Committee for Risk Assessment

RAC

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

Adopted

26 November 2021

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26 November 2021

CLH-O-0000007049-71-01/F

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

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

Chemical name: **bifenox (ISO); methyl 5-(2,4-dichlorophenoxy)-2-nitrobenzoate**

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

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

The proposal was submitted by **Poland** and received by RAC on **24 November 2020.**

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

PROCESS FOR ADOPTION OF THE OPINION

Poland has submitted a CLH dossier containing a proposal together with the justification and background information documented in a CLH report. The CLH report was made publicly available in accordance with the requirements of the CLP Regulation at ***http://echa.europa.eu/harmonised-classification-and-labelling-consultation/*** on **11 January 2021**. Concerned parties and Member State Competent Authorities (MSCA) were invited to submit comments and contributions by **12 March 2021**.

ADOPTION OF THE OPINION OF RAC

Rapporteur appointed by RAC: **Ignacio de la Flor Tejero**

Co-Rapporteur appointed by RAC: **Ralf Stahlmann, supported by Anna Sonnenburg**

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

The RAC opinion on the proposed harmonised classification and labelling was adopted on **26 November 2021** by **consensus.**

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

	Index No	Chemical name	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors and ATE	Notes
					Hazard Class and Category Code(s)	Hazard statement Code(s)	Pictogram, Signal Word Code(s)	Hazard statement Code(s)	Suppl. Hazard statement Code(s)		
Current Annex VI entry	No current Annex VI entry										
Dossier submitters proposal	TBD	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	
RAC opinion	TBD	bifenox (ISO); methyl 5-(2,4-dichlorophenoxy)-2-nitrobenzoate	255-894-7	42576-02-3	Acute Tox. 4 Aquatic Acute 1 Aquatic Chronic 1	H302 H400 H410	GHS07 GHS09 Wng	H302 H410		oral: ATE = 1500 mg/kg bw M = 1000 M = 1000	
Resulting Annex VI entry if agreed by COM	TBD	bifenox (ISO); methyl 5-(2,4-dichlorophenoxy)-2-nitrobenzoate	255-894-7	42576-02-3	Acute Tox. 4 Aquatic Acute 1 Aquatic Chronic 1	H302 H400 H410	GHS07 GHS09 Wng	H302 H410		oral: ATE = 1500 mg/kg bw M = 1000 M = 1000	

GROUNDS FOR ADOPTION OF THE OPINION

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.* It 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).

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**.

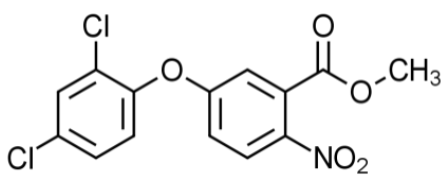

Figure: Chemical structure of bifenox

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 **no classification is warranted 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**.

HUMAN HEALTH HAZARD EVALUATION

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**.

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

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.**

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**.

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.**

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.**

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**.

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) 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)	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 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.**

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	Mouse lymphoma L5178Y cells (tk+/- system) Conditions:	Bifenox was negative with respect to the mutant frequency in the LK5178Y	Anonymous, 2005b

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
	Purity: 99.1%	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.	TK+/- mammalian cell mutagenicity test. **Negative**	
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	Anonymous, 1985

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
			low. No chromosomal aberrations were observed. Cyclophosphamide induced a significant increase in aberration frequencies. **Negative**	
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**.

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).

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

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	<u>Top dose F1/ F2</u>: 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) <u>low dose F1</u>: 2 pups of 1 litter with multiple malformations in low dose <u>top dose F1</u>: 1 pup with multiple malformations in high dose F2: no malformations	<u>Top dose</u>: 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	<u>Control:</u> foetal incidence of supernumerary ribs: 1 % large fontanelles: 2 % <u>225 mg/kg bw/d</u>: foetal incidence of supernumerary ribs: 2.2 % large fontanelles:	<u>Control:</u> 7/25 not pregnant 17 live litters <u>225 mg/kg bw/d</u>: 5/25 not pregnant +35 % water consumption GD13-19 20 live litters <u>900 mg/kg bw/d</u>: 3/25 not pregnant	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

Study	Effects on offspring	Effects on maternal animals	Limitations/ Remarks
as suspension in 1 % aq. methylcellulose gavage	2.6 % 900 mg/kg bw/d: foetal incidence of supernumerary ribs: 0.8 % large fontanelles: 2 % 3600 mg/kg bw/d: foetal incidence of supernumerary ribs: 6.4 % (HCD 0.9 - 7.1 %) large fontanelles: 10.1 %	+35 % water consumption GD13-19 1 total resorption 21 live litters 3600 mg/kg bw/d: 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*	gravid uterine weights and sex ratio of foetuses not reported
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	Control: incidence of hyoid, alae, angled: 6.2 % (litter) 0.9 % (foetal) 2 mg/kg bw/d: incidence of hyoid, alae, angled: 12.5 % (litter) 1.9 % (foetal) 20 mg/kg bw/d: incidence of hyoid, alae, angled: 10.5 % (litter) 1.4 % (foetal) 200 mg/kg bw/d: ↓ body weight (-8.3 %) incidence of hyoid, alae, angled: 27 % (litter) 4 % (foetal) (HCD 0-35 % litter, 0-5.3 % foetal)	Control: 3/20 not pregnant 16 live litters 2 mg/kg bw/d: 4/20 not pregnant 16 live litters 20 mg/kg bw/d: ↑* number of dams with soft or liquid faeces; alopecia 19 live litters 200 mg/kg bw/d: 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 %	Control: 1/1 foetus/litter with fused ribs 3 litters with visceral variations 11 litters with skeletal variations 5 mg/kg bw/d: 0/0 foetuses/litters with malformations	Control: 1 abortion 59/11 live foetuses/litters body weight GD6: 4260 ± 462 g body weight GD29: 4401 ± 443 g 5 mg/kg bw/d: 3 abortions	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**

Study	Effects on offspring	Effects on maternal animals	Limitations/ Remarks
0, 5, 50, 160, 500, 1000 mg/kg bw/d no vehicle reported gavage	4 litters with visceral variations 9 litters with skeletal variations <u>50 mg/kg bw/d</u>: 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 <u>160 mg/kg bw/d</u>: 2/2 foetuses/litters with malformations (1 midline closure defect, 1 fused sternebrae) 4 litters with visceral variations 11 litters with skeletal variations	62/9 live foetuses/litters -18 % food consumption body weight GD6: 4017 ± 358 g (-4.4 %) body weight GD29: 4192 ± 304 g (-4.8 %) <u>50 mg/kg bw/d</u>: 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 %) <u>160 mg/kg bw/d</u>: 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	**more than one malformation** deviations from TG: unusual dose spacing 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: <u>controls</u>: 11 litters of 13 females <u>10 mg/kg bw/d</u>: 8 litters of 10 females <u>100 mg/kg bw/d</u>: 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

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**.

ENVIRONMENTAL HAZARD EVALUATION

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) = 0.00189 mg/L (mm)	-	Wenzel (2016c)
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 and 123-3	*Lemna gibba*	bifenox	NOE_rC (14d, static) = 0.00045 mg/L (mm)	-	Hoberg (1998)
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* stud*y.* 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**.**

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**.

ANNEXES:

Annex 1 The Background Document (BD) gives the detailed scientific grounds for the opinion. The BD is based on the CLH report prepared by the Dossier Submitter; the evaluation performed by RAC is contained in 'RAC boxes'.

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