**Committee for Risk Assessment**

**RAC**

Annex 2

# Response to comments document (RCOM)

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

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

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

CLH-O-0000007049-71-01/F

**Adopted**

**26 November 2021**

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**ANNEX 2 - COMMENTS AND RESPONSE TO COMMENTS ON CLH PROPOSAL ON METHYL 5-(2,4-DICHLOROPHENOXY)-2-NITROBENZOATE; BIFENOX**

**COMMENTS AND RESPONSE TO COMMENTS ON CLH: PROPOSAL AND JUSTIFICATION**

Comments provided during consultation are made available in the table below as submitted through the web form. Any attachments received are referred to in this table and listed underneath, or have been copied directly into the table.

All comments and attachments including confidential information received during the consultation have been provided in full to the dossier submitter (Member State Competent Authority), the Committees and to the European Commission. Non-confidential attachments that have not been copied into the table directly are published after the consultation and are also published together with the opinion (after adoption) on ECHA's website. Dossier submitters who are manufacturers, importers or downstream users, will only receive the comments and non-confidential attachments, and not the confidential information received from other parties. Journal articles are not confidential; however they are not published on the website due to Intellectual Property Rights.

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**Substance name: bifenox (ISO); methyl 5-(2,4-dichlorophenoxy)-2-nitrobenzoate**
**EC number: 255-894-7**
**CAS number: 42576-02-3**
**Dossier submitter: Poland**

**GENERAL COMMENTS**

| Date | Country | Organisation | Type of Organisation | Comment number |
|---|---|---|---|---|
| 10.03.2021 | Germany | | MemberState | 1 |
| Comment received | | | | |
| see attached document<br><br>ECHA note – An attachment was submitted with the comment above. Refer to public attachment DE-CA Comments CLH-bifenox_final.docx | | | | |
| Dossier Submitter's Response | | | | |
| **General comments:**<br><br>**DS:** Although definitive comment must come from the GLP monitoring authority on this point, it is noted that the studies were conducted in 2008, ca. 10 years prior to the 2019 allegations. We can only take it in good faith that issue apparent in 2019 did not stretch back to 2008.<br><br>**Acute oral toxicity:**<br>• "Which vehicle was actually used in the rat acute oral toxicity study (Anonymous, 1985a)?"<br>**DS:** This was aqueous 1% methylcellulose.<br>• "Which kind of day does this statement refer to? Day of <u>treatment</u> (in this case, an acute toxic potential of the test substance could be excluded) or day of <u>gestation</u> (in this case, an acute toxic potential might exist since dosing started only on day 6 of gestation)?"<br>**DS:** The days referred-to are <u>gestation</u> days.<br><br>**Germ cell mutagenicity:**<br>"In particular, the available *in vivo* data should be considered supplementary only: In the Mouse Micronucleus Study (Leuschner 2003), bone marrow exposure is not demonstrated (no change in the ratio of PNE/NCE, no | | | | |

signs of systemic toxicity even in the highest dose, no ADME data in mice). Furthermore, statistical power of the result is limited because only a total of 2000 erythrocytes were counted for each animal, but 4000 are required as compared to OECD TG 474 (2016). The second *in vivo* study (Rat Bone Marrow Chromosome Aberration (Schreiner 1981) is also not sufficiently reliable because of the low statistical power (only 50 metaphases were analysed, but according to OECD TG 475 (2016), at least 200 metaphases should be analysed for each animal for structural chromosomal aberrations including and excluding gaps)."

**DS:** Bone marrow exposure is inferable in both sexes of rats and mice from at least the 13 week rat (2500 mg/kg bw/day Blair 1982), and 24 month mouse study (147-179 mg/kg bw/day, Manus, 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. This would indicate pertubation of marrow function by the presence of test item.

As regards a concern of "reduced statistical power" of the assay (because only 2000 erythrocytes counted per animal), it should be remembered that both sexes were used in the assay, and, that bone marrow exposure was highly likely for both sexes. In the repeat dose toxicology studies there were no sex-related toxicological differences seen that would indicate any sex would be significantly more refractory to bone marrow micronuclei formation over the other under the conditions employed in Leuschner, 2003. Hence with both sexes together the total number of erythrocytes counted on study would be equal to that, had just one sex been tested and 4000 erythrocytes per animal counted. As such in this particular case (because the material is not deemed more or less relevant to one specific sex specific), that only 2000 erythrocytes/animal were counted, makes no impact on the required sensitivity, because the data from both sexes is relevant. It would be very difficult to justifiy the use of animals in a repeat of the study in such circumstances.

"Regarding the available *in vitro* data, only two available studies should be considered reliable (Ames (Schreib 2015), Chromosome aberration (Hofman-Huether 2016)). For the other *in vitro* studies, several deviations are identified, e.g.

- HPRT test (Wallner 2016): According to OECD TG 476, the highest concentration tested should aim to achieve between 20 and 10% 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."

**DS:** Doses used were limited by test material solubility with precipitates being observed at at least the top dose tested in each experiment (200 or 250 and sometimes down 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.

"In particular, because of the similarity to nitrofen (another herbicide of the diphenyl ether class classified as Carc. 1 B, H351 and Repr. 1 B, H360D according to Reg. (EC) No 1272/2008 and additionally assumed to be mutagenic), data (e.g. bone marrow exposure in mice) and a weight of evidence analysis, taking into account all uncertainties, are required to definitively conclude on the mutagenic potential of bifenox."

**DS:** On detailed examination none the concerns raised here for the mouse micronucleus assay or the HPRT assay stand up to scrutiny. There are no reasonable uncertainties and the genotoxicity data is clearly "conclusive but not sufficient for classification".

NB A brief examination of the carcinogenicity and reprotoxicity profiles of bifenox and nitrofen immediately show that there no grounds to entertain any association with nitrofen. The genotoxicity data for nitrofen show some equivocal activity in vitro at best. Bifenox by comparison is very clearly negative both in vivo and in vitro.

**Carcinogenicity:**

"We agree that based on the available data on carcinogenicity in rats and mice, the effects do not trigger classification for carcinogenicity. However, further information would be required to conclude on the carcinogenic properties of bifenox with certainty. In order to take account of this uncertainty, we are of the opinion that in table 7 of the CLH report the reason for non-classification shall say "data inconclusive" and not "conclusive but not sufficient for classification".
Justification: In both studies on carcinogenicity (rats and mice), the maximal tolerated dose (MTD) was not reached. The available studies did not report any clear toxicological effects. However, according to OECD Guidance Document 116 (ENV/JM/MONO(2011)47), selection of adequately high dose levels is essential to avoid false negatives."

**DS:** The dose levels chosen in full compliance with the OECD guidance document cited above, with the reasonable expectation of reaching a MTD, while avoiding severe toxicity, given the results of the associated 90 day studies. Toxicity does always manifest in a manner proportional to dose or time, and often it manifests as a tipping point , or never reaches "MTD levels. Despite expectations from the 90 day studies, the expected level of toxicity in chronic studies does not always manifest. However if a NOAEL can be set (as was for both studies in question), this is an indication that adversity will have manifest in both species, and their systems stressed to at least some extent. In addtion, the top dose in the rat carcinogenicity study was ca. 250 mg/kg bw/day, and that in the mouse was ca 150 mg/kg bw/day, meaning substantial doses were received by the animals. In light of this, pragmatism needs to be applied if repeating the studies will really result relevant hazard information that will impact on rsik assesments or risk management.

"In addition, the available studies on carcinogenicity are not fully reliable (we do not agree with Klimisch 1). E.g., the study in mice (1982) was not conducted under GLP according to section 3.9.1.2 of document CLH_REP_Annex1_PL_SPS-018553-20 V2.docx. Other deviations are mentioned as well. Furthermore, 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 of the performing laboratory, but compared with published literature findings, they were considered not significant. All these considerations on significance might be questionable due to the low dose of administration (MTD not reached).
Thus, as also noted in the EFSA Conclusion 2007 (EFSA Scientific Report (2007) 119, 1-84, Conclusion on the peer review of bifenox), data are of limited quality to conclude sufficiently on the carcinogenic profile of the substance."

**DS:** The rat study in question was conducted to GLP and has a quality assurance statement.
Although missed in the original evaluations (probably due to relevant statements coming after the main report), the mouse study self certifies as GLP (as is the practice in the USA) without explicitly claiming such. The Quality Assurance statement on page 45 of the report makes reference to the FDA GLP code FDA 21 CFR part 58.35.

"Moreover, nitrofen, another herbicide of the diphenyl ether class, is classified as Carc. 1 B, H351 and Repr. 1 B, H360D according to Reg. (EC) No 1272/2008, and a comparison to nitrofen should be

performed using respective QSAR or read-across tools to exclude such properties, especially due to the plausible identified alert in carcinogenicity (e.g. using Derek Nexus as performed by DE). Thus, due to remaining uncertainties, data or expert justification is needed to finally conclude on the carcinogenic properties of bifenox."

**DS:** A brief examination of the carcinogenicity and reprotoxicity profiles of bifenox and nitrofen immediately show that there no grounds to entertain any association with nitrofen.

"Finally, with regard to the carcinogenicity study in rats it could still be worthwhile for the assessment of the observed islet cell tumours to know the exact study start. When looking at the background data for this tumour type (tables 24.2 and 24.3), it seems that the background incidence gradually increased over time. Hence, knowledge of the exact study start may be beneficial to compare tumour incidences of the rat study with the most appropriate background data provided by the laboratory."

**DS:** The Study appears to have an "initiation date" of March 1979.

**Reproductive toxicity:**

Adverse effects on sexual function and fertility

**DS:** In as far as in might be relevant, this information was actually publically available in the DAR conclusion for the study: "Conclusion: a reproductive NOAEL= 750 ppm (148 mg/kg/day) was based on decreased pup and litter weight in F1 and F2 generation at top dose (878 mg/kg bw/d). These reproductive effects occurred in the presence of slight parental (systemic) toxicity as suggested by the decreased body weight gain seen at 4500 ppm (343 mg/kg bw/d). NOAEL parental toxicity= 750 ppm (44.5 mg/kg bw/d).

…

Mean test substance intake (mg/kg bw/d) during pre-mating growth period

| | 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 |
| **F1**week 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 |

"

**Should further information be required please see the embedded file.**

Adverse effects on development

**DS: See embedded file.**

**Hazardous to the aquatic environment:**

Chapter 11.1 - Rapid degradability of organic substances:

**DS:** Available data from aquatic simulation studies support Bifenox meeting the criteria of rapid degradation (i.e. half-life of < 16 days). Results from the aerobic mineralization and water-sediment studies are summarized below. In addition, results of the adsorption/desorption study are provided for additional context.
In the OECD 309 (aerobic mineralization) study, Bifenox DT50 values of 4.5 and 3.7 days at the low- and high-test concentration, respectively, were measured in the water phase. The major metabolite Bifenox acid was also measured in the aerobic mineralization study at a maximum occurrence of 75.2% AR on day 14 at the low concentration and 81.8% AR on day 14 and day 91 at the high concentration. Since only marginal degradation of the metabolite Bifenox acid was measured during this study, no DT50 could be calculated.

In the OECD 308 (water-sediment) study, Bifenox degraded rapidly from the water compartment in both test systems, with persistence DT50 values in the water compartment and whole system of under 0.1 days. The maximum persistence DT50 of Bifenox acid in the water compartment is 2.54 days. Aminobifenox had maximum persistence DT50 values in the water, sediment, and whole system of 3.0 days, 38.11 days, and 102.14 days, respectively. Aminobifenox acid occurred as a major metabolite in the water compartment only.

Mineralization to $CO_2$ in both the OECD 308 and OECD 309 studies was limited. In the OECD 308 study, $CO_2$ reached a maximum of 1.8% AR across both systems. In the OECD 309 study, mineralization reached 15.2% and 7.0% AR at the low and high concentrations, respectively, at the end of incubation.

Adsorption/desorption test
Two OECD 106 (adsorption/desorption) studies showed that Bifenox sorbs strongly to soil, with Kfoc values between 4,400 and 23,000 L/kg (n=6). Bifenox is thus characterized as immobile. Given this high Kfoc, Bifenox can be expected to have limited bioavailability

- Chapter 11.5.3 – Acute toxicity to aquatic plants:
  **DS:** The correct endpoint is $E_rC_{50}$ = 0.00476 mg/L (from recovery).
- Chapter 11.6.2 – Reproductive and development toxicity to *Daphnia magna*:
  **DS:** DS agrees to the endpoint of $NOEC_{body\ length}$ = 0.15 µg/L

RAC's response

Thank you for your comments and response. RAC has carefully considered the points made.

RAC agrees that in depth study summaries allow better analysis and conclusion on classification of a substance.

RAC agrees to the endpoint $NOEC_{body\ length}$ for *Daphnia magna*.

RAC clarifies that in accordance to the CLP Report Annex the lowest acute value for *M.spicatum* water-sediment test is ErC50 (fresh weight) = 0.000488 mg/L. The ErC50 value after recovery for the same endpoint is 0.00476 and not 0.000476 mg/L. RAC agrees with the comment that the lowest endpoint should be used for classification ErC50 = 0.000488 fresh weight, instead of 0.000629 mg/L total shoot length. In addition, RAC

concludes that this test is not valid for classification since exposure via sediment cannot be discarded.

RAC appreciates the further efforts of the DS in explaining degradation data.

**OTHER HAZARDS AND ENDPOINTS – Hazardous to the Aquatic Environment**

| Date | Country | Organisation | Type of Organisation | Comment number |
|---|---|---|---|---|
| 12.03.2021 | Belgium | | MemberState | 2 |

Comment received

BE CA support the classification with Aquatic acute 1, H400 based on the lowest 72hErC50 of 0.000420 mg/L for algae (Scenedesmus subspicatus). A M-factor of 1000 is also supported.

Furthermore we agree with the conclusion that the substance is not rapidly degradable:
- Not readily biodegradable: 11.8-14.0 % ThCO2 after 28 d
- Not ultimately degraded in a surface water simulation test. Although the DT50 in fresh water was determined to be 3.7 - 4.5d, CO2-formation was low (7% - 15.2%)
- The substance is hydrolytically stable (the longest half-life >1yr)

The classification as Aquatic chronic 1, H410 and corresponding M-factor of 1000 is supported.
This proposal is based on the OECD 308 (sediment-free) study with the aquatic plant Myriophillum spicatum where a NOEC of 0.000058 mg/L for all growth parameters was determined.
Beside NOECs, also14d ErC10s for main shoot length, total shoot length, wet weight, dry weight and number of whorls were determined in this study. According to the ECHA Guidance on the application of the CLP criteria (v5.0, July 2017) EC10 values are are preferred over NOEC values in chronic toxicity studies when both are available for the same endpoint. Although not leading to a more stringent classification (because both in the same order of magnitude), the lowest ErC10 of 0.000025 mg/L was determined for inhibition of specific growth rate (dry weight).

Furthermore, the adequacy of the available chronic fish data is questionable.
- The OECD 204 with Oncorhynchus mykiss cannot be considered as a chronic test since it does not examine a sensitive stage in the fish life-cycle. It should only be considered when provision of further information on possible short-term effects is requested.
- US EPA study with Lepomis macrochirus is conducted according to US-EPA, Methods for acute tests with fish, macroinvertebrates and amphibians (1975). It is stated in the CLH report that it does not cover the life stages of the species. It is however not clear from the annex I to the CLH report which life stage was covered in the study.
If no adequate data are available for all trophic levels, the surrogate approach should be considered, but no clear LC50 was determined neither for fish (> 0.27 mg/L) nor for Daphnia (>0.66 mg/L).

Some editorial or/and minor comments :

Table 29: Summary of relevant information on rapid degradability: Kinetic evaluation of Water/ sediment study (Knoch, 1992): Metabolites:
Aminobifenox bound to sediment at up to 64 67% AR and at 6.4% AR in the water phase

11.5.3 Acute (short-term) toxicity to algae or other aquatic plants
"The ErC50 value was determined to be 0.000488 mg a.s./L and 0.000476 mg/L, respectively". From annex I to the CLH report it is clear that the ErC50 of 0.000488 mg/L was determined for Myriophylum spicatum (OECD 239: water-sediment) for fresh weight. It is however not clear for which growth rate parameter ErC50 of 0.000476 mg/L was assessed.

Dossier Submitter's Response

Thank you for your comment.
Regarding the importance of ErC10 and NOEC values, having regard CLP Regulation (Annex I, section 4.1.2.7.2) it has be acknowledged that the NOEC value and the $EC_x$ (e.g. $EC_{10}$) value can be used as equivalent. As you have noticed both values leads to the same classification, although the EC10 value is lower.
Corrigendum to the acute (short-term) toxicity to algae or other aquatic plants.
"The ErC50 value was determined to be 0.000488 mg a.s./L and 0.00476 mg/L (when considering a 21 day recovery), respectively". The value is 4.76 µg/L in the report. There is a typo in the summary table of the AIR3 dossier and in CLH report. It should be 0.00476 mg/L not 0.000476 mg/L.

Regarding the chronic fish data, DS is aware that the chronic fish study is not appropriate to assess the chronic risk. In the context of the re-registration of Bifenox in the EU, the registrant performed a new fish early life stage test (according to OECD 210), which is close to finalisation (finalisation expected in May 2021). The lowest endpoints are as follow:

Overall NOEC (based on cumulative mortality) = 0.0133 mg/L
Overall EC10 (based on cumulative mortality) = 0.0128 mg/L
Overall EC20 (based on cumulative mortality) = 0.0233 mg/L

The report can be submitted on request, but the data indicate that the results do not change the outcome of the previous hazard evaluation.

RAC's response

RAC agrees with the comments provided by the MS in relation to the non validity of the chronic tests presented for fish as well as on the preference of EC10 values over NOECs. Tests performed according to OECD 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 does not have access to the chronic fish study. Nevertheless fish does not seem to be the most sensitive thropic level to this substance.

| Date | Country | Organisation | Type of Organisation | Comment number |
|---|---|---|---|---|
| 12.03.2021 | United Kingdom | Health and Safety Executive | National Authority | 3 |

Comment received

Bifenox (EC: 255-894-7; CAS: 42576-02-3)
Please can the DS clarify whether the OECD TG 238 validity criteria for the control growth were met in the key chronic study with Myriophyllum? This is relevant as the proposed aquatic chronic classification is based on the NOEC from this study. Regarding the most relevant long-term endpoint, we note the use of EC10 or EC20 long term values for are preferred for hazard classification. Various EC10 endpoints for the most sensitive growth endpoints measured in the study are within the same concentration range as the NOEC so these support the proposed chronic M-factor. However, EC20 values may be more appropriate than EC10 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 EC20 values from the study would lead to a chronic M-factor of 100, compared with the proposed M-factor of 1000.

Dossier Submitter's Response

Thank you for your comment. Yes, study fulfilled the validity criteria according to OECD 238:
• Doubling of the main shoot length of the control during the test period was 10.9 d (required < 14 days).
• 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.
• All replicates of the control and solvent control group were kept sterile over the exposure period of 14 days without any apparent contamination by other organisms, e.g. algae, fungi, bacteria (required > 50 % of the replicates).

RAC's response

RAC considers the M.spicatum test leading to the chronic classification of Bifenox valid and prefers the EC10 = 0.025µg/L (0.004 - 0.178) value over the EC20 = 0.109µg/L (0.017 - 0.746). RAC considers the confient interval of the EC10 adequate and hence the value reliable. Further, RAC also notes that the test fulfils validity criteria 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".

PUBLIC ATTACHMENTS
1. DE-CA Comments CLH-bifenox_final.docx [Please refer to comment No. 1]