Committee for Risk Assessment

RAC

Annex 1

Background document

to the Opinion proposing harmonised classification and labelling at EU level of

bifenox (ISO); methyl 5-(2,4-dichlorophenoxy)-2-nitrobenzoate

EC Number: 255-894-7
CAS Number: 42576-02-3

CLH-O-0000007049-71-01/F

The background document is a compilation of information considered relevant by the dossier submitter or by RAC for the proposed classification. It includes the proposal of the dossier submitter and the conclusion of RAC. It is based on the official CLH report submitted to consultation. RAC has not changed the text of this CLH report but inserted text which is specifically marked as 'RAC evaluation'. Only the RAC text reflects the view of RAC.

Adopted

26 November 2021

ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON BIFENOX (ISO); METHYL 5-(2,4-DICHLOROPHENOXY)-2-NITROBENZOATE

CLH report

Proposal for Harmonised Classification and Labelling

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

International Chemical Identification:

bifenox (ISO); methyl 5-(2,4-dichlorophenoxy)-2-nitrobenzoate

EC Number: **255-894-7**

CAS Number: **42576-02-3**

Index Number: **-**

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Polish Competent Authority

Bureau for Chemical Substances

Version number: 02 **Date: 2020**

ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON BIFENOX (ISO); METHYL 5-(2,4-DICHLOROPHENOXY)-2-NITROBENZOATE

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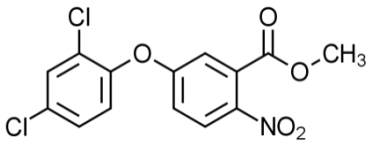
1 IDENTITY OF THE SUBSTANCE

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)	bifenox (ISO); methyl 5-(2,4-dichlorophenoxy)-2-nitrobenzoate;
Other names (usual name, trade name, abbreviation)	-
ISO common name (if available and appropriate)	bifenox
EC number (if available and appropriate)	255-894-7
EC name (if available and appropriate)	methyl 5-(2,4-dichlorophenoxy)-2-nitrobenzoate
CAS number (if available)	42576-02-3
Other identity code (if available)	CIPAC number 413
Molecular formula	$C_{14}H_9Cl_2NO_5$
Structural formula	
SMILES notation (if available)	-
Molecular weight or molecular weight range	342.14 g/mol
Information on optical activity and typical ratio of (stereo) isomers (if applicable and appropriate)	Not relevant
Description of the manufacturing process and identity of the source (for UVCB substances only)	Not relevant
Degree of purity (%) (if relevant for the entry in Annex VI)	Not relevant

1.2 Composition of the substance

Table 2: Constituents (non-confidential information)

Constituent (Name and numerical identifier)	**Concentration range (% w/w minimum and maximum in multi-constituent substances)**	**Current CLH in Annex VI Table 3.1 (CLP)**	**Current self-classification and labelling (CLP)**
bifenox	**> 97%**	**None**	**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
None				

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
None				

2 PROPOSED HARMONISED CLASSIFICATION AND LABELLING

2.1 Proposed harmonised classification and labelling according to the CLP criteria

Table 6:

	Index No	International Chemical Identification	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors	Notes
					Hazard Class and Category Code(s)	Hazard statement Code(s)	Pictogram, Signal Word Code(s)	Hazard statement Code(s)	Suppl. Hazard statement Code(s)		
Current Annex VI entry	**None**										
Dossier submitters proposal		bifenox (ISO);methyl 5-(2,4-dichlorophenoxy)-2-nitrobenzoate;	255-894-7	42576-02-3	Aquatic Acute 1 Aquatic Chronic 1	H400 H410	GHS09 Wng	H410	-	M = 1000 M = 1000	
Resulting Annex VI entry if agreed by RAC and COM		methyl 5-(2,4-dichlorophenoxy)-2-nitrobenzoate; bifenox	255-894-7	42576-02-3	Aquatic Acute 1 Aquatic Chronic 1	H400 H410	GHS09 Wng	H410	-	M = 1000 M = 1000	

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

Hazard class	Reason for no classification	Within the scope of public consultation
Explosives	Conclusive but not sufficient for classification	Yes
Flammable gases (including chemically unstable gases)	Conclusive but not sufficient for classification	No
Oxidising gases	Conclusive but not sufficient for classification	No
Gases under pressure	Conclusive but not sufficient for classification	No
Flammable liquids	Conclusive but not sufficient for classification	No
Flammable solids	Conclusive but not sufficient for classification	Yes
Self-reactive substances	Conclusive but not sufficient for classification	Yes
Pyrophoric liquids	Conclusive but not sufficient for classification	No
Pyrophoric solids	Conclusive but not sufficient for classification	Yes
Self-heating substances	Conclusive but not sufficient for classification	Yes
Substances which in contact with water emit flammable gases	Conclusive but not sufficient for classification	Yes
Oxidising liquids	Conclusive but not sufficient for classification	No
Oxidising solids	Conclusive but not sufficient for classification	Yes
Organic peroxides	Conclusive but not sufficient for classification	Yes
Corrosive to metals	Conclusive but not sufficient for classification	Yes
Acute toxicity via oral route	Conclusive but not sufficient for classification	Yes
Acute toxicity via dermal route	Conclusive but not sufficient for classification	Yes
Acute toxicity via inhalation route	Conclusive but not sufficient for classification	Yes
Skin corrosion/irritation	Conclusive but not sufficient for classification	Yes
Serious eye damage/eye irritation	Conclusive but not sufficient for classification	Yes
Respiratory sensitisation	Conclusive but not sufficient for classification	No
Skin sensitisation	Conclusive but not sufficient for classification	Yes

Hazard class	Reason for no classification	Within the scope of public consultation
Germ cell mutagenicity	Conclusive but not sufficient for classification	Yes
Carcinogenicity	Conclusive but not sufficient for classification	Yes
Reproductive toxicity	Conclusive but not sufficient for classification	Yes
Specific target organ toxicity-single exposure	Conclusive but not sufficient for classification	Yes
Specific target organ toxicity-repeated exposure	Conclusive but not sufficient for classification	Yes
Aspiration hazard	Conclusive but not sufficient for classification	Yes
Hazardous to the aquatic environment	harmonised classification proposed H400, M = 1 000 H410, M = 1 000	Yes
Hazardous to the ozone layer	Conclusive but not sufficient for classification	Yes

3 HISTORY OF THE PREVIOUS CLASSIFICATION AND LABELLING

No classification according to Dangerous Product Regulations incl. EC guidelines (67/548/EEC and 1999/45/EC). A harmonised classification for methyl 5-(2,4-dichlorophenoxy)-2-nitrobenzoate (bifenox) is not available in Annex VI of the Regulation (EC) No 1272/2008.

RAC general comment

Bifenox, also in the form of potassium or ammonium salts, is an active substance (herbicide) in many plant protection products against dicotyledonus weeds and other plants such as *Lamium spp*., *Viola arvensis*, *Veronica serpyllifolia*, and *Matricaria spp.* Bifenox is used on various crops: spring and winter cereals like barley, wheat, oats, spelt, triticale and also grasses, grassland, decorative lawns and turf.

It was included in Annex I of Council Directive 91/414/EEC (Commission Directive 2008/66/EC of 30 June 2008) in 2009 and is an approved active substance under Regulation (EC) 1107/2009 (PPPR). The initial DAR submitted by Belgium as rapporteur member state (RMS) was peer reviewed by EFSA in 2007 (the EFSA conclusion was published in 2008). Data submitted for bifenox to the RMS (Poland) for preparation of the RAR were used in the CLH report.

In vitro, in rat and human liver microsomes, the hydrolysis product bifenox-acid was shown to be the main metabolite in both species (Anonymous, 2015). In an *in vivo* toxicokinetic study in male and female rats, after a single oral gavage dose of 90 mg/kg bw bifenox-suspension in aqueous gum tragacanth approximately 30 % of the dose in male and 53 % in female rats was recovered in the urine as bifenox-acid, while 63 % and 46 %, respectively, were excreted *via* the faeces predominantly as unchanged bifenox (Anonymous, 1986). After a single oral administration of 900 mg/kg bw, around 12 %

and 20 % of the dose for males and females, respectively, were found in the urine and 85 % and 83 %, respectively, in the faeces. Thus, excretion was predominantly *via* faeces within the first 48 hours after administration. Percentages between 0.1 and 0.9 % of both administered doses were recovered in tissues and carcass 48 hours after exposure indicating a low potential for bioaccumulation. Similar percentages of excreted bifenox and metabolites were measured in a repeated dose study over 14 days with the lower dose, and in a study with bile duct-cannulated rats after single oral gavage of the same two doses in the same vehicle (Anonymous, 2016). Biliary excretion accounted for around 16 % in males and 18 % in females with most of the biliary excretion occurring during the first 24 hours after administration. Overall, based on mean urinary excretion values, oral absorption of bifenox was calculated to be 25 %. RAC notes that bifenox is reported to be a solid that is essentially insoluble in water (0.398 mg/L at 25 °C according to GESTIS Substance Database), thus absorption from aqueous suspensions may be limited.

Of note, peak blood concentration values were around 2-fold higher in males after both doses (135 µg/mL and 185 µg/mL after 90 and 900 mg/kg bw, respectively, in males vs. 57 and 75 µg/mL, respectively, in females). Peaks occurred 2 hours later in males than in females. Mean half-lives were shorter in males with 39 and 56 hours after 90 and 900 mg/kg bw, respectively, as compared to 66 and 70 hours, respectively, in females (Anonymous, 1986).

4 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

Bifenox is an active substance in the meaning of Directive 91/414/EEC and Regulation 1107/2009. In accordance with article 36(2) of the CLP Regulation, bifenox shall be subjected to harmonised classification and labelling.

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

5 IDENTIFIED USES

Bifenox was included in Annex I of Council Directive 91/414/EEC (Commission Directive 2008/66/EC of 30 June 2008). This active substance is an approved active substance under Regulation (EC) 1107/2009 (repealing Commission Directive 91/414/EEC) as specified in Commission Implementing Regulation (EU) 540/2011 of 25 May 2011 and Commission Implementation Regulation No. 1124/2013 amending Commission Implementation Regulation No 540/2011.

Bifenox, also in the form of potassium or ammonium salts, is an active substance (herbicide) in many plant protection products against dicotyledonus weeds and other plants as Lamium spp., Viola arvensis, Veronica serpyllifolia, Matricaria spp.. Bifenox is used for great deal of various crops, spring and winter cereals like barley, wheat, oats, spelt, triticale and also grasses, grassland, decorative lawn and turf.

6 DATA SOURCES

Data submitted with the dossier supporting the application for renewal of the regulatory approval of bifenox under Commission Implementing Regulation (EU) 844/2012 of 18 September 2012 submitted to the RMS Poland are used in this CLH report.

7 PHYSICOCHEMICAL PROPERTIES

Table 8: Summary of physicochemical properties

Property	Value	Reference	Comment (e.g. measured or estimated)
Physical state at 20°C and 101,3 kPa	Pale yellow crystalline solid with no characteristic odour		
Melting/freezing point	Bifenox purified active substance melts in the range 86.0°C – 87.7°C.	Ristorcelli & Bates (2000)	Measured
	Bifenox technical active substance melts in the range 86.2°C – 87.3°C.		
Boiling point	Bifenox purified active substance does not boil. It decomposes from 398.6°C.	Ristorcelli & Bates (2000)	Measured
	Bifenox technical active substance does not boil. It decomposes from 392.8°C.		
Relative density	The relative density (D_4^{20}) of Bifenox purified active substance is 1.541.	Ristorcelli & Bates (2000)	Measured
	The relative density (D_4^{20}) of Bifenox technical active substance is 1.498.		
Vapour pressure	at 20°C, 4.74×10^{-8} Pa at 25°C, 1.85×10^{-7} Pa	Bates (2001a)	Measured
	$< 10^{-7}$ torr (25°C) corresponds to $< 1.33 \times 10^{-5}$ Pa (25°C)	Teeter (1986)	Measured
	Henry's law constant: $K > 1.62 \times 10^{-4}$ Pa m^3 mol^{-1} at 20°C	Bascou (2001a)	Calculated
	Henry's law constant: $K > 1.13 \times 10^{-7}$ atm m^3 $g.mol^{-1}$ at 25°C	Guyot (1988)	Calculated
Surface tension	Surface tension of 90% technical	Bascou (1998)	Measured

Property	Value	Reference	Comment (e.g. measured or estimated)
	Bifenox solubility in water was found to be 72.5 mN/m at 20°C. The substance can be regarded as not surface active.		
Water solubility	< 0.1 mg/L at 20°C (pH 4 - 9)	Bates (2001b)	Measured
Solubility in organic solvents	at 20°C Acetone: 580 ± 14 g/L Acetonitrile: 330 ± 5.8 g/L Dichloromethane: > 1000 g/L Ethyl acetate: 440 ± 29 g/L n- Heptane: 3.1 ± 0.28 g/L Methanol: 23 ± 1.0 g/L n-Octanol: 10 ± 0.33 g/L Toluene: 320 ± 49 g/L	Bates (2001b)	Measured
Partition coefficient n-octanol/water	log P_{ow}: 3.64 (within a 95% confidence range of 3.55 to 3.73) Guideline EC method A.8 OECD 117	Bates (2000c)	Measured
	Bifenox acid: Log P_{ow}: 2.64 (pH 7; at 20°C ± 1°C) (Mean log P_{OW} of two runs; the result within a range of ± 0.1 log units (absolute deviation)). Guideline EC method A.8 OECD 117	Birnschein (2016a)	Measured
	Aminobifenox: Log P_{ow}: 4.86 (pH at 20°C ± 1°C) (Mean log P_{OW} of two runs; the result within a range of ± 0.1 log units (absolute deviation)). Guideline EC method A.8 OECD 117	Birnschein (2016b)	Measured
	Aminobifenox acid: Log P_{ow}: 2.75 (pH 7; at 20°C ± 1°C) (Mean log P_{OW} of two	Birnschein (2016c)	Measured

Property	Value	Reference	Comment (e.g. measured or estimated)
	runs; the result within a range of ± 0.1 log units (absolute deviation)). Guideline EC method A.8 OECD 117		
	Log P_{ow}: 1.54 (pH 4; at 20°C) Log P_{ow}: -0.82 (pH 7; at 20°C) Log P_{ow}: -1.07 (pH 10; at 20°C) Guideline EC method A.8 OECD 107 OPPTS 830.7550	Document MII, Section 1 by European Union 2,4-D Task Force 2012 dated 27 February 2012- For further information please refer to Document-B.	Measured
	The results not available Guideline EC method A.8 OECD 107 OPPTS 830.7550	Baer (2016)	Measured
Flash point	Not relevant to be measured since the substance melts. No auto-ignition occurred before the melting point of ca 89°C was reached.	Francois (1998)	Estimated
Flammability	Bifenox is not highly flammable (substance melts, no flame is observed, flash point >100°C)	Francois (2000)	Estimated
Explosive properties	Bifenox technical does not have shock sensibility and thermal sensibility to explosion.	Francois (1998)	Estimated
Self-ignition temperature	No auto ignition occurred since the substance melts (self-ignition temperature >400°C).	Francois (1998)	Measured
Oxidising properties	Bifenox technical active substance has no oxidising properties.	Francois (1998)	Measured
	Bifenox technical active substance has no oxidising properties.	Franke (2006)	Measured
Granulometry			Not available
Stability in organic solvents and identity of relevant degradation products			
Dissociation constant	Bifenox is neither an	Bascou (2001b)	Not available

Property	Value	Reference	Comment (e.g. measured or estimated)
	acid with pKa < 2 nor a base with pKa > 2. The substance does not dissociate		
Viscosity	-	-	Not available

8 EVALUATION OF PHYSICAL HAZARDS

8.1 Explosives

Table 9: Summary table of studies on explosive properties

Method	Results	Remarks	Reference
Guideline: EEC A.14	Bifenox technical does not have shock sensibility, friction sensibility and thermal sensibility to explosion.	Estimated	Francois (1998)

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

Bifenox technical does not have shock sensibility and thermal sensibility to explosion.

8.1.2 Comparison with the CLP criteria

Bifenox does not meet the criteria.

8.1.3 Conclusion on classification and labelling for explosive properties

Bifenox does not require classification and labelling with regard to explosive properties according to Annex I part 2 of the CLP regulation.

8.2 Flammable gases (including chemically unstable gases)

Not relevant

8.3 Oxidising gases

Not relevant

8.4 Gases under pressure

Not relevant

8.5 Flammable liquids

Not relevant

8.6 Flammable solids

Table 10: Summary table of studies on flammable solids

Method	Results	Remarks	Reference
Guideline: EEC A.10	Bifenox is not highly flammable (substance melts, no flame is observed, flash point >100°C)	Estimated - numerical results are inaccessible	Francois (2000)

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

Bifenox is not highly flammable.

8.6.2 Comparison with the CLP criteria

Bifenox does not meet the criteria.

8.6.3 Conclusion on classification and labelling for flammable solids

Bifenox does not require classification and labelling with regard to flammability.

8.7 Self-reactive substances

Table 11: Summary table of studies on self-reactivity

Method	Results	Remarks	Reference
Guideline: EEC A.16	No auto ignition occurred since the substance melts.	Measured	Francois (1998)

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

No auto ignition occurred since the substance melts.

8.7.2 Comparison with the CLP criteria

Bifenox does not meet the criteria.

8.7.3 Conclusion on classification and labelling for self-reactive substances

Bifenox does not require classification and labelling with regard to self-reactive substances.

8.8 Pyrophoric liquids

Not relevant.

8.9 Pyrophoric solids

Not relevant.

8.10 Self-heating substances

Not relevant.

8.11 Substances which in contact with water emit flammable gases

Not relevant.

8.12 Oxidising liquids

Not relevant.

8.13 Oxidising solids

Table 12: Summary table of studies on oxidising solids

Method	Results	Remarks	Reference
Guideline: EEC A.21	Bifenox technical active substance has no oxidising properties.	Measured	Francois (1998)
Guideline: EEC A.17	Bifenox technical active substance has no oxidising properties.	Measured	Franke (2006)

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

Bifenox technical active substance has no oxidising properties.

8.13.2 Comparison with the CLP criteria

Bifenox does not meet the criteria.

8.13.3 Conclusion on classification and labelling for oxidising solids

Bifenox does not require classification and labelling with regard to oxidising properties.

8.14 Organic peroxides

Not relevant.

8.15 Corrosive to metals

Not relevant.

RAC evaluation of physical hazards

Summary of the Dossier Submitter's proposal

Since bifenox is a solid, hazard classes for gases and liquids do not apply.

Explosives

The DS summarised one EEC A.14 test (Francois, 1998) which was negative for shock sensitivity, friction sensitivity, and thermal sensitivity to explosion. They concluded that bifenox does not meet the classification criteria.

Flammable solids

The DS summarised one EEC A.10 test (Francois, 2000) which was negative because the substance melts. No classification for this hazard class was proposed.

Self-reactive substances

No auto-ignition was observed in one A.16 test (Francois, 1998) because the substance melts. Thus, according to the DS, bifenox does not meet the criteria and no classification was proposed.

Pyrophoric Solids, Self-heating substances, Substances which in contact with water emit flammable gases, Corrosive to metals

The DS stated that these hazard classes were not relevant for bifenox but provided no justification.

Oxidising solids

One EEC A.21 test (Francois, 1998) and one EEC A.17 (Franke, 2006) test are available in which bifenox did not show oxidising properties. The DS proposed no classification.

Comments received during consultation

No comments were received for Physical Hazards during consultation.

Assessment and comparison with the classification criteria

RAC agrees that as a solid, bifenox does not meet the criteria for hazard classes for gases and liquids. Furthermore, it is not an organic peroxide.

Explosives

The DS summarised one EEC A.14 test which was negative. However, this test is not sufficient for classification according to UN RTDG. Structural features associated with explosive properties as laid out in table A6.1 in Appendix 6 of the UN RTDG are unsaturated C-C bonds (e.g. acetylenes, acetylides, and 1,2-dienes), C-metal or N-metal bonds, contiguous nitrogen or oxygen atoms, N-O bonds including nitro-compounds, N- or O-halogen bonds. Although C-C double bonds are present in the molecule, none of the examples given in table A6.1 refer to structures similar to aromatic rings. However, bifenox contains a nitro group (see below). Thus, it cannot be concluded that bifenox does not meet the criteria for classification. RAC proposes **no classification due to inconclusive data**.

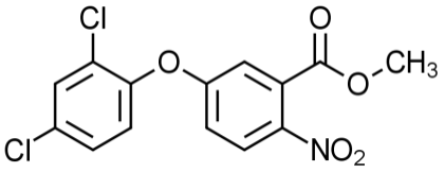


Figure: Chemical structure of bifenox

Flammable solids

The DS summarised one EEC A.10 test which was negative because the substance melts. If negative, this test is equivalent to the UN RTDG N.1 test method. Thus, RAC concurs with the DS that **no classification for this hazard class is warranted**.

Self-reactive substances

No auto-ignition was observed in one A.16 test. However, according to CLP Guidance the determination of auto-ignition temperature is not relevant for self-reactive substances. Substances must not be considered for this hazard class if they do not contain structural features associated with explosive (see above) or self-reactive properties. Structural features associated with self-reactive properties are (according to Table A6.2, Appendix 6, UN RTDG): e.g. aminonitriles, haloanilines, organic salts of oxidising acids, S-O double bonds, phosphites, epoxides and aziridines, olefines and cyanates. RAC considers aromatic rings excluded from the definition of olefins (cyclic or acyclic alkenes). However, at least one structural feature for explosive properties is present in the molecule. RAC proposes **no classification due to inconclusive data**.

Pyrophoric Solids, Self-heating substances

According to the annex provided with the CLH report, bifenox is known to be stable in contact with air at room temperature for prolonged periods of time. Furthermore, its melting point is below 160 °C (86-87.7 °C) Thus, **no classification is warranted for the pyrophoric solids and self-heating substances hazards**.

Substances which in contact with water emit flammable gases

Bifenox does not contain metals or metalloids and hence **does not meet the classification criteria for this hazard class**.

Corrosive to metals

According to the annex provided with the CLH report, bifenox does not contain any chemical groups which could initiate an irreversible electrochemical reaction with metals leading to significant damage or destruction. Thus, **no classification is warranted for this hazard class**.

Oxidising solids

One EEC A.21 test and one EEC A.17 test are available in which bifenox did not show oxidising properties. These are not sufficient for classification. However, bifenox does not contain fluorine, chlorine or oxygen atoms that are bound to any element other than carbon or hydrogen. Thus, it **does not meet the criteria for oxidising solids**.

9 TOXICOKINETICS (ABSORPTION, METABOLISM, DISTRIBUTION AND ELIMINATION)

9.1 Absorption, distribution, metabolism and excretion by oral exposure

The rate and extension of absorption, distribution, metabolism and excretion after oral exposure of Bifenox has been evaluated from a study conducted in rats. This study already had been submitted in the context of the inclusion of the active substance Bifenox in Annex I of the Council Directive 91/414/EEC.

Metabolism pattern of Bifenox has been investigated with radiolabelled [^{14}C]-Bifenox on the dichlorophenyl ring. Cleavage of the ether bond and then breaking of the molecule into 2 ring moieties was not expected to occur during the metabolism process. Therefore, only one ring was uniformly labelled. This assumption was confirmed by the *in vivo* metabolism study (Anonymus, 1986), which is summarized below. In this study, more than 95% of the radioactivity recovered was associated with Bifenox or identified metabolites containing the two rings.

As addressed under Regulation (EU) 283/2013, a study comparing *in vitro* metabolism has been investigated to verify rat metabolism as a suitable model. The study compares human and rat metabolism of uniformly ring labelled [^{14}C]-Bifenox *in vitro* in liver microsomes. The results from the *in vitro* study are summarized under Anonymus, 2015.

Evaluation of this study lead to the conclusion, that the metabolism of Bifenox in human and rats is comparable, based on similar metabolites. Therefore, results from the ADME study (Anonymus 1986) may be considered to be toxicologically relevant for humans. This study has been re-evaluated, in order to examine ADME endpoints after oral exposure of Bifenox. The endpoints from the rat metabolism study are summarized as follows:

Rate and extent of absorption:	25% (based on urinary excretion within 48 h)
Distribution:	Highest levels in excretory organs
Potential for accumulation:	No evidence for accumulation
Rate and extent of excretion:	29.1 - 52.6% via urine; 63 - 46% via faeces within 48 h
Metabolism in animals:	Nitro-reduction and O-demethylation leading to Bifenox acid and Aminobifenox
Toxicologically significant compounds:	Bifenox

Oral absorption occurred within 48 hours after a single oral gavage and was sex and dose dependent. Bioavailability reached 29% and 53% in male and female rats. An increased dose was followed by a reduced urinary excretion, suggesting saturation of absorption. Based these results, oral absorption is estimated to be 25%. The only tissues showing signs of accumulation after seven days were the kidneys and liver. No evidence of retention in tissues was observed. Bifenox will be found almost metabolized in faeces, but completely metabolized in urine. Metabolism occurred by nitro-reduction and O-demethylation leading to the metabolites Aminobifenox (in faeces) and Bifenox acid (in urine).

Table 13: Summary table of toxicokinetic studies

Method	Results	Remarks	Reference
GLP / Guidelines not applicable	Transformations of [^{14}C]-Bifenox in the test system were dominated by protein reactivity and/or ester hydrolysing enzymes. NADPH dependent	*In vitro*, liver microsomes	Anonymus, 2015

Method	Results	Remarks	Reference
	metabolism could not be observed. Reactions in rat and human liver microsomes were similar, no unique human metabolite was observed.		
GLP / OECD 417	Based on urinary excretion, the extent of bioavailability reaches 29% and 53% in male and female rats. Bifenox undergoes a nitro-reduction and O-demethylation leading to formation of Bifenox acid and Aminobifenox.	*In vivo*, rat	Anonymus, 1986

9.2 Absorption, distribution, metabolism and excretion by other routes

Executive Summary

The absorption and excretion of radio-labelled [^{14}C]-Bifenox after single oral doses to bile duct-cannulated male and female Sprague Dawley CD rats at 90 mg/kg bw and 900 mg/kg bw were investigated in this toxicokinetics study.

After single oral doses of [^{14}C]-Bifenox to bile duct-cannulated rats, excretion of radioactivity was rapid with more than 85% of the dose excreted within the first 24 hours post dosing. Biliary excretion accounted for ≤ 18% of the dose at the 90 mg/kg dose level and ≤ 8.0% of the dose at the 900 mg/kg dose level.

Excretion of radioactivity was predominantly via the faeces and accounted for 42.8 – 72.9% dose during 0 – 48 hours at the 90 mg/kg dose level and 89.7 – 96.5% dose at the 900 mg/kg dose level. Urinary excretion during 0 – 48 hours accounted for 13.9 – 32.0% dose at the 90 mg/kg dose level and 4.8 – 9.1% dose at the 900 mg/kg dose level. A sex difference was noted in the routes of excretion at the 90 mg/kg dose level as a higher proportion of radioactivity was eliminated in the urine in females, whilst a greater proportion of radioactivity was eliminated in the faeces of males.

The extent of absorption was assessed as the sum of total radioactivity measured in bile, urine, liver and carcass. On this basis it was estimated that the extent of absorption was 30.3% and 50.3% of the dose for males and females, respectively at the 90 mg/kg dose level and 16.2 % and 12.9% dose for males and females at the 900 mg/kg dose level. At 48 hours, retention of radioactivity in residual carcass and tissues was low, accounting for 0.5 – 0.8% dose at the 90 mg/kg dose level and 0.1 – 0.9% at the 900 mg/kg dose level.

This metabolism study in the Sprague Dawley CD rats is acceptable and satisfies the guideline requirement for a toxicokinetic study OECD 417 and US EPA OPPTS 870.7485 in Sprague Dawley CD rats.

Method	Results	Remarks	Reference
GLP / OECD 417	Oral absorption 90 mg/kg bw: 30.3% male - 50.3% female Oral absorption 900 mg/kg bw: **12.9% male - 16.2% female**	In vivo, rat	Anonymus, 2016

During the course of the study, all animals were routinely observed for behavioural changes, ill health or reactions to treatment. On the day of dosing, all animals were observed immediately after dosing,

again within 2 hours after dosing and on at least one other occasion during the working day. On all other days after dosing, all animals were observed on at least one occasion.

After single oral doses of [14C]-Bifenox to bile duct-cannulated rats at dose levels of 90 and 900 mg/kg bw, excretion of radioactivity was rapid with more than 85% of the dose excreted within the first 24 hours post dose. Biliary excretion accounted for ≤ 17.8% dose at the 90 mg/kg dose level and ≤ 8.0% dose at the 900 mg/kg dose level.

Excretion of radioactivity was predominantly via the faeces and accounted for 42.8 – 72.9% dose during 0 48 hours at the 90 mg/kg dose level and 89.7 – 96.5% dose at the 900 mg/kg dose level. Urinary excretion during 0 – 48 hours accounted for 13.9 – 32.0% dose at the 90 mg/kg dose level and 4.8 – 9.1% dose at the 900 mg/kg dose level. A sex difference was noted in the routes of excretion at the 90 mg/kg dose level as a higher proportion of radioactivity was eliminated in the urine in females, whilst a greater proportion of radioactivity was eliminated in the faeces of males.

At 48 hours, retention of radioactivity in residual carcass and tissues was low, accounting for 0.5 – 0.8% dose at the 90 mg/kg dose level and 0.1 – 0.9% at the 900 mg/kg dose level.

The extent of absorption was assessed as the sum of total radioactivity measured in bile, urine, liver and carcass. On this basis, it was estimated that the extent of absorption was 30.3% and 50.3% dose for males and females respectively at the 90 mg/kg dose level and 16.2 % and 12.9% dose for males and females respectively at the 900 mg/kg dose level.

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

Toxicokinetic data for Bifenox have no impact on classification.

10 EVALUATION OF HEALTH HAZARDS

Acute toxicity

10.1 Acute toxicity - oral route

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

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance,	Dose levels, duration of exposure	Value LD_{50}	Reference
GLP, EPA 81-1 870.1100	Rat	Bifenox Lot no. 3123142017 Purity: 97%	at a level of 5000 mg/kg bw. Signs of toxicity and body weights were recorded up to 14 days after dosing.	LD_{50} > 5000 mg/kg bw (male and female)	Anonymus, 1985a
Not confirming GLP or Guidelines, data gaps for endpoints. The study was considered to be not valid.	Mouse	Bifenox Batch #. MCTR-126-78 Purity not stated	between at 0, 316, 1000, 3160, 10000 mg/kg bw Animals were observed for clinical signs	LD_{50} > 1540 mg/kg bw (male), LD_{50} > 1780 mg/kg bw (female)	Anonymus, 1978

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance,	Dose levels, duration of exposure	Value LD_{50}	Reference
			of toxicity up to 14 days after dosing.		

10.1.1 Study 1

The first study does not provide a statement of GLP but the study report claims GLP compliance. The study has been performed in accordance with EPA Guideline 81-1 870.1100 and an internal quality assurance statement is provided. Furthermore, the study was accepted in the DAR (2006). In order to assess the current reliability of this study, the ToxRTool (Toxicological data reliability Assessment Tool) has been used for evaluation. Consequently, this study is categorized into Klimisch category 1: reliable without restriction. Therefore, it is concluded that this study is valid, even without a statement of GLP and is considered for classification.

Bifenox (lot no. 3123142017, purity 97%) was administered orally by gavage, to five male and five female Sprague-Dawley rats, at a level of 5000 mg/kg bw. Signs of toxicity and body weights were recorded up to 14 days after dosing. Gross pathological examinations were performed on all main study animals.

No mortality was recorded during the study. The majority of the animals showed expected body weight gain throughout the study. Clinical signs included faecal staining, soft stool and hypoactivity (1-2 animals) 24 hours after dosing. 2 days after oral administration, some animals showed alopecia of abdomen, chest and/or hind leg. Reduced food consumption was observed in some cases. At necropsy, some animals showed red foci and discoloration of lungs.

The results on bodyweight gain and mortality are summarized in Table 15.

Table 15 Summary of body weight gains and mortalities at 5000 mg/kg Bifenox

Animal No.	Body weight gain [g]					Mortality (day)
	Pre-test	Day 7	Change*	Day 14	Change*	
Males						
2066	256	285	29	327	71	14**
2072	264	307	43	365	101	14**
2076	262	263	1	329	67	14**
2081	255	265	10	346	91	14**
2096	250	250	0	316	66	14**
Females						
2108	242	260	18	270	28	14**
2115	230	246	16	261	31	14**
2119	242	270	28	290	48	14**
2126	235	259	24	280	45	14**
2130	236	250	14	275	39	14**

* Change from pre-fasted weight
** At terminal sacrifice – not substance related

The acute oral median lethal dose (LD50) of Bifenox in rats was greater than 5000 mg/kg body weight. Based on the acute oral LD50 value, Bifenox does not require classification and labelling according to Regulation (EC) 1272/2008.

10.1.1 Study 2

The second study does not provide a statement of GLP, not even a statement on internal quality control. Reporting is very brief and no information on test item purity is given. Furthermore, this study was not performed in accordance with any guidelines and it was not accepted in the DAR (2006).

In order to assess the current reliability of this study, the ToxRTool (Toxicological data reliability Assessment Tool) has been used for evaluation. Consequently, this study is categorized into Klimisch category 3: not reliable. Therefore, this study is judged not to be valid and is not considered for classification.

This study does not fully confirm to the directive EEC 92/69 method B1 or GLP and it provides only incomplete data for body weight, clinical signs and necropsy findings. Based on these data gaps and the statement in the DAR (2006), the study is considered not to be valid and the results will not be included for classification according to Regulation (EC) 1272/2008.

Bifenox was suspended in corn oil and administered orally by gavage, to mice (5 per dose-group and sex). The dose groups were between at 0, 316, 1000, 3160, 10000 mg/kg bw. Animals were observed for clinical signs of toxicity up to 14 days after dosing. Body weights were recorded at the beginning and at the end of the study. Gross pathological examinations were performed at the end of the observation period.

On day two, mortality was observed at 1000 mg/kg bw in 1/5 males. At a dose level of 3160 mg/kg bw, 3/5 males and 3/5 females died on day 1, 2/5 males and 2/5 females died on day 2. At a dose level of 10000 mg/kg bw 3/5 males and 4/5 females died on day 1, 1/5 males and 1/5 females on day 2. There was no significant change in body weight gain compared to normal. Clinical signs included inactivity, unsteady gait and shivering. During necropsy, no gross lesions were observed. The results on mortality are summarised in Table 3.1.1-2.

Table 16 Mortalities in the acute oral toxicity study in mice

Group	Dose (mg/kg)	Mortalities in males	Mortalities in females
1	0	0/5	0/5
2	316	0/5	0/5
3	1000	1/5	0/5
4	3160	5/5	5/5
5	10000	4/5	5/5

Based on deaths occurring in the 14 days of treatment and using a modification of the method of Horn (Biometrics. 12. p. 311, 1956), LD50 values of 1540 mg/kg (95% confidence limits: 833-2850) and 1780 mg/kg (95% confidence limits: not determined due to steep response slope) were calculated for male and female mice, respectively.

Signs of toxicity judged to be related to treatment included inactivity, unsteady gait and shivering.

Necropsy findings among the surviving animals showed no gross lessions. Among mice that died, gas in the stomach and intestines was noted.

Following the oral administration of graded doses of the test compound to fasted young mice, LD50 values of 1540 mg/kg and 1780 mg/kg were calculated for males and females, respectively. The gastrointenstinal tract may be a possible site of toxicity.

There are no human data on acute oral toxicity of bifenox.

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

Based on the study no 1 (rat) confirm to the directive EEC 92/69 method B1. bifenox is of low (no mortal cases) toxicity by the oral route in rats (LD_{50} > 5000 mg/kg bw).

The study no 2 (mouse) does not fully confirm to the directive EEC 92/69 method B1. The purity of the tested substance was not determined in this study. The test material (bifenox) was suspended in corn oil. There are incomplete raw data for body weight, clinical signs and necropsy findings. Additionaly the lack of information about the solvent and purity of the test substance in the test no 2 (mouse) comparing to test 1 (rat).

However, based on the LD_{50} found in mice the classification *Xn (Harmful), R22 – Harmful if swallowed* has been attributed to the substance in EFSA Scientific Report (2007)[1].

According to DAR (2006)[2] it has been assuming that the diference in the test results is propably related to the solvent used in this study as suggested by absence of toxic effects seen in the micronucleus test performed in mice (Anonymus, 2003) with bifenox suspended in hydroxypropyl-cellulose (aqueous suspension).

Considering all test results of studies number 1 and 2 in oral way and arguments presented above the test on mice could have been omitted for classification.

Taking into account all data bifenox is not classified in acute toxicity by the oral way.

10.1.2 Comparison with the CLP criteria

CLP Criteria (oral route):
Category 1: $0 < ATE \leq 5$
Category 2: $5 < ATE \leq 50$
Category 3: $50 < ATE \leq 300$
Category 4: $300 < ATE \leq 2\ 000$

Bifenox does not meet the CLP criteria classified in acute toxicity (oral).

10.1.3 Conclusion on classification and labelling for acute oral toxicity

Bifenox does not require classification and labelling with regard to acute oral toxicity.

10.2 Acute toxicity - dermal route

Table 15: Summary table of animal studies on acute dermal toxicity

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance,	Dose levels duration of exposure	Value LD_{50}	Reference
GLP, EPA 81-2 870.1200	Rabbit	Bifenox Lot no.	dose level of 2000 mg/kg bw	LD_{50} > 2000 mg/kg bw	Anonymus, 1985b (DAR,

[1] EFSA Scientific Report (2007) 119, 1-84, Conclusion on the peer review of bifenox
[2] DAR – Draft Assessment Report (Belgium, February 2006)

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance,	Dose levels duration of exposure	Value LD_{50}	Reference
		3123142017 Purity: 97%	Animals were observed for clinical signs for at least 14 days after treatment		2006)

There are no human data on acute dermal toxicity of bifenox.

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

There was no mortality. Most of the animals were free of significant signs although a single occurrence of ocular discharge was seen, as were occasional observations of nasal discharge and food consumption decrease. No abnormalities were seen at the majority of animals during necropsy.

The acute dermal median lethal dose (LD_{50}) of Bifenox in rabbits was greater than 2000 mg/kg body weight. Based on the acute dermal LD_{50} value, Bifenox does not require classification and labelling according to Regulation (EC) 1272/2008.

Bifenox is of low toxicity by the oral route in rats (LD_{50} > 2000 mg/kg bw).

10.2.2 Comparison with the CLP criteria

CLP Criteria (skin route):

Category 1: $0 < ATE \leq 50$

Category 2: $50 < ATE \leq 200$

Category 3: $200 < ATE \leq 1\,000$

Category 4: $1\,000 < ATE \leq 2\,000$

Based on the acute dermal LD_{50} value, Bifenox does not meet the criteria and does not require classification and labelling according to Regulation (EC) 1272/2008. .

10.2.3 Conclusion on classification and labelling for acute dermal toxicity

Bifenox does not require classification and labelling with regard to acute dermal toxicity.

10.3 Acute toxicity - inhalation route

Table 16: Summary table of animal studies on acute inhalation toxicity

Method, guideline, deviations if any	**Species, strain, sex, no/group**	**Test substance, , form and particle size (MMAD)**	**Dose levels, duration of exposure**	**Value** LC_{50}	**Reference**
GLP, EPA 81-3, EC Directive 92/69/EEC, 93/21/EEC B.2 and OECD 403	Rat	Bifenox Lot no. 3123142017 Purity: 97%	dust atmosphere concentration was at 0.91 mg/L, which was the maximum attainable exposure concentration with a MMAD of 2.7 µm and a SGD of 1.6	LC_{50} > 0.91 mg/L (maximal attainable concentration)	Anonymus, 1985

There are no human data on acute inhalation toxicity of bifenox.

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

No mortality was observed throughout study performance. Clinical signs included activity and white material on fur, were exhibited during exposure. Upon removal from the chamber and during the 2-hour post-exposure observation period, increased secretory response, moist rales, yellow/brown ano-genital staining and soft stool were exhibited by the test animals. During the first days of test week 1, rats exhibited yellow ano-genial staining and slightly increased secretory responses. There were no significant changes in body weight gain. During necropsy, no compound related findings were observed.

The acute (4-hour) inhalation LC_{50} for rats exposed to Bifenox was greater than 0.91 mg/L (LD_{50} of 245 mg/kg bw). Based on the acute inhalation LC_{50} value, Bifenox does not require classification and labelling according to Regulation (EC) 1272/2008.

Bifenox is of low toxicity by the inhalation route in rats ($LC_{50\ (4h)}$ > 0.91 mg/L). There were no mortalities at the maximum attainable concentration of 0.91 mg/L air.

10.3.2 Comparison with the CLP criteria

CLP criteria for inhalation route (dusts and mists)
Category 1: $0 < ATE \leq 0.05$
Category 2: $0.05 < ATE \leq 0.5$
Category 3: $0.5 < ATE \leq 1.0$
Category 4: $1.0 < ATE \leq 5.0$

The dust atmosphere concentration was at 0.91 mg/L, which was the maximum attainable exposure concentration No mortality was observed throughout study performance.

The acute (4-hour) inhalation LC50 for rats exposed to Bifenox was greater than 0.91 mg/L (LD50 of 245 mg/kg bw).

10.3.3 Conclusion on classification and labelling for acute inhalation toxicity

Based on the acute inhalation LC50 value, Bifenox does not require classification and labelling according to Regulation (EC) 1272/2008.

Bifenox does not require classification and labelling with regard to acute inhalation toxicity.

RAC evaluation of acute toxicity

Summary of the Dossier Submitter's proposal

No cases of acute intoxication or poisoning incidents were reported for bifenox in humans.

Oral

The DS summarised two acute oral toxicity studies: one in rats and the other in mice (Anonymous 1985a, 1978). The study in rats was reported to be performed according to EPA 81-1 870.1100, conforming to the directive EEC 92/69 method B1 and GLP standards using 97 % pure substance in aqueous vehicle. The LD_{50} for both sexes was greater than 5000 mg/kg bw. The other study performed in mice did not fully conform to the directive EEC 92/69 method B1 or GLP. The study report provided incomplete data for clinical evaluation and was deemed not reliable by the DS. In this study, bifenox (purity unknown) in corn oil was used and LD_{50} values of 1540 mg/kg bw for males and 1780 mg/kg bw for females were calculated.

In the rat study, no mortality occurred, while clinical signs were comprised of faecal staining, soft stool and hypoactivity, alopecia of the abdomen, chest and/or hind leg, as well as reduced food consumption. During necropsy, some animals showed red foci and discoloration of the lungs.

In the mouse study, mortality occurred starting from day two following exposure in 1/5 male rats at 1000 mg/kg bw. Clinical signs included inactivity, unsteady gait and shivering. During necropsy, no gross lesions but gas in the stomach and intestines were observed among animals that died.

Dermal

The DS summarised one acute dermal toxicity study performed in rabbits according to EPA 81-2 870.1200, complying to GLP standards (Anonymous, 1985b). It was considered reliable by application of ToxRTool. No mortalities occurred in any animal at the dose of 2000 mg/kg bw. No dermal irritation was observed and clinical signs comprised infrequent occurrences of ocular discharge, nasal discharge and decrease in food

consumption. No gross pathological abnormalities were observed in the majority of animals during necropsy.

Inhalation

One acute inhalation toxicity in rats was available (Anonymous, 1985). It was conducted according to EPA 81-3, EC Directive 92/69/EEC, 93/21/EEC B.2, and OECD TG 403, complying with GLP standards. The maximum attainable exposure concentration was a dust atmosphere concentration of 0.91 mg/L, as a single four-hour whole body exposure in a chamber.

No mortality occurred. Clinical signs during exposure comprised altered activity (RAC notes that the CLH report and study summary did not provide any details but only stated "activity" as a clinical sign), and white material on the fur during exposure. In the 2 hours post exposure, increased secretory response, moist rales, yellow/brown ano-genital staining, and soft stool were observed. Therefore, the $LC_{50(4h)}$ for this study was > 0.91 mg/L. During the first days of test week 1, rats exhibited yellow ano-genial staining and slightly increased secretory responses. No significant changes in body weight gain were recorded and no compound-related findings were observed during necropsy.

Conclusion on classification

Based on the first **acute oral toxicity** study in rats that yielded LD_{50} values > 5000 mg/kg bw, thus exceeding the guidance range for oral toxicity (Category 4: 1 000 < ATE ≤ 2 000), bifenox does not meet the criteria and the DS proposed no classification for bifenox.

For **acute dermal toxicity**, based on the fact that no treatment related mortalities were observed above the cut-off value for Cat 4 (2000 mg/kg bw) the DS proposed no classification.

For **acute toxicity via inhalation**, the DS proposed no classification since no mortalities were observed at the maximum attainable concentration.

Comments received during consultation

One Member State Competent Authority (MSCA) commented on acute oral hazard class and supported the proposed no classification with an ATE > 2000 mg/kg bw. Another study in mice (mouse micronucleus test, Anonymous, 2003) was taken into consideration. In this study, bifenox in hydroxypropyl cellulose vehicle in doses up to 2000 mg/kg bw resulted in no mortalities in male and female mice, thus suggesting an LD_{50} >2000 mg/kg. On the other hand, the MSCA also noted that EFSA in 2007 concluded that based on the mouse acute oral toxicity study, Acute Tox 4, H302 classification for bifenox was warranted.

Assessment and comparison with the classification criteria

One **acute oral toxicity** study in rats performed according to EPA Guideline 81-1 870.1100 yielded LD_{50} values that exceeded the boundaries for category 4 for acute oral toxicity classification. The study in mice used a test substance of unknown purity and yielded calculated LD_{50} values of 1540 mg/kg bw for males and 1780 mg/kg bw for females. Moreover, in a mouse micronucleus test, no mortalities occurred at doses of 2000 mg/kg bw, supporting the results of the rat study. However, RAC notes that in both

the rat study and the micronucleus test in mice, (carboxy)methylcellulose was used as the vehicle, in which the highly lipophilic bifenox is not soluble. In contrast, the acute oral toxicity study in mice used corn oil as the vehicle, which seems more appropriate.

Therefore, RAC considers the acute oral toxicity study in mice more suitable for classification. Based on the results from this study, RAC proposes classification as **Acute Tox 4, H302** with a rounded **ATE of 1500 mg/kg bw**.

RAC concurs with the DS that **no classification for acute dermal toxicity** is warranted based on the guideline and GLP compliant study presented in the CLH dossier in which no mortalities were observed at doses relevant for classification.

For **acute inhalation toxicity**, one guideline and GLP compliant study in rats using milled 97 % pure substance with (guideline compliant) mean mass aerodynamic diameters (MMAD) of 2.7 µm and a 4-hour exposure in a chamber was presented. No mortalities were observed during or after exposure, thus, the LC_{50} for this study was above the highest concentration tested (0.91 mg/L). However, RAC notes that no information was provided on how the concentration of test substance in the chamber was measured and how the maximum attainable concentration was determined. Industry provided additional information and explained that higher concentrations of the dust are expected to agglomerate so that particle sizes would have exceeded the MMAD required by the test guideline. Therefore, RAC concurs with the DS that **no classification** for this hazard class is warranted.

10.4 Skin corrosion/irritation

Table 17: Summary table of animal studies on skin corrosion/irritation

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance,	Dose levels duration of exposure	Results -Observations and time point of onset -Mean scores/animal -Reversibility	Reference
GLP, EPA 81-5 870.2500	Rabbit	Bifenox lot no. 3123142017, purity 97%	0.5 g dermal dose The area was examined at 1, 24, 48 and 72 hours after the removal of the patch and scored according to Draize (1959)	The only irritation noted was very slight erythema in one animal at 0.5 h. Not irritating	Anonymus 1985c

There are no human data on skin corrosion/irritation of Bifenox.

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

The potential of Bifenox to irritate skin was tested in New Zealand White albino rabbits. The animals were exposed for 4 h to 0.5 g of the test material. Bifenox was not irritating to the skin of rabbits.

Table 18: Dermal irritation responses in rabbits according to the Draize scheme

Animal	Erythema score						Oedema score					
No. Sex	1 m	2 m	3 m	4 f	5 f	6 f	1 m	2 m	3 m	4 f	5 f	6 f
after 30 min.	0	0	0	0	0	1	0	0	0	0	0	0
24 h	0	0	0	0	0	0	0	0	0	0	0	0
48 h	0	0	0	0	0	0	0	0	0	0	0	0
72 h	0	0	0	0	0	0	0	0	0	0	0	0
Irritation Index*	0						0					

* (Mean scores 24 – 72 h)

Based on mean skin irritation scores 24 to 72 hours after removal of test substance, Bifenox is not a skin irritant and will not require classification and labelling.

10.4.2 Comparison with the CLP criteria

CLP criteria for skin irritation category:

(1) Mean score of ≥ 2,3 and ≤ 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 reactions; 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.

Bifenox was essentially non-irritating to the skin of rabbits. The only irritation noted was very slight erythema in one animal at 0.5 h.

Bifenox does not meet the criteria and does not require classification and labelling according to CLP Regulation.

10.4.3 Conclusion on classification and labelling for skin corrosion/irritation

Bifenox does not require classification and labelling with regard to skin irritation.

RAC evaluation of skin corrosion/irritation

Summary of the Dossier Submitter's proposal

No human data are available on skin corrosion or irritation.

The DS summarised one EPA 81-5 870.2500 study conducted according to GLP standards with bifenox at a dose of 0.5 g applied for four hours in New Zealand White rabbits (Anonymous, 1985c). Except for slight erythema observed in one female rabbit at 0.5 h after application, no signs of erythema or oedema were observed in any of the remaining animals at any of the observation points. No clinical signs were reported.

Since no signs of toxicity or dermal irritation were observed in a reliable, GLP compliant, EPA 81-5 870.2500 study, the DS concluded that **no classification** was warranted for skin corrosion/irritation.

Comments received during consultation

No comments regarding this endpoint were received during consultation.

Assessment and comparison with the classification criteria

One EPA 81-5 870.2500 study was summarised in the CLH report. Bifenox technical grade (purity 97 %) moistened with 0.9 % saline was applied to the backs of six NZW rabbits, three males and three females, at a dose of 0.5 g and covered with a gauze and tape for 4 hours. Except for one female rabbit showing slight erythema, no irritation or corrosion responses were observed at any of the readings 24 to 72 hours post-exposure. Mean scores for erythema and oedema were 0 for all animals. However, in the study summary no data was provided on the area of exposure nor on the condition of application whether an occlusive or semi-occlusive patch was used. According to the guideline, an area of approximately 6 cm^2 should be covered with the test substance under a semi-occlusive dressing. Since no deviations from the guideline were noted RAC assumes that these provisions were not violated.

Thus, RAC concurs with the DS that in accordance with the CLP Regulation **no classification** for skin corrosion/irritation is warranted.

10.5 Serious eye damage/eye irritation

Table 19: Summary table of animal studies on serious eye damage/eye irritation

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance,	Dose levels duration of exposure	Results - Observations and time point of onset - Mean scores/animal - Reversibility	Reference
GLP, EPA 81-4 870.2400	Rabbit	Bifenox lot no. 3123142017, purity 97%	dose of 29.7 mg (equivalent to 0.1 mL) The treated eyes of the rabbits were observed and scored for ocular irritation approx. 1, 24, 48, and 72 hours and 7 days after instillation of the test material.	Slight conjunctival irritation (redness, chemosis, discharge) and iridial changes Slightly irritating	Anonymus, 1985d

Slight conjunctival irritation (redness, chemosis, discharge) and iridial changes were noted in all treated eyes at one hour. No corneal opacity or ulceration was observed. All nine animals were free of ocular irritation within 24 h to 7 days after instillation of the test article. Comparable results were obtained for washed and unwashed eyes.

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

The potential of Bifenox to irritate eyes was tested in New Zealand White albino rabbits. The animals were exposed to 0.1 mL (equivalent to 29.7 mg) of the test material. Bifenox produced only mild and reversible ocular irritation**.**

Table 20: Eye irritation mean scores (24-72 h) in rabbits according to the Draize scheme

	Mean scores (24 - 72 h) – Animal number								
	# 1	# 2	# 3	# 4	# 5	# 6	# 7	# 8	# 9
Corneal opacity	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Iritis	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Chemosis conjunctivae	0.3	0.3	0.0	0.3	0.3	0.3	0.0	0.3	0.0
Redness conjunctivae	0.7	0.7	0.0	1.3	0.3	0.3	0.0	1.0	0.0
Reversibility (day)	3	3	0	7	2	3	0	7	0

Based on mean eye irritation scores 24 to 72 hours after removal of test substance, bifenox is not an eye irritant and does not require classification and labelling. All effects reverses within an observation period.

10.5.2 Comparison with the CLP criteria

CLP criteria for Category for reversible eye effects:

at least in 2 of 3 tested animals, a positive response of:

(1) corneal opacity ≥ 1 and/or
(2) iritis ≥ 1, and/or
(3) conjunctival redness ≥ 2 and/or
(4) 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

Instillation of Bifenox technical into the eyes of New Zealand white rabbits produced mild and reversible ocular irritation. Bifenox does not meet the criteria classification.

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

Bifenox does not require classification and labelling with regard to eye irritation.

RAC evaluation of serious eye damage/irritation

Summary of the Dossier Submitter's proposal

No human data are available on eye irritation or damage.

The DS summarised one study following EPA 81-4 870.2400 that was conducted according to GLP standards (Anonymous, 1985d).

In this study, a single 29.7 mg dose equivalent to 0.1 mL was instilled into the elevated lower lid of the right eye of nine New Zealand White albino rabbits (five males and four females). Ocular irritation was observed and scored over 24-72 h. Slight conjunctival redness and chemosis of the eye were observed in all animals one hour after exposure (mean score, 1). All effects resolved within 24 hours to 7 days after instillation of the test substance. Results were comparable for washed and unwashed eyes. Thus, mean values for conjunctival chemosis were 0.3 for 6/9 animals, and 1.3 for 1/9 animals for conjunctival redness, and 0 for iritis and corneal opacity in all animals.

Since mean scores for conjunctival redness, conjunctival chemosis, iritis, and corneal opacity were below guidance values in the study presented, the DS concluded that no classification was warranted for Serious Eye Damage/Eye Irritation.

Comments received during consultation

No comments regarding this endpoint were received during consultation.

Assessment and comparison with the classification criteria

One EPA 81-4 870.2400 study was summarised in the CLH report. Based on the scores reported in this study, only slight/mild conjunctival irritation (redness - grade 2 in one animal, grade 1 for all other animals, chemosis – grade 1, discharge) and iridial changes,

that were described as slight deepening of rugae or slight hyperaemia (effects not included in the Draize system), were observed in all treated eyes at one hour. All lesions were reversible and disappeared within 24 h to 7 days. No corneal opacity or ulceration was observed in any animal at any observation point.

According to CLP guidance, a substance is classified as eye irritant when it evokes in at least in 2 of 3 tested animals, a positive response of: (a) corneal opacity ≥ 1 and/or (b) iritis ≥ 1, and/or (c) conjunctival redness ≥ 2 and/or (d) conjunctival oedema (chemosis) ≥ 2. None of these criteria were fulfilled by bifenox in this study.

Thus, RAC concurs with the DS that in accordance with the CLP Regulation **no classification** for Serious Eye Damage/ Eye Irritation is warranted.

10.6 Respiratory sensitisation

There are no experimental data and no observations or indications for respiratory sensitisation.

10.7 Skin sensitisation

Table 21: 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
GLP, OECD 406	Guinea pig	Bifenox batch # 0546/7, purity 98.2%	0.1 mL of a 5% mixture of Bifenox the skin reaction results from the first stage were evaluated at 25 and 48 hours, of the second stage at 49 and 72 hours after begin of exposure.	No skin irritations were noted after challenge Non-sensitizing (M&K)	Anonymus, 2001
Not confirming GLP, OECD 406, EC B.06 Dir. 67/548/EEC V	Guinea pig	bifenox lot no. 353-12-1, purity 98%	0.5 mL test article (50% w/v in acetone) Cutaneous reactions were evaluated 24, 48 and 72 hours after challenge application	No skin-sensitizing properties Non-sensitizing (Buehler)	Anonymus, 1985

In order to assess the current reliability of the first study (Anonymus, 2001), the ToxRTool (Toxicological data reliability Assessment Tool) has been used for evaluation. Consequently, this study is categorized into Klimisch category 1: reliable without restriction.

Intracutaneous injections of 0.1 mL of a 5% mixture of Bifenox in sesame oil resulted in discrete or patchy erythema in all treated animals after 25 and 48 hours. Topical application of 2 mL of a 30% mixture of Bifenox in sesame oil result in discrete or patchy erythema or moderate and confluent erythema in all treated animals after 49 and 72 hours. No skin irritations were noted after challenge with the 5% mixture of Bifenox in sesame oil. At no time were adverse reactions noted at the vehicle control sites in test animals or at any sites of control animals.

Under the conditions of the Magnusson Kligman test, Bifenox did not exhibit skin-sensitizing properties in guinea pigs and was considered to be non-sensitizing.

According to the criteria laid down in Regulation (EC) 1272/2008, the test substance Bifenox does not require classification for skin sensitizing properties.

The second study does not provide a statement of GLP but the study report claims GLP compliance. However, the study has been performed in accordance with OECD Guideline 406 and an internal quality assurance statement is provided. Furthermore, the study was accepted in the DAR (2006).

Under the conditions of the Buehler test, Bifenox did not exhibit skin-sensitizing properties in guinea pigs and was considered to be non-sensitizing.

According to the criteria laid down in Regulation (EC) 1272/2008 the test substance Bifenox does not require classification for skin sensitizing properties.

There are no observations or indications for skin sensitisation in humans. No incidences of allergic reaction reported.

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

The dermal sensitisation of Bifenox was evaluated by means of the Magnusson and Kligman maximisation test (Anonymus, 2001) in 15 female Dunkin-Hartley guinea pigs (10 test and 5 control animals). Concentrations used were determined from a series of "sighting tests" to determine the highest concentrations for the relevant endpoints which were 5% for intra dermal application, 30% for topical induction and 5% for topical challenge. Following challenge with the test material no skin responses were noted. At no time any adverse reactions were noted at the vehicle control sites in test animals or at any sites of control animals. Under the conditions of this assay, Bifenox did not exhibit sensitizing properties.

The use of 10 animals and 5 controls is the minimum in the Guinea-Pig Maximisation Test (GPMT), but then it is not possible to claim that the substance is a sensitizer. In this case, according to the test method it is recommended to use additional animals to test 20 animals with 10 controls.

Another sensitization assay was performed according to the Buehler method (Saeber, 1985). A group of 10 female Hartley guinea pigs were exposed to Bifenox, 10 animals were used as control group. The animals received once weekly during a 3-week induction period an application of 0.5 mL test article to the clipped skin on the left shoulder region for 6 hours. Thereafter, the dressing was removed. The resulting dermal reactions were assessed 24 hours later for erythema and oedema according to the Draize scale. Control animals were treated similarly without test substance. Following a rest of 2 weeks, a challenge cutaneous application of 0.5 mL test article to

the clipped skin of the right flank for 6 hours. At no time any adverse reactions were noted at the vehicle control sites in test animals or at any sites of control animals. Under the conditions of the Buehler test, Bifenox did not exhibit skin-sensitizing properties in guinea pigs and was considered to be non-sensitizing.

10.7.2 Comparison with the CLP criteria

Under the conditions of the Magnusson Kligman test and the Buehler method, Bifenox did not exhibit skin-sensitizing properties in guinea pigs and was considered to be non-sensitizing.

10.7.3 Conclusion on classification and labelling for skin sensitisation

Bifenox does not require any hazard classification for skin sensitizing properties

RAC evaluation of skin sensitisation

Summary of the Dossier Submitter's proposal

No human data are available to address the skin sensitisation potential of bifenox. The DS summarised a GPMT according to OECD TG 406 and GLP standards (Anonymous, 2001), and a Buehler assay (Anonymous, 1985; the same TG). Both tests gave negative results under the conditions employed.

Thus, the DS proposed no classification for Skin Sensitisation.

Comments received during consultation

One MSCA commented on this hazard class during the consultation. They supported no classification and listed a number of deviations from the test guideline for the reported Buehler assay.

Assessment and comparison with the classification criteria

No human data are available.

In a GLP compliant GPMT, 10 female guinea pigs received an intradermal induction concentration of 5 % bifenox in sesame oil that was irritating to the skin as evidenced by patchy erythema in all treated animals. A topical induction concentration of 30 % bifenox in sesame oil was also irritating, as required by the guideline. No skin irritation or sensitisation reactions were observed after topical challenge with 5 % bifenox in sesame oil while animals of a positive control group reacted to 2 % benzocaine. A series of previous sighting tests to determine concentrations used in the main study were mentioned in the study summary but no details (concentrations used, number of animals) were reported. During RAC CLH process, further details were provided on the positive controls and sighting tests. According to these, 100 % of animals showed a positive reaction to 2 % benzocaine in a positive control test series conducted in the year of the study. In the sighting test, 8 animals were treated with concentrations of 0.5, 1, 5, 10, 15 and 30 % bifenox. Patchy erythema was observed from concentrations of 10 % in animals that were depilated prior to treatment. Thus, 5 % was chosen as the highest

non-irritating substance concentration.

RAC notes that according to OECD TG 406, in case it cannot be concluded that a substance has sensitising properties, additional animals are recommended to be tested to give a total of 20 test and 10 control animals, which was not done in this case. However, RAC considers this a minor deviation.

The Buehler assay was performed which complied with GLP standards according to the study summary but was reported as non-conforming to GLP in the CLH report. Ten female guinea pigs were used in both the treatment and control groups. Treated animals received three topical induction applications with 50 % (w/v) bifenox in acetone and a challenge application after 2 weeks of rest with the same substance concentration. No skin reactions were reported at all. The study summary concluded that bifenox was negative for skin sensitising properties under the test conditions.

RAC reiterates the deviations from the guideline already posed by the commenting MSCA:

- both induction and challenge concentrations were the same although the guideline requires the induction concentration to be mildly irritative and the challenge concentration non-irritating
- only 10 instead of 20 animals were used
- no positive control or any skin reactions were described in the study summary.

Overall, RAC considers that the Buehler assay was not reliable.

Conclusion on classification

Based on the negative GPMT that was reliable with a minor deviation from the guideline, RAC concurs with the DS that **no classification for Skin Sensitisation is warranted**.

10.8 Germ cell mutagenicity

Table 22: Summary table of mutagenicity/genotoxicity tests *in vitro*

Method, guideline, deviations if any	**Test substance,**	**Relevant information about the study including rationale for dose selection (as applicable)**	**Observations**	**Reference**
Bacterial mutagenicity GLP / OECD 471, EC 440/2008 B. 13/14, EPA, OPPTS 870.5100, 712-C-98-247	Bifenox D-20140741 Purity: 98%	*S. typhimurium* (TA100, TA1535, TA98, TA1537 and TA1538), *E. coli* WP2 uvrA **Conditions:** Plate incorporation assay, with and without S9 mix 3.16-5000 µg/plate	Bifenox technical did not cause gene mutations by base pair changes or frameshifts in the genome of the tester strains used. Negative	Anonymus, 2015
Mammalian cell gene mutation GLP / OECD 476, EC 440/2008 B. 17, EPA, OPPTS 870.5300, 712-C-98-221	Bifenox Batch no. D-20140741 Purity: 98%	*V79 Chinese Hamster cells* HPRT locus assay **Conditions:** 0.25 - 250 µg/mL without S9 mix; 0.5 - 250 µg/mL with S9 mix	Bifenox technical did not cause gene mutations in the genome of V79 Chines Hamster cells.	Anonymus, 2016

Method, guideline, deviations if any	**Test substance,**	**Relevant information about the study including rationale for dose selection (as applicable)**	**Observations**	**Reference**
			Negative	
Chromosomal aberration GLP / OECD 473, EC 440/2008 B. 10, EPA, OPPTS 870.5375, 712-C-98-223	Bifenox D-20140741 Purity: 98%	*V79 Chinese Hamster cells* **Conditions:** Two experiments (4 and 21 h), 5-500 µg/mL with and without S9 mix	Bifenox technical did not induce structural chromosomal aberrations in the V79 Chinese hamster cell line Negative	Anonymus, 2016 (amended 2017)
Bacterial mutagenicity GLP / OECD 471	Bifenox Batch no. 10830 Purity: 99.1%	*S. typhimurium* (strain TA100, TA1535, TA98, TA1537, TA1538 and TA102) **Conditions:** Plate incorporation assay and a preincubation method at 3.16 to 316 µg/plate	Bifenox did not induce gene mutation towards *S. Typhimurium under the experimental condition* Negative	Anonymus, 2005a
Bacterial mutagenicity No GLP statement / assay was conducted according to standard procedures (Ames et al, 1975) on which OECD 471 is based / test is judged to be valid	Bifenox Batch no. not stated Purity: 99.5%	*S. typhimurium* (TA100, TA1535, TA98, TA1537 and TA1538), *E. coli* WP2 uvrA **Conditions:** Plate incorporation assay, with and without S9 mix 10-5000 µg/plate	Bifenox did not increase the reversion rate in the different *S. Typhimurium under the experimental condition* Negative	Anonymus, 1982
Chromosomal aberration No GLP statement / assay was conducted according to standard procedures on which OECD 473 is based / test is judged to be valid	Bifenox Lot no. 3123142024 Purity: 97%	CHO cells **Conditions:** 25 - 2510 µg/mL With and without S9 mix	Bifenox was tested at 25, 75, 250 and 750 µg/ml without S9mix for 8 hr. None of the concentrations induced aberration frequencies different from the negative control. Mitomycin induced a significant increase in aberration frequency. After 18 hr exposure, negative results were seen. With S9mix, bifenox was tested at 125, 250, 400, 1260 and 2510 µg/ml for 2 hr + 8 hr (growth period).	Anonymus, 1985

Method, guideline, deviations if any	**Test substance,**	**Relevant information about the study including rationale for dose selection (as applicable)**	**Observations**	**Reference**
			Very low mitotic index was reported at 1260 µg/ml. No chromosomal aberrations were observed. When the growth period was 17 hr, mitotic indexes at concentrations > 400 µg/ml were extremely low. No chromosomal aberrations were observed. Cyclophosphamide induced a significant increase in aberration frequencies. Negative	
Mammalian cell gene mutation GLP / OECD 476	Bifenox Batch no. 10830 Purity: 99.1%	Mouse lymphoma L5178Y cells ($tk^{+/-}$ system) **Conditions:** 19.53 to 312.5 µg/mL with and w/o S9 mix; in the second experiment w/o S9 mix concentrations ranging from 9.77 to 156.25 µg/mL were used.	bifenox was negative with respect to the mutant frequency in the LK5178Y TK+/- mammalian cell mutagenicity test Negative	Anonymus, 2005b
Mammalian cell gene mutation No GLP statement / assay was conducted according to standard procedures on which OECD 476 is based / test is judged to be valid	Bifenox MCTR-12-79 (MRI #248) Purity not stated	Mouse lymphoma L5178Y cells ($tk^{+/-}$ system) **Conditions:** w/o S9 mix: 133-1000 µg/mL, with S9 mix: 18 - 133 µg/mL	Bifenox did not induce mutation in the TK locus of L5178Y TK+/- cells when tested in the presence and absence of metabolic activation system Negative	Anonymus, 1979
Mammalian cell gene mutation GLP/assay was conducted according to standard procedures on which OECD 476 is based/test is judged to be valid	Bifenox Batch no. and purity not stated	CHO-cells (HGPRT system) **Conditions:** 50 - 500 µg/mL with S9 mix, 30 – 250 µg/mL without S9 mix	Bifenox were negative in the CHO/HGPRT mammalian cell forward gene mutation test Negative	Anonymus, 1983

Method, guideline, deviations if any	Test substance,	Relevant information about the study including rationale for dose selection (as applicable)	Observations	Reference
UDS assay GLP/assay was conducted according to standard procedures on which OECD 482 is based / test is judged to be valid	Bifenox Lot no. 16230 Purity not stated	Rat hepatocytes **Conditions:** 8 doses from 100 µg/mL to 0.5 µg/mL	Bifenox was considered to be inactive in the primary rat hepatocytes UDS assay Negative	Anonymus, 1981

Table 23: Summary table of mutagenicity/genotoxicity tests in mammalian somatic or germ cells in vivo

Method, guideline, deviations if any	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
Mouse micronucleus GLP / OECD 474	Bifenox Batch no. 20010903 Purity: 97.3%	Mouse bone marrow **Route of administration:** Oral gavage **Dose range tested** 500, 1000, 2000 mg/kg bw	No signs of systemic toxicity tested up to the highest reasonable dose of 2000 mg/kg bw showed no mutagenic properties Negative	Anonymus, 2003
Metaphase analysis No GLP statement / assay was conducted according to standard procedures on which OECD 475 is based / test is judged to be valid	Bifenox Lot no 16230 Purity 93.8%	Rat bone marrow **Route of administration:** Oral gavage, 5 days **Dose range tested** 500, 1000, 1500 mg/kg bw	Bifenox did not induce any remarkable pharmacological effects. Cytotoxicity was not observed although bifenox was detected in blood. No clastogenic activity was seen with bifenox. Severe cytotoxicity was observed with cyclophosphamide, which was clastogenic. Negative	Anonymus, 1981

There are no human data on mutagenicity.

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

Bifenox is structurally related to the genotoxic carcinogen nitrofen. The genotoxic activity of nitrofen is believed to be mediated by enzymatic reduction and activation of its nitro group to

highly reactive electrophilic intermediates, which can react with DNA-bases to form DNA adducts. However, it was shown that bifenox is not genotoxic although sharingmost of its structural features with nitrofen. The inactivity of bifenox may be explained by a steric interference of carboxyl-moiety in ortho position next to the nitro group with enzymes (acetyltransferases, sulfotransferases), which active the N hydroxylamine intermediate to highly reactive O-conjugates. Similar to bifenox, 3-nitro-2-naphtoic acid is not mutagenic, whereas its isomer 8-nitro-1-naphtoic acid or 2-nitronaphtalene is mutagenic.

A number of studies had been investigated in order to evaluate genotoxic effects of Bifenox in vitro or in vivo in somatic cells. There was no evidence or signs of genotoxic effects from the active substance, an in vivo study in germ cells has therefore been concluded not to be relevant in accordance with Regulation (EU) No. 283/2013.

Bifenox was concluded to be non-genotoxic.

10.8.2 Comparison with the CLP criteria

Category 1

There is no evidence or indication that Bifenox induces heritable mutations in germ cells of humans.

There are no epidemiological studies indicating that Bifenox induces heritable mutations in germ cells of humans.

There are no positive results from *in vivo* heritable cell mutagenicity tests or *in vivo* somatic cell mutagenicity tests in mammals. In fact these tests gave negative results.

Category 2

There is no concern for humans. Bifenox is not considered to induce heritable mutations in humans because:

No positive evidence was obtained from experiments in mammals.

No positive results were obtained from *in vitro* mammalian mutagenicity assays.

In fact, the negative *in vivo* results confirm the negative *in vitro* results. There is no evidence that Bifenox may cause genotoxic effects.

Under the conditions of the *in vivo* and *in vitro* assays that were conducted with Bifenox, no genotoxic potential from the substance was revealed. Bifenox is concluded to be non-genotoxic

10.8.3 Conclusion on classification and labelling for germ cell mutagenicity

Bifenox does not require labelling as mutagenic.

RAC evaluation of germ cell mutagenicity
Summary of the Dossier Submitter's proposal The DS evaluated several *in vitro* and *in vivo* studies to assess the genotoxicity of bifenox.

In vitro

The DS summarised three Ames tests performed on strains of *Salmonella typhimurium* and *Escherichia coli* with and without metabolic activation that were negative. A fourth Ames test was reported in the study summaries provided with the annex to the CLH report but was not assessed by the DS.

The four studies on mammalian cell gene mutation *in vitro* (one HPRT test on Chinese hamster V79 cells, one HPRT test on Chinese hamster ovary cells and two TK locus assays on Mouse lymphoma cells) did not show any mutagenic potential caused by bifenox. Two mammalian chromosome aberration tests (one on Chinese hamster V79 cells, one on Chinese hamster ovary cells) were negative and no chromosomal aberrations were observed but the test on Chinese hamster ovary cells showed a very low mitotic index at 1260 µg/mL. When the growth period was 17 h, mitotic indexes at concentrations > 400 µg/mL were extremely low.

In the *in vitro* UDS assay using rat hepatocytes, bifenox was considered to be inactive.

In vivo

Both *in vivo* tests (one micronucleus test with mouse bone marrow and one metaphase analysis with rat bone marrow) were negative. Cytotoxicity was not observed, although bifenox was detected in the blood. Bifenox showed no mutagenic properties and no clastogenic activity.

Furthermore, the DS stated that bifenox is structurally related to the genotoxic carcinogen nitrofen. The genotoxic activity of nitrofen is believed to be mediated by enzymatic reduction and activation of its nitro group to highly reactive electrophilic intermediates, which can react with DNA-bases to form DNA adducts. However, it was shown that bifenox is not genotoxic, despite sharing most of its structural features with nitrofen. The inactivity of bifenox may be explained by a steric interference of carboxyl-moiety in the *ortho* position next to the nitro group with enzymes (acetyltransferases, sulfotransferases), which activate the N-hydroxylamine intermediate to highly reactive O-conjugates. Similar to bifenox, 3-nitro-2-naphthoic acid is not mutagenic, whereas its isomer 8-nitro-1-naphthoic acid or 2-nitronaphthalene is mutagenic.

Based on the available *in vitro* and *in vivo* data on the genotoxicity of bifenox, the DS summarised that there was no evidence or signs of genotoxic effects from the active substance. An *in vivo* study in germ cells has therefore been concluded not to be relevant in accordance with Regulation (EU) No. 283/2013.

The DS concluded that no classification of bifenox for germ cell mutagenicity is warranted.

Comments received during consultation

One MSCA commented on this hazard class and stated that the available *in vivo* data should be considered supplementary only: In the mouse micronucleus study (2003), bone marrow exposure was not demonstrated (no change in the ratio of PNE/NCE, no signs of systemic toxicity even at the highest dose, no ADME data in mice). Furthermore, the statistical power of the result is limited because only a total of 2000 erythrocytes were counted for each animal, but 4000 are required by OECD TG 474 (2016). The second *in vivo* study (rat bone marrow chromosome aberration (1981)) is also not sufficiently reliable because of the low number (50) of metaphases analysed (at least 200 should be analysed according to OECD

TG 475 (2016)).

Regarding the available *in vitro* data, only two available studies should be considered reliable (Ames test (2015), chromosome aberration study (2016)). For the other *in vitro* studies, several deviations are identified, e.g.

- HPRT test (2016): According to OECD TG 476, the highest concentration tested should aim to achieve between 20 and 10 % relative survival (RS). This was not the case in the study (45 % RS without S9-mix, 74 % RS with S9-mix). Furthermore, results with S9-mix might indicate an increase in mutation frequency.
- The maximum test concentration of 5 mg/plate not reached due to precipitation in one Ames test (2005a). However, precipitation did not occur in any other Ames test. Negative results were not confirmed by a repeated experiment in two studies (1982; 1979).
- UDS assay (1981): the OECD test guideline 482 for the *in vitro* UDS assay was deleted in 2014 due to limited performance and is no longer recommended.

The DS argued that bone marrow exposure can be inferred in both sexes of rats and mice from at least the 13-week rat study (2500 mg/kg bw/day; 1982), and the 24-month mouse study (147-179 mg/kg bw/day; 1982). In particular, there were unusually high increases in reticulocytes in the 13-week rat study compared to the magnitude of other red blood cell disturbances and likewise an unusually large decrease in reticulocytes in the 24-month mouse study. The DS suggested that this would indicate perturbation of marrow function by the test item.

As regards the concern of reduced statistical power of the assay, the DS stated that although only 2000 erythrocytes/animal were counted, this does not impact the required sensitivity because animals of both sexes were used in the study and thus 4000 erythrocytes were counted in total.

Regarding the HPRT test (2016), the doses used were limited by test material solubility, with precipitates being observed in at least the top dose tested in each experiment (200 or 250 µg/mL and sometimes as low as 100 µg/mL). In these circumstances there is no requirement for the cytotoxicity condition to be met. The study has no limitations and is clearly negative.

Overall, the DS argued that the genotoxicity data are clearly "conclusive but not sufficient for classification".

Assessment and comparison with the classification criteria

Eleven studies have been evaluated which address the genotoxic effects of bifenox: nine *in vitro* studies (four Ames tests, four mammalian cell gene mutation tests and one UDS assay) and two *in vivo* studies (one mouse micronucleus assay and one metaphase analysis in rats). All available studies on genotoxicity/germ cell mutagenicity *in vitro* and *in vivo* are listed in the tables below.

***Table**: Summary table of genotoxicity/ germ cell mutagenicity tests in vitro (modified from Table 22 in CLH report)*

Method, guideline, deviations if any	Test substance (Batch No; purity)	Relevant information about the study including rationale for dose selection (as applicable)	Observations /Results	Reference
Bacterial Reverse Mutation Test (Ames test)				
Bacterial mutagenicity GLP / OECD 471, EC 440/2008 B. 13/14, EPA, OPPTS 870.5100, 712-C-98-247	bifenox D-20140741 Purity: 98%	*S. typhimurium* (TA100, TA1535, TA98, TA1537 and TA1538), *E. coli* WP2 *uvr*A Conditions: Plate incorporation assay, with and without S9 mix 3.16-5000 µg/plate	bifenox technical did not cause gene mutations by base pair changes or frameshifts in the genome of the tester strains used. Negative	Anonymous , 2015
Bacterial mutagenicity GLP / OECD 471	bifenox Batch no. 10830 Purity: 99.1%	*S. typhimurium* (strain TA100, TA1535, TA98, TA1537, TA1538 and TA102) Conditions: Plate incorporation assay and a preincubation method at 3.16 to 316 µg/plate	Bifenox did not induce gene mutation towards *S. typhimurium* under the experimental conditions. Negative	Anonymous , 2005a
Bacterial mutagenicity No GLP statement / assay was conducted according to standard procedures (Ames *et al.,* 1975) on which OECD 471 is based / test is judged to be valid	bifenox Batch no. not stated Purity: 99.5%	*S. typhimurium* (TA100, TA1535, TA98, TA1537 and TA1538), E. coli WP2 *uvr*A Conditions: Plate incorporation assay, with and without S9 mix 10-5000 µg/plate	Bifenox did not increase the reversion rate in the different *S. typhimurium* under the experimental conditions. Negative	Anonymous , 1982
Bacterial mutagenicity No GLP statement / plate incorporation assay was conducted according to standard procedures (Ames *et al.,* 1975) on which OECD 471 is based / test is judged to be valid	bifenox Batch no. MCTR-12-79 (MRI #248) Purity not stated	*S. typhimurium* (TA100, TA1535, TA98, TA1537 and TA1538) Conditions: Plate incorporation assay, with and without S9 mix 10000, 5000, 2500, 500 or 100 µg/plate	Bifenox did not increase the reversion rate in the different *S. typhimurium* strains under these experimental conditions. Negative	Anonymous , 1979
Mammalian Cell Gene Mutation Test				

Mammalian cell gene mutation GLP / OECD 476, EC 440/2008 B. 17, EPA, OPPTS 870.5300, 712-C-98-221	bifenox Batch no. D-20140741 Purity: 98%	V79 Chinese Hamster cells HPRT locus assay Conditions: 0.25 - 250 µg/mL without S9 mix; 0.5 - 250 µg/mL with S9 mix	Bifenox technical did not cause gene mutations in the genome of V79 Chines Hamster cells. Negative	Anonymous, 2016
Mammalian cell gene mutation GLP / OECD 476	bifenox Batch no. 10830 Purity: 99.1%	Mouse lymphoma L5178Y cells (tk+/- system) Conditions: 19.53 to 312.5 µg/mL with and w/o S9 mix; in the second experiment w/o S9 mix concentrations ranging from 9.77 to 156.25 µg/mL were used.	Bifenox was negative with respect to the mutant frequency in the LK5178Y TK+/- mammalian cell mutagenicity test. Negative	Anonymous, 2005b
Mammalian cell gene mutation No GLP statement / assay was conducted according to standard procedures on which OECD 476 is based / test is judged to be valid	bifenox MCTR-12-79 (MRI #248) Purity not stated	Mouse lymphoma L5178Y cells (tk+/- system) Conditions: w/o S9 mix: 133-1000 µg/mL, with S9 mix: 18 - 133 µg/mL	Bifenox did not induce mutation in the TK locus of L5178Y TK+/- cells when tested in the presence and absence of metabolic activation system. Negative	Anonymous, 1979
Mammalian cell gene mutation GLP/assay was conducted according to standard procedures on which OECD 476 is based/test is judged to be valid	bifenox Batch no. and purity not stated	CHO-cells (HGPRT system) Conditions: 50 - 500 µg/mL with S9 mix, 30 – 250 µg/mL without S9 mix	Results for bifenox were negative in the CHO/HGPRT mammalian cell forward gene mutation test. Negative	Anonymous, 1983
Mammalian Chromosome Aberrations Test				
Chromosomal aberration GLP / OECD 473, EC 440/2008 B. 10, EPA, OPPTS 870.5375, 712-C-98-223	bifenox D-20140741 Purity: 98%	V79 Chinese Hamster cells Conditions: Two experiments (4 and 21 h), 5-500 µg/mL with and without S9 mix	Bifenox technical did not induce structural chromosomal aberrations in the V79 Chinese hamster cell line. Negative	Anonymous, 2016 (amended 2017)

Chromosomal aberration No GLP statement / assay was conducted according to standard procedures on which OECD 473 is based / test is judged to be valid	bifenox Lot no. 3123142024 Purity: 97%	CHO cells Conditions: 25 - 2510 µg/mL With and without S9 mix	Bifenox was tested at 25, 75, 250 and 750 µg/mL without S9 mix for 8 hr. None of the concentrations induced aberration frequencies different from the negative control. Mitomycin induced a significant increase in aberration frequency. After 18 hr exposure, negative results were seen. With S9 mix, bifenox was tested at 125, 250, 400, 1260 and 2510 µg/mL for 2 hr + 8 hr (growth period). Very low mitotic index was reported at 1260 µg/mL. No chromosomal aberrations were observed. When the growth period was 17 hr, mitotic indexes at concentrations > 400 µg/mL were extremely low. No chromosomal aberrations were observed. Cyclophosphamide induced a significant increase in aberration frequencies. Negative	Anonymous, 1985
Unscheduled DNA Synthesis Assay				
UDS assay GLP/assay was conducted according to standard procedures on which OECD 482 is based / test is judged to be valid	bifenox Lot no. 16230 Purity not stated	Rat hepatocytes Conditions: 8 doses from 100 µg/mL to 0.5 µg/mL	Bifenox was considered to be inactive in the primary rat hepatocytes UDS assay. Negative	Anonymous, 1981

***Table**: Summary table of genotoxicity/ mutagenicity tests in mammalian somatic or germ cells in vivo (modified from Table 23 in CLH report)*

Method, guideline, deviations if any	Test substance	Relevant information about the study (as applicable)	Observations/Results	Reference
Micronucleus Test				
Mouse micronucleus GLP / OECD 474	bifenox Batch no. 20010903 Purity: 97.3%	Mouse bone marrow Route of administration: Oral gavage Dose range tested 500, 1000, 2000 mg/kg bw	No signs of systemic toxicity up to the highest reasonable dose of 2000 mg/kg bw and no mutagenic responses Negative	Anonymous, 2003
Bone Marrow Cytogenetic Test – Chromosomal Analysis				

Metaphase analysis No GLP statement / assay was conducted according to standard procedures on which OECD 475 is based	bifenox Lot no 16230 Purity 93.8%	Rat bone marrow Route of administration: Oral gavage, 5 days Dose range tested 500, 1000, 1500 mg/kg bw	Bifenox did not induce any remarkable pharmacological effects. Cytotoxicity was not observed although bifenox was detected in blood. No clastogenic activity was seen with bifenox. Severe cytotoxicity was observed with cyclophosphamide, which was clastogenic. Negative	Anonymous, 1981

In vitro

Bifenox did not cause gene mutations in the genome of *Salmonella typhimurium* and *Escherichia coli* with and without metabolic activation in the Ames test from 2015. A further Ames test (Anonymous, 2005a) performed on *S*. *typhimurium* strains was also negative, but the maximum test concentration of 5 mg/plate was not reached due to precipitation. The precipitation was not observed in any other Ames test. In the bacterial mutagenicity assays in 1982 and 1979, bifenox was considered to be non-mutagenic. However, both assays have several limitations. In the Ames test from 1982, phenotypic characteristics of strains were not checked, the study was not repeated, plates were duplicated (triplicates are required by the guideline), the negative control was absent, and the preparation of S9 was not reported. The Ames test from 1979 had limitations due to absence of study replication, no reported standard deviations and not tested TA 102 and *E.coli* strains. Overall, one guideline compliant negative Ames test was available for bifenox.

The study on mammalian cell gene mutation in Chinese hamster V79 cells (2016) showed no mutagenic potential of bifenox. Furthermore, the mouse lymphoma asays (Anonymous, 2005b and 1979) with and without metabolic activation were also negative, but the study of 1979 has several minor limitations: the absence of mycoplasma was not verified, the colony sizing was not performed, and the study was not repeated. In a further CHO/HGPRT mammalian cell forward gene mutation assay from 1983, bifenox did not cause gene mutations in the genome of Chinese hamster ovary cells. However, the experiment was not repeated but all treatment groups were tested in duplicate. Moreover, the absence of mycoplasma was not verified and the origin of S9 was not given. Overall, there were two guideline compliant mammalian cell gene mutation assays (one HGPRT test, and one MLA $TK^{+/-}$ test) that were both negative.

In the *in vitro* mammalian chromosomal aberration assay in Chinese hamster V79 cells in 2016, bifenox did not induce structural chromosomal aberrations and was considered to be non-clastogenic. This result is supported by the negative mammalian chromosomal aberration assay in Chinese hamster ovary cells (Anonymous, 1985). No chromosomal aberrations were observed. However, it should be mentioned that bifenox showed a very low mitotic index at 1260 µg/mL. When the growth period was 17 h, the mitotic indexes at concentrations > 400 µg/mL were extremely low. The study had further deviations from the test guideline: the assay was not repeated, only percentage of abnormal cells excluding gaps were reported, only 15 metaphases/culture were examined for positive control, the exposure time with S9 was too short (2 h instead of 3-6 hours) and the harvest was at 8/10 h and 18/19 h while the guideline requires harvest after 1.5 normal cell cycles (cell cycle length for CHO cells is approx. 24 hours).

In the primary rat hepatocyte unscheduled DNA synthesis assay (Anonymous, 1981), bifenox

was considered to be inactive, but the experiment was not repeated. Furthermore, it should be mentioned that the study was performed according to the OECD test guideline 482, which was deleted in 2014 due to limited performance and is no longer recommended.

Thus, one negative guideline compliant reliable chromosomal aberration test for bifenox was available.

In vivo

In the mouse micronucleus test from 2003, bifenox showed no mutagenic properties up to the highest reasonable dose of 2000 mg/kg bw at the sampling times of 24 and 48 hr. However, bone marrow exposure was not shown and the statistical power of the result is limited due to the reduced count of 2000 erythrocytes for each animal. OECD TG 474 (2016) requires 4000 erythrocytes/animal.

In the metaphase analysis from 1981, bifenox did not significantly increase clastogenic events in rats treated for 5 consecutive days up to the highest dose tested. Cytotoxicity was not observed although bifenox was detected in blood. But the study reliability is limited: only 50 metaphases/rat instead of 200 were examined, the animals were treated for 5 days instead of a single dose as preferred by OECD TG 475, the mitotic index was not measured, and the samples were taken 6 hours instead of 12 to 18 hours after latest dose. Moreover, bone marrow exposure was not demonstrated.

Conclusion on classification

There are no human epidemiological data available for bifenox. In one publication from the open literature presented in the annex to the CLH report, a product containing 45 % bifenox induced statistically significantly decreased mitotic indices and a delay in cell cycle in lymphocytes of two healthy donors. However, the study population was too small to draw firm conclusions for classification purposes. The animal studies did not show any indication that bifenox could induce heritable mutations in the germ cells of humans. None of the *in vitro* tests showed a mutagenic or clastogenic potential for bifenox.

However, as was elaborated by the MSCA during consultation, several tests had limitations to their reliability. Nevertheless, one Ames test, two mammalian cell gene mutation assays, and one *in vitro* chromosomal aberration test were guideline compliant and gave consistent negative results.

Regarding the *in vivo* assays, RAC considers the changes in reticulocyte counts in rat and mice (sub-)chronic toxicity studies indicate a possible bone marrow disturbance but are not sufficient to conclude that the bone marrow was indeed exposed in the *in vivo* mutagenicity studies. Exposure times are considerably shorter in the latter and no systemic toxicity was reported in either study. However, the concern for bifenox is lowered by several reliable negative *in vitro* assays.

The criteria for classification for mutagenicity were not met on the basis of the available data. Therefore, RAC supports the DS' proposal that **no classification for germ cell mutagenicity is warranted**.

10.9 Carcinogenicity

Table 24: 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**
Oral toxicity/ carcinogenicity, 104 weeks, Rat GLP / not fully compliant to OECD 453 (MTD not reached)	Bifenox Batch, # 353-12-1 purity 98% 50 Charles River Sprague Dawley CD 500, 1580, 5000 ppm corresponding to Males: 18.9, 59, 188 mg/kg bw/d Females 24.6, 77, 252 mg/kg bw/d	No significant adverse effects Not carcinogenic NOEL: 252 mg/kg bw/day (top dose tested)	Anonymus, 1987
Carcinogenicity, 24 months, Mice GLP / not fully compliant to OECD 451 (MTD not reached)	Bifenox Batch # 16230, purity 98.3%, Males: 7, 30, 147 mg/kg bw/d Females: 9, 35, 179 mg/kg bw/d	Small effects on haematological parameters at highest dose level Not carcinogenic NOEL: 30 mg/kg bw/day	Anonymus, 1982

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

In order to identify adverse parameters after chronic exposure towards the active substance Bifenox on target organs or dose-response relationships, long-term studies conducted in rats or mice were re-evaluated. Those studies already were submitted in the context of the inclusion of the active substance Bifenox in Annex I of the Council Directive 91/414/EEC.

Long-term exposure in rats or mice did not reveal significant substance related effects up to the top dose levels of 252 mg/kg bw/day for rats. For mice, the NOAEL was set at 30 mg/kg bw/day, based on small effects on haematological parameters.

The endpoints were discussed in the conclusion on the peer review of Bifenox by the EFSA and were corrected to original study results. Effects that were seen in rats at 252 mg/kg bw/day were considered not to be relevant. Initially the NOAEL was determined to be 59 mg/kg bw/day, based on reduced body weight gain in 6% of male and female rats. However, this effect was statistically not significant.

Target/critical effect: Reduced platelets and reticulocytes at terminal sacrifice (mice). Reduced body weight gain and decreased food consumption (rat).

Lowest relevant NOAEL: 30 mg/kg bw/day, 2-year mice, 252 mg/kg bw/day, 2-year rats

Carcinogenicity: Not carcinogenic in rat or mice up to the highest dose tested

Bifenox is not carcinogenic. This conclusion is supported by the absence of genotoxic activity of Bifenox, *tested in vivo* and *in vitro*. The results of the long-term toxicity studies are summarized in Table 24.

Rats

In a 104 weeks study in rats, reduced body weight gain and decreased food consumptions was noted, however, those effects were not significant. Islet cell adenoma and/or adenocarcinoma of the pancreas were observed for male rats at the low and intermediate dose reaching statistical significance when compared with control. However, there was no evidence of a trend across the treatment groups. The low dose for females was found to have significantly more tumours than the controls, but again no trend across the treatment groups was apparent. Comparison with data from open literature indicates that for this common type of tumour, the marginally significant increase at low and intermediate dose in male rats results probably from a random occurrence of a low concurrent control rate.

Tumours of the pancreas were not directly responsible for animal deaths. Islet cell adenoma and/or adenocarcinoma of the pancreas were observed for male rats at the low and intermediate dose reaching statistical significance when compared with control. However, there was no evidence of a trend across the treatment groups. The low dose for females was found to have significantly more tumours than the controls, but again no trend across the treatment groups was apparent.

No statistical significance was found in the incidence of malignant islet cell tumours in any group of male or female rats when compared to control, nor was there any significant trend in malignant islet cell tumour incidence with increasing dosage. A statistical significance was found in a pair wise comparison for combined benign and malignant islet cell in male ($p = 0.05$) and female ($p = 0.03$) rats receiving 500 ppm and in male rats ($p = 0.04$) receiving 1580 ppm, but the dose response trend between control and exposure groups indicated a lack of statistical significance.

When comparing the combined incidence of tumours in the study with the historical control data based on adenoma and adenocarcinoma incidences provided by the laboratory (background data from the study report), the results at 500 and 1580 ppm are outside the concurrent historical control data. However, according to the open literature the incidence of islet cell adenomas is within 1.67-25.71% and 1.43-14.29% for male rats and female rats (CD Sprague Dawley rats). For carcinoma the incidence is 0.77-14% and 0.77-4.29% for male and female rats respectively. Islet cell tumours subclassified as adenoma and adenocarcinomas, increase in incidence with age and are more frequently observed in males than in females.

Background data from the study report on islet cell tumours in CD Sprague Dawley rats was evaluated as demonstrated in Table 24.1.

Table 24.1 Incidence of islet cell tumors in background data from CD Sprague Dawley rats

Study number		**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Number of islet cell tumors	Male	12	14	10	2	13	7	19	7
	Female	5	4	5	4	3	3	7	3
Number of pancreas examined	Male	100	100	50	49	100	50	100	50
	Female	100	99	50	50	100	50	100	50

Table 24.2: Further details for the incidence of islet cell tumors in background data from CD Sprague Dawley rats reported in Anonymus(1987) for the same test laboratory and strain from 1980 to 1982. See table below for data relating 1982 to 1984

Study number	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**			
Study start	Feb-80	Apr-80	Sep-80	Jun-80	Apr-81	Feb-81	Jul-81	Jan-82			
Animal supplier	crusa	crusa	crusa	crusa	crusa	crusa	crusa	crusa		**% range**	
Study duration	115	115	105	108	104	104	105	104	**mean**	**Min**	**Max**
	Males										
Number of animals	100	100	50	50	100	50	100	50			
Number examined	100	96	50	49	100	50	97	50			
Islet cell tumours incidence	12	14	10	2	13	7	19	7			
%	12.0	14.6	20.0	4.1	13.0	14.0	19.6	14.0	**14.19**	**4.1**	**20.0**
	Females										
Number of animals	100	100	50	50	100	50	100	50			
Number examined	100	99	50	50	100	50	100	50			
Islet cell tumours incidence	5	4	5	4	3	2	7	3			
%	5.0	4.0	10.0	8.0	3.0	4.0	7.0	6.0	**5.51**	**3.0**	**10.0**

crusa = Charles River USA

Table 24.3: Incidence of islet cell tumors in background data from CD Sprague Dawley rats for the same test laboratory and strain for the period 1982 to 1984

Study number	**8**	**n/a**	**n/a**	**n/a**	**n/a**	**n/a**	**n/a**	**n/a**	**n/a**	**n/a**	**n/a**	**n/a**	**n/a**	**n/a**	**n/a**	**n/a**	**n/a**			
Code number	cdr8201	cdr8203	cdr8207	cdr8207b	cdr8210a	cdr8210b	cdr8301	cdr8304	cdr8305	cdr8307	cdr8311	cdr8312	cdr8406	cdr8409	cdr8409	cdr8410a	cdr8410b			
Study start	Jan-82	Mar-82	Jul-82	Jul-82	Oct-82	Oct-82	Jan-83	Apr-83	May-83	Jul-83	Nov-83	Dec-83	Jun-84	Sep-84	Sep-84	Oct-84	Oct-84			
Animal supplier	crusa	crusa	crusa	crusa	crusa	crusa	crusa	crusa	crusa	crusa	crusa	crusa	crusa	crusa	crusa	crusa	crusa		**% range**	
Study duration	104	111	104	104	110	106	105	105	110	106	106	106	106	106	106	106	106	**Mean**	**Min**	**Max**
	Males																			
Number of animals	50	105	60	50	50	50	50	55	50	50	50	55	100	50	50	50	55			
Number examined	50	104	60	50	50	50	49	55	50	49	50	55	100	50	50	50	55			
Islet cell tumours incidence	7	17	9	8	7	6	6	10	10	9	8	11	22	7	15	15	12			
%	14.0	16.3	15.0	16.0	14.0	12.0	12.2	18.2	20.0	18.4	16.0	20.0	22.0	14.0	30.0	30.0	21.8	**18.2**	**12**	**30**
	Females																			
Number of animals	50	105	61	50	50	50	50	55	50	50	50	55	100	50	50	50	55			
Number examined	50	105	61	50	50	49	50	55	50	50	50	55	100	50	50	50	55			
Islet cell tumours incidence	3	7	5	4	2	1	5	1	11	4	5	13	7	6	5	8	1			
%	6.0	6.7	8.2	8.0	4.0	2.0	10.0	1.8	22.0	8.0	10.0	23.6	7.0	12.0	10.0	16.0	1.8	**16**	**1.8**	**23.6**

crusa = Charles River USA

Data from open literature

Data from open literature examined for spontaneous pancreas tumour occurrence in rats

Islet cell tumours (endocrine tumours), subclassified as adenoma and adenocarcinomas, increase in incidence with age and are more frequently observed in males than females. The incidence of spontaneous tumours has been reported by different authors. The reported incidence of Islet cell tumours of rats has ranged from 0 to 17.6% in two small series of 71 male Sprague Dawley Hap and 108 females Sprague Dawley CD rats. The histological type of tumours of the Islet cell of the pancreas in rats is similar from that in humans, but in rats, the spontaneous incidence of Islet cell tumours is higher than that of exocrine tumours (Longnecker and Millar, 1990).

A survey of the incidence of spontaneous pancreatic tumours in CD rats from two-year carcinogenicity studies over a 15-year period was carried out. The survey revealed Islet cell adenomas to be the most common of pancreatic tumours with a higher incidence in untreated males (11.7% in comparison to females 5.5%) (Majeed, 1997).

A more recent compilation of spontaneous neoplastic lesions in Crl:CD (SD) BR rats from control groups (Charles River laboratories), in a total number of 1531 pancreases from male rats, 106 has Islet cell adenoma and 47 had carcinoma. In 1729 pancreases of female rats, 59 had Islet cell adenoma and 19 carcinoma. In this study minimum and maximum percent found were 1.67-25.71% and 1.43-14.29% for Islet cell adenoma in male and female rats, and 0.77-14% and 0.77-4.29% for carcinoma in male and female rats respectively (Giknis and Clifford, 2001).

In 2001, a study compared the effects of ad libitum (AL) overfeeding and moderate or marked dietary restriction (DR) on age related degenerative and proliferative changes of the endocrine pancreas in Sprague Dawley rats. In AL-fed rats, early changes in the islet morphology occurred, which resulted in a high incidence of islet fibrosis, focal hyperplasia and adenomas by two years. Compared to AL-fed rats, DR-fed rats had smaller pancreas, smaller pancreatic islets, smaller insulin secreting cell volumes, a lower degree of Islet fibrosis and a lower Islet cell BrdU labelling index, which correlated with a lower incidence of Islet adenoma and carcinoma at study termination. Moderate and marked degrees of DR delayed the onset and severity of Islet hyperplasia and fibrosis in a temporal and dose related manner (Molon-Noblot et al, 2001).

Table 24.4: Incidence of islet cell tumors in CD Sprague Dawley rats from open literature

Reference		**Gikins and Clifford, 2001[3]**	**Majeed, 1997[4]**	**Longnecker and Millar, 1990[5]**
Species / Strain		Crl:CD Sprague Dawley	CD Sprague Dawley	CD Sprague Dawley
Incidence Islet tumours	Male	Adenocarcinoma: 0.8-14% Adenoma: 1.7-25.7%	11.7%	0-17%
	Females	Adenocarcinoma: 0.8-4.3% Adenoma: 1.4-14.3%	5.5%	

[3] Gikins, M.L.A., Clifford, C.B., (2001) Compilation of Spontaneous Neoplastic Lesions And Survival in Crl:CD (SD) BR Rats From Control Groups, Charles River Laboratories

[4] Majeed, S.K., (1997) Studies of the incidence of spontaneous pancreatic tumors in ageing CD rats, Arzeneimittel-Forschung, 47 (7), 879-884

[5] Longnecker, D.S. and Millar, P.M., (1990) Pathology of tumours in laboratory animals. Vol I. Tumors of the rat, IARC scientific publications (99)

Table 24.5: Elaboration of the public domain historical control data cited in connection with Ruckman et al., 1987.

Reference		**Lang, 1992** elaborated on instead of Gifkins and Clifford, (2001)*	**Majeed, 1997**	**Longnecker and Millar, 1990**
Strain		Crl:CD Sprague Dawley, bred at Portland Michig Source Charles River Breeding Laboratories, Portage, Michigan, USA.	CD Sprague Dawley, (Charles River UK Ltd., Margate, Kent, UK)	CD Sprague Dawley ***Crl: COBS(r) CR(r) SD.*** These rats have been produced continuously at the Charles River Breeding Laboratories (CRBL) Wilmington, Massachusetts since 1955
Incidence Islet tumours	Male	Adenocarcinoma: 1.6-8.2% Adenoma: 2.9 -24.0%	11.7%	The reported incidence of islet cell tumours of rat has ranged from 0 to 17.6% in two small series of 71 male Sprague-Dawley Hap and 108 female
	Females	Adenocarcinoma: 1.4-8.2% Adenoma: 1.4-8.6%	5.5%	
Reference		Lang PL (1992) Spontaneous Neoplastic Lesions and Selected Non-neoplastic Lesions in the Crl:CD®BR Rat Published by Charles River Laboratories (February 1992)	Majeed, S. K. (1997). Studies of the incidence of spontaneous pancreatic tumours in ageing CD rats. Arzneimittel-forschung, 47(7), 879-884	Longnecker DS and Millar PM. (1994). Pathology of Tumours in Laboratory Animals. Vol. I. Tumours of the Rat. pp241-257 IARC Sci. Publ. No. 99, Publ. Lyon 1990 citing Anver MR, Cohen BJ, Lattuada CP, Foster SJ (1982) Age associated lesion in barrier-reared male Sprague-Dawley rate: A comparison between Hap:(SD) and Crl: COBS(r) CR(r) SD stocks. Exp. Agric. Res., 8(1), 3-22
Period covered		Studies with Start dates ranging from Dec 1985 to Feb 1989	1970 - 1995	Assumed to be 1980-1982.

***Although Gifkins and Clifford, (2001) was cited, Lang, 1992 also published by Charles River Laboratories, is more temporally relevant.**

For this common type of tumour, the marginally significant increases at 500 and 1580 ppm in male rats results from a random occurrence of a low concurrent control rats.

For mammary adenocarcinoma, there was no significant trend in tumour incidence with increasing dose. The intermediate dose group however, showed a slight increase from the controls, but this difference did not attain statistical significance ($p = 0.08$). No significant treatment effects were found when the combined category of any mammary tumour was considered.

A NOAEL of 5000 ppm = 252 mg/kg bw/day in rats was set, because the effects were statistically not significant or lacked dose response.

Table 24.6 Data from Lang, P. L. (1992). Spontaneous neoplastic lesions and selected non-neoplastic lesions in the Crl: CD® BR rat. *Charles River Laboratories.*[6]

TABLE 5a (Continued)
NEOPLASMS
24 MONTH STUDIES
MALE CD® RATS

	# groups in which	total if	percent	# groups using	minimum	maximum
LOCATION & TUMOR	organ examined	lesions	of total	this diagnosis	% found	% found
LIVER	19					
nodular hepatocellular proliferation		9	0.72	2	8.0	10.2
hepatocellular adenoma		53	4.21	18	1.3	18.2
hepatocellular carcinoma		33	2.62	12	1.1	9.1
cholangioma		1	0.08	1	-	1.4
cholangiocellular carcinoma		2	0.16	2	1.0	2.0
carcinosarcoma		1	0.08	1	-	2.0
PANCREAS (EXOCRINE)	19					
acinar cell adenoma		7	0.56	7	1.3	2.0
sarcoma (NOS)		1	0.08	1	-	1.8
URINARY SYSTEM						
KIDNEY	19					
renal cell adenoma		3	0.24	3	1.4	2.1
renal adenocarcinoma		4	0.32	4	1.0	2.0
transitional cell carcinoma		2	0.16	2	1.4	2.0
hemangiosarcoma		1	0.08	1	-	2.1
lipoma		1	0.08	1	-	1.3
liposarcoma		1	0.08	1	-	2.1
lipomatous tumour (M)		1	0.08	1	-	1.0
mixed cell tumor (M)		3	0.24	2	2.0	3.0
mixed mesenchymal tumor (NOS)		1	0.08	1	-	1.4
URINARY BLADDER	19					
transitional cell papilloma		1	0.08	1	-	1.0
transitional cell carcinoma		3	0.24	3	1.4	1.5
mesothelioma		1	0.08	1	-	1.0
REPRODUCTIVE SYSTEM						
TESTIS	19					
interstitial (leydig) cell tumor (B)		59	4.68	18	1.4	10.0
interstitial cell tumor (M)		1	0.08	1	-	1.4
mesothelioma (M)		2	0.16	2	1.0	1.4
PROSTATE	19					
carcinoma (M)		3	0.24	3	1.0	1.8
lipoma		1	0.08	1	-	1.4
mesothelioma (M)		1	0.08	1	-	1.0
ENDOCRINE SYSTEM						
PANCREAS (ENDOCRINE)	19					
islet cell adenoma		103	8.29	17	2.9	24.0
islet cell carcinoma		25	2.01	10	1.6	8.2
mesothelioma		1	0.08	1	-	1.0
PITUITARY GLAND	19					
adenoma, pars intermedia		4	0.32	2	1.0	4.9
adenoma, pars distalis		750	60.68	19	37.1	81.3
carcinoma,						
pars distalis		79	6.39	10	1.0	33.3
craniopharyngioma		1	0.08	1	-	1.9
hemangioma		1	0.08	1	-	1.9

6 https://www.criver.com/sites/default/files/Technical%20Resources/Spontaneous%20Neoplastic%20Lesions%20and%20Selected%20Non-neoplastic%20Lesions%20in%20the%20Crl-CD%20BR%20Rat%20-%20February%201992.pdf

TABLE 5b (Continued)
NEOPLASMS
24 MONTH STUDIES
FEMALE CD® RATS

LOCATION & TUMOR	# groups in which tissue examined	total X lesions	percent of total	k groups using this diagnosis	minimum % found	maximum % found
URINARY BLADDER	19					
polyp		1	0.08	1	--	1.4
transitional cell papilloma		1	0.08	1	--	1.4
transitional cell carcinoma		1	0.08	1	--	1.4
REPRODUCTIVE SYSTEM						
UTERUS/CERVIX	19					
adenocarcinoma (M)		4	0.32	4	1.0	1.4
endometrial stromal						
polyp		51	4.05	15	1.1	10.0
fibroma		1	0.08	1	--	1.4
leiomyoma		3	0.24	1	--	5.5
endometrial stromal sarcoma		3	0.24	3	1.4	1.6
leiomyosarcoma		2	0.16	1	--	3.6
hemangiosarcoma		1	0.08	1	--	1.4
sarcoma (NOS)		1	0.08	1	--	1.4
fibroma, cervix		1	0.08	1	--	1.4
leiomyosarcoma, cervix		1	0.08	1	-	1.4
squamous cell carcinoma, vagina/cervix		2	0.16	2	1.4	1.6
squamous cell carcinoma, vagina		4	0.32	3	1.4	2.9
stromal polyp, vagina		3	0.24	3	1.4	1.6
fibroma, vagina		4	0.32	4	1.4	2.0
hemangioma, vagina		1	0.08	1	--	1.4
OVARY	19					
granulosa/theca cell tumor		13	1.04	9	1.4	3.2
papillary adenoma		1	0.08	1	1.4	1.4
tubular adenoma		1	0.08	1	1.0	1.0
sex cord stromal tumor (B)		3	0.24	3	1.0	2.0
ENDOCRINE SYSTEM						
PANCREAS (ENDOCRINE)	19					
islet cell adenoma		48	3.82	17	1.4	8.6
islet cell carcinoma		18	1.43	8	1.4	8.2
PITUITARY GLAND	19					
microadenoma, pars intermedia		1	0.08	1	--	1.0
adenoma, pars distalis		902	72.10	19	31.4	88.8
carcinoma, pars distalis		131	10.47	14	1.3	57.1
THYROID GLAND	19					
follicular cell adenoma		32	2.58	15	1.0	14.5
follicular cell carcinoma		13	1.05	9	1.0	5.8
C-cell adenoma		91	7.33	19	1.0	17.1
medullary carcinoma		42	3.38	11	2.1	13.1
PARATHYROID GLAND	19					
adenoma (B)		6	0.53	5	1.6	4.0

Data from Majeed, S. K. (1997). Studies of the incidence of spontaneous pancreatic tumours in ageing CD rats. Arzneimittel-forschung, 47(7), 879-884.

The Majeed (1997) paper included data from 33618 Sprague-Dawley CD rats (soured from Charles River UK Ltd., Margate, Kent, UK) from studies of up to 104 weeks duration. All rats in this survey were fed similar diet and kept under similar housing conditions. Among these were 4655 untreated males and 4385 untreated females. In addition, data from 15709 rats from 13 week studies, 7627 rats from 26 week studies, 2899 rats from 52 week studies and 1533 rats from 78 week studies were also included. All studies were performed during the period 1970-1995. The data from untreated and treated animals are included for comparative purposes as none of the studies included showed any evidence of a treatment effect on the pancreas. Sections of visceral organs, bone marrow, brain, spinal cord and peripheral nerve were routinely prepared from all rats and stained with haematoxylin end eosin. A small proportion of islet cell tumours were also stained with strept-Avidin Biotin Complex, as anti-insulin stain for beta cells.

Table 24.7: Incidence of pancreatic tumours in <u>untreated</u> CD rats up to 78 weeks of age:

Type of tumour	Male	Female
Islet cell adenoma	8 (3%)	-
Islet cell carcinoma	-	-
Exocrine adenoma	:	-
Exocrine carcinoma	-	
Mixed Islet-acinar cell adenoma	-	-
Multiple Islet cell adenoma	-	-
Number of rats examined	277	283

Table 24.8: Incidence of pancreatic tumours in <u>treated</u> CD rats up to 78 weeks of age:

Type of tumour	Male	Female
Islet cell adenoma	-	4 (1%)
Islet cell carcinoma	3 (0.6%)	-
Exocrine adenoma	2 (0.4%)	-
Exocrine carcinoma	1 (0.2%)	-
Mixed Islet-acinar cell adenoma	-	-
Multiple Islet cell adenoma	-	-
Number of rats examined	489	488

Table 24.9: Incidence of pancreatic tumours in <u>untreated</u> CD rats up to 104 weeks of age:

Type of tumour	Male	Female
Islet cell adenoma	543 (11.66%)	239 (5.45%)
Islet cell carcinoma	112 (2.40%)	46 (1.040%)
Exocrine adenoma	94 (2%)	20 (0.45%)
Exocrine carcinoma	4 (0.08%)	1 (0.02%)
Mixed Islet-acinar cell adenoma	4 (0.08%)	2 (0.04%)
Multiple Islet cell adenoma	20 (0.4%)	-

Number of rats examined	4655	4385

Table 24.10: Incidence of pancreatic tumours in <u>treated</u> CD rats up to 104 weeks of age:

Type of tumour	Male	Female
Islet cell adenoma	1457 (11.45%)	442 (3.71%)
Islet cell carcinoma	293 (2.30%)	141 (1.18%)
Exocrine adenoma	275 (2.15%)	42 (0.35%)
Exocrine carcinoma	53 (0.41%)	11 (0.09%)
Mixed Islet-acinar cell adenoma	22 (0.2%)	6 (0.05%)
Multiple Islet cell adenoma	4 (0.03%)	16 (0.09%)
Number of rats examined	12718	11860

Table 24.11: Data from Anonymus (1982) Age associated lesion in barrier-reared male Sprague-Dawley rate: A comparison between Hap:(SD) and Crl: COBS(r) CR(r) SD stocks. *Exp. Agric. Res.*, 8(1), 3-22

AGE-ASSOCIATED LESIONS IN RATS 7

Table 1 Continued

	Age (Months)										
	6-11	12-17		18-23		24-29			30-38	Incidence	
Type of Neoplasm	HAP	CD	HAP	CD	HAP	CD	HAP	Sig.	CD	CD	HAP
ALIMENTARY SYSTEM											
Liver:											
Neoplastic nodule	0/13 (0)	0/20 (0)	0/13 (0)	0/12 (0)	0/10 (0)	2/27 (7.4)	0/34 (0)	–	2/45 (4.4)	4/104 (3.8)	0/70 (0)
Hepatocellular carcinoma	0/13 (0)	0/20 (0)	0/13 (0)	0/12 (0)	0/10 (0)	2/27 (7.4)	1/34 (2.9)	–	1/45 (2.2)	3/104 (2.9)	1/70 (1.4)
Hemangioma	0/13 (0)	0/20 (0)	0/13 (0)	0/12 (0)	0/10 (0)	0/27 (0)	1/34 (2.9)	–	1/45 (2.2)	1/104 (1.0)	1/70 (1.4)
Capillary hemangiosarcoma	0/13 (0)	0/20 (0)	0/13 (0)	0/12 (0)	0/10 (0)	0/27 (0)	1/34 (2.9)	–	0/45 (0)	0/104 (0)	1/70 (1.4)
Pancreas:											
Islet cell tumor	0/13 (0)	1/21 (4.8)	0/13 (0)	0/12 (0)	0/11 (0)	9/32 (28.1)	0/34 (0)	**	9/43 (20.9)	19/108 (17.6)	0/71 (0)
Mouth:											

Mice

In a 24 months study in mice, the MTD was not reached. No clear toxic effects were demonstrated on mortality, clinical signs, body weight, food consumption or haematology through the course of the study. Slight changes on haematological parameters (Leucocyte and RBC count) and slightly increased liver and kidney weight were observed at the top dose level.

Long-term oral exposure of Bifenox did not reveal any statistically significant substance related effects. Based on small effects on haematological parameters in mice at terminal sacrifice, the lowest relevant NOAEL was determined to be 30 mg/kg bw/day.

Islet cell adenoma or carcinoma were observed in the pancreas of male rats (Anonymus, 1987) at lower dose levels. Comparing these results to historical data of data from open literature, it was concluded that statistical significant results were due to a random occurrence of a low concurrent control group and related to the treatment with Bifenox.

There was no evidence of carcinogenic potential from Bifenox. Therefore, Bifenox is considered not to be carcinogenic. This conclusion is supported by the absence of genotoxic activity of Bifenox, tested *in vivo* and *in vitro*.

Based on the data derived from these studies, Bifenox is not carcinogenic.

Hepatic neoplasms were diagnosed as carcinoma and adenomas and were encountered more frequently at 24 months in top dose group male mice (combined hepatocellular adenoma and carcinoma 31.57%), but this incidence was not unusually high for mice of this age and strain. Statistical analyses were performed which examined incidence of hepatocellular carcinoma, adenoma and carcinoma or adenoma in each sex separately. Of the tests employed, none indicated statistical significance at the $p < 0.05$ levels in males. In females the trend test was weakly positive when hepatocellular carcinomas alone were examined or when carcinomas were combined with adenomas ($p = 0.45$ and $p = 0.41$). Because of the small numbers of tumours involved, this finding is considered to represent a statistical aberration rather than evidence for oncogenicity. Anonymus (1990) reported a mean incidence of 42.2% for male B6C3F1 control mice spontaneous liver tumours. Anonymus (1981) report a mean incidence for five independent laboratories for spontaneous liver tumours in mal B6C3F1 mice of 32.1%.

Malignant lymphomas occurred more frequently in exposed than in unexposed females. In several instances, the line between hyperplasia and neoplasia was not clear. In no sex or dose group was the incidence of this class of tumour unusual for this age and strain of mouse.

Lung tumours were fairly frequent occurrences. The incidence of hepatic neoplasms in the males and malignant lymphomas in females as not unusual for this age and strain of mouse, statistical analysis provided no substantive evidence for oncogenicity

Haematology at 12 months was without significant observations. At terminal sacrifice reduced platelet counts were noted in males only which reached significance at the highest dose level. In females this parameter was unaffected or increased. Also at the highest dose level significantly reduced reticulocyte counts were noted in females while in males a non-significant reduction was noted.

In both rat and mice, no clear toxic effects were demonstrated on mortality, clinical signs, body weight, food consumption or haematology through the course of two long-term studies.

10.9.2 Comparison with the CLP criteria

Application of the classification criteria of Annex I to Regulation (EC) 1272/2008 to the available body of genotoxicity and carcinogenicity data for Bifenox indicate that a classification into category 1A can be ruled out because the substance is not known to cause cancer in humans.

For classification into category 1B (presumed human carcinogen) requires animal experiments for which there is sufficient evidence to demonstrate animal carcinogenicity.

For classification into category 2 (suspected human carcinogen) requires animal experiments for which there is limited evidence to demonstrate animal carcinogenicity.

Based on the available studies there were no substance related carcinogenic effects from Bifenox evidenced in rats or mice.

Based on the criteria laid down in Regulation (EC) 1272/2008, Bifenox is not carcinogenic.

10.9.3 Conclusion on classification and labelling for carcinogenicity

Bifenox is not carcinogenic in rats or mice. This conclusion is supported by the absence of genotoxic activity of Bifenox and published data on background tumour incidences.

Bifenox does not require classification for carcinogenicity.

RAC evaluation of carcinogenicity

Summary of the Dossier Submitter's proposal

In order to identify any carcinogenic potential of bifenox after chronic exposure, two long-term studies– one in Sprague Dawley CD rats (Anonymous, 1987) and one in B6C3F1 mice (Anonymous, 1982)- were evaluated.

In rats, an increased incidence of islet cell tumours compared to controls was found in males and females. When comparing the combined incidence of tumours in the study with the historical control data based on adenoma and adenocarcinoma incidences provided by the laboratory (background data from the study report), the results at 500 and 1580 ppm (low and mid dose) were also outside the concurrent historical control data. However, according to the open literature, the incidence of islet cell adenomas is within 1.67-25.71 % and 1.43-14.29 % for male and female CD Sprague Dawley rats, respectively. For carcinoma, the incidence is 0.77-14% and 0.77-4.29 % for male and female rats, respectively. The DS stated that islet cell tumours subclassified as adenoma and adenocarcinoma increase in incidence with age and are more frequently observed in males than in females. They concluded, that for this common type of tumour, the marginally significant increases at 500 and 1580 ppm in male rats result from a random occurrence of a low incidence in control rats.

In mice, hepatic neoplasms were diagnosed as carcinomas and adenomas and were encountered mostly at 24 months in top dose group mice (combined hepatocellular adenoma and carcinoma 31.57 % in males). This finding was not statistically significant for hepatocellular carcinoma, adenoma, and carcinoma/adenoma combined and were not considered as unusual for mice of this age and strain. For females, the statistical analysis showed a significant increase of hepatocellular carcinomas. Due to the small numbers of tumours involved, the DS considered this finding to represent a statistical aberration rather than evidence for carcinogenicity. Further, a mean incidence of 42.2 % for spontaneous liver tumours in male B6C3F1 control mice was reported in a publication on the NTP database (Haseman, Hailey, and Morris 1998 – RAC notes that the reference was given as "Anonymous 1990" and "Anonymous 1989" in the annex to the CLH report). Data from five independent laboratories show a mean incidence for spontaneous liver tumours in male B6C3F1 mice of 32.1 % (Tarone, Chu, and Ward 1981 – reported as "Anonymous 1981" in the CLH report). Therefore, the DS considered these findings not to be of toxicological significance.

In summary, bifenox showed no relevant carcinogenic effects in either species. Thus, the DS proposed no classification for carcinogenicity.

Comments received during consultation

One MSCA commented on this hazard class and agreed that the available data in rats and mice do not trigger classification for carcinogenicity. However, they considered further information was necessary to conclude on the carcinogenic properties of bifenox with certainty and stated that the data should be considered inconclusive. They raised the following points:

- In both studies on carcinogenicity (rats and mice), the maximum tolerated dose

(MTD) was not reached. The available studies did not report any clear toxicological effects.

- The available studies on carcinogenicity are not fully reliable, e.g., the study in mice (1982) was not conducted under GLP. Other deviations are mentioned as well.
- There were some positive findings in rats as well in mice, which were discussed but considered not relevant. In rats, there were findings outside the histological control data range of the performing laboratory, but compared with published literature findings they were considered not significant.
- The EFSA Conclusion 2007 (EFSA Scientific Report (2007) 119, 1-84, Conclusion on the peer review of bifenox), noted that data are of limited quality to conclude sufficiently on the carcinogenic profile of the substance.

In their response, the DS considered doses chosen appropriate, referring to the results from repeated dose toxicity studies and declared that 250 mg/kg bw/d in rats and 150 mg/kg bw/d in mice are considerably high doses. They also clarified that the study report for the mouse carcinogenicity study had a self-certified GLP status referring to respective FDA regulations.

Assessment and comparison with the classification criteria

No human data are available.

Study in Rats

In a 104-week combined carcinogenicity/long-term toxicity feeding study - OECD TG 453 (1981), 50 Charles River Sprague Dawley CD rats/sex/dose received bifenox in their diet.

The dose levels were set at 500, 1580, and 5000 ppm, corresponding to 18.9, 59, and 188 mg/kg bw/d for males and 24.6, 77, 252 mg/kg bw/d for females. Four satellite groups of 20 males and 20 females were included for blood sampling and for interim sacrifice after 52 weeks of treatment.

The only clinical findings were a reduced body weight gain at the top dose below 10 % as compared to controls (6 % in males and females) and a reduced food consumption in males. These findings were considered not to be toxicologically relevant (see table below).

Table: General results of the Bifenox rat study, 104 weeks

End point /dose	0		500		1580		5000 ppm	
Sex	m	f	m	f	m	f	m	f
Survival %	40	42	42	42	52	62	48	46
Bw gain week 0-26							↓6%	↓6%
Food consumption week 0- 26							(↓4%)	
Organ weight relative								
Liver interim sacrifice			(↑6%)	(↑9%)		(↑15%)	(↑6%)	(↑9%)

In terms of carcinogenicity, no statistical elevation of tumour findings has been reported. Further, no statistical significance was found in the incidence of malignant islet cell tumours in any group of male or female rats when compared to controls, nor was there

any significant trend in malignant islet cell tumour incidence with increasing dosage but results in the low and mid dose groups were outside concurrent historical control ranges (see table below).

***Table**: Incidence of islet cell tumours*

Dose	0		500		1580		5000 ppm	
Sex	m	f	m	f	m	f	m	f
Islet cell adenocarcinoma								
Killed or dying during study	1/32	0/29	0/29	0/29	0/25	1/19	2/27	0/28
At terminal sacrifice	0/18	0/21	2/21	2/21	3/25	2/31	1/23	2/22
Total number adenocarcinoma	1/50	0/50	2/50	2/50	4/50	3/50	4/50	2/50
Islet cell Adenoma								
Killed or dying during study	4/32	1/29	6/29	1/29	3/25	1/19	1/27	0/28
Terminal sacrifice	1/18	1/21	3/21	5/21	9/25	2/31	5/23	2/22
Total No adenoma	5/50	2/50	9/50	6/50	12/50	4/50	6/50	3/50
Total adenoma + carcinoma	6/50	2/50	11/50 *	8/50 *	15/50 *	6/50 *	10/50	5/50
	12%	4%	22%	16%	30%	12%	20%	10%

*outside the HCD range for the period 1980-1982, but within the HCD range for the period 1982-1984

Combined incidences for islet cell adenoma and carcinoma were increased above control values (12 % and 4 % for males and females, respectively) for the low dose males and females (22 % and 16 %, respectively) and for mid dose males and females (30 % and 12 %, respectively) but not at the high dose. According to the literature, this tumour type is a relatively common background finding in rats with higher control incidences in males. No clear dose-dependence was observed and the incidences in low and mid dose groups were only outside of one of the two HCD ranges provided. These were 4.1-20 % (mean 14.2 %) for males in the period of 1980-1982, and 3-10 % (mean 5.5 %) for females in the same period. For the years 1982-1984, HCD ranges were 12-30 % (mean 18.2 %) for males, and 1.8-23.6 % (mean 16 %) for females. However, only combined HCD for all kinds of islet tumours were provided and incidences seemed to have increased over time. According to the study report, the study had been conducted from 1984 to 1986. Thus, the HCD for the later period are more relevant. Overall, RAC considers the result of the study equivocal.

Study in Mice

In a 24-month carcinogenicity study, B6C3F1 mice (60/sex/dose) received bifenox in their diet. The dose levels were set at 50, 200 and 1000 ppm, corresponding to 7, 30 and 147 mg/kg bw/d for males and 9, 35, 179 mg/kg bw/d for females. This study was not performed according to OECD guideline 451 (1981): three females were pregnant and allowed to litter, mice were observed weekly for clinical signs instead of daily, and haematology was performed on ten animals only. Further it is mentioned, that the MTD was not reached and a number of animals escaped or were withdrawn from the study but no explanation was given as to why they were removed.

Minor effects on haematological parameters were observed (reduced platelets and reticulocyte counts) at 1000 ppm. An increase in relative kidney weights was reported for female mice at the mid and high doses, which was statistically significant at terminal

sacrifice (+8 % and +18 % as compared to controls, respectively, at terminal sacrifice and +13.5 % and +21.3 %, respectively, at interim sacrifice). Absolute kidney weights were increased similarly.

Neoplastic findings are summarised in the table below.

***Table**: Effects observed in the mice study - Tumour pathology data*

End point /dose	0 ppm		50 ppm		200 ppm		1000 ppm	
	m	f	m	f	m	f	m	f
Scheduled + unscheduled death days 1-367								
N° examined mice	12	12	13	15	13	12	11	13
N° mice with tumours	1	0	0	2	0	0	2	2
Lung adenoma	1	0	0	0	0	0	1	0
Hepatocellular carcinoma	0	0	0	1	0	0	1	1
Haemopoietic system								
Malignant lymphoma	0	0	0	1	0	0	0	1
Scheduled +unscheduled death days 1-737								
N° examined mice	58	52	60	58	58	56	57	58
N° mice with tumours	25	17	20	22	24	27	29	27
Liver								
Hepatocellular adenoma	5	1	3	3	8	0	7	3
Hepatocellular carcinoma	4	1	9	0	6	0	11	2*
Haemangiosarcoma	4	0	1	0	0	0	0	1
Haemopoietic system								
Malignant lymphoma	4	6	2	12	4	12	5	13
Spleen haemangiosarcoma	0	0	1	0	0	1	2	3
Lungs								
Adenoma	1	0	0	0	0	0	1	0
Metastatic	0	0	0	0	0	1	1	1
Alveolar/bronchiolar adenoma	2	3	2	0	4	2	2	1
Alveolar/bronchiolar carcinoma	2	1	4	0	3	3	3	1

* statistically significant (Gehan-Breslow trend test)

In females, malignant lymphomas occurred more frequently in exposed than in unexposed females. However, this finding was not statistically significant and no clear dose-dependence was observed.

Moreover, in top dose females there was a statistically significant increase in hepatocellular carcinoma, although with a low incidence (2/58 vs 1/52 in controls). An

increase in hepatocellular carcinomas was also observed in males without a clear dose-dependence but with higher incidences (11/57 at the top dose vs. 4/58 in controls). The DS reported historical control data sets for hepatocellular adenoma and carcinoma. RAC notes that the data set from the NTP program comprised studies from 1990 to 1997, while the study with bifenox was conducted between 1980 and 1982. In the second publication (Tarone *et al,* 1981), a mean incidence for spontaneous liver tumours in five independent laboratories was reported as 32.1 %. RAC notes that in this review paper, historical control data for lymphomas were also mentioned but these were not reported in the CLH report or annex. These ranged from 5 - 45 % with a mean of 24.4 %. However, RAC notes that although contemporary with the study, historical control data from other laboratories than that performing the study in question are of limited relevance.
Spleen haemangiosarcoma occurred in both top dose males and females at higher incidences compared to controls (2/57 in males and 3/58 in females vs none in controls). No historical control data were available for this tumour type.
Overall, given the deviations from the guideline, the overall poor reporting, and a lack of any clinical signs even at the top dose, RAC considers the study rather unreliable.
Conclusion on classification

In summary, RAC notes that doses chosen in both studies did not lead to relevant clinical signs, reporting was limited in the mouse study (which overall was of a rather low reliability), no clear dose-dependence was observed for any tumour type in both species (but again – dose-dependence may have been masked by too low doses).

Thus, based on the available data, RAC concurs with the DS that **no classification** for carcinogenicity is warranted. However, the studies presented had several limitations and no clear conclusion could be drawn. Thus, RAC considers the data to be inconclusive.

10.10 Reproductive toxicity

10.10.1Adverse effects on sexual function and fertility

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

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
Rat, 2-generation reproduction and chronic toxicity GLP / not fully compliant to OECD 416 28 Sprague Dawley rats of Charles River	Bifenox Batch # 9401021 Purity: 99% Dose levels: 125, 750, 4500 ppm Concentrations were	4500 ppm Parental (systemic): decreased body weight gain Reproductive: decreased pup and litter weight at weanling in F1 and F2 generation NOAEL:	Anonymus, 1995

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference**
CD; 24 male and female F1 weanlings were selected for rearing to maturity and mating to produce F2 generation.	within ± 10% of nominal concentration at all dose levels	Chronic toxicity: 44.5 mg/kg bw/day Reproduction: 148 mg/kg bw/day	

There are no observations in humans regarding reproductive toxicity of Bifenox.

Parental toxicity was evidenced either by reduced body weight gain and/or reduced food utilisation efficiency (food conversion to bodyweight) at the top dose in the premating and/or gestational phases. As a result maternal body weights at the top dose were lower than all other groups at the start of lactation. During lactation bodyweight gain in top dose dams was increased over control as was food efficiency. As result top dose dams at least partially recouped the weight differential to controls, seemingly at the expense of lactational bodyweight gain in the pups. Hence reduced body weight gain in pups was more likely due to dams recouping reduced bodyweight gains up to the beginning of lactation, rather than direct toxicity of the milk to pups. i.e. results in pups due to maternal toxicity.

Maternal body weight data, fertility and developmental parameters are provided as far as they were available in the report, in the three tables below. With respect to providing corrected maternal body weights, there were no gravid uterine weights provided in the 2 generation study report, however body weight gains for various periods have been presented as a % proportion of the starting bodyweights for the period in question (highlighted rows).

Historical control data: No historical control data was cited against findings from this study in the evaluation that would require further elaboration.

Table 25.1: Parameters of maternal toxicity in the two-generation rat study with bifenox. Anonymus (1995): maternal toxicity. Blank cell = zero incidence

Generation	**Dose Level**	**0 ppm**	**125 ppm**	**750 ppm**	**4500 ppm**
		Mortality			
F0		1			
F1				1	
		Clinical observations and Necropsy findings			
F0	Hair Loss / scabbing	3	6		2
	Red liquid evident from Vagina				1
	Ear torn/black/encrusted				1
	Mass (no further info provided)				1
	Uterus:dilated	2	2		
	Vagina:mass	1			
	Cervix: enlarged		1		1
	Lungs: dark				1
F1	Scabbing/staining/coat scruffy	1		2	1
	Lump/mass	1		1	
	Hair loss	4	1		2

	Pale/breathing difficulty			1	
	Eyelid encrusted/lacrimation		1	3	
	Red liquid from vagina/nervous/ agitated/piloerection			1	
	Incisors short/chipped				1
	Hind limbs swollen/purple/red	1			
	Uterus dilated	1		1	
	Red staining around mouth and nose/lungs dark/blood in thoracic cavity			1	
		Maternal body weight, body weight gain and food consumption			
F0 premating	Body weight (g±SD) Week 0	142±14	140±12	145±13	140±14
	Weight gain (g) Week 0-9	126	132 (+5%)	132 (+5%)	117 (-7%)
	Weight gain, Week 0-9, corrected for maternal body weight by expression as a percentage of starting body weight (%change versus control)	88.7%	94.3% (+6.3%)	91.0% (+2.6%)	81.4% (-8.2%)
	Total Food consumption Week 0-9 (total g/animal)	1119	1348	1343	1295
	Food conversion to body weight (% w/w)	11.3	9.8	9.8	9.0
F1 premating	Body weight (g±SD) Week 3	69±15	67±16	75±16	56±10
	Weight gain (g) Week 3-15 (%change vs control)	227	238 (+5%)	236 (+4%)	220 (-3%)
	Weight gain, Week 3-15, corrected for maternal body weight by expression as a percentage of starting body weight (%change versus control)	329.0%	355.2% (+8.0%)	314.7% (-4.4%)	392.9% (+19.4%)
	Total Food consumption Week 3-15 (total g/animal)	1782	1811	1816	1716
	Food conversion to body weight (% w/w)	12.7	13.1	13.0	12.8
F0 gestation	Body weight (g±SD) D0	305±30	318±34	319±30	298±30
	Weight gain (g) D 0-20	156	154 (-1%)	147 (-6%)	137 (-12%)
	Weight gain, D 0-20, corrected for maternal body weight by expression as a percentage of starting body weight (%change versus control)	51.1%	48.4% (-5.3%)	46.1% (-9.9%)	46.0% (-10%)
	Total Food consumption D 0-20 (total g/animal)	613	653	630	620
	Food conversion to body weight (% w/w)	2.0	2.1	2.0	2.1
F1 gestation	Body weight (g±SD) D0	297±28	299±25	301±30	275±30
	Weight gain (g) D 0-20	157	157	147 (-5%)	147 (-6%)

	Weight gain, D 0-20, corrected for maternal body weight by expression as a percentage of starting body weight (%change versus control)	52.9%	52.5% (-0.7%)	48.8% (-7.6%)	53.5% (+1.1%)
	Total Food consumption D 0-20 (total g/animal)	629	650	608	638
	Food conversion to body weight (% w/w)	25.0	24.2	24.2	23.0
F0 lactation	Body weight (g±SD) D 0	337±38	344±39	346±32	329±39
	Body weight gain (g) D 0-21	10	16	11	18 (+5%)
	Weight gain, D 0-21, corrected for maternal body weight by expression as a percentage of starting body weight (% change versus control)	3.0%	4.7% (56.7%)	3.2% (7%)	5.5% (84.4%)
	Total Food consumption D 0-21 (total g/animal)	1534	1544	1583	1455
	Food conversion to body weight (% w/w)	0.65	1.04	0.69	1.24
F1 lactation	Body weight (g±SD) D 0	332±37	339±34	336±40	325±40
	Body weight gain (g) D 0-21	9	7	20	14
	Weight gain, D 0-21, corrected for maternal body weight by expression as a percentage of starting body weight (%change versus control)	2.7%	2.1% (-23.8%)	6.0% (+119.6%	4.3% (+58.9%)
	Total Food consumption D 0-21 (total g/animal)	1461	1517	1525	1454
	Food conversion to body weight (% w/w)	0.6	0.5	1.3	1.0

Table 25.2: Fertility, gestational and birth indices and pup bodyweight parameters

Dose Level	**0 ppm**	**125 ppm**	**750 ppm**	**4500 ppm**
	F0 Dams Fertility, gestational and birth indices and pup bodyweight parameters			
Median number of nights to positive mating sign	2	2.5	2.5	2
Number passing one oestrus	0	0	1	1
Male fertility Index (%)[a]	68	75	79	86
Female fertility index (%)[a]	71	82	82	86
Duration of gestation				
21 days	2	5	3	5
22 days	17	16	16	19
23 days	1	2	4	0
Mean duration gestation	22.0	21.9	22.0	21.8
Gestation index[b] % dams producing live litters (N)	100 (20)	100 (23)	100 (23)	100 (24)
Mean implant sites±SD	17.2±3.3	17.3±1.4	17.5±1.3	16.1±2.6
Mean pups born/litter ±SD	15.5±3.4	15.8±1.7	15.4±2.6	14.8±2.5
	Mean live pups ±SD			
Day 0 lactation	15.4±3.4	15.6±1.8	15.1±2.8	14.6±2.4

Day 4 lactation	13.8±3.3	14.4±2.9	14.7±2.7	14.4±2.5
Day 21 lactation	13.6±3.3	13.3±3.1	13.8±2.8	13.0±3.4
	F1 pup indices			
	Birth index[c]			
Mean litter index (%)	90	90	88	92
Number losing >2 pups	3	6	6	4
Number of litters	20	23	23	24
	Live Birth index[d]			
Mean litter index (%)	99	99	97	99
Number losing >1 pups	0	1	2	1
Number of litters	20	23	23	24
	Viability index Day 0-4[e]			
Mean litter index (%)	91	86	89	92
Number losing >3 pups	4	4	4	2
Number of litters	20	23	23	24
	Lactation index Days 4-21[f]			
Mean litter index (%)	98	94	99	98
Number losing >1 pups	1	2	1	2
Number of litters	20	23	22	24
	Overall survival index Birth to Day 21[g]			
Mean litter index (%)	89	81	86	88
Number losing >4 pups	3	4	4	2
Number of litters	20	23	23	24
	Group mean litter weight (g±SD)			
Lactation Day 1	97±20	93±17	97±17	92±15
Lactation Day 21	621±118	604±104	635±108	478±96***
	Mean litter pup weight (g±SD)			
Lactation Day 1 males	6.7±0.9	6.6±0.7	6.8±0.7	6.5±1.0
Lactation Day 21 males	48.6±9.2	48.2±9.7	47.6±6.5	38.9±6.4***
Lactation Day 1 females	6.3±0.9	6.3±0.7	6.5±0.6	6.2±0.7
Lactation Day 21 females	46.0±9.0	46.2±9.2	46.3±5.4	36.9±5.9***
	F1 Dams Fertility, gestational and birth indices and pup bodyweight parameters			
Median number of nights to positive mating sign	3	3	3	2.5
Number passing one oestrus	0	0	0	3
Male fertility Index (%)[a]	92	88	83	88
Female fertility index (%)[a]	92	92	92	92
Duration of gestation				
21 days	5	5	4	11
22 days	17	16	16	10
23 days	0	0	2	1
Mean duration gestation	21.8	21.8	21.9	21.5
Gestation index[b] % dams producing live litters (N)	100 (22)	100 (22)	100 (22)	100 (22)
Mean implant sites±SD	15.8±2.8	15.8±2.6	16.3±2.7	15.1±2.1
Mean pups born/litter ±SD	14.3±2.9	14.7±2.6	14.3±2.7	13.2±2.0
	Mean live pups ±SD			
Day 0 lactation	14.3±2.9	14.5±2.6	14.1±2.5	13.2±2.1

Day 4 lactation	13.6±2.9	13.2±3.3	13.6±2.5	12.9±2.3
Day 21 lactation	13.3±2.8	12.9±3.2	13.4±2.6	12.4±2.2
	F2 pup indices			
	Birth index[c]			
Mean litter index (%)	91	93	89	89
Number losing >2 pups	6	3	6	4
Number of litters	22	22	21	22
	Live Birth index[d]			
Mean litter index (%)	100	99	99	99
Number losing >1 pups	0	0	1	0
Number of litters	22	22	21	22
	Viability index Days 0-4[e]			
Mean litter index (%)	91	92	89	84
Number losing >3 pups	0	3	2	3
Number of litters	22	22	22	22
	Lactation index Days 4-21[f]			
Mean litter index (%)	98	98	98	96
Number losing >1 pups	0	1	1	2
Number of litters	21	21	19	19
	Overall survival index Birth to Day 21[g]			
Mean litter index (%)	89	89	81	80
Number losing >4 pups	0	3	1	3
Number of litters	22	21	20	22
	Group mean litter weight (g±SD)			
Lactation Day 1	90±16	94±15	94±15	86±16
Lactation Day 21	604±100	599±113	628±78	470±65***
	Mean litter pup weight (g±SD)			
Lactation Day 1 males	6.7±0.7	6.8±0.8	7.0±0.8	6.7±0.5
Lactation Day 21 males	47.7±7.6	48.9±8.0	48.9±7.9	39.3±5.1***
Lactation Day 1 females	6.3±0.7	6.7±1.7	6.6±0.7	6.4±0.5
Lactation Day 21 females	45.3±7.2	46.5±7.7	46.9±7.5	37.5±4.9***

*** Statistically significantly different from controls (P<0.001)
a = Number of pregnant females or siring males / number paired
b = Number bearing live pups / number pregnant
c = Total number of pups born (live and dead) / Number of implantation scars
d = Number of pups live on Day 0 of lactation / Total number born
e = Number of pups live on Day 4 of lactation / Number live on Day 0
f = Number of pups live on day 21 of lactation / Number live on Day 4
g = Number of pups live on Day 21 of lactation / Total number of pups born (live and dead)

Table 25.3: Developmental toxicity and other findings in pups

Dose Level	**0 ppm**	**125 ppm**	**750 ppm**	**4500 ppm**
	F1 pups			
Litters (pups) with malformations	**0**	**1 (2)[a]**	**0**	**1(1)[d]**
Litters (pups) with other findings (but no malformations)	**1(1)[c]**	**0**	**1 (1)[b]**	**1(2)[e]**
	F2 pups			

Litters (pups) with malformations	**0**	**0**	**0**	**0**
Litters (pups) with other findings (but no malformations)	**0**	**0**	**0**	**2(2)[f,g]**

[a]Two pups in F0parent-F1Litter 152 (125 p.p.m. Bifenox), died shortly after birth, with multiple abnormalities including misshapen cranium, shortened lower jaw, open eyes, cleft palate, fused digits and subcutaneous oedema.
[b]One pup in F0parent-F1Litter 180 (750 p.p.m. Bifenox) with a small kidney at necropsy.
[c]One pup in F0parent-F1Litter 139 (Control), killed Day 19, with body tremors, piloerection, encrusted eyes and apparent hind limb weakness/ataxia.
[d]One pup in F0parent-F1Litter 212 (4500 p.p.m. Bifenox), killed Day 23, with ataxia, hydrocephalus and one eye apparently absent.
[e]One pup in F0parent-F1Litter 223 (4500 p.p.m. Bifenox), died Day 19, with piloerection prior to death. Second pup with piloerection and swollen abdomen Day 21.
[f]One pup in F1parent-F2Litter 479 (4500 p.p.m. Bifenox), killed Day 16, with a firm, lobular mass on the lower lip.
[g]One pup in F1parent-F2Litter 496 (4500 p.p.m. Bifenox), small with brown fluid in one kidney at necropsy.

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

In a 2-generation rat study, the MTD was not reached. Two rats died during the course of the study, without association to treatment. Clinical signs and necropsy findings did not indicate any association with treatment.

Mating performance, fertility and duration of gestation were not considered to be affected by treatment. Litter size, pup survival and mean number of implantation was slightly reduced at 4500 ppm in both generations. The differences did not reach statistical significance and were considered incidental.

During the lactation period, the mean body weight gain of litter and pups was lower at top dose and on day 21 weights were approximately 80% of control. The reduction in litter and pup weights at 4500 ppm were statistically significant lower than controls but were accompanied by (slight) parental toxicity (evidenced by reduced body weight gain at top dose). Based on this, these effects were not considered to be relevant for a classification with regard to reproduction toxicity. Fertility parameters were not affected. In the parental generation females reveal slight reduction of body weight gain even in the mid dose during gestation (up to 12%) and especially a significant reduction of body weight gain during lactation from Day 1 to Day 14 (up to 42%).

The NOAEL is based on statistically significant reduction of litter/pup weight at top dose, clearly evident at slight parental toxicity and was also not considered to be relevant for a classification in view of the accompanying parental toxicity. Abnormalities among pups did not suggest any association with treatment.

A reproductive NOAEL = 750 ppm (148 mg/kg bw/day) was based on decreased pup and litter weight in F_1 and F_2 generation at 4500 ppm. The reproductive effects occurred in the presence of slight parental (systemic) toxicity as suggested by the decreased body weight gain seen at 4500 ppm. NOAEL parental toxicity = 750 ppm (44.5 mg/kg bw/day).

10.10.3 Comparison with the CLP criteria

Bifenox was thoroughly evaluated for fertility and reproductive toxic potential in one rat two-generation study. The NOAEL is based on statistically significant reduction of litter/pup weight at top dose, clearly evident at slight parental toxicity and was also not considered to be relevant for a

classification in view of the accompanying parental toxicity. Abnormalities among pups did not suggest any association with treatment. According to CLP criteria adverse effects on reproduction seen only at very high dose levels in animal studies (for example doses that induce prostration, severe inappetence, excessive mortality) would not normally lead to classification, unless other information is available, e.g. toxicokinetics information indicating that humans may be more susceptible than animals. The reduction in litter and pup weights at the highest dose level (4500 ppm) were statistically significant lower than controls although but were accompanied by (slight) parental toxicity (evidenced by reduced body weight gain at top dose). A significant toxic effect in the offspring, e.g. irreversible effects, was not noted. In conclusion, Bifenox did not adversely affect fertility and general reproductive performance in the two-generation rat study at the reproductive NOAEL of 148 mg/kg bw/day.

Therefore, it can be concluded that from the discussed rat study, no evidence of a reproductive toxic effect of Bifenox can be derived and there is no classification required for fertility or reproductive toxicity.

10.10.4 Adverse effects on development

Table 26: 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, developmental toxicity GLP / not fully compliant to OECD 414	Bifenox Batch # 353-12-1 Purity: 98% Dose levels: 225, 900, 3600 mg/kg bw/day	NOAEL 3600 mg/kg bw/d (top dose tested) Maternal: mortality and clinical signs marginally higher incidence of foetuses/litters with a large fontanel at the top dose, but size not consistent and hence not considered treatment related. The top dose was also 3.6 x the normal limit dose, again casting doubt on the usefulness of the finding as a relevant hazard indicator. NOEL maternal + developmental: 900 mg/kg bw/day	Anonymus. 1987
Rabbit, developmental toxicity GLP / not fully compliant to OECD 414	Bifenox Batch # 312165 Purity: 98% Dose levels: 2, 20, 200 mg/kg bw/day	200 mg/kg bw/d Maternal: mortality, clinical signs, reduced body weight gain and food consumption Developmental: slightly increased incidence of hyoid alae angulated, but well within the historical control range, and made prominent by an abnormally low concurrent control incidence. Not reproduced in the subsequent study. NOEL maternal + developmental: 20 mg/kg bw/day	Anonymus, 1986
Rabbit developmental toxicity GLP / OECD 414	Bifenox Batch # 3123142024 Purity: 97%	160 mg/kg bw/d Maternal: clinical signs, very slight body weight	Anonymus, 1986

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
	Dose levels: 5, 50, 160, 500, 1000 mg/kg bw/day	decrease (NS) reduced food consumption Developmental: No adverse effects NOEL maternal + developmental: 50 mg/kg bw/day	

There are no observations in humans regarding reproductive toxicity of Bifenox.

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

At the top dose severe maternal toxicity might be characterized by at least 2 treatment related maternal deaths and clinical signs of salivation, staining of mouth, patchy hair loss. The patchy hair loss might indicate poor general condition. It is noteworthy that the top dose was 3.6 fold greater than todays permitted limit dose, hence the relevance of results from this group to hazard assessment is questionable.

Data available in the report pertaining to maternal toxicity is provided in the table below.

The extra details on the historical control data presented were requested from current incarnation of the test laboratory. The laboratory could not provide exact study details to the data cited, but did state that: “The HCD presented in the report would have been matched to the study for species, strain, supplier and performing laboratory.”

Table 26.1: Maternal toxicity findings and key fetal findings including those for which historical control data was cited in the report, for the developmental rat toxicity study by Anonymus (1987)

Endpoint/dose (mg/kg bw/d)	0	225	900	3600
N° mated females	25	25	25	25
Mortality	0	0	0	5
Not pregnant	7	5	3	5
Clinical signs	none	none	none	Salivation, staining mouth, patchy hair loss
	Water consumption (g/animal/day)			
D 13	27	35	33	38
D 14-16	32	37	37	42
D 17-19	33	39	40	38

Avg D 13-19 (% change vs control)	27.9	37.6 (+35%)	37.7 (+35%	39.7 (+42%)
	Food consumption (g/animal/day)			
D 6-9	21	20	20	19
D 10-13	24	25	25	23
D 14-16	25	26	26	25
D 17-19	25	26	26	27
Avg D 6-19	23.6	24.1	24.2	23.4
Body weight (g)				
D 6	228.9	231.5	230.2	235.7
D 20	345.8	348.0	343.0	351.3
Body weight gain (g) D 6-20	116.9	116.5	112.8	115.6
Maternal Reproductive performance				
Total resorptions			1	
N° females with live young	17	20	21	15
N° corpora lutea	13.1	13.4	13.6	13.5
N° implants	12.3	12.3	12.2	12.8
N° dead implants	0.9	0.5	1.1	1.1
Pre implantation loss %	6.1	10	9.1	4.8
Post implantation loss %	7.8	3.7	11.4	8.5
Mean litter weight (g)	39.19	40.43	38.29	40.64
Mean fetal weight (g)	3.45	3.41	3.5	3.46
Fœtal examination:				
N fetuses (litters) examined	98 (17)	119 (20)	115 (21)	88 (15)
	Skeletal evaluation: % fetal incidence (N litters affected)			
Number of ribs				
12/12	-	-	-	1.1 (1)
13/13	98.0 (17)	98.8 (20)	95.6 (21)	92.2 (15)
13/14	-	0.6 (1)	0.8 (1)	5.3 (3)
14/14	1.0 (1)	1.6 (2)	-	1.1 (1)
Historical control data extra 14th rib:	10 studies performed in 1985: mean fetal incidence: 2.9% with a range of 0.9-7.1%			
Small fontanelles	2.0 (1)	2.1 (2)	3.6 (3)	1.1 (1)
Medium fontanelles	96 (17)	95.3 (20)	94.4 (21)	88.8 (15)

Large fontanelles	2 (2)	2.6 (3)	2 (3)	10.1 (6)
Frontonasal suture enlarged	1.0 (1)	5.4 (4)	2.6 (3)	1.3 (1)
Incomplete ossification of frontal/parietal/squamosal/jugal/nasal bone (s)	1.0 (1)	3.3 (4)	1.0 (1)	3.2 (3)
Incomplete ossification of interparietal bone	5.1 (5)	16.7 (12)	12.4 (7)	12.1 (5)
Incomplete ossification of supraoccipital bone	8.0 (6)	11.5 (11)	6.2 (7)	7.2 (5)
Patchy/incomplete ossification of one or more cranial bones	1.0 (1)	0.7 (1)	-	-
Incomplete ossification/absence of hyoid bone	7.0 (6)	14.0 (12)	12.2 (11)	14.4 (8)

The results reported in the table are not statistically significant.

Developmental toxicity studies have been investigated in order to assess effects on embryonic and foetal development, maternal toxicity and to establish dose-response relationships in dams and offspring in rats and rabbits,

Bifenox was thoroughly evaluated for a developmental toxic potential in one rat and two rabbit studies. The study directors concluded in all these studies that there is no evidence of a developmental toxic potential of Bifenox.

In this evaluation a few parameters which showed some degree of variability (see table below) are discussed with regard to their possible interference with the overall conclusion.

In the rat study, the extra 14th rib incidences were within the historical data range given in the report (up to 7.1 %) so that they are due to variability and not to treatment. Furthermore, the other findings of extra 13th and combined 13th and 14th ribs were not dose-related and thus most likely due to variation which supports an absence of an effect of Bifenox on the rib development.

The litter incidences of small, medium and large fontanelles appear to show a slight increase in "large fontanelles" at the highest dose. However, the size definitions are not explained and may only be subjective, and in the absence of any other head bone variations, this increase is of doubtful relevance. Furthermore a size reduction might rather be expected at this maternal-toxic dose and not an enlargement. However, the incidence of small size fontanelles was not increased by treatment. In addition this apparent increase also occurred at 3.6 x the limit dose, at which strong maternal toxicity was also observed (salivation, staining of mouth, patchy hair loss), hence usefulness of this parameter as an indicator of a pertinent developmental hazard is doubtful.

The incidences of the finding incomplete ossification/absence of hyoid bone did not show a dose-response relationship, since the incidence in the low dose was as high as that of the highest dose, whereas the incidence in the 900 mg/kg bw group was lower again despite a 4-fold higher dose compared to the low dose.

Overall, therefore, the findings from the rat developmental toxicity study are not sufficient evidence of a developmental toxicity hazard by bifenox.

Table 26.2: Selected findings in the rat developmental toxicity study

	Finding in % fetal incidence (number of litters affected)			
Dose (mg/kg bw/day)	**0**	**225**	**900**	**3600**
Number of fetuses (litters) examined	95 (17)	118 (20)	118 (21)	88 (15)
Size of fontanelle				
small	2.0 (1)	2.1 (2)	3.6 (3)	1.1 (1)
medium	96.1 (17)	95.3 (20)	94.4 (21)	88.8 (15)
large	2.0 (2)	2.6 (3)	2.0 (3)	10.1 (6)
Incomplete ossification/absence of hyoid bone	7.0 (6)	14.0 (12)	12.2 (11)	14.4 (8)
Number of ribs				
12/12	-	-	-	1.1 (1)
13/13	98.0 (17)	98.8 (20)	95.6 (21)	92.2 (15)
13/14	2.0 (2)	0.6 (1)	3.6 (3)	1.3 (1)
14/14	-	0.6 (1)	0.8 (1)	5.3 (3)
Historical incidence 14th rib (min – max)	0.9 - 7.1 %			

In the rabbit studies (Anonymus, 1986) at the top dose there were maternal deaths, gastric ulcerations, perturbations to the condition of the faeces, and resorptions and abortions indicating that this dose produced severe toxicity.

Data available in the report pertaining to maternal toxicity is provided in the two tables below (Table 26.3 and Table 26.4).

The extra details on the historical control data are presented in the Table 26.5.

Table 26.3: Maternal toxicity findings and key fetal findings including those for which historical control data was cited in the report for the developmental rabbit toxicity study by Anonymus (1986).

Endpoints/ dose	**0**	**2**	**20**	**200 mg/kg bw/d**
	Mortality and clinical signs			
Mortality				3[a]
Dried feces	2/13	0/0	2/11	8**/41**
No feces present	1/2	0/0	1/1	2/9**
Soft or liquid feces	1/1	3/8c	8**/18**	1/3
Alopecia	3/18	4/19	8/52**	3/21
	Necropsy observations			
Aborted	0	0	0	3
Gastric ulceration[b]	0	0	0	6
Kidneys light brown in colour	0	0	0	1
Kidney cortex light brown in colour	0	0	0	1
Liver pale brown in colour	0	0	0	1
Urine red brown in colour	0	0	0	1
Left uterine horn filled with dark red fluid	0	0	0	1
	Bodyweight and bodyweight gain			
Body weight (kg) Day 29	4.27±0.46	4.25±0.41	4.38±0.50	4.12±0.52

Body weight gain (kg) Day 6-29	0.30±0.18	0.23±0.19	0.30±0.14	0.13±0.38 (↓ Day 6-12)
Food consumption Day 6-29 (g/animal/day)	146.1±30	147.1±33.3	147.9±34.8	135.2±39.7
Food conversion to body weight (% w/w)	0.21	0.16	0.20	0.10
	Reproductive data			
N° gravid females	20	20	20	20
Pregnant rabbits (n°)	17	16	20	16
Corpora lutea mean	9.8	11	10.7	9.4
Implantations mean	7.2	7	7.9	7.8
Litter size mean	6.6	6.6	7.5	6.8
N° live/death fetuses	106/0	106/0	143/0	75/0
N°early/late resorption	8/2	5/1	5/2	8/3
Resorptions mean	0.6±1.3	0.4±0.5	0.4±0.8	1±1.4
Live fetal bw/litter	46.06	48.44	46.05	42.75
% Resorbed conceptuses/litter	7.7±16	6.3±9.8	3.5±8	13.5±17.2
N° litters evaluated	16	16	19	11
	Skeletal alterations: litter/fetal incidence n° (%)			
Hyoid, Alae, angulated	1/1 (6.2/0.9%)	2/2 (12.5/1.9)	2/2 (10.5/1.4)	3/3 (27%/4%)
	Historical data from laboratory			
Hyoid, Alae, angulated (See final table below for a breakdown of studies from which it was derived)	Litter incidence 0 – 35% Fetal incidence 0 – 5.3%			

[a]One rabbit was sacrificed moribund on day 18 of gestation prior to sacrifice, this rabbit was observed to have corneal opacity, lacrimation, ataxia, decreased motor activity, increased sensitivity to touch in the abdominal area and a lack of muscular control in the hindlegs.

[b]ulcerations in cardiac, pyloric and/or fundic regions

/ Rabbits / days

** Significantly different from vehicle control value, at P≤0.01.

↓ Statistically significantly different from control at at P≤0.05; () not significantly different from control

Table 26.4: Uterine contents and litter data in individual rabbits which died, were sacrificed moribund, aborted or delivered naturally

Dose Group (mg/kg bw/day animal number)	Day of termination or death	Corpora lutea			Implantations			Embryos or fetuses[a]				Resorptions[b]			
		R	L	T	R	L	T	R	L	A/Del	T	R	L	A/Del	T
0 (vehicle) 10409	Delivered and sacrificed on day 28 of gestation	6	5	11	2	4	6	0	1[c]	2[c]	3[c]	1(LR)	1(LR)	1(LR)	3(LR)
20 10452	Delivered and sacrificed on day 29 of gestation	5	5	10	5	5	10	3[d]	3[d]	4[d]	10[d]	0	0	0	0
200 10461	Aborted and sacrificed on day 26 of gestation	5	6	11	4	5	9	0	0	8[e]	8[e]	0	0	-	-[e]
200 10463	Moribund sacrificed on day 18 of presumed gestation	6	5	11	0	0	0	0	0	-	0	0	0	-	0
200 10464	Aborted and sacrificed on day 24 of gestation	4	7	11	4	5	9	0	0	-[f]	-[f]	0	0	6(LR)[f]	-[f]
200 10469	Aborted and sacrificed on day 24 of gestation	5	5	10	1	0	1	0	0	1[g]	1[g]	0	0	0	0
200 10472	Found dead on day 14 of gestation	5	4	9	5	4	9	0	0	-	0	5	4	-	9
200 10480	Found dead on day 20 of gestation	4	7	11	4	7	11	3	5	-	8[b]	1(LR)	2(LR)	-	3(LR)

R Right; L Left; A Aborted; T Total; LR Late resorption

[a] Live unless noted otherwise

[b] Early unless noted otherwise

[c] Two delivered pups and one late resorption were found in the cage pan. One fetus was found in utero. All conceptuses appeared to have been alive at the time of delivery and normal for their developmental ages.

[d] Four delivered pups and one placenta were found in the cage pan. One delivered pup was observed to have a cannibalized tail. All remaining fetuses and delivered pups appeared to have been alive at the time of delivery and normal for their developmental ages.

[e] Eight aborted fetuses (four with placentas attached) and five placentas were found in the cage pan. Aborted fetuses appeared to have been alive at the time of abortion and normal for their developmental ages. Remaining conceptus was presumed to have been cannibalized.

[f] Six late resorptions were found in the cage pan. Remaining conceptuses were presumed to have been cannibalized.

[g] One aborted fetus was found in the cage pan. Aborted fetus appeared to have been alive at the time of abortion and normal for its developmental age.

[h] Fetuses found in utero appeared to have been alive at the time of maternal death and normal for their developmental ages.

Historical control data for New Zealand White Rabbits sourced from Hazleton Research Animals, Denver, Pennsylvania, USA and used at **Argus Research Laboratories**, Pensylvania, USA

Table 26.5: Data is from studies conducted within 2.5 year of the conduct of Dearlove (1986)

Study Code	**1**	**2**	**6**	**7**	**10**	**12**	**13**	**14**	**16**	**17**	**18**	**23**	**24**	**25**	**26**	**27**			
Study type	Seg II	Seg II	Seg II	Seg II	Seg II	Seg II	Seg II	Seg II	Seg II	Seg II	Seg II	Seg II	Seg II	Seg II	Seg II	Seg II			
Vehicle	Propanol/ water	0.5% CMC	3.0% cornstarch suspension	3.0% cornstarch suspension	0.25% CMC	None - sham	Water	Water	Tween80 and CMC	Water	Water	0.5% CMC	0.5 hydroxypropyl cellose	Not known	0.5% Tween and 0.7% CMC	Corn oil			
Dose vol (mL/Kg))	2	5	10	10	10/5	None - sham	10	0.75	5	Drinking water	5	5	5	0.83	5	2			
Route	Topical	Oral	Oral	Oral	Oral	Intra uterine implant	Oral	IV	Oral	Drinking water	Oral	Oral	Oral	I.V.	Oral	Oral			
First date of C-section	31/07/1984	31/01/1984	26/06/1984	09/10/1984	07/11/1983	21/05/1984	31/03/1984	21/08/1984	13/09/1983	17/04/1984	15/08/1983	26/02/1985	17/06/1985	22/02/1985	03/12/1984	23/04/1985			
Litters	20	20	12	17	12	15	17	17	16	15	16	15	20	19	18	14			
Fetuses	138	97	98	112	87	90	150	110	96	99	133	105	135	135	111	101			
Hyoid Angulated																	**mean**	**Min**	**Max**
Litters	0	0	0	6	0	2	0	2	0	0	3	3	1	5	3	3			
%	0	0	0	35	0	13	0	12	0	0	19	20	5	26	17	21	**10.6**	**0**	**35**
Fetuses	0	0	0	6	0	2	0	2	0	0	3	3	1	6	3	3			
%	0	0	0	5.3	0	2.2	0	1.8	0	0	2.2	2.8	0.7	4.4	2.6	3	**1.6**	**0**	**5.3**

In the rabbit studies no evidence of a developmental toxic potential was obvious. If were not considered incidental, the frequency of hyoid alae, angulated' was within the litter incidence of historical background data and a treatment relationship is considered unlikely, not least given the ten fold increases between dose levels. Furthermore, the highest dose was severely maternally-toxic (as evidenced by the death and occurrence of moribund animals and gastric ulceration) and this dose, in principle this dose should not be used for evaluation. The finding was also not observed in a second rabbit developmental toxicity study. Overall therefore, a relevance of this finding for human safety is doubtful particularly in absence of any other relevant findings and it's non-repeatability in other rabbit studies.

Table 26.6: Selected findings in the rabbit developmental toxicity study

Dose (mg/kg bw)	**0**	**2**	**20**	**200**
Litters evaluated	16	16	19	11
Fetuses evaluated	106	106	143	75
Hyoid alae, angulated				
Number of litter (%)	1 (6.2)	2 (12.5)	2 (10.5)	3 (27.3)
Number of fetuses (%)	1 (0.9)	2 (1.9)	2 (1.4)	3 (4.0)
Historical control data (HCD)				
Number of litters (%) Number of foetuses (%)	29 (8.63) 32 (1.29)			

Therefore it can be concluded that from the discussed rat and rabbit studies no evidence of an intri developmental toxic effect of Bifenox can be derived and there is no classification required for developmental toxicity. This is also supported by the fact that a published mouse developmental toxicity study with Bifenox did not reveal evidence of a developmental toxic effect.

The overall NOAEL for maternal toxicity was determined to be 50 mg/kg bw/day from a developmental study in rabbits (Anonymus, 1986). The NOAEL for developmental toxicity from the same study was 160 mg/kg bw/day based on the slight increased incidence of hyoid alae angulated at 200 mg/kg bw/day.

Table 26.7: Summary of findings (maternal and fetal) from the rabbit developmental toxicity study (Anonymus, 1986)

Dose level [ppm]	**0**	**5**	**50**	**160**	**500**	**1000**
Mortality	0/16	0/16	1/16	0/16	14/16	16/16
Abortion or signs of imminent abortion	1	3	0	2	1	Group died
	Clinical signs					
Soft faeces:	0	1	0	0	1	
Hypoactive:	0	0	0	4	13	10
Slightly hypoactive:	0	0	0	2	15	14
Thin:	0	0	0	1	5	1
Ashen or pale appearance:	0	0	0	1	9	4
Ataxia:	0	0	0	1	2	3
Tremors:	0	0	0	0	1	6
	Body weight					
Body weight (day 6) [g]	4260±462	4017±358	4139±350	4078±331	4110±375	4063±356
Body weight (day 11) [g]	4273±442	4064±332	4201±357	4141±319	3667±431	3379±374b

Body weight (final) [g]	4401±443	4192±304	4305±414	4144±363	4246a	Group died
Body weight gain [g]	281 ± 248	311 ± 134	284 ± 243	229 ± 282 (-19%)		
Food consumption (G.-days 20-24) [mg]	580 + 347	479 + 239 (-18%)	495 + 260 (-15%)	410 + 321 (-30%)		
	Reproductive indices and fetal paramaters					
Corpora lutea[b] / dam	12	11	13	12	16	
Implantations[b] / dam	6	7	6	8	12	
Implantation efficiency	53.0	71.0	66.3	71.7	75.0	
Mean early resorptions (%)	1 (19.9)	1 (22.3)	1 (10.6)	2 (16.3)	0 (0.0)	
Mean late resorptions (%)	0 (0.0)	0 (0.0)	0 (5.2)	0 (0.7)	0 (0.0)	
Mean total resorptions (%)	1 (19.9)	1 (22.3)	1 (15.8)	2 (17.0)	0 (0.0)	
Mean number dead foetuses (%)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	
Mean number live foetuses (%)	5 (80.1)	6 (77.8)	7 (84.2)	7 (83.0)	12 (100.0)	
Mean number of litters with viable foetuses	11	9	13	11	1	
Mean number of male foetuses (%)	3 (56.1)	3 (41.4)	4 (43.7)	4 (46.8)	7 (58.3)	
Mean viable fetal weights (g)	46.7	45.5	41.5	40.6	35.1	
Males	47.8	45.3	42.2	43.1	33.4	
Females	45.0	44.5	41.1	39.5	37.5	
	Malformations and variations					
	Fetal external malformations					
Fetuses evaluated N	59	62	96	82	12	
Live N	59	62	96	82	12	
Dead N	0	0	0	0	0	
Short tail						
Fetal incidence N(%)	0	0	1 (1.0)	0	0	
Litter incidence N(%)	0	0	1 (7.7	0	0	
	Hyperflexion forepaw (both)					
Fetal incidence N(%)	0	0	1 (1.0)	0	0	
Litter incidence N(%)	0	0	1 (7.7	0	0	
	Midline closure effect[c]					
Fetal incidence N(%)	0	0	0	1(1.2)	0	
Litter incidence N(%)	0	0	0	1(9.1)	0	
	Fetal visceral variations					
Litters evaluated N	11	9	13	11	1	
Fetuses evaluated (total)[d] N	59	62	96	82	12	
Fetuses evaluated (intact)[d] N	33	35	52	44		
Live N	59	62	96	82	12	
Dead N	0	0	0	0	0	
	Dark area, right side, maxilla, approximately 4 mm in length[d]					
Fetal incidence N(%)	0	0	1 (1.0)	0	0	
Litter incidence N(%)	0	0	1(7.7)	0	0	
	Left carotid arising from innominate artery					
Fetal incidence N(%)	0	0	1 (1.0)	0	1 (8.3)	

Litter incidence N(%)	0	0	1 (7.7)	0	1 (100.0)	
	Lung – intermediate lobe agenesis					
Fetal incidence N(%)	11 (18.6)	7 (11.3)	6 (6.3)	8 (9.8)	1 (8.3)	
Litter incidence N(%)	3 (27.7)	4 (44.4)	3 (23.1)	4 (36.4)	1 (100.0)	
	Lung – intermediate lobe hypoplastic					
Fetal incidence N(%)	0	1 (1.6)	1 (1.0)	0	0	
Litter incidence N(%)	0	1 (11.1)	1 (7.7)	0	0	
	Fetal visceral malformations					
Litters evaluated N	11	9	13	11	1	
Fetuses evaluated N	59	62	96	82	12	
Live N	59	62	96	82	12	
Dead N	0	0	0	0	0	
	Heart and/or great vessel anomaly[e]					
Fetal incidence N(%)	0	0	2 (2.1)	0	0	
Litter incidence N(%)	0	0	1 (7.7)	0	0	
	Midline closure effect[c] (also in external malformations above)					
Fetal incidence N(%)	0	0	0	1 (1.2)	0	
Litter incidence N(%)	0	0	0	1 (9.1)	0	
	Fetal skeletal variations					
Litters evaluated N	11	9	13	11	1	
Fetuses evaluated (total)[d] N	59	62	96	82	12	
Fetuses evaluated (intact)[d] N	33	35	52	44		
Live N	59	62	96	82	12	
Dead N	0	0	0	0	0	
	Skull-accessory bone					
Fetal incidence N(%)	1 (3.0)	0	2 (3.9)	1 (2.3)	0	
Litter incidence N(%)	1(9.1)	0	2 (15.4)	1 (9.1)	0	
	Frontal – incompletely					
Fetal incidence N(%)	0	0	2 (3.9)	0	0	
Litter incidence N(%)	0	0	1 (7.7)	0	0	
	Interparietal – incompletely ossified					
Fetal incidence N(%)	0	0	1 (1.9)	0	0	
Litter incidence N(%)	0	0	1 (7.7)	0	0	
	Thoracic vertebral centra misaligned					
Fetal incidence N(%)	0	1 (1.6)	0	0	0	
Litter incidence N(%)	0	1 (11.1)	0	0	0	
	Lumbar arches interrupted ossification					
Fetal incidence N(%)	0	0	1 (1.0)	1 (1.2)	0	
Litter incidence N(%)	0	0	1 (7.7)	1 (9.1)	0	
	Caudal vertebrae – misaligned					
Fetal incidence N(%)	0	0	1 (1.0)	0	0	
Litter incidence N(%)	0	0	1 (7.7)	0	0	
	26 presacral vertebra count					
Fetal incidence N(%)	3 (5.1)	6 (9.7)	8 (8.3)	8 (9.8)	0	

Litter incidence N(%)	3 (27.3)	2 (22.2)	6 (46.2)	5 (45.5)	0	
	Centra bipartites					
Fetal incidence N(%)	0	0	1 (1.0)	0	0	
Litter incidence N(%)	0	0	1 (7.7)	0	0	
	Sternebra 5th not ossified					
Fetal incidence N(%)	9 (15.3)	13 (21.0)	10 (10.4)	12 (14.6)	1 (8.3)	
Litter incidence N(%)	6 (54.5)	5 (55.6)	6 (46.2)	6 (54.5)	1 (100.0)	
	Sternebra 5th bipartite					
Fetal incidence N(%)	1 (1.7)	0	1 (1.0)	0	0	
Litter incidence N(%)	1 (9.1)	0	1 (7.7)	0	0	
	Sternebra 6th bipartite					
Fetal incidence N(%)	1 (1.7)	0	2 (2.1)	2 (2.4)	0	
Litter incidence N(%)	1 (9.1)	0	2 (15.4)	2 (18.2)	0	
	Sternebra 6th not ossified					
Fetal incidence N(%)	1 (1.7)	1 (1.6)	4 (4.2)	5 (6.1)	0	
Litter incidence N(%)	1 (9.1)	1 (11.1)	3 (23.1)	4 (36.4)	0	
	Sternebra 2nd not ossified					
Fetal incidence N(%)	0	0	0	1 (1.2)	0	
Litter incidence N(%)	0	0	0	1 (9.1)	0	
	Sternebra 2nd bipartite					
Fetal incidence N(%)	0	0	0	1 (1.2)	0	
Litter incidence N(%)	0	0	0	1 (9.1)	0	
	Sternebrae Misaligned					
Fetal incidence N(%)	0	0	0	1 (1.2)	0	
Litter incidence N(%)	0	0	0	1 (9.1)	0	
	13th full rib –unilateral					
Fetal incidence N(%)	9 (15.3)	0	7 (7.3)	8 (9.8)	3 (25.0)	
Litter incidence N(%)	6 (54.5)	0	6 (46.2)	5 (45.5)	1 (100.0)	
	13th Rudimentary rib – unilateral					
Fetal incidence N(%)	12 (20.3)	6 (9.7)	22 (22.9)	11.8(13.4)	1 (8.3)	
Litter incidence N(%)	7 (63.6)	5 (55.6)	9 69.2()	8 (72.7)	1 (100.0)	
	13th full ribs –bilateral					
Fetal incidence N(%)	10 (16.9)	12 (19.4)	29 (30.2)	20 (24.4)	1 (8.3)	
Litter incidence N(%)	7 (63.6)	5 (55.6)	12 (92.3)	8 (72.7)	1 (100.0)	
	Ribs 13th Floating					
Fetal incidence N(%)	3 (5.1)	3 (4.8)	1 (1.0)	5 (6.1)	0	
Litter incidence N(%)	2 (18.2)	2 (22.2)	1 (7.7)	4 (36.4)	0	
	13 Rudimentary ribs - bilateral					
Fetal incidence N(%)	1 (1.7)	1 (1.6)	6 (6.3)	8 (9.8)	1 (8.3)	
Litter incidence N(%)	1 (9.1)	1 (11.1)	5 (38.5)	6 (54.4)	1 (100.0)	
	Ribs - forked					
Fetal incidence N(%)	1 (1.6)	1 (1.0)	1 (1.0)	1 (1.2)	0	
Litter incidence N(%)	1 (11.1)	1 (7.7)	1 (7.7)	1 (9.1)	0	
	Ribs – interrupted ossification					
Fetal incidence N(%)	0	0	0	1 (1.2)	0	
Litter incidence N(%)	0	0	0	1 (9.1)	0	
	Rib(s) – extra					

Fetal incidence N(%)	0	0	0	1 (1.2)	0	
Litter incidence N(%)	0	0	0	1 (9.1)	0	
	First thoracic rib(s) small					
Fetal incidence N(%)	0	0	1 (1.0)	0	0	
Litter incidence N(%)	0	0	1 (7.7)	0	0	
	Metacarpals and phalanges less than 19 count					
Fetal incidence N(%)	0	0	1 (1.0)	0	0	
Litter incidence N(%)	0	0	1 (7.7)	0	0	
	Metacarpals and phalanges misaligned					
Fetal incidence N(%)	0	0	0	0	2 (16.7)	
Litter incidence N(%)	0	0	0	0	1 (100.0)	
	Skeletal malformations					
	Centra fused					
Fetal incidence N(%)	0	0	2 (2.1)	0	0	
Litter incidence N(%)	0	0	1 (7.7)	0	0	
	Vertebral anomaly with associated rib anomaly					
Fetal incidence N(%)	0	0	1 (1.0)	0	0	
Litter incidence N(%)	0	0	1 (7.7)	0	0	
	Stenebrae fused					
Fetal incidence N(%)	0	0	0	1 (1.2)	0	
Litter incidence N(%)	0	0	0	1 (9.1)	0	
	Ribs fused					
Fetal incidence N(%)	1 (1.7)	0	0	0	0	
Litter incidence N(%)	1 (9.1)	0	0	0	0	
	Summary of External, visceral and skeletal findings by fetus					
Fetuses evaluated N	59	62	96	82	12	
Fetuses with any malformation N (%)	1 (1.7)	0	6 (6.3)	2 (2.4)	0	
Litters evaluated N	11	9	13	11	1	
Litters with any malformation N	1	0	3	2	0	
Percent with any malformation	9.0	0	23.1	18.2	0	
	Total findings by litter					
	External					
Litters with variants N (%)	0	0	0	0	0	
Litters with malformations N (%)	0	0	2 (15.4)	1 (9.1)	0	
	Visceral					
Litters with variants N (%)	3 (21.3)	4 (44.4)	5 (38.5)	4 (36.4)	1 (100.0)	
Litters with malformations N (%)	0	0	1 (7.7)	1 (9.1)	0	
	Skeletal					
Litters with variants N (%)	11 (100.0)	9 (100.0)	13 (100.0)	11 (100.0)	1 (100.0)	
Litters with malformations N (%)	1 (9.1)	0	1 (7.7)	2 (18.2)	0	

[a] Only one survivor by this point
[b] all animals started losing bodyweight from the beginning of dosing and were lost between day 8 and 24
[c] liver protruding through umbilicus
[d] Fetuses evaluated (Total) includes all foetuses, those without heads and those with heads (foetuses evaluated intact); whereas, foetuses evaluated (intact) include only foetuses with heads.
[e] one dextrocardia, one major heart anomaly.

Mean number of corpora lutea and implantations as well as foetal viability and foetal sex distribution were comparable for all groups. Mean foetal body weights decreased across groups in a dose related, but not statistically significant manner. Foetal skeletal and visceral variations were noted in all groups with foetuses available for examination. The incidence was not dose related. A single malformation was observed in the control group. Six foetuses in three litters at 50 mg/kg bw/day had malformations and two foetuses in two litters at 160 mg/kg bw/day. The single litter available for evaluation at 500 mg/kg bw/day had no malformations.

Signs of slight maternal toxicity were noted at 160 mg/kg bw/day. At 50 mg/kg bw/day, one female was found dead during the treatment period (prior to dosing on Day 15). No other evidence of maternal toxicity was observed. No abortions occurred in this group. Body weights and food consumption were comparable to the control values. Foetal malformations in this group included hyperflexed paws, two heart anomalies (in one litter), and one litter containing foetuses with fused vertebral centra and one foetus with multiple anomalies including the vertebral column.

Marked maternal toxicity resulted from doses at and above 500 mg/kg/day and included increased incidences of death, abortion, and reduced body weight gain and food consumption.

The incidence of spontaneous abortions did not occur in a dose related pattern; therefore, it is not clear whether the three abortions are attributable to treatment with Bifenox technical. Except for these three abortions, females had no observable signs of adult or foetal toxicity or teratogenicity at 160 mg/kg bw/day. Foetal malformation did not occur in a dose related pattern.

No increased incidence of the variation (hyoid alae angulated) noted in the Anonymus (1986) study was noted.

On the basis of these observations, the maternal no-effect level (NOEL) was proposed to be 50 mg/kg/day.

However, without any effects noted on foetuses it is considered that the developmental NOAEL is 160 mg/kg bw/day.

10.10.6 Comparison with the CLP criteria

Category 1 Known or presumed human reproductive toxicants

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

Application of the classification criteria of Annex I to Regulation (EC) 1272/2008 to the available body of reproductive toxicity data for Bifenox indicate that a classification into category 1 can be ruled out because the substance is not known to cause reproductive toxicity in humans. Furthermore, there is no evidence that Bifenox adversely affects sexual function and fertility or development in the absence of other toxic effects.

Category 2 Suspected human reproductive toxicants

Substances are classified in Category 2 for reproductive toxicity when there is some evidence from humans or experimental animals, possibly supplemented with other information, of an adverse effect on sexual function and fertility, or on development, and where the evidence is not sufficiently convincing to place the substance in Category 1. If deficiencies in the study make the quality of evidence less convincing, Category 2 could be the more appropriate classification.

Such effects shall have been observed in the absence of other toxic effects, or if occurring together with other toxic effects the adverse effect on reproduction is considered not to be a secondary non-specific consequence of the other toxic effects.

Bifenox did not adversely affect fertility or general reproductive performance. There were no teratogenic effects evidenced in rats or rabbits induced by Bifenox.

10.10.7 Adverse effects on or via lactation

Table 27: 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, 2-generation reproduction and chronic toxicity GLP / not fully compliant to OECD 416	Bifenox Batch # 9401021 Purity: 99% Dose levels: 125, 750, 4500 ppm	4500 ppm Parental (systemic): decreased body weight gain Reproductive: decreased pup and litter weight at weanling in F1 and F2 generation NOAEL: Chronic toxicity: 44.5 mg/kg bw/day Reproduction: 148 mg/kg bw/day	Anonymus, 1995

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

In a 2-generation study in the rat during the lactation period, the mean body weight gain of litter and pups was lower at top dose and on day 21 weights were approximately 80% of control. The reduction in litter and pup weights at 4500 ppm were statistically significant lower than controls but were accompanied by (slight) parental toxicity (evidenced by reduced body weight gain at top dose). There was no treatment related incidence of abnormal lactation or pups suckling abnormally, and weight disturbance was considered to be due to systemic toxicity of material passed through the milk. Based on this, these effects were not considered to be relevant for a classification with regard to reproduction toxicity.

10.10.9 Comparison with the CLP criteria

There are no clear evidence of adverce effects in the offspring due to transfer bifenox in the milk nor evidences that bifenox interferes with lactation. Adverse effect on the quality of the milk has not been shown. Absorption, metabolism, distribution and excretion studies have not indicated the likelihood that the substance is present in potentially toxic levels in breast milk and are hence not considered to be relevant for a classification with regard to effects on or via lactation.

10.10.10 Conclusion on classification and labelling for reproductive toxicity

Bifenox does not require classification for reproductive toxicity.

RAC evaluation of reproductive toxicity

Summary of the Dossier Submitter's proposal

Fertility

The DS summarised one two-generation reproductive toxicity study in rats (Anonymous, 1995) that was not fully compliant with OECD TG 416 (deviations from the guideline were not specified). For the F0 generation, the DS stated that 28 rats per sex and dose group were fed diets containing 0, 125, 750, and 4500 ppm of bifenox (purity: 99 %). For the F1 parental generation, 24 male and 24 female weanlings were selected to produce the F2 generation.

A slight reduction in body weight gain was observed in F0 and F1 high dose females during the premating and gestation periods. However, weight gains were increased compared to controls during lactation for both generations. Thus, final body weights of high dose dams were similar to controls. No treatment related clinical signs were noted. No statistically significant changes in fertility indices, duration of gestation, gestation indices, mean number of implantation sites, or mean number of pups born per litter were observed in either generation. A non-statistically significant reduction in viability indices on days 0 to 4 and in survival indices throughout lactation was reported for the mid and high dose F2 pups. Moreover, pup body weights were statistically significantly reduced in high dose F1 and F2 pups on day 21 of lactation (in both males and females). The DS attributed these reductions to the increased weight gain of dams during lactation but provided no further elaboration on this hypothesis.

In contrast, in their conclusion on this endpoint the DS pointed out that there was a significant reduction in body weight gain of dams during lactation days 1 to 14 of up to 42 %. They therefore considered effects on pup body weight not relevant for classification based on maternal toxicity. RAC notes that this number was not extractable from the data tables provided with the study summary but could be confirmed with data from the full study report.

Since no effects were observed in any of the reported fertility parameters, the DS concluded that no classification for effects on fertility is warranted.

Development

The DS summarised one developmental toxicity study in rats (Anonymous, 1987) and two developmental toxicity studies in rabbits (both with reference listed as Anonymous, 1986). In all three studies, the top doses led to mortalities and severe maternal toxicity.

In rats, reproductive performance was not affected by treatment. The only findings in foetuses were an increased number of large fontanelles at the top dose (3600 mg/kg bw/d) and a dose-related increase in supernumerary ribs (within the historical control range). For the fontanelle findings, no size definition was available and they were deemed of doubtful relevance by the DS since no other head bone variations were

observed. Moreover, the high dose was well above the limit dose.

In the first rabbit study, reproductive parameters were not affected but only 17, 16, 20, and 16 rabbits were pregnant in control, 2, 20, and 200 mg/kg bw/d dose groups, respectively. In the high dose group, 3 dams died, and 3 additional dams aborted and were sacrificed preterm. In this dose group, body weight gain was markedly reduced (by 57 %, the reduction occurred over GD 6-12). The only finding in foetuses was a skeletal alteration termed as "hyoid, alae, angulated" at the top dose which occurred at percentages within the provided historical control range. The DS noted that dose spacing was unusual in this study, with a tenfold increase between dose groups.

In the second rabbit study, both of the highest dose groups (500 and 1000 mg/kg bw/d) were severely affected by mortality. All dams died in the top dose group and 14/16 dams died in the second highest dose group. In the latter, one dam aborted. Thus, only one viable litter was produced in this group. Only single incidences of skeletal and visceral malformations were observed with no dose-dependence (only taking into account the dose groups without maternal mortality, i.e. 0, 5, 50, and 160 mg/kg bw/d). Some skeletal and visceral variations (delayed ossification, supernumerary ribs, intermediate lung lobe agenesis) were also observed but again at single incidences and/or without a dose-response relationship. The DS concluded that none of the findings was treatment related.

The DS considered the available data on developmental toxicity reported in rats and rabbits not sufficient for classification. They also mentioned a published study in mice, that supported their assessment, but did not report any details. Thus, the DS proposed **no classification** for developmental effects.

Effects on or via lactation

No details whatsoever were provided in the two-generation study on quality or quantity of the milk, and no data on the transfer of bifenox to milk are available. Therefore, the DS argued that it was not possible to link effects seen on high dose pup weight in this study to a transfer of bifenox to the milk or an effect of bifenox on lactation performance. Thus, they concluded that **no classification for effects on or via lactation** is warranted.

Comments received during consultation

One Member State Competent Authority (MSCA) commented on this hazard class and supported no classification for both fertility and development. They requested more details on achieved doses in the two-generation study and on a study mentioned in the CLH report concerning developmental toxicity, which the DS provided. These details are included in the assessment section below.

Assessment and comparison with the classification criteria

Fertility

No human data on adverse effects of bifenox on sexual function and fertility are available.

In a two-generation reproductive toxicity study, Sprague Dawley CD rats received bifenox at concentrations of 0, 125, 750, and 4500 ppm in their diets. Doses achieved are summarised in the table below.

***Table**: Mean test substance intakes for different periods of the 2-generation study in rats in mg/kg bw/d. Table as provided by the DS during consultation.*

	125 ppm		**750 ppm**		**4500ppm**	
	m	**f**	**m**	**f**	**m**	**f**
F0 week 1-10	8.5±2	11.7±1.4	56.8±11	69.4±7.7	343±64	421±49
F0 week 13-16	7.3±0.4		44.5±2.3		276±12.3	
Week1, 2, 3 of gestation		10.9±0.25		63.6±0.57		405±6.2
Week1, 2, 3 of lactation		24.4±7.6		149±45		878±228
F1week 4-15	11.4±4.2	13±3.7	68.6±25	76.6±21	441±166	501±159
F1 week 18-21	7.5±0.22		44.7±1.7		291±11	
Week1, 2, 3 of gestation		11.3±0.9		63.6±3.2		436±5.5
Week1, 2, 3 of lactation		24.8±7.4		148±45		904±232

The study was claimed to have been "not fully compliant" with OECD TG 416 but the only deviation listed was the reduction of the mating period to one week. Moreover, RAC notes that no results concerning male reproductive organ weights or sperm parameters were reported in the study summary provided with the CLH report. The only parameter mentioned was the male fertility index, which was unusually low in the F0 generation controls (68 %). Based on this, there was an increase in the male fertility index in this generation. Due to the low control value, this effect is considered of no toxicological relevance. In the F1 generation, fertility indices for control males and females were 92 %. While female fertility indices were not affected by treatment, male fertility indices were reduced in all treated F1 groups, the largest reduction being in the mid dose group (83 %). However, other parameters (gestation index, live birth index, viability index on day 4, lactation index, and overall survival index) were not affected by treatment.

In the full study report, no female reproductive organ weights nor histopathological examinations were reported. For male reproductive organs, there was a slight dose-dependent increase in absolute epididymis weights (no relative weights reported) of F0 males that was not statistically significant. In F1 males this increase was also seen but without dose-dependence. Seminal vesicle weights were slightly and non-dose-dependently increased in F0 males, while in F1 males the increase was dose-dependent and statistically significant in high dose group. Overall, the slight weight changes were not reproduced within the generations.

Maternal body weight gains were slightly reduced in high dose females of both generations during premating and gestation (up to 12 % as compared to controls in F0 during gestation) but increased considerably during lactation. Absolute body weight values were only reported for females on premating week 0, GD0, and LD0 in the study summary available to RAC. For F1 females, body weight was reduced by 7.4 % as compared to controls on GD0. No gravid uterus weights were reported and no corrected body weights were provided. Body weights of high dose F1 and F2 pups were statistically significantly reduced at lactation day 21 but no information was provided on milk quality or quantity nor clinical signs in dams to which this reduction could have been attributed.

Single incidences of malformations were observed at the low dose (two pups from one litter) and high dose (one pup) F1 offspring but none were seen in F2 pups. Other findings occurred randomly and at single incidences and thus were not considered treatment related.

The following relevant results were extracted from the study reports of repeated dose toxicity studies:

- No organ weight changes were observed in the 28-day dermal toxicity study in rats, and no histopathological examinations were performed on these organs.
- In dogs, absolute and relative testes weights were increased in treated groups at terminal sacrifice to a similar extent in all three dose groups. Absolute and relative uterus weights were lower in low and high dose females but higher in mid dose females at interim sacrifice, while at terminal examination both mid and high dose females had slightly but not dose-dependently increased absolute and relative uterus weights. Absolute and relative weights of ovaries were dose-dependently increased at terminal sacrifice. Histopathology did not reveal any treatment related effects, single incidences of histological alterations were scattered over all dose groups including controls.
- In the 90-day oral toxicity study in mice, relative and absolute epididymis weights were dose-dependently decreased but without statistical significance nor histopathological correlates. For females no reproductive organ weights nor histopathological data were recorded.
- In the 90-day oral toxicity study in rats, the only alterations reported were an inversely dose-related decrease in relative weight of ovaries and increased relative testes weights at the mid and high dose in males. However, the mid dose was associated with reduced body weights >10 % in males and mortality in females, and the high dose was associated with mortality and reduced body weights >20 % in both males and females.
- In the mouse carcinogenicity study that was of overall low reliability, testes weights were slightly but not dose-dependently decreased at interim kill but were similarly increased at terminal kill. No reproductive organ weights were available for females. Histopathological changes in male and female reproductive organs at terminal sacrifice were consistent with the age of the animals and were observed in all dose groups, including controls.
- No treatment related histopathological alterations nor reproductive organ weight changes (uterus and prostate weights not recorded) were observed in the rat carcinogenicity study.

Overall, repeat dose toxicity studies did not show any consistent alterations in reproductive organ weights nor histopathological alterations that could have been be attributed to treatment.

Conclusion on classification

Overall, no effects on male or female fertility parameters (as far as recorded) nor statistically significant male reproductive organ changes were observed under the test conditions of a rat 2-generation reproductive toxicity study. Moreover, no reproducible changes in reproductive organ weights nor any treatment-related histopathological changes were recorded in repeated dose toxicity studies.

Thus, RAC concurs with the DS that based on the available data **no classification for**

effects on fertility is warranted.

Development

Effects relevant for classification for developmental effects are compiled in the table below.

***Table**: Effects observed in reproductive toxicity studies relevant for classification for developmental toxicity.*

Study	Effects on offspring	Effects on maternal animals	Limitations/ Remarks
2-generation study (1995) in rats (SD Charles River CD) OECD TG 416 with deviations purity: 99.2 % males (F0): 0, 8.5, 56.8, 343 mg/kg bw/d females (F0): 0, 11.7, 69.4, 421 mg/kg bw/d weeks 0-10 (for more details see table in fertility section) via diet	Top dose F1/ F2: statistically significantly reduced pup body weight on lactation day 21 in males and females (by 23 and 22 %, respectively, expressed as mean litter weight, for litter weight development see table below) low dose F1: 2 pups of 1 litter with multiple malformations in low dose top dose F1: 1 pup with multiple malformations in high dose F2: no malformations	Top dose: slightly ↓ body weight gain in both F0 and F1 dams during premating and gestation (by a maximum of 12 %) ↑ body weight gain over control values during lactation (LD14-21) slightly ↓ body weight during gestation GD0:-2.3 % F0, -7.4 % F1 GD7: -5 % F0, -8 % F1, GD14 -5.4 % F0, -8.4 % F1, GD20 -5.6 % F0, -7 % F1 and first two thirds of lactation LD0 -2.4 % F0, -2.1 % F1, LD7 -5 % F0, -6.8 % F1, LD14 -8.5 % F0, -4.3 % F1 (F1 controls did not gain weight from LD7 to LD14) both F0 and F1 high dose females reached control weights by LD21 *no other absolute weight values were reported in the study (except for starting bw)*	Deviations from TG: mating period shortened to 1 week no record of male reproductive organ weights, sperm parameters, and gravid uterine weights food consumption slightly reduced in F0 high dose females during lactation (mean -5%) and F1 females LD14-LD21 (-5.5 %)
Prenatal developmental toxicity study (1987) in rats (Crl: COBS CD (SD) BR) OECD TG 414 with deviations purity: 98 % 0, 225, 900, 3600 mg/kg bw/d as suspension in 1 % aq. methylcellulose gavage	Control: foetal incidence of supernumerary ribs: 1 % large fontanelles: 2 % 225 mg/kg bw/d: foetal incidence of supernumerary ribs: 2.2 % large fontanelles: 2.6 % 900 mg/kg bw/d: foetal incidence of supernumerary ribs:	Control: 7/25 not pregnant 17 live litters 225 mg/kg bw/d: 5/25 not pregnant +35 % water consumption GD13-19 20 live litters 900 mg/kg bw/d: 3/25 not pregnant +35 % water consumption GD13-19 1 total resorption 21 live litters	Deviations from TG: dose spacing top dose well above limit dose (dose selection based on preliminary study in which 3000 mg/kg bw/d resulted in reduced maternal weight gain) dosing at GD 6-15 gravid uterine weights and sex ratio of foetuses not reported

	0.8 % large fontanelles: 2 % <u>3600 mg/kg bw/d</u>: foetal incidence of supernumerary ribs: 6.4 % (HCD 0.9 - 7.1 %) large fontanelles: 10.1 %	<u>3600 mg/kg bw/d</u>: mortality 5/25 5/25 not pregnant salivation, stained mouth, patchy hair loss +42 % water consumption GD13-19 15 live litters *no changes in body weight or body weight gains in any dose group compared to controls*	
Developmental toxicity study in rabbits (1986) (NZW) OECD TG 414 with deviations purity: 98.2 % 0,20, 200 mg/kg bw/d in 0.5 % aq. carboxymethyl cellulose gavage	<u>Control:</u> incidence of hyoid, alae, angled: 6.2 % (litter) 0.9 % (foetal) <u>2 mg/kg bw/d</u>: incidence of hyoid, alae, angled: 12.5 % (litter) 1.9 % (foetal) <u>20 mg/kg bw/d</u>: incidence of hyoid, alae, angled: 10.5 % (litter) 1.4 % (foetal) <u>200 mg/kg bw/d</u>: ↓ body weight (-8.3 %) incidence of hyoid, alae, angled: 27 % (litter) 4 % (foetal) (HCD 0-35 % litter, 0-5.3 % foetal)	<u>Control:</u> 3/20 not pregnant 16 live litters <u>2 mg/kg bw/d</u>: 4/20 not pregnant 16 live litters <u>20 mg/kg bw/d</u>: ↑* number of dams with soft or liquid faeces; alopecia 19 live litters <u>200 mg/kg bw/d</u>: mortality 4/20 ↑* number of dams with dried or no faeces gastric ulcerations in 6/20 ↓ food consumption (-7.5 %) ↓ food conversion to body weight % w/w (-52 %) ↓ body weight gain (-57 %) ↓ body weight GD29 (-3.5 %, no values for other days reported) 3 abortions 4/20 not pregnant 11 live litters	Deviations from TG: unusual dose spacing dosing GD 6-18 low number of pregnant rabbits/ litters gravid uterine weights and sex ratio of foetuses not reported
Developmental toxicity study in rabbits (1986) (NZW) OECD TG 414 with deviations bifenox technical purity: 97 % 0, 5, 50, 160, 500, 1000 mg/kg bw/d no vehicle reported gavage	<u>Control</u>: 1/1 foetus/litter with fused ribs 3 litters with visceral variations 11 litters with skeletal variations <u>5 mg/kg bw/d</u>: 0/0 foetuses/litters with malformations 4 litters with visceral variations 9 litters with skeletal variations	<u>Control</u>: 1 abortion 59/11 live foetuses/litters body weight GD6: 4260 ± 462 g body weight GD29: 4401 ± 443 g <u>5 mg/kg bw/d</u>: 3 abortions 62/9 live foetuses/litters -18 % food consumption body weight GD6: 4017 ± 358 g (-4.4 %) body weight GD29:	Two highest dose groups excluded from assessment due to (almost) 100 % mortality rate all litters showed skeletal variations **it was not clear from the presented data if foetuses with malformations had more than one malformation** deviations from TG: unusual dose spacing

	50 mg/kg bw/d: 6/3 foetuses/litters with malformations (2 fused centra, 1 vertebral anomaly, 2 heart anomaly, 1 hyperflexion of both forepaws) 5 litters with visceral variations 13 litters with skeletal variations 160 mg/kg bw/d: 2/2 foetuses/litters with malformations (1 midline closure defect, 1 fused sternebrae) 4 litters with visceral variations 11 litters with skeletal variations	4192 ± 304 g (-4.8 %) 50 mg/kg bw/d: mortality 1/16 96/13 live foetuses/litters -15 % food consumption body weight GD6: 4139 ± 350 g (-2.8 %) body weight GD29: 4305 ± 414 g (-2.1 %) 160 mg/kg bw/d: 2 abortions 82/11 live foetuses/litters 6/16 hypoactive 1/16 thin, pale, ataxia (not clear if the same animal) -30 % food consumption body weight GD6: 4078 ± 331 g (-4.3 %) body weight GD29: 4144 ± 363 g (-5.8 %) -19 % body weight gain	low number of animals and litters dosing GD 6-19 gravid uterine weights not reported
Teratogenicity study in rodents (Sprague Dawley rats and Swiss mice) Francis (1986) J Env Sci Health Part B 21(4):303-317 bifenox synthesised purity (alleged): > 99 % mice: 0, 10, 100 mg/kg bw/d GD 5-14 in corn oil p.o. rats: 0, 100 mg/mL in corn oil at GD 9, 10, 11, or 12 p.o.	Mice: 2 foetuses with exencephaly in one treated litter (dose not specified) no results for orally exposed rats reported	Mice: controls: 11 litters of 13 females 10 mg/kg bw/d: 8 litters of 10 females 100 mg/kg bw/d: 7 litters of 7 females no results for orally exposed rats reported	No guideline followed non-GLP non-standard dosing only one or two doses different number of animals per dose group no information on housing conditions or clinical signs in dams experiments with topical dosing on the tails at varying doses were not considered for assessment **study was considered of low quality and not relevant for classification purposes**

* *statistically significant*

***Table**: Body weight development of pups (as mean litter weight) of top dose group and respective dams as compared to controls (%) as well as viability indices (%) for all groups in 2-generation reproductive toxicity study in rats.*

	F1 pups	**F0 females**	**F2 pups**	**F1 females**
Live birth index 0-125-750-4500 ppm	99-99-97-99		100-99-99-99	
bw LD0/LD1 4500 ppm	-5.2	-2.4	-4.4	-2.1

bw LD4 4500 ppm	-6	no data	-6.1	no data
Viability index LD0-4 0-125-750-4500 ppm	91-86-89-92		91-92-89-84	
bw LD7 4500 ppm	-8.9	-5	-9.5	-6.8
bw LD14 4500 ppm	-15.4	-8.5	-12.9	-4.3
Lactation index LD4-21 0-125-750-4500 ppm	98-94-99-98		98-98-98-96	
bw LD21 4500 ppm	-23*	+/- 0	-22.2*	+/- 0
Survival index LD0-21 0-125-750-4500 ppm	89-81-86-88		89-89-81-80	

*statistically significant

Conclusion on classification

According to the CLP criteria, a classification of a substance in Category 1B is based on data that provide *clear* evidence of an adverse effect on development in the absence of other toxic effect, or if occurring together with other toxic effects the adverse effect on reproduction is considered not to be a secondary non-specific consequence of other toxic effects.

Substances are classified in Category 2 for reproductive toxicity when there is *some* evidence from human or experimental animals of an adverse effect on development, and where the evidence is not sufficiently convincing to place the substance in Category 1.

No human data is available on reproductive toxicity of bifenox. There were some effects observed on foetal development in rat and rabbit developmental toxicity studies but these were accompanied by maternal toxicity (as evidenced by mortality in the respective dose groups) and the incidences were within historical control ranges (where data were provided). Moreover, these effects were not consistently observed in the studies. Malformations consisted of scattered single incidences of various types of external, skeletal, and visceral changes.

There was an effect on pup body weight seen in the 2-generation toxicity study in rats during lactation. High dose pup body weight was lower as compared to controls over the whole lactation period. The difference was statistically significant only on lactation day 21 in both F1 and F2 pups (see table above). Possibly, pups began to consume their mothers' diet and the decreased weight in the late days of lactation was due to a direct effect of the substance. Moreover, no effects were observed on the number of live pups at birth, viability index, lactation index, and overall pup survival in F1 pups and only slight, non-significant reductions of the viability index and overall survival in F2 pups. Thus, RAC considers the effects not sufficient for classification.

Overall, studies were not compliant with current guidelines regarding exposure period, dose spacing, and reporting of parameters. Moreover, bifenox was suspended in aqueous (carboxy)methyl cellulose in the rat developmental toxicity study and the first rabbit developmental toxicity study (no vehicle was reported for the second rabbit study). The choice of the vehicle was not justified in the study summaries available to RAC and seemed unusual given the fact that bifenox is lipophilic (with logKow between 3.64 and

4.48 according to Bates (2000c) and GESTIS Substance Database). It was not reported in the study summaries whether the homogeneity and stability of the test substance was ensured in the suspensions prior to administration.

Additional data from a published study were considered of low quality due to poor reporting and overall non-standard protocol and were not used in the assessment.

Overall, RAC concurs with the DS that **no classification for developmental effects is warranted**.

Effects on or via lactation

In the 2-generation reproductive toxicity study in rats, statistically significantly reduced body weights were observed in high dose F1 and F2 pups at LD21 as compared to respective controls. However, no data are available on the quality or quantity of the milk after exposure to bifenox. It is possible that the effect on pup body weight was due to pups beginning to consume their mothers' feed in the late days of lactation. Moreover, the bioavailability of bifenox is assumed to be limited. Only marginal amounts of the substance were recovered from the tissues 48 hours after oral administration. Thus, overall a transfer to milk at relevant concentrations seems unlikely. Therefore, RAC concurs with the DS that **no classification for effects on or via lactation is warranted**.

10.11 Specific target organ toxicity-single exposure

In acute animal testing bifenox was of minimal oral toxicity. Alopecia was observed in rats after dosing with 5000 mg/kg bw. Gas in the stomach and intestines was noted among mice that died in the acute toxicity test. Other signs noted in mice included inactivity, unsteady gait and shivering. However, from subchronrc animal testing data it is assumed that blood parameters may be affected adversely (i.e. reduced RBCcount) after poisoning. Thus signs of anemia and cyanosis may not be excluded.

No specific data on humans are available.

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

Adverse effects, that were noted in acute toxicity studies in experimental animals included faecal staining, soft stool and hypoactivity. Those clinical findings were related to acute systemic toxicity due to a single exposure at very high dose levels and were fully reversible within the period of the experiment. Neither the adverse effects nor necropsy findings in those acute toxicity studies could be related to a specific target organ toxicity. Bifenox is of low toxicity.

No human data available on specific target organ toxicity of Bifenox. No clinical cases are reporterted. There are no observations in humans indicating any adverse effects upon a single dose of Bifenox. Bifenox is of low toxicity.

10.11.2Comparison with the CLP criteria

Specific target organ toxicity (single exposure) is defined as specific, non lethal target organ toxicity arising from a single exposure to a substance or mixture. All significant health effects that can impair function, both reversible and irreversible, immediate and/or delayed.

No avers effects were noted in acute studies in experimental animals and there are no observations in humans indicating any adverse effects upon a single dose of Bifenox. Bifenox is of low toxicity.

10.11.3Conclusion on classification and labelling for STOT SE

Bifenox does not require classification for specific target organ toxicity – single exposure.

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

Summary of the Dossier Submitter's proposal

No human data are available to indicate specific target organ toxicity after single exposure to bifenox. Adverse effects noted in acute toxicity studies included faecal staining, soft stools, hypoactivity, unsteady gait, and shivering. However, no specific target organ toxicity was observed and effects occurred at doses above the guidance values for STOT-SE classification.

Thus, the DS proposed **no classification** for STOT-SE.

Comments received during consultation

One MSCA commented on this hazard class and supported no classification.

Assessment and comparison with the classification criteria

No human data are available.

The acute oral toxicity study in rats was performed as a limit test at a dose of 5000 mg/kg bw. Results from this study are therefore not useful for STOT-SE classification (cut-off value for the oral route is 2000 mg/kg bw). In mice, inactivity, unsteady gait, and shivering were reported as clinical signs but it was not clear from the study summary available to RAC at which doses these effects occurred.

The acute dermal toxicity study in rabbits was also performed as a limit test with a dose of 2000 mg/kg bw, which is at the boundary for STOT-SE 2 classification. Nasal and ocular discharge and decreased food consumption were occasionally observed. These effects were not sufficiently severe to trigger classification.

In the acute inhalation toxicity study, at a concentration of 0.91 mg/L as a dust, rats exhibited altered activity, ano-genital staining, soft stool, and increased secretory responses. Since no compound-related findings were observed during necropsy, none of the effects can be attributed to specific target organ toxicity.

In the preliminary study to the mouse micronucleus test (Anonymous, 2003), no signs of systemic toxicity were observed at doses of 30, 100, 100 or 2000 mg/kg bw according to the study summary. For the main study with doses of 500, 1000 and 2000 mg/kg bw, no clinical signs are mentioned in the very short study summary.

Conclusion on classification

Overall, effects observed in acute toxicity studies do not provide sufficient concern to trigger classification, and no systemic toxicity was observed in a mouse micronucleus test with a single gavage dose up to 2000 mg/kg bw.

Thus, RAC concurs with the DS that **no classification** for STOT-SE is warranted.

10.12 Specific target organ toxicity-repeated exposure

Table 28: 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**	Targets/main effects	**Reference**
Oral repeated dose toxicity			
Oral 90-day study in rats GLP / study in accordance with OECD 408	**Dose levels** 0, 300, 900 and 2500	Liver toxicity, slight blood toxicity (at top dose), kidney toxicity **NOAEL** [mg/kg bw/day] 300	Anonymus, 1982
Oral 52 weeks study in dogs GLP / study in accordance with OECD 409	**Dose levels** 20, 145 and 1000	Blood toxicity, liver toxicity, liver fibrosis **NOAEL** [mg/kg bw/day] 145	Anonymus 1986
Dermal repeated dose toxicity			
Percutaneous 28-day study in rats GLP / EC Directive 92/69/EC, OECD 410	**Dose levels** 15, 150 and 1000	Slight bw gain reduction, slight food consumption reduction, liver necrosis **NOAEL** [mg/kg bw/day] 150	Anonymus 2002

There are no observations in humans regarding repeated dose toxicity of Bifenox.

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

Animals repeatedly exposed to Bifenox in their diet developed mild signs of porphyria as suggested by small-altered blood parameters, kidney toxicity and some altered clinical chemistry, which could suggest hepatotoxicity Those effects are considered to be treatment related and occurred only at the highest dose levels of 900 mg/kg bw/day in rats (90 days) and 1000 mg/kg bw/day in dogs (90-days) or 47 mg/kg bw/day in mice (2 years).

Dermal repeated exposure revealed minor treatment related effects at the top dose (1000 mg/kg bw/d) that suggested hepatotoxicity are confirmed by systemic effects seen after oral repeated exposure.

Those effects occurred in all studies at the highest dose levels. Administration at lower dose levels did not exhibit any significant adverse effects due to the low toxicity of Bifenox.

10.12.2Comparison with the CLP criteria

The experimentally observed LOAELs exceeded the trigger values for classification regarding STOT RE of 100 mg/kg bw/day upon 90-day oral administration and 600 mg/kg bw/d upon 28-day dermal exposure.

10.12.3Conclusion on classification and labelling for STOT RE

Bifenox does not require classification for specific target organ toxicity upon repeated exposure.

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

Summary of the Dossier Submitter's proposal

No human data are available to indicate specific target organ toxicity after repeated exposure to bifenox. Adverse effects noted in repeat dose toxicity studies included mild signs of porphyria as evidenced by altered blood parameters, as well as kidney toxicity and liver toxicity as evidenced by altered clinical chemistry parameters. However, the DS concluded that these effects occurred only at doses above the guidance values for STOT-RE classification and therefore do not trigger classification.

The DS proposed **no classification** for STOT-RE.

Comments received during consultation

One MSCA commented on this hazard class and supported no classification.

Assessment and comparison with the classification criteria

No human data are available.

The DS summarised two repeat dose oral toxicity studies. One in rats with 90 days of exposure (Anonymous, 1982), and one 1-year study in dogs (Anonymous, 1986). Additionally, the DS summarised a dermal 28-day study in rats (Anonymous, 2002). All three studies were conducted according to OECD guidelines and GLP standards. RAC notes that in the oral rat study, the lowest dose group was three times the upper guidance value for STOT-RE category 2. Thus, the study results are not informative for specific target organ toxicity in the scope of a STOT-RE classification.

The carcinogenicity study in mice (Anonymous, 1982) was briefly mentioned in the STOT-RE section of the CLH report but it was not assessed for this endpoint, stating that effects occurred only at the highest dose level above the upper guidance value for category 2. The rat carcinogenicity study (Anonymous, 1987) employed doses above the converted cut-off value for STOT-RE category 2 classification. The study summary for the 2-generation

reproductive toxicity study in rats (Anonymous, 1995) provided only a statement that food consumption and body weight were reduced in high dose F0 males and females (343 and 421 mg/kg bw/d, respectively) during premating (9 weeks), thus at doses above the converted upper guidance value for category 2.

Another 90-day study (in mice) was reported in the study summaries provided with the annex but were not mentioned in the CLH report (Anonymous, 1979).

Relevant repeated dose toxicity studies are summarised in the table below.

***Table**: Repeat dose toxicity studies with bifenox employing doses relevant for STOT-RE classification. Effects provided for relevant doses only.*

Study	**Doses (mg/kg bw/d)/ no. of animals per group/ test substance and exposure method**	**Effects**	**Limitations/ Remarks**
Anonymous 1979 oral 90-day study in mice (B6C3F1) non-GLP / OECD TG 408	0, 23, 57.5, and 115* 25/sex/dose bifenox (purity: 98.3 %) via diet *doses calculated based on the NOAEL given in the study summary	dose-dependent stat. sign. increase in rel. and abs. liver weights for males: rel.: 13 %, 18 %, 29 %, respectively vs ctrl. abs.: 17 %, 21 %, 35 % vs. ctrl. in females, rel. and abs. liver weight only stat. sign. increased in top dose hepatocellular hypertrophy in 10/10 m and 3/10 f at the high dose	no haematological or clinical chemistry measurements, organ weights only for liver, kidney, testes, and epididymis histopathology limited to liver and kidney
Anonymous 1986 Oral 52 weeks study in dogs GLP / OECD TG 409	0, 20, 145, and 1000 6/sex/dose interim sacrifice at 26 weeks: 2/sex/dose bifenox (purity: 98 %) in a gelatine capsule	non-significant but dose-dependent increase in rel. and abs. weight of ovaries after one year of exposure	converted guidance values for 52-weeks oral exposure: Cat 1. ≤ 2.5 mg/kg bw/d Cat 2. ≤ 25 mg/kg bw/d converted guidance values for 26-weeks oral exposure: Cat 1. ≤ 5 mg/kg bw/d Cat 2. ≤ 50 mg/kg bw/d
Anonymous 1982 Oral 104-weeks study in mice (Charles River CD SD) GLP/ OECD TG 453 (1981)	0, 7, 30, 147 for males 0, 9, 35, 179 for females 60/sex/dose interim sacrifice at 1 year: 10/sex/dose bifenox (purity: 98.3 %) via the diet	low dose males, terminal sacrifice: 2/60 kidney cortex atrophy vs 0/58 in ctrl (1/58 at mid dose, 6/57 at high dose) 25/60 convoluted tubule hypertrophy vs 5/58 in ctrl (39/58 at mid dose, 42/57 at high dose)	converted guidance values for 104-weeks oral exposure: Cat 1. ≤ 1.25 mg/kg bw/d Cat 2. ≤ 12.5 mg/kg bw/d non-neoplastic results only for terminal sacrifice

		platelet count -16 % reticulocytes +7 % (no other haematology parameters reported in the summary) low dose females: 8/58 convoluted tubule hypertrophy vs 0/52 in ctrl (2/56 at mid dose, 4/58 at high dose) 35/58 urinary bladder multifocal inflammation vs 25/52 in ctrl 33/58 liver inflammation vs 21/52 in ctrl platelet count +67 % reticulocytes +1 % (no other haematology parameters reported in the summary)	haematology only for 10/sex/dose 3 females were pregnant only weekly observations instead of daily inflammation in females may have been be due to an infection overall low reliability, as described in more detail in carcinogenicity section
Anonymous 2002 Percutaneous 28-day study in rats (SD Crl:CD) GLP / EC Directive 92/69/EC, OECD 410	0, 15, 150, and 1000 5 sex/dose bifenox (purity: 98.2 %) suspended in arachis oil for 6 h/d on approx. 10 % of the body surface under semi-occlusive dressing	no clinical signs and no alteration of haematological or biochemical parameters at low or mid dose stat. sign. increased rel. liver weight in males (13 %) at high dose, slight increase at mid dose, absolute liver weight increased non stat. sign. in mid and high dose males and low and high dose females	converted guidance values for 28-day dermal exposure: Cat 1. ≤ 60 mg/kg bw/d Cat 2. ≤ 600 mg/kg bw/d 3 animals were exposed for 24 h on day 2 due to an error

In the 90-day mouse study, animals received bifenox *via* the diet in doses up to 115 mg/kg bw/d. There was a dose-dependent, statistically significant increase in relative and absolute liver weights in males of all dose groups, and females of the top dose as well as hepatocellular hypertrophy in 10/10 males and 3/10 females of the high dose group. No investigations of haematological parameters or clinical chemistry were performed. Body weights were slightly increased in some weeks in high dose males only, but the changes did not exceed 10 % as compared to controls according to the study summary. At terminal sacrifice, body weights were similar in all dose groups for both males and females. The described histopathological effects were considered adaptive by the examining pathologists. They were described as minimal or equivocal. Based on this, RAC considered them not sufficiently severe to trigger classification.

RAC notes that in the 1-year study in dogs, the measured parameters were reported only as percentage changes compared to controls, some parameters were not reported at all for some dose groups (i.e. marked as "no data available" in the results table), no absolute values were given and no absolute organ weights were reported in the study summary available but were extracted by RAC from the full study report. The only effect at a dose

relevant for classification was a non-statistically significant increase in relative and absolute weight of ovaries at the low dose. This effect itself does not trigger classification but was also observed in the higher dose groups above the guidance values and was dose-dependent. However, due to the low number of animals examined (n = 4 per dose group) the biological relevance of this finding is questionable. Moreover, it is already considered in the weight of evidence approach for fertility effects in the reproductive toxicity section.

In the study summary of the carcinogenicity study in mice, reporting of haematological parameters was confined to the platelet count and percentage of reticulocytes at terminal sacrifice from 10 animals/sex/dose. Some dose-dependent changes in the kidney were observed in males, but the only dose relevant for STOT-RE classification was the lowest dose. Thus, no firm conclusion on the relevance of these effects for classification can be drawn. Inflammation of the urinary bladder and liver was reported in low dose females at a high incidence but also in the control and other dose group females, indicating an infection rather than a substance related effect. Overall, the study seems unreliable, with 3 of the females being pregnant, and some animals escaping or being withdrawn from the study without any obvious explanation.

For the 28-day dermal study in rats, some alterations in liver weights of mid and high dose males and low and high dose females were recorded. Since a statistically significant effect was only reported for relative liver weight for males at the top dose (1000 mg/kg bw/d), which exceeds the upper converted guidance value for category 2, this finding is not relevant for classification.

Conclusion on classification

Overall, no effects were observed in repeat dose toxicity studies that would trigger classification. However, RAC notes that the studies and respective study summaries provided with the annex to the CLH report did not provide sufficient detail to thoroughly assess potential target organ toxicity. Therefore, RAC consulted the full study reports. Most of the studies employed too high doses for STOT-RE classification purposes. However, given the lack of effects raising concerns at doses above the guidance values for classification, it seems sufficiently safe to assume that no classifiable effects would have been triggered at relevant doses.

Based on the available data, RAC concurs with the DS that **no classification for STOT-RE is warranted.**

10.13 Aspiration hazard

Not relevant. Substance is a solid at RTP.

11 EVALUATION OF ENVIRONMENTAL HAZARDS

11.1 Rapid degradability of organic substances

Table 29: Summary of relevant information on rapid degradability

Method	Results	Remarks	Reference
Kinetic evaluation of aerobic degradation in soil (Simmonds and Burr, 1999) FOCUS 2006 & 2014	DT_{50} soil (persistence) at 20°C and 45% MWHC: - chloro phenyl label: Bifenox: 5.65 d (DFOP) Bifenox acid: 64.55 (SFO) - nitro phenyl label: Bifenox: 5.44 d (DFOP) Bifenox acid: 75.22 (SFO) DT_{90} soil (persistence) at 20°C and 45% MWHC: - chloro phenyl label: Bifenox: 111.70 d (DFOP) Bifenox acid: 214.4 (SFO) - nitro phenyl label: Bifenox: 116.90 d (DFOP) Bifenox acid: 249.90 (SFO)	^{14}C-chloro phenyl-Bifenox and ^{14}C-nitro phenyl Bifenox Soil: loam	O'Brien, 2016a
Kinetic evaluation of degradation in soil (Simmonds and Burr, 2000) FOCUS 2006 & 2014	DT_{50} & DT_{90} soil (persistence) at 20°C and 45% MWHC: - sandy loam, high microbial content: Bifenox: DT_{50} soil 14.64 d, DT_{90} soil 84.92 d (FOMC) Bifenox acid: DT_{50} soil 60.0 d, DT_{90} soil 199.30 d (SFO) - clay loam Bifenox: DT_{50} soil 3.96 d, DT_{90} soil 13.17 d (SFO) Bifenox acid: DT_{50} soil 86.47, DT_{90} soil 287.24 (SFO) - sandy loam, low microbial content Bifenox: DT_{50} soil 8.97 d, DT_{90} soil 29.81 d (SFO) Bifenox acid: DT_{50} soil 165.27, DT_{90} soil : 549.01 (SFO)	^{14}C-chloro phenyl-Bifenox and ^{14}C-nitro phenyl Bifenox Soils: sandy loam (high microbial content), clay loam, sandy loam (low microbial content)	O'Brien, 2016b
Kinetic evaluation of degradation in soil (metabolite, Heintze, 2003) FOCUS 2006 & 2014	DT_{50} & DT_{90} soil (persistence) at 20°C and 45% MWHC: Bifenox acid: - 2.2 loamy sand: DT_{50} soil 22.65, DT_{90} soil : 75.26 (SFO) - 2.3 sandy loam: DT_{50} soil 90.52, DT_{90} soil : 300.7 (SFO)	Bifenox acid Purity: 97.5% Soil: 2.2 loamy sand, 2.3 sandy loam and 3A loam	O'Brien, 2016c

Method	Results	Remarks	Reference
	- 3A loam: DT_{50} soil 26.53, DT_{90} soil : 88.13 (SFO)		
Kinetic evaluation of degradation in soil (metabolite, Morlock, 2005a and b) FOCUS 2006 & 2014	DT_{50} & DT_{90} soil (persistence) at 20°C and 45% MWHC: Aminobifenox - 2.2 loamy sand: DT_{50} soil 5.75, DT_{90} soil : 37.70 (FOMC) - 2.3 sandy loam: DT_{50} soil 4.89, DT_{90} soil : 16.24 (SFO) - 3A loam: DT_{50} soil 6.37, DT_{90} soil : 21.17 (SFO) - Aminobifenox acid - 2.2 loamy sand: DT_{50} soil 1.22, DT_{90} soil : 28.81 (FOMC) - 2.3 sandy loam: DT_{50} soil 1.58, DT_{90} soil : 20.06 (DFOP) - 3A loam: DT_{50} soil 0.55, DT_{90} soil : 10.94 (DFOP)	Aminobifenox and Aminobifenox acid Purity: 97.5% Aminobifenox and 97% Aminobifenox acid Soil: 2.2 loamy sand, 2.3 sandy loam and 3A loam	O'Brien, 2016d
Anaerobic degradation in soil OECD 307 (2002)	DT_{50} in soil (anaerobic): Metabolites: Bifenox acid under initial aerobic conditions. Under anaerobic conditions aminobifenox acid formed in major amounts of 29.3% AR.	[dichlorophenyl ring –U-^{14}C] Bifenox Soil: sandy loam	Hüben, 2016a
Soil photolysis US EPA 161-3 (1982)	DT_{50}: 41.3 days (irradiated) Metabolite: Bifenox acid 16.5% AR after 30 d (irradiated)	^{14}C-dichloro-Bifenox Purity: > 99% Soil: loam soil	Oddy and Roach, 1999
Field soil dissipation SETAC 1995, EPA OPPTS 835.6100, OECD 217	DT_{50} & DT_{90} soil (persistence), not normalised: Bifenox - N. Germany: DT_{50} soil 51, DT_{90} soil : 282 (DFOP) - N. France: DT_{50} soil 8.8, DT_{90} soil : 99.1 (DFOP) - S. France: DT_{50} soil 34.5, DT_{90} soil : 115 (SFO) - Spain: DT_{50} soil 22.9, DT_{90} soil : 75.9 (SFO) - S. Germany: DT_{50} soil 43.7, DT_{90} soil : 145 (SFO)	Formulated Bifenox, 480 g/L 5 sites: - N Germany (loamy sand) - N France (silt clay loam) - S France (loam) - Spain (clay loam) - S Germany (silt loam)	Gezahegne, 2016
Adsorption/ desorption OECD 106	Strong absorption to soil. K_{fOC} 4477-8070 L/kg 1/n 1.055-1.117	^{14}C-chlorophenyl Bifenox Soils: sandy loam, loamy sand and clay loam	Giraud, 1983
Adsorption/ desorption OECD 106	Strong absorption to soil. K_{fOC} 4400-23000 L/kg (n=3) 1/n 0.77-0.99 (n=3)	^{14}C-nitrophenyl Bifenox Soils: sand, sandy loam, silt loam, sandy clay loam	Spare, 1984
Adsorption/ desorption	Bifenox acid: moderately absorbed to soil,	^{14}C-chlorophenyl	Matla and Vonk, 1992

Method	Results	Remarks	Reference
(metabolite) Dutch batch adsorption guidelines	K_{fOC} 130-155 L/kg, 1/n 0.79-0.89 Aminobifenox: strongly absorbed to soil, K_{fOC} 3697-5024 L/kg, 1/n 0.70-0.77	Bifenox acid ^{14}C-chlorophenyl Aminobifenox Soils: sand (high humic content), loam, sand soil (low humic content)	
Adsorption/ desorption (metabolite) OECD 106	Aminobifenox acid: moderately to strongly absorbed to soil, K_{fOC} 417-3756 L/kg, 1/n 0.72-1.01	Aminobifenox acid (cold) Soils: fine sand, loam, sandy loam	Morlock, 2005c
Hydrolysis study US EPA 161-1	Half lives (1st order): -pH 4 buffer; 50ºC: stable -pH 5 buffer; 50ºC stable -pH 7 buffer; 25ºC 265 d -pH 9 buffer; 25ºC 4 d Metabolites: Bifenox acid > 10% (21.6% at pH 7 and 102.1% at pH 9 at end of incubation)	^{14}C-chlorophenyl Bifenox Purity: 99.2%	Crowe, 2000a
Hydrolysis study OECD 111 (2004)	pH 4, 7 and 9 at 50°C for 5 days: stable	^{14}C-dichlorophenyl bifenox acid Purity: > 98.8%	Hüben, 2016b
Direct photochemical degradation in water OECD 316 (2008)	Half lives (h): 22.9 to 80.3 in irradiated samples. Main photolysis products: 2,4-dichlorophenol, max. 11.5% AR after 48 hours, max. 6.6% AR at study end (168 h). Methyl-5-hydroxy-2-nitrobenzoate, max. 42.9% AR after 72 hours, max. 22.3% AR at study end (168 h).	^{14}C-chlorophenyl Bifenox Purity: 99.9%	Hüben, 2016c
Ready biodegradability OECD 301 B	11.8-14.0 % $ThCO_2$ after 28 d. The result of the study showed that Bifenox is not biodegradable within 28 days.	Bifenox, not specified	Lebertz, 1989
Aerobic mineralization in surface water of Bifenox OECD 309 (2004)	The DT_{50} were 4.5 d (lowest test concentration) and 3.7 d (highest test concentration) in water phase. Metabolite: Bifenox acid was identified in relevant amounts (max. 48.0% AR)	^{14}C-dichlorophenyl bifenox Purity: 100%	Traub, 2015
Kinetic evaluation of Water/ sediment study (Knoch, 1992) FOCUS 2006, 2014	-DT_{50} system (persistence): 0.02 d (FOMC) and 0.06 d (SFO) - DT_{50} water (persistence) 0.01 d (FOMC) and 0.07 d (SFO) - DT_{50} sediment (persistence) 0.02 d (FOMC) and 0.05 d (SFO) Metabolites: Aminobifenox bound to sediment at up to 64% AR and at 6.4% AR in the water phase. - DT_{50} system (persistence): 102.14 and	^{14}C-dichlorophenyl bifenox Purity: > 98.9% Systems: two (silty loam sand and silty loam)	Sulc, 2016

Method	Results	Remarks	Reference
	93.88 d - DT_{50} water (persistence): 0.83 and 3.00 d - DT_{50} sediment (persistence): 38.11 and 25.01 d) Bifenox acid one time > 5% (7.8%) in water. - DT_{50} water (persistence): 2.54 and 0.33 d, SFO Aminobifenox acid > 10% or > 5% for two successive time intervals in water phase.		
Degradation in air BBA Guideline part IV, 6-1 (phase 2)	No significant volatilisation of Bifenox from plant surfaces (up to 0.8 and 1.3% AR). No volatile metabolites found.	[ring-^{14}C] Bifenox System: volatilisation chamber, French beans	Kubiak, 1994a, b
Degradation in air BBA Guideline part IV, 6-1 (phase 2)	No significant volatilisation of Bifenox from soil surfaces (< 1.0%AR). No volatile metabolites found.	[ring-^{14}C] Bifenox System: volatilisation chamber, soil surface (sandy soil)	Jendrzejczak et al., 1994a, b
Transport via air	Bifenox displays low overall persistence, limited transfer potential and low travel distance (estimated at 89 km)	Bifenox	O'Brien, 2015

11.1.1 Ready biodegradability

The biodegradability of Bifenox was investigated in one ready biodegradability study (28 days). The 10% level was not reached within the 10 days from the beginning of the study. The biodegradation after 28 days was 14.0 and 11.8% for the 10 and 20 mg/L test concentrations, respectively. The result of the study showed that Bifenox is not biodegradable within 28 days, the criterion of 'rapid degradability' is not met.

11.1.2 BOD_5/COD

Please refer to point 11.1.1.

11.1.3 Hydrolysis

In the available hydrolysis study with Bifenox, the preliminary test showed Bifenox was stable at pH 4 and 5 at 50°C. In the main test at 25°C, the corresponding first order hydrolysis rate constant was determined and equivalent to a DT_{50} of 265 days and 4 days at pH 7 and 9, respectively. Bifenox acid was the only metabolite detected and occurred at maximum amounts at end of incubation of 21.6% AR at pH 7 and 102.1% AR at pH 9.

In the aqueous hydrolysis study conducted with the metabolite bifenox acid, the substance was determined to be hydrolytically stable at pH 4, 7 and 9 over a period of 5 days at 50°C and, therefore, no additional testing was required or was performed. The DT_{50} (25°C) is estimated to be > 1 year.

11.1.4 Other convincing scientific evidence

The adsorption and desorption in soil of Bifenox has been evaluated in two batch adsorption studies with ^{14}C-chlorophenyl and ^{14}C-nitrophenyl Bifenox. The studies showed that Bifenox is strongly adsorbed to

soil particles. The Freundlich adsorption coefficient K_{fOC} was found to be in the range of 4477 to 8070 L/kg (n=3) in the first study and ranging between 4400 L/kg and 23000 L/kg (n=3) in the second study. Therefore, Bifenox is considered to be immobile in soil.

The adsorption and desorption in soil of the major Bifenox metabolite formed under aerobic conditions, i.e. bifenox acid, and also of aminobifenox (formed in minor amounts under anaerobic conditions) has been evaluated in one study with three soils. It was found that bifenox acid is weakly adsorbed to soil particles. The K_{fOC} values in humic sand, loam and low humic sand were determined to be 145, 155 and 130 L/kg, respectively. Aminobifenox is strongly adsorbed to soil with high K_{fOC} values of 4611, 5024 and 3697 L/kg, respectively. In an additional study the adsorption properties of aminobifenox acid (major metabolite under anaerobic conditions) were determined in a laboratory experiment in three soils. It was found that aminobifenox acid was moderately to strongly adsorbed to the test soils with K_{fOC} values of 3756 L/kg (silty sand), 417 L/kg (sandy loam) and 636 L/kg (loamy sand).

Bifenox has a vapour pressure of 4.74×10^{-8} Pa at 20°C and a Henry law constant of $> 1.62 \times 10^{-4}$ Pa m^3 mol^{-1}. Bifenox may thus be considered as not volatile from soil or plant surfaces. Additionally, metabolism degradation studies in soil, water and water/sediment systems indicated there are no volatile breakdown products of concern from Bifenox. And moreover, the results of volatilisation studies from plant and soil surfaces conducted with Bifenox under controlled conditions showed negligible volatilisation of the substance from either surface.

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

In a field soil dissipation study, the residue levels of Bifenox and its metabolite bifenox acid were determined and additionally analysis for Nitrofen was made in five soil trials at sites in northern Europe (N-Germany, S-Germany and N-France) and southern Europe (S-France and Spain). A single autumn application of Fox was applied to bare soil at a nominal application rate of 757.5 g a.s./ha. Immediately after application, the soil surface was covered with a sand layer of 0.5 cm thickness. Soil samples were collected at day 0 (0-30 cm) and 11 additional timings (0-100 cm) over the course of 1 year.

In treated soil samples of all trials, residues of Bifenox were determined between 0.21 mg/kg and 0.52 mg/kg (as wet weight) 0 days after application (DAA) in the 0 to 10 cm layer. In all trials, residue amounts decreased to negligible levels by study end and were seldom found in relevant amounts in lower soil layers throughout the study.

Residues of bifenox acid ranged from < LOD to 0.018 mg/kg at 0 DAA. The residues of bifenox acid increased to a maximum between 0.14 mg/kg (Trial 1 at 90 +/- 3 DAA) to 0.23 mg/kg mg/kg (Trial 5 at 60 +/- 3 DAA) in the 0 to 10 cm layer. In the 10 to 20 cm layer a maximum from 0.035 mg/kg (Trial 2 at 120 +/- 3 DAA) to 0.095 mg/kg (Trial 4 at 60 +/- 3 DAA) was observed. Bifenox acid was not detected in any samples from soil layers below 30 or 40 cm, depending on the trial site. In all trials, no residues of nitrofen were detected (LOD < 0.03 mg/kg).

A kinetic evaluation of the five available trial datasets was performed to derive trigger and modelling endpoints of Bifenox and its metabolite bifenox acid according to the guidance of the FOCUS work group on degradation kinetics and EFSA. The derived DT_{50} / $DegT_{50}$ values (trigger and modelling endpoints – modelling endpoints normalised to 20°C and pF2) for Bifenox ranged from 8.8 (SFO) to 51 days (DFOP) and from 11.2 (SFO) to 23.3 days (SFO), respectively. For the metabolite bifenox acid, the derived DT_{50} / $DegT_{50}$ values (trigger and modelling endpoints – modelling endpoints normalised to 20°C and pF2) were ranging from 60.8 to 110 days (both SFO) and from 24.4 to 43.4 days (both SFO), respectively.

11.1.4.2 Inherent and enhanced ready biodegradability tests

No information available or required. Please refer to point 11.1.1.

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

In a water/sediment study with ^{14}C dichlorophenyl Bifenox, two natural systems 'Bickenbach' and 'Unter Widdersheim' were used. Bifenox was applied to the test systems at initial concentration of 0.33 mg/L. Bifenox rapidly disappeared from the two water/sediment systems within the first day.

Aminobifenox, the major metabolite was bound to the sediment in amounts up to 67% AR of the applied parent compound. No other metabolite was detected in the sediment throughout the study. Aminobifenox occurred to 6.4% AR in the water phase. Aminobifenox acid appeared in one system at a maximum of 12.7% AR in water 24 hours after treatment. This level decreased to 5.2% within the following 24 hours and did not reach amounts above 10% thereafter. In the other system, a maximum amount of 10.6% AR was found on day 14 and decreased to 3.1% AR at the consecutive sampling event. However, at day 105 still 5% AR found in the water accounted for this metabolite. Bifenox acid accounted for maximum 7.8% AR in water 48 hours after application and did not exceed 5% at any other sampling time.

Kinetic re-evaluation of the DT values was performed and the half-lives of Bifenox in the total system were 0.02 and 0.06 days in the 'Bickenbach' and 'Unter Widdersheim' system, respectively. For the metabolite bifenox acid, maximum DT_{50} value for the water compartment was 2.54 days (Bickenbach system), and since there was no occurrence in the sediment phase, this endpoint can be extrapolated to the total system as well. For the metabolite aminobifenox acid, for the whole system maximum DT_{50} value was 30.75 days (Bickenbach system). For the metabolite Aminobifenox acid, no appropriate kinetic fitting could be found.

Two studies on the route and rate of degradation of Bifenox were conducted. In the first study with radio-labelled [chloro phenyl-^{14}C] Bifenox and [nitro phenyl-^{14}C] Bifenox, there were no notable differences in the metabolism and rate of degradation of Bifenox between the two labels. Bifenox was moderately quickly degraded in one soil declining from mean 87.01-90.16% AR at day 0 to 9.04-9.59% AR after 120 days. One major metabolite was detected. Bifenox acid was observed accounting for maximum on day 14 of 63.8% AR (chloro phenyl label) and 60.87% AR (nitro phenyl label) and decreased to ca. 27% AR at study end forming bound residues (maximum 46% AR) and CO_2 (maximum ca. 11% AR). As minor metabolites, aminobifenox and aminobifenox acid occurred to a maximum levels of 1.15% AR (day 2) and 0.84% AR (day 90), respectively with no remarkable difference between the labels. In second study in three soils with radio-labelled [chloro phenyl-^{14}C] Bifenox, the active substance was moderately quickly degraded in the soils at 20°C declining from 98.07-95.01% AR at day 0 to 2.00-3.78% AR after 181 days. At 10°C, Bifenox degraded from 95.86% AR at day 0 to 10.80% AR at day 181. One major metabolite was detected. Bifenox acid was observed accounting for maximum on day 10 at 78.71% AR (clay loam) and decreased to 23.11% AR at study end forming bound residues (maximum ca. 52% AR) and CO_2 (maximum ca. 19% AR). As minor metabolites, aminobifenox and aminobifenox acid occurred to a maximum levels of 0.59% AR (day 181) and 2.58% AR (day 120), respectively with no remarkable difference between the soils. Incubation at 10°C resulted in a slower degradation of Bifenox and subsequently lower metabolite amounts formed.

In one study, the rate of degradation of bifenox acid (non-radio-labelled) was investigated. Bifenox acid was moderately to quickly degraded in the soils at 20°C declining from mean of 98.5-107.7% applied at day 0 to 2.84-35.8% applied after 120 days. The results were corrected for recovery of fortified samples at each sampling time.

Kinetic re-evaluation of the DT_{50} values was performed and DT_{50} values (at 20°C, not normalised to pF2) of Bifenox ranged from 3.96 to 14.64 days (n=5) and respective DT_{90} values ranged from 13.17 to 116.90 days.

For Bifenox acid the DT_{50} values (at 20°C, not normalised to pF2) ranged from 22.65 to 165.27 days (n=8) and respective DT_{90} values ranged from 75.26 to 549.01 days. For Aminobifenox the DT_{50} values (at 20°C, not normalised to pF2) ranged from 4.89 to 6.37 days (n=3) and respective DT_{90} values ranged from 16.24 to 37.70 days. For Aminobifenox acid the DT_{50} values (at 20°C, not normalised to pF2) ranged from 0.55 to 1.58 days (n=3) and respective DT_{90} values ranged from 10.94 to 28.81 days.

Soil metabolism in anaerobic (flooded) conditions was evaluated considering the intended uses of Bifenox with application in the autumn to winter cereals and oilseed rape. In a study with radio-labelled

[chloro phenyl-^{14}C] Bifenox, incubation of a treated soil for 6 days under aerobic conditions was followed by an anaerobic phase lasting up to 120 days. The amounts of Bifenox decreased quickly under aerobic conditions to 33.9% AR by day 6 after treatment and afterwards, under anaerobic conditions, decreased further and was not detected after 90 d of incubation. Bifenox acid built up during the aerobic incubation phase and increased to a concentration of 49.3% until 6 days after application of Bifenox. Afterwards, under anaerobic conditions, this major aerobic metabolite was further degraded to aminobifenox acid. No bifenox acid was detected any more from day 30 onwards. Beginning from day 14 after application, aminobifenox acid was detected indicating that amination takes place under anaerobic conditions. The amounts of aminobifenox acid increased slowly reaching maximum of 29.3% AR after 90 days of incubation and thereafter decreasing to 24.0% AR at the final sampling at day 120. Aminobifenox was detected in some samples but without a clear temporal trend and not at consecutive samplings with a maximal value of 8.6% AR observed after 30 days. Nitrofen was never detected (LOQ of the method 0.01 mg/kg; LOD: 0.003 mg/kg). Bound radioactive residues increased throughout the incubation, reaching a maximum value of 54.4% AR at the end of incubation (120 d). Only negligible amounts (< 1% AR) of volatile degradation products were found in the trapping solutions.

An additional non-GLP study on anaerobic soil degradation with non-radio labelled Bifenox demonstrated similar results to the above discussed GLP study with radio-labelled material. The initial aerobic phase was 7 days and was followed by a 90-day anaerobic phase. Analysis was made for the metabolites bifenox acid (maximum 49% of applied amount) and aminobifenox acid (maximum 8.9% of applied amount). Additionally, this study included detailed analysis for the metabolite nitrofen which was not found in any of the samples under either initial aerobic or main anaerobic conditions.

11.1.4.4 Photochemical degradation

The aqueous phototransformation of the test item Bifenox was studied under continuous artificial light for up to 168 hours in sterile aqueous media. The direct photolysis rate constant of Bifenox was determined using a single first order (SFO) kinetic model (KinGUI version 1.1). The $DegT_{50}$ values were 63.4, 80.3, 22.9 and 43.9 hours for [dichlorophenol-^{14}C] label (middle rate), [dichlorophenol-^{14}C] label (low rate), [benzoyl-^{14}C] label (middle rate) and [benzoyl-^{14}C] label (low rate), respectively. 2,4-Dichlorophenol as major metabolite of [dichlorophenyl-^{14}C]-labelled reached maximum levels of 11.5% AR and 6.2% AR after 48 h irradiation and decreased to ranges of 6.6% AR and 5.8% AR at the final sampling point at 168 h at middle and low concentration, respectively.

Methyl-5-hydroxy-2-nitrobenzoate as major metabolite of [benzoyl-^{14}C]-labelled-Bifenox reached maximum levels of 37.6% AR after 48 h and 42.9% AR after 72 h irradiation and decreased to ranges of 17.7% AR to 22.3% AR at the final sampling point at 168 h at middle and low concentration, respectively.

The quantum yield was calculated after measuring the spectral photon irradiance of the light source and the molar decadic absorption coefficient and ranged from 7.60×10^{-5} to 2.67×10^{-4}.

Soil photolysis was found not to have any significant contribution to the degradation of Bifenox on soil surfaces. The metabolism observed was similar between irradiated and non-irradiated soils with 16.5 and 28.2% AR recovered as bifenox acid after 30 days. According to first order kinetics, DT_{50} / DT_{90} values of Bifenox determined for irradiated soils were, respectively, 41.3 / 137.2 days.

11.2 Environmental transformation of metals or inorganic metals compounds

Not relevant.

11.3 Environmental fate and other relevant information

No further data.

11.4 Bioaccumulation

Table 30: Summary of relevant information on bioaccumulation

Method	Results	Remarks	Reference
ASTM, proposed standard practise for conducting bioconcentration with fish, 1977 - 1979	BCF = 1500 (whole fish) Clearance time CT_{50} = 1.4 days	$^{-14}$C-bifenox by bluegill sunfish (*Lepomis macrochirus)*	Anonymus (1986)

11.4.1 Estimated bioaccumulation

On the base on the experimental study the bioconcentration factor (BCF) was determined to be 1500, a bioconcentration study was conducted with bluegill sunfish (*Lepomis macrochirus*). A rapid elimination of ^{14}C Bifenox related residues from fish was recognized, the DT_{50} for clearance was 1.4 days.

The agreed BCF value in the EFSA Conclusion (2007) from this study was 1500.

11.4.2 Measured partition coefficient and bioaccumulation test data

The water partitioning coefficient value of bifenox (log P_{OW}) 3.64 (range 3.55 to 3.73, 20 – 25 °C, pH unadjusted)[7] is confirmed in the DAR document 2016 (CA 8.2.2.3). Bifenox rapidly degrades in water/sediment systems (DT_{50} = 0.03 days, DT_{90} = 0.44; geometric mean, whole system, n = 2. The values of partition coefficient are less than cut-of value (CLP criteria: K_{OW}≥4 for chronic categories).

According to CLP using a cut-off value of log K ow ≥ 4 is intended to identify only those substances with a real potential to bioconcentrate. The log P_{OW} value is used to classification if experimentally determined BCF is not available.

Taking into account above-mentioned not rapidly degradability and the experimentally determined BCF value for bifenox, the partition coefficient value (log P_{OW}) can be omitted in the consideration of the bifenox classification.

11.5 Acute aquatic hazard

Table 31: Summary of relevant information on acute aquatic toxicity

Method	Species	Test material	Results	Remarks	Reference
OECD 203	Rainbow trout (*Oncorhynchus mykiss*)	Bifenox	LC_{50} (96 h, flow-through) = 0.67 mg/L (nom.)	-	Anonymus (1993)
US-EPA (1975)	Bluegill sunfish (*Lepomis macrochirus*)	Bifenox	LC_{50} (96 h, flow-through) > 0.27 mg/L (m.m.)	-	Anonymus (1985a)
US-EPA (1975)	*Daphnia magna*	Bifenox	EC_{50} (48 h, flow-through) > 0.66 mg/L	-	Anonymus (1985b)

[7] EFSA Scientific Report (2007) 119, 1-84, Conclusion on the peer review of bifenox (https://efsa.onlinelibrary.wiley.com/doi/epdf/10.2903/j.efsa.2008.119r)

			(m.m.)		
OECD 201	*Scenedesmus subspicatus*	Bifenox	E_rC_{50} (72 h, static) = 0.00042 mg/L (m.m.)	-	Odin-Feurtet (1998a)
OECD 201	*Naviculla pelliculosa*	Bifenox	E_rC_{50} (72 h, static) = 0.0380 mg/L (m.m.)	-	Hoberg (1999)
FIFRA 122-2 and 123-3	*Lemna gibba*	Bifenox	E_rC_{50} (14 d, static) = 0.0028 mg/L (m.m.)	-	Hoberg (1998)
OECD 238	*Myriophyllum spicatum*	Bifenox	E_rC_{50} (14 d, semi-static) = 0.00189 mg/L (m.m.)	-	Wenzel (2016c)
OECD 239	*Myriophyllum spicatum*	Bifenox	E_rC_{50} (14 d, semi-static) = 0.000629 mg/L (m.m.)	Water-sediment	Wenzel (2016h)

11.5.1 Acute (short-term) toxicity to fish

The lowest LC_{50} (96 h) of Bifenox for fish was determined to be 0.67 mg/L from a study with rainbow trout. The LC_{50} (96 h) of Bifenox for bluegill sunfish based on measured concentrations was determined to be > 0.27 mg/L.

11.5.2 Acute (short-term) toxicity to aquatic invertebrates

The EC_{50} (48 h) of Bifenox for *Daphnia magna* was calculated to be > 0.66 mg/L.

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

Effects of Bifenox on algal growth were investigated in several studies submitted during the first EU evaluation for Annex I inclusion of Bifenox. They were conducted according to OECD guideline 201 (1984) and in compliance with Good Laboratory Practice (GLP) regulations; however, not all of the studies fulfil the validity criteria according to the current guideline version (2011). Only studies still considered valid are summarized in Table 31 above. The re-calculated EC_{50} values for Bifenox from a study with *Scenedesmus subspicatus* are 0.000420 mg/L for growth rate and 0.000272 mg/L for yield. This study with *Scenedesmus subspicatus* provides the lowest acute endpoint. A second algal species (*Naviculla pelliculosa)* was tested since Bifenox is an herbicide. The E_rC_{50} of 0.038 mg/L value was considerably higher.

The E_rC_{50} derived from a study with *Lemna gibba* was 0.0028 mg/L, the E_bC_{50} was 0.0021 mg/L. Effects of Bifenox on the dicotyledonous aquatic macrophyte *Myriophyllum* have also been investigated since Bifenox is an herbicide to control dicotyledonous weeds. The E_rC_{50} value was determined to be 0.000488 mg a.s./L and 0.000476 mg/L, respectively. The presence of sediment in the method can effect the reliability of the results especially as bifenox appears to adsorb strongly to soil/sediment. The water-sediment *Myriophyllum spicatum* test method complements the sediment-free *Myriophyllum spicatum* Toxicity Test.

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

Further acute toxicity studies are not available and are considered not necessary.

11.6 Long-term aquatic hazard

Table 32: Summary of relevant information on chronic aquatic toxicity

Method	Species	Test material	Results[1]	Remarks	Reference

OECD 204	Rainbow trout (*Oncorhynchus mykiss*)	Bifenox	NOEC (21 d, flow-through) = 0.0091 mg/L (m.m.)	-	Anonymus (1991)
US-EPA (1975)	Bluegill sunfish (*Lepomis macrochirus*)	Bifenox	NOEC (14 d, flow-through) = 0.13 mg/L (m.m.)	-	Anonymus (1981)
OECD 211	*Daphnia magna*	Bifenox	NOEC (21 d, static) = 0.015 mg/L (m.m.)	3 d exposure, 18 d recovery	Anonymus (1999)
OECD 211	*Daphnia magna*	Bifenox	NOEC (21 d, static) = 0.00033 mg/L (m.m.)	-	Anonymus (1990)
BBA Guideline Proposal (1995)	*Chironomus riparius*	Bifenox	NOEC (28 d, static) = 0.015 mg/L (nom.)	Water-sediment	Anonymus (1996)
OECD 201	*Scenedesmus subspicatus*	Bifenox	NOE_rC (72h, static) < 0.000250 mg/L (m.m.)	-	Odin-Feurtet (1998a)
OECD 201	*Naviculla pelliculosa*	Bifenox	NOE_rC (72 h, static) = 0.00016 mg/L (m.m.)	-	Hoberg (1999)
FIFRA 122-2 and 123-3	*Lemna gibba*	Bifenox	NOE_rC (14 d, static) = 0.00045 mg/L (m.m.)	-	Hoberg (1998)
OECD 238	*Myriophyllum spicatum*	Bifenox	NOE_rC (14 d, semi-static) = 0.000058 mg/L (m.m.)	-	Wenzel (2016c)
OECD 239	*Myriophyllum spicatum*	Bifenox	NOE_rC (14 d, semi-static) < 0.000064 mg/L (m.m.)	Water-sediment	Wenzel (2016h)

[1] Indicate if the results are based on the measured or on the nominal concentration

11.6.1 Chronic toxicity to fish

The lowest NOEC (21 d) of 0.0091 mg/L for Bifenox (*Oncorhynchus mykiss)* was determined from a prolonged toxicity study with rainbow trout. Chronic toxicity data from OECD TG 204 is not considered suitable for chronic classification under CLP (Guidance on the Application of CLP Criteria).

The second study results NOEC (14 d, flow-through) of 0.13 mg/L (m.m.) for Bifenox (*Lepomis macrochirus).* The test results do not cover the life stages of the used species. The NOEC value is higher than for rainbow trout therefore it is not considered to classification.

11.6.2 Chronic toxicity to aquatic invertebrates

The lowest NOEC (21 d) for reproduction of 0.00033 mg/L for Bifenox was determined from a chronic study with *Daphnia magna.*

11.6.3 Chronic toxicity to algae or other aquatic plants

Effects of Bifenox on algal growth were investigated in several studies submitted during the first EU evaluation for Annex I inclusion of Bifenox. They were conducted according to OECD guideline 201 (1984) and in compliance with Good Laboratory Practice (GLP) regulations; however, not all of the studies fulfil the validity criteria according to the current guideline version (2011). Only studies still considered valid are summarized in Table 32 above. The lowest NOE_rC for algae of < 0.00025 mg/L for Bifenox was determined from a study with *Scenedesmus subspicatus.* A second algal species (*Naviculla pelliculosa) was tested since Bifenox is an herbicide. The* NOE_rC of 0.00016 mg/L was considerably

lower. Results obtained in a water-sediment test system for *Chironomus riparius* due to feasible adsorption of bifenox with demonstrated strong absorption to soil may be tough to interpretation.

The NOE_rC derived from a study with *Lemna gibba* was 0.00045 mg/L. Effects of Bifenox on the dicotyledonous aquatic macrophyte *Myriophyllum* have also been investigated since Bifenox is an herbicide to control dicotyledonous weeds. The lowest definitive NOE_rC for aquatic plants of 0.000058 mg/L for Bifenox was determined from a study with *Myriophyllum spicatum*. This study provides the lowest definitive chronic endpoint to be considered for classification and labelling. Results obtained in a test system only in water phase, without sediment (OECD 238) enable clearer interpretation than in case of the presence of sediment (OECD 239) where for poorly soluble substances adsorption can be confused with degradation. Proceses of bifenox adsorption on sediment can have direct impact on test results and interpretation could be more complicated.

11.6.4 Chronic toxicity to other aquatic organisms

A NOEC (28 d) for reproduction of 0.015 mg/L for Bifenox was determined from a development study with *Chironomus riparius*.

11.7 Comparison with the CLP criteria

11.7.1 Acute aquatic hazard

Bifenox is of high acute toxicity (endpoints < 1 mg/L) to fish, invertebrates, algae and macrophytes (lowest E_rC_{50} = 0.000420 mg/L, *Scenedesmus subspicatus)* and fulfils the criteria for the proposed classification as Category Acute 1 (H400 - Very toxic to aquatic life) according to Regulation EC 1272/2008.

The corresponding M factor for an acute endpoint between 0.0001 and 0.001 mg/L to be considered for mixture toxicity is 1000.

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

Substances that rapidly degrade can be quickly removed from the environment. Bifenox is not rapidly degradable in the environment.

Experimentally determined BCF 1500 for bifenox indicated for its bioaccumulation potential (comparing to CLP criteria: BCF≥500) is clearly above criteria. An octanol-water partition coefficient value (log P_{OW}) is assumed 3.64 (CLP criteria K_{OW} ≥4). According to CLP criteria an experimentally determined BCF value provides a better measure and shall be used in preference if available. BCF 1500 is indicative of the potential to bioconcentrate for classification purposes in chronic aquatic hazarad.

Bifenox is of high chronic toxicity (endpoints < 0.1 mg/L) to fish, invertebrates, algae and macrophytes (lowest NOEC = 0.000058 mg/L, Myriophyllum spicatum) and fulfils the criteria for the proposed classification as Category Chronic 1 (H410 - Very toxic to aquatic life with long lasting effects) according to Regulation EC 1272/2008 for a non-rapidly degradable substance.

The corresponding M factor for a chronic endpoint between 0.00001 and 0.0001 mg/L of a non-rapidly degradable substance to be considered for mixture toxicity is 1000.

11.8 CONCLUSION ON CLASSIFICATION AND LABELLING FOR ENVIRONMENTAL HAZARDS

Classification according to Regulation (EC) No 1272/2008 [CLP]

Acute aquatic toxicity: Category 1 - H400

Chronic aquatic toxicity: Category 1 - H410

M factor = 1000 (acute) and M factor = 1000 (chronic)

Labelling according to Regulation (EC) No. 1272/2008 [CLP]

GHS Pictogram:

GHS09

Signal word:

Warning

Hazard statements:

H410 - Very toxic to aquatic life with long lasting effects

RAC evaluation of aquatic hazards (acute and chronic)

Summary of the Dossier Submitter's proposal

There is no current harmonised classification for methyl 5-(2,4-dichlorophenoxy)-2-nitrobenzoate (bifenox) in Annex VI of Regulation (EC) No 1272/2008.

Degradation

Photolysis

The aqueous phototransformation of bifenox was studied following OECD TG 316 under continuous artificial light for up to 168 hours in sterile aqueous media. The direct photolysis rate constant of bifenox was determined using a single first order (SFO) kinetic model (KinGUI version 1.1). The DT_{50} values were 63.4, 80.3, 22.9 and 43.9 hours for [dichlorophenol-14C] label (middle rate), [dichlorophenol-^{14}C] label (low rate), [benzoyl-14C] label (middle rate) and [benzoyl-14C] label (low rate), respectively. Various metabolites were formed: 2,4-dichlorophenol, methyl-5-hydroxy-2-nitrobenzoate.

Hydrolysis

In the available hydrolysis study following US EPA 161-1, the preliminary test showed bifenox was stable at pH 4 and 5 at 50°C. In the main test at 25°C, the corresponding first order hydrolysis rate constant was determined and equivalent to a DT_{50} of 265 days and 4 days at pH 7 and 9, respectively. Bifenox acid was the only metabolite detected and occurred at maximum amounts at the end of incubation of 21.6% Applied Radioactivity (AR) at pH 7 and 102.1% AR at pH 9.

In the aqueous hydrolysis study conducted with the metabolite bifenox acid following OECD TG 111, the substance was determined to be hydrolytically stable at pH 4, 7 and 9 over a period of 5 days at 50°C and, therefore, no additional testing was required or was performed. The DT_{50} (25°C) is estimated to be > 1 year.

Ready Biodegradation

The biodegradability of bifenox was investigated in one ready biodegradability study following OECD TG 301B. The 10% level was not reached within the 10 days from the beginning of the study. The biodegradation after 28 days was 14.0% and 11.8% for the 10 and 20 mg/L test concentrations, respectively. The result of the study showed that bifenox is not readily biodegradable.

Water and water/sediment

In a water/sediment study with ^{14}C dichlorophenyl bifenox, two natural systems 'Bickenbach' and 'Unter Widdersheim' were used. Bifenox was applied to the test systems at initial concentration of 0.33 mg/L and rapidly disappeared from the two water systems within the first day. Kinetic re-evaluation of the DT values was performed and the half-lives of bifenox in the total system were 0.02 and 0.06 days in the 'Bickenbach' and 'Unter Widdersheim' system, respectively.

Two metabolites were found. Aminobifenox, the major metabolite was bound to the sediment in amounts up to 67% AR of the applied parent compound. No other metabolite was detected in the sediment throughout the study. Bifenox acid accounted for maximum 7.8% AR in water 48 hours after application and did not exceed 5% at any other sampling time. For the metabolite bifenox acid, maximum DT_{50} value for the water compartment was 2.54 days (Bickenbach system), and since there was no occurrence in the sediment phase, this endpoint can be extrapolated to the total system as well. For the metabolite aminobifenox acid, for the whole system the maximum DT_{50} value was 30.75 days (Bickenbach system). For the metabolite Aminobifenox acid, no appropriate kinetic fitting could be found.

Soil degradation

Two studies on the route and rate of degradation of bifenox were conducted. In the first study with radio-labelled [chloro phenyl-^{14}C] bifenox and [nitro phenyl-^{14}C] bifenox, there were no notable differences in the metabolism and rate of degradation of bifenox between the two labels. Bifenox was moderately quickly degraded in one soil declining from mean 87.01-90.16% AR at day 0 to 9.04-9.59% AR after 120 days. One major metabolite was detected, bifenox acid, accounting for maximum on day 14 of 63.8% AR (chloro phenyl label) and 60.87% AR (nitro phenyl label) and decreased to ca. 27% AR at study end forming bound residues (maximum 46% AR) and CO_2 (maximum ca. 11% AR). As minor metabolites, aminobifenox and aminobifenox acid were produced.

In the second study, in three soils with radio-labelled [chloro phenyl-^{14}C] bifenox, the active substance was moderately quickly degraded in the soils at 20°C declining from 98.07-95.01% AR at day 0 to 2.00-3.78% AR after 181 days. At 10°C, bifenox degraded from 95.86% AR at day 0 to 10.80% AR at day 181. One major metabolite was detected. Bifenox acid was observed accounting for maximum on day 10 at 78.71% AR (clay loam) and decreased to 23.11% AR at study end forming bound residues (maximum ca. 52% AR) and CO_2 (maximum ca. 19% AR). As minor metabolites, aminobifenox and aminobifenox acid occurred.

Kinetic re-evaluation of the DT_{50} values was performed and DT_{50} values (at 20°C, not normalised to pF2) of bifenox ranged from 3.96 to 14.64 days (n=5). For bifenox acid, the DT_{50} values (at 20°C, not normalised to pF2) ranged from 22.65 to 165.27 days (n=8), and for Aminobifenox acid the DT_{50} values (at 20°C, not normalised to pF2) ranged from 0.55 to 1.58 days (n=3) and respective DT_{90} values ranged from 10.94 to 28.81 days.

Based on the above data, the DS considers bifenox as non-rapidly degradable.

Bioaccumulation

A bioconcentration study was conducted with bluegill sunfish (*Lepomis macrochirus*) during a 28-day exposure and 14-day depuration period. Bifenox in acetone was continuously dosed to the water flow to a nominal final concentration of 5.0 µg/L. A control only spiked with acetone was set up accordingly. In this study, a BCF = 1500 L/kg was determined. A rapid elimination of ^{14}C bifenox related residues from fish was recognized, the DT_{50} for clearance was 1.4 days. The agreed BCF value in the EFSA Conclusion (2007) from this study was 1500.

The water/octanol partitioning coefficient value for bifenox (log P_{ow}) = 3.64 (range 3.55 to 3.73, 20 – 25 °C, pH unadjusted). This value is less than the CLP cut-off value of 4.

According to CLP criteria an experimentally determined BCF value provides a better measure of bioaccumulation and shall be used in preference if available. A BCF of 1500 is indicative of the potential to bioconcentrate for classification purposes.

Aquatic toxicity

Acute aquatic hazard

Method	Species	Test material (purity > 97%)	Results	Remarks	Reference
OECD TG 203	Rainbow trout (*Oncorhynchus mykiss*)	bifenox	LC_{50} (96h, flow-through) = 0.67 mg/L (nom)	-	Handley *et al*. (1993)
US-EPA (1975)	Bluegill sunfish (*Lepomis macrochirus*)	bifenox	LC_{50} (96h, flow-through) > 0.27 mg/L (mm)	-	Surprenant (1985a)
US-EPA (1975)	*Daphnia magna*	bifenox	EC_{50} (48h, flow-through) > 0.66 mg/L (mm)	-	Surprenant (1985b)
OECD TG 201	*Scenedesmus subspicatus*	bifenox	E_rC_{50} (72h, static) = 0.00042 mg/L (mm)	-	Odin-Feurtet (1998a)
OECD TG 201	*Naviculla pelliculosa*	bifenox	E_rC_{50} (72h, static) = 0.0380 mg/L (mm)	-	Hoberg (1999)
FIFRA 122-2 and 123-3	*Lemna gibba*	bifenox	E_rC_{50} (14d, static) = 0.0028 mg/L (mm)	-	Hoberg (1998)
OECD TG 238	*Myriophyllum spicatum*	bifenox	E_rC_{50} (14d, semi-static) =	-	Wenzel (2016c)

			0.00189 mg/L (mm)		
OECD TG 239	*Myriophyllum spicatum*	bifenox	E_rC_{50} (14d, semi-static) = 0.000629 mg/L (mm)	Water-sediment	Wenzel (2016h)

Nom – nominal concentrations, mm – mean measured concentrations

The lowest LC_{50} (96h) of bifenox for fish was determined to be 0.67 mg/L (nominal) from a study with rainbow trout under flow-through conditions. The LC_{50} (96h) of bifenox for bluegill sunfish based on measured concentrations was determined to be > 0.27 mg/L. In this test, bifenox concentrations were on average 27, 23, 22, 33 and 43% of the nominal concentrations 1.0, 0.65, 0.42, 0.27 and 0.18 mg/L, respectively. Due to the degradation of bifenox the evaluation of the results is based on mean measured concentrations.

The acute toxicity of bifenox to *Daphnia magna* was investigated under dynamic conditions for 48 hours. Nominal test concentrations of bifenox primarily solved in acetone were 0.062, 0.12, 0.25, 0.50 and 1.0 mg/L. A control without addition of any further compounds and a solvent control were set up accordingly. The bifenox concentrations in the test solutions were on average 0.018, 0.035, 0.074, 0.16 and 0.35 mg/L. The EC_{50} (48h) of bifenox was calculated to be > 0.66 mg/L. This value is higher than the range of test concentrations and exceeds the solubility of bifenox.

Effects of bifenox on algal growth were investigated in several studies submitted during the first EU evaluation for Annex I inclusion of bifenox. They were conducted according to OECD TG 201 (1984) and in compliance with Good Laboratory Practice (GLP) regulations; however, not all of the studies fulfil the validity criteria according to the current guideline version (2011). Only studies still considered valid are summarised in the table above.

In an algae test, subcultures of *S. subspicatus* were exposed to nominal concentrations of 0.25, 1.0 and 1.5 µg/L bifenox dissolved in dimethylformamide (3 replicates per test concentration). At test termination, bifenox concentrations were reduced to 60, 84 and 93% compared to nominal values. This study provides the lowest acute endpoint based on measured concentrations. The re-calculated EC_{50} values for bifenox from a study with *Scenedesmus subspicatus* are 0.000420 mg/L for growth rate and 0.000272 mg/L for yield.

A second algal species (*Naviculla pelliculosa*) was tested. The E_rC_{50} of 0.038 mg/L (mean measured concentrations) value is considerably higher.

Further, from a study with *Lemna gibba* an E_rC_{50} = 0.0028 mg/L was obtained, the E_bC_{50} was 0.0021 mg/L. In this test, colonies of *Lemna gibba* were transferred to nominal concentrations of 0.63, 1.3, 2.5, 5.0 and 10.0 µg a.s./L bifenox solved in DMF. The test media were renewed on day 3, 6, 9 and 12. The mean measured concentrations of bifenox, considering freshly prepared and aged solutions were 0.45, 1.1, 2.2, 4.5, and 9.6 µg a.s./L. Results presented are based on mean measured concentrations.

Effects of bifenox on the dicotyledonous aquatic macrophyte *Myriophyllum spicatum* have also been investigated since bifenox is a herbicide used to control dicotyledonous weeds. In a sediment-free semi-static test based on OECD TG 238, *M. spicatum* was exposed to a range of concentrations 0.080, 0.240, 0.710, 2.05, 5.95, 17.2 and 50.0 µg bifenox/L, nominal, and 0.058, 0.197, 0.544, 1.47, 4.37, 10.2 and 22.1 µg bifenox/L mean measured. Effective concentrations ($EC_{10, 20, 50}$) were calculated for the growth rate and yield of the measured parameters main shoot length, total shoot length, fresh and dry weights, as well

as number of whorls. The E_rC_{50} = 0.00189 mg/L (mean measured concentrations) was determined for total shot length. Further, the test resulted in an E_rC_{50} = 0.000661 mg/L, fresh weight, and E_rC_{50} = 0.00182, dry weight. Fresh weight was the most sensitive growth rate parameter.

In a second study, the effects of bifenox on the growth of *M. spicatum* were determined in a water-sediment system following OECD TG 239, under sterile conditions over 14 days. 5 nominal concentration were chosen resulting in mean measured concentrations: 0.064, 0.173, 0.559, 1.90 and 6.84 µg bifenox/L. The growth parameters: main shoot length, length of lateral branches, development of fresh and dry weight were recorded. Since the measured concentrations of the test item in the water phase were below 80% of the nominal concentrations at test end, concentrations were determined in sediment. At test start bifenox was only found in concentrations above the LOQ in sediment of the highest treatment (0.840 µg/kg sediment dw). After one day, bifenox was above the LOQ in the two highest test concentrations (0.899 and 3.51 µg/kg sediment dw) and after seven days in the three highest treatments (0.845, 3.25 and 8.26 µg/kg sediment dw). After 14 days 2.12 and 6.54 µg/kg sediment dw were analysed in sediment of the two highest test concentrations. Fresh weight was the most sensitive parameter. The E_rC_{50} for fresh weight was 0.000488 mg bifenox/L. For dry weight an E_rC_{50} value of 0.00152 mg bifenox/L was found. The E_rC_{50} value for total shoot length was 0.000629 mg/L.

Chronic Aquatic Toxicity

Method	Species	Test material (purity>97%)	Results	Remarks	Reference
OECD TG 204	Rainbow trout (*Oncorhynchus mykiss*)	bifenox	NOEC (21d, flow-through) = 0.0091 mg/L (mm)	-	Handley *et al*. (1991)
US-EPA (1975)	Bluegill sunfish (*Lepomis macrochirus*)	bifenox	NOEC (14d, flow-through) = 0.13 mg/L (mm)	-	Forbis & Boudreau (1981)
OECD TG 211	*Daphnia magna*	bifenox	NOEC (21d, static) = 0.015 mg/L (mm)	3 d exposure, 18 d recovery	Odin-Feurtet (1999)
OECD TG 211	*Daphnia magna*	bifenox	NOEC (21d, static) = 0.00033 mg/L (mm) (reproduction) 0.00015 mg a.s./L (mm) body length	-	Young (1990)
BBA Guideline Proposal (1995)	*Chironomus riparius*	bifenox	NOEC (28d, static) = 0.015 mg/L (nom.)	Water-sediment	Mc Elligott (1996)
OECD TG 201	*Scenedesmus subspicatus*	bifenox	NOE_rC (72h, static) < 0.000250 mg/L (mm)	-	Odin-Feurtet (1998a)
OECD TG 201	*Naviculla pelliculosa*	bifenox	NOE_rC (72h, static) = 0.00016 mg/L (mm)	-	Hoberg (1999)
FIFRA 122-2	*Lemna gibba*	bifenox	NOE_rC (14d, static) = 0.00045	-	Hoberg (1998)

and 123-3			mg/L (mm)		
OECD TG 238	*Myriophyllum spicatum*	bifenox	NOE_rC (14d, semi-static) = 0.000058 mg/L (mm)	-	Wenzel (2016c)
OECD TG 239	*Myriophyllum spicatum*	bifenox	NOE_rC (14d, semi-static) < 0.000064 mg/L (mm)	Water-sediment	Wenzel (2016h)

Nom – nominal concentrations, mm – mean measured concentrations

The lowest NOEC (21 d mortality) of 0.0091 mg/L for bifenox was determined from a prolonged toxicity study with rainbow trout according to OECD TG 204.

In another study of 14 days of duration done according to US-EPA 1975 a NOEC (mortality) = 0.13 mg/L was obtained for *Lepomis macrochirus.*

For invertebrates, the reproductive toxicity of bifenox technical on *Daphnia magna* was investigated for 18 days after a 3-days exposure period in a study resulting in a NOEC = 0.015 mg/L.

In a second study, the reproductive toxicity of bifenox technical on *Daphnia magna* was investigated during a 21-days exposure period to concentrations of 0 (control), 0.12, 0.24, 0.95 and 1.9 µg/L under semi-static conditions. The mean measured concentrations of bifenox, were 0.066, 0.15, 0.33, 0.72 and 1.4 µg/L. Due to the degradation of bifenox during the study period, the evaluation of the results refers to measured concentrations. A NOEC (21d) of 0.00015 mg a.s./L was obtained due to the reduction in body length. The NOEC (21d) based on the reproduction rate was 0.00033 mg/L.

For the case of chronic toxicity to algae and aquatic plants, the same studies, but considering chronic endpoints are available. The lowest NOE_rC for algae of < 0.00025 mg/L for bifenox was determined from a study with *Scenedesmus subspicatus*. In this study, an E_rC_{10} of 0.00024mg/L was obtained. For the second algal species (*Naviculla pelliculosa*), a NOE_rC of 0.00016 mg/L and an EC_{10} = 0.0038 mg/L were obtained.

The NOE_rC derived from a study with *Lemna gibba* was 0.00045 mg/L. An E_rC_{10} = 0.0014 mg bifenox/L (number of fronds) was obtained for this species.

In the case of *Myriophyllum spicatum,* for the test done according to OECD TG 238 (sediment-free), the lowest E_rC_{10} (dry weight) = 0.000025 mg/L. An E_rC_{10} = 0.000066 mg/L fresh weight and an E_rC_{10} = 0.000137 mg/L for total shoot length were obtained. The NOE_rC = 0.000058 mg/L.

In the *M. spicatum* water-sediment toxicity test (OECD TG 239) the following endpoints were obtained: E_rC_{10} (fresh weight) = 0.000057 mg/L, E_rC_{10} (dry weight) = 0.000082 mg/L and E_rC_{10} (total shoot length) = 0.000098 mg/L. The DS indicates that the results obtained in the test system only in the water phase, without sediment (OECD TG 238), enabled clearer interpretation than in the presence of sediment (OECD TG 239) where for poorly soluble substances adsorption can be confused with degradation. In addition, bifenox adsorption on sediment can have direct impact on test results.

Results obtained in a water-sediment test system for *Chironomus riparius* due to feasible adsorption of bifenox with demonstrated strong absorption to soil may be tough to interpretation.

Based on the above data the DS concludes that bifenox is of high acute toxicity (endpoints < 1 mg/L) to fish, invertebrates, algae and macrophytes (lowest E_rC_{50} = 0.00042 mg/L, *Scenedesmus subspicatus*) and fulfils the criteria for the proposed classification as Aquatic Acute 1 (H400 - Very toxic to aquatic life) according to Regulation EC 1272/2008 with a corresponding M-factor of 1000.

For chronic toxicity the DS considers bifenox not rapidly degradable and having a potential to bioaccumulate based on a BFC value of 1500. Bifenox is of high chronic toxicity (endpoints < 0.1 mg/L) to fish, invertebrates, algae and macrophytes (lowest NOEC = 0.000058 mg/L, *Myriophyllum spicatum*) and fulfils the criteria for the proposed classification as Aquatic Chronic 1 (H410 – Very toxic to aquatic life with long lasting effects) according to Regulation EC 1272/2008 for a non-rapidly degradable substance with a corresponding M-factor of 1000.

Comments received during consultation

Three Member States commented on the proposed classification. Two of them supported the proposed classification but had various comments:

For chronic classification, the E_rC_{10} of 0.000025 mg/L dry weight for *M. spicatum*, is preferred to the proposed NOEC = 0.000058 mg/L. This is based on ECHA's CLP Guidance (v5.0, July 2017) which indicates that EC_{10} values are preferred over NOEC values for chronic toxicity studies when both are available for the same endpoint.

Furthermore, the adequacy of the available chronic fish data was questioned. Since there are no adequate tests for chronic fish toxicity, the surrogate approach was suggested. In its response, the DS indicated that in the context of the re-assessment of bifenox as a pesticide in the EU, the applicant performed a new fish early life stage test (according to OECD TG 210), which is close to finalisation (finalisation expected in May 2021). According to the DS, this data indicates that the results do not change the outcome of the previous hazard evaluation. The lowest endpoints are as follow:

- Overall NOEC (based on cumulative mortality) = 0.0133 mg/L
- Overall EC_{10} (based on cumulative mortality) = 0.0128 mg/L
- Overall EC_{20} (based on cumulative mortality) = 0.0233 mg/L

In another comment, it was questioned why the endpoint $NOEC_{reproduction}$ = 0.33 µg/L was given preference over the lower endpoint $NOEC_{body\ length}$ = 0.15 µg/L in the case of *Daphnia magna*. The DS agrees with considering the NOEC body length.

In addition, it was questioned why for *M. spicatum* the E_rC_{50} (shoot length) was given preference over the more sensitive E_rC_{50} (fresh weight).

The other MS requested that the DS clarify the OECD TG 238 validity criteria for the control growth in the key *M. spicatum* study. The DS indicated that the validity criteria were met. Regarding the most relevant long-term endpoint, the MS noted that the use of EC_{10} or EC_{20} long term values are preferred for hazard classification instead of the NOEC. In this sense, they also mentioned that EC_{20} values may be more appropriate than EC_{10} values depending on the coefficients of variation in control plants which should be lower than the effect level being estimated (OECD TG 238 section 3). The most sensitive EC_{20} values from the study would lead to a chronic M-factor of 100, compared with the proposed M-factor of 1000.

RAC agrees with the comments provided in relation to the non-validity of the chronic test presented for fish as well as on the general preference of EC_{10} values over NOECs when both

are available in the same test. Tests performed according to OECD TG 204 (Fish, Prolonged Toxicity Test: 14-Day Study (OECD 1984)) or similar guidelines such as US-EPA, Methods for acute tests with fish, macroinvertebrates, and amphibians (1975), used for *Lepomis macrochirus* cannot be considered suitable long-term tests. They are, in effect, prolonged acute studies with fish mortality as the major endpoint examined.

RAC considers the *M. spicatum* test leading to the chronic classification of bifenox valid and prefers the EC_{10} = 0.025 µg/L (0.004 - 0.178) value over the EC_{20} = 0.109 µg/L (0.017 - 0.746). The EC_{10} has an adequate confident interval and hence is considered reliable. Furthermore, RAC also notes that the test fulfils the validity criteria, including the one related to the coefficient of variation: "*The mean coefficient of variation for yield based on measurements of shoot fresh weight and the measurement variables relevant for the test evaluation in the control cultures did not exceed 35 % between replicates*". RAC notes that the lowest acute and chronic endpoints should be used for classification.

RAC supports the use of the $NOEC_{body\ length}$ = 0.15 µg/L in the case of *Daphnia magna.*

RAC does not have access to the new chronic fish study. Nevertheless, fish do not seem to be the most sensitive trophic level to bifenox.

Assessment and comparison with the classification criteria

Degradation

RAC agrees with the DS and considers bifenox as not rapidly degradable based on:

- The substance is not readily biodegradable in OECD TG 301B test. The biodegradation after 28 days was 14.0% and 11.8%.
- In water studies, bifenox quickly disappears from the test system. DT_{50} values of 4.5 and 3.7 days were found at two concentrations tested. Little mineralisation occurs. Metabolite bifenox acid exhibited marginal degradation in the test.
- In water/sediment studies bifenox disappears fast from the test system, DT_{50}: 0.02 and 0.06 days but transforms into various metabolites and little mineralisation occurs. It cannot be demonstrated that these metabolites are not hazardous to the aquatic environment.
- In the hydrolysis test, at 25°C, the corresponding first order hydrolysis rate constant was determined and equivalent to a DT_{50} of 265 days and 4 days at pH 7 and 9, respectively. Data on hydrolysis might be considered for classification purposes only when the longest half-life t½ determined within the pH range 4-9 is shorter than 16 days.
- Bifenox degrades fast in soil, DT_{50} = 3.96 to 14.64 days. However, it transforms into metabolites for which no aquatic toxicity data is available.

Bioaccumulation

RAC agrees with the DS and considers bifenox to be bioaccumulative based on the experimental BCF of 1500 L/kg, which is above the CLP criterion of 500 L/kg. In accordance with the CLP criteria and guidance a measured BCF value is preferred over the log k_{ow} which for bifenox ranges between 3.64 (based on the EFSA Report) and 4.48 (based on the GESTIS Database and EPIWEB 4.1). RAC notes that the log k_{ow} range is both below and above the CLP cut-off value of 4.

Acute aquatic toxicity

RAC questions the reliability of the acute fish study performed with *Lepomis macrochirus*. A LC_{50} > 0.27 mg/L is reported, whereas the range of concentrations tested go from 0.18 to 1.0 mg/L. The bifenox measured concentration was 43 % of nominal for the highest concentration (0.43 mg/L). It is not clear to RAC why an unbounded LC_{50} is presented.

RAC also notes the uncertainty related to the EC_{50} (48h) for *Daphnia magna* which was calculated to be > 0.66 mg/L since this value is higher than the range of tested concentrations and exceeds the solubility of bifenox.

In addition, RAC considers that the lowest endpoint for *M. spicatum* is the E_rC_{50} (fresh weight) of 0.000488 mg/L. For algae, the lowest endpoint corresponds to an E_rC_{50} of 0.00042 mg/L for *Scenedesmus subspicatus*.

RAC considers there is appropriate aquatic acute toxicity data for fish, invertebrates, algae and macrophytes. The lowest endpoints for each trophic level are:

- Fish: LC_{50} (96h) = 0.67 mg/L nominal. This value could be lower if concentrations had been measured. Yet RAC notes fish is not the most sensitive organisms.
- Invertebrates (*Daphnia magna*): EC_{50} > 0.66 mg/L (mm)
- Algae/Plants (*Scenedesmus subspicatus*): E_rC_{50} = 0.00042 mg/L (mm)

RAC agrees with the DS that the lowest endpoint for acute toxicity corresponds to the *Scenedesmus subspicatus* E_rC_{50} = 0.00042 mg/L. RAC agrees with the DS that bifenox fulfils the criteria for the proposed classification as Aquatic Acute 1 (H400) and according to table 4.1.3 of CLP, M = 1000.

Chronic aquatic toxicity

RAC disagrees with the DS and considers the studies available for fish not valid for chronic toxicity assessment. Tests performed according to OECD TG 204 (Fish, Prolonged Toxicity Test: 14-Day Study (OECD 1984)) or similar guidelines such as US-EPA, Methods for acute tests with fish, macroinvertebrates, and amphibians (1975), used for *Lepomis macrochirus* cannot be considered suitable long-term tests. They are, in effect, prolonged acute studies with fish mortality as the major endpoint examined.

For invertebrates, RAC considers the reproductive toxicity of bifenox technical on *Daphnia magna* by Odin-Feurtet (1999) invalid since there was only a 3-day exposure period. Furthermore, RAC considers the study by Young (1990) valid and RAC is if the opinion that the EC_{10} of 0.000296 mg/L is a preferred value over the NOEC.

There is also a *Chironomus riparius* test where toxicity via sediment cannot be excluded. Hence the test is not suitable for classification.

For *M. spicatum* there are two tests available: OECD TG 238 (without sediment) and OECD TG 239 (with sediment). RAC considers that a test with sediment should not preferentially be used for classification in this case since exposure via sediment cannot be discarded given the adsorption potential of bifenox with k_{oc} values ranging from 4400 to 23000 L/kg. In relation to the test performed according to OECD TG 238, RAC notes that the test duration is 14 days, a time where multiple generation might not be covered for this dicotyledonous species. This would be a normal prerequisite for chronic aquatic toxicity testing. However, the substance is a herbicide for dicotyledonous weeds and had severe effects in the test. RAC

concludes that the data of this test is relevant for both acute and chronic classification. There are multiple endpoints available in the test but RAC considers that the lowest toxicity value for dry weight E_rC_{10} = 0.000025 mg/L should be used for classification. This value should be used in preference over the NOE_rC = 0.000058 mg/L, as chosen by the DS.

The lowest endpoints for chronic toxicity are:

- Fish: No data available. RAC could not evaluate the reported chronic fish study in the RCOM.
- Invertebrates (*Daphnia magna*): EC_{10} = 0.000296 mg/L
- Alga/plants (*M. spicatum*): E_rC_{10} (dry weight) = 0.000025 mg/L

RAC considers bifenox non rapidly degradable and bioaccumulative. There is adequate chronic data available for invertebrates, algae and macrophytes. The lowest endpoint corresponds to the E_rC_{10} = 0.000025 mg/L for *M. spicatum* which leads to the classification as Aquatic Chronic 1 (H410) according to Regulation EC 1272/2008 for a non-rapidly degradable substance, with M = 1000 for a chronic endpoint between 0.00001 and 0.0001 mg/L.

Since there is no adequate chronic data for fish, RAC has also applied the surrogate approach. Using the acute LC_{50} of 0.67 mg/L classification as Aquatic Chronic 1, M-factor = 1 is derived. This is a less strict classification than using available chronic data, so this approach is not used.

In conclusion, RAC proposes to **classify bifenox as Aquatic Acute 1 (H400), M = 1000 and Aquatic Chronic 1 (H410), M = 1000.** This is in agreement with the DS proposal although RAC has used different endpoint values**.**

12 EVALUATION OF ADDITIONAL HAZARDS

12.1 Hazardous to the ozone layer

Table 33: Summary table of data concerning hazardous properties of the substance for the ozone layer

Type of study/data	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
Degradation in air BBA Guideline part IV, 6-1 (phase 2)	[ring-^{14}C] Bifenox System: volatilisation chamber, French beans	No significant volatilisation of Bifenox from plant surfaces (up to 0.8 and 1.3% AR). No volatile metabolites found.		Kubiak, 1994a,b
Degradation in air BBA Guideline part IV, 6-1 (phase 2)	[ring-^{14}C] Bifenox System: volatilisation chamber, soil surface (sandy soil)	No significant volatilisation of Bifenox from soil surfaces (< 1.0%AR). No volatile metabolites found.		Jendrzejczak et al., 1994a,b
Transport via air	Bifenox	Bifenox displays low overall persistence, limited transfer potential and low travel distance (estimated at 89 km)		O'Brien, 2015

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

Bifenox has a vapour pressure of 4.74×10^{-8} Pa at 20°C and a Henry law constant of $> 1.62 \times 10^{-4}$ Pa m^3 mol^{-1}. Bifenox may thus be considered as not volatile from soil or plant surfaces. Additionally, metabolism degradation studies in soil, water and water/sediment systems indicated there are no volatile breakdown products of concern from Bifenox. And moreover, the results of volatilisation studies from plant and soil surfaces conducted with Bifenox under controlled conditions showed negligible volatilisation of the substance from either surface.

The estimated half life in the atmosphere of 10.19 days based on the Atkinson method was calculated wit AOPWIN v 1.92a. However, it was shown via a multimedia model that when other factors are taken into account, overall persistence of Bifenox in air is lower, i.e. 8 days. Of more relevance, the characteristic travel distance estimated at 89 km is quiet low when compared to a theoretical travel distance of 1000 km that is proposed to be represented by the estimated DT_{50} in air of 2 days. In addition, when compared to known POP-like chemicals, the model shows that Bifenox does not share their characteristics but displays instead a low overall persistence, limited transfer potential and low travel distance.

Overall, considering the negligible volatilisation potential from experimental evidence, a contamination of air in amounts that can be considered of relevance to the environment are considered very unlikely. Additionally, the calculation of half-life in the atmosphere based on the Atkinson method only includes reaction with OH radicals and no ozone reaction estimation. When considering also reactions with ozone, the half-life in reality would be expected to be lower.

Therefore, no effects or hazards to the ozone layer from Bifenox are to be expected.

12.1.2 Comparison with the CLP criteria

There is no calculation of the ozone depleting potential of Bifenox available. However, this is considered as not relevant, please refer to point 12.1.1.

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

Bifenox is not considered as hazardous to the ozone layer. The available evidence concerning its properties and its predicted or observed environmental fate and behaviour indicate that it will not present a danger to the structure and/or the functioning of the stratospheric ozone layer.

RAC evaluation of hazards to the ozone layer

Summary of the Dossier Submitter's proposal

Bifenox has a vapour pressure of 4.74×10^{-8} Pa at 20°C and a Henry law constant of $> 1.62 \times 10^{-4}$ Pa m^3 mol^{-1}. Bifenox may thus be considered as not volatile from soil or plant surfaces. Additionally, degradation studies in soil, water and water/sediment systems indicated there are no volatile breakdown products of concern from bifenox. Further, the results of volatilisation studies from plant and soil surfaces conducted with bifenox under controlled conditions showed negligible volatilisation of the substance from either surface.

The estimated half-life in the atmosphere of 10.19 days was calculated with AOPWIN v 1.92a. However, it was shown via a multimedia model that when other factors are taken into account, overall persistence of bifenox in air is lower, i.e., 8 days. Of more relevance, the

characteristic travel distance estimated at 89 km is quiet low. In addition, when compared to known POP-like chemicals, the model shows that bifenox does not share their characteristics but displays instead a low overall persistence, limited transfer potential and low travel distance.

Overall, bifenox is not considered as hazardous to the ozone layer. The available evidence concerning its properties and its predicted or observed environmental fate and behaviour indicate that it will not present a danger to the structure and/or the functioning of the stratospheric ozone layer.

Comments received during consultation

No comments were received.

Assessment and comparison with the classification criteria

Evidence based on its properties and its fate and behaviour show bifenox will not present a danger to the structure and/or the functioning of the stratospheric ozone layer.

In conclusion, RAC agrees with the DS that **bifenox does not warrant classification as Hazardous to the ozone layer**.

13 ADDITIONAL LABELLING

Not relevant.

14 REFERENCES

14.1 Physicochemical Properties

Author(s)	Year	Title Owner Report No. Source (where different from owner) GLP or GEP status Published or not	Owner
Baer, C.	2016	Study plan: PARTITION COEFFICIENT OF 5-METHYL- 2-HYDROXY NITROBENZOATE ADAMA Agan Ltd, 90019750 GLP/GEP: yes Published: no	ADM
Bascou, J.P.	2001a	Bifenox Henry´s law constant ADAMA Agan Ltd Report-no. ACS/EDRA/BAJ/01023 GLP/GEP: no Published: no	ADM
Bascou, J.P.	2001b	Bifenox pH and dissociation constant ADAMA Agan Ltd Report-no. 00-139 GLP: yes Published: no	ADM
Bascou, J.P.	1998	Bifenox technical grade active ingredient: Surface tension ADAMA Agan Ltd Report-no. 98-129 GLP: yes Published: no	ADM
Bates, M.	2001a	Bifenox: Vapour pressure ADAMA Agan Ltd Report-no. 1905/4-D2141 GLP: yes Published: no	ADM
Bates, M.	2001b	Bifenox: Water and solvent solubility ADAMA Agan Ltd Report-no. 448792, 1905/3-D2141 GLP: yes Published: no	ADM
Bates, M	2000c	Bifenox: n-Octanol: Water Partition Coefficient ADAMA Agan Ltd Report-no. 1905/2-D214 GLP: yes Published: no	ADM
Birnschein, K.	2016a	DRAFT REPORT: PARTITION COEFFICIENT OF BIFENOX ACID (HPLC METHOD) ADAMA Agan Ltd Report-no. S16-00565 GLP/GEP: yes Published: no	ADM
Birnschein, K.	2016b	DRAFT REPORT: PARTITION COEFFICIENT OF AMINO-BIFENOX (HPLC METHOD) ADAMA Agan Ltd Report-no. S16-00566 GLP/GEP: yes Published: no	ADM
Birnschein, K.	2016c	DRAFT REPORT: PARTITION COEFFICIENT OF AMINO-BIFENOX ACID (HPLC METHOD) ADAMA Agan Ltd Report-no. S16-00564 GLP/GEP: yes Published: no	ADM

Author(s)	Year	Title Owner Report No. Source (where different from owner) GLP or GEP status Published or not	Owner
Francois, J.M.	2000	Bifenox - Determination of the flammability ADAMA Agan Ltd Report-no. 00-331-SEC GLP: yes Published: no	ADM
Francois, J.M.	1998	Determination of the explosion properties, ability for self heating and oxidizing properties of technical bifenox ADAMA Agan Ltd Report-no. 98-226-SEC, 98-133 GLP: yes Published: no	AMD
Franke, J.	2006	Oxidizing Properties A.17. Feinchemie Schwebda GmbH Report-no. 20060054.01 GLP: yes Published: no	ADM
Guyot, C.N.	1988	Bifenox - Henry´s Law Constant ADAMA Agan Ltd Report-no. 440111, 793C10 GLP/GEP: no Published: no	ADM
Ristorcelli, D., Bates, M.	2000	Bifenox: Physical characteristics ADAMA Agan Ltd Report-no. 447998, 1905/1-D2141 GLP: yes Published: no	ADM
Teeter, D.	1986	Determination of ambient vapour pressure of Bifenox ADAMA Agan Ltd Report-no. 440110, 34611 GLP/GEP: no Published: no	ADM

14.2 Health Hazard

Author(s)	Year	Title Owner Report No. Source (where different from owner) GLP or GEP status Published or not	Owner
Anonymus.	1985a	ACUTE ORAL TOXICITY STUDY ON RATS ADAMA Agan Ltd, 440124, 5799-85 GLP: yes Published: no	ADM
Anonymus	1985b	ACUTE DERMAL TOXICITY STUDY IN RABBITS ADAMA Agan Ltd, 440126, 5800-85 GLP: yes Published: no	ADM
Anonymus	1985c	PRIMARY DERMAL IRRITATION STUDY IN RABBITS ADAMA Agan Ltd, 440128, 5801-85 GLP: yes Published: no	ADM
Anonymus	1985d	EYE IRRITATION STUDY IN RABBITS - EPA ADAMA Agan Ltd, 440129, 5802-85 GLP: yes Published: no	ADM
Anonymus .	1995	BIFENOX TWO GENERATION REPRODUCTION STUDY IN RATS ADAMA Agan Ltd, 600885, 11324 GLP: yes Published: no	ADM
Anonymus .	1987	EFFECT OF BIFENOX ON PREGNANCY OF THE RAT ADAMA Agan Ltd, 412538, RNP 242/861056 GLP: yes Published: no	ADM
Anonymus	1982	13-WEEK DIETARY TOXICITY STUDY IN RATS MCTR-299-79 ADAMA Agan Ltd, 440132, 450 035 GLP: yes Published: no	ADM
Anonymus .	2015	COMPARATIVE IN VITRO METABOLISM OF [14C]-BIFENOX USING RAT AND HUMAN LIVER MICROSOMES ADAMA Agan Ltd, 20664, 90017812 GLP: yes Published: no	ADM
Anonymus .	1986	DEVELOPMENTAL TOXICITY (EMBRYO/FETAL TOXICITY AND TERATOGENIC POTENTIAL) STUDY OF BIFENOX TECHNICAL ADMINISTERED ORALLY (STORMACH TUBE) TO NEW ZEALAND WHITE RABBITS ADAMA Agan Ltd, 218-003 GLP: yes Published: no	ADM
Anonymus	1992	REVIEW OF THE KIDNEY SLIDES FROM STUDY: 24-MONTH CARCINOGENICITY STUDY IN MICE WITH BIFENOX 440135, Report n° R-21063 GLP: Not applicable	
Anonymus .	1983	CHO/HGPRT MAMMALIAN CELL FORWARD GENE MUTATION ASSAY PH 314-DC-001-83 BIFENOX-TECHNICAL ADAMA Agan Ltd, 440140, PH 314-DC-001-83 GLP: yes Published: no	ADM
Anonymus.	1986	BIFENOX ORAL TOXICITY STUDY IN BEAGLE DOGS (REPEATED DAILY DOSAGE FOR 52 WEEKS) ADAMA Agan Ltd, 410810, RNP 218/85998 GLP: yes Published: no	ADM

Haworth, S.R.	1979	SALMONELLA/MAMMALIAN-MICROSOME PLATE INCORPORATION MUTAGENESIS ASSAY ADAMA Agan Ltd, 440138, 595-248-1 GLP/GEP: no Published: no	ADM
Anonymus	1982	BIFENOX - MUTAGENICITY STUDY USING BACTERIAL STAINS ADAMA Agan Ltd, 440137, 82-095 GLP/GEP: no Published: no	ADM
Anonymus .	2016	IN VITRO MAMMALIAN CHROMOSOME ABERRATION TEST IN CHINESE HAMSTER V79 CELLS WITH BIFENOX TECHNICAL ADAMA Agan Ltd, 160132, 90019295 GLP: yes Published: no	ADM
Anonymus.	1978	ACUTE TOXICITY STUDY IN MICE BIFENOX (MCTR-126-78) ADAMA Agan Ltd, 440125, 20982 GLP/GEP: no Published: no	ADM
Anonymus.	2002	BIFENOX: TWENTY-EIGHT DAY REPEATED DOSE DERMAL TOXICITY STUDY IN THE RAT ADAMA Agan Ltd, 644/061 GLP: yes Published: no	ADM
Anonymus .	1979	EVALUATION OF COMPOUND MCTR-12-79 (MRI #248) FOR MUTAGENIC POTENTIAL EMPLOYING THE L5178Y TK+/- MUTAGENESIS ASSAY ADAMA Agan Ltd. 406963, 595-248-7 GLP/GEP: no Published: no	ADM
Anonymus	1986	BIOKINETICS AND METABOLISM IN THE MALE AND FEMALE RAT ADAMA Agan Ltd, 26/08/25 not available GLP: yes Published: no	ADM
Anonymus	2001	EXAMINATION OF BIFENOX IN THE SKIN SENSITISATION TEST IN GUINEA PIGS ACCORDING TO MAGNUSSON AND KLIGMAN (MAXIMISATION TEST) ADAMA Agan Ltd, 14261/01 GLP: yes Published: no	ADM
Anonymus	2005a	MUTAGENICITY STUDY OF BIFENOX IN THE SALMONELLA TYPHIMURIUM REVERSE MUTATION ASSAY (IN VITRO) ADAMA Agan Ltd, 18627/04 GLP: yes Published: no	ADM
Anonymus	2005b	MUTAGENICITY STUDY OF BIFENOX IN THE MOUSE LYMPHOMA FORWARD MUTATION ASSAY - IN VITRO - ADAMA Agan Ltd, 18628/04 GLP: yes Published: no	ADM
Anonymus	2003	MICRONUCLEUS TEST OF BIFENOX TECH. IN BONE MARROW CELLS OF THE NMRI MOUSE BY ORAL ADMINISTRATION" ADAMA Agan Ltd, 17124/1/03 GLP: yes Published: no	ADM
Anonymus	1982	24-MONTH CARCINOGENICITY STUDY IN MICE BIFENOX (MCTR-1-79) ADAMA Agan Ltd, 404836, 21063 GLP: yes Published: no	ADM
Anonymus	1986	RABBIT TERATOLOGY STUDY BIFENOX TECHNICAL REVISED FINAL REPORT ADAMA Agan Ltd, 656-125 GLP: yes Published: no	ADM
Anonymus	1981	EVALUATION OF BIFENOX TECHNICAL (LOT #16230) (MCTR-1-79) IN THE PRIMARY RAT HEPATOCYTE UNSCHEDULED DNA SYNTHESIS ASSAY ADAMA Agan Ltd, 440147, 2314-80 GLP: yes Published: no	ADM

Anonymus	1987	BIFENOX POTENTIAL TUMORIGENIC AND TOXIC EFFECTS IN PROLONGED DIETARY ADMINISTATION TO RATS ADAMA Agan Ltd, 440137, RNP 220/87642 GLP: yes Published: no	ADM
Anonymus	2016	REVERSE MUTATION ASSAY USING BACTERIA (SALMONELLA TYPHIMURIUM AND ESCHERICHIA COLI) WITH BIFENOX TECHNICAL ADAMA Agan Ltd, 160130, 90019663 GLP: yes Published: no	ADM
Anonymus	1981	METAPHASE ANALYSES OF RAT BONE MARROW CELLS TREATED IN VIVO WITH BIFENOX TECHNICAL ADAMA Agan Ltd, 440144, 2312-80 GLP: yes Published: no	ADM
Anonymus	1985	DELAYED CONTACT HYPERSENSITIVITY IN THE GUINEA-PIG WITH BIFENOX, TECHNICAL ADAMA Agan Ltd, 440130, 84676D/RNP 229/SS (G) GLP: yes Published: no	ADM
Anonymus	1985	AN ACUTE INHALATION TOXICITY STUDY OF BIFENOX (TECHNICAL) IN THE RAT ADAMA Agan Ltd, 440127, 85-7809 GLP: yes Published: no	ADM
Anonymus	1985	IN VITRO CHROMOSOMAL ABERRATION ASSAY ON BIFENOX TECHNICAL ADAMA Agan Ltd, 440142, 850027 GLP/GEP: no Published: no	ADM
Anonymus	2016	IN VITRO MAMMALIAN CELL GENE MUTATION TEST (HPRT-LOCUS) IN CHINESE HAMSTER V79 CELLS WITH BIFENOX TECHNICAL ADAMA Agan Ltd, 160131, 90018294 GLP: yes Published: no	ADM

14.3 Environmental Hazard

Author(s)	Year	Title Owner Report No. Source (where different from owner) GLP or GEP status Published or not	Owner
Crowe, A.	2000a	[14C]-BIFENOX: HYDROLYSIS UNDER LABORATORY CONDITIONS AT PH 4, 5, 7 AND 9 ADAMA Agan Ltd Report-no. RNP 636/002253, 202529 GLP: yes Published: no	ADM
Anonymus	1981	DYNAMIC TOXICITY OF BIFENOX TO BLUEGILL SUNFISH (LEPOMIS MACROCHIRUS) ADAMA Agan Ltd Report-no. 440247, 27115 GLP: yes Published: no	ADM
Anonymus	1986	UPTAKE, DEPURATION AND BIOCONCENTRATION OF 14C-BIFENOX BY BLUEGILL SUNFISH (LEPOMIS MACROCHIRUS) ADAMA Agan Ltd Report-no. 440205 GLP: yes Published: no	ADM
Gezahegne, W.	2016	FIELD SOIL DISSIPATION STUDY WITH ONE AUTUMN APPLICATION OF FOX (AG-B2-480 SC) A FORMULATED PRODUCT CONTAINING BIFENOX ON 3 SITES IN NORTH EUROPE AND 2 SITES IN SOUTH EUROPE IN 2014-2015 ADAMA Agan Ltd, S14-04459, R-90017806 GLP: yes Published: no	ADM
Giraud, J.P.	1983	BIFENOX SOIL SORPTION STUDY ADAMA Agan Ltd Report-no. 440155, AG/CRLD/An/102.83 GLP/GEP: no Published: no	ADM
Anonymus	1991	THE PROLONGED TOXICITY OF BIFENOX TO RAINBOW TROUT (ONCORHYNCHUS MYKISS) ADAMA Agan Ltd Report-no. 426191, 282/113 GLP: yes Published: no	ADM
Anonymus	1993	THE ACUTE TOXICITY OF BIFENOX TO RAINBOW TROUT (ONCORHYNCHUS MYKISS) ADAMA Agan Ltd Report-no. 432068, 282/388 GLP: yes Published: no	ADM
Hoberg, J.R.	1999	BIFENOX TECHNICAL - TOXICITY TO THE FRESHWATER DIATOM, NAVICULA PELLICULOSA ADAMA Agan Ltd Report-no. 604634, 10566.6577 GLP: yes Published: no	ADM
Hoberg, J.R.	1998	BIFENOX - TOXICITY TO THE DUCKWEED, LEMNA GIBBA ADAMA Agan Ltd Report-no. 603461, 98-10-7499 GLP: yes Published: no	ADM

Author(s)	Year	Title Owner Report No. Source (where different from owner) GLP or GEP status Published or not	Owner
Hüben, M.	2016a	ANAEROBIC TRANSFORMATION OF BIFENOX IN SOIL ADAMA Agricultural Solutions Ltd., Israel, ADM-001/5-31, 90017808 GLP: yes Published: no	ADM
Hüben, M.	2016b	HYDROLYSIS OF BIFENOX ACID AS A FUNCTION OF PH ADAMA Agricultural Solutions Ltd., Israel, ADM-002/1-35, 90018351 GLP: yes Published: no	ADM
Hüben, M.	2016c	PHOTOTRANSFORMATION OF BIFENOX IN WATER - DIRECT PHOTOLYSIS ADAMA Agricultural Solutions Ltd., Israel, ADM-001/5-40, 90017809 GLP: yes Published: no	ADM
Jendrzejczak, N.H., Maestracci, M.P., Turier, G.P.	1994a	SOIL SURFACE VOLATILITY STUDY OF MCPP-P AND BIFENOX FORMULATED AS EXP04404 (OFFICIAL GERMAN REFERENCE N°RPA44040H) ADAMA Agan Ltd Report-no. 94-39, 9416036 GLP: yes Published: no	ADM
Jendrzejczak, N.H., Maestracci, M.P., Turier, G.P.	1994b	SOIL SURFACE VOLATILITY STUDY OF MCPP-P AND BIFENOX FORMULATED AS EXP30535 (OFFICIAL GERMAN REFERENCE N°RPA30535H) ADAMA Agan Ltd Report-no. 94-38, 9416286 GLP: yes Published: no	ADM
Kubiak, R.	1994a	INVESTIGATION OF THE VOLATILIZATION OF 14C-MCCPP-P AND 14C-BIFENOX FORMULATED ACCORDING TO FOXTRIL SUPER (RPA 30535H) FROM PLANT SURFACES UNDER LABORATORY CONDITIONS ADAMA Agan Ltd Report-no. 94-26, RPA15 GLP: yes Published: no	ADM
Kubiak, R.	1994b	INVESTIGATION OF THE VOLATIZATION OF 14C-MCPP-P AND 14C BIFENOX FORMULATED ACCORDING TO VERIGAL D (RPA44040H) FORM PLANT SURFACES UNDER LABORATORY CONDITIONS ADAMA Agan Ltd Report-no. 94-25, RPA14 GLP: yes Published: no	ADM

Author(s)	Year	Title Owner Report No. Source (where different from owner) GLP or GEP status Published or not	Owner
Lebertz, H.	1989	STUDY ON THE BIODEGRADABILITY OF BIFENOX: ACCORDING TO MODIFIED STURM TEST (OECD GUIDELINE 301 B FOR TESTING CHEMICALS AND THE 6TH AMENDMENT OF THE COUNCIL DIRECTIVE 67/548/EEC, 1984) ADAMA Agan Ltd Report-no. 440153, R 67 006 04 GLP: yes Published: no	ADM
Matla Y.A., Vonk J.W.	1992	ADSORPTION OF BIFENOX ACID (5-(2,4-DICHLOROPHENXY)-2-NITROBENZOIC ACID) AND AMINO-BIFENOX (METHYL 5-(2,4-DICHLOROPHENOXY)-2-AMINOBENZOATE) TO SOIL PARTICLES IN THREE SOIL TYPES ADAMA Agan Ltd Report-no. 200131, IMW-R 92/202 GLP: yes Published: no	ADM
Anonymus	1996	BIFENOX TOXICITY TO THE SEDIMENT DWELLING CHIRONOMID LARVAE (CHIRONOMUS RIPARIUS) UNDER STATIC CONDITIONS ADAMA Agan Ltd Report-no. 601283, SA 95480 GLP: yes Published: no	ADM
Morlock, G.	2005c	DETERMINATION OF THE ADSORPTION/DESORPTION BEHAVIOUR OF AMINOBIFENOX ACID IN THREE DIFFERENT SOILS ADAMA Agan Ltd, 20051049/01-PCAD GLP: yes Published: no	ADM
O'Brien, K.	2015	STATEMENT ON LONG-RANGE TRANSPORT OF BIFENOX IN AIR ADAMA Agan Ltd, 2491115-CA-070302-01 GLP/GEP: no Published: no	ADM

Author(s)	Year	Title Owner Report No. Source (where different from owner) GLP or GEP status Published or not	Owner
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15 ANNEXES

Annex I to the CLH report