CLH report

Proposal for Harmonised Classification and Labelling

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

Chemical name: pethoxamid (ISO); 2-chloro-N-(2-ethoxyethyl)-N-(2-methyl-1-phenylprop-1-enyl)acetamide

EC Number:

CAS Number: **106700-29-2**

Index Number: **616-145-00-3**

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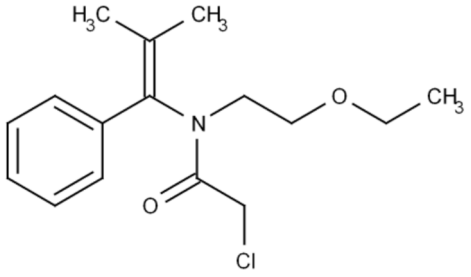
CONTENTS

1 IDENTITY OF THE SUBSTANCE

1.1 Name and other identifiers of the substance

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

Name(s) in the IUPAC nomenclature or other international chemical name(s)	2-Chloro-*N*-(2-ethoxyethyl)-*N*-(2-methyl-1-phenylprop-1-enyl) acetamide
Other names (usual name, trade name, abbreviation)	-
ISO common name (if available and appropriate)	Pethoxamid
EC number (if available and appropriate)	
EC name (if available and appropriate)	-
CAS number (if available)	106700-29-2
Other identity code (if available)	CIPAC number 665
Molecular formula	$C_{16}H_{22}ClNO_2$
Structural formula	
SMILES notation (if available)	CCOCCN(C(=O)CCl)C(=C(C)C)C1=CC=CC=C1
Molecular weight or molecular weight range	295.8
Information on optical activity and typical ratio of (stereo) isomers (if applicable and appropriate)	-
Description of the manufacturing process and identity of the source (for UVCB substances only)	-
Degree of purity (%) (if relevant for the entry in Annex VI)	*[The minimum and maximum values should be specified.]*

1.2 Composition of the substance

Pethoxamid as manufactured includes no isomers or additives.

Table 2: Constituents (non-confidential information)

Constituent (Name and numerical identifier)	Concentration range (% w/w minimum and maximum in multi-constituent substances)	Current CLH in Annex VI Table 3.1 (CLP)	Current self-classification and labelling (CLP)
Pethoxamid (CAS: 106700-29-2)	Minimum content of Pethoxamid in Pethoxamid Technical is 945 g/kg (94.5%) based on full scale production. Nominal content of Pethoxamid in Pethoxamid Technical is 973 g/kg (97.3%) (range 960 to 982 g/kg, mean 973 g/kg, SD 8.6) based on full scale production.	Acute Tox 4*, H302 Skin Sens., 1 , H317 Aquatic Acute 1, H400 Aquatic Chronic 1, H410	Acute Tox 4, H302 Skin Sens., 1 , H317 Aquatic Acute 1, H400 Aquatic Chronic 1, H410

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

Impurity (Name and numerical identifier)	Concentration range (% w/w minimum and maximum)	Current CLH in Annex VI Table 3.1 (CLP)	Current self-classification and labelling (CLP)	The impurity contributes to the classification and labelling
Toluene	< 3g/kg (0.3%)	Flam. Liq. 2, H225 Skin Irrit. 2, H315 Asp. Tox. 1, H304 STOT SE 3, H336 STOT RE 2*, H373** Repr. 2, H361***		

Toluene has an existing entry in Annex VI of CLP. However, given the concentration at which this impurity is present and the available data on pethoxamid, this is not considered to impact on the classification proposed in this dossier.

A number of confidential impurities are present; however none of these are relevant for the classification of the substance.

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

Additive (Name and numerical identifier)	Function	Concentration range (% w/w minimum and maximum)	Current CLH in Annex VI Table 3.1 (CLP)	Current self-classification and labelling (CLP)	The additive contributes to the classification and labelling
None	-	-	-	-	-

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

Identification of test substance	Purity	Impurities and additives (identity, %, classification if available)	Other information	The study(ies) in which the test substance is used
-	-	-	The purity of pethoxamid tested in the studies ranged from 92.6% - 98.6% w/w.	-

Identification of test substance	Purity	Impurities and additives (identity, %, classification if available)	Other information	The study(ies) in which the test substance is used
			Information on the actual purity used is provided in the relevant summaries and tables of this report. The tested material in all cases is considered to be equivalent to and representative of that specified above.	

2 PROPOSED HARMONISED CLASSIFICATION AND LABELLING

2.1 Proposed harmonised classification and labelling according to the CLP criteria

Table 6:

	Index No	International Chemical Identification	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors 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	616-145-00-3	pethoxamid (ISO); 2-chloro-N-(2-ethoxyethyl)-N-(2-methyl-1-phenylprop-1-enyl)acetamide		106700-29-2	Acute Tox. Cat 4* Skin Sen. Cat 1 Aquatic Acute 1 Aquatic Chronic 1	H302 H317 H400 H410	GHS07 GHS09 Wng	H302 H317 H410		M=100	
Dossier submitters proposal	616-145-00-3	pethoxamid (ISO); 2-chloro-N-(2-ethoxyethyl)-N-(2-methyl-1-phenylprop-1-enyl)acetamide		106700-29-2	**Retain** Aquatic Acute 1 Aquatic Chronic 1 **Modify** Acute Tox. Cat 4 Skin Sen. Cat 1A	**Retain** H302 H317 H400 H410	**Retain** GHS07 GHS09 Wng	**Retain** H302 H317 H410		**Retain** M=100 **Add** oral: ATE = 983 mg/kg bw M=10	
Resulting Annex VI entry if agreed by RAC and COM	616-145-00-3	pethoxamid (ISO); 2-chloro-N-(2-ethoxyethyl)-N-(2-methyl-1-phenylprop-1-enyl)acetamide		106700-29-2	Acute Tox. Cat 4 Skin Sen. Cat 1A Aquatic Acute 1 Aquatic Chronic 1	H302 H317 H400 H410	GHS07 GHS09 Wng	H302 H317 H410		oral: ATE = 983 mg/kg bw M=100 M=10	

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

Hazard class	Reason for no classification	Within the scope of consultation
Explosives	Data lacking	Yes
Flammable gases (including chemically unstable gases)	Hazard class not applicable (solid)	-
Oxidising gases	Hazard class not applicable (solid)	-
Gases under pressure	Hazard class not applicable (solid)	-
Flammable liquids	Hazard class not applicable (solid)	-
Flammable solids	Data conclusive but not sufficient for classification	Yes
Self-reactive substances	Data lacking	Yes
Pyrophoric liquids	Hazard class not applicable (solid)	-
Pyrophoric solids	Data conclusive but not sufficient for classification	Yes
Self-heating substances	Data lacking	Yes
Substances which in contact with water emit flammable gases	Data conclusive but not sufficient for classification	Yes
Oxidising liquids	Hazard class not applicable (solid)	-
Oxidising solids	Data conclusive but not sufficient for classification	Yes
Organic peroxides	Data conclusive but not sufficient for classification	Yes
Corrosive to metals	Data lacking	Yes
Acute toxicity via oral route	**Harmonised classification proposed**	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	Yes
Skin sensitisation	**Harmonised classification proposed**	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	Hazard class not applicable (solid)	-
Hazardous to the aquatic environment	**Harmonised classification proposed**	Yes
Hazardous to the ozone layer	Data conclusive but not sufficient for classification	Yes

3 HISTORY OF THE PREVIOUS CLASSIFICATION AND LABELLING

Pethoxamid was included in ATP01 (Regulation (EC) 790/2009) of Regulation (EC) 1272/2008.

Classification:
Acute Tox 4*, H302
Skin Sens., 1, H317
Aquatic Acute 1, H400
Aquatic Chronic 1, H410

The data and the carcinogenic properties of pethoxamid were extensively discussed in the EFSA peer review of the pesticide risk assessment of the active substance pethoxamid (EFSA, 2017), mainly considering whether or not a phenobarbital mode of action was sufficiently demonstrated, and whether it can be considered relevant to humans or not. During the meeting, a slight majority of the experts considered that pethoxamid should not be classified as carcinogenic. After further consideration of the existing knowledge, the opinions were evenly divided in a post-meeting consultation. As a consequence, EFSA considers that the proposed classification Carcinogen category 2 should apply to pethoxamid (EFSA, 2017). It is noted that the Rapporteur Member State (RMS) disagreed, and is of the opinion that classification for carcinogenic effects is not necessary. Subsequent to this decision, additional mechanistic data has been generated that support the proposed phenobarbital mode of action, and discount alternatives.

Proposed classification according to Regulation (EC) No 1272/2008 on the classification (CLH Regulation), labelling and packaging of substances and mixtures.

Classification:
Acute Tox 4, H302
Skin Sens., 1A , H317
Aquatic Acute 1, H400
Aquatic Chronic 1, H410

Justification:
H302: based on the results of the acute oral toxicity study in rats showing an LD50 of 983 mg/kg bw (Anonymous, 1994 – 63 PXA). In addition, the removal of the minimum classification for acute toxicity is also proposed as modification of the existing entry.

H317: based on the results of the skin sensitization study in guinea pigs (maximisation test) showing a clear evidence of skin sensitizing effects (Anonymous, 1998 -68 PXA). Therefore, a 1A sub-categorisation is proposed as a modification of the existing entry.

H400/H410: Based on the study with the algae *Pseudokirchneriella subcapitata* (Anonymous, 1999 -158 PXA, and Anonymous, 2016-158 PXA supplementary report 1 and 2) with the active substance an endpoint for acute and chronic classification was derived. Based on the results on growth rate an E_rC_{50} of 0.00408 mg a.s./L and an E_rC_{10} of 0.00119 mg a.s./L was determined. The active substance is classified as “not ready biodegradation”. The active substance, therefore, is proposed classification as aquatic hazardous, acute and chronic 1.

4 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

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

5 IDENTIFIED USES

Pethoxamid (Date of approval: 1st December 2018; Expiration of approval: 30th November 2033)[1] is intended to be used as a preemergence herbicide in soybeans and both a pre-emergence and early postemergence herbicide in maize for the control of mono and dicotyledonous weeds.

Pethoxamid, a member of the chemical class of the chloroacetamides, is a soil-active and selective herbicidal compound, taken up primarily by the roots, but also by the hypocotyls and the cotyledons of young seedlings.

[1] EU Pesticides Database (v.2.2) Active substance (europa.eu)

Although the precise mode of action of pethoxamid at molecular level seems to be not completely decoded, pethoxamid is assumed to act as an inhibitor of enzyme(s) involved in the de-novo biosynthesis of fatty acids with an alkyl chain longer than C18, mainly on the elongation step.

6 DATA SOURCES

The present evaluation exclusively relies on data submitted in the context of the application for approval as an active substance under Regulation 1107/2009.

7 PHYSICOCHEMICAL PROPERTIES

The physico-chemical properties of pethoxamid are summarised below. Reference should be made to the Draft Renewal Assessment Report (dRAR) Volume 3, Annex B.2; Physical and Chemical properties, August 2016 (dRAR, 2016).

All studies were conducted to appropriate quality standards and were considered adequate during the peer review.

Table 8: Summary of physicochemical properties

Property	Value	Reference	Comment (e.g. measured or estimated)
Physical state at 20°C and 101,3 kPa	Pethoxamid pure grade is a 'glassy' large crystalline solid being off white (Munsell N 8.5/68.4% R). Pethoxamid technical grade is a crystalline red-brown (Munsell 5 YR 4/4) solid. Under some circumstances technical grade pethoxamid can also be a viscous liquid. Pethoxamid pure grade has no odour, while Pethoxamid technical grade has a sweet odour.	Anonymous, (1999) 33 PXA	ASTM D1535-98 Batch: TP-980714 Purity: ≥ 99.9% Batch: TP-960418A Purity: ≥ 99.9% Batch: TB- 960306-C, Purity: 94.8%
Melting/freezing point	Melting point 37-38°C.	Anonymous, (1999) 33 PXA	EEC A1 (melting point apparatus) Batch: TP- 980714 Purity: ≥99.9%
Boiling point	N/A (possible chemical change from ca. 135°C followed by decomposition from ca. 200°C.)	Anonymous, (1999) 33 PXA	EEC A2, ASTM E537-6 (differential scanning calorimetry) Batch: TP- 980714 Purity: ≥99.9%
Relative density	1.19	Anonymous, (1999) 33 PXA	EEC A3 (pycnometer method) Batch: TP- 980714 Purity: ≥99.9%
Vapour pressure	2.8×10^{-3} Pa at 25°C 1.6×10^{-3} Pa at 20°C	Anonymous, (2015a) 1403 PXA Anonymous,(2015a)	EEC A4 (vapour pressure balance), OECD 104 Batch: P1351- BKA- 89 Purity: 99.8%

Property	Value	Reference	Comment (e.g. measured or estimated)
	Calculated using the data and equations from study 1403 PXA	1415 PXA	
	Using the vapour pressure and water solubility values the Henry's law constant was determined as 1.18 x 10^{-3} Pa m³/mol at 20°C.	Anonymous,(2015a) 1415 PXA	Henry's law calculation Batch: P1351- BKA- 89 Purity: 99.8%
	Henry's law constant 7.5 x 10^{-11} atm-m^3/mol at 25°C	Anonymous, (1999) 33 PXA	Bond contribution method.
Surface tension	90% saturated solution 53.0 mN/m at 20.0°C. Pethoxamid is surface active.	Anonymous,(2015b) 1401 PXA	EEC A5, OECD 115 Batch: P1351-BKA-89 Purity: 99.8%
	53.2 mN/m for a 90% saturated aqueous solution at 19.5°C.	Anonymous, (1999) 33 PXA	EEC A5 (harmonised ring) Batch: TB- 960306-C Purity: 94.8%
Water solubility	0.400 g/L at 20°C (distilled water pH 7.0-7.2)	Anonymous, (2015c) 1404 PXA + 1404 amdt 1 PXA	EEC A6 (Shake flask method), OECD 105 Determination by RP HPLC/UV Batch: P1351- BKA- 89 Purity: 99.8%
	0.401 g/L at 20°C	Anonymous, (1999) 33 PXA	EEC A6 (Shake flask method) Determination by HPLC Batch: TP- 980714 Purity: ≥99.9%
Partition coefficient n-octanol/water	Log P_{ow} = 2.963 ± 0.02 at 20°C (pH 5) Investigation into the effect of pH was not necessary because compound is not ionized between pH 4 and 10.	Anonymous, (1996) 41 PXA	EEC A8, OECD 107 (Shake flask method) Batch: TP- 940421 Purity: ≥99.9%
Flash point	182°C at 1008 mbar (100800 Pa)	Anonymous, (1999) 33 PXA	EEC A9 (closed cup) Batch: TB- 960306-C Purity: 94.8%
Flammability	Due to the low melting point of pethoxamid, it is not possible to perform this test. Therefore, the flash point test was performed for liquids.	-	-
Explosive properties	Tested for heat, shock and friction sensitivity. Pethoxamid is not explosive.	Anonymous, (1999) 33 PXA	EEC A14 Batch: TB- 960306-C Purity: 94.8%
Self-ignition temperature	Auto-flammability: 299°C at 1015 mbar (101500 Pa)	Anonymous, (1999) 33 PXA	EEC A15, ASTM E659-78 Batch: TB- 960306-C Purity: 94.8%

Property	Value	Reference	Comment (e.g. measured or estimated)
Oxidising properties	Pethoxamid was found to be non-oxidising.	Anonymous,(2015d) 1402 PXA	EEC A17 Batch: P1351-JaKT2- 23-6 Purity: 96.2%
	This test could not be performed due to the low melting point of pethoxamid.	Anonymous, (1999) 33 PXA	EEC A17
Granulometry	No data	-	-
Stability in organic solvents and identity of relevant degradation products	Solubility determined at 20°C Acetone >250 g/L Dichloromethane >250 g/L Ethyl acetate >250 g/L Isopropyl alcohol >250 g/L n-Heptane 114-133 g/L n-octanol >250 g/L Toluene >250 g/L	Anonymous, (2013), 792 PXA	CIPAC MT 181 Batch: P1351- JaK-T2-23-6 Purity: 95.8%
Dissociation constant	This value was not determined because Pethoxamid is not ionized between a pH of 4 and 10. Therefore, the pKa is not of environmental relevance	N/A	N/A
Viscosity	N/A (solid)	-	-

Pethoxamid pure grade is a 'glassy' large crystalline solid being off white with no odour. Pethoxamid technical grade is a crystalline red-brown solid and has a sweet odour. Pure grade pethoxamid has a melting point of 37-38°C and decomposes at 200°C. It is moderately soluble in water and readily soluble in organic solvents. The UV/VIS absorption maximum is at 243 nm (ε = 12200 L mol-1 cm-1), and there is still some absorption at 290 nm (ε = 85 L mol-1 cm-1). The flash point is 182°C at 1008 mbar, it is not flammable, not explosive and non-oxidising.

8 EVALUATION OF PHYSICAL HAZARDS

8.1 Explosives

Table 9: Summary table of studies on explosive properties

Method	Results	Remarks	Reference
ECC A14	ECC A.14 is not comparable to the test methods in Part I of the UNRTDG. Therefore data lacking.	-	(1999) 33 PXA

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

The explosive properties of pethoxamid have been tested according ECC A14. ECC A.14 is not comparable to the test methods in Part I of the UNRTDG.
Data lacking.

8.1.2 Comparison with the CLP criteria

A substance is considered for classification as an explosive substance where a positive result is obtained in the test series indicated in figure 2.1.2 of Annex I of the CLP regulation.

ECC A.14 is not comparable to the test methods in Part I of the UNRTDG.
Data lacking.

8.1.3 Conclusion on classification and labelling for explosive properties

Data lacking.

8.2 Flammable gases (including chemically unstable gases)

Not applicable.

8.3 Oxidising gases

Not applicable.

8.4 Gases under pressure

Not applicable.

8.5 Flammable liquids

Not applicable.

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

Not applicable.

8.5.2 Comparison with the CLP criteria

Not applicable.

8.5.3 Conclusion on classification and labelling for flammable liquids

Not applicable.

8.6 Flammable solids

Table 10: Summary table of studies on flammable solids

Method	Results	Remarks	Reference
EEC A9 (closed cup)	182°C at 1008 mbar (100800 Pa)	-	(1999) 33 PXA

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

Due to the low melting point of pethoxamid, it is not possible to perform the test to assess flammable solids. Therefore, the flash point test was performed for liquids. Flash point was 182°C at 1008 mbar (100800 Pa).

8.6.2 Comparison with the CLP criteria

A substance is classified as a flammable liquid when the flash point is <60°C. It is concluded that pethoxamid is not highly flammable as it does not meet the criteria for classification as flammable liquid.

8.6.3 Conclusion on classification and labelling for flammable solids

Not classified (conclusive but not sufficient for classification).

8.7 Self-reactive substances

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

The substance shall not be considered for classification as 'self-reactive', if it is (will be) classified as explosive, oxidising liquid or solid or organic peroxide. No valid data for explosive properties, no data for self-reactive substances available. Data lacking.

8.7.2 Comparison with the CLP criteria

The substance shall not be considered for classification as 'self-reactive', if it is (will be) classified as explosive, oxidising liquid or solid or organic peroxide. No valid data for explosive properties, no data for self-reactive substances available. Data lacking.

8.7.3 Conclusion on classification and labelling for self-reactive substances

Data lacking.

8.8 Pyrophoric liquids

Not applicable.

8.9 Pyrophoric solids

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

A study was not necessary due to practical experience in handling and use.

8.9.2 Comparison with the CLP criteria

The substance is known to be stable in contact with air at room temperature for prolonged periods of time, therefore the criteria for classification are not met.

8.9.3 Conclusion on classification and labelling for pyrophoric solids

Not classified (conclusive but not sufficient for classification).

8.10 Self-heating substances

Table 11: Summary table of studies on self-heating substances

Method	Results	Remarks	Reference
EEC A15 (autoflammability)	According to the CLP	-	(1999)

Method	Results	Remarks	Reference
	Regulation, self-heating properties are tested using methods given in Part III, sub-section 33.3.1.6 of the UN RTGD; results from test method EC A.15 are not sufficient to conclude on this hazard class. Therefore data lacking.		33 PXA

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

According to the CLP Regulation, self-heating properties are tested using methods given in Part III, sub-section 33.3.1.6 of the UN RTGD; results from test method EC A.15 are not sufficient to conclude on this hazard class. Therefore data lacking.

8.10.2 Comparison with the CLP criteria

According to the CLP Regulation, self-heating properties are tested using methods given in Part III, sub-section 33.3.1.6 of the UN RTGD; results from test method EC A.15 are not sufficient to conclude on this hazard class. Therefore data lacking.

8.10.3 Conclusion on classification and labelling for self-heating substances

Data lacking.

8.11 Substances which in contact with water emit flammable gases

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

No test is necessary since pethoxamid does not contain metals or metalloids. Further experience in handling and use indicates that it will not emit flammable gases on contact with water.

8.11.2 Comparison with the CLP criteria

The substance does not meet the criteria for classification.

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

Not classified (conclusive but not sufficient for classification).

8.12 Oxidising liquids

Not applicable.

8.13 Oxidising solids

Table 12: Summary table of studies on oxidising solids

Method	Results	Remarks	Reference
EEC A17	According to the CLP Regulation, oxidising properties are tested using UN test O.1 or O.3	-	(2015d) 1402 PXA

Method	Results	Remarks	Reference
	see CLP Annex I, 2.14.2.1. Results from test method EC A.17 are not sufficient to conclude on this hazard class. The hazard class can be assessed also based on the screening procedure see criteria in CLP Annex I, 2.14.4.1. 2.14.4.1.For organic substances or mixtures the classification procedure for this class shall not apply if: (a) the substance or mixture does not contain oxygen, fluorine or chlorine; or (b) the substance or mixture contains oxygen, fluorine or chlorine and these elements are chemically bonded only to carbon or hydrogen. In this case (b) applies. Therefore, the criteria for classification are not met.		

8.13.1 Short summary and overall relevance of the provided information on oxidising solids

According to the CLP Regulation, oxidising properties are tested using UN test O.1 or O.3 see CLP Annex I, 2.14.2.1. Results from test method EC A.17 are not sufficient to conclude on this hazard class. The hazard class can be assessed also based on the screening procedure see criteria in CLP Annex I, 2.14.4.1.

2.14.4.1. For organic substances or mixtures the classification procedure for this class shall not apply if:

(a) the substance or mixture does not contain oxygen, fluorine or chlorine; or

(b) the substance or mixture contains oxygen, fluorine or chlorine and these elements are chemically bonded only to carbon or hydrogen.

In this case (b) applies. Therefore, the criteria for classification are not met.

8.13.2 Comparison with the CLP criteria

According to the CLP Regulation, oxidising properties are tested using UN test O.1 or O.3 see CLP Annex I, 2.14.2.1. Results from test method EC A.17 are not sufficient to conclude on this hazard class. The hazard class can be assessed also based on the screening procedure see criteria in CLP Annex I, 2.14.4.1.

2.14.4.1. For organic substances or mixtures the classification procedure for this class shall not apply if:

(a) the substance or mixture does not contain oxygen, fluorine or chlorine; or

(b) the substance or mixture contains oxygen, fluorine or chlorine and these elements are chemically bonded only to carbon or hydrogen.

In this case (b) applies. Therefore, the criteria for classification are not met.

8.13.3 Conclusion on classification and labelling for oxidising solids

Not classified (conclusive but not sufficient for classification).

8.14 Organic peroxides

Not applicable, pethoxamid does not contain peroxo moieties.

8.15 Corrosive to metals

8.15.1 Short summary and overall relevance of the provided information on corrosive to metals

The screening procedure for this hazard class is based on melting point and the chemical nature of the substance. According to the CLP guidance 2.16.4.1, solids having a melting point lower than 55 °C as well as substances or mixtures containing halogen should be tested. Both is the case with pethoxamid. No test has been provided.

8.15.2 Comparison with the CLP criteria

The screening procedure for this hazard class is based on melting point and the chemical nature of the substance. According to the CLP guidance 2.16.4.1, solids having a melting point lower than 55 °C as well as substances or mixtures containing halogen should be tested. Both is the case with pethoxamid. No test has been provided.

8.15.3 Conclusion on classification and labelling for corrosive to metals

Data lacking.

9 TOXICOKINETICS (ABSORPTION, METABOLISM, DISTRIBUTION AND ELIMINATION)

Table 13: Summary table of toxicokinetic studies

Method	Results	Remarks	Reference
Metabolism in the rat OECD 417 (1984) GLP	Extensive absorption (>80%) is demonstrated; a significant proportion of absorbed radioactivity (74%) was excreted in bile. There is no potential for accumulation, but evidence of erythrocyte binding. Toxicologically significant compounds were identified as pethoxamid and MET-42, the main metabolite. The study was performed with a single (ring) label, however there is no evidence of cleavage and this was therefore considered appropriate.	Weight variation of animals exceeds 20%	Anonymous, 2000 60 PXA

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

The absorption, distribution, excretion and metabolism of pethoxamid in rats were investigated with the active substance radiolabelled in the phenyl ring (C-label) (Anonymous, 2000- 60 PXA).

The biliary excretion investigation indicated greater than 80% absorption following oral administration at a low (8 mg/kg bw) dose level. A comparison of the data from the low (8 mg/kg bw) and high dose (300 mg/kg

bw) oral studies on intact animals show that the excretion pattern was similar to that of the bile-duct cannulated animals and hence the absorption would also be similar; these data also indicate the presence of enterohepatic recirculation. A comparison of the areas under the curves (AUC) at the low and high doses indicated that the pharmacokinetic parameters were not dose-dependent, which is in agreement with the excretion data. Similarly, the Cmax and AUC data for males and females showed no significant differences.

Tissue distribution after a single dose was similar for males and females and the pattern of distribution in the two dose levels was proportionately similar. There was significant uptake in the red blood cells and a terminal half-life for whole-blood concentrations of 122–149 hours compared to 41–47 hours for plasma radioactivity. There was no suggestion of accumulation in tissues, however, there was a possible indication of binding to red blood cells.

In the low and high single dose studies and the multiple dose (8 mg/kg bw/d for 14 days), elimination mostly occurred during 0-48 hours. The majority of the radioactivity for all three dosing regimes was excreted via the faeces (53-63 % within 96 hours).

Pethoxamid was extensively metabolised by glutathione-S-transferase to the cysteine conjugates, which were then further oxidised to sulphoxides and sulphones. Further metabolism of these moieties also occurred.

There were no findings from the toxicokinetic studies that might influence the proposed classification of pethoxamid.

10 EVALUATION OF HEALTH HAZARDS

The acute toxicity of pethoxamid has been investigated by the oral, dermal and inhalation routes. *In vitro* and *in vivo* irritation studies are available, and skin sensitisation has been investigated in a guinea pig maximisation test.

Acute toxicity

10.1 OECD Acute toxicity - oral route

The acute oral toxicity of pethoxamid was investigated in a traditional acute oral toxicity study in rats.

Table 14: Summary table of animal studies on acute oral toxicity

Method, guideline, deviations if any	Species, strain, sex, no/group	Dose levels, Test substance details	Value LD50	Remarks	Reference
Acute oral toxicity study OECD 401 GLP	Rat Hsd/Ola:Sprague Dawley (CD) 2/sex/group (prelim study) 5/sex/group (main study) Observation period: 14 days	Prelim study: 2500 mg/kg bw Main Study: 800, 1260, 2000 mg/kg bw administered as supplied Dose volume: 0.72, 1.13, 1.8 ml/kg Batch: TB-930727 Purity: 95%	**Males :** 983 (623 – 1360) mg/kg bw **Females:** 1472 (1039-2235) mg/kg bw	Recovery of surviving rats was complete by day 3 (females at 800 mg/kg bw), day 4 (1260 mg/kg bw) or day 6 (males at 800 mg/kg bw). At 2000 mg/kg bw, the surviving female showed clinical signs until day 15.	Anonymous, (1994a) 63 PXA

Table 15: 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 relevant studies				

Table 16: 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 relevant studies				

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

In an acute oral toxicity study in rats (OECD 401), a dose of 2500 mg/kg bw was initially administered by oral gavage to 2 males and 2 females in a preliminary study. At 2500 mg/kg bw 3/4 rats died (or were sacrificed for humane reasons) on day 2 after dosing. In the main study, groups of fasted rats (5 males and 5 females) received doses of 800, 1260 or 2000 mg/kg bw. At 2000 mg/kg bw, deaths occurred in 5/5 males and 4/5 females between days 1 and 3. At 1260 mg/kg bw, deaths occurred in 3/5 males and 2/5 females on days 2 or 3. At 800 mg/kg bw, deaths occurred in 2/5 males on day 2, no females died prior to scheduled termination.

Clinical signs included piloerection, abnormal body carriage, abnormal gait, lethargy, decreased respiratory rate, partially closed eyelids, pallor of extremities, soft/liquid faeces and increased salivation in all or the majority of rats. Unsteadiness, body tremors, dilation of pupils, cold to touch and ano-genital staining was observed in rats at 1260 and 2000 mg/kg bw. In the one surviving rat dosed at 2000 mg/kg bw, clinical signs persisted until day 15. Recovery of surviving rats was complete by day 3 (females at 800 mg/kg bw), day 4 (all rats at 1260 mg/kg bw) or day 6 (males at 800 mg/kg bw). The mean body weights of surviving animals increased within the normal range by day 15. There were no abnormalities at macroscopic examination of animals, which survived until day 15.

However, in the mouse carcinogenicity study (see Section 10.9) the top dose was 983mg/kg bw/day which is equivalent to the lowest LD50 available. Despite long-term survivability is given at this dose in mice, proposed ATE for oral toxicity is still being based the most sensitive species (the rat).

10.1.2 Comparison with the CLP criteria

In accordance with the CLP criteria, substances are classified for acute oral toxicity if the LD50 value is ≤2000 mg/kg bw. In order to be classified with acute toxicity category 4 (oral), the lowest category for this endpoint, the LD50 must fall between the following range: 300 < LD50 ≤ 2000 mg/kg bw. Since, in the available study, the LD50 value of pethoxamid was 1196 mg/kg bw (combined sexes), it is concluded that the substance does meet the criteria for classification (Category 4). Accordingly, based on the LD50 for male rats, an oral ATE = 983 mg/kg bw is proposed.

10.1.3 Conclusion on classification and labelling for acute oral toxicity

Acute Tox 4, H302 Harmful if swallowed; ATE = 983mg/kg bw

10.2 Acute toxicity - dermal route

The acute dermal toxicity of pethoxamid was investigated in one study in rats.

Table 17: Summary table of animal studies on acute dermal toxicity

Method, guideline, deviations if any	Species, strain, sex, no/group	Dose levels, Test substance details	Value LD50	Remarks	Reference
Acute dermal toxicity study OECD 402 GLP	Rat Hsd/Ola:Sprague Dawley (CD) 5/sex/group	2000 mg/kg bw administered as supplied Dose volume: 1.8 ml/kg	> 2000 mg/kg bw	No deaths. No signs of systemic toxicity or skin irritation. No adverse	Anonymous, (1994b) 64 PXA

Method, guideline, deviations if any	Species, strain, sex, no/group	Dose levels, Test substance details	Value LD50	Remarks	Reference
	Observation period: 14 days	Applied for 24 h Batch: TB-930727 Purity: 95%		macroscopic necropsy findings.	

Table 18: 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 relevant studies				

Table 19: 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 relevant studies				

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

In an acute dermal toxicity study, rats were exposed to a single limit dose of 2000 mg/kg pethoxamid for 24 hours under a semi-occlusive dressing. The application area comprised at least 10% of the total body surface area. At the end of the 24-hour exposure period, the dressing was removed and the application site was rinsed with warm water. Skin effects were monitored soon after removal of the dressing and daily thereafter. There were no deaths, signs of systemic toxicity or local skin effects observed. The mean body weights of the animals increased within the normal range throughout the study period and there were no adverse macroscopic findings at necropsy on day 15.

10.2.2 Comparison with the CLP criteria

In accordance with the CLP criteria, substances are classified for acute dermal toxicity if the LD50 value is ≤ 2000 mg/kg. Since in the available study the LD50 value of pethoxamid was > 2000 mg/kg, it is concluded that the substance does not meet the criteria for classification.

10.2.3 Conclusion on classification and labelling for acute dermal toxicity

Not classified (conclusive but not sufficient for classification).

10.3 Acute toxicity - inhalation route

A limit test at the maximum attainable concentration has been conducted in rats to investigate the acute toxicity of pethoxamid by the inhalation route.

Table 20: Summary table of animal studies on acute inhalation toxicity

Method, guideline, deviations if any	Species, strain, sex, no/group	Dose levels, Test substance details	Value LD50	Remarks	Reference
Acute inhalation toxicity study OECD 403	Rat Sprague Dawley 5/sex/group	Aerosol Control: air Test: 4.16 mg/L (analysed	LD50 > 4.16 mg/L (highest attainable concentration)	No deaths Clinical signs included matted fur, partially	Anonymous, (1994) 65 PXA

Method, guideline, deviations if any	Species, strain, sex, no/group	Dose levels, Test substance details	Value LD50	Remarks	Reference
GLP	4 h whole body exposure Observation period: 14 days	concentration), 4.38 mg/L (gravimetric concentration) MMAD = 3.3µm GSD = 2.14 Batch: TB-930727 Purity: 95%		closed eyes, wetness/staining around the eyes, snout and mouth, exaggerated respiratory movements (seen in 1 male), and were observed from 1 hour to 8 days after exposure. There were no treatment related macroscopic or microscopic necropsy findings.	

Table 21: 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 relevant studies				

Table 22: 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 relevant studies				

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

In an acute inhalation study, rats were exposed in a whole body system for four hours to a maximum attainable concentration of pethoxamid as a liquid droplet aerosol (analysed concentration of 4.16 mg/L air). There were no deaths. General indications of toxicity (partially closed eyes, wet /stained fur, matted fur) and respiratory effects that are commonly associated with the inhalation route of exposure were observed between one hour and 8 days after the exposure in the treated group. No clinical symptoms were recorded in the controls. All treated animals showed recovery from day 6 in males or day 8 in females. Body weights increased as expected from day two onwards and there were no treatment related macroscopic or microscopic findings at necropsy. Lung weight to body weight ratios in the treated rats were similar to the ratios for the control rats.

10.3.2 Comparison with the CLP criteria

In accordance with the CLP criteria, a substance is classified for acute inhalation toxicity if the 4 h LC50 value is less than 5 mg/L. Since in the available study the LC50 value of pethoxamid was > 4.16 mg/L (the highest attainable analysed concentration), it is concluded that the substance is of low acute toxicity by the inhalation route and does not meet the criteria for classification.

10.3.3 Conclusion on classification and labelling for acute inhalation toxicity

Not classified (conclusive but not sufficient for classification).

10.4 Skin corrosion/irritation

The skin irritation potential of pethoxamid was assessed in a GLP compliant rabbit study.

Table 23: Summary table of animal studies on skin corrosion/irritation

Method, guideline, deviations if any	Species, strain, sex, no/group	Dose levels duration of exposure	Results -Observations and time point of onset -Mean scores/animal -Reversibility	Reference
Acute dermal irritation in rabbits OECD 404 GLP	Rabbit New Zealand White 6 males Observation period: 5 days	0.5 mL applied to intact skin for 4 hours under a semi-occlusive dressing. Batch: TB-930727 Purity: 95%	No signs of toxicity. Very slight erythema present on day 1 in 6/6 animals (approximately 30 min after removal of the dressings). Very slight erythema present in 1 rabbit only, on days 2, 3 & 4. Very slight oedema present in 1 rabbit only, on days 2 & 3. All reactions had resolved by Day 5. Mean scores (averaged over 24, 48 & 72 hours) for each animal: 0, 0, 0, 0, 1, 0 for erythema 0, 0, 0, 0, 0.66, 0 for oedema Not a skin irritant.	Anonymous, (1994a) 66 PXA

Table 24: 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 relevant studies				

Table 25: 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 relevant studies				

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

In a rabbit skin irritation study, 6 male rabbits were exposed to 0.5 mL pethoxamid under a semi-occlusive dressing for 4 hours. Very slight erythema (score 1) was present on day 1 in all 6 animals (approximately 30 mins after removal of the dressings). Very slight erythema and very slight oedema was present in 1/6 rabbits on days 2 & 3. All reactions had resolved by Day 5. No other cutaneous reactions and no signs of toxicity were observed during the study. Mean scores over 24, 48 and 72 hours for each animal were 0.0, 0.0, 0.0, 0.0, 1.0 and 0.0 for erythema and 0.0, 0.0, 0.0, 0.0, 0.66 and 0.0 for oedema.

10.4.2 Comparison with the CLP criteria

A substance is classified as a skin irritant category 2 if any of the following criteria are met:

(1) mean value of ≥ 2.3 - ≤ 4.0 for erythema/eschar or for oedema in at least 2 of 3 tested animals from gradings at 24, 48 and 72 hours after patch removal or, if reactions are delayed, from grades on 3 consecutive days after the onset of skin reactions; or

(2) inflammation that persists to the end of the observation period, normally 14 days in at least 2 animals, particularly taking into account alopecia (limited area), hyperkeratosis, hyperplasia, and scaling; or

(3) in some cases where there is pronounced variability of response among animals, with very definite positive effects related to chemical exposure in a single animal but less than the criteria above.

Since, in the available study, the average scores for each animal were 0 or 1 for erythema and 0 or 0.66 for oedema, none of these criteria was met. It is concluded that pethoxamid is without skin irritating properties and does not meet the criteria for classification.

10.4.3 Conclusion on classification and labelling for skin corrosion/irritation

Not classified (conclusive but not sufficient for classification).

10.5 Serious eye damage/eye irritation

The eye irritation potential of pethoxamid has been investigated in a GLP-compliant test in rabbits.

Table 26: Summary table of animal studies on serious eye damage/eye irritation

Method, guideline, deviations if any	**Species, strain, sex, no/group**	**Dose levels duration of exposure**	**Results** **- Observations and time point of onset** **- Mean scores/animal** **- Reversibility**	**Reference**
Acute eye irritation in rabbits OECD 405 GLP	Rabbit New Zealand White 6 rabbits (1 rabbit treated initially) Observation period: 7 days	0.1 mL instilled in one eye (after warming in a water bath) Batch: TB-930727 Purity: 95%	1 hour after instillation: dulling of the cornea (present in 5/6), conjunctival reddening, chemosis and discharge (present in 6/6 rabbits). Mean scores (averaged over 24, 48 & 72 hours) for each animal: 0, 0, 0, 0, 0, 0 for corneal opacity 0, 0, 0, 0, 0, 0 for iris lesions 0.3, 0, 0.3, 0, 0.3, 0 for conjunctival chemosis 0.3, 0.3, 0.3, 0, 0.3, 0.3 for conjunctival redness 0, 0, 0, 0, 0, 0 for conjunctival discharge All reactions reversible within 48 hours after application.	Anonymous, (1994b) 67 PXA

Table 27: 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 relevant studies				

Table 28: 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 relevant studies				

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

In a rabbit eye irritation study, pethoxamid was warmed in a water bath and 0.1 mL place into the lower everted lid of one eye of 6 rabbits. One rabbit was treated first to assess the severity of responses seen. Animals were observed for 7 days and ocular responses assessed using a handheld light. There were no signs of gross toxicity, adverse clinical signs or abnormal behaviour following administration of the test substance.

Dulling of the cornea was seen in 5/6 animals at 1 hour after instillation. Diffuse conjunctival redness (grade 2), obvious conjunctival chemosis (grade 2) and conjunctival discharge with moistening of the eyelids (grade 2/3) were noted in all six animals at 1 hour after instillation.

At 24 hours after instillation, slight conjunctival chemosis (grade 1) was noted in 3/6 rabbits and slight conjunctival redness (grade 1) was noted in 5/6 rabbits. There were no signs of ocular irritation in any animals at 48 hours after instillation. The mean scores calculated for each animal over 24, 48 and 72 hours were 0.0, 0.0, 0.0, 0.0, 0.0 and 0.0 for corneal opacity and iris lesions, 0.3, 0.0, 0.3, 0.0, 0.3, 0.0 for conjunctival chemosis and 0.3, 0.3, 0.3, 0, 0.3, 0.3 for conjunctival redness. The ocular reactions were fully reversible within 48 hours after application.

10.5.2 Comparison with the CLP criteria

In accordance with CLP, a substance is classified as an eye irritant category 2 if, when applied to the eye of an animal, the substance produces in at least 2 of 3 tested animals a positive response of:

- corneal opacity ≥ 1 and/or
- iritis ≥ 1, and/or
- conjunctival redness ≥ 2 and/or
- 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.

In the rabbit test with pethoxamid, the individual and overall mean eye irritation scores (24 to 72 hours) were 0.0 for corneal opacity and iris lesions. The mean scores for conjunctival redness and chemosis for each animal were 0.3 or 0.0 and were thus less than those that trigger classification. Furthermore, the reactions seen were fully reversible within 48 hours. It is concluded that pethoxamid is without eye irritating properties and does not meet the criteria for classification.

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

Not classified (conclusive but not sufficient for classification).

10.6 Respiratory sensitisation

Table 29: Summary table of animal studies on respiratory sensitisation

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance,	Dose levels, duration of exposure	Results	Reference
No relevant studies					

Table 30: Summary table of human data on respiratory sensitisation

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

Table 31: Summary table of other studies relevant for respiratory sensitisation

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

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

No studies available.

10.6.2 Conclusion on classification and labelling for respiratory sensitisation

Because of the lack of data, a definitive conclusion on respiratory sensitisation cannot be made.

10.7 Skin sensitisation

The skin sensitisation potential of pethoxamid was assessed in a GLP compliant guinea-pig maximisation test. This included a concurrent positive control, hexyl cinnamic aldehyde (HCA), a known sensitiser, to confirm the sensitivity of the method.

Table 32: Summary table of animal studies on skin sensitisation

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
Guinea-pig maximisation test (GPMT) OECD 406 GLP	Guinea pigs Dunkin-Hartley 60 Males, 4 groups: Gp 1 (control) - 20 Gp 2 (Test) - 20 Gp 3 (control for positive control) 10 Gp 4 (HCA) - 10	Intra-dermal induction: 0.5% in Alembicol D adjuvant/0.9% aqueous NaCl Topical induction: as supplied Topical Challenge: 25% and 12.5% in Alembicol D Batch: TB-960306 Purity: 95%	Responses after challenge: Dermal reactions seen in 19/20 treated animals (Gp 2) were more marked than in the control group (Gp 1). **19/20 positive responses**. HCA: Marked dermal reactions were observed in 10/10 animals in the positive control group (Gp 4) compared to 0/10 in control group (Gp 3), confirming the sensitivity of the test.	Anonymous, (1998) 68 PXA

Table 33: Summary table of human data on skin sensitisation

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

Table 34: 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 relevant studies				

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

In the available guinea-pig maximisation test (GPMT), the induction and challenge concentrations of pethoxamid used were determined from a preliminary study where a range of dilutions of pethoxamid were assessed. Alembicol D (a product of coconut oil) was used as a vehicle for intra-dermal injection and topical challenge. In the preliminary test, two animals were used to determine the highest concentration of pethoxamid that caused irritation suitable for the intra-dermal induction and 4 animals were used to determine the maximum non –irritant concentration for the challenge application. These animals were pre-treated with Freund's complete adjuvant, 50:50 with water by intra-dermal injection approximately 1 week prior to the preliminary tests. Based on the results, a concentration of 0.5% (v/v) in Alembicol D was selected for intradermal induction, test substance as supplied for the topical induction and concentrations of 25 and 12.5% (v/v) in Alembicol D for topical challenge.

In the main study, animals in the test and control groups showed slight irritation at the sites of injections with pethoxamid 0.5% (v/v) in Alembicol D or Alembicol D only. The test animals all showed slight (11/20), well-defined (8/20) or moderate erythema (1/20) following topical induction. No erythema was seen in the control guinea pigs. Following the challenge exposure, 4/20 control animals showed slight erythema at 24 or 48 hours. In the test group, 19 /20 animals showed skin reactions at 24 and/or 48 hours, which were more marked than those seen for controls and are considered positive responses. The remaining animal showed a similar response to the controls and is a negative response. As 95% of animals gave a positive response in this test, it is concluded that a skin sensitisation potential was demonstrated under the conditions of the study.

10.7.2 Comparison with the CLP criteria

The sub-categorisation of skin sensitisers on the basis of a GPMT is illustrated in the table below.

Concentration for intradermal induction (% v/v)	Incidence sensitised guinea pigs (%)	Potency	Predicted subcategory
≤ 0.1	≥ 60	Extreme	1A
≤ 0.1	≥ 30 - < 60	Strong	1A
>0.1 - ≤ 1.0	≥ 60	Strong	1A
>0.1 - ≤ 1.0	≥ 30 - < 60	Moderate	1B
> 1.0	≥ 30	Moderate	1B

In the case of the GPMT study with pethoxamid, 95% of the test animals responded to a 0.5% intradermal induction dose and therefore classification in sub-category 1A is warranted.

10.7.3 Conclusion on classification and labelling for skin sensitisation

Skin Sens 1A; H317 – May cause an allergic skin reaction.

10.8 Germ cell mutagenicity

The genotoxic potential of pethoxamid has been investigated in a series of five *in vitro* studies and two *in vivo* studies, a mouse micronucleus test and a unscheduled DNA synthesis evaluation in the rat liver. Two new *in vitro* studies, a reverse mutation test in bacteria and a mammalian cell gene mutation assay (mouse lymphoma cells), utilised a new reference source of pethoxamid and demonstrated equivalence with the material tested in the older studies.

In vitro

The potential of pethoxamid to induce gene mutations in bacterial cells, gene mutation/clastogenicity in mammalian cells and clastogenicity/aneuploidy in mammalian cells has been investigated in *in vitro* studies.

Table 35: Summary table of mutagenicity/genotoxicity tests *in vitro*

Method, guideline, deviations if any	Test substance,	Test system (Organism, strain)	Conc tested (range)	Results		Remarks (information on cytotoxicity)	Reference
				-S9	+S9		
Bacterial reverse mutation assay OECD 471 / OECD472 Does not include full range of tester strains GLP	Pethoxamid Batch: TB-930727 Purity: 95% Solvent: DMSO	*Salmonella typhimurium* strains TA1535, TA1537, TA98 and TA100.	-/+ S9 mix: 50, 150, 500, 1500, 5000 µg/plate	-ve	-ve	Toxicity was observed following treatment with 5000 µg/plate.	Anonymous, (1994) 75 PXA
Bacterial reverse mutation assay OECD 471 GLP	Pethoxamid technical Batch: P1351-BKA-65 Purity: 93.5% Solvent: DMSO	*Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537 and *E. coli* WP2 uvrA.	-/+ S9 mix: Experiments I and II: 3.16, 10.0, 31.6, 100, 316, 1000, 2500, 5000 µg/plate.	-ve	-ve	Experiment I toxic effects ≥1000 µg/plate (-S9) and ≥2500 µg/plate (+S9), depending on strain. Experiment II toxic effects ≥316 µg/plate (-S9) and ≥1000 µg/plate (+S9), depending on strain.	Anonymous, (2012) 647 PXA
In vitro chromosome aberration test in human lymphocytes. OECD 473 GLP	Pethoxamid Batch: TB-930727 Purity: 95% Solvent: DMSO	Cultured human lymphocytes. Without S9 exposure 18h. With S9 mix exposure 3h.	Test 1: - S9 mix: 2.0, 7.8, 15.6 µg/ml + S9 mix: 3.9, 15.6, 31.3 µg/ml Test 2: - S9 mix: 3.75, 20, 37.5 µg/ml + S9 mix: 7.5, 45, 80 µg/ml	+ve	+ve	Test 1: No cells survived, +/- S9 mix, at concentrations of ≥62.5 µg/ml. Test 2: Death of all cells was seen at a concentration of ≥75 µg/ml in the absence of S9 mix, and at 200 µg/ml in the presence of S9 mix.	Anonymous, (1994) 76 PXA

Method, guideline, deviations if any	Test substance,	Test system (Organism, strain)	Conc tested (range)	Results		Remarks (information on cytotoxicity)	Reference
				-S9	+S9		
In vitro mammalian cell gene mutation assay (MLA assay; thymidine kinase locus TK+/-) OECD 476 GLP	Pethoxamid technical Batch: P1351-BKA-65 Purity: 94.5% Solvent: RPMI (+5% horse serum) cell culture medium	Mouse lymphoma cell line L5178Y	Experiment I without metabolic activation: 25, 50, 100, 150, 200, 250, 300, 350 µM with metabolic activation: 50, 100, 150, 200, 250, 300, 350, 400, 450 µM Experiment II without metabolic activation: 0.5, 1, 5, 10, 25, 50, 75, 100 µM with metabolic activation: 60, 120, 170, 230, 280, 330, 360, 390 µM	-ve	-ve	Growth inhibition was observed in experiment I and II without and with metabolic activation. In experiment I without metabolic activation the relative total growth (RTG) was 16.7% for the highest concentration (350 µM) evaluated. The highest concentration evaluated with metabolic activation was 450 µM with a RTG of 8.7%. In experiment II without metabolic activation the relative total growth (RTG) was 11.6% for the highest concentration (100 µM) evaluated. The highest concentration evaluated with metabolic activation was 390 µM with a RTG of 10.0%.	Anonymous, (2015) 1449 PXA
In vitro mammalian cell gene mutation assay in V79 (HGPRT Test) OECD 476 GLP	Pethoxamid Batch: 0592 Purity: 98.6% Solvent: DMSO	Cultured mammalian cells (V79). Without S9 exposure 24h. With S9 mix exposure 2h.	- S9 mix: 1, 3, 10, 20, 30 µg/ml + S9 mix: 10, 30, 100, 200, 300 µg/ml	-ve	-ve	Cytotoxicity was observed starting at concentrations between 20 and 30 µg/ml without S9 mix and between 100 and 200 µg/ml with S9 mix.	Anonymous, (1992) 77 PXA

Five well-conducted, reliable *in vitro* studies have been conducted to investigate the *in vitro* genotoxic potential of pethoxamid in bacterial and mammalian cells. Cytotoxicity, which was evident in each test, indicated that adequate test concentrations had been used in all studies. Four of the five *in vitro* tests demonstrated negative results.

In the chromosome aberration test in human lymphocytes there were potential clastogenic effects with and without metabolic activation. In the first test no cells survived, both in the presence and absence of S9 mix, at concentrations of 62.5 µg/ml and above. In the second test, death of all cells was seen at a concentration of 75 µg/ml and above in the absence of S9 mix, and at 200 µg/ml in the presence of S9 mix. In the first test, in the absence of S9 mix, a statistically significant increase in chromosomal aberrations occurred at the highest dose level, 15.6 µg/ml, indicative of a clastogenic activity. In the presence of S9 mix, a statistically significant increase in chromosomal aberrations (gaps only) occurred at the intermediate dose level. In the second test, in the absence of S9 mix, statistically significant increases in chromosomal aberrations occurred at the intermediate (20 µg/ml) and high dose levels (37.5 µg/ml). In the presence of S9 mix, a statistically significant

increase in chromosomal aberrations occurred at all dose levels analysed. The positive control compounds produced clear increases in chromosome aberrations.

In vivo

A micronucleus test has been conducted to investigate the potential of pethoxamid to induce chromosomal damage in mice. An unscheduled DNA synthesis test was conducted in rats.

Table 36: Summary table of mutagenicity/genotoxicity tests in mammalian somatic or germ cells *in vivo*

Method, guideline, deviations if any	**Test substance,**	**Species, strain, sex**	**Relevant information about the study (as applicable)**	**Results**	**Reference**
Mouse micronucleus study OECD 474 1000 cells assessed current (2016) requirement 4000 per animal. Note both sexes used only 1 sex required. Information demonstrating bone marrow exposure to the test substance based on ADME studies. GLP	Pethoxamid Batch: TB-930727 Purity: 95% Vehicle: 1% aqueous methyl cellulose + 0.5% Tween 80	Mouse, CD-1 Swiss origin. Males and females	Single dose of 0, 320, 640 or 1280 mg/kg bw via oral gavage. Groups of 5 females and 5 males (where possible) were killed 24, 48 and 72 hours after treatment.	Eight female and 3 male mice died after treatment with the high dose. Clinical signs, within the first hour after dosing in all groups, and piloerection. Hunched posture also observed in the intermediate and high dose. Recovery within 3 h for low and intermediate dose group. High dose group also showed lethargy and ptosis, recovery of surviving mice by end of Day 1. No significant increase in the number of micronucleated immature erythrocytes observed at 24, 48, or 72 h. No effect on the proportion of immature erythrocytes, indicating no bone marrow toxicity.	Anonymous, (1994) 78 PXA
In vivo rat liver DNA repair test OECD 486 and OECD 482 GLP	Pethoxamid Batch: TB-930727 Purity: 95% Vehicle: 1% aqueous methyl cellulose + 0.5% Tween 80.	Rat, Albino Hsd/Ola Sprague-Dawley Males	Single doses of 600, 1200 (group, not evaluated for UDS) and 2000 mg/kg bw by oral gavage. Hepatocytes isolated 2 and 14 hours after the administration. 4 animals assessed at each timepoint.	High dose is limit dose for this study type. Slight piloerection observed at 600 and 2000 mg/kg bw, recovered by 2 h. Pethoxamid did not cause any significant increase in either the gross or net nuclear grain count at both dose levels at the 2 hours expression time. At the 14 hours expression time, statistically significant increases in gross nuclear grain counts were obtained at 600 and 2000 mg/kg bw but not in net nuclear grain counts, therefore considered not to indicate unscheduled DNA synthesis (considered indicative of an increased cell proliferation). Pethoxamid did not elicit unscheduled DNA synthesis in the rat liver.	Anonymous, (1994) 79 PXA

An *in vivo* micronucleus test was conducted in CD-1 mice. A single dose of 0, 320, 640 or 1280 mg/kg bw was administered. Groups of 5 females and 5 males were killed after 24, 48 and 72 hours. Exposure of the bone marrow to the test substance was demonstrated in an ADME study in rats (Anonymous, 2000 – 60 PXA), where pethoxamid could be detected in bone tissue after a single oral dose of 300 mg/kg bw, providing evidence of bone marrow exposure to the test substance. The vehicle (1% aqueous methyl cellulose and 0.5% Tween 80) served as the negative control and Mitomycin C as the positive control. The animals were sacrificed 24, 48 and 72 hours after test substance administration and bone marrow smears were prepared from each animal. For each animal, 1000 polychromatic erythrocytes were evaluated for micronuclei, therefore 10000 were scored per test group. The positive control Mitomycin C was only assessed at the 24 hour time point and showed the expected increase in micronuclei. No significant increase in the number of micronucleated immature erythrocytes was observed at 24, 48, or 72 hours. No effect was observed on the proportion of immature erythrocytes, indicating no bone marrow toxicity. The positive control group did show an increased frequency of micronucleated cells. In conclusion, pethoxamid did not show any evidence of chromosomal or other damage leading to micronucleus formation in this *in vivo* test.

In an *in vivo* investigation of unscheduled DNA synthesis, single doses of 600, 1200 (provisional group, not evaluated for unscheduled DNA synthesis) and 2000 mg pethoxamid/kg bw was administered. Groups of 4 rats were administered the test compound orally by intragastric gavage. Hepatocytes were isolated at 2 and 14 hours after the administration. Positive controls were included. Gross nuclear grain counts (silver grains overlying the nucleus) and net nuclear grain counts (cytoplasmatic grain count subtracted from gross nuclear grain count) were assessed. On completion of the grain count analysis, the stained autoradiographs from the 14 hours expression were recorded for S-phase cells (for each animal 1000 hepatocytes from several randomly fields of view). Pethoxamid did not cause any significant increase in either the gross or net nuclear grain count at both dose levels at the 2 hours expression time. At the 14 hours expression time, statistically significant increases in gross nuclear grain counts were obtained at dose levels of 600 and 2000 mg/kg bw but not in net nuclear grain counts, i.e. not indicative for unscheduled DNA synthesis. Positive control group animals showed the expected significant increase in the gross and net nuclear grain count. During analysis, it was noted that some slices had an unusually high number of S phase cells (easily recognizable by their heavily grained appearance). Therefore for all groups (including the provisional group) the S-phase cells after 14 hours of expression were determined. Only the group which received 600 mg/kg bw showed statistical significance. The increases of S-phase cells are indicative of an increased cell proliferation. In conclusion, pethoxamid did not elicit unscheduled DNA synthesis in the rat liver in this *in vivo* test system.

Table 37: 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 relevant studies				

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

Pethoxamid has been tested for genotoxic potential in a battery of *in vitro* assays and two *in vivo* studies. Both *in vivo* studies were found to be negative, with the *in vivo* mouse micronucleus study supporting the conclusion that the clastogenic effects observed a single *in vitro* study were not manifest *in vivo.* Four further *in vitro* assays were negative. In the peer review of the pesticide risk assessment of the active substance pethoxamid, EFSA states that 'On the basis of the available data, pethoxamid is concluded as unlikely to be genotoxic (EFSA, 2017).

10.8.2 Comparison with the CLP criteria

Pethoxamid has been assessed for a genotoxic potential in five *in vitro* assays and two *in vivo* guideline and GLP compliant assays. No germ cell mutagenicity studies are available with pethoxamid.

Category 2: Substances which cause concern for humans owing to the possibility that they may induce heritable mutations in the germ cells of humans

The classification in Category 2 is based on:

Positive evidence obtained from experiments in mammals and/or in some cases from in vitro experiments, obtained from:

- *somatic cell mutagenicity tests in vivo, in mammals; or*
- *other in vivo somatic cell genotoxicity tests which are supported by positive results from in vitro mutagenicity assays.*

Pethoxamid does not meet the criteria for Category 2 mutagen because four of five *in vitro* tests demonstrated negative results, only the in vitro chromosomal aberration test indicated potential clastogenic effects. In addition, both in vivo studies were found negative, including a mouse micronucleus study, supporting the conclusion that clastogenic effects are not manifest *in vivo*.

10.8.3 Conclusion on classification and labelling for germ cell mutagenicity

Not classified (conclusive but not sufficient for classification).

10.9 Carcinogenicity

The carcinogenic potential of pethoxamid in animals has been investigated in rats and mice.

Table 38: 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**
Rat chronic and carcinogenicity study via the oral (dietary) route OECD 453 + electron microscopy of liver. Deviations: MCH not presented; uterus not weighed; Coagulating gland and peripheral nerve not preserved. GLP Rat Crl: CD BR (IGS)	Pethoxamid Batch: TB-960306 Purity: 95.0% Dose levels: 0, 25, 400, 1600 ppm in diet for 104 weeks	1600 ppm (70 mg/kg bw/day in males and 99 mg/kg bw/day in females) ↓ Body weight gain: Week 0-4 15% males, 15% females; Week 0-88 13% males, 23% females ↓ Food consumption: Week 1-4 8% males, 6% females; Week 1-104 6% males Clinical chemistry: ↑ Cholesterol All timepoints 17-42% males, 33-46% females; ↓ Globulin First year of study 9-13% females; ↑ γ GT activity All timepoints 1.5-8 x control in males ↑ Liver weights: Adjusted weight 10 and 12% males Weeks 27 and 53; 17 and 16% females Weeks 27 and 105 ↑ Thyroid weights: Adjusted weight 50% and 31% males Weeks 53 and 79. No similar change Week 27 or 105. *Non-neoplastic findings* Liver pathology: Centrilobular hepatocyte hypertrophy 11/50 males, 8/50 females (0/50 controls); Concentric intracytoplasmic inclusions 10/50 males (0/50 controls); Cystic degeneration 24/50 males (12/50 controls); Clear cell hepatocytes 15/50 males (6/50 controls). Thyroid pathology: Not statistically significant. Follicular cell hyperplasia 4/50 males (0/50 controls); Follicular cell cystic hyperplasia 7/50 males (4/50 controls). 400 ppm (17.0 mg/kg bw/day in males and 23.3 mg/kg bw/day in females) ↓ Body weight gain: Week 0-88 11% females (not statistically significant but considered treatment related) ↓ Food consumption: Week 1-4 4% males	Anonymous, (2000a) 80 PXA (Anonymous; 2000-amdt-1; 80 PXA amdt-1) (Anonymous, 2003; 80 PXA suppl-1) (Anonymous; 2016; 80 PXA suppl-2) (Anonymous; 2003; 203 PXA)

<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>Main groups: 50 /sex/group

Up to 10 rats/sex/group examined after the completion of 26, 52 and 78 weeks of treatment.</td><td></td><td>↑ Thyroid weights: Adjusted weight 39% and 49% males Weeks 53 and 79.

<u>25 ppm (1.0 mg/kg bw/day in males and 1.4 mg/kg bw/day in females)</u>
↑ Thyroid weights: Adjusted weight 38% males Week 53.

The NOAEL was considered to be 25 ppm (1.0 mg/kg bw/day), based on the decreased body weight gain in females at 400 ppm.

Neoplastic findings
<table>
<tr><td>Finding</td><td colspan="4">Dietary Concentration (ppm)</td></tr>
<tr><td>MALES</td><td>0 (control)</td><td>25</td><td>400</td><td>1600</td></tr>
<tr><td>Thyroid follicular cell adenoma</td><td>2/50</td><td>2/50</td><td>2/49</td><td>9/50$</td></tr>
<tr><td>Thyroid follicular cell carcinoma</td><td>2/50</td><td>0/50</td><td>0/49</td><td>0/50</td></tr>
<tr><td>Pancreas islet cell adenoma</td><td>4/49</td><td>8/50</td><td>6/50</td><td>8/50</td></tr>
<tr><td>Pancreas islet cell carcinoma</td><td>0/49</td><td>2/50</td><td>1/50</td><td>2/50</td></tr>
</table>
Main study animals. $ trend test statistically significant

The only statistically significant evidence of tumourigenicity was a higher incidence of thyroid follicular cell adenoma in males at the high dose level of 1600 ppm. Mechanistic studies demonstrate that the mode of action is not relevant to humans.</td><td></td></tr>
<tr><td>Mouse carcinogenicity study via the oral (dietary) route.

OECD 453, however OECD 451 acceptable for second rodent species

Deviations from OECD 453: Slight exceedance of weight variation in females; no organ weights of spleen, uterus; no preservation of</td><td>Pethoxamid
Batch: TB-960306
Purity: 95.0% and
Batch: TB-960306-C
Purity: 94.8%.

Dose levels: 0, 30, 400, 5000 ppm in diet.</td><td><u>5000 ppm (982 mg/kg bw/day in males, 1068 mg/kg bw/day in females)</u>
↓ Bodyweight at termination: 16% males, 17% females
↓ Body weight gain: 40% males to Week 95; 34% females to Week 92
↑ Total food consumed: 21% males
↑ Liver weight: Terminal adjusted 77% males, 36% females
↑ Kidney weight: Terminal adjusted 23% females
↑ Thyroid weight: Terminal adjusted 29% females
↑ Adrenal weight: Terminal adjusted 26% males

Non-neoplastic findings
Liver pathology: Hepatocyte hypertrophy 39/50 males (generalised), 42/50 females (periportal) 0/50 in controls.
Kidney pathology <u>both sexes</u>: Cortical tubules basophilic 43/50 males (33/50 controls), 41/50 females (12/50 controls); Medullary tubules dilated with eosinophilic casts: 42/50 males (27/50 controls), 32/50 females (14/50 controls); Cortical mineralisation 46/50 males (32/50 controls), 26/50 females (3/50 controls); Medullary mineralisation 44/50 males (6/50 controls), 31/50 females (0/50 controls); Papillary mineralisation 36/50 males (11/50 controls), 30/50 females (3/50 controls).
Kidney pathology <u>males only</u>: Cortical tubular cell hypertrophy (slight) 8/50 (0/50 controls); Cortical fibrosis with tubular collapse</td><td>Anonymous; (2000b)

82 PXA

(Anonymous; 2016; 82 PXA suppl-1)

(Anonymous; 2001; 83 PXA)

(Anonymous; 2001; 1484 PXA)

(Anonymous; 2001; 1241 PXA)</td></tr>
</table>

<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>the coagulating gland, peripheral nerves; haematology-only blood smears; no clinical biochemistry.

GLP

Mouse
Crl: CD-1 BR

50/sex/group

Treated until one group in each sex reached 50% survival
i.e. up to 92 week (females) or 95 week (males).

Additional 10/sex/group treated for up to 52 weeks.</td><td></td><td>and basophilia 37/50 (13/50 controls); Cortical cysts 31/50 (20/50 controls).
Duodenum pathology: Swelling/rarefaction of villous epithelium 42/49 males (0/48 controls), 18/49 females (0/45 controls); Villous hypertrophy 27/49 males (0/48 controls).
Jejunum pathology: Swelling/rarefaction of villous epithelium 35/49 males (0/48 controls), 14/49 females (0/46 controls); Villous hypertrophy 16/49 males (0/48 controls).

<u>400 ppm (56.8 mg/kg bw/day in males, 68 mg/kg bw/day in females)</u>
↑ Liver weight: Terminal adjusted 14% males (not statistically significant), 10% females
↑ Kidney weight: Terminal adjusted 11% females

Non-neoplastic findings
Duodenum pathology: Swelling/rarefaction of villous epithelium 29/47 males (0/48 controls), 12/50 females (0/45 controls); Villous hypertrophy 9/47 males (0/48 controls).
Jejunum pathology: Swelling/rarefaction of villous epithelium 25/48 males (0/48 controls), 7/50 females (0/46 controls); Villous hypertrophy 8/48 males (0/48 controls).

<u>30 ppm (4.0 mg/kg bw/day in males, 5.0 mg/kg bw/day in females)</u>
Non-neoplastic findings
Duodenum Pathology: Swelling/rarefaction of villous epithelium 6/47 males at termination, 5/8 males at interim kill (0/48, 0/8 controls, respectively).

The LOAEL was considered to be < 30 ppm (<4 mg/kg bw/day).

Neoplastic findings:

Treatment-related increase in hepatocellular adenomas in male mice at 5000 ppm. Slightly higher (not statistically significant) incidence of hepatocellular carcinomas within historical control incidence.
<table>
<tr><td>Finding</td><td colspan="4">Dietary Concentration (ppm)</td></tr>
<tr><td>MALES</td><td>0 (control)</td><td>30</td><td>400</td><td>5000</td></tr>
<tr><td>Hepatocellular adenoma</td><td>19/50</td><td>15/50</td><td>18/50</td><td>34/50**</td></tr>
<tr><td>Hepatocellular carcinoma</td><td>3/50</td><td>3/50</td><td>4/50</td><td>6/50</td></tr>
</table>
Statistical significance: **P<0.01

The only evidence of tumourigenicity was an increased number of males showing benign hepatocellular liver tumours at the high dose of 5000 ppm. The no effect level for tumourigenicity was set at 400 ppm (56.8 mg/kg bw/day). Mechanistic studies demonstrate a phenobarbitone-like mode of action for pethoxamid.</td><td></td></tr>
</table>

Table 39: Summary table of human data on carcinogenicity

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

Table 40: Summary table of other studies relevant for carcinogenicity

Type of study/data	Test substance, species, strain, sex, no/group	Relevant information about the study (as applicable)	Observations	Reference
Additional study on cell proliferation in the livers taken from Carcinogenicity Study by dietary Administration to CD-1 Mice for at least 80 Weeks (Anonymous, 2000b; 82 PXA) GLP	Pethoxamid Batch: TB-960306 Purity: 95.0% and Batch: TB-960306-C Purity: 94.8%. Mouse Crl: CD-1 BR 8-10 male mice/group Dose levels: 0, 30, 400, 5000 ppm in diet for at least 80 weeks.	Original paraffin blocks used in the carcinogenicity study were subjected to immunohistochemistry for proliferating cell nuclear antigen (PCNA). PCNA labeling index (LI) was determined for each animal by counting the number of PCNA-positive (S-phase) cells per approximately 1000 hepatocytes.	Pethoxamid appeared to have no influence on cell proliferation in the liver when administered in feed to male mice at 30, 400 and 5000 ppm for 52 or 95 weeks.	Anonymous; (2001a) 1241 PXA
Evaluation of thyroid function using perchlorate discharge test. Non- guideline study. GLP	Pethoxamid Batch: TB-9603061 Purity: 94.8% Positive controls: Phenobarbital and Propylthiouracil Rat Sprague Dawley 16 males/group Dose levels: 0, 1600, 5000 ppm in diet for 28 days (0, 155, 462 mg/kg bw/day).	Blood samples taken before treatment and on days 12 and 24 for measurement of T3, T4 and TSH. After 28 days of treatment, sodium ^{125}Iodide was given i.p. to each animal. 6 h later potassium perchlorate and saline were given to 6 animals/group i.p. 2½ min later the rat was anaesthetised and blood sampled to measure radioactivity. Animals were killed, the thyroid removed and weighed and the total amount of radioactivity in the thyroid measured.	Elevated TSH, only statistically significant in the 1600 ppm group on day 12. No effects on T3 or T4. Pethoxamid did not cause a significant discharge of thyroid radioactivity by perchlorate; thus, the activity of thyroid peroxidases was not reduced. Comparing the data for pethoxamid with that for propylthiouracil, it suggests that pethoxamid did not directly affect the thyroid function. The data obtained for pethoxamid at the high dose level are similar to that for phenobarbitone (TSH levels, thyroid and whole-blood radioactivity); thus, the mechanism of action of pethoxamid is similar to that of phenobarbitone.	Anonymous; (2000) 94 PXA
Hepatic drug-metabolising enzyme induction and cell	Pethoxamid Batch: TB-9603061 Purity: 95% Mouse ICR (crj:CD-1)	Hepatic enzyme activity, PCNA labeling index and cell to cell communication in liver by evaluation of gap junction protein connexion 32 (Cx 32)	Liver weights increased at 5000 ppm. Microsomal protein and PROD activity increased in 5000 pm group. Cytochrome P-450 isoenzyme contents of CYP1A, CYP2B, CYP3A2	Anonymous; (2001b) 98 PXA

Type of study/data	Test substance, species, strain, sex, no/group	Relevant information about the study (as applicable)	Observations	Reference
proliferation study. Non- guideline study. GLP	18 males/group Dose levels: 0, 30, 400, 5000 ppm in diet for 14 days (0, 3.92, 49.1, 541 mg/kg bw/day).	spots per hepatocyte assessed. Measured after 3, 7 and 14 days.	and CYP4A1 increased in 5000 pm group. CYP2B most affected. Increase in PROD in the 400 ppm group. PCNA labeling index increased in 5000 ppm group after 3 and 7 days, but not after 14 days. Decrease in Cx 32 spots (20-30%) in 400 and 5000 ppm groups. Decrease in Cx 32 spots in 30 ppm group after 3 and 7 days, but not 14 days. Based on the results observed, the overall profile of effects are suggestive that pethoxamid may be a phenobarbital-type enzyme inducer which can increase cell proliferation in the liver during the initial stages of exposure when administered at 5000 ppm in the diet. In addition, the test substance may inhibit gap junctional intercellular communication (CJIC) in the liver.	
Evaluation of liver and thyroid effects and their potential reversibility after dietary exposure in mice and rats. Non- guideline study. GLP	Pethoxamid Technical Batch: P1351-Jak-T2-23-6 Purity: 92.6% Mice: CRL:CD 1(ICR) 8 male mice/group Mice dosed at 0, 400 and 5000 ppm for a period of 7 days (0, 82.1, 972 mg/kg bw/day main group). Rats: CRL: CD® Sprague Dawley Rats dosed at 0, 400 and 1600 ppm for a period of 14 days (0, 31.2, 131.6 mg/kg bw/day main group). 15 male rats/group	Mice: Livers weighed and histopathology (general and IHC) performed. BrdU administered to mice by osmotic pump, containing 15 mg/mL BrdU implanted subcutaneously. Incorporation of BrdU into hepatocytes was then measured using a mouse monoclonal anti-BrdU antibody and light microscopy. Rats: Serum thyroid hormones assessed. Thyroids and livers harvested. Thyroids weighed and histopathology (general and IHC) performed. Rats received 10 µg/hr BrdU. Incorporation of BrdU into hepatocytes was then measured using a mouse monoclonal anti-BrdU antibody and light microscopy.	Mice: <u>5000 ppm</u> ↑ Liver weight (absolute and relative) Demonstrated hepatocellular hypertrophy and an increase of the number of BrdU positive cells. Increases were completely reversible in recovery animals evaluated on Day 49. <u>400 ppm</u> minimal effects. Rats: <u>1600 ppm</u> ↑ Thyroid weight (absolute and relative) An increase of the BrdU labelling index in the thyroid gland. Increases were completely reversible in recovery animals evaluated on Day 56. Thyroid follicular epithelium hypertrophy (grade 1) was observed in 2/8. No observed in recovery animals. There were no noteworthy effects of pethoxamid on circulating thyroid hormone <u>400 ppm</u> minimal effects. Conclusion: Pethoxamid administered to mice in their diet at concentrations of 5000 ppm caused reversible changes in liver	Anonymous; (2016) 1538 PXA

Type of study/data	Test substance, species, strain, sex, no/group	Relevant information about the study (as applicable)	Observations	Reference
	Recovery after 42 days two groups per species (control and high dose).		weight and centrilobular hepatocytes including hypertrophy as well as increased hepatocyte proliferation. Pethoxamid administered to rats in their diet at concentrations of 1600 ppm caused increased absolute and relative thyroid weights and reversible increases in follicular thyroid cell proliferation.	
Ex vivo evaluation of liver microsomal Cytochrome P450 induction and UGT expression in rodents Non- guideline study. GLP	Liver samples for this study taken from Anonymous, 2016 (1538 PXA)	Evaluation of effect of Pethoxamid on liver microsomal cytochrome P450 (CYP) enzyme activity and mRNA levels in male mice. Evaluation of effect of Pethoxamid on liver microsomal uridine diphosphate glucuronosyltransferase (UGT) activity and mRNA levels toward the thyroid hormone thyroxine (T4) in male rats.	Mice: <u>5000 ppm</u> ↑ Cytochrome b5 content: 1.39-fold, ↑ Cytochrome P450 content 1.50-fold ↑ 7-ethoxyreorufin-O-dealkylation (Cyp1a1/2) activity: 1.54-fold ↑ testosterone 16β-hydroxylase (Cyp2b10) activity: 1.70-fold ↑ Cyp1a2 mRNA levels: 1.97-fold ↑ Cyp2b10 mRNA levels: 115-fold ↑ Cyp3a11 mRNA levels: 6.90-fold ↑ Cyp4a10 mRNA levels: 9.00-fold Following a 42 day recovery period, measured values were generally comparable between mice previously treated at 5000 ppm pethoxamid and concurrent controls. <u>400 ppm</u> ↑ Cytochrome P450 content: 1.22- fold ↑ 7-ethoxyreorufin-O-dealkylation (Cyp1a1/2) activity: 1.69-fold ↑ testosterone 16β-hydroxylase (Cyp2b10) activity: 1.46-fold ↑ testosterone 6β-hydroxylase (Cyp3a11/13) activity: 4.39-fold ↑ Cyp2b10 mRNA levels: 12.4-fold Rats: <u>1600 ppm</u> ↑ Cytochrome b5 content: 1.22-fold ↑ Cytochrome P450 content: 1. 63-fold ↑ thyroxine glucuronidase (UGT1A1/6) activity: 1.62-fold ↑ UGT1A1 mRNA levels: 1.23-fold ↑ UGT1A6 mRNA levels 3.78-fold Following a 42 day recovery period, measured values were generally comparable between rats previously treated at 1600 ppm pethoxamid and concurrent controls. <u>400 ppm</u> ↑ Cytochrome P450 content: 1.17-fold ↑ UGT1A6 mRNA levels: 1.82- fold	Anonymous; (2016) 1539 PXA

Type of study/data	Test substance, species, strain, sex, no/group	Relevant information about the study (as applicable)	Observations	Reference
			The most pronounced effects of pethoxamid were the statistically significant and dose-dependent increases in UGT1A6 mRNA levels in male rats and the statistically significant and dose-dependent increases in Cyp3a11/13 activity and Cyp2b10, Cyp3a11 and Cyp4a10 mRNA levels in male mice.	
Mode of action and human relevance analysis of rodent-specific tumors	Pethoxamid:	Position paper	Based on results from the repeat-dose toxicity and mechanistic studies, the data strongly support the MoA for pethoxamid induced male mouse liver tumours involving the activation of the nuclear hormone receptor CAR, which mediates the induction of replicative DNA synthesis in the liver. The proposed mouse liver tumor MoA satisfies the conditions of dose and temporal concordance, biological plausibility, coherence, strength, consistency and specificity as described in the IPCS framework. The proposed MoA for the male rat thyroid follicular cell adenomas is based on activation of CAR along with the induction of UGT. This results in disrupted thyroid hormone homeostasis, leading to thyroid follicular cell adenomas in the male rat. The increased tumour incidence in carcinogenicity studies performed with pethoxamid are not of relevance to humans.	Anonymous; (2016) 1540 PXA
Inhibition of thyroperoxidase (TPO) activity *in vitro* Non guideline study. GLP	Pethoxamid Technical. Batch: 21082018 Purity: 97.7% Positive control: TPO inhibitor 6-propyl-2-thiouracil (PTU)	The guaiacol assay of TPO activity was used for this study. Final concentrations: 0, 0.01, 0.1, 0.3, 1, 3, 10, 30, 100, 300 and 1000 µM tested in pooled thyroid microsomes prepared from male Sprague Dawley rats.	Pethoxamid did not inhibit TPO activity at any of the tested concentrations.	Anonymous; (2019) 2018TOX-PXA4481
90-day rat study to evaluate potential mechanisms underlying the observed	Pethoxamid Batch: 21082018 Purity: 99.4% Rat Crl:CD(SD)	Thyroid hormone assessed on Days -3, 15, 29, 57, and 89. Total T3 and T4, TSH and rT3 levels analysed.	<u>Pethoxamid</u> ↓ Body weight in the 5000 ppm group, 7.5% lower than controls at the end of study. ↑ Mean TSH values in the 1600 and 5000 ppm groups on Days 15, 29, 57,	Anonymous; (2020) 2018TOX-PXA4560

Type of study/data	Test substance, species, strain, sex, no/group	Relevant information about the study (as applicable)	Observations	Reference
thyroid gland changes. General design similar to OECD 408. GLP	Sprague Dawley Dose levels: 0, 400, 1600, 5000 ppm (0, 24, 96, 308 mg/kg bw/day). Positive control: Phenobarbital 1000 ppm. 15 males/group termination after 90 days 5 males/group termination after 30 days	At termination liver, thyroid and pituitary weighed and histopathology performed. Hepatic UDP-glucuronosyltransferase (UGT) activity (T3-glucuronidation and T4-glucuronidation) and protein concentration assessed	and 89 ↓ Time-dependent decrease in total T4 relative to pretreatment values during the first 29 days → Total T3 or rT3 ↑ Thyroid weight in the 5000 ppm group on Days 30 (absolute 31% and relative to body weight 58%) and 93/94 (absolute 27% and relative to body weight 39%) ↑ Liver weight in the 5000 ppm group on Day 30 (absolute 6% and relative to body weight 30%) and in the 1600 (absolute 15% and relative to body weight 16%) and 5000 ppm (absolute 35% and relative to body weight 47%) groups on Day 93/94. ↑ Thyroid follicular cell hypertrophy in the 1600 (3/14 compared with 0/15 in control) and 5000 ppm (15/15 compared with 0/15 in control) groups on Day 93/94 ↑ Hepatocellular hypertrophy in the 5000 ppm group on Days 30 (3/5 compared with 0/5 in control) and 93/94 (5/15 compared with 0/15 in control) ↑ T4-glucuronidation activity was observed in the 1600 and 5000 ppm groups on Days 30 (2.7 and 4.9 fold increase in the 1600 and 5000 ppm groups respectively) and 93/94 (1.9 and 3.2 fold increase in the 1600 and 5000 ppm groups respectively) ↑ T3-glucuronidation activity was elevated in the 1600 and 5000 ppm groups on Day 30 (1.9 and 1.7 fold increase in the 1600 and 5000 ppm groups respectively) and in the 5000 ppm group on Day 93/94 (1.0 and 1.9 fold increase in the 1600 and 5000 ppm groups respectively) <u>PB</u> ↑ Mean TSH values on Days 15, 29, 57, and 89 ↓ Total T4 on Day 15 and 29 ↑ Total T3 or rT3 on Day 89 ↑ Thyroid weight on Days 30 (absolute 53% and relative to body weight 69%) and 93/94 (absolute 36% and relative to body weight 36%) ↑ Liver weight on Day 30 (absolute 17% and relative to body weight 32%) and Day 93/94 (absolute 52% and relative to body weight 52%) ↑ Thyroid follicular cell hypertrophy	

Type of study/data	Test substance, species, strain, sex, no/group	Relevant information about the study (as applicable)	Observations	Reference
			on Day 30 (2/5 compared with 0/5 in control) and hypertrophy (15/15 compared with 0/15 in control) and hyperplasia (5/15 compared with 0/15 in control) on Day 93/94 ↑ Hepatocellular hypertrophy Days 30 (5/5 compared with 0/5 in control) and 93/94 (15/15 compared with 0/15 in control) ↑ T4-glucuronidation activity was observed on Days 30 (4.1 fold increase) and 93/94 (2.4 fold increase) ↑ T3-glucuronidation activity on Days 30 (3.7 fold increase) and 93/94 (1.9 fold increase) Overall, it can be concluded that liver enzyme induction, leading to an increase in T4 glucuronidation and clearance of T4, elicited a feedback response on the thyroid via an increase in TSH. Further, the increased TSH and associated thyroid follicular cell hypertrophy resulted in functional compensation by the thyroid in the Pethoxamid-treated rats. The results with pethoxamid were consistent with the results of the positive control PB, which was included in this study.	
Biliary Excretion of [^{125}I]Thyroxine and Metabolites in Rats. Non guideline study. GLP	Pethoxamid Batch: 21082018 Purity: 99.4% Rat Sprague Dawley Dose levels: 0, 300 mg/kg bw/day. Positive control: Phenobarbital (PB), 100 mg/kg bw/day. 6 bile-duct and jugular vein cannulated male/group termination after 7 days.	Bile-duct cannulated rats were pre-treated with control, PB (positive control), or pethoxamid once daily for 7 consecutive days. On Day 8, ~15 minutes prior to the [^{125}I]T4 dose, the rats were dosed with 2 mg/kg of potassium iodide. A single IV dose of [^{125}I]T4 in sterile saline was administered by intravenous injection to animal. Blood was collected from the jugular cannula for serum at 6 time points following IV dose administration. Bile was collected at 2 time points (0-2 and 2-4 hours) following IV dose administration.	<u>Pethoxamid</u> ↑ Liver weight ↓ Serum total radioactivity Cmax and AUC_{0-4} ↑ % administered radioactivity in bile ↑ T4 Glucuronide in bile <u>PB</u> ↑ Liver weight ↓ Serum total radioactivity Cmax and AUC_{0-4} ↑ % administered radioactivity in bile ↑ T4 Glucuronide in bile Similar response in pethoxamid and PB-treated animals. Overall, data indicates greater clearance of thyroxine due to liver induced T4 glucuronidation in the pethoxamid-treated rats compared to controls. The results were consistent with those for the phenobarbital positive control.	Anonymous; (2019) 2018MET-PXA4538

Type of study/data	Test substance, species, strain, sex, no/group	Relevant information about the study (as applicable)	Observations	Reference
In vitro mRNA and DNA synthesis induction in cultured mouse and human hepatocytes. Non guideline study. GLP	Pethoxamid Batch: 21082018 Purity: 97.7% Primary hepatocytes isolated from male CD-1 mice and primary cryopreserved hepatocytes from three male human donors. Dose levels: Mouse hepatocytes were exposed to pethoxamid (1, 3, 10 and 20 µM). Human hepatocytes were exposed to pethoxamid (1, 3, 10 and 20 µM, donor 385) and (0.3, 1, 3 and 10 µM, donors 8210 and 8219). Positive control: Phenobarbital (PB), 1000 µM. Epidermal growth factor (EGF; 25 ng/mL) used as positive control for replicative DNA synthesis.	Isolated primary male CD-1 mouse hepatocytes or male primary human hepatocytes (3 donors) were exposed in culture to pethoxamid, PB or EGF for approximately 96 hours after which cell cytotoxicity was evaluated by quantification of ATP levels, or the cells were harvested and processed for mRNA analysis of Cyp2b10 and Cyp3a11 (mouse) and CYP2B6 and CYP3A4 (human), or cells were processed for assessment of replicative DNA synthesis. Constitutive androstane receptor (CAR) and pregnane-X-receptor (PXR) activation was assessed by downstream Taqman® mRNA analysis (Cyp2b10 and Cyp3a11 in mouse, respectively; and CYP2B6 and CYP3A4 in human, respectively). Cell proliferation (measured as the change in replicative DNA synthesis (RDS) [S-phase of the cell cycle]) in both mouse and human hepatocytes assessed.	This study was designed to evaluate the hypothesis that pethoxamid induces mouse liver tumors via a PB-like MoA. Cyp2B and Cyp3A expression is induced by CAR and to a lesser degree pregnane X receptor (PXR). In rodents, activation of CAR by PB leads to hepatocellular tumors, which is not evident in hamsters, guinea pigs or primates including humans (Elcombe et al., 2014). While CAR/PXR induced gene expression is conserved across species, difference in replicative DNA synthesis (RDS) occurs in rodent hepatocytes, and not in human hepatocytes. Cultures of primary hepatocytes isolated from male CD-1 mice and cryopreserved human hepatocytes from three individual male donors were used to investigate the potential of pethoxamid to activate CAR and PXR. After a 96-hour treatment period, cells were harvested and processed for mRNA analysis of Cyp2b10 and Cyp3a11 for mice and Cyp2B6 and Cyp3A4 for human cells, corresponding to CAR and PXR receptor activation, respectively. Cell proliferation was measured as the change in both mouse and human hepatocytes following incubation with BrdU and subsequent immunohistochemical staining to determine the number of cells in S phase. PB was included as a positive control for the activation of CAR and PXR; epidermal growth factor (EGF) was included as a positive control for the induction of cell proliferation. An assessment of cytotoxicity was also performed. PB, the positive control, induced Cyp2b10 and Cyp3a11 mRNA in mouse hepatocytes, compared with concurrent vehicle controls, respectively (Table 10). Pethoxamid induced Cyp2b10 or Cypb3a11 mRNA in mouse hepatocytes to a somewhat lesser degree relative to PB. Therefore, pethoxamid was considered to be a weak activator of CAR and PXR in vitro, relative to PB in mouse hepatocytes.	Anonymous; (2019) 2018TOX-PXA4482

Type of study/data	Test substance, species, strain, sex, no/group	Relevant information about the study (as applicable)	Observations	Reference
			In human hepatocytes, PB treatment resulted in marked induction of Cyp2B6 mRNA and Cyp3A4 mRNA (Table 11). In cultures treated with pethoxamid, overt cytotoxicity was observed at 10–20 µM, depending on the specific human donor hepatocytes. Cyp2B6 mRNA was induced at 10 or 20 µM. Cyp3A4 mRNA was similarly induced. In cultures of primary male CD-1 mouse hepatocytes, PB and pethoxamid both induced RDS at all concentrations tested (Table 12). Neither PB nor pethoxamid induced RDS in human hepatocytes. The positive RDS control, EGF, induced RDS in both mouse hepatocytes and human hepatocytes. Overall, pethoxamid is a weak activator of CAR and/or PXR in vitro. Pethoxamid has been shown to induce RDS in mouse hepatocytes, but not in human hepatocytes. The responses observed with pethoxamid are similar to that of PB. These data are supportive of the lack of human relevance for mouse liver tumor formation following treatment with pethoxamid.	
Pethoxamid: Human Relevance Framework Assessment of Induced Rodent Liver and Thyroid Tumors	Pethoxamid:	Position paper *NB: an updated position paper to assess the mode of action and human relevance of the rodent liver and thyroid tumors to include the additional data generated to further characterise the mechanistic bases for the increased tumor incidence.*	Review of all available data from toxicological studies and mode of action (MoA) studies strongly demonstrate that the hepatocellular adenomas/carcinomas and thyroid follicular cell adenomas observed in rodents treated with high doses of pethoxamid are not relevant to humans. In an in vitro species comparison assay, pethoxamid induced cell proliferation in mouse hepatocytes, but not in human hepatocytes. Based on the difference in biological response in humans and rodents to CAR activation, any hepatocellular adenomas developed in mice through activation of these nuclear receptors by pethoxamid in mice, are not of relevance to humans. Therefore, it can be concluded that pethoxamid does not pose a hepatic carcinogenic hazard in humans.	Anonymous; (2020) FMC-54841

Type of study/data	Test substance, species, strain, sex, no/group	Relevant information about the study (as applicable)	Observations	Reference
			Overall, it is considered that a concordant and highly plausible MoA has been established for pethoxamid-induced rat thyroid follicular cell adenomas and that this MoA is not relevant to humans. In conclusion, the extensive experimental data demonstrate that pethoxamid does not pose a carcinogenic hazard to humans.	

10.9.1 Short summary and overall relevance of the provided information on carcinogenicity

Pethoxamid has been assessed for a genotoxic potential in five *in vitro* and two *in vivo* guideline and GLP compliant assays. It is concluded that based on a weight of evidence analysis of the genotoxicity data package that pethoxamid is non-genotoxic.

In a lifetime carcinogenicity study, groups of 50 CD-1 mice/sex/dose were administered pethoxamid via the diet for up to 95 weeks (males) or 92 weeks (females) at 0, 30, 400 or 5000 ppm (Anonymous, 2000b; 82 PXA). No treatment-related effects were observed on clinical signs or longevity. At 5000 ppm, body weight and body weight gain were both reduced at 92/95 weeks, in both sexes. An increase in liver and kidney weight was observed at 400 and 5000 ppm. Histopathological findings included generalized hepatocyte hypertrophy in males and periportal hepatocyte hypertrophy in females. Hepatocyte hypertrophy was also observed in interim kill mice at 52 weeks. In an additional investigation of cell proliferation in the livers taken from this study, pethoxamid appears to have no influence on cell proliferation in the liver at 52 or 95 weeks.

An increased number of hepatocellular adenomas was observed in male mice at the high dose of 5000 ppm. Although the number of hepatocellular carcinomomas was slightly higher than the concurrent control incidence (not statistically significant), it was well within the historical control incidence for the same strain in the same laboratory.

Finding	Dietary Concentration (ppm)				Historical control incidence (range) 16 studies 1996-1999
MALES	0 (control)	30	400	5000	
Hepatocellular adenoma %	19/50 38%	15/50 30%	18/50 36%	34/50** 68%	199/867 22.95% (8.3%-42%)
Hepatocellular carcinoma %	3/50 6%	3/50 6%	4/50 8%	6/50 12%	70/867 8.07% (3.6%-22%)

Statistical significance: **p<0.01

A review of the data and an assessment of the relevance of the mouse liver tumors to humans has been conducted following the IPCS and ILSI/HESI framework and is presented in the position paper "Pethoxamid: Human Relevance Framework Assessment of Induced Rodent Liver and Thyroid Tumors" (Anonymous, 2020; FMC-54841).

Additional mechanistic data has been generated to support the earlier assessment; PB was included in these more recent studies to ensure a direct within study comparison to pethoxamid. The relevance of these tumor types to humans has also been examined in detail in an earlier review by Anonymous (2016; 1540 PXA). The weight of evidence is supported by the investigative studies summarised in Table 40.

The human relevance framework assessment of liver tumor mode of action (MoA) in rodents concludes that the available data for pethoxamid support a MoA in rodents involving the following key events:

Molecular Initiating Event: Constitutive androstane receptor (CAR) activation
Key Event 1: Altered gene expression specific to CAR activation in hepatocytes
Key Event 2: Increased cell mitogenic proliferation in hepatocytes
Key Event 3: Increased preneoplastic foci in hepatocytes
Adverse Outcome: Increased hepatocellular adenoma

The available data for pethoxamid supports the proposed CAR-mediated MoA. The induction of liver tumors in mice is attributed to activation of the nuclear receptor CAR, ultimately leading to tumorigenesis. This is a well-accepted MoA for the induction of hepatocellular adenomas in mice for the central nervous system depressant phenobarbital (PB), the most commonly cited substance that has been shown to operate through this MoA. The postulated mouse tumor MoA for the liver was tested against the Bradford Hill criteria and was found to satisfy the conditions of dose and temporal concordance, biological plausibility, coherence, strength, consistency, and specificity that fits with a well-established MoA for liver tumors. Concordance analyses of the pethoxamid data exhibit strong dose and temporal concordance in the proposed key events resulting in liver tumors in male mice. Clear thresholds for mouse tumor development were identified (Anonymous, 2000a; 80 PXA). Alternative MoAs considered were genotoxicity, PPAR activation (peroxisome proliferation), AhR activation, estrogenic stimulation and cytotoxicity, among other MoAs. However, none of these alternative MoAs were consistent with the data available following exposure with pethoxamid.

In an *in vitro* species comparison assay, pethoxamid induced cell proliferation in mouse hepatocytes, but not in human hepatocytes. Based on the difference in biological response in humans and rodents to CAR activation, any hepatocellular adenomas developed in mice through activation of these nuclear receptors by pethoxamid in mice, are not of relevance to humans. Therefore, it can be concluded that pethoxamid does not pose a hepatic carcinogenic hazard in humans.

In a combined chronic toxicity and carcinogenicity study groups of 50 Crl:CD rats/sex/dose were administered pethoxamid via the diet for up to 104 weeks at 0, 25, 400 or 1600 ppm (Anonymous, 2000a; 80 PXA). Satellite groups were killed at 27, 53 and 79 weeks and subject to histopathological examination. Body weight gain was reduced in both sexes at 1600 ppm and correlated with reduced food consumption. An increase in relative liver weight was recorded at 1600 ppm, in both sexes at various time points, although at 104 weeks, only males demonstrated an increased liver weight versus controls. The incidence of centrilobular hepatocyte hypertrophy was elevated at 1600 ppm, in both sexes at 26, 52, 78 and 104 weeks. In males, there was a dose-related increase in relative thyroid weight, which at 53 weeks was statistically significant at all doses. At 1600 ppm, a slightly higher incidence of follicular cell adenomas of the thyroid was seen in male rats. The incidence falls outside the background control range. The test for trend was statistically significant. The adenomas were all within a similar morphological and size range and occurred generally singly in the affected animals.

Finding	**Dietary Concentration (ppm)**				**Historical control incidence (range) 16 studies 1996-1999**
MALES	0 (control)	25	400	1600	
Thyroid follicular cell adenoma %	2/50 4%	2/50 4%	2/49 4%	9/50[$] 18%	30/954 3.14% (0%-12%)
Thyroid follicular cell carcinoma %	2/50 4%	0/50 0%	0/49 0%	0/50 0%	7/954 0.73% (0%-5.1%)

[$] Trend test statistically significant

A review of the data and an assessment of the relevance of the rat thyroid follicular cell tumors to humans has been conducted following the IPCS and ILSI/HESI framework and is presented in the position paper "Pethoxamid: Human Relevance Framework Assessment of Induced Rodent Liver and Thyroid Tumors" (Anonymous, 2020; FMC-54841). Additional mechanistic data has been generated to support the earlier assessment; PB was included in these more recent studies to ensure a direct within study comparison to pethoxamid. The relevance of these tumor types to humans has also been examined in detail in an earlier

review by (Anonymous 2016; 1540 PXA). The weight of evidence is supported by the investigative studies summarised in Table 40.

The human relevance framework assessment of thyroid tumor MoA in rats concludes that the available data for pethoxamid support a MoA in rats involving the following key events:

Molecular Initiating Event: CAR activation
Key Event 1: Upregulation of UDP-glucuronosyltransferases (UDPGT's) in liver
Key Event 2: Increased T4/T3 catabolism and excretion in bile
Key Event 3: Decreased (initially) serum T4 / T3
Key Event 4: Increased serum thyroid stimulating hormone (TSH)
Key Event 5: Thyroid hypertrophy and hyperplasia of follicular cells
Adverse Outcome: Increased thyroid follicular cell adenoma

The available data for pethoxamid supports the proposed MoA for the development of thyroid follicular adenomas in male rats. Data from studies with a number of chemicals of various classes and diverse pharmacology, have demonstrated the same MoA. PB is representative of the class of xenobiotics that alters thyroid hormone homeostasis and promotes thyroid tumor development in rats at dose levels associated with high levels of enzyme induction. The induction of thyroid tumors is observed in PB-treated rats, whereas thyroid tumors are not seen in mice treated with PB. Pethoxamid shows a similar pattern of tumorigenesis as PB. The postulated rat tumor MoA for the thyroid gland was tested against the Bradford Hill criteria and was found to satisfy the conditions of dose and temporal concordance, biological plausibility, coherence, strength, consistency, and specificity that fits with a well-established MoA for thyroid follicular cell tumors. Clear thresholds for thyroid tumor development were identified (Anonymous, 2000b; 82 PXA). Alternative MoAs considered were genotoxicity, sodium iodide symporter inhibition, TPO inhibition, thyroid hormone transport disruption, enhanced cellular transport of thyroid hormones, sulfotransferase inhibition, deiodinase inhibition and thyroid receptor interaction, among other MoAs. These alternate MoAs were not consistent with the data available following exposure with pethoxamid. Therefore, they were considered not to be feasible. There is sufficient quantitative evidence on the basic physiological processes in the general literature to conclude that thyroid tumors, induced by a process involving increased hepatic clearance of thyroid hormone and altered thyroid homeostasis in rodents, will not lead to an increase in susceptibility to thyroid tumor development in humans. Overall, it is considered that a concordant and highly plausible MoA has been established for pethoxamid-induced rat thyroid follicular cell adenomas and that this MoA is not relevant to humans.

In conclusion, the extensive experimental data demonstrate that pethoxamid does not pose a carcinogenic hazard to humans.

Table 41: Compilation of factors to be taken into consideration in the hazard assessment

Species and strain	Tumour type and background incidence	Multi-site responses	Progression of lesions to malignancy	Reduced tumour latency	Responses in single or both sexes	Confounding effect by excessive toxicity?	Route of exposure	MoA and relevance to humans
Rat	Thyroid follicular cell adenoma 9/50 (18%) at 1600 ppm. Historical control overall mean 3.14% (range 0 – 12%)	No	No	No	Males	No	Oral, diet	Not relevant
Mouse	Hepatocellular adenoma 34/50	No	No	No	Males	No	Oral, diet	Not relevant

Species and strain	Tumour type and background incidence	Multi-site responses	Progression of lesions to malignancy	Reduced tumour latency	Responses in single or both sexes	Confounding effect by excessive toxicity?	Route of exposure	MoA and relevance to humans
	(68%) at 5000 ppm. Historical control overall mean 22.95% (range 8.3 – 42%)							

10.9.2 Comparison with the CLP criteria

The criteria for Category 1 (known or presumed human carcinogen) is based on human evidence or on animal data where there is sufficient evidence to demonstrate animal carcinogenicity. There are no data on humans. Treatment related increases in tumours were observed in both available studies conducted in rats and mice. Additional considerations indicate that these increases were limited to one sex only, which cannot be explained by differences in ADME behaviour of pethoxamid.

Without further investigations these data would show limited evidence of carcinogenicity in experimental animals. However, the underlying MoA that has been identified and is considered not relevant to humans. Therefore, no classification is warranted for pethoxamid.

Important factors taken into considerations:

Category 2 (Suspected Human Carcinogen) is based on human evidence and/or animal data where there is insufficient data to place the compound into Category 1, but there may be limited data from human or animal studies of an effect. Pethoxamid demonstrated a higher incidence of thyroid follicular cell adenoma in male rats as well as benign liver tumours in male mice at the highest dose. However, mechanistic studies demonstrate a MoA, which is not considered to be relevant to humans. Therefore, no classification as category 2 carcinogen is warranted.

(1) *Tumour type and background incidence*: Higher (not statistically significant) incidences of thyroid follicular cell adenomas but no increase in carcinomas in male rats at high dose have been observed, which are slightly above the HCD and show a significant trend. In addition, slightly higher pancreas islet cell tumours were observed in rats at high- and low-, but no at mid-dose levels. These changes are slightly above the HCD (not statically significant, no trend) but due to a lack of dose-dependency and statistically significance considered as incidental and not related to treatment. Also, an increased number of male mice showing hepatocellular adenomas at high dose (statistically significant) and a slight (not statistically significant) number of malignant hepatocellular tumours were found.

(2) *Multi-site responses:* In rats neoplastic findings were confined to the thyroid and pancreas, whereas in mice the liver was the only affected organ.

(3) *Progression of lesions to malignancy*: In rat, only benign tumours were observed in the thyroid and incidences in pancreatic islet cell carcinoma were low and not dose-dependent. In mice, the increased incidences of hepatocellular carcinomas were not significant but dose-dependent.

(4) *Whether responses are in single or both sexes:* In rats as well as mice, only males were affected. However, ADME studies did not indicate a significant difference in the toxicokinetic profile of pethoxamid between males and females.

(5) *Whether responses are in a single species or several species:* Tumours occurred in mice and rats.

(6) *Routes of exposure:* In both, the rat chronic carcinogenicity study as well as the mouse carcinogenicity study, pethoxamid was administered via the oral (dietary) route.

(7) *Mode of action and its relevance for humans:* A phenobarbital-like mode of action via activation of CAR is proposed for the induction of hepatocellular adenomas in male mice which are only seen at the very high dose level of 982 mg/kg bw/day. However, this dose level also corresponds to LD50 observed in rats. Based on differences in the biological response between rodents and humans to CAR activation, the weight of evidence indicates that a phenobarbital mode of action is not of relevance for humans (ECHA Guidance on the Application of the CLP Criteria, Version 5.0; July 2017). These include certain thyroid tumours in rodents mediated by UDP glucuronyltransferase (UGT) induction. Studies have been conducted which establish the mode of action of pethoxamid induced thyroid tumors is consistent with this mechanism, and therefore the increase in follicular cell tumours in the male rat is considered not relevant to humans and pethoxamid should not be classified as a carcinogen.

Pethoxamid is listed in Annex VI of Regulation (EC) 1272/20083 and no classification for carcinogenicity is included. As a consequence, peer review of pethoxamid concludes that: "Thyroid tumours were observed in high dose rats and liver tumours were observed in high-dose mice. New mechanistic studies were provided to support a phenobarbital mode of action via an activation of the constitutive androstane receptor (CAR) in the nucleus (with induction of UDP-glucuronosyltransferase (UGT)). These data and the carcinogenic properties of pethoxamid (thyroid tumours in rats and liver tumours in mice) were extensively discussed by the experts, mainly considering whether or not a phenobarbital mode of action was sufficiently demonstrated, and whether it can be considered relevant to humans or not. During the meeting, a slight majority of the experts considered that pethoxamid should not be classified as carcinogenic. After further consideration of the existing knowledge, the opinions were evenly divided in a post-meeting consultation. As a consequence, EFSA considers that the proposed classification Carcinogen category 2 should apply to pethoxamid. It is noted that the RMS disagreed and is of the opinion that classification for carcinogenic effects is not necessary." (EFSA, 2017).

Subsequent to this decision, additional mechanistic data has been generated that support the proposed phenobarbital mode of action, and discount alternatives, summarised in Table 40.

The data available from the two oncogenic studies and associated mode of action studies in the rat and mouse do not, therefore, support a classification for carcinogenicity for pethoxamid.

10.9.3 Conclusion on classification and labelling for carcinogenicity

Not classified (conclusive but not sufficient for classification).

10.10 Reproductive toxicity

The reproductive toxicity of pethoxamid has been investigated in a two-generation study in rats and developmental toxicity studies in rats and rabbits.

10.10.1 Adverse effects on sexual function and fertility

A two-generation study in rats is available to investigate the effects of pethoxamid on sexual function and fertility.

Table 42: 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**
Rat preliminary study of effects on reproductive performance.	Pethoxamid Batch: TB-960306 Purity: 95.0% Dose levels: 0, 25, 100,	**F0 Parental Toxicity** 1600 ppm (127-172 mg/kg bw/day) No effects. **Selected F1 Toxicity** 1600 ppm (240-296 mg/kg bw/day) ↓ Body weight: Week 4 16.5% males, 10.6% females	Anonymous;

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
Non guideline preliminary study. GLP Rat Sprague Dawley 6/sex/group	400, 1600 ppm in diet F0: 15 days prior and through pairing, gestation and lactation Selected F1: Continuously until approximately 6 weeks of age	↑ Liver weight (relative to body weight): 24.2% males; 12.0% females 400 ppm No effects. **Offspring Toxicity** 1600 ppm ↓ Body weight: Day 1 14.5% males, 15.6% females; Day 21 17.1% males, 15.6% females. ↓ Body weight gain from day 4 [values not reported; calculated male 3 g gain in control Day 1 to 4 compared with 1.3 g gain at 1600 ppm, female 2.7 g gain in control Day 1 to 4 compared with 1.7 g gain at 1600 ppm] ↑ Liver weight (absolute): 4.7% males, 8.7% females, unselected F1 pups. 400 ppm ↓ Body weight gain from day 4 [values not reported; calculated male 3 g gain in control Day 1 to 4 compared with 1.9 g gain at 400 ppm, female 2.7 g gain in control Day 1 to 4 compared with 1.8 g gain at 400 ppm] 100 ppm No effects. Note: no statistical analysis, body weight gain values not reported.	(1998a) 84 PXA
Rat two-generation reproductive study. Guideline OPPTS 870 3800. Study design compatible with OECD 416, includes oestrus cyclicity, sperm evaluation, sexual development land marks. GLP Rat Sprague Dawley 28/sex/group	Pethoxamid Batch: TB-960306 Purity: 95.0% Dose levels: 0, 25, 200, 1600 ppm in diet F0 and F1: 10 weeks prior and through pairing, gestation and lactation	**F0 Parental toxicity:** 1600 ppm (pre-mating: 85-170 mg/kg bw/day males; 117-181 mg/kg bw/day females) ↓ Body weight gain during gestation: 10.7% Day 0-20 of gestation ↑ Liver weight (absolute; g): 12.9% males, 12.1% females ↑ Liver weight (relative to body weight): 18.3% males; 11.9% females ↓ Spleen weight (relative to body weight): 10% females 200 ppm (pre-mating: 11-22 mg/kg bw/day males; 14-24 mg/kg bw/day females) No effects. **F1 Parental toxicity** 1600 ppm (pre-mating: 97-291 mg/kg bw/day males; 123-303 mg/kg bw/day females) ↓ Body weight Week 10: 9.8% males; 10.1% females ↓ Body weight gain Week 0-10: 10.7% males; 12.3% females ↑ Liver weight (relative to body weight): 15.3% males; 8.5% females 200 ppm (pre-mating: 12-34 mg/kg bw/day males; 16-36 mg/kg bw/day females) ↓ Body weight Week 10: 6.71% females ↓ Body weight gain Week 0-10: 9.84% females ↓ Thymus weight (absolute): 15% females ↑ Seminal vesicles (relative to body weight): 12% males These effects were not considered to be treatment related. **Fertility and Reproductive Performance** No adverse effects.	Anonymous; (2000) 85 PXA

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
		F1 Offspring Toxicity: 1600 ppm ↓ Body weight Day 21: 6.4% males, 6.8% females ↓ Body weight gain Day 1-21: 7.9% males, 7.7% females ↑ Liver weight (relative to body weight): 19% males, 17% females ↓ Spleen weight (relative to body weight): 11% males, 9% females 200 ppm ↑ Liver weight females: 9% (relative to body weight) , 6% (absolute) These effects were not considered to be treatment related. **F2 Offspring Toxicity** 1600 ppm ↓ Body weight Day 21: 11.7% males, 11.6% females ↓ Body weight gain Day 1-21: 12.3% males, 12.4% females ↑ Liver weight (relative to body weight): 19% males, 16% females 200 ppm ↑ Liver weight (relative to body weight): 6% males 25 ppm No effects. Parental NOAEL: 200 ppm (11 mg/kg bw/day) Reproductive NOAEL: 1600 ppm (85 mg/kg bw/day) Offspring NOAEL: 200 ppm (11 mg/kg bw/day)	

Table 43: 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 relevant studies				

Table 44: Summary table of other studies relevant for toxicity on sexual function and fertility

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

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

In the rat two-generation reproduction study, clear parental toxicity (reductions in body weight and body weight gain during gestation; increases in liver weight and decreased spleen weight) was observed at the highest dose level of 1600 ppm. No effects were observed at the intermediate dose level of 200 ppm and this was the NOAEL for parental toxicity. Despite the parental toxicity, there was no evidence for any effect of pethoxamid on sexual function and fertility in the rat at the highest dose level tested of 1600 ppm (approximately 112 mg/kg bw/day).

Systemic toxicity was also observed in the pups at 1600 ppm, with lower body weight and body weight gains evident at the end of the lactation period. The relevant offspring NOAEL was 200 ppm (14 mg/kg bw/day).

In summary, there were no adverse effects of pethoxamid on the sexual function and fertility of parental rats at dose levels that induced toxicity. There was evidence of effects of pethoxamid on the body weights of the offspring at maternally toxic dose levels.

10.10.3 Comparison with the CLP criteria

Adverse effects on sexual function and fertility:

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

There were no adverse effects on sexual function and fertility in the rat to warrant classification of pethoxamid as a potential human reproductive toxicant.

This is in agreement with the EFSA conclusions (EFSA, 2017): 'Pethoxamid is not classified or proposed to be classified as toxic for reproduction category 2, in accordance with the provisions of Regulation (EC) No 1272/2008'.

10.10.4 Adverse effects on development

The developmental toxicity of pethoxamid has been investigated in rats and rabbits. Systemic toxicity was also observed in the pups at 1600 ppm, with lower body weight and body weight gains evident at the end of the lactation period. The relevant offspring NOAEL was 200 ppm (14 mg/kg bw/day). There was evidence of effects of pethoxamid on the body weights of the offspring at maternally toxic dose levels. Developmental toxicity includes, in its widest sense, any effect which interferes with normal development of the conceptus, either before or after birth, and resulting from exposure of either parent prior to conception, or exposure of the developing offspring during prenatal development, or postnatally, to the time of sexual maturation.

Table 45: Summary table of animal studies on adverse effects on development

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference**
Rat preliminary embryo-fetal development study. Non guideline preliminary study. GLP Rat CD (Sprague Dawley origin) sexually mature, pregnant	Pethoxamid Batch: TB-951005 Purity: 95% Vehicle: 1% w/v methyl cellulose + 0.5% w/v Tween 80 Dose levels: 0, 8, 80, 400, 800 mg/kg bw/day	<u>800 mg/kg bw/day</u> Maternal mortality: 2/6 females died on Day 9, and 1/6 female killed in extremis on Day 10 ↓ Bodyweight Day 20: 11.0% (surviving 3 females only) ↑ Salivation: 6/6 females <u>400 mg/kg bw/day</u> ↑ Salivation: 6/6 females No other effects <u>80 mg/kg bw/day</u> ↑ Salivation: 6/6 females, however at lower incidence than higher doses No other effects. <u>8 mg/kg bw/day</u> No effects	Anonymous; (1996) 86 PXA

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
6/group	Exposure: Days 6-15 of gestation	NOEL: 400 mg/kg bw/day	
Rat embryo-fetal development study. No guideline mentioned but consistent with OECD 414 except: Shorter administration period (day 6 to 15 of gestation only) and 40% (10/25) of the dams of the high dose group died. However considered acceptable. GLP Rat CD (Sprague Dawley origin) sexually mature, pregnant 25/group	Pethoxamid Batch: TB-951005 Purity 95% Vehicle: 1% w/v methyl cellulose + 0.5% w/v Tween 80 Dose levels: 0, 8, 80, 600 mg/kg bw/day Exposure: Days 6-15 of gestation	<u>600 mg/kg bw/day</u> Maternal mortality: 10/25 killed in extremis or found dead Day 8-17 Signs prior to termination included piloerection, hunched posture, unresponsive to stimuli ↓ Bodyweight Day 9: 1%. No weight gain for first 3 days after dosing, but overall weight gain to Day 20 similar to control ↑ Salivation: 25/25 females <u>80 mg/kg bw/day</u> ↑ Salivation: 24/25 females No other effects <u>8 mg/kg bw/day</u> No effects Maternal NOEL: 80 mg/kg bw/day Fetal NOEL: 600 mg/kg bw/day	Anonymous; (1997a) 87 PXA
Rat developmental toxicity study. OECD 414 (1981) OPPTS 870.3700 (1998). GLP Rat Crl:CD(SD) sexually mature, pregnant 25/group	Pethoxamid technical Batch: P1351-JaK-T2-23-6 Purity: 95.80% Vehicle: 1% w/v methyl cellulose + 0.5% v/v Tween 80 Doses levels: 0, 10, 75, 500/350/250 mg/kg bw/day	<u>500/350/250 mg/kg bw/day</u> **Maternal** Maternal mortality: Due to mortality and/or adverse clinical signs of toxicity in 7 rats at the 500 mg/kg bw/day within 3-7 days of dosing, dose level reduced to 350 mg/kg bw/day. Mortality occurred in 5 additional rats at 350 mg/kg bw/day, which resulted in a subsequent reduction of the dose level to 250 mg/kg bw/day. Clinical signs: 21/30 to 26/30 hunched posture; light brown faeces and dehydration.4/30 to 9/30 moderate dehydration; slightly pale and/or pale ears; ungroomed coat; ptosis; thin body condition; urine staining; slight excess salivation; decreased motor activity; pale extremities; coldness to the touch; scant faeces; and ataxia. ↓ Body weight Day 21: 10.5% ↓ Body weight gain: 24.0% Day 0-21; 28.4% Day 6-21 ↓ Food consumption: 17.0% Day 6-21 ↓ Uterine weight: 8%	Anonymous; (2014a) 1138 PXA

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
30 high dose group	Exposure: Days 6-20 of gestation	**Fetal:** ↓ Fetal weight: 10.1% (combined sexes) No fetal gross external, soft tissue or skeletal alterations (malformations or variations) considered to be test substance-related. <u>75 mg/kg bw/day</u> No effects <u>10 mg/kg bw/day</u> No effects Maternal NOEL: 75 mg/kg bw/day Fetal NOEL: 75 mg/kg bw/day	
Rabbit tolerability study. Non guideline preliminary study. GLP Rabbit: NZW, females	Pethoxamid Batch: TB-960306 Purity: 95.0% Vehicle: 1% w/v methyl cellulose + 0.5% w/v Tween 80 2 animals dosed 50 mg/kg for 2 days, dose doubled every 2 days to 800 mg/kg bw on days 9 and 10 2 further mated females dosed for 7 days at 300 mg/kg bw/day	<u>Escalating dose to 800 mg/kg bw/day</u> Maternal mortality: One animal found dead, the other killed *in extremis* after two doses of 800 mg/kg bw/day. Following treatment with 400 mg/kg bw/day both females showed ↓ body weight, ↓ food consumption, ↓decreased water intake and ↓ faecal output. No effects at 200 mg/kg bw/day. <u>300 mg/kg bw/day for 7 days in mated females</u> ↓ Bodyweight: Average 0.48 kg body weight loss Day 0-7. Note only one female pregnant Conclusion: Dosing for preliminary embryo-fetal development study should be ≤300 mg/kg bw/day.	Anonymous; (1997b) 89 PXA
Rabbit preliminary embryo-fetal developmental toxicity study. Non guideline preliminary study. GLP Rabbit: NZW pregnant	Pethoxamid Batch :TB-960306 Purity: 95.1% Vehicle: 1% w/v methyl cellulose + 0.5% w/v Tween 80 Dose levels: 0, 30, 100, 300 mg/kg bw/day	<u>300 mg/kg bw/day</u> ↓ Bodyweight: Marked decrease in the two days following the start of dosing. The body weight change during the treatment period was negative. Overall weight gain during gestation 45% lower than control ↓ Food consumption: In 3/4 females. Approximately 46% of control during first half of treatment period. Approximately 12% of control during second half of treatment ↓ Faecal output: In 3/4 females No effects on litter parameters <u>100 mg/kg bw/day</u> No treatment related effects	Anonymous; (1998b) 90 PXA

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
4/group	Exposure: Days 6-19 of gestation	30 mg/kg bw/day No treatment related effects Conclusion: Marked effects on dams at 300 mg/kg bw/day.	
Rabbit embryo-fetal development toxicity study No guideline mentioned but consistent with OECD 414 except: Shorter administration period (day 6 to 19 of gestation only). GLP Rabbit: NZW pregnant 20/group	Pethoxamid Batch: TB-960306 Purity: 95.1% Vehicle: 1% w/v methyl cellulose + 0.5% w/v Tween 80 Dose levels: 0, 12.5, 50, or 200 mg/kg bw/day Exposure: Days 6-19 of gestation	200 mg/kg bw/day ↓ Body weight: No weight gain mid treatment then weight loss. Group mean body weight gain approximately 47% of control at end of treatment period. [note overall weight gain for gestation period similar to control.] ↓ Food consumption: Approximately 76% of control during second half of treatment period No effects on litter parameters or upon growth or development of foetuses in utero 50 mg/kg bw/day ↓ Food consumption: Slightly lower than control during second half of treatment period (approximately 90% of control) 12.5 mg/kg bw/day No treatment related effects Maternal NOAEL: 50 mg/kg bw/day Foetal NOEL: 200 mg/kg bw/day	Anonymous; (1998c) 88 PXA
Rabbit developmental toxicity study OECD 414 (1981) OPPTS 870.3700 (1998). GLP Rabbit: Hra:(NZW)SPF pregnant 25/group	Pethoxamid technical Batch: P1351-JaK-T2-23-6 Purity: 95.80% Vehicle: 1% w/v methyl cellulose + 0.5% v/v Tween 80 Dose levels: 0, 12.5, 50, 200 mg/kg bw/day Exposure: Days 6 to 28 of gestation	200 mg/kg bw/day **Maternal:** ↑ Abortions: 4/25 (0/25 controls) Clinical observations in rabbits observed to abort: scant faeces, ungroomed coat, thin body condition, mild dehydration and red substance in the cage pan ↓ Body weight: 8.1% Day 29 ↓ Body weight gain: 58.3% Day 0-29; 74.3% Day 6-29 ↓ Food consumption: 28.7% Day 6-29 ↓ Gravid uterine weight: 12.2% **Fetal:** ↓ Fetal weight: 18.7% (combined sexes) ↑ Incidence of supernumerary thoracic ribs: mean value 12.64 (control 12.38) ↑ Number of thoracic vertebrae mean value 12.70 (control 12.43) ↓ Number of lumbar vertebrae mean value 6.30 (control 6.56) 50 mg/kg bw/day No treatment related effects 12.5 mg/kg bw/day No treatment related effects Maternal NOEL: 50 mg/kg bw/day Fetal NOEL: 50 mg/kg bw/day	Anonymous; (2014b) 1139 PXA

Table 46: 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 relevant studies				

Table 47: 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 relevant studies				

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

There are two GLP compliant developmental toxicity studies in each of the rat and rabbit. Additional, rat and rabbit developmental toxicity studies were undertaken for data purposes outside the E.U, to ensure studies had been conducted according to modern test guidelines. In particular, these more recent studies included a longer period of test substance administration.

In the first rat developmental toxicity study, animals received the test compound at dosages of 0, 8, 80 or 600 mg/kg bw/day by oral gavage from Day 6 to 15 of gestation. Significant maternal toxicity was seen at 600 mg/kg bw/day. The dose of 8 mg/kg bw/day was considered to be the maternal NOEL, based on the salivation which occurred from 80 mg/kg bw/day. The NOEL for developmental toxicity was 600 mg/kg bw/day. Although, more than 10% of the dams died at the high dose, the study was considered to be acceptable because a high number of dams (15) were available for the investigations at the end of the study, and there was no evidence of developmental toxicity. In the preliminary study, at 400 mg/kg bw/day, no substance related effects (apart from salivation) were observed on the dams and up to the highest dose (800 mg/kg bw/day), no developmental toxicity was seen.

In the more recent rat developmental toxicity study, female Crl:CD(SD) rats (25 or 30/group) were orally administered pethoxamid or the vehicle control once daily by oral gavage on gestation days 6 through 20 at dose levels of 0, 10, 75 or 500 mg/kg bw/day. Due to mortality and/or adverse clinical signs of toxicity in rats at the 500 mg/kg bw/day dose level within the first 3 to 10 days of dosing, the high-dose level was reduced to 350 mg/kg bw/day. Mortality occurred in three additional rats at the 350 mg/kg bw/day dose level, which resulted in a subsequent reduction of the dose level to 250 mg/kg bw/day. There were two additional deaths in the 250 mg/kg bw/day dose group that occurred on gestation day 21 after the last dose on gestation day 20. Based on effects on mortality, clinical signs, body weight and food consumption in maternal females at the high dose, the NOAEL for maternal toxicity was 75 mg/kg bw/day. Based on reductions in gravid uterine weight and fetal body weight the NOAEL for developmental end-points was established at 75 mg/kg bw/day. Based on results of the study pethoxamid was considered not to be a developmental toxicant.

In the initial rabbit developmental toxicity study, four groups of 20 pregnant New Zealand White rabbits, received the test compound at doses of 0, 12.5, 50 or 200 mg/kg bw/day, administered by oral gavage from Day 6 to 19 of gestation. At 200 mg/kg bw/day, a reduced body weight gain was observed during the treatment period and at 50 mg/kg bw/day and above a lower food intake was observed during the treatment period. Therefore, the dose of 50 mg/kg bw/day was considered to be the maternal NOAEL. No dose was associated with adverse effects on *in utero* survival or embryo-fetal development; thus the NOAEL (NOEL) for developmental toxicity is 200 mg/kg bw/day.

In the more recent rabbit developmental toxicity study, 25 pregnant New Zealand White rabbits were administered pethoxamid or the vehicle control substance once daily by oral gavage on Days 6 through 28 of gestation at dose levels of 0, 12.5, 50, or 200 mg/kg bw/day. An increased incidence in the number of rabbits that aborted and were subsequently euthanized during the study, reductions in body weight, body weight gain and food consumption values occurred in the 200 mg/kg bw/day dose group. On the basis of these data, the maternal NOAEL for pethoxamid was 50 mg/kg bw/day. In the 200 mg/kg bw/day dose group, fetuses showed

decreased weight and statistically significant increases in the incidence of supernumerary thoracic ribs with associated statistically significant increases and decreases in the numbers of thoracic and lumbar vertebrae, respectively, a common variation observed at maternally toxic doses. The developmental NOEL was also 50 mg/kg bw/day. Based on results of that study pethoxamid was considered not to be a selective developmental toxicant.

In summary, pethoxamid has been investigated extensively in the pregnant rat and pregnant rabbit. Maternal toxicity was demonstrated at high doses but none of the four main studies produced any indication of adverse effects on fetal development.

In addition, body weights as well as bodyweight gains in pethoxamid-treated F1 as well as F2 pubs were found to be significantly lower at day 21 in the two-generation reproduction toxicity study (around -7% in F1 pups and -12% in F2 pups) in rat as compared to control (see table Section 10.10.8). However, these changes are considered as a consequence of direct food consumption.

10.10.6 Comparison with the CLP criteria

In the classification system, adverse effects on development of the offspring include any effect which interferes with normal development of the conceptus, either before or after birth, and resulting from exposure of either parent prior to conception, or exposure of the developing offspring during prenatal development, or postnatally, to the time of sexual maturation. Such effects shall have been observed in the absence of other toxic effects, or shall not be secondary non-specific consequences of the other toxic effects.

In two developmental toxicity studies in rats, based on effects on mortality, clinical signs, decreased body weight gain and food consumption in maternal females at the high doses, the NOAEL for maternal toxicity was 75 mg/kg bw/day or 80 mg/kg bw/day. At these doses, there was no evidence of intra-uterine toxicity to the embryos / fetuses or an induction of malformations. The studies did not demonstrate any developmental toxicity potential of pethoxamid in rats.

Doses up to 200 mg/kg bw/d have been investigated in two oral developmental toxicity studies in rabbits. Based on effects on decreased body weight gain and food consumption in maternal females at the high doses, the NOAEL for maternal toxicity was 50 mg/kg bw/day. At this dose, there was no evidence of intra-uterine toxicity to the embryos / fetuses or an induction of malformations. The studies did not demonstrate any developmental toxicity potential of pethoxamid in rabbits.

In a rat two-generation reproductive study F1 and F2 pups showed a significantly decreased body weight at day 21 and a significantly decreased body weight gain during the last week before weaning (days 14 to 21) at the highest dose. According to the CLP Regulation point 3.7.1.4, any effect which interferes with normal development of the pups until the time of sexual maturation should be taken into account. However, the body weight decrements evident at day 21 are considered a consequence of direct consumption of the diet and a consequence of systemic toxicity. Necropsy of F1 and F2 pups that died before weaning revealed absence of milk in the stomach as the only consistent finding. Necropsy of F1 and F2 pups at 25 days of age revealed no evident changes that could be related to treatment.

According to point 3.7.2.3.4 of the CLP Regulation, which states that “Classification is not necessarily the outcome in the case of minor developmental changes, when there is only a small reduction in foetal/pup body weight or retardation of ossification when seen in association with maternal toxicity”. Therefore, pethoxamid did not meet the criteria for classification for developmental toxicity in these studies.

10.10.7 Adverse effects on or via lactation

Information on the potential for pethoxamid to induce adverse effects on or via lactation is provided by a two-generation study in rats (described in section 10.10.1).

Table 48: 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
Rat preliminary study of effects on reproductive performance. Non guideline preliminary study. GLP	Rat Sprague Dawley Pethoxamid Batch: TB-960306 Purity: 95.0% Dose levels: 0, 25, 100, 400, 1600 ppm in diet F0: 15 days prior and through pairing, gestation and lactation 6/sex/group Selected F1: Continuously until approximately 6 weeks of age	**Offspring Toxicity** 1600 ppm ↓ Body weight: Day 1 14.5% males, 15.6% females; Day 21 17.1% males, 15.6% females. ↓ Body weight gain from day 4 [values not reported; calculated male 3 g gain in control Day 1 to 4 compared with 1.3 g gain at 1600 ppm, female 2.7 g gain in control Day 1 to 4 compared with 1.7 g gain at 1600 ppm] ↑ Liver weight (absolute): 4.7% males, 8.7% females, unselected F1 pups. 400 ppm ↓ Body weight gain from day 4 [values not reported; calculated male 3 g gain in control Day 1 to 4 compared with 1.9 g gain at 400 ppm, female 2.7 g gain in control Day 1 to 4 compared with 1.8 g gain at 400 ppm] 100 ppm No effects. Note: no statistical analysis, body weight gain values not reported.	Anonymous; (1998a) 84 PXA
Rat two-generation reproductive study. Guideline OPPTS 870 3800. Study design compatible with OECD 416, includes oestrus cyclicity, sperm evaluation, sexual development land marks. GLP	Rat Sprague Dawley Pethoxamid Batch: TB-960306 Purity: 95.0% Dose levels: 0, 25, 200, 1600 ppm in diet F0 and F1: 10 weeks prior and through pairing, gestation and lactation 28/sex/group	**F1 Offspring Toxicity:** 1600 ppm ↓ Body weight Day 21: 6.4% males, 6.8% females ↓ Body weight gain Day 1-21: 7.9% males, 7.7% females ↑ Liver weight (relative to body weight): 19% males, 17% females ↓ Spleen weight (relative to body weight): 11% males, 9% females 200 ppm ↑ Liver weight females: 9% (relative to body weight) , 6% (absolute). Not considered treatment related. **F2 Offspring Toxicity** 1600 ppm ↓ Body weight Day 21: 11.7% males, 11.6% females ↓ Body weight gain Day 1-21: 12.3% males, 12.4% females ↑ Liver weight (relative to body weight): 19% males, 16% females 200 ppm ↑ Liver weight (relative to body weight): 6% males Not considered treatment related. 25 ppm No effects. Offspring NOAEL: 200 ppm (11 mg/kg bw/day)	Anonymous; (2000) 85 PXA

Table 49: 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 relevant studies				

Table 50: 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
No relevant studies				

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

The two-generation reproduction toxicity study of pethoxamid provides the relevant information to assess potential impaired nursing behaviour, decreased pup viability during lactation or effects on the offspring due to transfer of the chemical or metabolites via the milk. The key data are summarised in the text table below:

Parameter	F1 pups		F2 pups	
	Control	1600 ppm	Control	1600 ppm
Live born index (%)	89	93	91	95
Pup viability to day 4 (%)	82	94	80	92
Lactation index Day 4-21 (%)	99	97	96	99
	MALES			
Pup weight at birth (g)	6.0	6.3	6.1	5.9
Pup weight Day 14 (g)	30.9	30.2	31.3	29.2
Pup weight Day 21 (g)	51.9	48.6* (-6,4%)	53.2	47.0** (-12%)
Pup weight gain Day 1-14 (g)	24.7	23.7	25.1	23.3
Pup weight gain Day 1-21 (g)	45.8	42.2* (-7,9%)	47.0	41.2** (-12%)
	FEMALES			
Pup weight at birth (g)	5.7	6.0	5.8	5.5
Pup weight Day 14 (g)	29.9	29.0	30.0	27.9
Pup weight Day 21 (g)	50.1	46.7* (-6,8%)	50.9	45.0** (-12%)
Pup weight gain Day 1-14 (g)	24.0	23.0	24.1	22.4
Pup weight gain Day 1-21 (g)	44.2	40.8* (-7,7%)	45.1	39.5** (-12%)

Statistical significance: * P≤0.05; **P≤0.01

There is no evidence in either generation of an effect on pup viability considering either live born index or survival to weaning (Day 21). There is a statistically significant decrease in body weight and body weight gain of pups on Day 21, in both generations. Evaluation of the data shows that both pup body weight on Day 14, and pup body weight gain from Day 1 to 14, are similar to control. As stated in the report of this study, in the

calculation of achieved dosage 'food intake between Days 14 and 20 of lactation includes diet eaten directly by the offspring', as well as the dam. Therefore, the body weight decrements evident at Day 21 are a consequence of direct consumption of the diet (no body weight decrement was evident at birth or up to Day 14) and considered to be a consequence of systemic toxicity, and do not indicate any adverse effect on the offspring due to transfer of the chemical via the milk or to the quality of the milk.

10.10.9 Comparison with the CLP criteria

The classification is intended to indicate when a substance may cause harm due to its effects on or via lactation and is independent of consideration of the reproductive or developmental toxicity of the substance. There were no effects to warrant classification of pethoxamid for effects on or via lactation.

10.10.10 Conclusion on classification and labelling for reproductive toxicity

Not classified (conclusive but not sufficient for classification).

10.11 Specific target organ toxicity-single exposure

The available acute studies that inform on specific target-organ toxicity following a single exposure are reported in section 10.1 to 10.3. An acute neurotoxicity study in rats is also available (summarised in the table below).

Table 51: Summary table of animal studies on STOT SE

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, route of exposure, dose levels, duration of exposure**	**Results**	**Reference**
Acute neurotoxicity study OECD 424 GLP Rat Crl:CD(SD) Dose range finding phase: 5/sex/group Main study phase: 10/sex/group	Pethoxamid Batch: P1351-JaK-T2-23-6 Purity: 95.8% Vehicle: 1% methylcellulose and 0.5% Tween® 80 Oral gavage, single administration Dose range finding phase: 0, 600, 800 mg/kg bw Main study phase: 0, 100, 300, 800 mg/kg bw Observation period: 7 days for dose range finding phase 15 days for main study phase	<u>Dose range finding phase:</u> <u>800 mg/kg bw</u> One male rat was observed with decreased motor activity, ptosis, pale right and left ears, mild and/or moderate dehydration, bradypnea and thin body condition. Clinical observations: hunched posture, vocalization to the touch, chromorhinorrhea, pale ears, red urine, coldness to the touch, ptosis, mild or moderate dehydration and whole body tremors ↓ Body weight: Males and females on Days 1 and 2 ↓ Food consumption (absolute and relative): Males Days 1 to 3 and females Day 1 to 2. <u>600 mg/kg bw</u> Clinical observations: hunched posture, vocalization to the touch, chromorhinorrhea, pale ears, red urine, coldness to the touch, ptosis, mild or moderate dehydration and whole body tremors ↓ Body weight: Males and females on Days 1 and 2 ↓ Food consumption (absolute and relative): Males and females on Day 1. <u>Main study phase:</u> <u>800 mg/kg bw</u> Mortality: Two female rats were found dead (on Days 2 and 3). Clinical signs: Decreased motor activity, ptosis, mild and moderate dehydration, hunched posture, pale ears and extremities, bradypnea, scant faeces and/or ungroomed coat were observed only in the two female rats that were found dead. ↓ Body weight: Males and females on Days 1 and 2 ↓ Food consumption (absolute and relative): Males and females Days 1 and 2 Functional observation battery: Adverse clinical signs during the FOB evaluation (i.e., hunched posture, bradypnea and pale right and left ears and extremities) were observed in one female rat that was subsequently found dead. <u>300 mg/kg bw</u> ↓ Body weight gain: Males and females on Days 1 and 2 ↓ Food consumption (relative): Males and females on Days 1 and 2. <u>100 mg/kg bw</u> No effects	Anonymous; (2014c) 1137 PXA

Table 52: Summary table of human data on STOT SE

Type of data/report	Test substance	Route of exposure Relevant information about the study (as applicable)	Observations	Reference
No evidence of specific target organ toxicity following single exposures				

Table 53: 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 relevant studies				

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

An acute neurotoxicity study was performed in two phases, a dose range finding (DRF) phase and a main study phase.

In the DRF phase, male and female rats (5/sex/group) were administered pethoxamid or the vehicle control substance once by oral gavage at dose levels of 0, 600, and 800 mg/kg bw. No mortality was observed. In the 800 mg/kg bw dose group, one male rat was observed with decreased motor activity, ptosis, pale right and left ears, mild and/or moderate dehydration, bradypnea and thin body condition. Body weight loss was observed in the 600 and 800 mg/kg bw dose groups in both sexes on Days 1 to 2 (males lost 2.6 g at 600 mg/kg bw and 11.6 g at 800 mg/kg bw compared with a gain of 6.0 g in the controls, statistically significant at 800 mg/kg bw; females lost 5.6 g at 600 mg/kg bw and 8.8 g at 800 mg/kg bw compared with a gain of 3.0 g in the controls, both statistically significant). There was also a reduction in absolute and relative food consumption values observed in male and female rats at 600 and 800 mg/kg bw on the day following dose administration compared with the vehicle control values females (absolute males 34% at 600 mg/kg bw and 57% at 800 mg/kg bw decrease compared with controls; both statistically significant) (relative males 33% at 600 mg/kg bw and 57% at 800 mg/kg bw decrease compared with controls; both statistically significant) (absolute females 44% at 600 mg/kg bw and 50% at 800 mg/kg bw decrease compared with controls; both statistically significant) (relative females 46% at 600 mg/kg bw and 52% at 800 mg/kg bw decrease compared with controls; both statistically significant). In the male rats at 800 mg/kg bw, absolute and relative food consumption values remained reduced on Days 2 to 3 (absolute 20% and relative 16% decrease compared with controls; not statistically significant). In the male and female rats, detailed clinical signs at 600 and 800 mg/kg bw that were considered to be related to the test substance included the following: hunched posture, vocalization to the touch, chromorhinorrhea, pale ears, red urine, coldness to the touch, ptosis, mild or moderate dehydration and whole body tremors. These detailed clinical signs were most apparent between the 12- and 16-hour time points.

In main study, male and female rats (10/sex/group) were administered pethoxamid or the vehicle control substance once by oral gavage on Day 1 at dose levels of 0, 100, 300, or 800 mg/kg bw. At 800 mg/kg bw, two female rats were found dead (on Days 2 and 3). Clinical signs (decreased motor activity, ptosis, mild and moderate dehydration, hunched posture, pale ears and extremities, bradypnea, scant faeces and/or ungroomed coat) were observed only in the two female rats that were found dead. At 800 mg/kg bw, body weight loss was observed in male and female rats on Days 1 to 2 after which body weight rebounded (males lost 2.9 g compared with a gain of 6.6 g in the controls, females lost 4.2 g compared with a gain of 4.1 g in the controls; both statistically significant). This was associated with absolute (g/day) and relative decreased food consumption (g/kg/day) in males (absolute 36% and relative 34% decrease compared with controls; both statistically significant) and females (absolute 45% and relative 46% decrease compared with controls; both statistically significant) from Day 1 to 2. At 300 mg/kg bw, decreased body weight gain was observed in male and female rats from Day 1 to 2 (males gained 2 g compared with a gain of 6.6 g in the controls, females gained 0.2 g compared with a gain of 4.1 g in the controls; statistically significant in males). This was associated with relative decreased food consumption (g/kg/day) in males and females (males 10% and females 22% decrease compared with controls; both statistically significant) from Day 1 to 2.

A functional observation battery (FOB), followed by motor activity evaluation was performed on all rats prior to dose administration, on the day of dose administration at 16-hour post-dose, 7 days after dose administration and 14 days after dose administration. Adverse clinical signs during the FOB evaluation (i.e., hunched posture, bradypnea and pale right and left ears and extremities) were observed in one female rat that was subsequently found dead. There were no statistically significant or biologically important effects of pethoxamid on the FOB parameters in the male or female rats at any time point that were considered to be test substance related. There were not treatment-related effects on motor activity. Absolute and relative brain weights were unaffected at all doses. There were no treatment-related gross or neurohistopathology findings.

In conclusion, in an acute oral neurotoxicity study, administration of pethoxamid at a dose of 800 mg/kg bw resulted in no evidence of neurotoxicity in either sex at the highest dose tested. Systemic toxicity was observed at 300 mg/kg bw and above (decreased body weight gain and relative food consumption at 300 mg/kg bw and mortality at 800 mg/kg bw).

In an acute oral study in rats (section 10.1), pethoxamid was administered at 800, 1260 or 2000 mg/kg bw. The observed clinical signs (piloerection, abnormal body carriage, abnormal gait, lethargy, decreased respiratory rate, partially closed eyelids, pallor of extremities, soft/liquid faeces, increased salivation, unsteadiness, body tremors, dilation of pupils, cold to touch and ano-genital staining) were indicative of general toxicity and did not give any indication of specific target-organ toxicity. Gross pathology did not reveal any adverse findings.

In an acute dermal study in rats (section 10.2), a dose of 2000 mg/kg bw did not induce any clinical signs of systemic toxicity and there were no macroscopic findings at necropsy.

In an acute inhalation toxicity study in rats (section 10.3), animals were exposed (whole body) to a liquid droplet aerosol of pethoxamid at a concentration of 4.16 mg/L for 4 hours. Clinical signs of toxicity included partially closed eyes, wet /stained fur, matted fur. These general indicators of toxicity and respiratory effects that are commonly associated with the inhalation route of exposure. All treated animals showed recovery from day 6 in males or day 8 in females. The results of this study do not provide specific evidence of an irritant effect on the respiratory tract of rats.

10.11.2 Comparison with the CLP criteria

STOT-SE categories 1 and 2 are assigned on the basis of clear evidence of significant or severe toxicity to a specific target organ that arises from a single exposure to a substance. STOT-SE category 3 is currently assigned for the transient effects of respiratory tract irritation and narcotic effects.

The available acute studies do not provide any indication that pethoxamid meets the classification criteria for specific target-organ toxicity category 1, 2 or 3 following a single exposure. No classification is proposed.

10.11.3 Conclusion on classification and labelling for STOT SE

Not classified (conclusive but not sufficient for classification).

10.12 Specific target organ toxicity-repeated exposure

The toxicity of pethoxamid following repeated exposure has been evaluated by the oral route of administration in rats, mice and dogs, including lifetime studies in rats and mice. This included a study investigating microscopic (via electron microscopy) changes in the liver from the 13 week mouse study, and a mechanistic study investigating thyroid function in the male rat. In addition, dermal toxicity was evaluated in rats in a 28-day study.

Table 54: Summary table of animal studies on STOT RE

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, route of exposure, dose levels, duration of exposure**	**Results**	**Reference**
Rat oral 28 day feeding study (via diet) OECD 407 Deviations: Some omissions in range of haematology parameters, organ weights recorded, tissues preserved and tissues examined microscopically. GLP Rat Crl:CD(SD)BR 5/sex/group	Pethoxamid Batch: TB-930727 Purity: 95.2%. Dose levels: 0, 500, 2500, 5000, 7500 ppm in diet for 28 days.	<u>7500 ppm (699 mg/kg bw/day males, 737 mg/kg bw/day females)</u> ↓ Body weight Week 4: 35.2% males, 20.5% females ↓ Body weight gain Week 0-4: 88.9% males, 64.4% females ↓ Food consumption Week 1-4: 28% males, 19% females ↓ Water consumption Week 3: 25% males (single value) Haematology:↓ Platelets 14.4% males; ↓ White blood cell counts 31.0% males; ↓ lymphocytes 36.3% males; ↓ haemoglobin 7.0% females; ↓ MCHC 4.7% females Clinical chemistry: ↑ Cholesterol 228% males, 173% females; ↓ Phosphorus 18.8% males; ↑ ALT 66.7% females; ↓ Glucose 16.1% males, 21.2% females; ↑ Globulin 11.4% males, 20.6% females ↑ Liver weights: Adjusted 63% males, 44% females Pathology findings: Centrilobular enlargement of hepatocytes 5/5 males and females (0/5 controls); Periportal hepatocytes with eosinophilic inclusions 4/5 males and 3/5 females (0/5 controls). <u>5000 ppm (482 mg/kg bw/day males, 535 mg/kg bw/day females)</u> ↓ Body weight Week 4:17.2% males, 8.8% females ↓ Body weight gain Week 0-4: 42.4% males 30.1% females ↓ Food consumption Week 1-4: 13% males Haematology:↓ Platelets 13.9% males; ↓ lymphocytes 30.3% males; ↓ haemoglobin 6.3% females Clinical chemistry: ↑ Cholesterol 162% males, 117% females; ↓ Phosphorus 10.4% males; ↓ Glucose 16.1% females; ↑ Globulin 20.0% males, 17.6% females ↑ Liver weights: Adjusted 69% males, 45% females Pathology findings: Centrilobular enlargement of hepatocytes 5/5 males and 3/5 females (0/5 controls); Periportal hepatocytes with eosinophilic inclusions 3/5 males and 1/5 females (0/5 controls). <u>2500 ppm (227 mg/kg bw/day males, 266 mg/kg bw/day females)</u> ↓ Body weight gain Week 0-4: 23.6% males ↓ Food consumption Week 1-4: 11% males Haematology:↓ Platelets 18.9% males Clinical chemistry: ↑ Cholesterol 121% males, 51.9% females ↑ Liver weights: Adjusted 58% males Pathology findings: Centrilobular enlargement of hepatocytes 1/5 males (0/5 controls); Periportal hepatocytes with eosinophilic inclusions 4/5 males (0/5 controls). <u>500 ppm (45.3 mg/kg bw/day males, 52.9 mg/kg bw/day females)</u> Clinical chemistry: ↑ Cholesterol 55.2% males **No NOAEL established.**	Anonymous; (1994) 69 PXA

Mouse oral 28 day feeding study (via diet) OECD 407 Deviations: no haematology or clinical chemistry. Additional liver enzyme investigations. Only macroscopic abnormalities and liver and thyroid examined microscopically. GLP Mouse Crl: CD-1 (ICR) 16/sex/group	Pethoxamid Batch: TB-930727 Purity: 95%. Dose levels: 0, 100, 500, 3000, 10000 ppm in diet for 28 days.	<u>10000 ppm (1786 mg/kg bw/day males, 2206 mg/kg bw/day females)</u> ↓ Body weight gain: Week 0-4 112% males; Week 0-1 weight loss 2.5 g males (controls gained 2.3 g), 1.2 g females (controls gained 0.6 g) ↓ Food consumption: Week 1 30% males, 24% females ↑ Liver weights: Adjusted 45% males, 27% females Hepatic enzyme activities: ↑ both sexes EROD, PROD, lauric acid 11 hydroxylase (LA11), p-nitrophenol UDP glucuronyltransferase (UDPGT) Pathology findings: Hepatocellular hypertrophy- Centrilobular 7/16 males (1/16 controls); generalised 9/16 males (0/16 controls); periportal 16/16 females (0/16 controls) <u>3000 ppm (539 mg/kg bw/day males, 679 mg/kg bw/day females)</u> ↓ Body weight gain: Week 0-4 52% males; Week 0-1 weight loss 0.2 g males (controls gained 2.3 g) ↓ Food consumption: Week 1 20% males, 14% females ↑ Liver weights: Adjusted 27% males, 16% females Hepatic enzyme activities: ↑ EROD, PROD both sexes; LA11 and UDPGT females only Pathology findings: Hepatocellular hypertrophy- Centrilobular 5/16 males (1/16 controls); generalised 5/16 males (0/16 controls); periportal 14/16 females (0/16 controls) <u>500 ppm (85 mg/kg bw/day males, 114 mg/kg bw/day females)</u> ↑ Liver weights: Adjusted 14% males Hepatic enzyme activities: ↑ PROD both sexes; EROD females only Pathology findings: Centrilobular hepatocellular hypertrophy 4/16 males (1/16 controls) <u>100 ppm (17 mg/kg bw/day males, 22 mg/kg bw/day females)</u> Hepatic enzyme activities: ↑ PROD both sexes **The NOAEL is 100 ppm corresponding to 17 mg/kg bw/day based on liver weight increase and liver histopathology findings at the dose level of 500 ppm (85 mg/kg bw/day).** **At 100 ppm, phenobarbitone-type liver enzyme induction was observed, but this was not considered an adverse effect.**	Anonymous; (1996a) 70 PXA

Dog maximum tolerated dose and four week constant dose study via capsule administration No guideline for this study type in non-rodents GLP Dog Beagle MTD phase: Dose given to initial 2 dogs increased every 3 to 4 days. Constant dose phase: Further pair of dogs	Pethoxamid Batch: TB 930727 Purity: 96.0% and Batch: TB 951005 Purity: 95.1%. Orally by capsule MTD phase: 25, 50, 100, 200, 400, 800, 1000 and 1600 mg/kg bw/day given for 3 to 4 days. Constant dose phase: 50, 200 and 800 mg/kg bw/day given to for 28 days. Due to adverse observations at 800 mg/kg bw/day, dosing suspended for 7 days and then restarted at 400 mg/kg bw/day for 28 days.	**<u>MTD phase:</u>** <u>25-1600 mg/kg bw/day</u> Liquid faces observed in both sexes at all dose levels. Mucoid/red stained faeces observed from 400 mg/kg bw/day. Vomiting observed from 800 mg/kg bw/day. Salivation observed in males from 1000 mg/kg bw/day. Female was subdued after 2 doses of 1600 mg/kg bw/day and dosing discontinued. ↓ Body weight: Slight in female from 800 mg/kg bw/day ↓ Food consumption: From 800 mg/kg bw/day ↑ Liver weights: Relative (to body weight) female Pathology findings: Centrilobular hepatocellular hypertrophy and prominent thyroid microfollicles (male only) **<u>Constant dose phase:</u>** <u>800 mg/kg bw/day</u> Liquid/red faeces, vomiting, salivation, subdued behaviour, marked loss of body weight and decreased food consumption at 800 mg/kg bw/day. Dosing suspended after 7 days. Following 7 days off dose, animals given 400 mg/kg bw/day for 28 days. <u>400 mg/kg bw/day</u> Vomiting, liquid/mucoid faeces, salivation and subdued behaviour. Decreased haemoglobin and related parameters. Haematology:↓ Slight in red cell parameters; ↑ platelets; ↑ reticulocytes Clinical chemistry: ↓ Slight cholesterol ↑ Liver weights: Relative (to body weight) female Hepatic enzyme activities: ↓ EROD male; PROD female; lauric acid 11 hydroxylase (LA11) male; lauric acid 12 hydroxylase (LA12) male and female; p-nitrophenol UDP glucuronyltransferase (UDPGT) male and female Pathology findings: Minimal centrilobular hepatocyte hypertrophy female only <u>200 mg/kg bw/day</u> Vomiting, liquid/mucoid faeces both male and female Subdued behaviour female ↑ Liver weights: Relative (to body weight) female Pathology findings: Minimal centrilobular hepatocellular hypertrophy, male and female <u>50 mg/kg bw/day</u> Liquid/mucoid faeces both dogs **Conclusion: Dosages of 400 mg/kg bw/day and above are not suitable for further investigations on dogs. No induction of drug metabolizing enzymes in dogs**	Anonymous; (1996b) 72 PXA

Rat oral 13 week feeding study (via diet) OECD 408 Deviations: No FOB or motor activity, thymus weight not recorded, no microscopic examination of accessory sex organs, skin or peripheral nerve. GLP Rat Crl: CD (BR) 10/sex/group	Pethoxamid Batch: TB-930727 Purity: 95.2%. Dose levels: 0, 100, 500, 2500, 5000 ppm in diet for 13 weeks	5000 ppm (388 mg/kg bw/day in males, 426 mg/kg bw/day in females) ↓ Body weight gain Week 0-13: 31% males, 25% females ↓ Food consumption: Week 1 49% males, 24% females; Week 1-13: 15% males ↓ Water consumption Week 12: 16% males Haematology: ↓ Platelets 11% males Clinical chemistry: ↑ Cholesterol 108% males, 75% females; ↓ Glucose 13% males; ↑ Total protein 11% males, 7% females Liver enzyme activity: ↑ cyt P450, EROD, PROD, LA11 and UDPGT in both sexes; LA12 in females only. Increase in PROD in females 791-fold. ↑ Liver weights: Adjusted 42% males, 42% females Liver pathology: Periportal hepatocyte margination of cytoplasm 10/10 males and 10/10 females (0/10 controls); Occasional concentric intracytoplasmic inclusions 7/10 males (0/10 controls); Minimally generalised hepatocyte enlargement 4/10 males and 4/10 females (0/10 controls); Fat deposition in periportal hepatocytes 6/10 males (0/10 controls). ↑ Thyroid weights: 15% absolute males; 34% adjusted females. Thyroid pathology: Follicular cell hypertrophy 9/10 males, 4/10 females (2/10 control males, 0/10 control females); Sparse colloid 7/10 males (1/10 controls). 2500 ppm (196 mg/kg bw/day in males, 207 mg/kg bw/day in females) ↓ Body weight gain Week 0-13: 24% males, 15% females ↓ Food consumption Week 1: 23% males ↓ Water consumption Week 12: 20% males Haematology: ↓ Platelets 12% males Clinical chemistry: ↑ Cholesterol 46% males, 37% females Liver enzyme activity: ↑ cyt P450, EROD, PROD, LA11 and UDPGT in both sexes; LA12 in females only. Increase in PROD in females 276-fold. ↑ Liver weights: Adjusted 27% males, 21% females Liver pathology: Periportal hepatocyte margination of cytoplasm 9/10 males (0/10 controls); Fat deposition in periportal hepatocytes 5/10 males (0/10 controls). Thyroid pathology: Follicular cell hypertrophy 7/10 males (2/10 control). 500 ppm (36.2 mg/kg bw/day in males, 41.6 mg/kg bw/day in females) ↓ Body weight gain Week 0-13: 12% males Liver enzyme activity: ↑ EROD, PROD, in both sexes; cyt P450, LA11, LA12 and UDPGT in females only. Increase in PROD in females 12.4-fold. No statistically significant increase in organ weights or pathology findings. 100 ppm (7.5 mg/kg bw/day in males, 8.0 mg/kg bw/day in females) No effects. **NOAEL 100 ppm (7.5 mg/kg bw/day) based on decreased body weight gain. The liver and thyroid findings at higher doses indicate an effect on the liver-thyroid axis known for phenobarbitone-type inducers of drug metabolizing enzymes in the liver.**	Anonymous; (1996) 61 PXA

90-day rat study to evaluate potential mechanisms underlying the observed thyroid gland changes. General design similar to OECD 408. Thyroid hormone assessed on Days -3, 15, 29, 57, and 89. Total T3 and T4, TSH and rT3 levels analysed. At termination liver, thyroid and pituitary weighed and histopathology performed. Hepatic UDP-glucuronosyltransferase (UGT) activity (T3-glucuronidation and T4-glucuronidation) and protein concentration assessed GLP Rat Crl:CD(SD) Sprague Dawley 15 males/group termination after 90 days 5 males/group termination after 30 days	Pethoxamid Batch: 21082018 Purity: 99.4% Dose levels: 0, 400, 1600, 5000 ppm (0, 24, 96, 308 mg/kg bw/day) in diet for 13 weeks Positive control: Phenobarbital 1000 ppm.	<u>Pethoxamid</u> <u>5000 ppm (308 mg/kg bw/day)</u> ↓ Body weight in the 5000 ppm group, 7.5% lower than controls at the end of study. ↑ Mean TSH values in the 5000 ppm groups on Days 15, 29, 57, and 89 ↓ Time-dependent decrease in total T4 relative to pretreatment values during the first 29 days ↑ Thyroid weight in the 5000 ppm group on Days 30 (absolute 31% and relative to body weight 58%) and 93/94 (absolute 27% and relative to body weight 39%) ↑ Liver weight in the 5000 ppm group on Day 30 (absolute 6% and relative to body weight 30%) and 5000 ppm (absolute 35% and relative to body weight 47%) group on Day 93/94. ↑ Thyroid follicular cell hypertrophy in the 5000 ppm group (15/15 compared with 0/15 in control) on Day 93/94 ↑ Hepatocellular hypertrophy in the 5000 ppm group on Days 30 (3/5 compared with 0/5 in control) and 93/94 (5/15 compared with 0/15 in control) ↑ T4-glucuronidation activity was observed in the 5000 ppm group on Days 30 (4.9 fold increase) and 93/94 (3.2 fold increase) ↑ T3-glucuronidation activity was elevated in the 5000 ppm group on Day 30 (1.7 fold increase) and on Day 93/94 (1.9 fold increase) <u>1600 ppm (96 mg/kg bw/day)</u> ↑ Mean TSH values in the 1600 ppm group on Days 15, 29, 57, and 89 ↑ Liver weight in the 1600 ppm group (absolute 15% and relative to body weight 16%) on Day 93/94. ↑ Thyroid follicular cell hypertrophy in the 1600 ppm group (3/14 compared with 0/15 in control) on Day 93/94 ↑ T4-glucuronidation activity was observed in the 1600 ppm group on Days 30 (2.7 fold increase) and 93/94 (1.9 fold increase) ↑ T3-glucuronidation activity was elevated in the 1600 ppm group on Day 30 (1.9 fold increase) <u>400 ppm (24 mg/kg bw/day)</u> No effects. <u>PB</u> Data shown in data summary table in Section 10.9 Overall, it can be concluded that liver enzyme induction, leading to an increase in T4 glucuronidation and clearance of T4, elicited a feedback response on the thyroid via an increase in TSH. Further, the increased TSH and associated thyroid follicular cell hypertrophy resulted in functional compensation by the thyroid in the Pethoxamid-treated rats. **The NOAEL (NOEL) was 400 ppm (24 mg/kg bw/day) based on increased liver weight of liver and thyroid follicular cell hypertrophy.**	Anonymous; (2020) 2018TOX-PXA4560

Mouse oral 13 week feeding study (via diet) OECD 408 Deviations: Uterus weight, thymus weight not included; several organs not examined microscopically and not all accessory sex organs preserved. Supplementary electron microscopy of liver. GLP Mouse Crl: CD-1 (ICR) BR 10/sex/group	Pethoxamid Batch: TB-960306 Purity: 95.0% Dose levels: 0, 50, 400, 3000, 10000 ppm in diet for 13 weeks	<u>10000 ppm (2354 mg/kg bw/day in males and 2492 mg/kg bw/day in females)</u> ↓ Body weight gain Week 0-1: loss of 2.7 g males, 1.1 g females; controls gained 2.3 and 1.2 g, respectively ↓ Body weight gain Week 0-12: 102% males, 80% females Haematology: ↓ Haemoglobin 11% males, 8% females; ↓ PCV 8% males; ↓ Red cell count 12% males, 8% females; ↑ MCV 5% males; ↓ MCHC 3% males; ↓ lymphoctytes 41% males (affected WBC). Clinical chemistry: ↑ Cholesterol 33% males, 108% females; ↓ Total protein 11% males, 6% females; ↓ Albumin 10% males, 13% females; Changes in plasma ion concentrations in males (↓ K and Ca; ↑ P and Cl). ↓ Urinary protein 75% females. ↑ Thyroid weights: Adjusted 23% females ↑ Liver weights: Adjusted 38% males, 48% females Liver pathology: Hepatocyte hypertrophy- centrilobular midzonal 8/10 males (0/10 controls); periportal 10/10 females (0/10 controls) ↓ Spleen weight: Absolute 33% males, 27% females Spleen pathology: Haemosiderosis 10/10 males (3/10 controls), 9/10 females (7/10 controls); Extramedullary haemopoiesis reduced severity; Reduced cellularity of the white pulp – marginal zone 9/10 males (2/10 control) Thymus pathology: Involution/atrophy 9/9 males (5/9 controls), 5/10 females (8/10 controls) GIT pathology: Villous epithelial cells swollen + cytoplasmic rarefaction in duodenum 10/10 males, 9/10 females; in jejunum 4/10 in males and females (no findings in control group). <u>3000 ppm (610 mg/kg bw/day in males and 724 mg/kg bw/day in females)</u> ↓ Body weight gain Week 0-12: 42% females Haematology: ↑ MCV 5% males Clinical chemistry: ↑ Cholesterol 33% males, 74% females; ↓ Albumin 10% males, 6% females; Changes in plasma ion concentrations in males (↓ Ca; ↑ Cl). ↑ Thyroid weights: Adjusted 20% females ↑ Liver weights: Adjusted 26% males, 22% females Liver pathology: Hepatocyte hypertrophy - periportal 10/10 females (0/10 controls) GIT pathology: Villous epithelial cells swollen + cytoplasmic rarefaction in duodenum 6/10 males (0/10 control). <u>400 ppm (70.5 mg/kg bw/day in males and 93 mg/kg bw/day in females)</u> No treatment-related effects. <u>50 ppm (9.1 mg/kg bw/day in males and 12.0 mg/kg bw/day in females)</u> No treatment-related effects. **The NOAEL (NOEL) was 400 ppm (70.5 mg/kg bw/day) based on decreased body weight gain, increased cholesterol, increased organ weights of liver and thyroid and hepatocyte hypertrophy.**	Anonymous; (1998) 71 PXA and Anonymous; L. (1997a) 1288 PXA

Dog 13 week study via capsule administration OECD 409 Deviations: Clinical chemistry: no ornithine decarboxylase GLP Dog Beagle 4/sex/group	Pethoxamid Batch: TB-960306 Purity: 95.0%. Dose levels: 0, 8, 50, 300 mg/kg bw/day for 13 weeks by oral capsule Due to poor clinical condition of 300 mg/kg bw/day animals, treatment stopped on Day 4 of Week 2. Following a recovery period of 4 days, animals dosed with 200 mg/kg bw/day for the remainder of the study.	<u>300/200 mg/kg bw/day</u> Clinical signs Week 12: Liquid faeces 22 incidences in males, 23 incidences in females; Salivation 14 incidences in males, 20 incidences in females; Vomiting 1 incidence in males, 7 incidences in females Body weight Week 0-1: loss of 0.3 kg males, 0.8 kg females (control weight gain 0.4 kg in both sexes). Lower terminal body weight 25% males 17% females, neither statistically significant ↓ Body weight gain Week 0-13: 76% males, 59% females ↓ Food consumption: Week 0-1: 15% males, 46% females; Week 3-13 6% males, 8% females Haematology Week 12: ↑ Platelets 34% males, 37% females; ↑ Reticulocytes 4.5-fold males, 5.5 fold females; ↓ Hb 15% males, 13% females; ↓ PCV 12% females; ↓ RBC 16% males 14% females Clinical chemistry: ↓ Albumin 16% males, 12% females; ↓ALT activity 37% females [↓ ALT both sexes approx. 50% week 6, but ↑ males week 12]. Organ weight differences considered to be due to the marked body weight difference of control and high dose group dogs. Pathology findings: Vacuolation cortical tubules in kidney 4/4 both sexes (2/4 controls); Thymus involution 3/4 both sexes (0/4 controls); Spleen minimal haemosiderosis 2/4 males, 3/4 females (1/4 controls) Glycogen depletion in the liver (4/4 both sexes); myeloid atrophy in the bone marrow (3/4 males, 4/4 females); reduced lymphoid cellularity in the lymph nodes (2/4 male), immaturity of prostate (3/4) and testes(2/4) with an absence of spermatozoa in the epididymides (2/4) and diffuse vacuolation of the zonae fasciculata and reticularis in adrenals (4/4 male) considered related to poor clinical condition, and not a direct effect. <u>50 mg/kg bw/day</u> Clinical signs Week 12: Liquid faeces 19 incidences in males, 17 incidences in females Haematology Week 12: ↓ RBC 8% females Clinical chemistry: ↓ Albumin 8% females <u>8 mg/kg bw/day</u> Clinical signs Week 12: Liquid faeces 6 incidences in males, 6 incidences in females. Haematology Week 12: ↓ RBC 11% females **NOAEL 8 mg/kg bw/day based on post dose liquid faeces**	Anonymous; (1997b) 73 PXA

Dog 52 week study via capsule administration OECD 452 GLP Dog Beagle 4/sex/group	Pethoxamid Batch: TB-960306 Purity: 95.0%. Dose levels: 0, 2, 20, 150 mg/kg bw/day for 12 months by oral capsule.	150 mg/kg bw/day One male and one female killed moribund weeks 47 and 38 respectively. Clinical observations in these animals were loss of appetite, bloody stool, salivation, dehydration signs, bradypnea, hypothermia, pale oral mucosa, pale conjunctiva, emaciation, decrease in spontaneous activity, prone and lateral position. Surviving animals had increased incidences of soft stool, diarrhoea and vomiting. ↓ Body weight Week 54: 16% females (surviving animals only). Final body weights of the sacrificed animals were 52 (male) and 64% (female) of their maximum values. Clinical chemistry Week 53: ↓ Albumin 4% males, 6% females; ↑ ALP activity 64% males, 151% females. Organ weights: ↑ Relative liver weight 38% males, 67% females; ↑ Relative kidney weights 59% females. GIT pathology: Stomach (pylorus) - micronecrosis mucosa 1/3 males, vacuolation and mononuclear cell infiltration in muscle layer 1/3 males, epithelium hyperplasia mucosa 1/3 males (0/4 in controls). Large intestines vacuolation and mononuclear cell infiltration in muscle layer 2/3 males (0/4 controls). 20 mg/kg bw/day Clinical signs: Soft stool days 162 to 293 and diarrhoea days 20 to 141. Organ weights: ↑ Relative liver weight 42% females. 2 mg/kg bw/day No treatment related effects. **Target organ gastrointestinal tract. The NOAEL in this study was 2 mg/kg bw/day based on increased liver weight in females and slightly increased frequency of diarrhoea**	Anonymous; (1999) 74 PXA

Rat chronic and carcinogenicity study via the oral (dietary) route OECD 453 + electron microscopy of liver. Deviations: MCH not presented; uterus not weighed; Coagulating gland and peripheral nerve not preserved. GLP Rat Crl: CD BR (IGS) Main groups: 50 /sex/group Up to 10 rats/sex/group examined after the completion of 26, 52 and 78 weeks of treatment.	Pethoxamid Batch: TB-960306 Purity: 95.0% Dose levels: 0, 25, 400, 1600 ppm in diet for 104 weeks	<u>1600 ppm (70 mg/kg bw/day in males and 99 mg/kg bw/day in females)</u> ↓ Body weight gain: Week 0-4 15% males, 15% females; Week 0-88 13% males, 23% females ↓ Food consumption: Week 1-4 8% males, 6% females; Week 1-104 6% males Clinical chemistry: ↑ Cholesterol All timepoints 17-42% males, 33-46% females; ↓ Globulin First year of study 9-13% females; ↑ ɣ GT activity All timepoints 1.5-8 x control in males ↑ Liver weights: Adjusted weight 10 and 12% males Weeks 27 and 53; 17 and 16% females Weeks 27 and 105 ↑ Thyroid weights: Adjusted weight 50% and 31% males Weeks 53 and 79. No similar change Week 27 or 105. *Non-neoplastic findings* Liver pathology: Centrilobular hepatocyte hypertrophy 11/50 males, 8/50 females (0/50 controls); Concentric intracytoplasmic inclusions 10/50 males (0/50 controls); Cystic degeneration 24/50 males (12/50 controls); Clear cell hepatocytes 15/50 males (6/50 controls). Thyroid pathology: <u>Not statistically significant</u>. Follicular cell hyperplasia 4/50 males (0/50 controls); Follicular cell cystic hyperplasia 7/50 males (4/50 controls). <u>400 ppm (17.0 mg/kg bw/day in males and 23.3 mg/kg bw/day in females)</u> ↓ Body weight gain: Week 0-88 11% females (not statistically significant but considered treatment related) ↓ Food consumption: Week 1-4 4% males ↑ Thyroid weights: Adjusted weight 39% and 49% males Weeks 53 and 79. <u>25 ppm (1.0 mg/kg bw/day in males and 1.4 mg/kg bw/day in females)</u> ↑ Thyroid weights: Adjusted weight 38% males Week 53. **The NOAEL was considered to be 25 ppm (1.0 mg/kg bw/day), based on the decreased body weight gain in females at 400 ppm.**	Anonymous; (2000a) 80 PXA (Anonymous; 2000-amdt-1; 80 PXA amdt-1) (Anonymous; 2003; 80 PXA suppl-1) (Anonymous; 2016; 80 PXA suppl-2) (Anonymous; 2003; 203 PXA)

Mouse carcinogenicity study via the oral (dietary) route. OECD 453, however OECD 451 acceptable for second rodent species Deviations from OECD 453: Slight exceedance of weight variation in females; no organ weights of spleen, uterus; no preservation of the coagulating gland, peripheral nerves; haematology-only blood smears; no clinical biochemistry. GLP Mouse Crl: CD-1 BR 50/sex/group Treated until one group in each sex reached 50% survival i.e. up to 92 week (females) or 95 week (males). Additional 10/sex/group treated for up to 52 weeks.	Pethoxamid Batch: TB-960306 Purity: 95.0% and Batch: TB-960306-C Purity: 94.8%. Dose levels: 0, 30, 400, 5000 ppm in diet.	<u>5000 ppm (982 mg/kg bw/day in males, 1068 mg/kg bw/day in females)</u> ↓ Bodyweight at termination: 16% males, 17% females ↓ Body weight gain: 40% males to Week 95; 34% females to Week 92 ↑ Total food consumed: 21% males ↑ Liver weight: Terminal adjusted 77% males, 36% females ↑ Kidney weight: Terminal adjusted 23% females ↑ Thyroid weight: Terminal adjusted 29% females ↑ Adrenal weight: Terminal adjusted 26% males *Non-neoplastic findings* Liver pathology: Hepatocyte hypertrophy 39/50 males (generalised), 42/50 females (periportal) 0/50 in controls. Kidney pathology <u>both sexes</u>: Cortical tubules basophilic 43/50 males (33/50 controls), 41/50 females (12/50 controls); Medullary tubules dilated with eosinophilic casts: 42/50 males (27/50 controls), 32/50 females (14/50 controls); Cortical mineralisation 46/50 males (32/50 controls), 26/50 females (3/50 controls); Medullary mineralisation 44/50 males (6/50 controls), 31/50 females (0/50 controls); Papillary mineralisation 36/50 males (11/50 controls), 30/50 females (3/50 controls). Kidney pathology <u>males only</u>: Cortical tubular cell hypertrophy (slight) 8/50 (0/50 controls); Cortical fibrosis with tubular collapse and basophilia 37/50 (13/50 controls); Cortical cysts 31/50 (20/50 controls). Duodenum pathology: Swelling/rarefaction of villous epithelium 42/49 males (0/48 controls), 18/49 females (0/45 controls); Villous hypertrophy 27/49 males (0/48 controls). Jejunum pathology: Swelling/rarefaction of villous epithelium 35/49 males (0/48 controls), 14/49 females (0/46 controls); Villous hypertrophy 16/49 males (0/48 controls). <u>400 ppm (56.8 mg/kg bw/day in males, 68 mg/kg bw/day in females)</u> ↑ Liver weight: Terminal adjusted 14% males (not statistically significant), 10% females ↑ Kidney weight: Terminal adjusted 11% females *Non-neoplastic findings* Duodenum pathology: Swelling/rarefaction of villous epithelium 29/47 males (0/48 controls), 12/50 females (0/45 controls); Villous hypertrophy 9/47 males (0/48 controls). Jejunum pathology: Swelling/rarefaction of villous epithelium 25/48 males (0/48 controls), 7/50 females (0/46 controls); Villous hypertrophy 8/48 males (0/48 controls). <u>30 ppm (4.0 mg/kg bw/day in males, 5.0 mg/kg bw/day in females)</u> *Non-neoplastic findings* Duodenum Pathology: Swelling/rarefaction of villous epithelium 6/47 males at termination, 5/8 males at interim kill (0/48, 0/8 controls, respectively). **The LOAEL was considered to be < 30 ppm (< 4 mg/kg bw/day).**	Anonymous; (2000b) 82 PXA (Anonymous; 2016; 82 PXA suppl-1) (Anonymous; 2001; 83 PXA) (Anonymous; 2001; 1484 PXA) (Anonymous; 2001; 1241 PXA)

Rat 28 day dermal administration toxicity study OECD 410 GLP Rat Crl: CD (SD) 10/sex/group	Pethoxamid technical Batch: P1351-JaK-T2-23-6 Purity: 95.80% Dose levels: 0, 100, 300, 1000 mg/kg bw/day for a 6-hour exposure period for 28 days. Test material applied neat and covered with a semi-occlusive wrap. After 6 hours, the wrap was removed and site washed.	1000 mg/kg bw/day Increase in the number of male and female rats with residue (presumed to be test substance) within the treated area. Increase in the number of male rats group observed with signs of irritation (incidence / animals); 21/7 erythema grade 1 and 45/4 flaking grade 1 [8/3 and 6/2 respectively in control group]. Decreased numbers of anagen-phase follicles (0.9 in males and 0.6 in females, 1.6 males and 2.9 females in controls) with some associated minimal to mild hyperkeratosis (1/10 in male and 6/10 in female, 0/10 in controls) were noted within sections of treated skin from the male and female rats. No systemic toxicity. 300 mg/kg bw/day Increase in the number of male and female rats with residue (presumed to be test substance) within the treated area. No systemic toxicity. 100 mg/kg bw/day Increase in the number of male and female rats with residue (presumed to be test substance) within the treated area. No systemic toxicity. **NOAEL for systemic toxicity is 1000 mg/kg bw/day**	Anonymous; (2014d) 1216 PXA

Table 55: Summary table of human data on STOT RE

Type of data/report	**Test substance**	**Route of exposure** **Relevant information about the study (as applicable)**	**Observations**	**Reference**
No relevant studies				

Table 56: Summary table of other studies relevant for STOT RE

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

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

Pethoxamid is generally of low toxicity in all species tested in repeat dose studies. Although there are 28 day studies in three species, the key studies are considered to be the 90 day studies. Data from longer term (1-2 years) has been included for completeness. Data from the multigeneration and developmental toxicity studies was taken into consideration, but did not indicate any specific target organ toxicity. The predominant effects were on body weight gain, as described for the rat 90 day study.

There is a consistent pattern of toxicity in rats and mice fed pethoxamid in the diet for 90 days. The main target organ is identified as the liver, and changes are characterised by altered clinical chemistry values (increased cholesterol) and increased activity of the hepatic enzymes. These changes are accompanied by increased liver weights and histopathology findings of hepatocyte hypertrophy. Changes in organ weight and histopathology occur after 90 days at and above dose levels of 196 mg/kg bw/day in male rats, 207 mg/kg bw/day in female rats; 610 mg/kg bw/day in male mice and 724 mg/kg bw/day in female. Changes such as liver hypertrophy in response to enzyme induction are considered to be indicative of an adaptive response, and not to be evidence of significant target organ toxicity.

Corresponding to the adaptive changes in the liver, there is evidence of changes in the thyroid (increased TSH, increased weight and follicular cell hypertrophy). It is well established that certain chemicals cause induction of liver enzymes and effect thyroid hormone homeostasis. A mechanistic study (see Section 10.9) demonstrated greater clearance of thyroxine due to liver induced T4 glucuronidation in the pethoxamid-treated rats compared to controls (Anonymous, 2019; 4538 PXA). Rodents are highly sensitive to disturbance of the thyroid hormone homeostasis. A review of the data and an assessment of the relevance of rat thyroid changes to humans has been conducted following the IPCS and ILSI/HESI framework and is presented in in the position paper "Pethoxamid: Human Relevance Framework Assessment of Induced Rodent Liver and Thyroid Tumors" (Anonymous, 2020; FMC-54841). There is sufficient quantitative evidence on the basic physiological processes in the general literature to conclude that thyroid tumors, induced by a process involving increased hepatic clearance of thyroid hormone and altered thyroid homeostasis in rodents, will not lead to an increase in susceptibility to thyroid tumor development in humans. Overall, it is considered that a concordant and highly plausible MoA has been established for pethoxamid-induced rat thyroid follicular cell adenomas and that this MoA is not relevant to humans.

In mice, additional histopathological changes were observed in spleen, thymus and duodenum. Effects in the spleen (haemosiderosis) and thymus (involution/atrophy of the thymus in males) were only seen in the 90 day study at a very high dose level, 2354 mg/kg bw/day in males and 2492 mg/kg bw/day in females, which is in excess of a normal limit dose for studies of this duration. Effects on the duodenum were seen at or above 610 mg/kg bw/day in males and 724 mg/kg bw/day in females in the 90 day study and at or above 4.0 mg/kg bw/day in males after 95 weeks and 68 mg/kg bw/day in females after 92 weeks in the lifetime study.

Pethoxamid was administered orally to dogs for 28 days during a dose range finding study with a limited number of animals, and in 90 days and 12 months guideline studies. Body weight was reduced at higher dose levels in all studies. In the 90-day study, liver weights were in increased (not statistically significant) in both sexes. After 1-year of administration, liver weights were markedly increased in males at 150 mg/kg bw/day and females starting at 20 mg/kg bw/day. However, there were no histopathological liver findings. Liver enzyme induction was analysed only during the 28 day dose range finding study, when no induction of hepatic enzymes could be detected.

Several changes were seen at high dose levels in the dog 90 day and 1 year studies. It should be noted that the high dose level in these studies was at or above a MTD. In the 90 day study, due to poor clinical condition animales treatment of animals with 300 mg/kg bw/day was stopped on Day 4 of Week 2 and following a recovery period of 4 days, 200 mg/kg bw/day was dosed for the remainder of the study. In the 1 year study, one male and one female at the high dose level of 150 mg/kg bw/day were killed moribund in weeks 47 and 38, respectively. Findings in the 90 day study included, increased vacuolation of the cortical tubules of the kidneys and haemosiderosis in the spleen (together with reduced red blood cell parameters, indicating anaemia). In the 1 year study, at the high dose of 150 mg/kg bw/day, the gastrointestinal tract was affected, showing vacuolation, atrophy and mononuclear cell infiltration in the muscle layer of stomach, small and large intestine.

In the 28-day dermal toxicity study in rats, pethoxamid was administered once daily for a 6-hour exposure period, for 28 consecutive days at dose levels of 0, 100, 300 or 1000 mg/kg bw/day. Local findings were confined to a slightly higher incidence of erythema (at the slight grade) and flaking at the dose site in males dosed at 1000 mg/kg bw/day, when compared with concurrent controls. There was no evidence of systemic toxicity. The systemic no-observed-adverse-effect-level (NOAEL) was, therefore, set at 1000 mg/kg bw/day for male and female rats.

10.12.2 Comparison with the CLP criteria

STOT-RE is assigned on the basis of a substance demonstrating evidence of significant or severe toxicity, generally at or below the oral guidance value of 100 mg/kg bw/day (for a classification in category 2) obtained in a 90-day rat study. The oral guidance value for a classification in category 1 is ≤ 10 mg/kg bw/day. The equivalent guidance values for a 28-day study are ≤ 300 mg/kg bw/day and ≤ 30 mg/kg bw/day, respectively; for a one-year study, they are ≤ 25 mg/kg bw/day and 2.5 mg/kg bw/day, respectively, and for a two-year study, ≤ 12.5 mg/kg bw/day and 1.25 mg/kg bw/day. For dermal exposure, the 90-day guidance value is ≤ 200 mg/kg bw/day in rats or rabbits. 'Significant' toxicity is taken to mean changes that clearly indicate functional

disturbance or morphological changes that are toxicologically relevant. 'Severe' toxicity is considered to be more profound or serious and indicates changes that are of a considerably adverse nature with a significant impact on health.

The effects reported in studies at doses below the guidance cut-off values for STOT-RE are summarised below.

Table 57: Extrapolation of equivalent effective dose for toxicity studies of greater or lesser duration than 90 days

Study	**(Adjusted) guidance value category 1 / 2 (mg/kg bw/day)**	**Effects at doses below guidance cut-off values**
28 day rat oral (dietary) study Anonymous, (1994) 69 PXA	30 / 300	Category 1: Lowest dose = 45.3 mg/kg bw/day in males and 52.9 mg/kg bw/day in females. Category 2: Increased cholesterol in males at 45.3 mg/kg bw/day (adaptive)
28 day rat dermal study Anonymous, (2014d) 1216 PXA	60 / 600	Category 1: Lowest dose = 100 mg/kg bw/day in males and females. Category 2: 300 mg/kg bw/day: No systemic effects
28 day mouse oral (dietary) study Anonymous, (1996a) 70 PXA	30 / 300	Category 1: At 17/22 mg/kg bw/day in males/females increased hepatic enzyme activity (adaptive) Category 2: At 85/114 mg/kg bw/day in males/females increased adjusted liver weight and hepatocellular hypertrophy and increased hepatic enzyme activity (adaptive)
MTD and 28 day dog oral (capsule) study Anonymous, (1996b) 72 PXA	30 / 300	Category 1: Lowest dose = 50 mg/kg bw/day in males and females. Category 2: At 200 mg/kg bw/day in males/females increased relative liver weight and hepatocellular hypertrophy (adaptive). Also vomiting, liquid/mucoid faeces and subdued behaviour
90 day rat oral (dietary) study Anonymous, (1996) 61 PXA	10 / 100	Category 1: 7.5/8.0 mg/kg bw/day in males/females = No treatment-related effects Category 2: 36.2/41.6 mg/kg bw/day in males/females = decreased body weight gain and increased hepatic enzyme activity (adaptive)
90 day male rat oral (dietary) study Anonymous, (2020) 2018TOX-PXA4560	10 / 100	Category 1: 24 mg/kg bw/day in males = No treatment-related effects Category 2: 96 mg/kg bw/day in males = Increased liver weight of liver and thyroid follicular cell hypertrophy (adaptive)
90 day mouse oral (dietary) study Anonymous, (1998) 71 PXA	10 / 100	Category 1: 9.1/12.0 mg/kg bw/day in males/females = No treatment-related effects Category 2: 70.5/93 mg/kg bw/day in males/females = No treatment-related effects.

Study	(Adjusted) guidance value category 1 / 2 (mg/kg bw/day)	Effects at doses below guidance cut-off values
90 day dog oral (capsule) study Anonymous, (1997b) 73 PXA	10 / 100	Category 1: 8.0 mg/kg bw/day in males/females = Liquid faeces in both sexes, decreased RBC count in females Category 2: 50.0 mg/kg bw/day in males/females = Liquid faeces in both sexes, decreased RBC count and decreased albumin in females
1 year dog oral (capsule) study Anonymous, (1999) 74 PXA	2.5 / 25	Category 1: 2.0 mg/kg bw/day in males/females = No treatment related effects Category 2: 20.0 mg/kg bw/day in males/females = Soft stools and diarrhoea in both sexes, increased relative liver weight in females (adaptive)
2 year rat oral (dietary) study Anonymous, (2000a) 80 PXA	2.5 / 25 (one-year) 1.25 / 12.5 (two-year)	Category 1: 1.0/1.4 mg/kg bw/day in males/females = increased thyroid weights in males week 53 (adaptive) Category 2: 17/23.3 mg/kg bw/day in males / females = increased thyroid weights in males week 53 and 79 (adaptive)
Chronic mouse oral (dietary) study. 95 weeks males 92 weeks females Anonymous.(2000b) 82 PXA	2.5 / 25 (one-year) 1.4 / 14.0 (92 or 95 weeks)	Category 1: Lowest dose = 4/5 mg/kg bw/day in males/females. Category 2: 4.0 mg/kg bw/day in males = low incidence of swelling/rarefaction of villous epithelium in duodenum at termination and 52 week interim kill.

Very few changes were seen at dose levels corresponding to the maximum values for category 1 or 2 STOT-RE and there were no changes which were considered to represent severe target organ toxicity.

In particular, there were no treatment-related effects in the definitive 90 day rat or mouse studies at or near the maximum reference dose of 10 mg/kg bw/day for category 1. No systemic adverse effects were recorded when pethoxamid was administered dermally to rats at the limit dose of 1000 mg/kg bw/day.

There were no treatment-related effects in the definitive 90 day mouse study at or near the maximum reference dose of 100 mg/kg bw/day for category 2 STOT-RE. The finding in the 28 and 90 day rat study at of decreased body weight gain and/or food consumption is a general non-specific effect, which does not, of itself, indicate significant toxicity of a target organ. Therefore, this finding was not considered to justify classification. Decreased platelets levels was observed in the rat 28 day study at 227mg/kg bw/day, in males only. The observation was not seen at relevant doses in studies of longer duration and therefore, due to the isolated nature of this finding, it do not represent a consistent observation that is relevant for classification.

Increased hepatic enzyme activity, clinical chemistry and organ weights, and pathological changes in the liver and thyroid, indicate adaptive responses to ingestion of the test material in the 28 day mouse and 28 day, 90 day and 2 year rat studies, are considered not to be evidence of significant target organ toxicity. Where, the response to a substance is considered to be purely adaptive, with no evidence of dysfunction, no classification is appropriate. Therefore, these findings are considered not to justify classification. Increased thyroid weights are consistent with the mechanism of increased metabolism of thyroid hormones, and thyroid stimulation, and are associated with the adaptive liver changes described. They are therefore, of insufficient concern for classification.

In the 28 day dog study, there were observations of vomiting, liquid/mucoid faeces and subdued behaviour in males and females at 200 mg/kg bw/day. These findings in the dog study, were considered to be not indicative of STOT-RE as they were not supported by any other evidence of toxicity or pathological findings. There was also increased relative (to body weight) liver weight and hepatocellular hypertrophy observed at this dose. These observations are considered to be an adaptive change, and there is no indication of organ dysfunction. These observations are therefore not adverse and do not support classification for classification.

In the 90 day dog study, there were three relevant findings at a dose level of 8.0 or 50 mg/kg bw/day; liquid faeces in both sexes, decreased RBC count in females and decreased albumin in females. These findings in the dog study were considered to be not of sufficient magnitude or severity to be indicative of STOT-RE. The very low incidence of liquid faeces in the dog (6 incidences in each sex) after 90 days at a dose level of 8.0 mg/kg bw/day were not supported by any other evidence of toxicity or pathological findings. The decreased RBC count and albumin level in the 90 day dog study were only observed in a single sex and were not observed at relevant doses in the 1 year study. Therefore, due to the isolated nature of these findings at these dose levels they do not represent 'significant' toxicity.

In the 52 week dog study, there was an observation of soft stool and diarrhoea in males and females at 20 mg/kg bw/day. These findings in the dog study were considered to be not of sufficient magnitude or severity to be indicative of STOT-RE as they were not supported by any other evidence of toxicity or pathological findings. There was also increased relative (to body weight) liver weight observed in females at this dose, which did not have any histopathological correlate and was considered to be an adaptive change. These observations are therefore, of insufficient concern for classification.

In the lifetime mouse study, swelling/rarefaction of villous epithelium in the duodenum was seen at 4.0 mg/kg bw/day after 52 weeks. This was above the corresponding reference dose of 2.5 mg/kg bw/day for category 1. The gastro-intestinal effects in the long-term toxicity study in mice are considered to be related to the metabolism in mice where it was shown that 33 to 66% of pethoxamid is excreted by faeces. This change had no effect on the survival, growth or development of the mice and the incidence of the change was lower after 95 weeks than after 52 weeks. The changes in the mouse duodenum are considered to indicate an adaptive change and not significant target organ toxicity and no classification is appropriate.

10.12.3 Conclusion on classification and labelling for STOT RE

Not classified (conclusive but not sufficient for classification).

10.13 Aspiration hazard

Table 58: Summary table of evidence for aspiration hazard

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

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

Pethoxamid is a solid and no data have been generated to address aspiration hazard.

10.13.2 Comparison with the CLP criteria

Pethoxamid is a solid, therefore there is no risk of aspiration hazard.

10.13.3 Conclusion on classification and labelling for aspiration hazard

Hazard class not applicable (solid).

11 EVALUATION OF ENVIRONMENTAL HAZARDS

11.1 Rapid degradability of organic substances

Table 59: Summary of relevant information on rapid degradability

Method	Results	Remarks	Reference
Ready biodegradability, OECD 301B	Pethoxamid is not readily biodegradable. Test material: Pethoxamid unlabelled Purity 94.8 % GLP: Yes Study considered valid Relevant for classification regarding degradability in aquatic environment (not rapidly degradable in the aquatic environment)		144 PXA, 1999
Aquatic hydrolysis, OECD 111	Pethoxamid is stable to hydrolysis at pH 4, 7 and 9 (buffer solutions, 50 °C and sterile conditions). Test material: Pethoxamid unlabelled Purity 99.9 % GLP: Yes Study considered valid Relevant for classification regarding degradability in aquatic environment (not rapidly degradable in the aquatic environment)	Legacy study broadly in line with OECD 111	42 PXA, 1999
Aerobic mineralisation in surface water, OECD 309	The degradation rate of radiolabelled pethoxamid was determined with the SFO model and the DT50 was 144 days. Test material: [U-Phenyl-^{14}C]-Pethoxamid Radiopurity > 99.9 % GLP: Yes Study considered valid Relevant for classification regarding degradability in aquatic environment (not rapidly degradable in the aquatic environment)		1443 PXA, 2015
Water/sediment study, OECD 308	The rate of degradation of radiolabelled pethoxamid in the total water/sediment system was determined using SFO kinetics. The DT50 was 7.0 days for the Golden Lake test system and 13.0 days for the Goose River test system. Test material: [U-Phenyl-^{14}C]-Pethoxamid Radiopurity 100 % GLP: Yes Study considered valid		1444 PXA, 2015

Method	Results	Remarks	Reference
	Relevant for classification regarding degradability in aquatic environment		
Aerobic transformation in soil, OECD 307	Refer to summary on water, water-sediment and soil degradation data provided below (Chapter 11.1.4.3). Test material: [U-Phenyl-^{14}C]-Pethoxamid Radiopurity > 98.5 % GLP: Yes Study considered valid Relevant for classification regarding degradability in aquatic environment (rapidly degradable in soil, DT50 < 16 days)	Legacy study broadly in line with OECD 307	134 PXA, 2000
Anaerobic transformation in soil, OECD 307	Refer to summary on water, water-sediment and soil degradation data provided below (Chapter 11.1.4.3). Test material: [U-phenyl-^{14}C]-Pethoxamid Radiopurity > 98.5 % GLP: Yes Study considered valid Relevant for classification regarding degradability in aquatic environment (rapidly degradable in soil, DT50 < 16 days)	Legacy study broadly in line with OECD 307	138 PXA, 2000
Terrestrial field dissipation, SETAC 1995	Refer to summary on water, water-sediment and soil degradation data provided below (Chapter 11.1.4.3). Test material: Pethoxamid formulated GLP: Yes Study considered valid Relevant for classification regarding degradability in aquatic environment (rapidly degradable in soil, DT50 < 16 days)		135 PXA, 2000
Direct photochemical degradation, OECD 316	Radiolabelled pethoxamid degraded moderately fast in aqueous pH 7 buffer when exposed to artificial sunlight, and averaged 20.3 % AR after 16 days of continuous light exposure. Pethoxamid was stable in dark control samples. The DT50 of pethoxamid was 7.7 days, equivalent to 13.9 days of summer sunlight (based on OECD values for 40 – 50 °N). The quantum yield of pethoxamid was determined to be 2.85×10^{-1}. Test material: [U-phenyl-^{14}C]-Pethoxamid Radiopurity 100 %		1442 PXA, 2015

Method	Results	Remarks	Reference
	GLP: Yes Study considered valid		
Soil photolysis, OECD draft (2002)	Refer to summary on photochemical degradation provided below (Chapter 11.1.4.4). Test material: [U-phenyl-^{14}C]-Pethoxamid Radiopurity > 97 % GLP: Yes Study considered valid	Legacy study broadly in line with OECD draft	139 PXA, 1999

11.1.1 Ready biodegradability

A readily biodegradability study (144 PXA, 1999) was conducted to current test method (OECD Test Guideline 301, 1992) and no major study deficiencies were identified. Data in 144 PXA (1999) indicate that pethoxamid is not readily biodegradable.

11.1.2 BOD_5/COD

No data available.

11.1.3 Hydrolysis

Although the aquatic hydrolysis study (42 PXA, 1999) was not conducted to the current test method (OECD Test Guideline 111, 2004), no major study deficiencies were identified. Data in 42 PXA (1999) indicate that pethoxamid is stable to hydrolysis at all pH values investigated (pH 4, 7 and 9; buffer solutions; 50 °C; sterile conditions).

11.1.4 Other convincing scientific evidence

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

No data available.

11.1.4.2 Inherent and enhanced ready biodegradability tests

No data available.

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

Water, water/sediment degradation data

In **natural water under aerobic conditions**, pethoxamid is initially metabolised via glutathione conjugation with subsequent loss of glycine and glutamic acid to form an intermediate cysteine conjugate, pethoxamide metabolite MET-30 (1443 PXA, 2015). In **water/sediment systems**, the cysteine conjugate is transitory and forms a thiol via beta lyase cleavage, with subsequent methylation to a methyl sulphide, pethoxamide metabolite MET-6, or transformation to pethoxamide metabolite MET-104 (1444 PXA, 2015).

Figure 11-1: Proposed route of degradation in aquatic systems

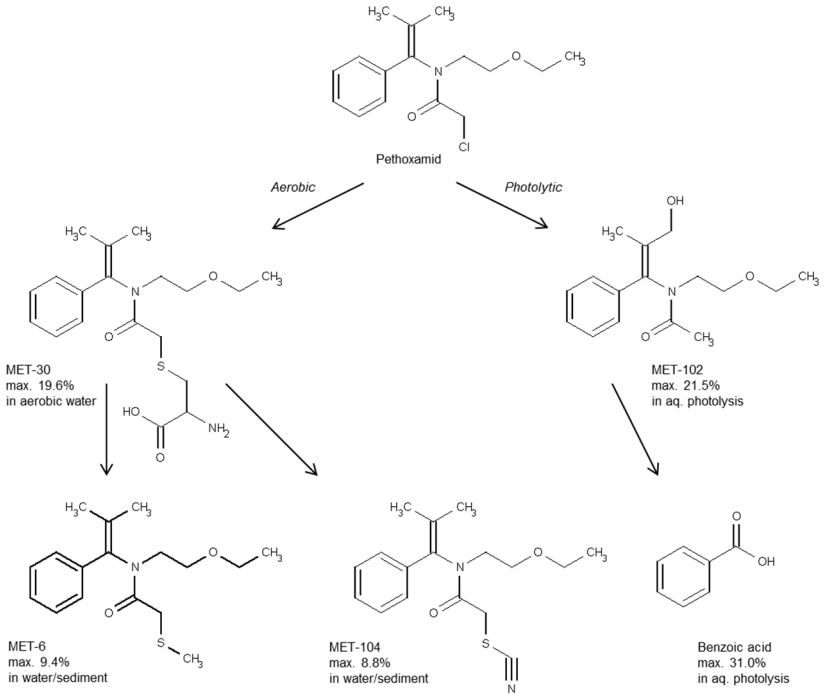


Table 60: Summary on maximum occurrence (% AR) of identified pethoxamide metabolites in aquatic laboratory studies conducted with pethoxamid

Compound	Aquatic hydrolysis (42 PXA, 1999)	Aquatic photolysis (1442 PXA, 2015)	Aerobic mineralisation in surface water (1443 PXA, 2015)	Water/sediment (1444 PXA, 2015)		
				Water phase	Sediment phase	Total system
Pethoxamid	na	na	na	na	14.6	na
MET-2	-	2.2	-	3.6	2.8	4.5
MET-3	-	-	-	0.3	0.5	0.8
MET-6	-	-	-	3.8	6.2	9.4
MET-22	-	-	-	2.3	2.2	3.3
MET-30	-	-	19.6	-	-	-
MET-42		-	-	2.0	1.1	2.2
MET-102	-	21.5	-	-	-	-
MET-104	-	-	-	0.3	8.8	8.8
Benzoic acid	-	31.6	-	-	-	-

na - not applicable

The **rate of degradation** of pethoxamid and its metabolites **in aquatic systems** has been assessed in laboratory studies and is summarised in the tables below.

Table 61: Summary of degradation of pethoxamid in aerobic water (OECD 309)

Water	pH	*DT50* water (d)	*DT90* water (d)	χ^2 (%)	Kinetic model	Reference
Lake Tuckahoe	7.5	144	480	2.9	SFO	1444 PXA, 2015

Table 62: Summary of degradation of pethoxamid in water/sediment (OECD 308)

Water / sediment system	pH water / sediment	*DegT50* system (d)	*DegT90* system (d)	Kinetic model	*DisT50* water (d)	*DisT90* water (d)	Kinetic model	*DisT50* sed. (d)	Kinetic model	Reference
Golden Lake	8.7 / 8.2	7.0	23.1	SFO	6.0	20.1	SFO	nd	na	1444 PXA, 2015
Goose River	8.1 / 7.8	13.0	43.1	SFO	10.6	35.2	SFO	nd	na	
Geometric mean (n = 2)		**9.5**	-	-	**8.0**	-	-	-	-	-

Table 63: Summary of degradation of pethoxamide metabolite MET-22 in total water/sediment (OECD 308)

Water / sediment system	pH water / sediment	*DegT50* system (d)	*DegT90* system (d)	ff	Kinetic model	Reference
Golden Lake	8.7 / 8.2	> 1000	> 1000	0.018	$P_{SFO} \rightarrow M_{SFO}$	1444 PXA, 2015
Goose River	8.1 / 7.8	> 1000	> 1000	0.023	$P_{SFO} \rightarrow M_{SFO}$	
Worst case (n = 2)		-	-	**0.023**	-	-

Soil degradation data

In soil under **aerobic conditions**, pethoxamid is initially metabolised via glutathione conjugation with subsequent loss of glycine and glutamic acid to form an intermediate cysteine conjugate, followed by formation of a thiol via beta lyase cleavage – all of which are transitory. Subsequent oxidation gives pethoxamide metabolite MET-101 or a sulfonic acid, pethoxamide metabolite MET-42. MET-42 is then degraded to pethoxamide metabolite MET-100 (134 PXA, 2000).

In soil under **anaerobic conditions**, pethoxamid is metabolised via reductive de-chlorination to give pethoxamide metabolite MET-22, with subsequent degradation to give pethoxamide metabolite MET-46. Several minor metabolites (< 5 % of AR) were detected, none of them was identified (138 PXA, 2000).

Figure 11-2: Proposed route of degradation in soil

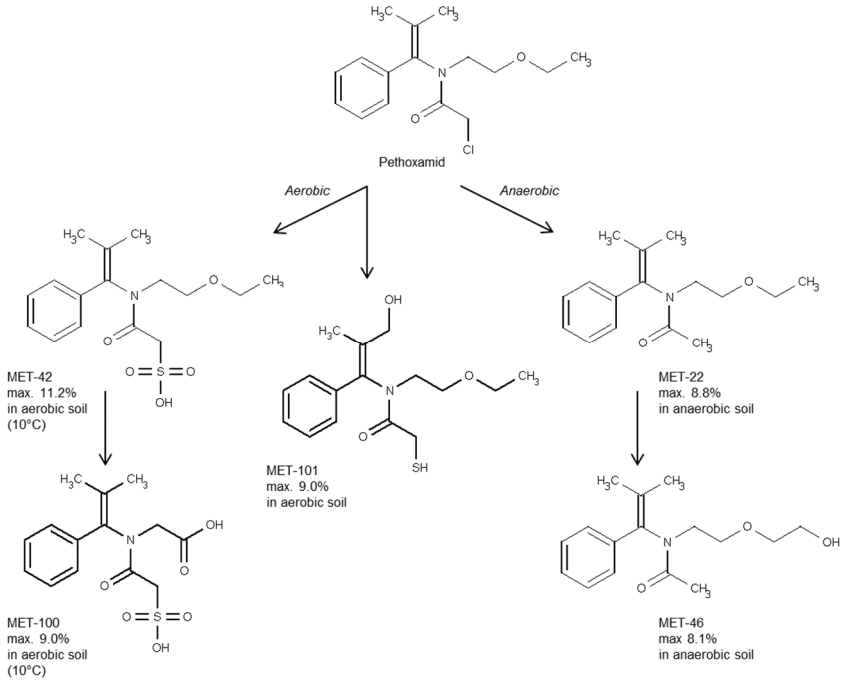


Table 64: Summary on maximum occurrence (% AR) of identified pethoxamid metabolites in laboratory soil route studies conducted with pethoxamid

Compound	Aerobic (134 PXA, 2000)	Anaerobic (138 PXA, 2000)	Soil photolysis (139 PXA, 1999)
MET-2	3.2	-	-
MET-3	1.1	-	-
MET-13	0.8	-	-
MET-22	-	8.8	-
MET-27	1.3	-	-
MET-42	11.2[a]	-	-
MET-46	-	8.1	-
MET-100	9.0[b]	-	-
MET-101	9.0	-	-

[a] 11.2 % in 10 °C study, 9.7 % in 20 °C study
[b] 9.0 % in 10 °C study, 4.4 % in 20 °C study

The **rate of degradation in soil** of pethoxamide and its metabolites has been assessed in laboratory studies and is summarised in the tables below.

Table 65: Summary of aerobic degradation rates for pethoxamid – laboratory studies (OECD 307)

Soil name	Soil type (USDA)	pH (H_2O)	Temp. (°C)	Water content (MWHC)	*DegT50* (d)	*DegT90* (d)	*DegT50* (d) 20 °C, pF2	χ^2 (%)	Kinetic model	Reference
PT 102	L	7.2	20	45 %	5.9	19.4	5.9	5.2	SFO	134 PXA, 2000
			10	45 %	15.0	49.8	-	2.7	SFO	
PT 103	SL	5.3	20	45 %	6.1	20.3	6.1	3.1	SFO	
PT 070	SiL	6.6	20	45 %	8.1	27.0	8.1	2.9	SFO	
SK 961089	CL	7.8	20	45 %	5.5	18.2	5.5	4.4	SFO	
Geometric mean (20 °C studies, n = 4)							**6.3**	-		
pH-dependency: y/n							n	-		

Table 66: Summary of aerobic degradation rates for pethoxamide metabolite MET-42 – laboratory studies (OECD 307)

Soil name	Soil type (USDA)	pH (H_2O)	Temp. (°C)	Water content (MWHC)	*DegT50* (d)	*DegT90* (d)	*ff*[a]	*DegT50* (d) 20 °C, pF2	χ^2 (%)	Kinetic model	Reference
PT 102	L	7.2	20	45 %	74.1	246	0.13	74.1	5.3	SFO	134 PXA, 2000*
			10	45 %	80.4	267	0.17	-	9.0	SFO	
PT 103	SL	5.3	20	45 %	82.0	272	0.05	82.0	9.6	SFO	
PT 070	SiL	6.6	20	45 %	79.9	265	0.07	79.9	8.0	SFO	
SK 961089	CL	7.8	20	45 %	31.5	105	0.10	31.5	11.1	SFO	
Arithmetic mean (20 °C studies, n = 4)							**0.09**	**-**	-		
Geometric mean (20 °C studies, n = 4)							**-**	**62.5**	-		
pH-dependency: y/n							n	-	-		

* Study performed with pethoxamid applied
[a] From parent

Table 67: Summary of aerobic degradation rates for pethoxamide metabolite MET-101 – laboratory studies (OECD 307)

Soil name	Soil type (USDA)	pH (H_2O)	Temp. (°C)	Water content (MWHC)	*DegT50* (d)	*DegT90* (d)	*ff*[a]	*DegT50* (d) 20 °C, pF2	χ^2 (%)	Kinetic model	Reference
PT 102	L	7.2	20	45 %	56.2	187	0.08	56.2	8.6	SFO	134 PXA, 2000*
			10	45 %	66.6	221	0.07	-	10.6	SFO	
PT 103	SL	5.3	20	45 %	106	352	0.11	106	8.1	SFO	
PT 070	SiL	6.6	20	45 %	33.8	112	0.12	33.8	8.1	SFO	
SK 961089	CL	7.8	20	45 %	27.3	90.8	0.07	27.3	11.6	SFO	
Arithmetic mean (20 °C studies, n = 4)							**0.10**	-	-		
Geometric mean (20 °C studies, n = 4)							-	**48.4**	-		
pH-dependency: y/n							n	-	-		

* Study performed with pethoxamid applied
[a] From parent

Table 68: Summary of aerobic degradation rates for pethoxamide metabolite MET-100 – laboratory studies (OECD 307)

Soil name	Soil type (USDA)	pH ($CaCl_2$)	Temp. (°C)	Water content (MWHC)	*DegT50* (d)	*DegT90* (d)	*ff*	*DegT50* (d) 20 °C, pF2	χ^2 (%)	Kinetic model	Reference
LUFA 2.2	LS	5.5	20	45 %	85.5	284	na	85.5	3.1	SFO	1264 PXA, 2014
LUFA 2.3	SL	6.8	20	45 %	9.1	30.1	na	8.5	12.7	SFO	
LUFA 2.4	L	7.2	20	45 %	9.2	30.6	na	7.8	16.0	SFO	
RefeSol 02-A	SiL	6.2	20	45 %	15.4	51.3	na	13.1	7.9	SFO	1531 PXA, 2016
RefeSol 03-G	SiL	5.2	20	45 %	13.3	44.0	na	13.3	6.2	SFO	
RefeSol 05-G	L	4.4	20	45 %	38.8	279	na	104[a]	2.9	HS	
Arithmetic mean							**na**	-	-		
Geometric mean (n = 6)							-	**21.6**	-		
pH-dependency: y/n							n	-	-		

na – not applicable
[a] HS slow rate *DegT50*

Table 69: Summary of aerobic degradation rates for pethoxamide metabolite MET-22 – laboratory studies (OECD 307)

Soil name	Soil type (USDA)	pH ($CaCl_2$)	Temp. (°C)	Water content (MWHC)	*DegT50* (d)	*DegT90* (d)	*ff*	*DegT50* (d) 20 °C, pF2	χ^2 (%)	Kinetic model	Reference
LUFA 2.2	LS	5.5	20	45 %	96.2	425	na	141[a]	3.0	HS	1263 PXA, 2014
LUFA 2.3	SL	6.8	20	45 %	17.1	82.5	na	23.1[b]	3.5	FOMC	
LUFA 2.4	L	7.2	20	45 %	19.2	63.9	na	16.3	4.6	SFO	
RefeSol 02-A	SiL	6.2	20	45 %	17.8	59.1	na	15.1	4.1	SFO	1530 PXA, 2016
RefeSol 03-G	SiL	5.2	20	45 %	15.8	52.4	na	15.8	3.2	SFO	

RefeSol 05-G	L	4.4	20	45 %	80.7	268	na	80.7	2.1	SFO	
Arithmetic mean							**na**	-	-		
Geometric mean (n = 6)							-	**31.7**	-		
pH-dependency: y/n							n	-	-		

na – not applicable
[a] HS slow rate *DegT50*
[b] FOMC-*DegT90* divided by 3.32

Table 70: Summary of aerobic degradation rates for pethoxamide metabolite MET-46 – laboratory studies (OECD 307)

Soil name	**Soil type (USDA)**	**pH ($CaCl_2$)**	**Temp. (°C)**	**Water content (MWHC)**	***DegT50* (d)**	***DegT90* (d)**	***ff***	***DegT50* (d) 20 °C, pF2**	**χ^2 (%)**	**Kinetic model**	**Reference**
LUFA 2.2	LS	5.5	20	45 %	0.2	0.8	na	0.2	9.1	SFO	1267 PXA, 2014
LUFA 2.3	SL	6.0	20	45 %	0.2	0.6	na	0.2	6.8	SFO	
LUFA 2.4	L	7.2	20	45 %	0.4	1.3	na	0.3	14.0	SFO	
Arithmetic mean							**na**	-	-		
Geometric mean (n = 3)							-	**0.2**	-		
pH-dependency: y/n							n	-	-		

na – not applicable

The **rate of degradation** of pethoxamid **in anaerobic soil** is rapid as well, but slightly slower than in aerobic soil (*DegT50* of 13.7 days *vs* 6.3 days) (138 PXA, 2000). Formation fraction of pethoxamide metabolite MET-22 was estimated to be 0.076 with MET-22 considered to be stable under conditions of anaerobic soil degradation.

Despite the fast degradation of pethoxamid in the laboratory, dissipation of pethoxamid was investigated in three **field dissipation studies** located in Spain and France (135 PXA, 2000). Based on best fit kinetics persistence trigger endpoints given in the table below were derived for pethoxamid.

Table 71: Summary of dissipation rates for pethoxamid in field studies – persistence triggering endpoints

Location	**Soil type (BBA)**	**pH (KCl)**	**Depth (cm)**	***DisT50* (d) actual**	***DisT90* (d) actual**	**χ^2 (%)**	**Kinetic model**	**Reference**
Spain	SCL	7.5	30	4.2	164	15.3	DFOP	135 PXA, 2000
N. France	SiSL	6.9	10	22.2	73.7	3.4	SFO	
Spain	SC	7.3	10	7.3	96.0	10.5	DFOP	
Maximum (n = 3)				**22.2**	**73.7**	**-**	**-**	**-**

Pethoxamide metabolite MET-42, which was included in the analysis as well, could not be kinetically assessed owing to low occurrence.

11.1.4.4 Photochemical degradation

In **water under photolytic conditions**, pethoxamid is degraded via reductive dechlorination, with subsequent oxidation to give pethoxamide metabolite MET-102. Further photolytic transformations produce the labile compound benzoic acid (1442 PXA, 2015). The quantum yield of pethoxamid was determined to be 2.85×10^{-1}.

Table 72: Summary of degradation of pethoxamid at conditions of aquatic photolysis (OECD 316)

Light Exposed	Suntest exposure days[a]		Sunlight equivalent days[b]		χ^2 (%)	Kinetic model	Reference
	DT50 (d)	*DT90* (d)	*DT50* (d)	*DT90* (d)			
pH 7 phosphate buffer	7.7	25.7	13.9	46.2	5.3	SFO	1442 PXA, 2015

[a] Continuous Suntest irradiation
[b] 40 – 50 °N, summer irradiation, 300 - 400 nm

The rate of degradation of pethoxamid by **photolysis on the soil surface** is relatively slow (*DegT50* of 53.0 days) and is not considered a significant dissipation pathway (139 PXA, 1999). Only minor metabolites (< 5 % of AR) are formed from the degradation of pethoxamid on soil under irradiation. None of them was identified.

11.2 Environmental transformation of metals or inorganic metals compounds

Not relevant.

11.3 Environmental fate and other relevant information

The **half-life in air** (for a 12 hour day, 1.6×10^5 OH radicals / cm^3) was estimated as 1.167 hours. As this is less than 2 days there is considered to be limited potential for long-range transport of pethoxamide (1423 PXA, 2015).

Henry's Law Constant of pethoxamid at 20 °C was calculated as 1.18×10^{-3} Pa m^3/mole (1415 PXA, 2015). Pethoxamid is therefore unlikely to partition from the water phase to air.

Adsorption in soil of pethoxamide and its metabolites MET-42, MET-100, MET-22 and MET-46 has been assessed in OECD 106 batch studies and is summarised in the tables below.

Table 73: Summary of soil adsorption for pethoxamide (OECD 106)

Soil name	Soil type (USDA)	OC (%)	pH ($CaCl_2$)	K_f (L/kg)	K_{foc} (L/kg)	1/*n* (-)	Reference
ND L	L	3.8	7.4	9.15	241	0.898	1344 PXA, 2014
CA L	L	0.9	7.0	1.91	212	0.908	
IL SiL	SiL	0.6	5.3	1.12	187	0.895	
ND SCL	SCL	2.6	6.4	5.42	208	0.874	
			Arithmetic mean (n = 4)		-	**0.89**	-
			Geometric mean (n = 4)		**211**	-	-
			pH-dependency: y/n		n	-	-

Table 74: Summary of soil adsorption for pethoxamide metabolite MET-42 (OECD 106)

Soil name	Soil type (USDA)	OC (%)	pH ($CaCl_2$)	K_d (L/kg)	K_{oc} (L/kg)	1/*n* (-)	Reference
LUFA 2.2	LS	1.7	5.5	0.09[a]	5	nd	1266 PXA, 2014
LUFA 2.3	SL	0.7	6.0	0.06[a]	9	nd	
LUFA 2.4	L	2.2	7.2	0.21[a]	10	nd	
				Arithmetic mean	-	**1***	-
				Geometric mean (n = 3)	**8**	-	-
				pH-dependency: y/n	n	-	-

* linear relation assumed
[a] K_d values below 0.3 L/kg (based on a soil to solution ratio of 1:1 and using the *indirect* method) cannot be determined precisely (OECD, 106). See text below.

Table 75: Summary of soil adsorption for pethoxamide metabolite MET-100 (OECD 106)

Soil name	Soil type (USDA)	OC (%)	pH ($CaCl_2$)	K_d (L/kg)	K_{oc} (L/kg)	1/*n* (-)	Reference
LUFA 2.2	LS	1.8	5.5	0.06[a]	4	nd	1261 PXA, 2014
LUFA 2.3	SL	0.9	6.8	0.03[a]	4	nd	
LUFA 2.4	L	2.3	7.2	0.03[a]	1	nd	
				Arithmetic mean	-	**1***	-
				Geometric mean (n = 3)	**3**	-	-
				pH-dependency: y/n	n	-	-

* linear relation assumed
[a] K_d values below 0.3 L/kg (based on a soil to solution ratio of 1:1 and using the *indirect* method) cannot be determined precisely (OECD, 106). See text below.

Table 76: Summary of soil adsorption for pethoxamide metabolite MET-22 (OECD 106)

				MET-22			
Soil name	Soil type (USDA)	OC (%)	pH ($CaCl_2$)	K_d (L/kg)	K_{oc} (L/kg)	1/*n* (-)	Reference
LUFA 2.2	LS	1.8	5.5	1.55	88	nd	1262 PXA, 2014
LUFA 2.3	SL	0.9	6.8	1.21	129	nd	
LUFA 2.4	L	2.3	7.2	2.27	100	nd	
				Arithmetic mean	-	**1***	-
				Geometric mean (n = 3)	**104**	-	-
				pH-dependency: y/n	n	-	-

* Linear relation assumed

Table 77: Summary of soil adsorption for pethoxamide metabolite MET-46 (OECD 106)

Soil name	Soil type (USDA)	OC (%)	pH ($CaCl_2$)	K_d (L/kg)	K_{oc} (L/kg)	1/*n* (-)	Reference
LUFA 2.2	LS	1.7	5.5	Not	Not	nd	1265 PXA, 2014
LUFA 2.3	SL	0.7	6.0	considered	considered	nd	
LUFA 2.4	L	2.2	7.2	reliable	reliable	nd	
				Arithmetic mean	-	**1***	-
				Geometric mean (n = 3)	**na**	-	-
				pH-dependency: y/n	na	-	-

* linear relation assumed

Based on results obtained in an **aged residue soil column leaching study**, aged residues of pethoxamid can be classified as having a moderate potential for leaching in soil (143 PXA, 1999).

In a **field dissipation study**, considered to give a worst-case for leaching, pethoxamid showed negligible potential for leaching to 100 cm depth with only 2 of 175 soil water samples from the treated plots containing residues (each at 0.1 µg/l). Pethoxamide metabolite MET-42 showed a significant potential to leach to 100 cm depth under the worst-case conditions of the study (140 PXA, 2000).

11.4 Bioaccumulation

Table 78: Summary of relevant information on bioaccumulation

Method	Results	Remarks	Reference
Partition coefficient n-octanol/water B.2.7/01 EEC A8/ OECD 107	Log P_{ow} = 2.963 ± 0.02 at 20°C (pH 5).	Complete phase separation reported. The effect of pH was not necessary because compound is not ionized between pH 4 and 10.	41 PXA (1996)

Method	Results	Remarks	Reference
(Shake flask method) GLP Batch: TP-940421 Purity: ≥99.9%			
TKC-94: Bioconcentration in Rainbow Trout. OECD 305. GLP Batch: CFQ10530 Radiochemical purity: >97%	Steady state BCF = 33 after 14 d (kinetic BCF = 47 – 50). Elimination >90% after 56 d.	Flow-through test. Conc. levels (nom.): 0.0015, 0.015 mg/L. The reported BCF value was normalised for 6% lipid content and not 5%. This deviation is not considered to impact the validity of the BCF value.	154 PXA (2000)

11.4.1 Estimated bioaccumulation

41 PXA (1996) conducted a GLP study to determine the partition coefficient between n-octanol and water using a shake flask method. The data showed that pethoxamid has a Log P_{ow} = 2.963 ± 0.02 at 20°C (pH 5). Pethoxamid has a LogP of <3.0. Please note that for pethoxamid as surface active substances it may be difficult to obtain reliable partitioning (log Pow) or bioconcentration factor (BCF) data for inclusion in current models used in performing environmental risk assessments.

11.4.2 Measured partition coefficient and bioaccumulation test data

Pethoxamid has a LogP of <3.0 and therefore bioconcentration in fish is not a data requirement in accordance with Commission Regulation (EU) No 283/2013. However, a study was previously conducted to address the potential risk, and was submitted during the EU review of pethoxamid for the inclusion into Annex I of EEC/91/414.

In a 28 day flow-through bioconcentration study, Rainbow trout (*Oncorhyncus mykiss*) were exposed to a nominal concentrations of 1.5 and 15 µg/L ^{14}C-pethoxamid (154 PXA, 2000). One treatment tank was employed for each treatment and the fish were exposed for a period of 28 days under flow-through conditions (uptake phase), followed by a period of 56 days in fresh water without test substance (depuration phase). Mean measured concentrations of ^{14}C-pethoxamid in the treatment tank was 1.64 and 16.1 µg/L for the nominal 1.5 and 15 µg/L treatments, respectively, indicating that the test substance remained stable in solution throughout the duration of the test.

Kinetic parameters derived from mean tissue radioactivity concentrations during the 28 day exposure period were similar for the nominal 1.5 and 15 µg/L treatments. Based on total radioactivity, calculated kinetic BCF's (whole fish) were 50 and 47 in the low and high concentrations, respectively. Mean steady state BCFs (whole fish), normalised for lipid content, were reported as 28 and 32 in the low and high concentrations, respectively (calculated as the mean of days 7-28).

In the dRAR (2017), steady state is considered to have been reached after 14 days. Therefore a mean steady state whole-fish BCFs of 33 is used, based on the average BCF after 14 days in the 15 µg/L treatment. It is noted in the dRAR (2017) that the reported BCF values were normalised for 6% lipid content and not 5%, as per current OECD Guideline requirements, however this deviation is not considered to impact the validity of the BCF value.

During the depuration period, mean concentrations of radioactivity in fish declined gradually. At the end of the 56 day depuration period, levels of radioactivity measured in fish were 5 – 26% of those measured on the last day of exposure. The reported DT_{90} (time for 90% depuration) was 64.4 and 50.9 days, in the 1.5 and 15

ug/L treatments, respectively. The dRAR (2017) states >90% depuration after 56 days, representing an average of the two values.

To conclude, pethoxamid does not meet classification criteria as a bioaccumulative substance.

11.5 Acute aquatic hazard

A summary of the suitable acute aquatic toxicity studies for pethoxamid, as reviewed under EU Regulation 1107/2009, is presented in the table below. The studies below conformed to GLP certification and are considered reliable for hazard classification purposes, or as useful supporting information, where stated. Additional information on the studies supporting pethoxamid is presented in the subsections below.

Table 79: Summary of relevant information on acute aquatic toxicity

Method	Species	Test material	Results[1]	Remarks	Reference
OECD 203 (1992)	*Oncorhynchus mykiss*	Pethoxamid techn. (TKC-94), Purity: 94.8 %. Lot-No. : TB-960306-C	96 h LC_{50}: **2.2 mg/L** (mm)	Static renewal, mortality	151 PXA plus 151 PXA amdt-1 (1999a)
OECD 203 (1992)	*Lepomis macrochirus*	Pethoxamid techn. (TKC-94), Purity: 94.8 %. Lot-No. : TB-960306-C	96 h LC_{50}: 6.6 mg/L (mm)	Static renewal, mortality	152 PXA plus 152 PXA amdt-1 (1999b)
OECD 203 (1992)	*Cyprinodon variegatus*	Pethoxamid techn., Purity: 95.8 % w/w. Batch No. : P1351-JaK-T2-23-6	96 h LC_{50}: 3.54 mg/L (mm)	Static, mortality	1177 PXA (2013)
OECD 202	*Daphnia magna*	Pethoxamid techn. (TKC-94), Lot-No.: TB-960306-C; purity: 94.8%	48 h EC_{50}: 23 mg/L (mm)	Static, immobility	155 PXA plus 155 PXA amdt-1 (1999a)
OPPTS 850.1035	*Americamysis bahia*	Pethoxamid techn., Purity: 95.8 % w/w. Batch No. : P1351-JaK-T2-23-6	96 h LC_{50}: 5.4 mg/L (mm)	Static, mortality	1176 PXA (2014a)
OPPTS 850.1025	*Crassostrea virginica*	Pethoxamid techn., Purity: 95.8 % w/w. Batch No. : P1351-JaK-T2-23-6	96 h EC_{50}: **3.28 mg/L** (mm)	Flow through, shell growth inhibition	1207 PXA (2014b)
OPPTS 850.1025	*Crassostrea virginica*	Pethoxamid techn., Purity: 96.2 % w/w. Batch No. : P1351-JaK-T2-23-6	96 h EC_{50}: 3.38 mg/L (mm)	Flow through, shell growth inhibition	1348 PXA (2014)
OECD 201	*Selenastrum capricornutum (Pseudokirch-neriella subcapitata)*	Pethoxamid techn. (TKC-94), Purity: 94.8 %. Lot-No. : TB-960306-C	72 h E_rC_{50}[2]: **0.00408 mg/L** (mm)	Static, growth rate	158 PXA (1999b) and 158 PXA suppl.-1 (2016a) and 158 PXA suppl.-2 (2016c)

OECD 201	*Anabaena flos-aquae*	Pethoxamid techn. (TKC-94), Purity: 94.8 %. Lot-No. : TB-960306-C	96 h E_rC_{50}[2] : 10.4 mg/L (mean measured)	Static, growth rate	157 PXA (1999c) and 157 PXA suppl.-1 (2016b) and 157 PXA suppl.-2 (2016d)

[1] Endpoints in bold are the critical endpoints for that organism group. The letters to the right of the numbers refer to what concentration of the active substance the endpoint is based on: n – nominal; mm – mean measured; im – initial measured
[2] Endpoints included in the RAR (2017) (based on re-calculation by 157 PXA suppl.-1 (2016b) and 158 PXA suppl.-2 (2016c), as indicated).
[3] Endpoints included in the original study report, reported in dRAR (2017).

11.5.1 Acute (short-term) todxicity to fish

Three reliable acute fish studies are available with reported 96 LC_{50}'s between 2.2 and 6.6 mg/L. The lowest reported LC_{50} was 2.2 mg/L for Rainbow trout (*Oncorhynchus mykiss*) (151 PXA, 1999a).

151 PXA (1999a)

The toxicity of pethoxamid to Rainbow trout (*Oncorhyncus mykiss*) was determined in a 96-hour acute toxicity test performed under static-renewal conditions (pethoxamid: batch TB-960306-C, purity 94.8%). The fish were exposed to nominal pethoxamid concentrations of 1, 1.8, 3.2, 5.8 and 10 mg/L alongside a dilution water control, with daily water renewal. Corresponding mean measured concentrations were 1.1, 1.7, 2.5, 4.7 and 8.3 mg/L (86 to 111 and 82 to 92% of nominal at 0 and 96 hours, respectively). Mortality in the control was less than 10% and dissolved oxygen, temperature and pH were maintained at acceptable levels throughout the test. Mean fish length was 3.6 cm as opposed to 4 to 8 cm normally used, however this deviation is not considered to impact the validity of the study.

Based on mean measured concentrations, the 96-hour LC_{50} for pethoxamid to *Oncorhyncus mykiss* was 2.2 mg/L with 95% confidence limits of 1.9 to 2.6 mg/L.

152 PXA (1999b)

The toxicity of pethoxamid to Bluegill sunfish (*Lepomis macrochirus*) was determined in a 96-hour acute toxicity test performed under static-renewal conditions (pethoxamid: batch TB-960306-C, purity 94.8%). The fish were exposed to nominal pethoxamid concentrations of 1, 1.8, 3.2, 5.8, 10 and 18 mg/L alongside a dilution water control, with daily water renewal. Corresponding mean measured concentrations were 0.81, 1.6, 2.7, 5.1, 8.5 and 15 mg/L (81 to 91 and 85 to 92% of nominal at 0 and 96 hours, respectively). Mortality in the control was less than 10% and dissolved oxygen, temperature and pH were maintained at acceptable levels throughout the test.

Based on mean measured concentrations, the 96-hour LC_{50} for pethoxamid to *Lepomis macrochirus* was 6.6 mg/L with 95% confidence limits of 5.1 to 8.5 mg/L.

1177 PXA (2013)

The toxicity of pethoxamid to Sheepshead minnow (*Cyprinodon variegatus*) was determined in a 96-hour acute toxicity test performed under static conditions (pethoxamid: batch P1351-JaK-T2-23-6, purity 95.8%). The fish were exposed to nominal pethoxamid concentrations of 0.65, 1.3, 2.5, 5.0 and 10 mg/L alongside a dilution water control. Corresponding mean measured concentrations were 0.608, 1.2, 2.36, 4.94 and 10.2 mg/L (98 to 102 and 87 to 95% of nominal at 0 and 96 hours, respectively). There were no mortalities in the control and dissolved oxygen, temperature and pH were maintained at acceptable levels throughout the test.

Based on mean measured concentrations, the 96-hour LC_{50} for pethoxamid to *Cyprinodon variegatus* was 3.54 mg/L with 95% confidence limits of 3.3 to 3.8 mg/L.

11.5.2 Acute (short-term) toxicity to aquatic invertebrates

Four acute studies on aquatic invertebrates are available with reported EC_{50}'s between 3.28 and 23 mg/L. The lowest reported EC_{50} was 3.28 mg/L based on shell growth inhibition in the Eastern oyster (*Crassostrea virginica*) (1207 PXA, 2014b)**.**

155 PXA (1999a)

The toxicity of pethoxamid to *Daphnia magna* was determined in a 48-hour acute toxicity test performed under static conditions (pethoxamid: batch TB-960306-C, purity 94.8%). Animals were exposed to nominal pethoxamid concentrations of 1, 1.8, 3.2, 5.8, 10, 18 and 32 mg/L alongside a dilution water control. Corresponding mean measured concentrations were 0.82, 1.6, 2.9, 5.1, 9.1, 17 and 29 mg/L (86 to 92 and 79 to 92% of nominal at 0 and 48 hours, respectively). Mortality in the control was less than 10% and dissolved oxygen, temperature and pH were maintained at acceptable levels throughout the test.

Based on mean measured concentrations, the 48-hour EC_{50} for pethoxamid to *Daphnia Magna* was 23 mg/L with 95% confidence limits of 20 to 25 mg/L.

1176 PXA (2014a)

The toxicity of pethoxamid to Mysid shrimp (*Americamysis bahia*) was determined in a 96-hour acute toxicity test performed under static conditions (pethoxamid: batch P1351-JaK-T2-23-6, purity 95.8%). Animals were exposed to nominal pethoxamid concentrations of 0.65, 1.3, 2.5, 5.0 and 10.0 mg/L alongside a dilution media control. Corresponding mean measured concentrations were 0.637, 1.26, 2.47, 4.96 and 10.3 mg/L (102 to 106 and 90 to 101% of nominal at 0 and 96 hours, respectively). The test media was laboratory saltwater at 19.5 to 20.2‰ salinity. There were four replicates per treatment each with five post-larvae mysids (<24-h old) which were fed daily on brine shrimp nauplii. Mysids were observed at 24-h intervals for mortality and sub-lethal effects. Mortality in the control was less than 10% and temperature, salinity and pH were maintained at acceptable levels throughout the test. Dissolved oxygen levels ranged from 42 to 110% saturation. Aeration was added to all test chambers following the 72-hour observations to account for low dissolved oxygen levels measured at this time point.

Based on mean measured concentrations, the 96-hour LC_{50} for pethoxamid to *Americamysis bahia* was 5.4 mg/L with 95% confidence limits of 4.62 to 6.31 mg/L.

1207 PXA (2014b)

The toxicity of pethoxamid to Eastern oyster (*Crassostrea virginica*) was determined in a 96-hour flow-through new shell growth test (pethoxamid: batch P1351-JaK-T2-23-6, purity 95.8%). Animals were exposed to nominal pethoxamid concentrations of 1.3, 2.2, 3.6, 6.0 and 10.0 mg/L alongside a dilution media control. Corresponding mean measured concentrations were 1.18, 2.01, 3.33, 5.53 and 9.53 mg/L (87 to 94 and 85 to 96% of nominal at 0 and 96 hours, respectively). The test media was laboratory saltwater at 19.6 to 20.0‰ salinity. There were two replicate chambers per treatment each with 10, previously acclimated, oysters with the ventral shell end ground away by 3-5 mm. A marine microalgal concentrate was added periodically during each day as a food source and was also provided via the diluter water. There was no mortality in the control or test substance treatments and temperature, dissolved oxygen, salinity and pH were maintained at acceptable levels throughout the test. Dissolved oxygen was slightly low (59% saturation) in one replicate but this was not considered biologically significant.

Based on mean measured concentrations, the 96-hour EC_{50} for new shell growth was 3.28 mg/L with 95% confidence limits of 2.73 to 3.82 mg/L.

1348 PXA (2014)

The toxicity of pethoxamid to Eastern oyster (*Crassostrea virginica*) was determined in a 96-hour flow-through new shell growth test (pethoxamid: batch P1351-JaK-T2-23-6, purity 96.2%). Animals were exposed to nominal pethoxamid concentrations of 0.42, 0.76, 1.4, 2.5, 4.4 and 8.0 mg/L alongside a dilution media control. Corresponding mean measured concentrations were 0.366, 0.668, 1.20, 2.12, 3.96 and 7.29 mg/L (81 to 89 and 85 to 91% of nominal at 0 and 96 hours, respectively). The test media was laboratory saltwater at 19.5 to 20.0‰ salinity. There were two replicate chambers per treatment each with 10, previously acclimated, oysters with the ventral shell end ground away by 3-5 mm. A marine microalgal concentrate was added

periodically during each day as a food source and was also provided via the diluter water. There was no mortality in the control or test substance treatments and temperature, dissolved oxygen, salinity and pH were maintained at acceptable levels throughout the test. All control validity criteria were met.

Based on mean measured concentrations, the 96-hour EC_{50} for new shell growth was 3.38 mg/L with 95% confidence limits of 3.03 to 3.74 mg/L.

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

Two acute studies on algae species are available. The most sensitive aquatic species in acute tests was the green algae (*Selenastrum capricornutum*, also known as *Pseudokirchneriella subcapitata*) with a reported growth rate (E_rC_{50}), the preferred endpoint for classification purposes, of 0.00408 mg/L (158 PXA 1999b and 158 PXA suppl.-1 2016a).

158 PXA (1999b) and 158 PXA suppl.-1 (2016a)

The toxicity of pethoxamid to green alga (*Selenastrum capricornutum, also known as Pseudokirchneriella subcapitata)* was determined in a 120-hour acute toxicity test performed under static conditions (pethoxamid: batch TB-960306-C, purity 94.8%). Algae were exposed to nominal pethoxamid concentrations 0.000625, 0.00125, 0.0025, 0.0050 and 0.010 mg/L alongside a culture medium control and a solvent control. Corresponding mean measured concentrations were 0.00058, 0.0012, 0.0024, 0.0046 and 0.0094 mg/L (92 to 103 and 85 to 95% of nominal at 0 and 120 hours, respectively). Temperature and pH were maintained at acceptable levels throughout the test. Subsequent analysis shows that the mean coefficient of variation for the section-by-section specific growth rate slightly exceeded the control validity criteria (37.7% for the period 0-72 hours) (158 PXA suppl.-2, 2016c). This was not deemed to affect the validity of the study and all other control validity criteria were met.

For classification purposes, only the 72-hour results are used.

The 72-hour EC_{50} and EC_{10} endpoints for biomass and growth rate have been subsequently recalculated from the original data (158 PXA suppl.-1 2016a):

The evaluation of the concentration-effect-relationships and the calculations of effect concentrations was performed with the original cell number values from GLP report 158 PXA (Table 1: Algal cell densities for control and test cultures) as outlined in the OECD guideline 201, as far as possible. The cell numbers of the treated cultures were compared with that of the pooled controls, since there was no statistically significant difference between control and solvent control.

At first, non-linear regression procedures, e.g. a 3-parametric cumulative normal distribution function according to Bruce and Versteeg (1992), were fitted to the data. Subsequently, other non-linear regression models provided by the computer programme ToxRat Professional® were used. If the requirements for a non-linear regression were not fulfilled, the EC values were calculated by linear regression (Probit analysis, based on normal distribution of the data (sigmoid normal), modified for continuous data).

For growth rate 72 h, all yield and biomass results, convergence criteria for non-linear regression models were not fulfilled and/or there were significant lacks of fit. Therefore, linear regression using Probit was performed. Individual replicate responses were used for the regression analysis.

For growth rate after 96 and 120 hours, convergence criteria for the 3-parametric logistic cumulative distribution function were fulfilled and there were no significant lacks of fit.

The re-calculated 72-hour E_bC_{50} and E_rC_{50} were very similar to the original report, at 0.00206 and 0.00408 mg/L, respectively (with 95% confidence limits of 0.00182 to 0.00232 and 0.00341 to 0.00495 mg/L, respectively). These re-calculated values are considered acceptable in the dRAR.

The acute E_rC_{50} of 0.00408 mg/L is considered reliable for acute classification purposes.

157 PXA (1999c) and 157 PXA suppl.-1 (2016b)

The toxicity of pethoxamid to blue-green alga (*Anabaena flos-aquae*) was determined in a 120-hour acute toxicity test performed under static conditions (pethoxamid: batch TB-960306-C, purity 94.8%). Algae were exposed to nominal pethoxamid concentrations 2.2, 4.6, 10, 22 and 46 mg/L alongside a culture medium

control. Corresponding mean measured concentrations were 1.6, 3.8, 8.6, 20 and 41 mg/L (81 to 89 and 67 to 92% of nominal at 0 and 120 hours, respectively). Temperature and pH were maintained at acceptable levels throughout the test. Subsequent analysis shows that some of the control validity criteria were not met (157 PXA suppl.-1, 2016d). Specifically, the cell density only increased by a factor of 7 between 0 and 72 hours, and the mean section-by-section specific growth rate exceeded the control validity criteria for all exposure periods. Due to lack of reliable 72-hour endpoints, this study is considered useful as supporting information only (consistent with the conclusion in the dRAR, 2017). Consequently, only the 96-hour endpoints are used (this is consistent with the conclusions in the dRAR, 2017).

The 96-hour EC_{50} for biomass and growth rate have been subsequently recalculated from the original data (157 PXA suppl.-1, 2016b). The re-calculated 96-hour E_bC_{50} and E_rC_{50} were similar to the original report at 8.99 and 10.4 mg/L, respectively (95% confidence intervals could not be calculated).

Due to lack of reliable 72-hour endpoints, this study is considered useful as supporting information only.

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

No additional studies.

11.6 Long-term aquatic hazard

A summary of the suitable aquatic toxicity studies for pethoxamid, as reviewed under EU Regulation 1107/2009, is presented in the table below. The studies below conformed to GLP certification and are considered reliable for hazard classification purposes, or as useful supporting information, where stated. Additional information on the studies supporting pethoxamid is presented in the subsections below. In line with the current CLP Guidance, preference is given to EC_{10} values for the chronic hazard classification over NOEC values and so EC_{10} values have been used where appropriate.

Table 80: Summary of relevant information on chronic aquatic toxicity

Method	Species	Test material	Results[1]	Remarks	Reference
OECD 210	*Oncorhynchus mykiss*	Pethoxamid techn., Purity: 95.8 % w/w, re-analysed 96.2 %. Batch No. : P1351-JaK-T2-23-6	94 d (60 d post hatch) NOEC: **0.0924 mg/L** (mm)	Flow-through, fry survival	1451 PXA (2015)
OECD 211	*Daphnia magna*	Pethoxamid techn. (TKC-94), Purity: 94.8 %. Lot-No. : TB-960306-C	21 d NOEC (survival, growth, repro): **2.8 mg/L** (mm)	Static renewal, survival, growth and reproduction	156 PXA (2000)
OECD 201	*Pseudokirchneriella subcapitata (Selenastrum capricornutum)*	Pethoxamid techn. (TKC-94), Purity: 94.8 %. Lot-No. : TB-960306-C	72 h E_rC_{50}[2]: 0.00408 mg/L (mm) 72 h E_rC_{10}[2]: **0.00119 mg/L** (mm) 120 h NOEC[3] (biomass and growth ate): 0.0012 mg/L (mm)	Static, growth rate	158 PXA (1999b) and 158 PXA suppl.-1 (2016a) and 158 PXA suppl.-2 (2016c)
OECD 201	*Anabaena flos-aquae*	Pethoxamid techn. (TKC-94), Purity: 94.8 %. Lot-No. : TB-960306-C	96 h E_rC_{50}[2]: 10.4 mg/L (mm) 96 h E_rC_{10}[2]: 8.39 mg/L (mm)	Static, growth rate	157 PXA (1999c) and 157 PXA suppl.-1 (2016b)

			120 h NOEC[3] (biomass and growth ate): 3.8 mg/L (mm)		and 157 PXA suppl.-2 (2016d)
OECD 221	*Lemna minor*	Pethoxamid techn. (TKC-94), Purity: 94.8 %. Lot-No. : TB-960306-C	14 d E_rC_{50}[2]: 0.0172 mg/L (mm) 14 d E_rC_{10}[2]: 0.0029 mg/L (mm) 14 d NOEC[3]: **0.001 mg/L** (mm)	Static renewal, growth rate	160 PXA (1999d) and 160 PXA suppl.-1 (2016e)

[1] Endpoints in bold are the critical endpoints for that organism group. The letters to the right of the numbers refer to what concentration of the active substance the endpoint is based on: n – nominal mm – mean measured im – initial measured
[2]Endpoints included in the dRAR (2017) (based on re-calculation by Wenzel 2016b and 2016c, as indicated).
[3] Endpoints included in the original study report and reported in dRAR (2017).

11.6.1 Chronic toxicity to fish

There is one reliable chronic fish study available with a reported NOEC of 0.0924 mg/L (1451 PXA, 2015).

1451 PXA (2015)

The toxicity of pethoxamid to Rainbow trout was determined in a 94 day test (60 days post-hatch) performed under flow-through conditions (pethoxamid: batch P1351-JaK-T2-23-6, purity 96.2%). Animals were exposed to nominal test concentrations 0.095, 0.19, 0.38, 0.75 and 1.5 mg/L, alongside a dilution control. Corresponding mean measured test concentrations were 0.0924, 0.177, 0.365, 0.722 and 1.44 mg/L, representing 97, 93, 96, 96 and 96% of the nominal concentration, respectively. The validity criteria for this study were considered to have been met. Temperature, pH and dissolved oxygen levels were maintained within acceptable levels, and in the control, mean hatching success was 76% and mean post-hatch survival was 84%.

On study day 42 (8 days post-hatch) the number of fry in each replicate was impartially reduced to 15 fry per replicate, which were released into their respective replicate growth chambers.

Statistically significant differences in post-hatch survival between the control and 0.177 and 0.365 mg/L concentrations were deemed to be not biologically relevant in the report because no significant effects were observed at 0.722 mg/L. However, in the dRAR it was proposed that these statistically significant effects should not be ignored. Therefore, based on mean measured concentrations and the most sensitive endpoint (post-hatch survival, before reduction) the chronic NOEC and LOEC for toxicity of pethoxamid to Rainbow trout are 0.0924 and 0.177 mg/L, respectively. Based on the nature of the data-set generated it was not possible to determine reliable EC_{10}/EC_{20} values.

11.6.2 Chronic toxicity to aquatic invertebrates

There is one reliable study available on chronic toxicity to aquatic invertebrates, with a reported NOEC and EC_{10} of 2.8 and 4.3 mg/L, respectively (156 PXA, 2000).

156 PXA (2000)

The toxicity of pethoxamid to *Daphnia Magna* was determined in a 21 day test performed under static-renewal conditions (pethoxamid: batch TB-960306-C, purity 94.8%). Groups of twenty five *Daphnia Magna* (ten individually housed plus three groups of five) were exposed to nominal test concentrations 1.4, 3.1, 6.8, 15 and 32 mg/L, alongside a dilution control. Corresponding mean measured test concentrations were 1.3, 2.8, 6.3, 13 and 29 mg/L (88 to 99 and 84 to 95% of nominal in fresh and expired solutions, respectively). Control mortality was <20% and all control validity criteria were met. Temperature, pH and dissolved oxygen were maintained at acceptable levels throughout the study.

Based on mean measured concentrations, the 21-day NOEC for survival, growth and reproduction (used in the risk assessment) and EC_{10}, for reporduction, were 2.8 and 4.3 mg/L, respectively.

11.6.3 Chronic toxicity to algae or other aquatic plants

There are three studies available on algae and aquatic plants. Based on the EC_{10} for growth rate (the preferred endpoint for classification purposes) the most sensitive species in chronic tests was the green algae (*Selenastrum capricornutum*, also known as *Pseudokirchneriella subcapitata*) with a reported E_rC_{10} of 0.00119 mg/L (158 PXA (1999b) and158 PXA suppl.-1 (2016a)).

158 PXA (1999b) and158 PXA suppl.-1 (2016a)

This study is described under Section 11.5.3. The 72-hour NOEC and E_rC_{10} values are relevant for chronic classification purposes. The original reported 72-hour NOEC based on biomass and growth rate was 0.0012 µg/L. The subsequently recalculated 72-hour E_rC_{10} was 0.00119 mg/L (158 PXA suppl.-1 2016a).

157 PXA (1999c) and 157 PXA suppl.-1 (2016b)

This study is described under Section 11.5.3. The original 96-hour NOEC based on biomass and growth rate was 3.8 mg/L. The subsequently recalculated 96-hour E_rC_{10} was 8.39 mg/L (157 PXA suppl.-1 2016b).

Due to lack of reliable 72-hour endpoints, this study is considered useful as supporting information only (consistent with the conclusion in the dRAR, 2017).

160 PXA (1999d) and 160 PXA suppl.-1 (2016e)

The toxicity of pethoxamid to the freshwater aquatic plant *Lemna minor* was determined in a 14-day static renewal test system, conducted according to the draft OECD guideline (1984) (pethoxamid: batch TB-960306-C, purity 94.8%). Lemna plants were exposed pethoxamid concentrations of 0.001, 0.0032, 0.01, 0.032 and 0.1 mg/L, plus a dilution water and solvent control. Corresponding mean measured concentrations of pethoxamid were 0.001, 0.0029, 0.0091, 0.028 and 0.085 mg/L (between 85 and 104% of mean measured). Temperature and pH remained within acceptable levels throughout the test.

Based on root length, the most sensitive parameter, the 14-day NOEC and LOEC were determined as 0.001 and 0.0029 mg/L, respectively.

The original data from this study have been subsequently re-evaluated (160 PXA suppl.-1 2016e) to determine EC_{10} and EC_{20} values for growth rate (frond number), yield and biomass. These values were not included in the original report. The 14 d E_rC_{10} for growth rate (the preferred endpoint for classification purposes) was 0.0029 mg/L (confidence limits 0.00203 – 0.0038 mg/L).

Note, endpoints were calculated for 14 days instead of 7 days as recommended in old and recent guidelines. The number of fronds should increase 7-fold within 7 days according to recent guidelines. In the study the frond number increased 6.4 and 5.8 fold within days 0 to 7 in the control and solvent control group, respectively. However, growth reached >30-fold in 14 days. Accordingly, in the dRAR (2017) evaluation, this study was deemed 'borderline acceptable'.

11.6.4 Chronic toxicity to other aquatic organisms

No additional studies.

11.7 Comparison with the CLP criteria

11.7.1 Acute aquatic hazard

Acute aquatic toxicity data on pethoxamid are available for fish, invertebrates and algae. Algae are the most acutely sensitive trophic group. The lowest reliable acute value is the 72-hour mean measured EC_{50} of 0.00408 mg/L for *Selenastrum capricornutum* (*Pseudokirchneriella subcapitata*), this is > 0.001 mg/L but ≤ 0.010 mg/L and therefore, pethoxamid should be classified as Aquatic Acute 1 with an Acute M-factor of 100.

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

Within the classification criteria, pethoxamid is considered 'not rapidly degradable'.

Pethoxamid has a LogP of <3.0, and therefore bioconcentration in fish is not a data requirement in accordance with Commission Regulation (EU) No 283/2013. However, a study was previously conducted (154 PXA, 2000) and reported a steady state BCF of 33 for pethoxamid. Therefore, pethoxamid does not meet classification criteria as a bioaccumulative substance.

Chronic/long-term aquatic toxicity data on pethoxamid are available for fish, invertebrates, algae and aquatic plants. Algae and aquatic plants are the most chronically sensitive group.

The lowest chronic value is the 14-day NOEC of 0.001 mg/L for *Lemna minor*. In the same study however, the 14-day E_rC_{10} for growth rate (the preferred endpoint for classification purposes) was 0.0029 mg/L. The green alga *Selenastrum capricornutum* (*Pseudokirchneriella subcapitata*) is similarly sensitive with NOEC and E_rC_{10} of 0.0012 and 0.00119 mg/L, respectively. Pethoxamid is 'not rapidly degradable' and based on any of these endpoints it should be classified as Aquatic Chronic 1. The choice of chronic M-factor depends on whether the NOEC for *Lemna minor* is chosen as the classification ranges are > 0.0001 to < 0.001 (M = 100) or > 0.001 to < 0.01 (M = 10) and the NOEC is right on this borderline. Since CLP guidance suggests the E_rC_{10} should be used where possible, it is recommended that the lowest E_rC_{10} for algae of 0.00119 mg/L should be used for chronic classification of pethoxamid. This is > 0.001 mg/L but ≤ 0.010 mg/L and therefore, pethoxamid should be classified as Aquatic Chronic 1 with a chronic M-factor of 10.

11.8 CONCLUSION ON CLASSIFICATION AND LABELLING FOR ENVIRONMENTAL HAZARDS

Aquatic Acute 1; H400: Very toxic to aquatic life

Acute M-factor = 100

Aquatic Chronic 1; H410: Very toxic to aquatic life with long lasting effects

Chronic M-factor = 10

12 EVALUATION OF ADDITIONAL HAZARDS

12.1 Hazardous to the ozone layer

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

Pethoxamid vapour pressure and Henry's law constant are 2.8 x 10^{-3} Pa at 25°C and 1.18 x 10^{-3} Pa m³/mol at 20°C respectively.

The atmospheric half-life of pethoxamid was estimated using the program AOPWIN (v 1.92), and was found to be 1.167 hours (1423 PXA, 201e). This indicates that the potential for long-range transport of pethoxamid is limited, and therefore it is highly unlikely that pethoxamid can deplete the stratospheric ozone layer.

Pethoxamid is not listed in Annex I to Regulation (EC) No. 1005/2009 (recognising the Montréal Protocol) and no Ozone Depleting Potential (ODP) is reported.

12.1.2 Comparison with the CLP criteria

A substance shall be classified as Hazardous to the Ozone Layer (Category 1) if the available evidence concerning its properties and its predicted or observed environmental fate and behaviour indicate that it may present a danger to the structure and/or the functioning of the stratospheric ozone layer.

The short atmospheric half-life of pethoxamid precludes an ozone-layer-depleting potential.

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

Not classified – conclusive but not sufficient for classification.

13 ADDITIONAL LABELLING

None.

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15 ANNEXES

ANNEX I to the CLH Report

Confidential Annex