Committee for Risk Assessment

RAC

Annex 1

Background document

to the 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

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

Adopted

16 March 2023

CLH report

Proposal for Harmonised Classification and Labelling

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

Chemical name:

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

EC Number: 285-503-5

CAS Number: 85114-00-7

Index Number: n.a.

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ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON 2-ETHYLHEXANOIC ACID, MONOESTER WITH PROPANE-1,2-DIOL

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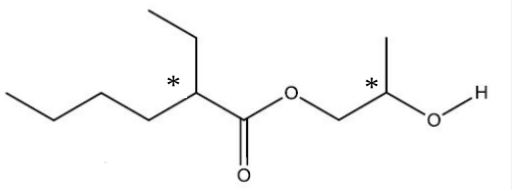
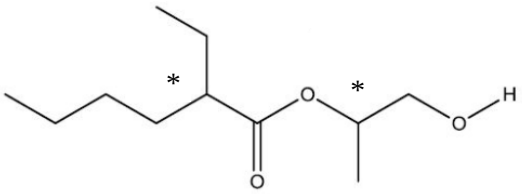
1 IDENTITY OF THE SUBSTANCE

1.1 Name and other identifiers of the substance

Table 1-1: Substance identity and information related to molecular and structural formula of the substance

Name(s) in the IUPAC nomenclature or other international chemical name(s)	2-ethylhexanoic acid, monoester with propane-1,2-diol
Other names (usual name, trade name, abbreviation)	Hexanoic acid, 2-ethyl-, monoester with 1,2-propanediol
ISO common name (if available and appropriate)	-
EC number (if available and appropriate)	285-503-5
EC name (if available and appropriate)	2-ethylhexanoic acid, monoester with propane-1,2-diol
CAS number (if available)	85114-00-7
Other identity code (if available)	
Molecular formula	$C_{11}H_{22}O_3$
Structural formula	Constituent 1:  Constituent 2: 
SMILES notation (if available)	Constituent 1: CC(O)COC(=O)C(CCCC)CC Constituent 2: CC(CO)OC(=O)C(CCCC)CC
Molecular weight or molecular weight range	202.29 g/mol
Information on optical activity and typical ratio of (stereo) isomers (if applicable and appropriate)	The substance is a multi-constituent. Under the REACH registration substance components are registered to consist of two isomers. The ratio of the two isomers is not publicly available. Constituent 1 and 2 each includes two asymmetric carbon atoms (*) resulting in a total of four stereoisomers for each of the constituents.
Description of the manufacturing process and identity of the source (for UVCB substances only)	The substance is not a UVCB

Degree of purity (%) (if relevant for the entry in Annex VI)	≥ 90 - ≤ 100 % (w/w) as sum of the content of constituent 1 and 2.

1.2 Composition of the substance

Table 1-2: Constituents (non-confidential information)

Constituent (Name and numerical identifier)	Concentration range (% w/w minimum and maximum in multi-constituent substances)	Current CLH in Annex VI Table 3 (CLP)	Current self-classification and labelling (CLP)
2-hydroxypropyl 2-ethylhexanoate CAS no. 58921-10-1	No data	None	"Not classified"
1-hydroxypropan-2-yl 2-ethylhexanoate CAS no. -	No data	None	None

Table 1-3: Impurities (non-confidential information) if relevant for the classification of the substance

Impurity (Name and numerical identifier)	Concentration range (% w/w minimum and maximum)	Current CLH in Annex VI Table 3 (CLP)	Current self-classification and labelling (CLP)	The impurity contributes to the classification and labelling
Impurities not relevant for the classification of the substance.				

Table 1-4: Additives (non-confidential information) if relevant for the classification of the substance

Additive (Name and numerical identifier)	Function	Concentration range (% w/w minimum and maximum)	Current CLH in Annex VI Table 3 (CLP)	Current self-classification and labelling (CLP)	The additive contributes to the classification and labelling
No additives have been identified					

2 PROPOSED HARMONISED CLASSIFICATION AND LABELLING

2.1 Proposed harmonised classification and labelling according to the CLP criteria

Table 2-1: For substance with no current entry in Annex VI of CLP

	Index No	Chemical name	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors and ATEs	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 submitter's proposal	TBD	2-ethylhexanoic acid, monoester with propane-1,2-diol	285-503-5	85114-00-7	Repr. 1B	H360D	GHS08	H360D			

Table 2-2: Reason for not proposing harmonised classification and status under consultation

Hazard class	**Reason for no classification**	**Within the scope of consultation**
Explosives	Hazard class not assessed in this dossier	No
Flammable gases (including chemically unstable gases)	Hazard class not assessed in this dossier	No
Oxidising gases	Hazard class not assessed in this dossier	No
Gases under pressure	Hazard class not assessed in this dossier	No
Flammable liquids	Hazard class not assessed in this dossier	No
Flammable solids	Hazard class not assessed in this dossier	No
Self-reactive substances	Hazard class not assessed in this dossier	No
Pyrophoric liquids	Hazard class not assessed in this dossier	No
Pyrophoric solids	Hazard class not assessed in this dossier	No
Self-heating substances	Hazard class not assessed in this dossier	No
Substances which in contact with water emit flammable gases	Hazard class not assessed in this dossier	No
Oxidising liquids	Hazard class not assessed in this dossier	No
Oxidising solids	Hazard class not assessed in this dossier	No
Organic peroxides	Hazard class not assessed in this dossier	No
Corrosive to metals	Hazard class not assessed in this dossier	No
Acute toxicity via oral route	Hazard class not assessed in this dossier	No
Acute toxicity via dermal route	Hazard class not assessed in this dossier	No
Acute toxicity via inhalation route	Hazard class not assessed in this dossier	No
Skin corrosion/irritation	Hazard class not assessed in this dossier	No
Serious eye damage/eye irritation	Hazard class not assessed in this dossier	No
Respiratory sensitisation	Hazard class not assessed in this dossier	No
Skin sensitisation	Hazard class not assessed in this dossier	No
Germ cell mutagenicity	Hazard class not assessed in this dossier	No
Carcinogenicity	Hazard class not assessed in this dossier	No
Reproductive toxicity	**Harmonised classification proposed (Repr. 1B, H360D)**	**Yes**
Specific target organ toxicity-single exposure	Hazard class not assessed in this dossier	No
Specific target organ toxicity-repeated exposure	Hazard class not assessed in this dossier	No
Aspiration hazard	Hazard class not assessed in this dossier	No
Hazardous to the aquatic environment	Hazard class not assessed in this dossier	No
Hazardous to the ozone layer	Hazard class not assessed in this dossier	No

3 HISTORY OF THE PREVIOUS CLASSIFICATION AND LABELLING

The substance, 2-ethylhexanoic acid, monoester with propane-1,2-diol (CAS no. 85114-00-7), has no current harmonised classification in Annex VI of the CLP regulation. A total of four notifiers have submitted one Joint Entry to ECHA and classify the substance as Repr. 2 (H361d).

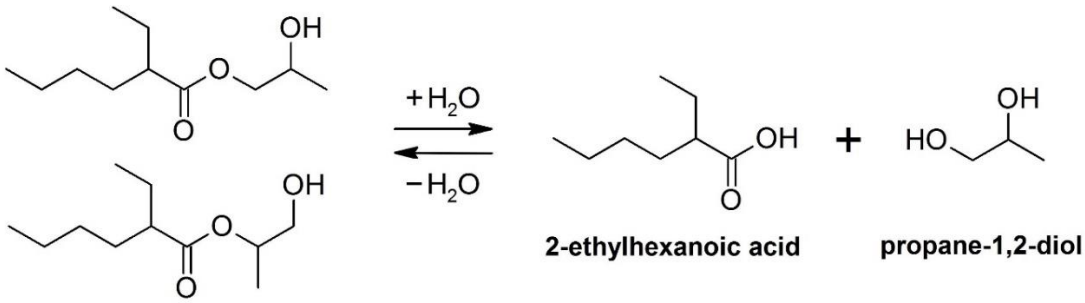
A potential metabolite generated by hydrolysis of the substance may be 2-ethylhexanoic acid (CAS no. 149-57-5), which was classified for reproductive toxicity under the former Dangerous Substance Directive (DSD) due to its developmental effects and later included in the CLP Annex VI (index no. 607-230-00-6) as Repr. 2 (H361d).

In addition, 2-ethylhexanoic acid has been subjected to substance evaluation due to a potential fertility concern[1]. However, the information requested during the evaluation did not confirm that concern.

After the substance evaluation a CLH dossier was elaborated on "2-ethylhexanoic acid and its salts with the exception of those specified elsewhere in this annex"[2]. The CLH dossier reviewing the most recent literature concluded that available literature did not warrant classification for sexual function and fertility or for effects on or via lactation. For developmental effects, it was proposed to maintain the classification of Repr. 2 (H361d). However, in their opinion[3] RAC concluded that the substance and its salts should be classified as Repr.1B (H360D). This decision was based on a weight of evidence assessment taking into account several animal studies examining the developmental toxicity of 2-ethylhexanoic acid in addition to further animal and human data on the structurally very closely related substance valproic acid, a known human teratogen.

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:



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).

[1] Substance evaluation - CoRAP - ECHA (europa.eu)

[2] CLH intentions until outcome 2-EHA and salts - ECHA (europa.eu)

[3] RAC opinion CLH report 2-EHA and salt (europa.eu)

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).

4 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

There is no requirement for justification that action is needed at Community level. According to Article 36(1) of the CLP regulation, reproductive toxicity is an endpoint for which harmonised classification and labelling (CLH) is warranted.

5 IDENTIFIED USES

Data in the publicly available part of the REACH registration dossier for 2-ethylhexanoic acid, monoester with propane-1,2-diol (September, 2021) identify the following uses: Registered uses for the substance include both consumers (coating products and inks and toner), professional workers (widespread uses), in formulation or re-packing at industrial sites and in manufacturing.

6 DATA SOURCES

The primary sources of information for this CLH proposal are the original prenatal developmental toxicity study reports, also included as key studies in the REACH registration of the substance:

Anonymous (2016). Test report. A prenatal developmental toxicity study (OECD TG 414) in rats.

Anonymous (2020). Test report. A prenatal developmental toxicity study (OECD TG 414) in mice.

Data from the REACH registration of the substance: https://echa.europa.eu/registration-dossier/-/registered-dossier/11954/3/1/6

For further data a systematic literature search was performed and completed in August 2021. The literature search included both scientific and other open literature. It was conducted using all identified chemical names related to the CAS no. 85114-00-7 and numerical identifiers.

Literature searches were performed using the Scientific and Technical information Network (STN) (e.g. TOXCENTER (Toxicology Center), EMBASE (Excerpta Medica), and Science Citation Index (SciSearch®).

Relevance of retrieved articles was first examined by title, then by abstract and lastly (where relevant) by review of the whole text. However, no further relevant literature on the substance was found from this search.

7 PHYSICOCHEMICAL PROPERTIES

Table 7-1: Summary of physicochemical properties

Property	Value	Reference	Comment (e.g. measured or estimated)
Physical state at 20°C and 101,3 kPa	Liquid	REACH registration dossier	
Melting/freezing point	-20 °C	REACH registration dossier	101.3 kPa
Boiling point	253 °C	REACH registration dossier	98.1 - 98.8 kPa
Relative density	0.942	REACH registration dossier	20.0±0.5 °C

Property	Value	Reference	Comment (e.g. measured or estimated)
Vapour pressure	1.2 Pa	REACH registration dossier	25 ºC
Surface tension	41.5 mN/m	REACH registration dossier	1g/L and 21.0±0.5 ºC
Water solubility	1.79 g/L	REACH registration dossier	20.0±0.5 ºC
Partition coefficient n-octanol/water	Log Kow = 2.98	REACH registration dossier	40 ºC
Flash point	126 ± 2 ºC	REACH registration dossier	101.3 kPa
Flammability	Non flammable	REACH registration dossier	Study waived with the reasoning: Based on experience in manufacture and use, pyrophoricity and flammability in contact with water are not demonstrated by the substance.
Explosive properties	Non explosive	REACH registration dossier	Study waived with the reasoning: No chemical groups associated with explosive properties present in the molecule of the substance.
Self-ignition temperature	360 ºC	REACH registration dossier	101.3 - 102.9 kPa
Oxidising properties	Non oxidising	REACH registration dossier	Study waived with the reasoning: The substance is incapable of reacting exothermically with combustible materials on the basis of chemical structure.
Granulometry	-	REACH registration dossier	The substance is a liquid.
Stability in organic solvents and identity of relevant degradation products	-	REACH registration dossier	Study waived with the reasoning: The stability of the substance in organic solvents is not considered to be critical.
Dissociation constant	-	REACH registration dossier	Study waived with the reasoning: The substance does not contain functional groups that can ionize and influence pH.
Viscosity	15.6 mPa s	REACH registration dossier	20.0

8 EVALUATION OF PHYSICAL HAZARDS

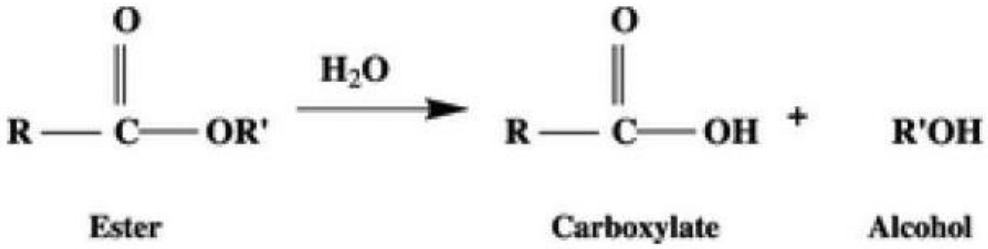
Hazard classes not assessed in this dossier.

9 TOXICOKINETICS (ABSORPTION, METABOLISM, DISTRIBUTION AND ELIMINATION)

No test data on metabolism of this substance have been found neither from literature search nor in the REACH registration of the substance.

However, in the REACH registration it is indicated that 2-ethylhexanoic acid, monoester with propane-1,2-diol *"is likely to undergo some degree of hydrolysis by esterases, particularly under acidic conditions to form **2-ethylhexanoic acid** and **propan-1,2-diol**".*

Such hydrolysis of the substance in the body is considered very likely, based on knowledge of human carboxylesterases:



RCOOR´:*2-ethylhexanoic acid, monoester with propane-1,2-diol*

Metabolites: RCOOH*: 2-ethylhexanoic acid* R'OH: *propan-1,2-diol*

According to Laizure *et al*. (2013) the carboxylesterases in mammals have been classified into five families, Ces1-Ces5 based on amino acid homology, but the majority identified fall into the Ces1 or Ces2 family. Humans follow a similar pattern with the two major carboxylesterases being human carboxylesterase 1 (hCE1) and human carboxylesterase 2 (hCE2). Though these carboxylesterases lack substrate specificity, and drug substrates are susceptible to hydrolysis by carboxylesterase (and often other esterases), usually one carboxylesterase predominates and serves as the major pathway of hydrolysis. Which carboxylesterase predominates is predictable based on the structure of the ester. Esters contain an acyl group (this becomes the carboxylic acid upon hydrolysis) and an alcohol group. The hCE1 enzyme prefers esters with a large, bulky acyl group and a small alcohol group, while hCE2 has the opposite preference, substrates with a small acyl group and a large alcohol group. The carboxylesterases are located in the cytoplasm and endoplasmic reticulum of numerous tissues including the liver, small intestine, kidney, and lungs, but the greatest quantities are found in the liver and small intestine where they contribute significantly to the first-pass metabolic hydrolysis (Laizure *et al.*, 2013).

So, although no quantitative data is available on the hydrolysis of 2-ethylhexanoic acid, monoester with propane-1,2-diol, the ubiquitous nature of active carboxylesterases in the human body would suggest hydrolysis of the substance to be a dominant metabolic route for the substance.

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

Based on general knowledge of human carboxylesterases the metabolism/hydrolysis of 2-ethylhexanoic acid, monoester with propane-1,2-diol into *2-ethylhexanoic acid* and *propan-1,2-diol*, is to be expected in the human body.

This is considered relevant for the classification of 2-ethylhexanoic acid, monoester with propane-1,2-diol, as a harmonised classification of 2-ethylhexanoic acid as Repr. 1B (H360D) has recently been adopted by RAC (ECHA, 2020).

10 EVALUATION OF HEALTH HAZARDS

Acute toxicity

10.1 Acute toxicity - oral route

Hazard class not assessed in this dossier.

10.2 Acute toxicity - dermal route

Hazard class not assessed in this dossier.

10.3 Acute toxicity - inhalation route

Hazard class not assessed in this dossier.

10.4 Skin corrosion/irritation

Hazard class not assessed in this dossier.

10.5 Serious eye damage/eye irritation

Hazard class not assessed in this dossier.

10.6 Respiratory sensitisation

Hazard class not assessed in this dossier.

10.7 Skin sensitisation

Hazard class not assessed in this dossier.

10.8 Germ cell mutagenicity

Hazard class not assessed in this dossier.

10.9 Carcinogenicity

Hazard class not assessed in this dossier.

10.10 Reproductive toxicity

10.10.1 Adverse effects on sexual function and fertility

No data on the substance specifically addressing adverse effects on sexual function and fertility are available.

However, fertility data for the potential metabolite *2-ethylhexanoic acid* (2-EHA) is available and in the CLH proposal for ”2-ethylhexanoic and its salts, with the exception of those specified elsewhere in Annex VI of CLP”(2019) the following summary was given based on the data:

“Regarding the adverse effects on fertility and sexual function of 2-EHA, an apparent reduction in sperm motility and a delay in fertilization were reported in a low-quality and non-GLP one-generation reproductive toxicity study with 2-EHA in Wistar rats administered in doses up to 600 mg/kg bw/d (Pennanen et al., 1993). Reduction of motile spermatozoa of 37% and 22% was seen at 100 and 600 mg/kg bw/d ($p<0.05$), respectively. Regarding the delay in fertilization, 2-EHA-treated female rats conceived in the course of three or four cycles while control animals did it in the course of two oestrus cycles. Moreover, all non-pregnant females belonged to treated groups. However, it has to be taken into consideration that effects on sexual function and fertility similar to those seen in the one generation reproductive toxicity study were not observed neither in the screening study nor in the EOGRTS performed in rats of the same strain at higher doses up to 800 mg/kg bw/d. Neither treatment-related effects on epididymal and testicular sperm parameters nor on fertility and reproductive performance of animals of the F0 generation and of cohort 1B of the F1 generation have been reported in these recently high-quality and GLP studies performed according to the OECD guidelines (Anonymous, 2015; 2016).

In conclusion, taking into account the three studies available with 2-EHA and considering the questionable quality of the one generation study and the lack of reproducibility of the effects observed, it has been considered that there is no animal evidence that 2-EHA interferes with sexual function or fertility.”

Based on this data, the RAC Opinion for sexual function and fertility of 2-ethylhexanoic acid was the following (ECHA, 2020):

"The slight delay in fertilisation in the one-generation study Pennanen et al. (1993) and slight changes in oestrous cyclicity in the EOGRTS study in Anonymous (2016) are probably related to treatment but are not considered sufficient to trigger classification. No other fertility related effects were observed in the generational or repeat dose studies. Thus, RAC agrees with the DS's proposal of no classification for sexual function and fertility".

Thus, the assumed metabolite 2-ethylhexanoic acid is not considered to cause adverse effects on sexual function and fertility.

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

No studies specifically addressing adverse effect on sexual function and fertility have been found on the substance.

The lack of any effects on sexual function and fertility is supported by data on *2-ethylhexanoic acid* (a potential metabolite from 2-ethylhexanoic acid, monoester with propane-1,2-diol). RAC has in their opinion on the classification proposal on 2-ethylhexanoic acid concluded that no classification for sexual function and fertility was warranted (ECHA, 2020).

10.10.3 Comparison with the CLP criteria

No data on concerning effects on sexual function and fertility has been identified for the substance. The available data on the potential metabolite, 2-ethylhexanoic acid, did not show effects on sexual function and fertility. Due to lack of relevant data, the substance 2-ethylhexanoic acid, monoester with propane-1,2-diol is not to be classified for effects on sexual function and fertility.

10.10.4 Adverse effects on development

Two OECD TG 414 studies using oral administration are available, one in mice and one in rats.

Table 10-1: Summary table of animal studies on adverse effects on development

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
Prenatal Development al Toxicity Study OECD TG 414 GLP Rats Sprague-Dawley Crl:CD® (SD) strain Females	*2-ethylhexanoic acid, monoester with propane-1,2-diol* Analytical purity: 95.8% (purity data from REACH-reg.) Vehicle: Corn oil Oral (gavage) Once daily Dose: 0, 100, 300, 1000 mg/kg bw/day	**Maternal toxicity** No adverse effects related to administration of the substance at any dose level were reported from the clinical observations. Body weight was unaffected by treatment at the end of the study. Gravid uterine weights and placental weights were unaffected by the treatment. Only statistically significant findings: *1000 mg/kg bw/day* ↓ Slightly, but statistically significant ($p<0.01$) loss in mean body weight (- 4g compared to 0 g in controls) on GD 6-7. ↓ Slightly, but statistically significant ($p<0.01$) lower mean food consumption (17 g/day compared to 20 g/day in control) on GD 6-9.	Anonymous, 2016

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
20/group Reliability 1	Days 6-19 of gestation	**Developmental toxicity** No statistical differences for any dose groups compared to controls were found for corpora lutea, implantation, early-, late-, and total resorptions or in pre- and post-implantation loss. Regarding live pups, fetal weight and sex ratio the following findings were reported: *1000 mg/kg bw/day* ↑ Number of live pups per litter (statistically significant, $p<0.05$) (16.0 pups/litter vs 14.3 pups/litter in controls). ↓ Mean fetal weight of the pups (11%) compared to controls (statistically significant, $p<0.05$). *300 mg/kg bw/day* ↓Male/female ratio of 45.6% (statistically significant, $p<0.01$) compared to controls (57.1%). Mean litter size was very close to that in the 1000 mg/kg bw/day group and yet the mean fetal weight at 300 mg/kg bw/day was similar to controls. *Malformations and variations:* *1000 mg/kg bw/day* Two fetuses in two litters had the major abnormality short/threadlike tail. There was an increased incidence of a spectrum of minor abnormalities/skeletal variants: large nasofrontal suture; thoracic vertebral abnormality; short supernumerary cervical rib and 14th rib; delayed/incomplete ossification/unossified cranial centres, cervical, thoracic and sacral caudal vertebrae, sternebra, pelvic bones, metacarpals/metatarsals and a decrease in ossified cervical vertebral centra; variation in lens shape; small/absent lobe of thyroid; partially undescended lobe of thymus; small/absent renal papilla and dilated ureter *300 mg/kg bw/day* Increased incidence of the minor abnormalities large nasofrontal suture; thoracic vertebral abnormality; delayed/incomplete ossification/unossified thoracic vertebrae and a decrease in ossified cervical vertebral centra *100 mg/kg bw/day* Increased incidence of the minor abnormalities large nasofrontal suture and variation in lens shape. The increased findings of large nasofrontal sutures (a minor, non-severe malformation) were considered by the authors of the test report as unusual and test item related. The findings occurred in a dose-related manner: 0 mg/kg bw/day: No fetuses (0 litters). 100 mg/kg bw/day: 5 fetuses (3 litters). 300 mg/kg bw/day:16 fetuses (7 litters)	

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
		1000 mg/kg bw/day: 107 fetuses (20 litters)	
Prenatal Developmental Toxicity Study OECD TG 414 GLP Mouse, Crl:CD-1(ICR) Females 24/group Reliability 1	*2-ethylhexanoic acid, monoester with propane-1,2-diol* Purity: * Vehicle: Corn oil Oral (gavage) Once daily Dose: 0, 100, 300, 1000 mg/kg bw/day Days 6-17 of gestation	**Maternal toxicity** No adverse effects related to administration of the substance at any dose level were reported by clinical observation of the animals throughout the study. Food consumption, thyroid hormones and body weight at the end of gestation were not different in any dose groups compared to controls. Statistical differences were observed in body weight changes. These were, however, transient and thus considered not to be related to the test substance. Gravid uterine weight and placenta weight were unaffected. **Developmental toxicity** No statistically significant differences for any dose groups compared to controls were found for corpora lutea, implantation, early/late/total resorptions or in pre- and post-implantation loss. Also, no statistical differences for any dose groups compared to controls were observed in litter size or sex ratios *1000 mg/kg bw/day* ↓ Fetal weight, ($p<0.001$) (male 13% reduction and female 15% reduction compared to controls, adjusted for litter size). *Malformations and variations:* *1000 mg/kg bw/day* Treatment related effects reported. ↑ Incidences of malformations of head, skull and brain - Head malformations (all exencephaly) were recorded in 8 fetuses (4 litters). Thus, statistically significant differences were observed in both % litters and % fetuses ($p<0.05$). - Brain malformations were recorded in 5 fetuses (2 litters) in the form of disorganized cranial structure. - Skull formations were recorded in a total of 6 fetuses (3 litters) in the form of small orbital sockets (3 fetuses) and open eyes (4 fetuses), misshapen frontal (3 fetuses), interparietal (3 fetuses), parietal (3 fetuses), squamosal regions (3 fetuses) and absent supraoccipital (3 fetuses). ↑ Incidences of skeletal variations, primarily unossified or incomplete ossification of the skeleton. A significant difference between control and 1000 mg/kg bw/day was observed for: - Bipartite ossification of the sternebra in 5 fetuses (4 litters). Statistically significant difference obtained in % litter ($p<0.05$) - Supernumerary rib present in the sternebra in 62 fetuses (19 litters). Statistically significant differences were obtained in both	Anonymous, 2020

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
		% litter and % fetal ($p<0.05$ and $p<0.001$, respectively) - Unossified vertebra – cervical centrum in 87 fetuses (20 litters) Statistically significant difference was obtained in both % litter and % fetal, ($p<0.001$). Other notable fetal variations in 1000 mg/kg bw/day were observed in skull, sternebra, vertebra, forelimb and hindlimb. *300 mg/kg bw/day* No treatment related effects reported. *100 mg/kg bw/day* No treatment related effects reported.	

*Data on purity in confidential Annex II

No further developmental studies for *2-ethylhexanoic acid, monoester with propane-1,2-diol* are available.

However, for the potential metabolite *2-ethylhexanoic acid*, the following overview on developmental studies in rats and mice, respectively, were given by RAC (ECHA, 2020) (Table 10-2 and Table 10-3):

Table 10-2: Overview of developmental studies in rats with *2 ethyl hexanoic acid* (ECHA, 2020)

Overview of developmental effects in rat studies with 2-EHA			
Study	**Dose, substance and vehicle, strain**	**Developmental findings**	**Maternal toxicity**
PNDT Anonymous (1988c)	500 mg/kg bw/d, 2-EHA in corn oil, Fischer 344	Dilated brain ventricles (variation), extra thoracic vertebra, reduced ossification, ↓ foetal weight (8%)	Clinical signs (hypoactivity, ataxia) at a low incidence; no effect on bw or fc
PNDT Anonymous (1997)	600 mg/kg bw/d, 2-EHA in olive oil, Wistar	↓ foetal weight (21%), tail malformations and absent caudal vertebrae (low incidence), extra thoracic and lumbar vertebrae, cervical and lumbar ribs, reduced ossification	No significant maternal toxicity
PNDT Pennanen *et al.* (1992)	600 mg/kg bw/d, sodium salt via drinking water, Han:Wistar	Clubfoot, dilated brain ventricles, wavy ribs, reduced ossification, ↓ foetal weight (9%)	Reduced corrected bw (ca. 20 g)
1-generation Pennanen *et al.* (1993)	600 mg/kg bw/d, sodium salt via drinking water, Han:Wistar	Kinky tail (from 300 mg/kg bw/d), ↓ pup weight (ca. 10%), developmental delay	Slightly reduced bw gain
EOGRTS Anonymous (2016)	800 mg/kg bw/d, 2-EHA via diet, Wistar	No developmental effects	Reduced bw gain
Developmental Narotsky *et al.* (1994)	900 mg/kg bw/d, 2-EHA in corn oil, Sprague-Dawley	↓ pup weight (13%), extra lumbar vertebra, cervical and lumbar ribs	Clinical signs (motor depression, rales), excessive mortality (related to respiratory effects)
Developmental Bui *et al.* (1998)	500 mg/kg bw/d, 2-EHA in corn oil, Sprague-Dawley	↑ resorptions, rib and tail anomalies, ↓ foetal weight (9%)	Reduced corrected bw gain (ca. 25 g)
Developmental, single dose Ritter *et al.* (1987)	1800 mg/kg bw, 2-EHA undiluted, Wistar	Limb, tail and cardiovascular defects, hydronephrosis	No information available

As indicated in Table 10-1 below, mice may be especially sensitive towards developmental effects from exposure to 2-ethylhexanoic acid (ECHA, 2020):

Table 10-3: Overview of observations of exencephaly in studies with *2-ethylhexanoic acid* in mice (ECHA, 2020)

Exencephaly in the mouse after i.p. injections on GD 7 and 8 (Hauck *et al.*, 1990)					
	Control	**(R)-2-EHA**	**(S)-2-EHA**	**(±)-2-EHA**	**(±)-2-EHA**
Dose (mg/kg bw)		**4 x 500**	**4 x 500**	**4 x 500**	**1 x 500**
Number of litters	10	17	9	20	14
Number of live foetuses	126	172	100	212	157
Embryolethality (%)	6	11	1	10	7
Exencephaly (%)	0	59	1	32	5
Foetal weight (g, ±SD)	1.14 (±0.05)	1.00 (±0.05)	1.16 (±0.10)	1.01 (±0.08)	1.17 (±0.09)

From these data it can be noted that the R-enantiomer of 2-ethylhexanoic acid was found to cause the adverse effects on brain development.

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

Two oral OECD TG 414 studies on the substance have been conducted, one in rats (Anonymous, 2016) and one in mice (Anonymous, 2020).

Prenatal developmental toxicity study (OECD TG 414) in rats (Anonymous, 2016)

In this GLP compliant OECD TG 414 study, groups of 20 female Sprague-Dawley rats were administered *2-ethylhexanoic acid, monoester with propane-1,2-diol* at dose levels of 0, 100, 300 or 1000 mg/kg bw/day (group 1, 2, 3 and 4) by oral gavage administration, from Day 6 until and including Day 19, after mating.

Maternal toxicity

There were no signs at routine examination that could be associated with treatment and no signs were observed in association with dose administration. Body weight, gravid uterine weight, food consumption and macroscopic evaluation were not adversely affected by treatment up to 1000 mg/kg bw/day, when compared with control animals. At 1000 mg/kg bw/day, slight mean body weight loss was recorded during days 6-7 of gestation and mean food consumption was slightly low during days 6-9 (Table 10-4).

Table 10-4: Body weight change for females during gestation (Anonymous, 2016)

Group /Sex		Days 0-3	Days 3-6	Days 6-7	Days 7-8	Days 8-9	Days 9-10	Days 10-11	Days 11-12	Days 12-13	Days 13-14	Days 14-15	Days 15-16
Statistical test:		Av	Av	Wi	Wi	Wi	Wi	Wi	Sh	Wi	Wi	Wi	Wi
1F	Mean	18	13	0	6	3	5	8	6	2	5	8	9
	SD	5.1	6.1	5.2	4.8	4.4	6.5	5.1	4.9	5.4	4.9	5.1	5.1
	N	20	20	20	20	20	20	20	20	20	20	20	20
2F	Mean	19	10	3	4	5	6	8	4	4	6	9	9
	SD	5.6	5.4	4.4	4.1	4.3	4.7	4.0	2.7	3.2	3.9	4.5	3.6
	N	20	20	20	20	20	20	20	20	20	20	20	20
3F	Mean	19	12	1	5	5	6	7	5	6*	5	9	10
	SD	6.1	6.9	4.1	5.2	5.0	3.8	3.5	6.1	4.5	4.1	4.3	4.2
	N	20	20	20	20	20	20	20	20	20	20	20	20
4F	Mean	19	10	-4**	4	6	7	8	5	6**	6	8	9
	SD	6.6	6.3	5.0	4.7	5.4	5.5	5.0	5.3	3.6	3.4	4.0	4.3
	N	20	20	20	20	20	20	20	20	20	20	20	20

Group /Sex		Days 16-17	Days 17-18	Days 18-19	Days 6-19	Days 19-20
Statistical test:		Wi	Wi	Wi	Wi	Wi
1F	Mean	13	14	15	95	18
	SD	5.2	5.9	3.2	13.9	4.9
	N	20	20	20	20	19
2F	Mean	16	15	13	102	18
	SD	3.8	5.8	4.7	7.4	6.7
	N	20	20	20	20	19
3F	Mean	14	16	15	104*	20
	SD	5.3	4.3	5.4	14.5	4.5
	N	20	20	20	20	19
4F	Mean	15	15	16	103*	18
	SD	4.3	4.1	4.3	12.1	8.7
	N	20	20	20	20	19

Av: Pre-treatment comparison of all groups using Analysis of variance followed by pairwise t-tests
Wi: Treated groups compared with Control using Williams' test
* $p<0.05$
** $p<0.01$

Developmental toxicity

Litter data as assessed by mean corpora lutea, implantations, early, late and total resorptions, sex ratio and pre- and post-implantation loss for animals receiving 100, 300 and 1000 mg/kg bw/day were not adversely affected by treatment. Placental and litter weight were similar to controls and were not affected by the administration. At 1000 mg/kg bw/day the litter size was significantly increased, and male, female andoverall fetal weights were statistically significantly lower when compared with controls (Tables 10-5 and 10-6).

Table 10-5: Litter data - corpora lutea, implantations, resorptions, life pups, sex ratio, implantation loss - group mean values on Day 20 of gestation (Anonymous, 2016)

Group		Corpora	Implantations	Resorptions			Live Young			Sex ratio	Implantation Loss (%)	
/Sex		Lutea		Early	Late	Total	Male	Female	Total	(%M)	Pre-	Post-
Statistical test:		Wi	Wi	Wc	Wc	Wc	Wi	Wi	Wi	Wa	Wa	Wa
1F	Mean	16.4	15.7	1.4	0.0	1.4	8.0	6.3	14.3	57.1	4.5	8.9
	SD	1.67	1.31				1.95	2.45	2.74			
	N	20	20	20	20	20	20	20	20	20	20	20
2F	Mean	17.3	16.2	0.8	0.4	1.2	7.8	7.3	15.0	52.7	6.1	7.4
	SD	1.71	1.66				2.05	2.79	2.79			
	N	20	20	20	20	20	20	20	20	20	20	20
3F	Mean	17.4	16.5	0.8	0.0	0.8	7.1	8.6*	15.7	45.6**	6.0	4.5
	SD	1.66	1.82				1.89	2.35	1.92			
	N	20	20	20	20	20	20	20	20	20	20	20
4F	Mean	17.2	16.6	0.6	0.1	0.7	8.4	7.6*	16.0*	52.8	3.7	3.9
	SD	1.84	1.88				1.90	2.04	1.82			
	N	20	20	20	20	20	20	20	20	20	20	20

Wa: Treated groups compared with Control using Wald's test.
Wc: Treated groups compared with Control using Wilcoxon rank sum test
Wi: Treated groups compared with Control using Williams' test
* $p<0.05$
** $p<0.01$

Table 10-6: Placental, litter and fetal weights - group mean values (g) on Day 20 of gestation (Anonymous, 2016)

Group /Sex		Placental Weight	Litter Weight	Litter Size	Male Fetal Weight	Female Fetal Weight	Overall Fetal Weight
Statistical test:		Wi	Sh	Wi	Wi	Sh	Wi
1F	Mean	0.55	53.13	14.30	3.82	3.58	3.72
	SD	0.065	10.469	2.736	0.270	0.319	0.282
	N	20	20	20	20	20	20
2F	Mean	0.56	56.73	15.00	3.85	3.62	3.74
	SD	0.057	12.443	2.791	0.427	0.430	0.412
	N	20	20	20	20	20	20
3F	Mean	0.54	58.48	15.70	3.84	3.65	3.73
	SD	0.047	7.116	1.922	0.230	0.223	0.224
	N	20	20	20	20	20	20
4F	Mean	0.54	52.53	15.95*	3.40**	3.19**	3.30**
	SD	0.073	6.168	1.820	0.239	0.224	0.231
	N	20	20	20	20	20	20

Sh: Treated groups compared with Control using Shirley's test
Wi: Treated groups compared with Control using Williams' test
* $p<0.05$
** $p<0.01$

Malformations and variations

Group 4, 1000 mg/kg bw/day

At this level there were two fetuses in two litters with the major abnormality short/threadlike tail. There was an increased incidence of a wide spectrum of minor abnormalities/skeletal variants: large nasofrontal suture; thoracic vertebral abnormality; short supernumerary cervical rib and 14th rib; delayed/incomplete ossification/unossified cranial centres, cervical, thoracic and sacral caudal vertebrae, sternebra, pelvic bones, metacarpals/metatarsals and a decrease in ossified cervical vertebral centra; variation in lens shape; small/absent lobe of thyroid; partially undescended lobe of thymus; small/absent renal papilla and dilated ureter when compared to concurrent control and Historical Control Data with the exception of delayed/incomplete ossification/unossified cervical vertebrae. These findings indicate a treatment related disturbance of development which is potentially adverse.

Group 3, 300 mg/kg bw/day

At 300 mg/kg bw/day there was an increased incidence of the minor abnormalities large nasofrontal suture; thoracic vertebral abnormality; delayed/incomplete ossification/unossified thoracic vertebrae and a decrease in ossified cervical vertebral centra when compared with concurrent control and Historical Control Data except for delayed/incomplete ossification/unossified thoracic vertebrae. These findings are considered not to represent an adverse effect on fetal development.

Group 2, 100 mg/kg bw/day

At 100 mg/kg bw/day there was an increased incidence of the minor abnormalities large nasofrontal suture and variation in lens shape compared to concurrent control and Historical Control Data. These findings are considered not to represent an adverse effect on fetal development.

The developmental effects described are based on the findings in Tables 10-7, 10-8 and 10-9 below:

Table 10-7: Observations of major anomalies (Anonymous, 2016)

		Fetuses				Litters			
Group		1	2	3	4	1	2	3	4
Number Examined		286	300	314	319	20	20	20	20
Total Number Affected		1	0	0	2	1	0	0	2
Lumbar (and abdominal)/Sacral/Caudal									
Skeletal	Termination vertebral column lumbar region	0	0	0	1	0	0	0	1
Visceral	Omphalocele	1	0	0	0	1	0	0	0
External	Imperforate anus	0	0	0	1	0	0	0	1
	Short/thread like tail	0	0	0	2	0	0	0	2
Appendicular									
External	Malrotated hindlimb(s)	1	0	0	0	1	0	0	0

Table 10-8: Observation regarding minor skeletal abnormalities (from Anonymous, 2016)

		Fetuses				Litters			
Group		1	2	3	4	1	2	3	4
Number Examined		143	150	158	160	20	20	20	20
Minor skeletal abnormalities									
Cranial	sutural bone	1	2	0	2	1	2	0	2
	fissure(s)	0	1	1	0	0	1	1	0
	interparietal fissure(s)	0	0	1	2	0	0	1	1
Vertebral element abnormality	thoracic	0	0	4	3	0	0	4	2
	lumbar	0	0	0	1	0	0	0	1
Ribs	medially thickend/kinked	0	0	0	3	0	0	0	2
Costal cartilage	misaligned	0	0	1	0	0	0	1	0
Total affected by one or more of the above		1	3	5	9	1	3	5	5
Rib and vertebral configuration									
Cervical rib	short supernumerary	1	1	2	5	1	1	2	4
13th rib	short	3	0	0	0	3	0	0	0
	interrupted ossification	0	1	0	0	0	1	0	0
Number of 14th ribs	short supernumerary	9	15	15	54	8	8	8	17
	full supernumerary	1	0	1	0	1	0	1	0
	total	10	15	16	54	9	8	9	17
Thoracolumbar vertebra(e)	20	1	2	0	4	1	2	0	2
Pelvic girdle	unilateral caudal shift	0	0	0	1	0	0	0	1
Delayed/Incomplete ossification/unossified									
Cranial	cranial centres	13	8	10	47	7	7	6	15
	large nasofrontal suture	0	5	16	107	0	3	7	20
	presphenoid	0	0	0	1	0	0	0	1
	hyoid	19	9	21	22	10	4	7	11
Vertebrae	cervical	0	2	1	4	0	2	1	4
	thoracic	7	11	16	27	7	8	12	12
	lumbar	0	0	1	1	0	0	1	1
	sacrocaudal	8	5	11	68	6	5	7	18
	caudal	0	0	0	1	0	0	0	1
Sternebrae	5th and/or 6th	112	101	124	160	20	19	19	20
	other	14	9	10	38	8	8	8	15
	total	113	101	125	160	20	19	19	20
Ribs	any	0	0	0	2	0	0	0	2
Appendicular	pelvic bones	3	4	5	47	3	4	4	15
	long bones	0	0	1	0	0	0	1	0
	metacarpals	4	0	0	27	3	0	0	12
	metatarsals	3	1	1	55	3	1	1	14
Increased ossification									
Cervical vertebral centra	more than 4 ossified	8	14	1	0	3	6	1	0

Note: Individual fetuses/litters may occur in more than one category.

Table 10-9: Observation regarding minor visceral abnormalities (Anonymous, 2016)

		Fetuses				Litters			
Group		1	2	3	4	1	2	3	4
Number Examined		143	150	156	159	20	20	20	20
Total Number Affected		20	36	22	57	12	16	11	18
Visceral abnormalities									
Brain	dilated interventricular foramen	0	1	1	0	0	1	1	0
Lens	variation in shape	2	5	1	13	2	3	1	10
Thyroid	small lobe	0	0	0	2	0	0	0	1
	absent lobe	0	0	0	2	0	0	0	2
Thymus	partially undescended lobe	3	3	3	11	3	3	3	8
	thymic remnant	0	0	0	1	0	0	0	1
Right subclavian artery	arises from aortic arch	0	1	0	1	0	1	0	1
Diaphragm	thinning with liver protrusion	2	2	0	0	2	2	0	0
Kidney(s)	small renal papilla	0	2	1	15	0	2	1	10
	absent renal papilla	0	0	0	2	0	0	0	2
Ureter(s)	dilated	1	2	1	4	1	1	1	4
Testis(es)	undescended	1	0	0	0	1	0	0	0
	malpositioned	4	1	0	1	4	1	0	1
Umbilical artery	left	2	1	2	2	2	1	2	2

Note: Individual fetuses/litters may occur in more than one category.

Prenatal developmental toxicity study (OECD TG 414) in mice (Anonymous, 2020).

In this GLP compliant OECD TG 414 study, groups of 24 female mice were administered *2-ethylhexanoic acid, monoester with propane-1,2-diol* at dose levels of 0, 100, 300 or 1000 mg/kg bw/day (Group 1, 2, 3 and 4) by oral gavage administration, from day 6 until and including day 17, after mating.

Maternal toxicity

There were no signs at routine examination that could be associated with treatment and no signs were observed in association with dose administration. Corrected weight changes[4] for animals administered 1000 mg/kg bw/day were 29% higher than control animals. However, statistical significance was not achieved. The non-significant corrected weight changes were observed with no substance-related effects on gravid uterus weights, carcass weights or weight changes and the corrected weight changes were thus considered incidental and unrelated to the test substance (Table 10-10).

Statistically significant differences were observed in food consumption on Gestation Day (GD) 6-7 in animals administered 100 mg/kg bw/day, and in body weight changes in animals administered 300 mg/kg bw/day on GD 9-12, and on GD 9-12 and GD 15-17 of animals administered 1000 mg/kg bw/day. However, these were transient and thus considered unrelated to the test substance (Table 10-12).

No maternal toxicity was linked to the test substance.

[4] carcass weight, equals terminal body weight minus uterine weight

Table 10-10: Female body Weight, GD 5-18 (Anonymous, 2020)

Test Article		Control	2-Ethylhexanoic acid, monoester with propane-1,2-diol		
Group		1	2	3	4
Dose level (mg/kg/day)		0	100	300	1000
		Data Presented in "g"			
Group/ Sex	Phase / Day	GE 5	GE 6	GE 7	GE 8	GE 9	GE 12
1/F	Mean	31.5	31.9	32.3	33.0	33.6	38.7
	SD	2.33	2.42	2.38	2.37	2.47	2.96
	N	23	23	23	23	23	23
2/F	Mean	31.8	32.1	32.6	33.2	34.1	39.8
	SD	2.54	2.49	2.53	2.70	2.83	3.35
	N	19	19	19	19	19	19
3/F	Mean	31.8	32.0	32.5	33.1	33.8	39.6
	SD	1.51	1.84	1.74	1.85	1.87	2.40
	N	24	24	24	24	24	24
4/F	Mean	31.1	31.5	32.0	32.4	33.2	39.1
	SD	2.59	2.44	2.48	2.62	2.74	3.22
	N	21	21	21	21	21	21
	Statistics	X1	A	A	A	A	A

GE = Gestation
X1 = No analysis required
A = ANOVA and Dunnett's

Summary of Body Weight

Test Article		Control	2-Ethylhexanoic acid, monoester with propane-1,2-diol		
Group		1	2	3	4
Dose level (mg/kg/day)		0	100	300	1000
		Data Presented in "g"			
Group/ Sex	Phase / Day	GE 15	GE 17	GE 18
1/F	Mean	46.9	55.0	58.6
	SD	4.05	5.49	6.37
	N	23	23	23
2/F	Mean	48.2	56.1	59.2
	SD	4.36	6.30	7.19
	N	19	19	19
3/F	Mean	48.1	55.4	59.6
	SD	3.35	4.35	5.03
	N	24	24	24
4/F	Mean	47.3	53.4	57.3
	SD	4.05	5.00	4.88
	N	21	21	21
	Statistics	A	A	A

GE = Gestation
A = ANOVA and Dunnett's

Table 10-11: Summary of food consumption (Anonymous, 2020)

Test Article		Control	2-Ethylhexanoic acid, monoester with propane-1,2-diol		
Group		1	2	3	4
Dose level (mg/kg/day)		0	100	300	1000
		Data Presented in "g/animal/day" Interval X to X			
Group/ Sex	Phase / Day	GE 6 - 7	GE 7 - 8	GE 8 - 9	GE 9 - 12	GE 12 - 15	GE 15 - 17
1/F	Mean	5.1	6.4	5.4	5.4	5.7	7.1
	SD	1.57	2.07	1.35	0.97	0.97	1.19
	N	23	23	22	23	23	22
2/F	Mean	7.0*	6.9	6.5	5.9	5.2	6.9
	SD	2.68	3.65	2.25	1.46	1.31	1.29
	N	17	19	19	19	19	19
3/F	Mean	6.0	6.3	6.0	5.7	6.0	7.6
	SD	1.36	2.78	1.50	1.02	0.92	1.21
	N	24	24	24	24	24	24
4/F	Mean	5.9	6.2	6.0	5.9	6.2	7.6
	SD	1.36	1.40	2.76	1.98	1.04	2.17
	N	20	21	20	21	21	20
	Statistics	AT	A	A	A	A	A

* P<=0.05
** P<=0.01
*** P<=0.001
GE = Gestation
A = ANOVA and Dunnett's
T = Rank-transformed data

Summary of Food Consumption

Test Article		Control	2-Ethylhexanoic acid, monoester with propane-1,2-diol		
Group		1	2	3	4
Dose level (mg/kg/day)		0	100	300	1000
		Data Presented in "g/animal/day" Interval X to X			
Group/ Sex	Phase / Day	GE 17 - 18	GE 6 - 18		
1/F	Mean	8.5	6.1		
	SD	2.70	1.00		
	N	23	21		
2/F	Mean	7.5	6.2		
	SD	2.19	0.94		
	N	19	17		
3/F	Mean	9.0	6.4		
	SD	2.17	0.91		
	N	24	24		
4/F	Mean	8.3	6.2		
	SD	2.52	0.70		
	N	21	18		
	Statistics	A	A		

GE = Gestation
A = ANOVA and Dunnett's

Developmental toxicity

Litter data as assessed by live and dead fetuses, sex ratio, pup weight, mean corpora lutea, implantations, early, late and total resorptions, sex ratio and pre- and post-implantation loss, for animals receiving 100, 300 and 1000 mg/kg bw/day were not adversely affected by treatment. One animal administered 1000 mg/kg bw/day was pregnant on GD 18 but had no viable fetuses. This was an isolated finding and thus considered incidental. In all dose groups placental and litter weight were similar to controls and were not affected by the administration (Tables 10-12 to 10-15).

Table 10-12: Pregnant females, corpora lutea, implantation sites, implantation loss (Anonymous, 2020)

	Group	Control	100 mg/kg	300 mg/kg	1000 mg/kg
Summary of Cesarean Section Data - Excluding Females with No Viable Fetuses					
Number of females pregnant at cesarean section	(n)	23	19	24	21
Corpora Lutea	(n)	23	19	24	21
	Mean	15.6	15.7	15.0	16.1
	SD	2.59	2.33	2.24	2.43
Implantation Sites	(n)	23	19	24	21
	Mean	14.1	14.6	14.3	14.8
	SD	2.55	2.34	1.94	1.37
Pre-implantation Loss	(n)	23	19	24	21
	Mean	1.5	1.2	0.7	1.3
	SD	1.38	2.43	0.95	1.85

Table 10-13: Pre-implantation loss, early-, late- and total resorptions (Anonymous, 2020)

	Group	Control	100 mg/kg	300 mg/kg	1000 mg/kg
Summary of Cesarean Section Data - Excluding Females with No Viable Fetuses					
Pre-implantation Loss (%)	(n)	23	19	24	21
	Mean	9.33	6.48	4.35	7.38
	SD	8.590	12.406	5.695	8.471
Early Resorptions	(n)	23	19	24	21
	Mean	0.8	0.5	0.7	0.7
	SD	1.41	0.96	0.91	0.90
Late Resorptions	(n)	23	19	24	21
	Mean	0.3	0.2	0.1	0.3
	SD	0.45	0.42	0.45	0.73
Total Resorptions	(n)	23	19	24	21
	Mean	1.0	0.7	0.8	1.0
	SD	1.40	0.99	1.05	1.24

Table 10-14: Post-implantation loss, dead and live fetuses (Anonymous, 2020)

	Group	Control	100 mg/kg	300 mg/kg	1000 mg/kg
Summary of Cesarean Section Data - Excluding Females with No Viable Fetuses					
Dead Fetuses	(n)	23	19	24	21
	Mean	0.0	0.3	0.0	0.0
	SD	0.00	1.16	0.00	0.22
Post-implantation Loss	(n)	23	19	24	21
	Mean	1.0	1.1	0.8	1.1
	SD	1.40	1.39	1.05	1.26
Post-implantation Loss (%)	(n)	23	19	24	21
	Mean	7.13	7.23	5.51	7.20
	SD	9.044	9.452	6.890	8.245
Live Fetuses	(n)	23	19	24	21
	Mean	13.3	13.5	13.4	13.7
	SD	2.53	2.63	1.74	1.55

Table 10-15: Fetal weight (Anonymous, 2020)

	Group	Control	100 mg/kg	300 mg/kg	1000 mg/kg
Summary of Mean Fetal Data					
Mean Fetal Weight	(n)	22@	19	24	21
(g)	Mean	1.359	1.347	1.367	1.164
	Adj Mean	1.354	1.352	1.365	1.167#H
	SD	0.1034	0.1053	0.0943	0.1026
Mean Weight	(n)	21@ [a]	19	24	21
- Male Fetuses (g)	Mean	1.387	1.378	1.399	1.194
	Adj Mean	1.382	1.382	1.397	1.197#H
	SD	0.1177	0.1131	0.1020	0.1076
Mean Weight	(n)	21@ [a]	19	24	21
- Female Fetuses	Mean	1.333	1.319	1.340	1.128
(g)	Adj Mean	1.328	1.324	1.338	1.131#H
	SD	0.1031	0.0924	0.0920	0.1000

@ Number examined reduced due to excluded data
#H = Dunnett Exact Homogeneous Test Significant: 0.001 level
[a] Sex not recorded for fetuses assigned for skeletal exams for Female M0001 (Group 1); therefore female excluded from mean calculations

Malformations and variations

Group 4, 1000 mg/kg bw/day

At this dose level a statistically significant ($p<0.001$) lower fetal weight (adjusted for litter size) compared with controls was observed (males: -13%, females: -15%, combined: -14%). The adverse finding is regarded as a test substance related effect.

High incidences of fetal malformations of the head, skull and brain in litters at this dose level were observed (see table 10-18 and 10-19 below). Skull malformations were observed in a total of 6/143 fetuses (litter incidence 14%) versus none observed in the control group. However, the malformations observed in the skull of the fetuses did not show any statistical differences from the control group. Malformations of the brain were reported to be a disorganisation of the cranial cavity structures. Such malformations were observed in 5/145 fetuses of two different litters (litter incidence 10%), versus no malformations in controls and the other dose levels. However, the malformation of the brain was not statistically significant. A statistically significant difference between control and 1000 mg/kg bw/day was observed in:

- Head malformation, exencephaly in 8/288 foetuses (litter incidence 19%), (statistical difference was obtained in litters with $p<0.005$).

Increased incidences of skeletal variations at dosing 1000 mg/kg bw/day were observed, primarily unossified or incomplete ossification of the skeleton (in skull, sternebra, cervical central arch, thoracic centrum and limbs).

Significant skeletal variations were only identified in fetuses maternally exposed to 1000 mg/kg bw/day and not when dosed 100 or 300 mg kg bw/day. A significant difference between control and 1000 mg/kg bw/day was observed in (please see fetal and litter incidences in table 10-17 and 10-19 below):

- Bipartite ossification of the sternebra (statistically significant difference obtained in % litter with $p<0.05$)
- Supernumerary rib present in the sternebra (statistically significant differences were obtained in both % litter and % fetal with $p<0.05$ and $p<0.001$, respectively)
- Unossified vertebra – cervical centrum (statistically significant difference was obtained in both % litter and % fetal with $p<0.001$).

Other notable fetal variations in 1000 mg/kg bw/day were observed in skull, sternebra, vertebra, forelimb and hindlimb.

Group 3, 300 mg/kg bw/day

No treatment related effects reported.

Group 2, 100 mg/kg bw/day

One incidence of head exencephaly was observed. An incidence of such malformation was however reported within Historical Control Data and together with the absence of a dose response (no incidences of such malformations at 300 mg/kg bw/day), the incidence of malformation in the head observed at 100 mg/kg bw/day was considered to be incidental for this one fetus.

Tabulated overviews of the observed fetal variations and malformations is given below in table 10-16, table 10-17 and table10-18:

Table 10-16: Overview of the fetal variations, including historical levels (Anonymous, 2020)

Variation	Control	0	100	300	1000	Historical control range Mean (SD) / range [No. affected]
				mg/kg/day		
Skull						
Hyoid - incomplete ossification	%Litter	0	0	0	10	0.80 (1.79) / 0 to 4 [1]
	%Fetal	0	0	0	1.47	0.17 (0.39) / 0 to 0.87 [1]
	Number of Litters/Fetus	0	0	0	2/2	
-Hyoid - unossified	%Litter	0	0	0	5	Not present in the historical control data
	%Fetal	0	0	0	2.38	
	Number of Litters/Fetus	0	0	0	1/3	
Mandible - incomplete ossification	%Litter	0	0	4	5	0.14 (0.3) / 0 to 0.68 [1]
	%Fetal	0	0	0.69	4.76	1 (2.24) 0 to 5 [1]
	Number of Litters/Fetus	0	0	1/1	1/6	
Parietal - incomplete ossification	%Litter	0	0	0	5	2 (2.74) / 0 to 5 [2]
	%Fetal	0	0	0	0.68	0.26 (0.37) / 0 to 0.79 [2]
	Number of Litters/Fetus	0	0	0	1/1	
Supraoccipital - incomplete ossification	%Litter	0	0	0	10	3 (4.47) / 0 to 10 [3]
	%Fetal	0	0	0	1.28	0.4 (0.6) / 0 to 1.36 [3]
	Number of Litters/Fetus	0	0	0	2/2	
Zygomatic arch - incomplete ossification	%Litter	0	0	0	5	Not present in the historical control data
	%Fetal	0	0	0	0.79	
	Number of Litters/Fetus	0	0	0	1/1	
Sternebra						
Bipartite ossification	%Litter	0	5	4	19*	6 (5.48) / 0 to 40 [6]
	%Fetal	0	0.88	1.04	3.74	1 (1.05) / 0 to 2.5 [8]
	Number of Litters/Fetus	0	1/1	1/2	4/5	
Incomplete ossification	%Litter	0	0	4	14	4.8 (3.56) / 0 to 10 [5]
	%Fetal	0	0	0.69	2.15	1.02 (0.63) / 0 to 1.45 [7]
	Number of Litters/Fetus	0	0	1/1	3/3	
Misaligned ossification centers	%Litter	13	21	8	33	46.2 (10.01) / 35 to 60 [48]
	%Fetal	1.71	4.00	1.22	5.81	10.32 (2.41) / 7.25 to 12.65 [71]
	Number of Litters/Fetus	3/3	4/5	2/2	7/8	
Supernumerary rib present	%Litter	57	32	71	90*	71.33 (14.57) / 55 to 83 [46]
	%Fetal	16.85	9.65	24.00	42.15#	34.18 (114.49) / 22.38 to 45.34 [139]
	Number of Litters/Fetus	13/25	6/14	17/39	19/62	

* Fisher 1 tail Ascending Test significant at the 0.05 level.
\# Wilcoxon rank Sum Test Significant at the 0.001 level.

Table 10-16 (continued): Overview of fetal variations, including historical levels

Variation	Control	0	100	300	1000 mg/kg/day	Historical control range Mean (SD) / range [No. affected]
Vertebra - cervical arch						
Additional ossification site	%Litter	0	5	4	14	Not present in the historical control data
	%Fetal	0	0.88	0.69	2.72	
	Number of Litters/Fetus	0	1/1	1/1	3/4	
Vertebra - cervical centrum						
Unossified	%Litter	26	26	29	95**	Not present in the historical control data
	%Fetal	9.16	6.42	8.37	60.71#	
	Number of Litters/Fetus	6/15	5/8	7/14	20/87	
Vertebra - thoracic centrum						
Unossified	%Litter	0	0	0	14	0.8 (1.79) / 0 to 4 [1]
	%Fetal	0	0	0	6.24	0.12 (0.28) / 0 to 0.62 [1]
	Number of Litters/Fetus	0	0	0	3/9	
Forelimb						
Metacarpal - unossified	%Litter	0	0	0	10	2.8 (4.090 / 0 to 9 [3]
	%Fetal	0	0	0	2.83	2.01 (2.76) / 0 to 5.07 [5]
	Number of Litters/Fetus	0	0	0	2/4	
Phalanx - unossified.	%Litter	0	0	0	5	23.4 (24.28) / 0 to 55 [25]
	%Fetal	0	0	0	4.08	10.59 (12.19) / 0 to 25.52 [50]
	Number of Litters/Fetus	0	0	0	1/6	
Hindlimb						
Metatarsal - unossified	%Litter	0	0	0	10	20 (28.06) / 0 to 65 [21]
	%Fetal	0	0	0	2.15	9.21 (12.46) / 0 to 24.83 [45]
	Number of Litters/Fetus	0	0	0	2/3	

** Fisher 1 tail Ascending Test significant at the 0.001 level.
# Wilcoxon rank Sum Test Significant at the 0.001 level.

Table 10-17: Observations regarding malformations (Anonymous, 2020)

Dose Group	Maternal animal ID	Fetus ID / Sex	Tissue	Malformation
0 (Control)	M0005	R8 Female	Mouth	Cleft Palate
	M0007	R5 Male	Eye	Partially opened - right
	M0016	R16 Male	Blood Vessel	Aortic arch - absent
			Blood Vessel	Subclavian artery - malpositioned left, arising from descending aorta
100 mg/kg/day	M0108	L1 Female	Head	Exencephaly
			Skull	Frontal - misshapen - bilateral
			Skull	Interparietal - misshapen
			Skull	Interparietal - split
			Skull	Orbital socket - small - bilateral
			Skull	Parietal - misshapen - bilateral
			Skull	Presphenoid - absent
			Skull	Squamosal - misshapen - bilateral
			Skull	Supraoccipital - absent
	M0114	L2 Male	Eye	Partially opened - left
300 mg/kg/day	M0202	L2 Male	Sternebra	Misshapen - 4, 5
	M0207	L3 Male	Limb	Malrotated hindlimb - right ankle joint
	M0224	L4 Male	Paw	Polydactyly hindlimb - right - one additional digit

Dose Group	Maternal animal ID	Fetus ID / Sex	Tissue	Malformation
1000 mg/kg/day	M0301	R10 Male	Brain	Cranial cavity structures - disorganized
			Head	Exencephaly
		R12 Female	Brain	Cranial cavity structures - disorganized
			Head	Exencephaly
	M0302	R10 Male	Mouth	Cleft palate
	M0304	R10 Female	Eye	Open - bilateral
			Head	Exencephaly
			Skull	Frontal - misshapen - bilateral
			Skull	Interparietal - misshapen
			Skull	Orbital socket - small - bilateral
			Skull	Parietal - misshapen - bilateral
			Skull	Presphenoid - absent
			Skull	Squamosal - misshapen - bilateral
			Skull	Supraoccipital - split
	M0309	L4 Female	Head	Exencephaly
			Skull	Frontal - misshapen - bilateral
			Skull	Interparietal - misshapen
			Skull	Orbital socket - small - bilateral
			Skull	Parietal - misshapen - bilateral
			Skull	Presphenoid - absent
			Skull	Squamosal - misshapen - bilateral
			Skull	Supraoccipital - absent
		L6 Male	Brain	Cranial cavity structures - disorganized
			Head	Exencephaly
		L7 Male	Brain	Cranial cavity structures - disorganized
			Eye	Open, left
			Head	Exencephaly
		R9 Female	Brain	Cranial cavity structures - disorganized
			Eye	Open - bilateral
			Head	Exencephaly
	M0322	L6 Male	Rib	Fused - right, 7/8, proximal
	M0324	L1 Male	Skull	Suture - sutural bone - large - interfrontal
			Skull	Suture - wide - interfrontal
		L3 Female	Eye	Open - bilateral
			Head	Exencephaly
			Mouth	Palate - high arched
			Skull	Frontal - misshapen - bilateral
			Skull	Interparietal - absent
			Skull	Nasal - misshapen - bilateral
			Skull	Orbital socket - small - bilateral
			Skull	Palatine - malpositioned - bilateral
			Skull	Palatine - small
			Skull	Parietal - misshapen - bilateral
			Skull	Presphenoid - absent
			Skull	Squamosal - misshapen - bilateral
			Skull	Supraoccipital - split
		R10 Male	Skull	Suture - sutural bone - large - interfrontal
			Skull	Suture - wide - interfrontal
			Vertebra	Cervical arch misshapen - right - 2 - neural arch
		R13 Female	Skull	Suture - wide - interfrontal

Table 10-18: Overview of notable abnormalities in fetuses/litters (table produced from the reporting in table 4.2, table 4.4 and table 8.10 from Anonymous, 2020)

Group / Parameter	Fetuses (observed/total no. examined fetus)				Litter (observed/total no. examined litters)			
	1	2	3	4	1	2	3	4
Malformations								
Exence-phaly	0/305	1/257	0/322	8/288	0/23	1/19	0/24	4/21
%	0	0.44	0	3.04*	0	5	0	19*
Skull, various malformations	0/152	1/129	0/161	6/143	0/23	1/19	0/24	3/21
%	0	0.77	0	26	0	5.3	0	14
Brain, disorganised cranial structures	0/153	0/128	0/161	5/145	0/23	0/19	0/24	2/21
%	0	0	0	3.97	0	0	0	10
Variations								
Bipartite ossification of the sternebra	0/152	1/129	2/161	5/143	0/23	1/19	1/24	4/21
%	0	0.88	1.04	3.74	0	5	4	19*
Supernumerary rib present in the sternebra	25/152	14/129	39/161	62/143	13/23	6/19	17/24	19/21
%	16.85	9.65	24	42.15**	57	32	71	90*
Unossified vertebra – cervical centrum	15/152	8/129	14/161	87/143	6/23	5/19	7/24	20/21
%	9.16	6.42	8.37	60.71**	26	26	29	95**

*p<0.05 **p<0.001

The statistically significant increased incidences of malformations, skeletal variations and lower fetal weight, observed in fetuses from animals administered 1000 mg/kg bw/day were considered to be treatment related.

These finding are regarded as similar with developmental adverse effects of 2-EHA (overview on developmental studies in rodents are given in Table 10-2 and Table 10-3). 2-EHA has for instance shown increased frequency of malformations, skeletal variations and reduced fetal weight. This supports the above findings as 2-ethylhexanoic acid monoester with propane-1,2-diol "*is likely to undergo some degree of hydrolysis by esterases, particularly under acidic conditions to form 2-ethylhexanoic acid and propan-1,2-diol*", as stated in the REACH registration.

10.10.6 Comparison with the CLP criteria

For potential classification for adverse effects on developmental toxicity in Category 1, the following criteria apply according to CLP Regulation:

Category 1

"Known or presumed human reproductive toxicant.

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

Category 1A

"Known human reproductive toxicant.

The classification of a substance in this Category 1A is largely based on evidence from humans."

As no human data are available on developmental toxicity of the substance the criteria for Category 1A are not fulfilled.

Category 1B

"Presumed human reproductive toxicant.

The classification of a substance in this Category 1B is largely based on data from animal studies. Such data shall provide clear evidence of an adverse effect on sexual function and fertility or on development in the absence of other toxic effects, or if occurring together with other toxic effects the adverse effect on reproduction is considered not to be a secondary non-specific consequence of other toxic effects. However, when there is mechanistic information that raises doubt about the relevance of the effect for humans, classification in Category 2 may be more appropriate."

Two GLP compliant OECD TG 414 prenatal developmental toxicity studies using oral administration to rats and mice, are available. In both studies adverse developmental effects were observed in the fetuses in the absence of any maternal toxicity, as no maternal toxicity was seen even at the highest dose level of 1000 mg/kg bw/day. Thus, any effects on fetal development in the studies cannot be considered as secondary to maternal toxicity.

In rats, an increased incidence of a wide spectrum of minor abnormalities/skeletal variants was found at the highest dose level of 1000 mg/kg bw/day. Most notably among these findings was skeletal abnormalities consisting of a large nasofrontal suture in 107 fetuses (20 litters) at 1000 mg/kg bw/day; in 16 fetuses (seven litters) at 300 mg/kg bw/day, and in five fetuses (three litters) at 100 mg/kg bw/day, indicating dose-response for this effect.

In mice, increased incidences of malformations of head, skull and brain, and skeletal variations were reported at the highest dose level of 1000 mg/kg bw/day. A significant difference was obtained between the control group and the dose group of 1000 mg/kg bw/day in exencephaly, bipartite ossification of the

sternebra, supernumerary rib present in the sternebra and unossified vertebra – cervical centrum. The most notable finding - exencephaly - was recorded in 8/288 fetuses from 4/21 litters.

While the finding of large nasofrontal sutures in rats was reported as minor non-adverse abnormalities, the finding of exencephaly in mice is to be considered as a severe adverse finding, and thus this finding constitutes a clear evidence of adverse developmental effects and the criteria for a Repr. 1B (H360D) classification are fulfilled.

The adverse developmental effects from 2-ethylhexanoic acid, monoester with propane-1,2-diol exposure are further supported by data on *2-ehtylhexanoic acid*, considered as a potential metabolite because of the ubiquitous nature of active carboxylesterases in the human body.

In rats, 2-ethylhexanoic acid exposure in two prenatal developmental toxicity studies was associated with a significantly increased occurrence of dilated brain ventricles at the highest oral dose levels of 500 and 600 mg/kg bw/day. There was a dose-response trend for this finding in both studies (ECHA, 2020). In another rat study, using only one maternal dose level of 500 mg/kg bw/day resulted in encephalocele in 14.1% of the fetuses (ECHA, 2020).

In mice maternal, i.p. injections of 2 x 500 mg 2-ethylhexanoic acid on GD 7 and 8 resulted in exencephaly in 59% of the fetuses in case of injection of the R-enantiomer of the substance and 1% of the fetuses in case of injection of the S-enantiomer. This suggests that the interaction of the enantiomers with chiral molecules (e.g. proteins) in the embryo may play a key role in the MoA (ECHA, 2020).

So overall, a very similar pattern of developmental effects is found for 2-ethylhexanoic acid, and the adverse effects caused by *2-ethylhexanoic acid, monoester with propane-1,2-diol* may likely be connected to the formation/content of the R-enantiomer of 2-ethylhexanoic acid.

Category 2

"Suspected human reproductive toxicant.

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

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

As the data are considered clearly sufficient for a Category 1B classification, a Category 2 classification is not considered relevant.

10.10.7 Adverse effects on or via lactation

The classification criteria for reproductive toxicity are established in Section 3.7.2 of the Regulation (EC) No. 1272/2008 (CLP Regulation) and documented in the ECHA Guidance on the Application of the CLP Criteria, Version 5.0, July 2017.

For the purpose of classification, the hazard class Reproductive Toxicity is differentiated into:

- adverse effects on sexual function and fertility, or development
- effects on or via lactation.

Effects on or via lactation are allocated to a separate single category. Substances which are absorbed by women and have been shown to interfere with lactation, or which may be present (including metabolites) in breast milk in amounts sufficient to cause concern for the health of a breastfed child, shall be classified and labelled to indicate this property hazardous to breastfed babies. This classification can be assigned on the:

(a) human evidence indicating a hazard to babies during the lactation period; and/or

(b) results of one or two generation studies in animals which provide clear evidence of adverse effect in the offspring due to transfer in the milk or adverse effect on the quality of the milk; and/or

(c) absorption, metabolism, distribution and excretion studies that indicate the likelihood that the substance is present in potentially toxic levels in breast milk.

No data are available to conclude on *2-ethylhexanoic acid, monoester with propane-1,2-diol*, for adverse effect on or via lactation. Therefore, no classification is proposed.

10.10.8 Conclusion on classification and labelling for reproductive toxicity

Based on reliable experimental animal data, *2-ethylhexanoic acid, monoester with propane-1,2-diol*, should be classified in **Category 1B (H360D)** for developmental toxicity.

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.

Additional key elements

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 below 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).

<u>90-day oral study in rats with 2-EHA-PG (Anonymous, 2016)</u>

In this GLP-compliant study, Crl:CD(SD) rats (10/sex/group) were administered 2-EHA-PG in corn oil via gavage at dose levels of 0, 250, 500 and 1 000 mg/kg bw/d. There was no mortality or clinical signs of toxicity, body weight was slightly reduced at the top dose (not statistically significant). Haematological examination revealed a mild decrease in haemoglobin and erythrocyte count in both sexes, clinical chemistry examination showed increased urea in males. Liver and kidney weights were increased in males and females. Histopathological changes were observed in the kidney (hyaline droplets and tubular dilatation with interstitial fibrosis in males), liver (hepatocellular hypertrophy in both sexes) and thyroid (follicular cell hypertrophy in both sexes). The NOAEL was set at

250 mg/kg bw/d primarily based on the kidney findings. No adverse effects on reproductive organ weights (testes, epididymides, ovaries, uterus and cervix) or histopathology (testes, epididymides, prostate, seminal vesicles, ovaries, uterus, uterine cervix, vagina) were detected in this study.

Multigeneration study in mice with PG (NTP, 1985)

The study was conducted by the National Toxicology Program (NTP) and employed the continuous breeding (RACB) protocol. In the RACB design, animals are cohabited following the first week of dosing and housed as breeding pairs throughout a 14-week dosing period. The litters are examined and killed as soon as the delivery is complete so that the female may be impregnated again immediately. As many as five litters can be evaluated during this test. At the end of the 14 weeks, the males and females are separated, and the last litter delivered is used for the second generation.

CD-1 mice (20/sex per dose group, 40/sex in the control) were exposed to PG via drinking water at concentrations of 0, 1, 2.5 and 5 % (equivalent to 0, 1 820, 4 800 and 10 100 mg/kg bw/d). Live litters born during the cohabitation phase were weighed, determined by sex and examined for external abnormalities. Offspring from the last litter of the control and high-dose groups were allowed to mature and reproductive performance was evaluated (cohabitation period in F1 lasted up to 1 week).

No treatment-related deaths or clinical signs of toxicity were noted in F0 during the cohabitation phase. PG had no significant effects on any of the following reproductive parameters in F0 animals: number of litters per pair, number of live pups per litter, sex ratio, pup weight, number of days to litter, dam weight at delivery. F0 parents were not necropsied.

Body weight of F1 animals was not affected. The mating index for control and treated groups was 85 %; the fertility index was 75 % for control and 80 % for the treated group. There were no significant differences in F2 litter size, number of live pups, sex ratio or pup weights. After the delivery of the F2 pups, the F1 adults were necropsied. In males, there were no significant differences in reproductive organ weights or sperm parameters. In females, there was no difference in oestrous cyclicity. No organs were examined histologically.

Assessment and comparison with the classification criteria

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

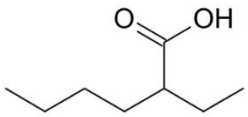
Two PNDT studies with 2-EHA-PG are available, one in rats and one in mice. The PNDT study in rats was conducted in 2015 (Anonymous, 2016). On a subsequent dossier compliance check ECHA requested a PNDT study in mice as a second species due to a concern for developmental toxicity raised by the rat study 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.

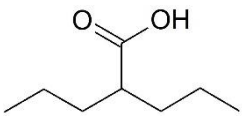
Developmental toxicity of 2-ethylhexanoic acid

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	0	1 (1)	0	8 (4)

affected foetuses/litter	0 %	0.4 %	0 %	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**.

10.11 Specific target organ toxicity-single exposure

Hazard class not assessed in this dossier.

10.12 Specific target organ toxicity-repeated exposure

Hazard class not assessed in this dossier.

10.13 Aspiration hazard

Hazard class not assessed in this dossier.

11 EVALUATION OF ENVIRONMENTAL HAZARDS

Not evaluated in this dossier.

12 EVALUATION OF ADDITIONAL HAZARDS

Not evaluated in this dossier.

13 ADDITIONAL LABELLING

Not applicable.

14 REFERENCES

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mechanisms. Pharmacology & Toxicology 69:310-321

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15 ANNEXES

Annex I to the CLH report. Proposal for Harmonised Classification and Labelling Based on Regulation (EC) No 1272/2008 (CLP Regulation), Annex VI, Part 2. Chemical Name: 2-ethylhexanoic acid, monoester with propane-1,2-diol. EC Number: 285-503-5. CAS Number: 85114-00-7. Index Number: 607-RST-VW-Y

Confidential Annex II to the CLH report. Confidential information to CLH report on 2-ethylhexanoic acid, monoester with propane-1,2-diol and Annex I to the CLH report.