Committee for Risk Assessment

RAC

Opinion

proposing harmonised classification and labelling
at EU level of

2-ethylhexanoic acid, monoester with propane-1,2-diol

EC Number: 285-503-5
CAS Number: 85114-00-7

CLH-O-0000007244-77-01/F

Adopted

16 March 2023

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16 March 2023

CLH-O-0000007244-77-01/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: **2-ethylhexanoic acid, monoester with propane-1,2-diol**

EC Number: **285-503-5**

CAS Number: **85114-00-7**

The proposal was submitted by **Spain** and received by RAC on **23 May 2022.**

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

PROCESS FOR ADOPTION OF THE OPINION

Spain 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 **14 June 2022**. Concerned parties and Member State Competent Authorities (MSCA) were invited to submit comments and contributions by **15 August 2022**.

ADOPTION OF THE OPINION OF RAC

Rapporteur, appointed by RAC: **Michal Martínek**

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 **16 March 2023** by **consensus**.

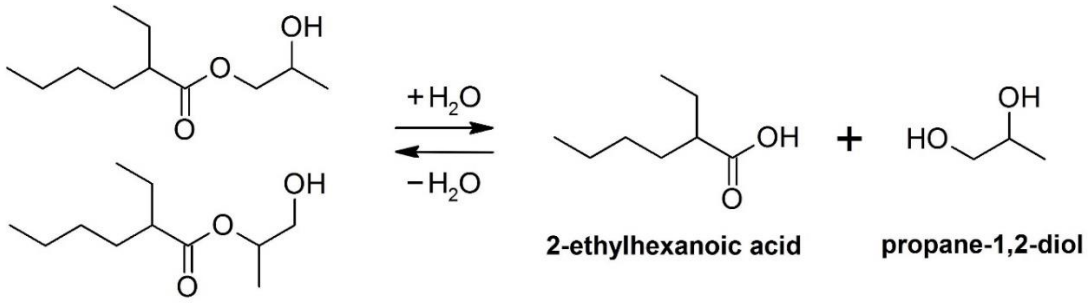
Classification and labelling in accordance with the CLP Regulation (Regulation (EC) 1272/2008)

	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	2-ethylhexanoic acid, monoester with propane-1,2-diol	285-503-5	85114-00-7	Repr. 1B	H360D	GHS08 Dgr	H360D			
RAC opinion	TBD	2-ethylhexanoic acid, monoester with propane-1,2-diol	285-503-5	85114-00-7	Repr. 1B	H360D	GHS08 Dgr	H360D			
Resulting Annex VI entry if agreed by COM	TBD	2-ethylhexanoic acid, monoester with propane-1,2-diol	285-503-5	85114-00-7	Repr. 1B	H360D	GHS08 Dgr	H360D			

GROUNDS FOR ADOPTION OF THE OPINION

RAC general comment

2-ethylhexanoic acid, monoester with propane-1,2-diol (hereafter 2-EHA-PG) is a liquid used as a coalescing agent. 2-EHA-PG is a mixture of two esters resulting from esterification at either of the two hydroxy groups of the diol. The hydrolysis/esterification reaction can be described by the following equation:



2-ethylhexanoic acid, monoester with propane-1,2-diol

No toxicokinetic data is available for 2-EHA-PG. Esters of carboxylic acids are usually metabolised via hydrolysis to the respective acid and alcohol, in this case to 2-ethylhexanoic acid (2-EHA) and propane-1,2-diol (propylene glycol, PG). Hydrolysis of carboxylic acid esters is catalysed by carboxylesterases (CES 1 and CES 2), which are highly expressed in several tissues including the liver and intestines (Wang *et al.*, 2018). The similarity between the developmental toxicity profiles of 2-EHA-PG and 2-EHA in rodents (skeletal variations in rats, exencephaly in mice) provides indirect evidence of 2-EHA formation after exposure to the ester. Information on toxicity of 2-EHA and PG is therefore considered relevant for the assessment of 2-EHA-PG.

2-EHA is also present in 2-EHA-PG as an impurity. 2-ethylhexanoic acid has a harmonised classification as Repr. 1B; H360D. The justification for this classification can be found in the respective RAC opinion (ECHA, 2020).

HUMAN HEALTH HAZARD EVALUATION

RAC evaluation of reproductive toxicity

Summary of the Dossier Submitter's proposal

The dossier submitter (DS) proposed a classification as Repr. 1B; H360D mainly based on exencephaly in a mouse prenatal developmental toxicity (PNDT) study with 2-EHA-PG, noting that this malformation was also seen in a mouse study with the presumed metabolite 2-EHA.

For sexual function and fertility and effects on or via lactation the DS proposed no classification due to lack of data.

Comments received during consultation

Two member state competent authorities supported the DS's proposal of Repr. 1B; H360D. Other parties did not provide comments.

Assessment and comparison with the classification criteria

In their assessment of sexual function and fertility the DS briefly summarised relevant studies with 2-EHA. RAC has identified additional relevant information, namely a 90-day oral study in rats with 2-EHA-PG (Anonymous, 2016) and a multigeneration study in mice with PG (NTP, 1985). Both studies are summarised in the Background Document (BD) based on a full study report to the 90-day study with 2-EHA-PG and published information on the multigeneration study with PG (NTP, 2004; Morrissey *et al.*, 1989).

Adverse effects on sexual function and fertility

No generational study with 2-EHA-PG is available.

No effects on reproductive organ weight or histopathology were observed in a 90-day rat study with 2-EHA-PG up to the top dose of 1 000 mg/kg bw/d. The study is described in more detail in the BD.

Generational studies are available for the hydrolysis products and presumed metabolites 2-EHA and PG. An extended one-generation reproductive toxicity study (EOGRTS) in rats with 2-EHA via dietary route was evaluated in detail by RAC in the opinion on 2-EHA and its salts (ECHA, 2020). Some slight effects were noted (e.g. changes in oestrous cyclicity) but it was concluded that these do not warrant classification. A multigeneration study with PG in mice via drinking water (continuous breeding protocol) conducted by NTP was negative. More information on the latter study can also be found in the BD.

Noting the absence of a generational study with 2-EHA-PG, RAC concludes that the available information **does not warrant classification of the substance for adverse effects on sexual function and fertility**.

Adverse effects on development

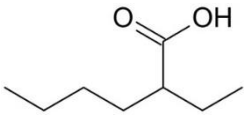
Two PNDT studies with 2-EHA-PG are available, one in rats and one in mice. The PNDT study in rats was conducted in 2015 (Anonymous, 2016). On a subsequent dossier compliance check ECHA requested a PNDT study in mice as a second species due to a concern for developmental toxicity raised by the rat study and by information on structurally related substances 2-EHA and 2-ethylhexyl 2-ethylhexanoate (ECHA, 2018). The mouse was considered a more appropriate second species than the rabbit in this case because a PNDT study in rabbits with the structurally related substance 2-EHA did not show evidence of developmental toxicity.

The RAC assessment begins with a brief summary of developmental toxicity of the presumed metabolite 2-EHA, followed by a description of the two PNDT studies with 2-EHA-PG.

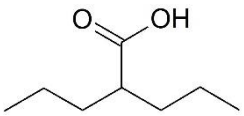
<u>Developmental toxicity of 2-ethylhexanoic acid</u>

The most consistent finding across the available standard PNDT studies with 2-EHA in rats is a range of skeletal variations (e.g. supernumerary vertebrae and ribs, reduced ossification). A PNDT study in rabbits was negative.

2-EHA is structurally related to the antiepileptic drug and a known human teratogen valproic acid. The structures of the two substances can be compared below.



2-ethylhexanoic acid



valproic acid

Use of valproic acid during pregnancy is associated with increased risk of several major congenital malformations including spina bifida. Importantly, the valproate-related malformations in humans are not reproduced in standard PNDT studies in rats. The main finding in rat PNDT studies with valproic acid is increased incidence of skeletal variations, and the overall pattern of developmental effects is similar to that seen in rat studies with 2-EHA.

Since neural tube defects are difficult to produce with valproic acid in rats and rabbits, one research group (Nau *et al.*, 1991) extensively used mice as a model for investigation of teratogenicity of valproate and its analogues. Under optimised treatment schedules (i.p. or s.c. injections during the critical windows) valproic acid induced a high incidence of spina bifida occulta (detected by measuring the distance between the ends of lumbar vertebral arches), a low incidence of spina bifida aperta and a high incidence of exencephaly. Studies with 2-EHA reported 32 % foetuses with exencephaly after a multiple i.p. treatment and 5 % after a single treatment at 430 mg/kg bw (expressed as free acid), compared to 0 % in the negative control and 44 % after a single treatment with valproic acid.

The similarity of the developmental toxicity profiles of 2-EHA and valproic acid in animal studies played a key role in the classification of 2-EHA as Repr. 1B; H360D (ECHA, 2020).

PNDT study in rats (Anonymous, 2016)

Pregnant Sprague-Dawley rats (20/group) were administered 2-EHA-PG in corn oil from GD 6 to 19 at dose levels of 0, 100, 300 and 1 000 mg/kg bw/d. The study was conducted according to OECD TG 414 and under GLP.

There was no significant maternal toxicity. Developmental findings at 1 000 mg/kg bw/d included reduced foetal weight (by 11 %), tail anomalies at a low incidence (2 foetuses from 2 litters), increased incidence of several skeletal variations (e.g. short supernumerary lumbar rib in 34 % of foetuses vs 6 % in the control), delayed ossification (skull, vertebrae, pelvis, phalanges) and increased incidence of small renal papilla (classified as a minor abnormality).

PNDT study in mice (Anonymous, 2020)

In this OECD TG 414 and GLP compliant study, pregnant Crl:CD-1 (ICR) mice were administered 2-EHA-PG in corn oil via gavage from GD 6 to 17 at dose levels of 0, 100, 300 and 1 000 mg/kg bw/d. The study was terminated on GD 18. Approximately half of the foetuses in each litter were examined for visceral abnormalities, the other half were processed for skeletal examination.

There was no maternal toxicity. The main developmental effects are summarised in the table below. Foetal weight was reduced by 14 % at the top dose. Exencephaly was found in 8 foetuses from 4 litters at 1 000 mg/kg bw/d and in 1 foetus at 100 mg/kg bw/d. A single case of exencephaly was present in historical control data (within 5 years before the current study, no further details). Thus, the single case at 100 mg/kg bw/d might be incidental.

The foetuses with exencephaly examined for visceral abnormalities were reported to have disorganised structure of the brain, those examined skeletally had skull malformations (misshapen, split and absent bones); these two findings are considered to be related to exencephaly.

As to variations, there were indications of a general ossification delay (involving the skull, sternebrae, vertebrae and phalanges) at the top dose as well as increased incidence of supernumerary (lumbar) rib.

PNDT study in mice (Anonymous, 2020)				
Dose (mg/kg bw/d)	**0**	**100**	**300**	**1 000**
Total no. of females	24	24	24	24
Non-pregnant females	1	5	0	2
Pregnant females	23	19	24	22
Pregnant with total litter loss	0	0	0	1
No. of pregnant females with live foetuses on GD 18	23	19	24	21
Post-implantation loss (%)	7.1	7.2	5.5	11.4 (7.2)[a]
Corrected body weight (g)	35.6	36.1	36.2	36.3
Mean litter size (live foetuses)	13.3	13.5	13.4	13.1 (13.7)[a]
Foetal weight (g)	1.36	1.35	1.37	1.16*
External examination: no. of foetuses	305	257	322	288
Exencephaly: foetuses (litters); % of affected foetuses/litter	0 0 %	1 (1) 0.4 %	0 0 %	8 (4) 3.0 %*
Skeletal examination: no. of foetuses	152	129	161	143
Wide interfrontal suture: foetuses (litters); % of affected foetuses/litter	0 0 %	0 0 %	0 0 %	3 (1) 2.0 %
Sternebra – bipartite ossification: foetuses (litters); % of affected foetuses/litter	0 0 %	1 (1) 0.9 %	2 (1) 1.0 %	5 (4) 3.7 %
Vertebra – cervical centrum unossified: foetuses (litters); % of affected foetuses/litter	15 (6) 9.2 %	8 (5) 6.4 %	14 (7) 8.4 %	87 (20) 60.7 %*
Vertebra – thoracic centrum unossified: foetuses (litters); % of affected foetuses/litter	0 0 %	0 0 %	0 0 %	9 (3) 6.2 %
Supernumerary (lumbar) rib: foetuses (litters); % of affected foetuses/litter	25 (13) 16.9 %	14 (6) 9.7 %	39 (17) 24.0 %	62 (19) 42.2 %*

* Statistically significant difference from control, $p \leq 0.05$

[a] Including (excluding) the dam with total litter loss; the dam with total litter loss had 15 early resorptions

Conclusion on developmental toxicity

No human data is available for 2-EHA-PG. The most concerning developmental finding in animals is exencephaly, a severe malformation, in the absence of maternal toxicity in the mouse PNDT study with 2-EHA-PG (Anonymous, 2020). The concern is further increased by occurrence of the same malformation in mouse studies with the structurally related substance valproic acid, a known human teratogen. Therefore, RAC agrees with the DS's proposal of Category 1B for developmental toxicity.

The rat PNDT study with 2-EHA-PG (Anonymous, 2016) also showed developmental effects, mainly skeletal variations, not secondary to maternal toxicity. This study is considered to provide additional support for classification.

Effects on or via lactation

There is no human data, no generational study with 2-EHA-PG nor any toxicokinetic information indicating presence of the substance in breast milk at potentially toxic levels. No classification was agreed by RAC for the presumed metabolite 2-EHA, for which a good-quality generational study was available (ECHA, 2020). Likewise, no effects potentially related to lactation are mentioned in the summary of the generational study with PG (NTP, 2004).

RAC concludes that classification for effects on or via lactation is not warranted, acknowledging that the information available on this endpoint is limited to generational studies with the two presumed metabolites (2-EHA and PG).

Overall conclusion on reproductive toxicity

RAC agrees with the DS's proposal of **Repr. 1B; H360D**.

Additional references

Anonymous (2016) RD 15134: Toxicity study by oral administration to Sprague-Dawley rats for 13 weeks. Envigo CRS Limited. Study number: NUG0004

ECHA (2018) Decision on a compliance check. Substance name: 2-ethylhexanoic acid, monoester with propane-1,2-diol. Decision number: CCH-D-2114449846-34-01/F. 26 November 2018

Morrissey *et al.* (1989) Results and evaluations of 48 continuous breeding reproduction studies conducted in mice. Fundamental and Applied Toxicology 13:747-777

Nau *et al.* (1991) Valproic acid-induced neural tube defects in mouse and human: aspects of chirality, alternative drug development, pharmacokinetics and possible mechanisms. Pharmacology & Toxicology 69:310-321

NTP (2004) NTP-CERHR monograph on the potential human reproductive and developmental effects of propylene glycol. NIH publication no. 04-4482. Online: https://ntp.niehs.nih.gov/ntp/ohat/egpg/propylene/pg_monograph.pdf (last accessed 15/11/2022)

NTP (1985) Propylene glycol: reproduction and fertility assessment in CD-1 mice when administered in drinking water. Cincinnati (OH): National Toxicology Program.

Wang *et al.* (2018) Human carboxylesterases: a comprehensive review. Acta Pharmaceutica Sinica B 8:699-712

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).