Committee for Risk Assessment

RAC

Annex 2

Response to comments document (RCOM)

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

piperonyl butoxide (ISO); 2-(2-butoxyethoxy)ethyl 6-propylpiperonyl ether

EC Number: 200-076-7
CAS Number: 51-03-6

CLH-O-0000006819-59-01/F

Adopted

11 June 2020

ANNEX 2 - COMMENTS AND RESPONSE TO COMMENTS ON CLH PROPOSAL ON PIPERONYL BUTOXIDE (ISO); 2-(2-BUTOXYETHOXY)ETHYL 6-PROPYLPIPERONYL ETHER

COMMENTS AND RESPONSE TO COMMENTS ON CLH: PROPOSAL AND JUSTIFICATION

Comments provided during public 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 public 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 public 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.

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Substance name: piperonyl butoxide (ISO); 2-(2-butoxyethoxy)ethyl 6-propylpiperonyl ether
EC number: 200-076-7
CAS number: 51-03-6
Dossier submitter: Greece

GENERAL COMMENTS

Date	Country	Organisation	Type of Organisation	Comment number
29.08.2019	France		MemberState	1
Comment received				
FR: In the table 3, we suggest to indicate "not classified" in the column "proposed classification" when "conclusive but not sufficient for classification" is mentioned in the column "reason for no classification" for hazards properties.				
Dossier Submitter's Response				
Comment noted and agreed.				
RAC's response				
Noted.				

Date	Country	Organisation	Type of Organisation	Comment number
30.08.2019	Germany	Endura S.p.A.	Company-Manufacturer	2
Comment received				
Endura S.p.A. agrees that the sub-acute dermal study may justify EUH066 even though the relevant findings were observed after repeated exposure for 6 h/day under semi-occlusive coverage. These conditions are not relevant for dermal exposure scenarios in real life. ECHA note – An attachment was submitted with the comment above. Refer to public attachment 20190829_PBO CLH Endura Comments.pdf				
Dossier Submitter's Response				
Point noted. However, EUH066 is a hazard statement describing a specific intrinsic health property regardless of potential exposure. According to point 1.2.4 of the CLP, EUH066 shall be assigned *"for substances and mixtures which may cause concern as a result of skin dryness, flaking or cracking but which do not meet the criteria for skin irritancy in section 3.2 of Annex I, based on either:*				

— practical observations; or *— relevant evidence concerning their predicted effects on the skin."* Dermal application of undiluted Piperonyl Butoxide to the skin of albino rabbits induced very slight erythema in four out of six animals (acute skin irritation/corrosion study, **Error! Reference source not found.**, 1991a). No oedema was formed. No irritation effects were observed 24h post exposure. Criteria for skin irritancy were not met. However, in the repeated dose dermal preliminary toxicity study in rabbits (**Error! Reference source not found.**, 1992) irreversible skin effects (erythema, edema, desquamation, fissuring, red raised areas) were observed from the lowest dose tested (100 mg/kg bw/day). The dossier submitter considers that the EUH066 statement reflects the toxicological profile of the substance.
RAC's response
Noted.

CARCINOGENICITY

Date	Country	Organisation	Type of Organisation	Comment number
21.08.2019	Germany		MemberState	3
Comment received				
Non-classification is not supported and category 2 is proposed. Carcinogenicity was intensively discussed during the biocide peer review procedure and it was concluded that "PBO should be considered as a potential carcinogen with a threshold mode of action." The following arguments should be in support of Carc. 2 rather than non-classification: - Although the MTD was exceeded in the rat study by Takahashi 1994a, this does not justify exclusion of this study from further WoE analysis. Mortality related to caecal haemorrhage was limited to wk 45-58 and males of the mid dose group (not reported in high dose and females). Hepatic adenoma and carcinoma were observed in this study from the mid-dose level of approx. 1000 mg/kg bw/d with adenoma only in the low dose group (500 mg/kg bw/d). Notably, the highest dose in the rat study Anonymous-10 was 500 mg/kg bw/d. Therefore, it should be concluded that hepatic neoplasia was observed in two species (rather than one species). - Tumor incidences observed in rat by Takahashi 1994a should be reported. In the independent review prepared for the PBO Task Force by W.H. Butler, visiting the laboratory, the following incidences were confirmed: adenoma in M/F: 0/0, 0/0, 8/1, 13/11; carcinoma in M/F: 0/0, 0/0, 3/0, 7/5 (according to Table II of the report by Butler). - GST-P positive foci in gpt delta rats were reported by: Matsushita K, Kijima A, Ishii Y, Takasu S, Jin M, Kuroda K, Kawaguchi H, Miyoshi N, Nohmi T, Ogawa K, Umemura T. Development of a Medium-term Animal Model Using gpt Delta Rats to Evaluate Chemical Carcinogenicity and Genotoxicity. At 12000 ppm in feed over 4 weeks, number and size of foci was increased significantly over controls (n=15, $p<0.01$). Mutation frequencies were unaltered, supporting the suggested non-genotoxic MoA. - While the involvement of CAR in the MoA was intensively studied, concerns about AhR activation during biocides peer review were not addressed. The data summarized by the DS also shows induction of *Cyp1a* mRNA as well as activity in mouse liver by PBO (Tables 37 and 38). Notably, this induction is still present in CAR/PXR double knock-out mice (Tables 39 and 40). Therefore, the effect of PBO is apparently not limited to CAR activation but likely includes activation of AhR related pathways. - Induction of *Cyp1a* and activation of AhR by PBO is supported by findings of: Kawai M, Saegusa Y, Jin M, Dewa Y, Nishimura J, Harada T, Shibutani M, Mitsumori K. Mechanistic				

study on hepatocarcinogenesis of piperonyl butoxide in mice. Toxicol Pathol. 2009 Oct;37(6):761-9. doi: 10.1177/0192623309344087.
- Therefore, the MoA analysis should be regarded as incomplete and there is good evidence for an alternative MoA which is considered relevant to humans (AhR pathway, OECD AOP No. 41: Sustained AhR Activation leading to Rodent Liver Tumors).

Overall, the German CA considers that there is evidence that hepatic neoplasia is induced in two rather than one species and that there is a plausible alternative mode of action with relevance to humans. Thus, classification as Cat. 2 rather than non-classification appears more appropriate.

Notice: A broader comparison on transcriptional effects of PBO and PB in vivo as provided in the CLH report can be obtained from: Kossler N, Matheis KA, Ostenfeldt N, Bach Toft D, Dhalluin S, Deschl U, Kalkuhl A. Identification of specific mRNA signatures as fingerprints for carcinogenesis in mice induced by genotoxic and nongenotoxic hepatocarcinogens. Toxicol Sci. 2015 Feb;143(2):277-95. doi: 10.1093/toxsci/kfu248.

Dossier Submitter's Response

Although there are some uncertainties, DS is not convinced that PBO fulfills the criteria for classification as a human carcinogen:

- Regarding consideration of PBO as carcinogenic in the rat, PB-like MoA for liver tumor formation could be equally applied.

- Reliability of the non-GLP, non-guideline carcinogenicity study in rat (Takahashi, 1994a) is questioned:

  - Hepatocellular adenomas and carcinomas occurred at the two highest doses (12000 and 24000 ppm). These doses are extremely high and above the MTD.
  - Decreased b.w. was 14.5% and 30% for males and females respectively at 12000 ppm and 22.8% and 50% for males and females respectively at 24000 ppm.
  - Survival was markedly decreased in males of the 12000 ppm group compromising the validity of the study. The authors also support that contamination with safrole, dihydrosafrole and isosafrole might have been responsible for the tumors observed. Tumor incidences (%) are presented in the table below:

Dose (ppm)*		**0**	**6000**	**12000**	**24000**
Adenomas	M	0	0	53.3%	20%
	F	0	0	16%	34.6%
Carcinomas	M	0	0	26.7%	80%
	F	0	0	0	57.7%

*Doses equivalent to 547/537, 1052/1061, 1877/2002 mg/kg bw/day for M/F respectively

- In the Anonymous – 10 chronic toxicity/carcinogenicity study, the liver was identified clearly as a target organ. However, there was no increased incidence of carcinomas up to the highest dose of 500 mg/kg bw/day. Two out of sixty (2/60) males treated with 500 mg/kg bw/day developed liver adenomas. No adenomas were observed in female animals. No historical control data available.

As shown in the table below (copy from CAR – Annex I to the CLH dossier), the top dose of 500 mg/Kg bw/day exceeded the maximum tolerated dose (MTD), thus the data derived from this group cannot be considered for classification.

Table A6.7/01-3b. Mean body weights at study weeks 78 and 104 (Anonymous-10)

Dose level (mg/Kg bw/day)	Group Mean Body Weights (g)					
	Male			Female		
	bw (g)	C 1	C2	bw (g)	C 1	C 2
Week 78						
Control 1	736	0.00	1.24	456	0.00	-6.94
Control 2	727	-1.22	0.00	490	7.46	0.00
30	740	0.54	1.79	478	4.82	-2.45
100	722	-1.90	-0.69	448	-1.75	-8.57
500	642	-12.77	-11.69	361	-20.83	-26.33
Week 104						
Control 1	674	0,00	1,51	438	0,00	-8,75
Control 2	664	-1,48	0,00	480	9,59	0,00
30	668	-0,89	0,60	486	10,96	1,25
100	618	-8,31	-6,93	448	2,28	-6,67
500	533	-20,92	-19,73	348	-20,55	-27,50

C1 - %difference from Control 1, C2 - % difference from Control 2

Overall, the DS considers that there is no treatment-related tumorigenesis in the rat.

- Regarding the induction of CYP1A mRNA and the involvement of AhR in PBO-treated wild type (w.t.) and CAR/PXR knock out (k.o.) mice:

  - Although CYP1A was still induced by PBO (4.6-fold increase) in CAR/PXR k.o. mice, the reduction of EROD enzyme activity from 646% (compared to control) in w.t. to 141% in CAR/PXR k.o. mice treated with PBO indicates no activation of AhR receptor.
  - The magnitude of CYP1A induction in w.t. PBO-treated mice (3.95 fold increase), is very low compared to the strong increase of CYP2B10 gene (1297 fold).
  - According to the Phase II mechanistic study, cells in S-phase were increased in both w.t. (5.95 fold increase) and CAR/PXR k.o. mice (4.03-fold increase). Although the mean labelling index was 10.182 in PBO treated w.t. mice vs 1.086 in PBO treated k.o. mice, the increase vs the control in both w.t. and k.o. mice was approximately of the same magnitude. However, these results are not in line with findings in liver weight and histopathology. The results of liver histopathology at phase II study termination indicate no effects in PBO-treated k.o. mice (10/10 PBO treated w.t. mice with hepatocellular hypertrophy vs 0/10 k.o. mice treated with PBO). Relative liver weight was also increased by 17% and 24% in w.t. mice treated with PBO for 7 and 14 days respectively. No statistically significant changes in liver weight were observed in k.o. PBO-treated mice.
  - A dose depended increase of replicative DNA synthesis was observed in PBO-treated mouse hepatocytes. Induction of human CAR was not followed by DNA replication, which is the prerequisite for tumor formation.
  - Regarding the publication by Kawai *et al.*, the induction of CYP1A and AhR mRNA does not necessarily indicates involvement of AhR receptor. Both genes are induced by activation of CAR receptor (Tolson A.H. & Wang H. *Adv Drug Deliv Rev*. 2010; 62(13):1238–1249).

In conclusion, tumorigenesis was observed in male mice (liver adenomas, carcinomas non-significant) and in female mice (liver adenomas only) in a dose-related manner in the

absence of systemic toxicity. Nevertheless, the experimental evidence provided shows that CAR/PXR activation is present. There are also data on AhR activation, bearing uncertainties whether this is really an alternative MOA (level of enzyme activation, no liver findings in CAR/PXR knock-out mice, no proliferation in human cells). Liver tumours in rats are observed in the presence of significant systemic toxicity. Therefore, the DS supports no classification for carcinogenicity.
RAC's response
Thank you for your comments and for the clarifications made. The considerations were mentioned in the RAC opinion.

Date	Country	Organisation	Type of Organisation	Comment number
30.08.2019	Germany	Endura S.p.A.	Company-Manufacturer	4
Comment received				
Endura S.p.,A. strongly supports to not classify PBO as Cat 2 for carcinogenicity. The CLH report recognises the Mode of Action (MoA) studies and concludes that liver adenomas in male mice observed after chronic PBO administration are not relevant for humans. The MoA involves CAR/PXR activation by PBO that occurs in murine, but not in human hepatocytes. Therefore, PBO should not be classified as Carc 2. ECHA note – An attachment was submitted with the comment above. Refer to public attachment 20190829_PBO CLH Endura Comments.pdf				
Dossier Submitter's Response				
Comment noted and agreed. For more details see response to comment 3.				
RAC's response				
Noted.				

MUTAGENICITY

Date	Country	Organisation	Type of Organisation	Comment number
21.08.2019	Germany		MemberState	5
Comment received				
The German CA agrees that the available data do not trigger classification with regard to germ cell mutagenicity.				
Dossier Submitter's Response				
Comment noted and agreed.				
RAC's response				
Noted.				

TOXICITY TO REPRODUCTION

Date	Country	Organisation	Type of Organisation	Comment number
21.08.2019	Germany		MemberState	6
Comment received				
The German CA agrees that the available data do not trigger classification with regard to reproductive toxicity.				
Dossier Submitter's Response				
Comment noted and agreed.				
RAC's response				
Noted.				

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Date	Country	Organisation	Type of Organisation	Comment number
30.08.2019	Sweden		MemberState	7

Comment received

Effects on sexual function and fertility
For transparency, the Swedish CA considers that below findings noted in the 24-month dietary study (Anonymous – 10, 1987) also should be included in the assessment of male reproductive toxicity:
• Statistically significantly increased dose-dependent incidence of smaller seminal vesicles was seen in 5%, 6.67%, 15%, 16.67% and 20% for the control group 1, control group 2, 30, 100 and 500 mg/kg bw/day;
• Statistically significantly increased incidences of bilateral testicular atrophy was reported in 18%, 15%, 33.3%, 46.67% and 43.33% for the control group 1, control group 2, 30, 100 and 500 mg/kg bw/day.

Adverse effects on or via lactation
The Swedish CA notes that an assessment of effects of piperonyl butoxide on or via lactation is lacking under heading 4.11 Reproductive toxicity. If criteria are not fulfilled this should also be stated by the DS in the CLH proposal.

Dossier Submitter's Response

Regarding the increased incidence of smaller seminal vesicles, these were only observed in animals found dead or sacrificed moribund during the study and were therefore not considered for classification purposes.

Regarding testicular atrophy, the following text is included in the CLH dossier under the Reproductive toxicity section: *"Histopathology of reproductive organs (testes and ovaries) is in line with observations in the chronic toxicity studies in rats (see Section 4.10.1.1) in which effects in ovaries (hyperplasia of Sertoli-like cells) were attributed to aging of the animals and testicular atrophy (grade: severe) was only observed at a dose above the MTD (500 mg/kg bw/day)."* Original data on testicular atrophy are included in the CLH report (Table 28). Moreover, after careful re-examination of the original study report (Anonymous - 10), it seems that the histopathological analysis was performed on the whole of study population (N = 60) including the animals found dead or sacrificed moribund during the study. In the animals surviving at study termination, the following incidences of atrophy (unilateral/bilateral) are observed: 3/18, 8/22, 3/13, 8/18, 7/22 at 0, 0, 30, 100, 500 mg/kg bw/day, respectively. The finding is not considered robust enough for classification.

Regarding lactation the only relevant finding is decreased body weight of pups from both breeding trials and both generations at 500 mg/kg bw/day dose. However, slight decrease in maternal body weight is also observed during lactation. There is no other observation in the study referring to the lactation period (e.g. clinical behaviour of pups etc) and no analytical measurement of PBO residues in milk.
Therefore, the two generation study results do not provide clear evidence of adverse effect in the offspring due to transfer in the milk or adverse effect on the quality of the milk. We agree that this assessment should have been included in the CLH report.

RAC's response

Thank you for your comment and clarifications. They were considered in the RAC opinion.

OTHER HAZARDS AND ENDPOINTS – Skin Hazard

Date	Country	Organisation	Type of Organisation	Comment number
21.08.2019	Germany		MemberState	8
Comment received				
The proposal for EUH066 is in agreement with the outcome of the discussion in the biocide review procedure and is supported.				
Dossier Submitter's Response				
Comment noted and agreed. For more details see response to comment 2.				
RAC's response				
Noted.				

OTHER HAZARDS AND ENDPOINTS – Specific Target Organ Toxicity Single Exposure

Date	Country	Organisation	Type of Organisation	Comment number
21.08.2019	Germany		MemberState	9
Comment received				
The proposal for STOT SE3, H335 is in agreement with the outcome of the discussion in the biocide review procedure and is supported.				
Dossier Submitter's Response				
Comment noted and agreed.				
RAC's response				
Noted.				

Date	Country	Organisation	Type of Organisation	Comment number
30.08.2019	Sweden		MemberState	10
Comment received				
The Swedish CA supports the classification of piperonyl butoxide as STOT SE 3, for respiratory tract irritation (RTI) effects. Since classification as STOT SE 3 in the current proposal is mainly based on human data, a more detailed description and discussion of the available epidemiological data would strengthen the CLH argumentation				
Dossier Submitter's Response				
Comment noted and agreed. A summary on the basis for STOT SE 3 classification, including information from epidemiology, is presented in section 2.2 of the CLH dossier. The references which support the DS proposal are available in the open literature.				
RAC's response				
Noted. Further evaluation of human data can be found in the RAC opinion.				

Date	Country	Organisation	Type of Organisation	Comment number
30.08.2019	Germany	Endura S.p.A.	Company-Manufacturer	11
Comment received				
Endura S.p.A. agrees that the acute inhalation study may justify H335. ECHA note – An attachment was submitted with the comment above. Refer to public attachment 20190829_PBO CLH Endura Comments.pdf				

Dossier Submitter's Response
Comment noted and agreed.
RAC's response
Noted.

Date	Country	Organisation	Type of Organisation	Comment number
25.07.2019	Finland		MemberState	12
Comment received				
Health effects following single exposure to piperonyl butoxide have been investigated in epidemiological studies and in two inhalation toxicity studies in rats. In humans, bronchospasm, cough/choke and dyspnea have been reported to be more likely if the exposure included piperonyl butoxide. According to other literature, pyrethrin-based products may exacerbate symptoms in asthmatics. The DS considers that these findings are probably related to the synergistic properties of piperonyl butoxide. In an acute inhalation toxicity study in rats, nasal discharge and labored breathing accompanied by red foci in the lungs were observed. In a 3-month inhalation toxicity study in rats, red nasal discharge and histopathological alterations in the larynx including slight squamous metaplasia with minimal hyperkeratosis and moderate inflammation were noted at 0.512 mg/l. No other effects on respiratory system were observed. Classification in STOT SE Category 3 (transient target organ effects, including respiratory tract irritation) is primarily based on human data. There are no validated animal models that deal specifically with respiratory tract irritation, but the CLP Regulation states that clinical findings and histopathology indicating respiratory irritant effects in animals can be used as part of weight of evidence evaluation. Considering all the available data on piperonyl butoxide, respiratory irritation has been observed in both human epidemiological data and animal studies, and in the absence of other more severe effects in the respiratory system. Therefore, classification as STOT SE Category 3 is justified. FI CA supports the proposed classification of STOT SE 3; H335 for piperonyl butoxide.				
Dossier Submitter's Response				
Comment noted and agreed.				
RAC's response				
Noted.				

OTHER HAZARDS AND ENDPOINTS – Hazardous to the Aquatic Environment

Date	Country	Organisation	Type of Organisation	Comment number
30.08.2019	Belgium		MemberState	13
Comment received				
Based on the available results in the CLH dossier, we support the proposed environmental classification for piperonyl butoxide as Aquatic Acute 1, 400 and Aquatic Chronic 1, H410. In view of above classification and toxicity bands the proposed M-factors are also supported: Macute = 1 : most sensitive species : invertebrates (Crassostrea virginica) with 96hEC50 = 0.23 mg/L Mchronic = 1 : not rapidly degradable substance, most sensitive species : invertebrates (Daphnia magna) with 21dNOEC= 0.030 mg/L.				

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Dossier Submitter's Response
Comment noted and agreed.
RAC's response
Thank you for your support.

Date	Country	Organisation	Type of Organisation	Comment number
30.08.2019	Germany	Endura S.p.A.	Company-Manufacturer	14
Comment received				
Endura S.p.A. agrees that the available information justifies H400 and Acute M-factor = 1. Endura S.p.A. agrees that the available information justifies H410 and Chronic M-factor = 1. ECHA note – An attachment was submitted with the comment above. Refer to public attachment 20190829_PBO CLH Endura Comments.pdf				
Dossier Submitter's Response				
Comment noted and agreed.				
RAC's response				
Thank you for the comment.				

PUBLIC ATTACHMENTS
1. 20190829_PBO CLH Endura Comments.pdf [Please refer to comment No. 2, 4, 11, 14]