-days to one-year in dogs. Furthermore one 4-week dermal study in rats was performed (see Table 10-36). Additional information is provided by the chronic / carcinogenicity studies in rats and mice, the findings in the parental animals and offspring in the 2-generation study, the developmental toxicity studies and by the subchronic neurotoxicity study in rats (see Table 10-38).

Table 10-36: Summary table of animal studies on STOT RE

Method, guideline, test substance purity	Species, strain, sex, no/group	Dose levels, duration of exposure	CLP guideline value for classification (mg/kg bw/day)	Results (% difference refers to difference from concurrent control unless otherwise stated)	Reference
Oral route					
Rat 28-day dietary toxicity study OECD 407 (1995) GLP Fluopicolide (purity 99.0%)	Sprague Dawley CRL:(IGS)CDBR rats 5/dose/sex	0, 20, 200, 2000 or 20,000 ppm Equivalent to: 1.78, 17.7, 179 and 1,770 mg/kg bw/day (combined sexes) 28 days	Cat 1 ≤ 30 Cat 2 ≤ 300	<u>There were no deaths or clinical signs of toxicity</u> <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 hepatocytic hypertrophy in 2/5 M (1 minimal & 1 slight) & 3/5 F (minimal)** <u>≥ 2,000 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 hepatocytic hypertrophy in 5/5 M (1 minimal & 4 slight) & 2/5 F (minimal)** <u>20,000 ppm (1,770 mg/kg bw/day)</u> ↓ bodyweight M ↑ water consumption ↓ feed intake ↓ ALT ↑ relative liver weights ↑ absolute liver weights Enlarged livers	Anonymous; 2000; M-199377-01-1

Method, guideline, test substance purity	**Species, strain, sex, no/group**	**Dose levels, duration of exposure**	**CLP guideline value for classification (mg/kg bw/day)**	**Results (% difference refers to difference from concurrent control unless otherwise stated)**	**Reference**
Mouse 28-day dietary toxicity study OECD 407 (1995) GLP Fluopicolide (purity 99.0%)	CD-1 mice 5/dose/sex	0, 6, 64, 640 or 6,400 ppm Equivalent to: 1.07, 11.6, 115 and 1,111 mg/kg bw/day (combined sexes) 28 days	Cat 1 ≤ 30 Cat 2 ≤ 300	There were no deaths or clinical signs of toxiicty 6 ppm (1.07 mg/kg bw/day) No effects observed 64 ppm (11.6 mg/kg bw/day) No effects observed ≥ 640 ppm (115 mg/kg bw/day) **↑ 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)** 6,400 ppm (1,111 mg/kg bw/day) ↑ 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)	Anonymous; 2000; M-197343-01-1

Method, guideline, test substance purity	**Species, strain, sex, no/group**	**Dose levels, duration of exposure**	**CLP guideline value for classification (mg/kg bw/day)**	**Results (% difference refers to difference from concurrent control unless otherwise stated)**	**Reference**
Mouse 28-day dietary mechanistic toxicity study No applicable guideline GLP Fluopicolide (purity 99.3%)	C57BL/6 mice 15/females/dose	0 or 3,200 ppm Equivalent to: 575 mg/kg bw/day 28 days	Cat 1 ≤ 30 Cat 2 ≤ 300	3,200 ppm (575 mg/kg bw/day) ↓ 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	Anonymous; 2004; M-229594-01-1
Dog 28-day oral gavage toxicity study OECD 409 (1998) GLP Fluopicolide (purity 96.9%)	Beagle dogs 2/sex/group	0, 10, 100 and 1,000 mg/kg bw/day 28 days	Cat 1 ≤ 30 Cat 2 ≤ 300	10 mg/kg bw/day No effects observed 100 mg/kg bw/day No effects observed 1,000 mg/kg bw/day ↑ cholesterol in blood in 1 M (+98% on day 29 compared with day 1) ↑ abs. (+34%) & rel. (+44%) liver weight (M)	Anonymous; 2000; M-197350-01-1
90-day dietary toxicity study in rats with 4-week recovery period OECD 407 (1995) GLP Fluopicolide (purity 96.9 and 97.5%)	Sprague Dawley rats 10/dose/sex	0, 100, 1,400 or 20,000 ppm equivalent to: 0, 7.9, 114 or 1,671 mg/kg bw/day (combined sexes) 13 weeks (+ 4 weeks for recovery group animals, high-dose and control)	Cat 1 ≤ 10 Cat 2 ≤ 100	100 ppm (7.9 mg/kg bw/day) No effects observed ≥ 1,400 ppm (114 mg/kg bw/day) ↑ 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) ↑ incidence hypertrophy of centrilobular hepatocytes (M) ↑ severity & incidence of trabecular hyperostosis of the bone joint (F) ↑ severity accumulation of hyaline droplets in the proximal kidney tubule (M)	Anonymous; 2000; M-197622-01-1

Method, guideline, test substance purity	Species, strain, sex, no/group	Dose levels, duration of exposure	CLP guideline value for classification (mg/kg bw/day)	Results (% difference refers to difference from concurrent control unless otherwise stated)	Reference
				↑ single cell death in the proximal kidney tubule epithelium (M) ↑ foci of basophilic (regenerating) tubules and granular casts (M) <u>20,000 ppm (1,671 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) <u>Recovery group 1,671 mg/kg bw/day</u> <u>Clinical signs: Hair loss in M & F and urogenital soiling in F</u> ↑ body-weight gain in M (+111%) & F (+300%) ↓ food consumption in M (-4%) Slightly ↑ urine volume in F Full or partial recovery of all other previous findings in the high-dose group	

Method, guideline, test substance purity	**Species, strain, sex, no/group**	**Dose levels, duration of exposure**	**CLP guideline value for classification (mg/kg bw/day)**	**Results (% difference refers to difference from concurrent control unless otherwise stated)**	**Reference**
90-day dietary toxicity study in mice OECD 408 (1998) GLP Fluopicolide (purity 97.3%)	Crl: CD-1 (ICR) BR mice 10/sex/ dose	0, 32, 320, 3,200 and 6,400 ppm Equivalent to: 0, 5.5, 53, 545 and 1,092 mg/kg bw/day (both sex combined) 13 weeks	Cat 1 ≤ 10 Cat 2 ≤ 100	32 ppm (5.5 mg/kg bw/day) No effects observed ≥ 320 ppm (53 mg/kg bw/day) ↑ **incidence hypertrophy of centrilobular hepatocytes in 9/10 M (6 minimal & 3 slight) & 2/10 F (minimal)** ≥ 3,200 ppm (545 mg/kg bw/day) ↓ body weight gain (F) ↑ALT (M/F) and AST (M) ↑ abs. & rel. liver weight (M/F) ↑ incidence of hepatocytic necrosis (F) 6,400 ppm (1,092 mg/kg bw/day) ↓ body weight gain (M) ↑AP (M) ↑ cholesterol and creatinine in blood (F) ↑ incidence of hepatocytic necrosis (M)	Anonymous; 2000; M-197623-01-1

Method, guideline, test substance purity	**Species, strain, sex, no/group**	**Dose levels, duration of exposure**	**CLP guideline value for classification (mg/kg bw/day)**	**Results (% difference refers to difference from concurrent control unless otherwise stated)**	**Reference**
90-day dietary toxicity study in mice OECD 408 (1998) GLP Fluopicolide (purity 95.9%)	C57BL/6JICO mice 10/sex/ dose	0, 50, 200, 800 and 3,200 ppm Equivalent to: 10.4/12.6, 37.8/52.8, 161/207, 770/965 mg/kg bw/day (M/F) 90 days	Cat 1 ≤ 10 Cat 2 ≤ 100	50 ppm (10.4/12.6 mg/kg bw/day) No effects observed ≥ 200 ppm (37.8/52.8 mg/kg bw/day) ↓ **cholesterol in blood in M (-26%**) & F (-21%**)** ≥ 800 ppm (161/207 mg/kg bw/day) ↓ albumin in blood (M/F) ↑ incidence centrilobular hepatocellular hypertrophy (M/F) ↑ rel. liver weight (M/F) ↑ abs. liver weight (F) 3,200 ppm (770/965 mg/kg bw/day) ↑ abs. liver weight (M) ↓ body weight gain (M/F) ↑ AP (M)	Anonymous; 2006; M-205579-02-1
Dog 90-day oral gavage toxicity study OECD 409 (1998) GLP Fluopicolide (purity 97.7%)	Beagle dogs 4/sex/group	0, 5, 70 or 1,000 mg/kg bw/day 13 weeks	Cat 1 ≤ 10 Cat 2 ≤ 100	5 mg/kg bw/day No effects observed 70 mg/kg bw/day No effects observed 1,000 mg/kg bw/day ↓ body weight gain (M/F) ↑ abs. & rel. liver weight (M/F)	Anonymous; 2000; M-199397-01-1
52-week toxicity study by oral route (gavage) in dogs OECD 452 (1981) GLP Fluopicolide (purity 95.9%)	Beagle dogs 5/sex/group	0, 70, 300 or 1,000 mg/kg/day 52 weeks	Cat 1 ≤ 2.5 Cat 2 ≤ 25	70 mg/kg bw/day No effects observed ≥ 300 mg/kg bw/day ↑ incidence of liver enlargement (M/F) 1,000 mg/kg bw/day ↓ bodyweight gain (M) ↑ cholesterol in blood (F)	Anonymous; 2002; M-216694-01-1

Method, guideline, test substance purity	Species, strain, sex, no/group	Dose levels, duration of exposure	CLP guideline value for classification (mg/kg bw/day)	Results (% difference refers to difference from concurrent control unless otherwise stated)	Reference
Dermal route					
Subacute dermal toxicity study in rats OECD 410 (1981) GLP Fluopicolide (purity 97.7%)	Wistar rats 10/dose/sex	0, 100, 250, 500, and 1,000 mg/kg bw/day semi-occlusive covering five days/ week for four weeks at	Cat 1 ≤ 30 Cat 2 ≤ 300	100, 250, 500 and 1,000 mg/kg bw/day No effects observed	Anonymous; 2003; M-220782-01-1

↑ / ↓ = 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

Table 10-37: Summary table of human data on STOT RE

Type of data/report	Test substance	Relevant information about the study (as applicable)	Observations	Reference
No human data				

Table 10-38: Summary table of other studies relevant for STOT RE

Method, guideline, test substance purity	Species, strain, sex, no/group	Dose levels, duration of exposure	CLP guideline value for classification (mg/kg bw/day)	Results (% difference refers to difference from concurrent control unless otherwise stated)	Reference
Chronic toxicity and carcinogenicity study in rats OECD 453 (1981) GLP Fluopicolide (purity 95.9%)	Crl: CD® (SD) IGS BR rats 60/sex/dose	0, 50, 200, 750 and 2,500 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)	Cat 1 ≤ 1.25 Cat 2 ≤ 12.5	50 ppm (2.1/2.8 mg/kg bw/day) No effects observed ≥ 200 ppm (8.4/10.8 mg/kg bw/day) **↓ bodyweight gain week 1 in F (-36%)** **↑ protein in blood in M in weeks 13 (+3%*) & 26 (+3%)** **↑ incidence centrilobular hepatocytic hypertrophy week 104 (9/60** M, slight)**	Anonymous; 2003; M-225616-01-1

Method, guideline, test substance purity	Species, strain, sex, no/group	Dose levels, duration of exposure	CLP guideline value for classification (mg/kg bw/day)	Results (% difference refers to difference from concurrent control unless otherwise stated)	Reference
				<u>≥ 750 ppm (31.5/41.0 mg/kg bw/day)</u> ↓ bodyweight gain week 1 (M) ↑ incidence yellow perigenital staining (F) ↑ cholesterol in blood (M) ↑ K^+ and/or Ca^{2+} in blood week 52 & 104 (M/F) ↑ abs. & rel. kidney weights week 52 & 104 (M) ↑ rel. liver weights week 52 (M) ↑ incidence centrilobular hepatocytic 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>2,500 ppm (109.4/142.2 mg/kg bw/day)</u> ↑ Brown staining on the dorsal body surface (F) ↓ bodyweight gain (M/F) ↓ RBC parameters (M/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)	

Method, guideline, test substance purity	Species, strain, sex, no/group	Dose levels, duration of exposure	CLP guideline value for classification (mg/kg bw/day)	Results (% difference refers to difference from concurrent control unless otherwise stated)	Reference
				↑ 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) ↑ incidenceof acinar atrophy with reduced colloid in prostate week 104 (M)	
Chronic toxicity and carcinogenicity study in mice OECD 451 (1981) GLP Fluopicolide (purity 95.9%)	C57BL/6 mice 60/sex/dose	0, 50, 400 and 3,200 ppm Equivalent to: 7.9/11.5, 64.5/91.9, 551/772.3 mg/kg bw/day (M/F) 78 weeks (52 week interim sacrifice)	Cat 1 ≤ 1.25 Cat 2 ≤ 12.5	<u>50 ppm (7.9/11.5 mg/kg bw/day)</u> No effects observed <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) <u>3,200 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)	Anonymous; 2003; M-225595-01-1;

Method, guideline, test substance purity	**Species, strain, sex, no/group**	**Dose levels, duration of exposure**	**CLP guideline value for classification (mg/kg bw/day)**	**Results (% difference refers to difference from concurrent control unless otherwise stated)**	**Reference**
Two-generation dietary study in rats[a] OECD 416 (1999) GLP Fluopicolide (purity 95.9%)	Crl: CD® (SD) IGS BR rats	0, 100, 500 and 2,000 ppm Equivalent to: <u>F0 pre-maiting (Week 1-10)</u> 7.4/8.1, 36.4/41.0, 147.3/159.7 mg/kg bw/day (M/F) <u>F0 gestation (GD 0-20)</u> 7.4, 38.1, 150.8 mg/kg bw/day <u>F0 lactation (LD 0-14)</u> 13.5, 70.5, 281.4 mg/kg bw/day <u>F1 pre-maiting (Week 1-10)</u> 8.8/9.4, 43.7/46.9, 179.9/193.9 mg/kg bw/day (M/F) <u>F1 gestation (GD 0-20)</u> 7.7, 39.2, 156.2 mg/kg bw/day <u>F1 lactation (LD 0-14)</u> 15.8, 74.8, 320.4 mg/kg bw/day	Cat 1 ≤ 10 Cat 2 ≤ 100	<u>100 ppm (7.4-15.8 mg/kg bw/day)</u> No effects on reproductive organs, liver & kidney observed <u>≥ 500 ppm (36.4-74.8 mg/kg bw/day)</u> ↑ **incidence of centrilobular hepatocyte hypertrophy (slight) in 9/28** F0 M & 8/24**F1 M** <u>2,000 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 scaring in the kidneys in both generations (M) ↑ incidence of cortical tubular dilatation and corticomedullary mineralization in kidneys of both generations (F)	Anonymous; 2004; M-247289-01-1

Method, guideline, test substance purity	**Species, strain, sex, no/group**	**Dose levels, duration of exposure**	**CLP guideline value for classification (mg/kg bw/day)**	**Results (% difference refers to difference from concurrent control unless otherwise stated)**	**Reference**
Developmental toxicity study in rats OECD 414 (1981) GLP Fluopicolide (purity 97.6-97.8%)	Hsd: Sprague Dawley SD 23 mated females/group	0, 5, 60 or 700 mg/kg bw/day GD 7-20	Cat 1 ≤ 60 Cat 2 ≤ 600	5 mg/kg bw/day No effects observed 60 mg/kg bw/day No effects observed 700 mg/kg bw/day ↓ bodyweight gain ↓ feed consumption ↓ foetal weights and crown-rump length ↑ incidence of minor skeletal defects and delayed ossification	Anonymous; M-202513-02-1
Developmental toxicity study in rabbits OECD 414 (1981) GLP Fluopicolide (purity 97.8%)	Chbb:HM(SPF) Himalayan rabbit	0, 5, 20 or 60 mg/kg bw/day GD 6-28	Cat 1 ≤ 36.5 Cat 2 ≤ 365	5 mg/kg bw/day No effects observed 20 mg/kg bw/day No effects observed 60 mg/kg bw/day **↑ mortality: 78% mortality (3/23 found dead on Days 24, 25 & 29 and 15/23 killed after abortion/premature delivery)** **↑ incidence of premature deliveries/abortions (12/23 dams aborted, 2/23 dams prematurely delivered a partially dead litter & 1 dam prematurely delivered a live litter)** **↑ clinical signs: ↓ defecation, ↓ hay consumption, hypoactivity, coat bristling, pultaceous faeces & discoloured urine (all animals)** **↓ bodyweight gain (-57% Days 0-29)** **↓ feed consumption (-43%* Days 23-26 & -54%* Days 26-29)** **↓ uterus weight (-19%)**	Anonymous; 2004; M-202513-02-1

Method, guideline, test substance purity	Species, strain, sex, no/group	Dose levels, duration of exposure	CLP guideline value for classification (mg/kg bw/day)	Results (% difference refers to difference from concurrent control unless otherwise stated)	Reference
				↓ foetal weights (-14%) and crown-rump length (-6%)	
Subchonic dietary neurotoxicity study in rats USEPA : OPPTS 870.6200 (1998) GLP Fluopicolide (purity 97.8%)	CD rats 10/sex/dose	0, 200, 1,400 or 10,000 ppm Equivalent to: 15.0/18.0, 107/125, 781/866 mg/kg bw/day (M/F) 13 weeks	Cat 1 ≤ 10 Cat 2 ≤ 100	200 ppm (15.0/18.0 mg/kg bw/day) No effects observed ≥ 1,400 ppm (107/125 mg/kg bw/day)[b] **↓ body weight gains in F (-13%*)** **↑ incidence of centrilobular hepatocyte hypertrophy in 9/10 M*** (slight)** **↑ incidence and/or severity of hyaline droplets in the cortical tubules in the kidneys in 10/10 M* (8 slight & 2 moderate)** 10,000 ppm (781/866 mg/kg bw/day) ↓ 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)	Anonymous; 2002; M-208051-01-1

[a] focused on specific organ effects, [b] adaptive and/or rat specific effects not relevant for classification
* p < 0.05 ; ** p < 0.01; *** p < 0.001 statistically different to controls

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

Repeated dose toxicity studies on fluopicolide (AE C638206) have been performed in rats, mice and dogs. The liver was identified as target organ in all species following repeated oral exposure to fluopicolide. In rats the kidney was additionally affected, especially in male animals. Effects on other organs were only observed at high dose levels not relevant for STOT RE classification (e.g. effects on blood related parameters, bones and adrenals in the subchronic rat study). The dog was less sensitive than the rodents and generally showed no effects at dose levels relevant for STOT RE classification. No adverse effects were observed after subacute exposure by the dermal route in rats up to 1,000 mg/kg bw/day.

Liver

The predominant effects on the liver at dose levels relevant for STOT RE classification observed in rats and mice are considered adaptive responses indicating induction of drug metabolizing enzymes in the liver consequent to the extensive hepatic metabolism of fluopicolide. These include increases in relative liver weights and increased incidences of minimal to slight centrilobular hepatocyte hypertrophy. In addition some slight and inconsistent changes in clinical chemical parameters were reported indicating an influence on liver function in a dose range relevant for STOT RE category 2. However, since these changes were slight and inconsistent (increased or decreased cholesterol level in blood, increased plasma transaminase activities (male mice in subacute study only) and increased protein concentration in blood (male rats in the chronic study week 13 and 26 only) they are not considered triggering a STOT RE classification.

Kidney

Kidney effects in a STOT RE classification relevant dose range were only observed in male rats and characterised by a minimally increased severity of hyaline droplets in the 28-day study and a slightly increased absolute kidney weight (+9%) in the chronic toxicity and carcinogencity study. Hyaline droplets have been recognized as indicator of changes associated with the accumulation of α2μ-globulin. Since the α2μ-globulin is an adult male rat-specific protein it is widely accepted that the renal effects induced in male rats by chemicals causing α2μ-globulin accumulation are unlikely to occur in humans[9]. Therefore, the available toxicity studies do not show significant or severe kidney effects that are relevant for humans at dose levels requiring classification as STOT RE.

10.12.2 Comparison with the CLP criteria

Classification for STOT-RE is warranted when repeated exposure to a substance results in ‘significant’ or ‘severe’ toxicity, generally at doses that are around or below the reference values assigned in the guidance on the application of the CLP criteria. For a 90-day oral study in the rat, the guidance cut-off value for category 2 is ≤ 100 mg/kg bw/day; this value is adjusted to ≤ 300 mg/kg bw/day for a 28-day study and ≤ 12.5 mg/kg bw/day for a one-year study. For category 1, the guidance cut-off value for an oral 90-day study in rats is ≤ 10 mg/kg bw/day. In the context of classification, ‘significant’ is taken to mean morphological changes that are toxicologically significant, or effects that clearly indicate functional disturbance. ‘Severe’ refers to more profound effects of an adverse nature or effects which significantly impact on health.

No effects were observed when fluopicolide was administered dermally to rats for 28 days at doses up to 1,000 mg/kg bw/day; therefore classification for STOT RE via the dermal route is not warranted.

9 Hard GC, Rodgers IS, Baetcke KP, Richards WL, McGaughy RE, Valcovic LR. Hazard evaluation of chemicals that cause accumulation of alpha 2u-globulin, hyaline droplet nephropathy, and tubule neoplasia in the kidneys of male rats. Environ Health Perspect. 1993 Mar;99:313-49.

In the available oral subacute and subchronic studies in rats, mice and dogs and in the subacute neurotoxicity study in rats no consistent changes in clinical biochemistry, haematology or urinalysis parameters that indicate severe organ dysfunction were seen in a dose range relevant for STOT RE classification. Severe organ damage apparent in microscopic examination following autopsy was only observed at very high doses. In addition, in the chronic toxicity and carcinogenicity studies in rats and mice and in the reprotoxicity rat studies no relevant effects occurred within the respective ranges of the CLP guideline values for classification for STOT RE. Histopathological changes observed in liver described as minimal to slight centrilobular hepatocyte hypertrophy are indicative of adaptive reversible changes and not considered adverse. Kidney effects observed in male rats in a STOT RE classification relevant dose range were graded as minimal to slight and assumed to be caused by an non-human relevant mode of action.

In the rabbit developmental toxicity study with doses of 0, 5, 20 or 60 mg/kg bw/day from Day 6-28 of gestation, at the hightest tested dose of 60 mg/kg bw/day severe maternal toxicity as evidenced by mortality, marked decreases in body weight gain and food consumption was observed resulting in high incidences of premature delivery. According to the CLP guideline values for classification for STOT RE these could be considered as results triggering STOT RE classification since the increased mortality occurred at a dose below 365 mg/kg bw/day which is the trigger value for STOT RE category 2 adapted to a exposure duration of 23 days. However, mortality was the main sign and no other consistent or siginificant organ damage was seen so that such a classification appears to be not appropriate. The kind of effect (mortality) is also not relevant for STOT SE classification, because STOT SE refers to non lethal effects. An acute toxicity classification is also not justified since only mortalities during the first 72 hours after first treatment in a repeated dose study should be considered for the assessment of acute toxicity (Guidance to Regulation (EC) No 1272/2008 on classification, labelling and packaging (CLP) of substances and mixtures Version 5.0 July 2017). Since the rabbit is known to be overly sensitive to some chemicals it is generally questionable if its mortality can be used for classification purposes. In conclusion, this study type and its results are not regarded as appropriate for an acute, STOT SE or possible STOT RE classification.

10.12.3 Conclusion on classification and labelling for STOT RE

In rodents and dogs, effects which could be relevant for STOT RE classification did neither occur in the short-term toxicity studies nor in the chronic toxicity and oncogenicity studies or in the reproduction and developmental toxicity studies, nor in a subchronic neurotoxicity study in rats with fluopicolide (AE C638206).

Overall, therefore, the data are conclusive, but do **not warrant** a STOT RE classification according to the CLP guidance (Guidance to Regulation (EC) No 1272/2008 on classification, labelling and packaging (CLP) of substances and mixtures Version 5.0 July 2017).

10.13 Aspiration hazard

Table 10-39: Summary table of evidence for aspiration hazard

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations	Reference
No specific studies on aspiration hazard are available, no evidence of this hazard for fluopicolide				

10.13.1 Short summary and overall relevance of the provided information on aspiration hazard

No specific studies on aspiration hazard are available. However, on the basis of existing animal studies and expert judgment that takes into account surface tension, water solubility, boiling point, volatility and chemical structure (fluopicolide is not an hydrocarbon, primary alcohol or ketone) aspiration hazard is not expected.

10.13.2 Comparison with the CLP criteria

An aspiration hazard is indicated at a kinematic viscosity of ≤ 20.5 mm^2/s at 40 °C. Measurements for kinetmatic viscosity are not available for fluopicolide. However, on the basis of existing animal studies and expert judgment that takes into account surface tension, water solubility, boiling point, volatility and chemical structure (fluopicolide is not an hydrocarbon, primary alcohol or ketone) aspiration hazard is not expected.

10.13.3Conclusion on classification and labelling for aspiration hazard

A classification for aspiration hazard according to the CLP guidance (Guidance to Regulation (EC) No 1272/2008 on classification, labelling and packaging (CLP) of substances and mixtures Version 5.0 July 2017) is **not warranted**.

11 EVALUATION OF ENVIRONMENTAL HAZARDS

Environmental hazards are **not** assessed in this dossier. Only health hazards are assessed.

11.1 Rapid degradability of organic substances

Please refer to Section 11.

11.1.1 Ready biodegradability

Please refer to Section 11.

11.1.2 BOD_5/COD

Please refer to Section 11.

11.1.3 Hydrolysis

Please refer to Section 11.

11.1.4 Other convincing scientific evidence

Please refer to Section 11.

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

Please refer to Section 11.

11.1.4.2 Inherent and enhanced ready biodegradability tests

Please refer to Section 11.

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

Please refer to Section 11.

11.1.4.4 Photochemical degradation

Please refer to Section 11.

11.2 Environmental transformation of metals or inorganic metals compounds

Please refer to Section 11.

11.2.1 Summary of data/information on environmental transformation

Please refer to Section 11.

11.3 Environmental fate and other relevant information

Please refer to Section 11.

11.4 Bioaccumulation

Please refer to Section 11.

11.4.1 Estimated bioaccumulation

Please refer to Section 11.

11.4.2 Measured partition coefficient and bioaccumulation test data

Please refer to Section 11.

11.5 Acute aquatic hazard

Please refer to Section 11.

11.5.1 Acute (short-term) toxicity to fish

Please refer to Section 11.

11.5.2 Acute (short-term) toxicity to aquatic invertebrates

Please refer to Section 11.

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

Please refer to Section 11.

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

Please refer to Section 11.

11.6 Long-term aquatic hazard

Please refer to Section 11.

11.6.1 Chronic toxicity to fish

Please refer to Section 11.

11.6.2 Chronic toxicity to aquatic invertebrates

Please refer to Section 11.

11.6.3 Chronic toxicity to algae or other aquatic plants

Please refer to Section 11.

11.6.4 Chronic toxicity to other aquatic organisms

Please refer to Section 11.

11.7 Comparison with the CLP criteria

Please refer to Section 11.

11.7.1 Acute aquatic hazard

Please refer to Section 11.

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

Please refer to Section 11.

11.8 CONCLUSION ON CLASSIFICATION AND LABELLING FOR ENVIRONMENTAL HAZARDS

Please refer to Section 11.

12 EVALUATION OF ADDITIONAL HAZARDS

Additional hazards are **not** assessed in this dossier. Only health hazards are assessed.

12.1 Hazardous to the ozone layer

Please refer to Section 12.

12.1.1 Short summary and overall relevance of the provided information on ozone layer hazard

Please refer to Section 12.

12.1.2 Comparison with the CLP criteria

Please refer to Section 12.

12.1.3 Conclusion on classification and labelling for hazardous to the ozone layer

Please refer to Section 12.

13 ADDITIONAL LABELLING

Not applicable

14 REFERENCES

A full reference list is provided in a separate confidential annex to this CLH report

15 ANNEXES

Annex I Annex I to CLH Report

Annex I to the CLH Report is provided separately.

Annex II Evaluation of the proposed MoA of fluopicolide according to the IPCS/human Framework method

Postulated MOA

The liver findings after fluopicolide treatment in mice were very similar to that demonstrated for phenobarbital and many other non-genotoxic compounds which cause liver tumours in rodents. *In vitro* studies in mouse and human hepatocytes showed that the key event of CAR/PXR activation which lead to liver cell proliferation and eventually to liver tumors in rodents, is not relevant to humans in which CAR induction and liver cell proliferation do not occur after exposure to fluopicolide. Thus, the postulated MOA for fluopicolide-induced liver tumours is mediated by CAR/PXR activation which in rodents leads to hyperplasia and eventually to liver tumours and is not relevant for humans.

Key events

The key and associative events as listed in Section 10.9.3.3 were demonstrated for the liver tumour MOA of fluopicolide.

Key event 1: Activation of CAR/PXR nuclear receptor

This MOA is starting with the activation of CAR and also PXR which was demonstrated in the described *in vivo* MOA study in which clear evidence of liver enzyme induction (AE 1) was seen. Total cytochrome P-450 content was markedly increased with the highest increases of enzymatic activity being PROD (+1143%) and BROD activities (+1785%) at 3,200 ppm (same dose as highest dose in chronic toxicity study).

This is further supported by the observed centrilobular hypertrophy (AE 2) in the liver of mice already observed at 400 ppm in the oncogenicity study, at 320 and 800 ppm in the subchronic studies and at 640 ppm in the subacute study. Therefore, it is assumed that liver enzyme induction starts at lower doses than the other effects of the MOA sequence. This supports the MOA as a consequence of liver-enzyme-mediated effects.

Key event 2: Altered gene expression secondary to CAR activation

The CAR/PXR activation subsequently leads to an increased activation of CYP2A and CYP3A enzymes, which was also demonstrated in the 28-day MOA study and the *in vitro* studies in mouse hepatocytes. The CAR/PXR activation is the first key event at an early time-point, with the subsequent events following later.

Key event 3: Increased hepatocellular proliferation

The activation of CAR and also PXR and subsequently increased activation of CYP2A and CYP3A enzymes was followed by cell proliferation as demonstrated in the *in vivo* and *in vitro* studies. A transiently increased cell proliferation was demonstrated after 7 days exposure, the mean BrdU labelling index was approximately 6.5-times higher in 3,200 ppm males and females than in controls. At terminal sacrifice afer 28-days, there was no increased hepatocellular proliferation in treated animals observed. The mean BrdU labeling index was even slightly lower in treated animals, when compared to controls. Moreover, the PCNA assessment on liver tissue from animals at 3,200 ppm in the second subchronic mouse study showed that fluopicolide did not produce hepatocellular proliferation on Day 90. This is completely consistent with the lack of cell proliferation

observed on Day 28 with the BrdU assessment. These findings emphasize that a transient liver cell proliferation followed by a return to control levels is a prerequisite for the development of hepatocellular adenoma following a long term exposure period to fluopicolide.

The *in vitro* studies supported the CAR-mediated action since hepatocellular proliferation was observed in WT cells but not in CARKO cells. Human hepatocytes did not display proliferation after fluopicolide thus demonstrating human non-relevance.

Key event 4: Increased clonal expansion, leading to altered foci

Markedly elevated incidences of altered foci (especially acidophilic) were observed in the chronic mouse study after 78 weeks at the highest tested dose of 3,200 ppm in males and females.

Key event 5: Increased incidence of hepatocellular tumours

This key event represents the occurrence of liver adenomas as observed in a mouse oncogenicity study due to the events described before.

In order to further corroborate the proposed liver tumour MOA the parameters dose and time concordance were evaluated.

Dose concordance

The repeated dose toxicity studies in mice demonstrated a clear dose-response relationship of the key events. In this regard the first key event was liver enzyme induction measured by PROD and BROD and/or liver hypertrophy and liverw eight increase, starting already at 400 ppm in the oncogenicity study, at 800 ppm in the subchronic study and at 640 ppm in the subacute study. This was followed by induction of altered liver foci and finally liver adenomas only at 3,200 ppm in the chronic study. Liver cell necrosis was never seen at any dose up to 6,400 ppm in the standard toxicity studies. An overview about the dose concordance of these events is given in the following table.

Table 15-1: Dose concordance of main liver effects

Study type / **Effects**	**Dose level [ppm]**										
	6	**32**	**50**	**64**	**200**	**320**	**400**	**640**	**800**	**3,200**	**6,400**
Subacute mouse toxicity											
Hepatocellular hypertrophy	NE	-	-	NE	-	-	-	↑ (m/f)	-	-	↑ (m/f)
Liver weight	NE	-	-	NE	-	-	-	↑ (f)	-	-	↑ (m/f)
Liver cell necrosis	NE	-	-	NE	-	-	-	NE	-	-	NE
Subchronic mouse toxicity (Crl: CD-1 (ICR) BR)											
Hepatocellular hypertrophy	-	NE	-	-	-	↑ (m/f)	-	-	-	↑ (m/f)	↑ (m/f)
Liver weight	-	NE	-	-	-	NE	-	-	-	↑ (m/f)	↑ (m/f)
Liver cell necrosis	-	NE	-	-	-	NE	-	-	-	↑ (f)	↑ (m/f)

Subchronic mouse toxicity (C57BL/6)											
Hepatocellular hypertrophy	-	-	NE	-	NE	-	-	-	↑ (m/f)	↑ (m/f)	-
Liver weight	-	-	NE	-	NE	-	-	-	↑ (m/f)	↑ (m/f)	-
Liver cell necrosis	-	-	NE	-	NE	-	-	-	NE	NE	-
Liver cell proliferation (PCNA)	-	-	-	-	-	-	-	-	-	NE	-
Mouse oncogencity study											
Hepatocellular hypertrophy (52 weeks)	-	-	NE	-	-	-	↑ (m/f)	-	-	↑ (m/f)	-
Hepatocellular hypertrophy (78 weeks)	-	-	NE	-	-	-	↑ (m/f)	-	-	↑ (m/f)	-
Altered foci (52 weeks)	-	-	NE	-	-	-	NE	-	-	NE	
Altered foci (78 weeks)	-	-	NE	-	-	-	NE	-	-	↑ (m/f)	-
Liver cell necrosis (52 weeks)	-	-	NE	-	-	-	NE	-	-	NE	-
Liver cell necrosis (78 weeks)	-	-	NE	-	-	-	NE	-	-	NE	-
Liver weight (52 weeks)	-	-	NE	-	-	-	↑ (m)	-	-	↑ (m/f)	-
Liver weight (78 weeks)	-	-	NE	-	-	-	↑ (m/f)	-	-	↑ (m/f)	-
Hepatocellular adenomas (52 weeks)	-	-	NE	-	-	-	NE	-	-	↑ (f)	-
Hepatocellular adenomas (78 weeks)	-	-	NE	-	-	-	NE	-	-	↑ (m/f)	-

m: male, f: female

- : dose not tested

NE: no effect

Temporal relationship

It is obvious from the fluopicolide study data that early key events occur before the formation of liver adenomas. Thus, activation of the nuclear CAR/PXR receptors and liver enzyme induction occurs in the studies as early as after already 7-day treatment as observed in the MOA study. Cell proliferation typically also occurs early in this MOA cascade, which is the case in the MOA study with fluopicolide in which liver cell proliferation and increased incidence of mitotic cells was seen after 7 days at 3,200 ppm. An increased incidence of mitotic cells was also seen after 28 days. However, at terminal sacrifice afer 28 days, there was no increased hepatocellular proliferation in treated animals observed. Moreover, the PCNA assessment on liver tissue from animals at 3,200 ppm in the second subchronic mouse study showed that fluopicolide did not produce hepatocellular proliferation on Day 90 which is in agreement with the transient induction of cell proliferation known from phenobarbital. The next step in this cascade of key events is the transition to hyperplasia and eventually to liver tumours. A significantly increased incidence of hepatocellular adenoma was observed at 3,200 ppm at 52 weeks in females only and at 78 weeks in both males and females in the mouse carcinogenesis study. Therefore, the time concordance observed for fluopicolide is in agreement with the postulated MOA. This can be seen in the following table which gives an overview of the time concordance of the relevant events and parameters.

Table 15-2: Time concordance of main events in liver

Time/ Effect	7 days (MOA study)	28 days (MOA study)	28 days (subacute study)	90 days (subchronic study)	90 days (subchronic study)	52 weeks (oncogenicity study)	78 weeks (oncogenicity study)
Cyp2b/Cyp3a (CAR/PXR activation)	↑ (f, 3200 ppm)	↑ (f, 3200 ppm)	-	-	-	-	-
Liver weight	↑ (f, 3200 ppm)	↑ (f, 3200 ppm)	↑ (f ≥640 ppm; m 6400 ppm)	↑ (m/f≥3200 ppm)	↑ (m/f ≥800 ppm)	↑ (m≥400 ppm; f 3200 ppm)	↑ (m/f≥400 ppm)
Liver cell hypertrophy	↑ (f, 3200 ppm)	↑ (f, 3200 ppm)	↑ (m/f≥ 640 ppm)	↑ (m/f≥320 ppm)	↑ (m/f ≥800 ppm)	↑ (m/f≥400 ppm)	↑ (m/f≥400 ppm)
Liver single cell necrosis	↑ (f, 3200 ppm)	NE	NE	↑ (f≥3200 ppm; m 6400 ppm)	NE	NE	NE
Liver increased mitotic cells	↑ (f, 3200 ppm)	↑ (f, 3200 ppm)	NE	NE	NE	NE	NE
Liver cell proliferation (PCNA/BrdU)	↑ (f, 3200 ppm)	NE	-	-	NE	-	-
Liver altered foci	NE	NE	NE	NE	NE	NE	↑ (m/f, 3200 ppm)
Liver adenomas	NE	NE	NE	NE	NE	↑ (f, 3200 ppm)	↑ (m/f, 3200 ppm)

m: male, f: female
- : dose not tested
NE: no effect

Strength, consistency and specificity of association of key events and tumour response

For a MOA to be accepted, it must be demonstrated that the key events are causally related to the formation of tumors, that the key events are actually required steps that lead to tumors, and that the data are reproducible. All of key events were observed in one or more studies. The early key events and subsequent events followed the dose and time concordance of the postulated MOA.

Biological plausibility and coherence

The liver is the most common target tissue in chronic toxicity and carcinogenicity studies since it is the first organ to be exposed after absorption and the major site of metabolization of xenobiotics. The MOA of liver tumour induction for fluopicolide is similar to the MOA of liver tumour induction of phenobarbital in rodents. Since this phenobarbital-type liver tumour MOA was intensively investigated and published it can be used as an example. Also phenobarbital leads to an activation of mainly CAR and CYP2B induction which is associated with liver weight increases, hepatocellular hypertrophy, proliferation of smooth endoplasmatic reticulum and cell proliferation as demonstrated by BrdU labelling and the eventual liver tumour formation. The importance of the CAR activation as the first key event in this cascade was proven in studies with fluopicolide and phenobarbital in CAR/PXR knockout mouse hepatocytes since in the CAR knockout cells no proliferation occurred. Moreover, neither fluopicolide nor phenobarbital induced replicative DNA synthesis in male or female human hepatocytes. Therefore, it can be assumed that the described MOA for liver tumour induction by fluopicolide and also the non-relevance for humans are well-supported.

Other modes of action

There was no evidence of a genotoxic potential of fluopicolide, so that this potential liver tumour MOA can be excluded.

Also PPARα or Arylhydrocarbon receptor mediated effects as possible alternative MOA could be excluded based on the results of the 28-day mechanistic study in mice since no relevant effects on EROD and Lauric acid, respectivel were noted.

Evidence of oxidative stress as possible MOA was not seen in the conducted toxicity studies with fluopicolide. Also liver cytotoxicity as a primary mode of action for the liver tumours can be excluded, since in the toxicity studies with fluopicolide in mice despite systemic effects at the highest doses, no typical signs of severe cytotoxicity in the liver, like inflammatory signs, broad hepatic necrosis, hepatocellular death, fibrosis, cirrhosis or severely increased transaminase activities were observed. Only some foci of single cell necrosis/apoptosis with minimal to moderate severity were seen in a few treated animals at interim sacrifice and at terminal sacrifice in the 28-day mechanistic study and in the first subchronic study at the highest tested dose levels.

Concordance table according to the 'International Programme on Chemical Safety (IPCS) Mode of Action Framework'

The described existing data were evaluated in this chapter according to the 'International Programme on Chemical Safety (IPCS) Mode of Action Framework' approach which leads to the conclusion that the liver adenomas after fluopicolide treatment in mice are not relevant to humans. A tabular summary is given in the following.

Table 15-3: Overview of fluopicolide liver tumour MOA

Key events	- CAR/PXR activation as demonstrated by increased PROD and BROD conversation activity (upregulated CYP2B and CYP3A protein/enzyme activity) - Transiently ncreased liver cell proliferation after CAR and PXR activation - CAR/PXR-mediated increased liver cell proliferation in rodents leads to regenerative hyperplasia and eventually to liver tumours
Concordance of dose-response relationship	- In repeated dose toxicity studies with fluopicolide, dose-dependent increases in liver weight and hepatocellular hypertrophy as evidence of enzyme induction - In the mouse oncogenicity study, liver adenomas occurred with increased incidences only at the highest dose at which a higher liver workload due to xenobiotic metabolism can be assumed
Temporal association	All phases of tumour development are in good agreement with the temporal succession of the MOA steps: - Early start of CAR/PXR activation after 7 days of treatment - Liver cell proliferation as demonstrated by BrdU incorporation or increased incidence of mitotic cells after 7- and 28-day treatment with fluopicolide - Some liver effects at doses of 3200 ppm already after 7 days and at later time points - altered liver foci and adenomas after longer treatment duration, after 52 weeks liver adenomas only in females and after 78 weeks in both male and female animals in the mouse oncogenicity study
Strength, consistency and specificity of association of tumour response with key events	- The importance of CAR/PXR activation as key event for the liver tumour induction was clearly demonstrated in special 28-day MOA studies in mice - In vitro studies in hepatocytes of wildtype mice and CAR/PXR-knockout mice showed cell proliferation in hepatocytes of wildtype mice only, but did not show a liver cell proliferating potential in hepatocytes of CAR/PXR KO mice clearly confirming that the liver tumour formation is CAR/PXR-mediated - In vitro studies in human hepatocytes with fluopicolide did not show a liver cell proliferating potential which confirms that human hepatocytes are not sensitive to this liver tumour MOA and thus that this MOA is not relevant to humans

Biological plausibility and coherence	- Succession of key events and liver tumour development in rodents is in agreement with knowledge about biological and morphological processes in the liver - The proposed MOA of rodent-specific liver tumour development is in agreement with broadly accepted knowledge that increased cell proliferation is the main underlying process which leads to development of tumours by non-genotoxic compounds - Well-documented support of the rodent-specific liver tumour MOA of fluopicolide by many other CAR/PXR-activating compounds, e.g. phenobarbital which cause liver tumours via a similar MOA, based on the literature - Since phenobarbital is used in human medicine since decades and did not lead to an increased liver tumour incidence in humans, this demonstrates that this MOA has no relevance to humans
Other possible MOAs	- The main other possible MOA, i.e. by genotoxicity, can be excluded since the genotoxicity testing of fluopicolide did not indicate a genotoxic potential - the 28-day mechanistic study demonstrated absence of a peroxisome-proliferating or AhR-mediating effect - Oxidative stress or severe liver cytotoxicity as a mode of action for the liver tumours alone can be excluded, since in the toxicity studies with fluopicolide in mice no signs of severe cytotoxicity, like inflammatory signs, broad hepatic necrosis, hepatocytic death, fibrosis, cirrhosis or severely increased transaminase activities were observed
Uncertainties, inconsistencies and data gaps	- No inconsistencies since there is a clear concordance between dose- and time-relationship of MOA key events and mouse liver adenoma increases - Although in vitro data from CAR/PXR -knockout hepatocytes are available which show clear involvement of CAR/PXR in the liver tumour MOA, in vivo studies in CAR/PXR-knockout animals would be additional information
Assessment of postulated mode of action	- High reliability of MOA since very good concordance between dose, temporality, and the expected sequence of events for tumourigenicity in the liver and agreement with broad database of published work in this area - Further support of high reliability of MOA due to very good agreement with the MOA of other compounds, like phenobarbital which showed the same concordance between dose, temporality, and the expected sequence of events for tumour induction in the rodent liver - This rodent-specific liver tumour MOA is not relevant to humans, based on the in vitro hepatocyte studies with human hepatocytes, which is also supported by published scientific research and on the fact that phenobarbital which has the same MOA and is used in human medicine since decades, epidemiologically did not show any effect on human tumour incidences