Committee for Risk Assessment

RAC

Annex 1

Background document

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

2-Ethylhexanoic acid and its salts, with the exception of those specified elsewhere in this Annex

EC Number: -

CAS Number: -

CLH-O-0000006817-63-01/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 public 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

11 June 2020

CLH report

Proposal for Harmonised Classification and Labelling

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

International Chemical Identification:

2-Ethylhexanoic acid and its salts, with the exception of those specified elsewhere in this Annex

EC Number: **n.a.**

CAS Number: **n.a.**

Index Number: **607-230-00-6**

Contact details for dossier submitter:

Ministry of Health, Consumer Affairs and Social Welfare
Paseo del Prado, 18-20
28071 – Madrid
Spain

Version number: 1 **Date: 16 April 2019**

ANNEX 1 – BACKGROUND DOCUMENT TO RAC OPINION ON 2-ETHYLHEXANOIC ACID AND ITS SALTS, WITH THE EXCEPTION OF THOSE SPECIFIED ELSEWHERE IN THIS ANNEX

CONTENTS

ANNEX 1 – BACKGROUND DOCUMENT TO RAC OPINION ON 2-ETHYLHEXANOIC ACID AND ITS SALTS, WITH THE EXCEPTION OF THOSE SPECIFIED ELSEWHERE IN THIS ANNEX

TABLES

ANNEX 1 – BACKGROUND DOCUMENT TO RAC OPINION ON 2-ETHYLHEXANOIC ACID AND ITS SALTS, WITH THE EXCEPTION OF THOSE SPECIFIED ELSEWHERE IN THIS ANNEX

ANNEX 1 – BACKGROUND DOCUMENT TO RAC OPINION ON 2-ETHYLHEXANOIC ACID AND ITS SALTS, WITH THE EXCEPTION OF THOSE SPECIFIED ELSEWHERE IN THIS ANNEX

1 IDENTITY OF THE SUBSTANCE

1.1 Name and other identifiers of the substance

This CLH proposal is related to the reproductive toxicity of the substances 2-ethylhexanoic acid (2-EHA) and its salts. The proposed Annex VI entry *"2-ethylhexanoic acid and its salts, with the exception of those specified elsewhere in this Annex"* includes, in principle, the acid and its salts that share the same carboxylate chemical structure, with a COO^- moiety as a functional group linked to a saturated branched aliphatic C_7 chain length (Figure 1). The salts only differ in the cation counterion.

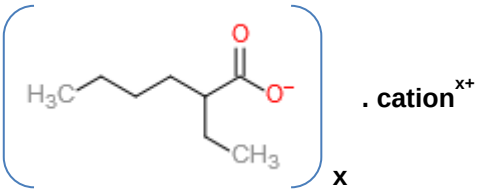


Figure 1. Salts common carboxylate chemical structure

Currently, there are 56 pre-registered salts of 2-EHA, 30 of them have only been notified to the C&L Inventory (18 as Repr.) and 13 are registered. In the framework of the ECHA Common Screening Approach for REACH and CLP processes 2014, eight of the registered salts of 2-EHA were manually screened by Spain, and in the 2016 screening round, an additional salt was screened as well (manually screened salts are grey-coloured in Table 2). The outcome of those screening activities was the same in all cases due to the concern for reproductive toxicity driven by the 2-EHA moiety. Thus, taking into account the harmonized classification of 2-EHA, the classification of the salts of 2-EHA as Repr. 2 (H361d) would be warranted, provided that the reproductive toxicity of the cation would not warrant category 1 classification and or additional classification on sexual function and fertility or effects on or via lactation. All the screened substances were self-classified by the registrants as Repr. 2 (H361d), but they lack a harmonized classification that is warranted for substances inducing reproductive toxicity in accordance with CLP Art. 36. Therefore, CLH was identified as the needed action at EU level for these substances.

There are 2-EHA salts where the cation itself is known to be more hazardous for reproductive toxicity than the 2-EHA anion (e.g. cobalt, lead). Thus, the cation toxicity shall always be evaluated and taken into account for the classification of the related salt. Because of this, we propose to include a note indicating the following: "*The classification for the hazard class(es) in this entry is based only on the hazardous properties of the part of the substance which is common to all members in the entry. The hazardous properties of any member in the entry also depends on the properties of the part of the substance which is not common to all members of the group; they must be evaluated to assess whether (a) more severe classification(s) (e.g. a higher category) or (b) a broader scope of the classification (additional differentiation, target organs and/or hazard statements) might apply for the hazard class(es) in the entry*".

Information on 2-ethylhexanoic acid and on the registered salts is shown in Table 2. Data on the registered nickel bis salt of 2-EHA is not included because it has already its own Annex VI entry that includes a higher classification for reproductive toxicity.

ANNEX 1 – BACKGROUND DOCUMENT TO RAC OPINION ON 2-ETHYLHEXANOIC ACID AND ITS SALTS, WITH THE EXCEPTION OF THOSE SPECIFIED ELSEWHERE IN THIS ANNEX

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

Name(s) in the IUPAC nomenclature or other international chemical name(s)	*2-Ethylhexanoic acid and it salts, with the exception of those specified elsewhere in this Annex*
Other names (usual name, trade name, abbreviation)	*n.a.*
ISO common name (if available and appropriate)	*n.a.*
EC number (if available and appropriate)	*n.a.*
EC name (if available and appropriate)	*n.a.*
CAS number (if available)	*n.a.*
Other identity code (if available)	*n.a.*
Molecular formula	*n.a.*
Structural formula	*n.a.*
SMILES notation (if available)	*n.a.*
Molecular weight or molecular weight range	*n.a.*

Table 2: Substance identity and information related to 2-ethylhexanoic acid and to its registered salts

Name(s) in the IUPAC nomenclature or other international chemical name(s)	Other names (usual name, trade name, abbreviation)	EC number (if available and appropriate)	EC name (if available and appropriate)	CAS number (if available)	Molecular formula	Molecular weight or molecular weight range	Index number in Annex VI of the CLP Regulation
2-Ethylhexanoic acid	*2-EHA*	*205-743-6*	*2-ethylhexanoic acid*	*149-57-5*	$C_8H_{16}O_2$	*144.2114*	*607-230-00-6*
Sodium 2-ethylhexanoate	*Hexanoic acid, 2-ethyl-, sodium salt*	*243-283-8*	*Sodium 2-ethylhexanoate*	*19766-89-3*	$C_8H_{16}O_2.Na$	*166.1933*	*n.a.*
Potassium 2-ethylhexanoate	*Hexanoic acid, 2-ethyl-, potassium salt* *2-Ethylhexanoic acid potassium salt*	*221-625-7*	*Potassium 2-ethylhexanoate*	*3164-85-0*	$C_8H_{16}O_2.K$	*182.3018*	*n.a.*
Calcium bis(2-ethylhexanoate)	*Hexanoic acid, 2-ethyl-, calcium salt*	*205-249-0*	*Calcium bis(2-ethylhexanoate)*	*136-51-6*	$C_8H_{16}O_2.1/2Ca$	*326.485*	*n.a.*
2-Ethylhexanoic acid, manganese salt	*n.a.*	*240-085-3*	*2-Ethylhexanoic acid, manganese salt*	*15956-58-8*	$C_8H_{16}O_2.xMn$	*341*	*n.a.*
Zinc bis(2-ethylhexanoate)	*Hexanoic acid, 2-ethyl-, zinc salt*	*205-251-1*	*Zinc bis(2-ethylhexanoate)*	*136-53-8*	$C_8H_{16}O_2.1/2Zn$	*351.816*	*n.a.*
Hexanoic acid, 2-ethyl-, zinc salt, basic	*n.a.*	*286-272-3*	*Hexanoic acid, 2-ethyl-, zinc salt, basic*	*85203-81-2*	*Not available*	*208.612*	*n.a.*
2-Ethylhexanoic acid, molybdenum salt	*Molybdenum 2-ethylhexanoate* *Hexanoic acid, 2-ethyl-, molybdenum salt*	*251-807-1*	*2-Ethylhexanoic acid, molybdenum salt*	*34041-09-3*	$C_8H_{16}O_2.xMo$	*≥ 239.1435*	*n.a.*
2-Ethylhexanoic acid, zirconium salt	*Hexanoic acid, 2-ethyl-, zirconium salt*	*245-018-1*	*2-Ethylhexanoic acid, zirconium salt*	*22464-99-9*	$C_8H_{16}O_2.xZr$	*377.631*	*n.a.*
Barium bis(2-ethylhexanoate)	*Hexanoic acid, 2-ethyl-, barium salt*	*219-535-8*	*Barium bis(2-ethylhexanoate)*	*2457-01-4*	$C_8H_{16}O_2.1/2Ba$	*423.734*	*056-002-00-7 (barium salts group entry)*
Tin bis(2-ethylhexanoate)	*Stannous octoate* *2-Ethylhexanoic acid, tin(II) salt* *Bis(2-ethylhexanoate)tin* *Ethylhexanoic acid tin(2+) salt* *Hexanoic acid, 2-ethyl, tin salt* *Hexanoic acid, 2-ethyl-, tin(2+) salt* *Metatin(TM) Catalyst S-26* *Stannous ethylhexanoate* *Stannous-2-ethyl hexanoate*	*206-108-6*	*Tin bis(2-ethylhexanoate)*	*301-10-0*	$C_{16}H_{30}O_4Sn$	*ca. 405.1*	*n.a.*

	Tin 2-ethylhexanoate *Tin II octoate* *Tin(II) 2-ethylhexanoate* *Tin(II) bis(2-ethylhexanoate)* *Tin(II) ethylhexanoate*						
Cobalt bis(2-ethylhexanoate)	*Cobalt octoate* *Cobalt-II-ethylhexanoat* *Cobaltoctoat* *Hexanoic acid, 2-Ethyl, Cobalt salt*	*205-250-6*	*Cobalt bis(2-ethylhexanoate)*	*136-52-7*	*$C_8H_{16}O_2.1/2Co$*	*345.34*	*n.a.*
1-(2-hydroxypropyl)-1,4-diazabicyclo[2.2.2]octan-1-ium 2-ethylhexanoate	*n.a.*	*413-670-8*	*Nitrilotriethyleneammoniopropane-2-ol 2-ethylhexanoate*	*103969-79-5*	*C17H34N2O3*	*314.46*	*613-184-00-8*

1.2 Composition of the substance

Information on the composition of 2-ethylhexanoic acid and on the registered salts is shown here.

Table 3: Constituents of the acid and its registered salts (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 3.1 (CLP)	Current self-classification and labelling (CLP)
2-Ethylhexanoic acid (EC no. 205-743-6)	Mono-constituent	**Repr. 2 (H361d)**	Not self-classified
Sodium 2-ethylhexanoate (EC no. 243-283-8)	Mono-constituent	n.a.	**Repr. 2 (H361)**
Potassium 2-ethylhexanoate (EC no. 221-625-7)	Mono-constituent	n.a.	Skin Irrit. 2 (H315) Eye Dam. 1 (H318) **Repr. 2 (H361d)**
Calcium bis(2-ethylhexanoate) (EC no. 205-249-0)	Mono-constituent	n.a.	Eye Dam. 1 (H318) **Repr. 2 (H361)**
2-Ethylhexanoic acid, manganese salt (EC no. 240-085-3)	Mono-constituent	n.a.	Eye Irrit. 2 (H319) **Repr. 2 (H361d)** STOT RE 2 (H373) Aquatic Chronic 2 (H411)
Zinc bis(2-ethylhexanoate) (EC no. 205-251-1)	Mono-constituent	n.a.	Eye Irrit. 2 (H319) **Repr. 2 (H361d)** Aquatic Chronic 3 (H412)
Hexanoic acid, 2-ethyl-, zinc salt, basic (EC no. 286-272-3)	Mono-constituent	n.a.	Eye Irrit. 2 (H319) **Repr. 2 (H361d)** Aquatic Chronic 3 (H412)
2-Ethylhexanoic acid, molybdenum salt (EC no. 251-807-1)	Mono-constituent	n.a.	**Repr. 2 (H361d)** Eye Irrit. 2 (H319)
2-Ethylhexanoic acid, zirconium salt (EC no. 245-018-1)	Mono-constituent	n.a.	**Repr. 2 (H361d)**
Barium bis(2-ethylhexanoate) (EC no. 219-535-8)	Mono-constituent	Acute Tox. 4* (H302) Acute Tox. 4* (H332)	Eye Damage 1 (H318) **Repr. 2 (H361d)**
Tin bis(2-ethylhexanoate) (EC no. 206-108-6)	Mono-constituent	n.a.	Skin Sens. 1B (H317) Eye Damage 1 (H318) **Repr. 2 (H361d)** Aquatic Chronic 3 (H412)
Cobalt bis(2-ethylhexanoate) (EC no. 205-250-6)	Mono-constituent	n.a.	Skin Sens. 1A (H317) Eye Irrit. 2 (H319) **Repr. 2 (H361d)** Aquatic Acute 1 (H400) Aquatic Chronic 3 (H412)
1-(2-hydroxypropyl)-1,4-diazabicyclo[2.2.2]octan-1-ium 2-ethylhexanoate	Mono-constituent	Eye Irrit. 2 (H319) Skin Sens. 1 (H317)	Aquatic Chronic 3 (H412)

** Based on registration data*

Table 4: 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 3.1 (CLP)	Current self-classification and labelling (CLP)	The impurity contributes to the classification and labelling

For the registered substances included in Table 3, impurities that may contribute to the classification and labelling have not been reported.

Table 5: 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 3.1 (CLP)	Current self-classification and labelling (CLP)	The additive contributes to the classification and labelling

Table 6: Test substances (non-confidential information)

Identification of test substance	Purity	Impurities and additives (identity, %, classification if available)	Other information	The study(ies) in which the test substance is used
2-Ethylhexanoic acid (EC no. 205-743-6)			Annex VI index no. 607-230-00-6 classified as Repr. 2 (H361d)	Toxicokinetics Reprotoxicity studies

2 PROPOSED HARMONISED CLASSIFICATION AND LABELLING

2.1 Proposed harmonised classification and labelling according to the CLP criteria

Table 7: Proposed harmonised classification and labelling according to the CLP criteria

	Index No	International Chemical Identification	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors	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	607-230-00-6	2-Ethylhexanoic acid	205-743-6	149-57-5	Repr. 2	H361d	GHS08 Wng	H361d	-	-	-
Dossier submitters proposal	**Retain:** 607-230-00-6	**Retain:** 2-Ethylhexanoic acid **Add:** and its salts, with the exception of those specified elsewhere in this Annex	**Delete:** 205-743-6	**Delete:** 149-57-5	**Retain:** Repr. 2	**Retain:** H361d	**Retain:** GHS08 Wng	**Retain:** H361d	-	-	**Add a new note:** The classification for the hazard class(es) in this entry is based only on the hazardous properties of the part of the substance which is common to all members in the entry. The hazardous properties of any member in the entry also depends on the properties of the part of the substance which is not common to all members of the group; they must be evaluated to assess whether (a) more severe

											classification(s) (e.g. a higher category) or (b) a broader scope of the classification (additional differentiation, target organs and/or hazard statements) might apply for the hazard class(es) in the entry.
Resulting Annex VI entry if agreed by RAC and COM	607-230-00-6	2- Ethylhexanoic acid and its salts, with the exception of those specified elsewhere in this Annex	-	-	Repr. 2	H361d	GHS08 Wng	H361d	-	-	The classification for the hazard class(es) in this entry is based only on the hazardous properties of the part of the substance which is common to all members in the entry. The hazardous properties of any member in the entry also depends on the properties of the part of the substance which is not common to all members of the group; they must be evaluated to assess whether (a) more severe classification(s) (e.g. a higher category) or (b) a broader scope of the classification

											(additional differentiation, target organs and/or hazard statements) might apply for the hazard class(es) in the entry.

Table 8: Reason for not proposing harmonised classification and status under public consultation

Hazard class	Reason for no classification	Within the scope of public 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. 2; H361d)*	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

Classification for reproductive toxicity of 2-ethylhexanoic acid (EC no. 205-743-6) was harmonized under the former Dangerous Substance Directive (DSD) as Repr. 2 (H361d) because of its developmental effects. It

was later included in the CLP00 Annex VI (index no. 607-230-00-6). Furthermore, it is relevant to mention that 2-EHA has been subjected to a substance evaluation process (CoRAP 2012) due to a potential fertility concern. The new information generated after ECHA decision on substance evaluation did not confirm that concern (see substance evaluation report in https://echa.europa.eu/documents/10162/ebaf3955-838a-6d94-592b-a68d28d51df3). Nevertheless, it is now proposed to have one Annex VI entry for 2-EHA and its salts as the data base for them is the same in this proposal. This proposal ensures that also the more recent data on 2-EHA are evaluated at EU level and compared with the current criteria for classification and labelling, i.e. the CLP criteria.

Of the registered salts of 2-EHA, nickel bis(2-ethylhexanoate), barium bis(2-ethylhexanoate) and 1-(2-hydroxypropyl)-1,4-diazabicyclo[2.2.2]octan-1-ium 2-ethylhexanoate are currently covered also by another entry in Annex VI. According to CLP Annex VI (1.1.1.5), individual substances may be covered by more than one group entry. In these cases, the classification of the substance reflects the classification for each of the two group entries, and in cases where different classifications for the same hazard are given, the most severe classification should be applied. E.g. the nickel salts of 2-EHA [nickel bis(2-ethylhexanoate), EC no. 224-699-9 (registered) and 2-ethylhexanoic acid nickel salt, EC no. 231-480-1 (non-registered)] are specifically included in Annex VI as part of a group of water soluble nickel compounds (index no. 028-054-00-0). This group entry includes a more severe classification for reproductive toxicity, i.e. Repr. 1B (H360D) (and other hazard classes). Therefore, the final classification for reproductive toxicity of nickel bis(2-ethylhexanoate) is the most severe between the two entries for each hazard class, which in this case coincide with the nickel salt group entry. Another example is 2-ethylhexanoic, lead salt (non-registered) that is also included in Annex VI as part of a group of the lead compounds (index no. 082-001-00-6) with a classification as Repr. 1A (H360Df) that should be applied to lead salt of 2-EHA.

Table 9: Resulting classification for a specific 2-EHA salt as defined by CLP Annex VI (1.1.1.5) if the current proposal is adopted#.

Substance	Group entry for 2-EHA acid and its salts after adoption of the current proposal (i.e classification based on the anion)	Existing harmonised classification based on group entry of the cation (Index number) (i.e classification based on the canion)	Resulting harmonised classification for the salt According to CLP Annex VI (1.1.1.5)
nickel bis(2-ethylhexanoate)	**Repr. 2 (H361d)**	Carc. 1A (H350i) Muta. 2 (H341) **Repr. 1B (H360D***)** STOT RE 1 (H372**) Resp. Sens. 1 (H334) Skin Sens. 1 (H317) Aquatic Acute 1 (H400) Aquatic Chronic 1 (H410) (028-054-00-0)	Carc. 1A (H350i) Muta. 2 (H341) **Repr. 1B (H360D***)** STOT RE 1 (H372**) Resp. Sens. 1 (H334) Skin Sens. 1 (H317) Aquatic Acute 1 (H400) Aquatic Chronic 1 (H410)
2-ethylhexanoic, lead salt	**Repr. 2 (H361d)**	**Repr. 1A (H360Df)** Acute Tox. 4* (H332) Acute Tox. 4* (H302) STOT RE 2* (H373**) Aquatic Acute 1 (H400) Aquatic Chronic 1 (H410) (082-001-00-6)	**Repr. 1A (H360Df)** Acute Tox. 4* (H332) Acute Tox. 4* (H302) STOT RE 2* (H373**) Aquatic Acute 1 (H400) Aquatic Chronic 1 (H410)

as usual, every additional information should be gathered to evaluate for self-classification for all other hazard classes not included in the Annex VI entry(ies).

If the reproductive toxicity of a specific cation salt of 2-EHA is not covered by another Annex VI entry, the reproductive toxicity of the cation and its contribution to the classification of the related cation salt of 2-EHA

must always be evaluated in accordance with CLP to assess whether a higher category (i.e. 1A or 1B) and/or additional hazards (i.e. adverse effects on sexual function and fertility or effects on or via lactation) might have to be applied. In addition, data relevant for other hazard classes than those included in CLP Annex VI for 2-EHA or for its specific salt need to be evaluated as part of the self-classification procedure in accordance with CLP.

4 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

There is no requirement for justification that action is needed at Community level.

RAC general comment

Current classification and aim of the CLH proposal

2-ethylhexanoic acid (2-EHA; also known as "octoic acid") has a harmonized classification as Repr. 2; H361d, transposed from the Dangerous Substance Directive. According to the available records, the classification was discussed by the Commission Working Group on the Classification and Labelling of Dangerous Substances in 1994. It seems that most of the developmental studies available at that time had been taken into consideration (including Anonymous, 1988c; Pennanen *et al.*, 1993; Ritter *et al.*, 1987) as well as the similarity to the human teratogen valproic acid.

The aim of the current CLH proposal is to re-evaluate the available information on reproductive toxicity of 2-EHA (including a recent generational study) and to extend the entry to include salts of 2-EHA, many of which have recently been registered under REACH. For salts, the evaluation is limited to the reproductive toxicity of the 2-EHA moiety; the properties of the cation have to be evaluated separately to assess whether a more severe classification and/or classification in additional differentiations of reproductive toxicity might apply.

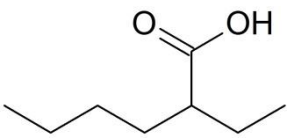
Substance evaluation

A need for new information on reproductive toxicity was identified during substance evaluation (2012-2014) as the only generational study available (Pennanen *et al.*, 1993) was a published non-guideline study of uncertain quality and it was considered to raise concerns regarding both fertility (reduced sperm motility and delayed fertilisation) and development (delay in the development of the grip and cliff avoidance reflexes). ECHA requested a new extended one-generation reproductive toxicity study (EOGRTS) including developmental neurotoxicity and immunotoxicity cohorts.

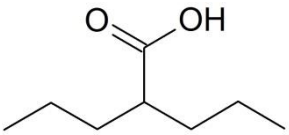
In 2017, the eMSCA concluded that the new EOGRTS (Anonymous, 2016) removed the initial concerns regarding fertility and developmental neurotoxicity and no follow-up action was needed.

Valproic acid

2-Ethylhexanoic acid bears structural similarity to valproic acid, an anticonvulsant and human teratogen self-classified as Repr. 1A; H360D (no Annex VI entry). The structures of both substances are shown below.



2-ethylhexanoic acid



valproic acid

Epidemiological data show an association between the use of valproic acid in pregnancy and occurrence of spina bifida and other defects (Tomson *et al.*, 2016), while standard rat prenatal developmental toxicity (PNDT) studies with valproic acid and sodium valproate show mainly reduced foetal weight, skeletal variations and a low incidence of skeletal malformations (Narotsky *et al.*, 1994; Binkerd *et al.*, 1988). Humans might be more sensitive to the teratogenicity of valproate than rats; the therapeutic dose for treatment of epilepsy associated with an increase in malformations is about 20-30 mg/kg bw/d (Nau *et al.*, 1991; Tomson *et al.*, 2016) while the threshold for developmental toxicity in rat studies is between 100 and 200 mg/kg bw/d (Binkerd *et al.*, 1988; Narotsky *et al.*, 1994). Part of this difference in sensitivity appears to be due to pharmacokinetic differences (Nau *et al.*, 1991).

5 IDENTIFIED USES

According to the information from registrations, uses of 2-EHA include: use as an intermediate in the manufacture of other substances, formulation of mixtures, use in laboratories and use as functional fluids (max. 15%).

Registration dossiers of the registered substances indicate a widespread use of 2-EHA salts. For most of these salts, identified life cycle stages include manufacture, formulation, industrial uses, professional uses, consumer uses and service life of articles. 2-EHA salts are reported to be present in coatings, inks, adhesives, sealants, elastomers, anti-freezing agents, lubricants and greases, heat transfer and hydraulic fluids. They are described to be used within polymer industry (including plastic, rubber and epoxy resin industry), in crude oil refining, as intermediates in chemical processes, as catalysts in PIR foams and as catalyst precursors.

6 DATA SOURCES

The following data sources have been taking into account for the compilation of this CLH report:

- REACH registration data
- The ECHA dissemination website
- Relevant studies found by systematic literature searches

7 PHYSICOCHEMICAL PROPERTIES

Table 10: Summary of physicochemical properties of 2-EHA and its registered salts

Property	2-EHA	Na-2-EHA	K-2-EHA	Ba-bis-2-EHA	Ca-bis-2-EHA	Mn-bis-2-EHA	Zn-basic-2-EHA	Zn-bis-2-EHA	Mo-bis-2-EHA	Zr-bis-2-EHA	Sn-bis-2-EHA	Co-bis-2-EHA	1-(2-hydroxypropyl)-1,4-diazabicyclo[2.2.2]octan-1-ium 2-EHA
Physical state at 20°C and 101,3 kPa	Liquid	Solid (powder)	Solid (crystalline)	Solid (powder)	Solid (pasty)	Solid (lump)	Liquid (viscous)	Liquid (highly viscous)	Liquid	Solid (lump)	Liquid (viscous)	Solid (waxy)	Liquid
Melting/freezing point	-57 ºC at 101.325 kPa	135 - 155 °C at 101.3 kPa	-	-	-	Decomposition at 140 °C	< -60 °C	< -60 °C	-	Decomposition at > 210 °C	9 °C	–53-58 °C	-
Boiling point	226-229 °C at 101.325 kPa	157 °C at 101.9 kPa	-	-	-	-	-	< 200 °C	250 °C at 101.3 kPa	-	-	-	> 250 °C at 101.3 kPa
Relative density	-	1.07 at 22 °C	343 g/L	1.39 g/mL	1.07 at 20 °C	1.15 at 20 °C	1.2 g/mL at 20 °C	1.18 g/mL at 20 °C	1.127 at 20 °C	1.4 at 20 °C	1.26 g/mL at 20 °C	1.25 at 20 °C	1.07 at 20 °C
Vapour pressure	0.04 hPa at 20 °C	< 1×10^{-6} Pa at 20 °C	-	-	-	-	-	-	-	-	0.3 Pa at 25 °C	-	< 6 Pa at 25 °C
Surface tension	n.a.	68.6 mN/m at 20 °C	47.63 mN/m	47.63 mN/m (R-A)	60.22 mN/m	60.22 mN/m (R-A)	n.a.	n.a.	60.22 mN/m (R-A)	60.22 mN/m (R-A)	55.9 mN/m.	64.43 mN/m at 20°C	69 mN/m at 20°C
Water solubility	1.4 g/L at 20 °C Soluble	> 1000 g/L Very soluble	>2134 g/L Very soluble	172 g/L Very soluble	80.37 g/L Very soluble	11.2 g/L Very soluble	3.2 g/L Soluble	5.8 g/L Soluble	0.09 g/L Slightly soluble	0.75 x10-6 g/L Insoluble	4.59 g/L Soluble	40.3 g/L at 20 °C Very soluble	> 1 g/L at 20 °C Soluble
Partition coefficient n-octanol/water (log value)	2.7 at 25 °C / pH = 4.7	1.3 at 23 °C	Waiving (inorganic)	Waiving (inorganic)	Waiving (inorganic)	Waiving (inorganic)	> 5.7	> 5.7 (R-A)	Waiving (inorganic)	Waiving (inorganic)	-	-	-
Flash point	118 ºC at 1013.25 hPa	-	-	-	-	-	-	-	112.5 °C at 1013 hPa	-	137 °C	-	135 °C
Flammability	-	Not highly flammable	-	-	-	-	-	-	-	-	Non flammable	Non flammable	-
Explosive properties	-	-	-	-	-	-	-	-	-	-	-	-	-

ANNEX 1 – BACKGROUND DOCUMENT TO RAC OPINION ON 2-ETHYLHEXANOIC ACID AND ITS SALTS, WITH THE EXCEPTION OF THOSE SPECIFIED ELSEWHERE IN THIS ANNEX

Property	2-EHA	Na-2-EHA	K-2-EHA	Ba-bis-2-EHA	Ca-bis-2-EHA	Mn-bis-2-EHA	Zn-basic-2-EHA	Zn-bis-2-EHA	Mo-bis-2-EHA	Zr-bis-2-EHA	Sn-bis-2-EHA	Co-bis-2-EHA	1-(2-hydroxypropyl)-1,4-diazabicyclo[2.2.2]octan-1-ium 2-EHA
Self-ignition temperature	-	-	-	-	-	-	-	-	-	-	> 400ºC	-	275 °C
Oxidising properties	-	-	-	-	-	-	-	-	-	-	No oxidising properties	-	-
Granulometry	-	D10 29.9 ± 0.3 µm D50 61.6 ± 0.5 µm D90 129.4 ± 10.4 µm	-	-	n.a. Very pasty solid	n.a. Agglomerate	-	-	-	D10 4.99 µm D50 26.75 µm D90 82.21 µm	-	-	-
Stability in organic solvents and identity of relevant degradation products	-	-	-	-	-	-	-	-	-	-	-	-	-
Dissociation constant	4.76 at 25 ºC	4.82 at 25 °C (calculated) (US EPA, 2002)	6.89 at 20 °C (US EPA, 2002)	-	8.45 at 20 °C (US EPA, 2002)	-	-	6.99 at 20 °C (US EPA, 2002)	-	5.81, 7.09, 7.65 and 8.24 at 20 °C (Zr (IV) 2-ethylhexanoate) (US EPA, 2002)	5.09 at 20 °C (US EPA, 2002)	6.41 at 20 °C (US EPA, 2002)	-
Viscosity	8.4 mPa×s at 20.3 ºC	-	-	-	-	-	10000 mPa×s at 20 °C	25800 mPa×s at 70 °C	162 mPa×s at 20 °C	-	306 mm²/s at 20°C	-	-

n.a. not applicable; R-A read-across

8 EVALUATION OF PHYSICAL HAZARDS

Not evaluated in this dossier.

9 TOXICOKINETICS (ABSORPTION, METABOLISM, DISTRIBUTION AND ELIMINATION)

Information in this section is limited to the information on 2-EHA since there are no toxicokinetics studies available for any of the registered salts of 2-EHA. In all cases, the information provided for the salts in the REACH registrations is covered by a combination of read-across from the substance dissociation products, i.e. the cation (usually a metal or its derivatives) and the 2-ethylhexanoic acid.

It is important to note that all of these salts have a common feature as they readily dissociate to the corresponding cation and 2-ethylhexanoate anion. In addition, further protonation at acidic pH may allow bioavailability of 2-ethylhexanoic acid. The information on 2-EHA is taken as the basis for this proposal, but as expressed in the proposed note to the Annex VI entry, the hazardous properties of the cationic species must be evaluated separately to conclude on the overall toxicity of the salt.

Non-human information

Regarding the toxicokinetics of 2-EHA, there is only one experimental study available. 2-EHA was investigated in female Fischer 344 rats, in a GLP study equivalent or similar to US EPA TSCA Health Effects Testing Guideline (CFR 40 798.7100), as it was reported in the registration dossier. The aim of this study was to provide information on the metabolic fate and elimination of 2-EHA after oral and dermal administration to rats. The study involved a series of individual studies using the following administration regimes (Anonymous, 1987; English *et al.*, 1998):

a. Single oral gavage at either 100 or 1000 mg radiolabelled 2-EHA/kg bw.

b. By gavage for 14 days with 100 mg unlabelled 2-EHA/kg bw/ day and with an equivalent dose of the radiolabelled 2-EHA on day 15.

c. Single dermal dose at either 100 or 1000 mg radiolabelled 2-EHA/kg bw by occlusive application for 96 hours.

d. Single intravenous application of 1 mg radiolabelled 2-EHA/kg bw.

All the studies were conducted with eight animals, except the 15-day study which was performed with four rats. The amount of administered radioactivity was about 10 µCi/animal in all cases.

In addition, a skin washing efficiency study was performed. For this purpose, four rats were dermally treated with 1000 mg undiluted radiolabelled 2-EHA/kg bw (about 10 μCi/animal). After 5 minutes, the test material was removed by aspiration and the application site was thoroughly washed.

For the absorption, distribution, metabolism and excretion (ADME) studies, excreta were collected at intervals for up to 96 hours after treatment and levels of radioactivity were quantified by liquid scintillation spectrometry in urine and faeces. Blood samples were obtained from the orbital sinus at intervals of up to 96 hours in the low oral and dermal dose groups and in the intravenous dose group. The total radioactivity was measured in the whole blood. The metabolites were analysed by HPLC and GC/MS in the urine samples, obtained from rats given radiolabelled 2-EHA by oral or dermal administration. Samples were collected within the first 96 hours at 24-hour intervals. Pulmonary excretion of 2-EHA metabolites was not investigated in this study.

The absorption after oral administration was rapid and extensive. A peak blood level of 85.1 µg equivalents 2-EHA/g blood were reached at either 15 or 30 minutes in individual animals following oral administration of 100 mg [^{14}C]2-EHA/kg bw. In the single oral studies, about 90% of the dose was recovered in the urine and faeces, primarily within the first 24 hours of administration. The greatest apparent difference between low- and high-dose administrations was in the percentage of radioactivity recovered in faeces, ca. 12% and 6%, respectively. In the repeated oral dose study, total recovery of the [^{14}C], about 75%, was markedly lower than that seen in the single gavage dose studies. Almost 15% of the dose was recovered in the faeces. As in

the single oral studies, the majority of the [^{14}C] was recovered within 24 hours of the final dose. Results suggest that biliary excretion or secretion into the lumen of the gastrointestinal tract took place and that the process was saturated at the high-dose level.

Dermal absorption was slower, with a peak blood level of 7.9 µg equivalents 2-EHA/g blood achieved 8 hours after application of 100 mg/kg bw (10-fold lower than peak levels after oral administration). The extent of dermal absorption was 70% relative to i.v. dosing. In both low- and high-dose level dermal studies, total recovery in the excreta was about 50% over 96 hours. Approximately 45% of the dose was recovered in the urine and 7.5% in the faeces at both dose levels.

In addition, dermal washing efficiency study resulted in recovery of all of the [^{14}C] applied to the skin (101.9%) during the washing procedure, with less than 0.2% of the applied radioactivity being found in the excreta over 96 hours.

2-EHA was rapidly eliminated following intravenous administration of 1 mg radiolabelled 2-EHA/kg bw. A mean of 70.2% of the injected radioactivity was recovered in the excreta over 96 hours. Radioactivity was rapidly excreted in the urine, with 64.2% excreted during the first 24 hours after dosing. Faecal elimination accounted for 2.9% in the same period. This is a further evidence of the biliary excretion or secretion into the lumen of the gastrointestinal tract. The organ distribution of [^{14}C]2-EHA was not determined.

Extensive metabolism of 2-EHA is evidenced by the small percentage of parent compound excreted and the number of urinary metabolites detected. Metabolites were likely to be formed by glucuronidation and/or cytochrome P450-dependent oxygenation (ω-oxygenation and ω-1-oxygenation), or β-oxidation. Analysis of metabolites revealed that 2-EHA was excreted via the urine, mainly as the glucuronide of 2-EHA. The extent of glucuronidation increased with increasing dose. Smaller amounts of unchanged 2-EHA were also detected. The other two major metabolites detected, 2-ethyl-6-hydroxyhexanoic acid and 2-ethyl-1,6-hexanedioic acid, are likely to arise from initial cytochrome P450-catalysed ω-oxygenation. Subsequently, they were partially conjugated with glucuronic acid. The detection of Δ^5-2-heptenone may support the role of β-oxidation as previously proposed by Albro (1975). Evidence of this route has also been reported by Walker and Mills (2001).

A largely similar metabolite profile was reported in a study with male Wistar rats, which were given 600 mg 2-EHA/kg bw in drinking water for nine weeks (Pennanen *et al*., 1991) and in a study with the related compound 2-ethylhexanol (Deisinger *et al*., 1994). This substance was reported to be metabolized mainly through the formation of 2-EHA.

In a further study performed *in vitro* in microsomes from rat, mouse and human liver, Pennanen *et al*. (1996) confirmed that the cytochrome P-450 isoenzymes are involved in the biotransformation of 2-EHA. The main metabolite produced in all microsomes was 2-ethyl-1,6-hexanedioic acid.

The glucuronidation of 2-EHA was studied in more detail by Hamdoune *et al*. (1995). The acid was found to be glucuronidated *in vitro* by liver microsomes from all investigated species (rat, rabbit, dog, guinea pig, rhesus monkey, man). Interspecies comparison showed that the most active glucuronidation of 2-EHA occurred in the dog and the rat. On the contrary, the lowest activities were observed in the man and the rabbit. Stereospecificity was detected in guinea pig and rabbit microsomes which glucuronidated the (R)-enantiomer to a greater extent. However, in the rest of the species, there were no differences in the glucuronidation of 2-EHA enantiomers.

Pennanen and Manninen (1991) investigated the distribution of [^{14}C]2-EHA in mice and rats. According to the available abstract, organ distribution of 2-EHA was studied by analysis of radioactivity after the administration of a single intraperitoneal dose of the radiolabelled substance in both species. The authors reported the highest uptake of [^{14}C]2-EHA in blood, liver and kidney of mice and rats. In contrast, low uptake of [^{14}C]2-EHA was seen in the brain. By 6 hours, the radioactivity decreased rapidly and was hardly measurable at 24 hours after the administration, which suggests that 2-EHA is rapidly cleared from tissues.

Further studies available as abstracts, showed that 2-EHA is able to cross the placenta and can be detected in the embryo at slightly lower concentrations to those detected in the dams (Collins *et al*., 1992, Scott *et al*. 1994). Scott *et al*. (1994) also observed that 2-EHA levels measured in the embryos correlated closely with the maternal plasma concentrations, but levels in the embryo were markedly lower.

Human information

There is scarce information on the toxicokinetics of 2-EHA in humans. Some *in vitro* studies have been performed in microsomes from humans and several animal species to investigate the metabolism of 2-EHA (Hamdoune *et al*., 1995). The human metabolism seems to show similar profile to the other species.

Oxidative and conjugated metabolites of 2-EHA, which is a known metabolite of important phtalates, have also been identified in urine of humans with high exposure to plasticizers (Walker and Mills, 2001).

Evaluation of worker exposure to 2-EHA via dermal and inhalation routes in Finnish sawmills showed a rapid urinary excretion of 2-EHA. In most cases, the highest urinary concentrations were found immediately after the work shift (Kröger *et al*., 1990).

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

Results from the toxicokinetic study in rats show that 2-EHA is rapidly and extensively absorbed after oral administration. Absorption following dermal exposure was slower and C_{max} (maximum concentration) was 10-fold lower than that seen after oral administration, at the same dose level. The extent of oral and dermal absorption is 90% and 70%, respectively.

In mice and rats, 2-EHA showed a preferential distribution in kidneys, liver and blood.

Available data indicate that 2-EHA undergoes extensive metabolism. Metabolites are likely to be formed by glucuronidation and/or cytochrome P450-dependent oxygenation, or β-oxidation. Analysis of metabolites revealed that 2-EHA was excreted via the urine, mainly as the glucuronide form. The extent of glucuronidation is increased with increasing dose. Human metabolism seems to show similar profile to other species. There is also evidence of the role of β-oxidation in humans.

Finally, 2-EHA exhibited a rapid elimination in rats after oral, intravenous and dermal administrations, predominantly in the urine within the first 24 hours, which is consistent with the rapid excretion of the substance observed in workers exposed by the dermal and inhalation routes.

10 EVALUATION OF HEALTH HAZARDS

In this proposal, the classification for reproductive toxicity of 2-EHA is reviewed in the light of the new data and of the CLP classification criteria. This evaluation and the resulting classification, as previously explained, shall be further applicable to the salts of 2-ethylhexanoic acid, except to those specified elsewhere in Annex VI.

Justification for the grouping approach

This CLH proposal is related to the reproductive toxicity of the substance 2-ethylhexanoic acid and its salts. The proposed Annex VI entry is named *"2-ethylhexanoic acid and its salts, with the exception of those specified elsewhere in this Annex"*.

For this CLH proposal, a grouping and read-across approach has been followed.

A group or category of substances may be defined for those members that have physicochemical, toxicological and ecotoxicological properties that are likely to be similar or follow a regular pattern as a result of structural similarity.

Applying the grouping concept means that information for physicochemical, human health and/or environmental properties may be predicted from information from tests conducted on reference substance(s) within the group through read-across.

The group considered for this CLH proposal covers the free acid (2-EHA) and its salts and it is based in the formation and bioavailability of 2-EHA for all group members. 2-EHA has currently an entry in Annex VI with the classification as Rep 2. (H316d) because of its developmental effects. The boundaries of the group have been defined establishing a high degree of structural similarity, since all the considered salts of 2-EHA share the same anionic moiety and they only differ in the cation counterion. In this context, all the potential

group members have been included in the group and the available data on some of the registered members have been taken into account in this proposal. There is no reason to include only certain salts and it could be perceived as if some salts were safer than those with a harmonized classification potentially leading to unjustified substitution.

The proposed read-across approach is considered according to the ECHA Guidance Document for categories, Guidance on information requirements and chemical safety assessment, Chapter R.6: QSARs and grouping of chemicals (ECHA, 2008). The Read-Across Assessment Framework (RAAF) (ECHA, 2017) has also been used as a reference.

Background

A read-across approach from 2-EHA has been proposed by the REACH registrants of 2-EHA metal salts for the vast majority of the human health endpoints, including reprotoxicity, during the registration phase for the registered substances that constitute the basis for this CLH dossier.

A comprehensive database exists for 2-EHA, considered as the source substance. Recently, new reprotoxicity data resulting from the REACH substance evaluation process have been added to this data set. On the other hand, there are no reproductive toxicity tests available for the registered salts (target substances).

Apart from the registered metal salts of 2-EHA, there is one tetralkyl-substituted ammonium salt registered following Article 24 of REACH Regulation (notified substances in accordance with Directive 67/548/EEC).

It has to be noted that a subcategory named 2-ethylhexanoate salts, including six of the 2-EHA metal salts (potassium, calcium, cobalt (2+), zinc basic, zirconium and tin (2+)), was already defined as part of the metal carboxylates category reported by The Metal Carboxylates Coalition for the assessment of these substances under the US High Production Volumen (HPV) Chemical Challenge Program in 2002 (US EPA, 2002). The main category of Metal Carboxylates comprised of 20 compounds, consisting of different metal salts of carboxylic acids. The justification for the category formation was based in the readily dissociation of all the substances to the corresponding metal and carboxylic acid.

Hypothesis for the category approach

This CLH proposal is related to the reproductive toxicity of the group of 2-ethylhexanoic acid and its salts. As a common feature, all of these salts readily dissociate to the corresponding cation and 2-ethylhexanoate anion. Further protonation at acidic pH may allow bioavailability of 2-ethylhexanoic acid that, currently has its own Annex VI entry (index no. 607-230-00-6) with the classification as Repr. 2 (H361d).

The read-across hypothesis is based in the formation and bioavailability of 2-EHA from all the salts. Thus, the rationale for the assessment of the reproductive toxicity is based on the existing data for 2-EHA.

The possible hazardous properties of the respective cationic moiety are not considered for this CLH proposal. Then, the resulting classification should be applied to all the 2-EHA salts, taking into account that the reproductive toxicity of the cationic part and its contribution to the classification of the salt of 2-EHA needs to be always assessed separately. Accordingly, the following note has been included as part of this proposal: "*The classification for the hazard class(es) in this entry is based only on the hazardous properties of the part of the substance which is common to all members in the entry. The hazardous properties of any member in the entry also depends on the properties of the part of the substance which is not common to all members of the group; they must be evaluated to assess whether (a) more severe classification(s) (e.g. a higher category) or (b) a broader scope of the classification (additional differentiation, target organs and/or hazard statements) might apply for the hazard class(es) in the entry.*".

Substance characterization

The substances characterization, including the impurity profiles has been clearly provided for the registered group members in the corresponding registration dossiers. In all the cases, they are registered as mono-constituent substances with a high degree of purity (see Table 3). The evidence for similarity between the source (2-EHA) and the target substances (its salts) purities is considered sufficient.

Structural similarity and structural differences within the group

Regarding the structural similarity within the group, the read-across hypothesis relies in the formation of 2-EHA from the salts. All salts share the same carboxylate chemical structure, with COO^- moiety as the functional group linked to the identical saturated branched aliphatic C_7 chain length (see Figure 2). They only differ in the cation counterion.

The source substance (2-EHA) is the free acid analogue with the same aliphatic chain substitution. As it represents the common (bio) transformation product, it has been included within the group.

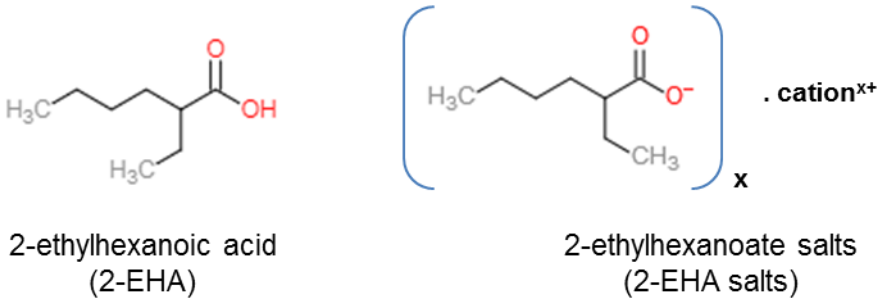


Figure 2. Category members chemical structure.

Link of structural similarities and structural differences with the proposed regular pattern

The group is structurally defined as substances that share the same carboxylate chemical structure, with COO^- moiety as the functional group linked to the identical saturated branched aliphatic C_7 chain length. They only differ in the cation counterion.

As previously mentioned, for this CLH proposal the toxicity for reproduction is focused solely on the acid moiety that is responsible for the observed developmental effects of 2-EHA. Although bioavailability studies are not available for any salt of 2-EHA, the dissociation constants of the salts indicate that in the neutral pH range, the substances will be mainly dissociated. At this respect, pKa values vary from 4.82 to 8.45 (US EPA, 2002). In addition, at the low pH of the stomach a complete dissociation and further protonation of the anion carboxylate is anticipated. Therefore, the free acid (2-EHA) is formed.

As possible hazardous properties of the respective cationic moiety are not considered for this CLH proposal, the reproductive effects expected for the salts are at least those caused by the 2-EHA.

Consistency of effects in the data matrix

A data matrix for the majority of the human health endpoints cannot be built since there is scarce information on the target substances themselves. Altogether, there are only three acute toxicity studies, several *in vivo* and *in vitro* studies for the dermal and ocular irritation effects, a sensitization study, a 14-day toxicity study, an *in vitro* gene mutation study in bacteria and a carcinogenicity study for the registered salts of 2-EHA. In relation to the reproductive toxicity endpoint, there is no information available on any of the target substances apart from 2-EHA. In the majority of cases, human health endpoints are covered by the read-across to 2-EHA and to the corresponding cation or its derivatives. At this respect, it has to be noted that data for cation (usually metals in their different forms) are extensive. Therefore, the influence of the cation on overall toxicity of the specific salts should be evaluated independently, see section 3 above.

In general, it is assumed that the toxicity is partially driven by the 2-EHA in addition to the cation toxicity, if any. A comprehensive database exists for 2-EHA. The information used for this proposal is the one included in the 2-EHA registration dossier.

Reliability and adequacy of the source study(ies)

As it has been previously explained, 2-EHA is considered the source substance for the minimum classification of the group for the reproductive toxicity endpoint.

Concerning fertility, 2-EHA was assessed under substance evaluation procedure (CoRAP 2012) because of a fertility concern. Following the substance evaluation decision, an oral combined repeated dose toxicity study with the reproduction/developmental toxicity screening test (OECD TG 422) and an extended one generation reproductive toxicity study (EOGRTS, OECD TG 443) were conducted according to GLP with 2-EHA in Wistar rats (Anonymous, 2015; 2016). After the evaluation of these new data, it has been considered that the new studies results provide sufficient and reliable information to conclude that 2-EHA does not show a specific effect on fertility and neurodevelopmental toxicity.

The information on the developmental effects of 2-EHA was considered reliable and adequate for the classification of the substance according to the former existing criteria. As a consequence, 2-EHA has currently an EU harmonised classification as toxic for reproduction, category 2 (H361d: suspected of damaging the unborn child) on the basis of observed developmental effects in prenatal developmental studies in rats, such as skeletal variations and malformations.

Nevertheless, the old and the new information available on the reproductive toxicity of 2-EHA is evaluated again according to the CLP criteria. The resulting classification should be applied to the acid and all the 2-EHA salts, taking into account that the cationic part needs to be always assessed separately. Accordingly, the following note has been included as part of this proposal: "*This entry is based solely on the data on adverse effects on reproduction induced by the anionic moiety of the salt, and the hazardous properties of the respective cationic moiety must always be evaluated in accordance with CLP Art. 5 to assess whether a higher category and/or additional hazards might have to be applied*".

Formation of common (identical) compound(s)

It is expected that the 2-EHA salts dissociate to the organic anion and the cation upon dissolution in aqueous media. The dissociation constants available, pKa values, vary from 4.82 to 8.45 (US EPA, 2002). This indicates that in the neutral pH range, the substances will be mainly dissociated. In addition, at the low pH of the stomach a complete dissociation and further protonation of the anion carboxylate moiety is anticipated. Therefore, the free acid (2-EHA) is formed and can be taken as the source substance for the salts of this carboxylic acid.

Carboxylic acid salts are ionic compounds usually soluble in water. Registration data from the registered salts but 2-ethylhexanoic acid, zirconium salt, show solubility in water in different degree, from the very soluble salts, i.e. sodium, potassium, calcium, manganese and barium, to the moderately/slightly soluble molybdenum salt (see Table 10).

Water solubility data may indicate differences in bioavailability of the toxicant. However, concerning the Zr and Mo salts of 2-EHA, it is important to keep in mind that water solubility tests (OECD TG 105) for these salts have been carried out by measuring metal concentration and not 2-EHA formation. In this context, formation of low-solubility metal oxide species after dissolution of the mentioned salts is expected. Consequently, the moderate to low solubility in water observed for these salts could be explained by the formation of insoluble metal compounds after salt dissociation.

In Figure 3 dissociation equilibrium of 2-EHA salts ($C_8H_{15}O_2$.(1/x)cation) and acid-base equilibrium of 2-EHA ($C_8H_{16}O_2$) is represented.

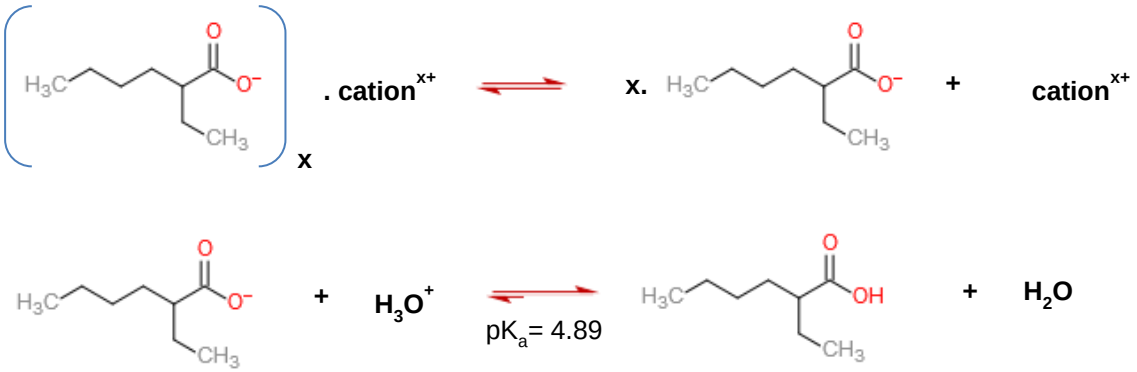


Figure 3. Dissociation equilibrium of 2-EHA salts and acid-base equilibrium of 2-EHA.

As 2-EHA is as weak acid (pK_a= 4.89), the conjugate carboxylate anion can be regarded as a strong base. Therefore, while reducing the pH, hydronium (H_3O^+, pK_a= -1.74) concentration will increase and readily react with carboxylate anions to form 2-EHA. This decrease in the concentration of carboxylate anions will shift equilibrium to favor solubility of the corresponding metal salts following Le Chatelier's principle.

The biological targets for the common compound(s)

The biological targets for the 2-EHA salts are those established for the acid, e.g. 2-EHA. Results from a toxicokinetic study in rats showed that 2-EHA was rapidly and extensively absorbed after oral administration and had a preferential distribution in the kidneys, liver and blood. The extent of oral and dermal absorption is 90% and 70%, respectively.

Information from the literature shows that 2-EHA is present after exposure to 2-EHA derivatives. The substance has been detected in urine of workers exposed to a wood preservative containing 26% sodium 2-ethylhexanoate (Kröger *et al.*, 1990).

In two subchronic (90 days) toxicity studies, the main observed effects of 2-EHA were associated with growth retardation, decreases in body weight, increases in absolute and relative liver weights and hepatocyte hypertrophy. The findings in the liver were considered to be primarily an adaptive change rather than a toxic effect.

Finally, the results obtained from reproductive and developmental studies showed that 2-EHA is harmful to the embryos and/or foetuses at dose levels without maternal toxicity. Developmental effects, such as skeletal variations (wavy ribs, reduced ossification) and skeletal malformations (clubfoot) were observed in rat following oral doses given on days 6-19 of gestation.

Exposure of the biological target(s) to the common compound(s)

Due to the fact that all the group members but 2-EHA itself are salts of 2-EHA, they are expected to be a relevant source of this organic acid. It is assumed that all the salts undergo rapid and complete dissociation with further carboxylate protonation. Consequently, organism exposure to 2-EHA and to the different cations is foreseen. As possible hazardous properties of the respective cationic moiety are not considered in this CLH proposal, in all cases the biological targets are expected to be exposed to the acid and, thus, at minimum the same adverse effects on reproductive toxicity are reasonably foreseen for all salts.

The impact of parent compounds

No information is available on the effects of the salts on the reproductive toxicity. Nevertheless, a rapid and complete dissociation of the salts of 2-EHA is expected even before absorption. Therefore, the impact of the non-dissociated salt of 2-EHA on the reproductive toxicity is expected to be negligible.

Formation and impact of non-common compounds

According to the available data on pK_a for the registered 2-EHA salts, a rapid and complete dissociation to 2-EHA and to the cation is expected. Since the acid moiety is identical, the non-common compounds are expected to be those derived from the cations.

As possible hazardous properties of the respective cationic moiety are not considered for this CLH proposal, the following note is proposed to be included: "*The classification for the hazard class(es) in this entry is based only on the hazardous properties of the part of the substance which is common to all members in the entry. The hazardous properties of any member in the entry also depends on the properties of the part of the substance which is not common to all members of the group; they must be evaluated to assess whether (a) more severe classification(s) (e.g. a higher category) or (b) a broader scope of the classification (additional differentiation, target organs and/or hazard statements) might apply for the hazard class(es) in the entry.*".

Bias that influences the prediction

The boundaries of the group have been defined establishing a high degree of structural similarity, since only the salts of 2-EHA and 2-EHA itself have been considered. In this context, all the potential group members have been included in the suggested group entry and the available data on the registered members have been taken into account in this proposal. These data include substance identification and physicochemical properties of the registered substances. They also include toxicological data of 2-EHA. In this context, the

source studies used for the basis of the prediction are considered to be reliable studies. Therefore, in principle, bias that influence the prediction is not expected.

Acute toxicity

10.1 Acute toxicity - oral route

Not evaluated in this dossier.

10.2 Acute toxicity - dermal route

Not evaluated in this dossier.

10.3 Acute toxicity - inhalation route

Not evaluated in this dossier.

10.4 Skin corrosion/irritation

Not evaluated in this dossier.

10.5 Serious eye damage/eye irritation

Not evaluated in this dossier.

10.6 Respiratory sensitisation

Not evaluated in this dossier.

10.7 Skin sensitisation

Not evaluated in this dossier.

10.8 Germ cell mutagenicity

Not evaluated in this dossier.

10.9 Carcinogenicity

Not evaluated in this dossier.

10.10 Reproductive toxicity

There are not available reproductive toxicity studies for any of the salts of 2-EHA. In all cases the information covers only the data on 2-EHA.

Concerning toxicity for reproduction, it is considered that the adverse effects are driven by the 2-ethylhexanoic acid, in addition effects that may be due to the cationic part of the substances should be evaluated.

10.10.1 Adverse effects on sexual function and fertility

Table 11: Summary table of animal studies on adverse effects on sexual function and fertility

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels, duration of exposure	Results	Reference
Oral extended one-generation reproductive toxicity study (OECD TG 443). Design includes the extension of cohort 1B to mate the F1 animals to produce the F2 generation and cohorts 2 (DNT) and 3 (DIT). GLP: Yes Rat/Wistar F0: 28 animals/sex/dose F1: 75 pups/sex/group Cohort 1A: 20 pups/sex/group Cohort 1B: 25 pups/sex/group Cohort 2A: 10 pups/sex/group Cohort 2B: 10 pups/sex/group Cohort 3: 10 pups/sex/group (an extra group of 6 male and female pups treated with cyclosporine A were included as positive control group for the determination of the KLH-specific IgM response). The evaluation of the potential developmental immunotoxicity by determining the titer of KLH-specific IgM antibody was performed in the serum of cohort 3 animals by ELISA. After at least 13 weeks of age, animals of cohort 1B were mated to produce the F2 generation.	2-EHA (purity 99.6%) Oral feed. Doses: 0, 80, 250, 800 mg/kg bw/d. Exposure: 2-week premating period, mating, gestation and lactation (females) and up to and including the day of sacrifice.	**F0 - Parental generation** **General toxicity** <u>Mortality and general clinical observations</u> During the post-mating phase, two male animals of the F0 high-dose group were sacrificed in a moribund condition. <u>Body weight and food consumption</u> (Tables 12 and 13) *80 mg/kg bw/d* ↓ Food consumption in females during the gestation period (GD 0-7) and during lactation period from PN days 4-7. *250 mg/kg bw/d* Females: ↓ Body weight gain from GD 0-7. ↑ Body weight gain on PN days 4-7. ↓ Food consumption in females during the gestation period (GD 0-7). *800 mg/kg bw/d* Males: ↓ Body weight on post-mating days 22, 29, 36 and 43. Body weight gain decreased during the premating period from days 0-7 and 0-14 and from post mating days 22 to 29. Females: ↓ Body weight gain during premating days 0-14. ↓ Body weight during GD 7, 14 and 21. ↓ Body weight gain from GD 0-7, GD 14-21 and GD 0-21. ↓ Body weight on PN days 4 and 21. ↓ Food consumption during the premating period in males and females, during three weeks gestation period and from PN days 4-7 and 14-21. <u>Haematology and clinical biochemistry</u> ↑ GGT activity in males and ↓ bilirubin in females at 800 mg/kg bw/d. No changes in TSH and T4 levels at any dose. <u>Urinalysis</u> *250 mg/kg bw/d* ↑ Amorphous material in males. *800 mg/kg bw/d* ↑ Amorphous material and ↓ pH in males. <u>Organ weights</u> (Table 18) *800 mg/kg bw/d* ↑ Absolute and relative weights of the liver in males and females along with microscopic findings (males). ↑ Relative weights of kidneys and thyroids in males. <u>Microscopic observations</u> (Table 21) 19/26 male animals at 800 mg/kg bw/d showed minimal to moderate accumulation of proteinaceous droplets in the tubuli of kidneys.	Anonymous, 2016

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels, duration of exposure	Results	Reference
		Fertility Fertility, parturition and sexual function (Tables 23 and 25-27) *80 mg/kg bw/d* Slight and not statistically significant ↓ in the number of implantations. Slight and not statistically significant ↑ in the number of implantations and post-implantation losses. *800 mg/kg bw/d* ↑ Mean length of the longest cycle (4.3 days versus 4 days in the control group) but within the range of historical control data. Considered as a fortuitous finding. Slight and not statistically significant ↓ in the number of implantations. Slight and not statistically significant ↑ in the number of implantations and post-implantation losses. No biologically relevant treatment-related effects were observed on fertility or reproductive performance. Gestation index was 100%. No statistically significant effects were observed on epididymal sperm motility, epididymal sperm count and epididymal sperm morphology. ***Cohort 1A*** **General toxicity** Mortality and general clinical observations One female of the low-dose group of cohort 1A was found dead on day 20 (at an age of 43 days) without clinical signs. This finding was not considered to be related to treatment. Body weight and food consumption (Tables 14 and 15) *80 mg/kg bw/d* ↑ Body weight gain in male animals from days 0-7, 21-28 and 35-42. ↓ Body weight gain in female animals from days 35-42. ↑ Food consumption in males from days 14-21. *250 mg/kg bw/d* ↑ Body weight gain in male animals from days 21-28. ↑ Body weight gain in female animals from days 0-7. *800 mg/kg bw/d* ↓ Body weight all days except for day 56, ↓ body weight gain from days 0-7 and 7-14 in males. ↓ Body weight gain in female animals from days 35-42. ↓ Food consumption in males from days 0-7, 7-14, 14-21 and 28-35, and in females from days 35-42. Haematology and clinical biochemistry *80 mg/kg bw/d* ↑ Prothrombin time in females.	

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels, duration of exposure	Results	Reference
		↑ Sodium values in males. *250 mg/kg bw/d* ↑ Prothrombin time in females. ↑ ALP in females. *800 mg/kg bw/d* ↓ MCH in males, ↓ MCV in females. ↓ Total protein and ↑ albumin/globulin ratio and sodium values in males. <u>Urinalysis</u> *250 mg/kg bw/d* ↓ Epithelial cells in males *800 mg/kg bw/d* ↓ pH and ketones in males <u>Organ weights and histopathology</u> (Table 19) *80 mg/kg bw/d* ↑ Absolute weight of the heart in males. ↓ Relative weight of the cauda epididymides. *800 mg/kg bw/d* ↑ Relative weights of heart, kidneys, liver and testes in males. ↑ Absolute and relative weights of the liver and in the relative weight of kidneys in females. <u>Microscopic observations</u> (Table 22) *250 mg/kg bw/d* 14/20 male animals showed minimal to moderate accumulation of proteinaceous droplets in the tubuli of kidneys. *800 mg/kg bw/d* 15/20 male animals showed minimal to moderate accumulation of proteinaceous droplets in the tubuli of kidneys; 9/20 male animals showed minimal to mild basophilic tubuli. **Fertility** <u>Fertility, parturition and sexual function</u> (Table 24 and 31) *800 mg/kg bw/d* ↑ Mean cycle length (4.70 days versus 4.16 days in the control group) but within the range of historical control data. Not considered to be treatment-related. ↓ Absolute number of growing follicles, but no effects in the development of antral and corpora lutea. This effect is not considered relevant. ***Cohort 1B*** **General toxicity** <u>Body weight and food consumption</u> (Tables 16 and 17) *80 mg/kg bw/d* ↓ Body weight gain in male animals from premating days 7-14. *250 mg/kg bw/d*	

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels, duration of exposure	Results	Reference
		↓ Body weight gain in male animals from premating days 7-14 and ↑ from days 63-70. ↓ Body weight gain in female animals from premating days 35-42 and ↑ from days 42-49. ↓ Food consumption in females from GD 0-7. *800 mg/kg bw/d* ↓ Body weight and food consumption in male animals during the major part of the premating and post-mating periods. ↓ Body weight gain in male animals from premating days 7-14, 14-21, 21-28, 35-42 and from post-mating days 89-96. ↓ Food consumption in female animals from premating days 35-42. ↓ Body weight gain in female animals from GD 7-14 and food consumption from GD 0-7 and 7-14. <u>Organ weights and histopathology</u> (Table 20) *250 mg/kg bw/d* ↑ Absolute and relative weights of the testes in males. *800 mg/kg bw/d* ↑ Absolute weight of the kidneys and relative weights of the kidneys, liver, testes and cauda epididymis in males. ↑ Relative weights of liver and kidneys in females. **Fertility** No biologically relevant treatment-related effects were observed on fertility or reproductive performance. Gestation index was 100% (Tables 28-30). **NOAEL for parental effects was established at 250 mg/kg bw/d, based on the effects on body weights, food consumption, kidney and liver weights and kidney pathology observed in animals of the highest dose.** **NOAEL for fertility effects was established at 800 mg/kg bw/d, due to the lack of effects.**	
Oral combined repeated dose toxicity study with the reproduction/developmental toxicity screening test (OECD TG 422) GLP: Yes Rat/Wistar 10 animals/sex/dose. Satellite groups of 6 extra animals/sex were added and	2-EHA (purity 99.8%) Oral feed. Doses males: 82-86, 248-253, 761-797 mg/kg bw/d females: 107-116, 308-351, 809-1146 mg/kg bw/d; PND 0-4: 190, 530 and 1371 mg/kg bw/d	**Parental generation** **General toxicity** *High-dose group* <u>Body weight and food consumption</u> ↓ Body weight (up to 10%) and food consumption in males and females. <u>Haematology and clinical biochemistry</u> ↓ MCV, MCHC and reticulocytes, ↑ total white blood cells, monocytes and in the absolute number of neutrophils, ↓ total protein and albumin concentrations	Anonymous, 2015

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels, duration of exposure	Results	Reference
pregnant females were sacrificed on gestation day 20 to gain knowledge on the possible mechanism of toxicity.	Exposure: 2-week premating period, mating and up to and including day 30 (males) and 2-week premating period, mating, gestation and lactation and up to and including the day of sacrifice (day 4 to 7 of lactation).	and ↑ albumin/globulin ratio (females). Organ weights ↑ Relative weight of the liver (males and females) and relative weight of the kidneys (males), ↓ absolute and relative weight of the thymus (females). Histopathology ↑ Incidence of proteinaceous droplets in the kidney renal tubuli (males). Changes in zinc (females) and metallothionein (MT) concentrations in liver and kidneys. **Fertility** Fertility, parturition and sexual function No treatment-related effects on fertility or reproductive performance were observed at any dose. **NOAEL for general toxicity of at least 248 mg/kg bw/d for males and 308 mg/kg bw/d for females, based on the effects on body weights, food consumption, organ weights, haematology, clinical chemistry and zinc and metallothionein concentrations observed at the highest dose.** **NOAEL for fertility was established at the highest dose tested.**	
One-generation reproductive toxicity study (no guideline) GLP: No Rat/Wistar 24 animals/sex/dose	2-EHA (purity 99.5%) (administered as sodium salt) Oral in drinking water. Doses. 0, 100, 300 and 600 mg/kg bw/d Exposure: Males 10 weeks and females for 2 weeks prior to mating, both sexes during mating period and females during gestation and lactation.	**F0 - Parental generation** **General toxicity** Mortality and general clinical observations There were no mortalities during the study. Body weight and food consumption (Tables 32 and 33) ↓ Maternal body weight (9-12%) from GD 7-21 and ↓ gestational weight gain (GD 0-21) ($p<0.01$) at 600 mg/kg bw/d. Organ weights (Tables 32 and 33) ↑ Relative weights of the right epididymides (12%) ($p<0.05$) at 600 mg/kg bw/d. Histopathology Epithelial hyperplasia in the vagina and slight dilation of the lumen in uterus (2/5 dams) at 300 and 600 mg/kg bw/d. **Fertility** Fertility parameters (Tables 34-36) ↓ Total number of spermatozoa in the cauda epididymides (14%) at 600 mg/kg bw/d but not statistically significant. ↓ Portion of motile spermatozoa at 100 mg/kg bw/d (37%) and at 600 mg/kg bw/d (22%) ($p<0.05$).	Pennanen *et al.*, 1993

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels, duration of exposure	Results	Reference
		↑ Morphologically abnormal spermatozoa (mostly agglutinations and abnormal heads) at 300 mg/kg bw/d (12.5% amorphous heads) and 600 mg/kg bw/d (20.8% amorphous heads), but not statistically significant. Dose-dependent delay in fertilization. No post-implantation losses were observed but a ↓ in average litter size (16%) of the F1 generation was observed at 600 mg/kg bw/d ($p<0.05$). This effect could be considered a fertility effect. **NOAEL of 300 mg/kg bw/d based on the delay in fertility recorded at 600 mg/kg bw/d.**	

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

Three studies on 2-EHA are available for examination of adverse effects on sexual function and fertility for the substances covered by this CLH proposal.

Oral extended one-generation reproductive toxicity study (OECD TG 443) (Anonymous, 2016)

A GLP extended one-generation reproductive toxicity study (OECD TG 443) was conducted with 2-EHA at doses of 0, 80, 250, 800 mg/kg bw/d in Wistar rats, following the information requirement included in the substance evaluation final decision under REACH Regulation. The initial study design included cohorts 2 and 3 to assess developmental neurotoxicity (DNT) and immunotoxicity (DIT). The extension of the cohort 1B to produce the second generation was left to the consideration of the Registrant who finally decided to produce the F2 generation to allow drawing a clear and reliable conclusion.

Parental (F0), cohort 1 (1A and 1B)

During the post-mating phase, two males of the highest dose in the F0 generation were sacrificed due to their moribund condition. Both animals were lethargic and pale and showed piloerection. In the low-dose group in the F1 generation, cohort 1A, one female was found dead without any relevant clinical signs.

Mainly males but also females showed slight but statistically significant reductions in body weight, body weight gain and food consumption at the highest dose tested in most parts of the F0 and F1 generations. Observed reduction on body weights and body weight gain were considered most probably related to lower food intake by the animals of the highest-dose group (Tables 12-17).

Table 12: Body weight (in grams) and food consumption data for F0 male animals from the EOGRTS (Anonymous, 2016)

		0 mg/kg bw/d	80 mg/kg bw/d	250 mg/kg bw/d	800 mg/kg bw/d
F0 - Mean body weight (pre-mating)	Day 0	373.39	375.26	375.03	372.82
	Day 7	395.18	395.31	396.19	387.03
	Day 14	411.74	410.14	411.04	400.13
F0 - Mean body weight (post-mating)	Day 1	421.00	422.18	419.55	408.49
	Day 8	434.28	433.64	432.21	418.76
	Day 15	447.00	444.54	445.54	429.20
	Day 22	456.72	454.91	454.68	**438.04* (-4.09%)**
	Day 29	468.62	467.00	467.48	**446.48* (-4.72%)**
	Day 36	474.73	474.28	473.79	**452.48* (-4.68%)**

	Day 43	478.10	479.40	478.62	**456.38* (-4.54%)**
F0 - Mean body weight gain (pre-mating)	D 0-7	21.79	20.05	21.16	**14.20** (-5.34%)**
	D 7-14	16.56	14.83	14.85	13.10
	D 0-14	38.35	34.88	36.01	**27.31** (-2.88%)**
F0 - Mean body weight gain (post-mating)	D 1-8	13.28	11.46	12.67	10.27
	D 8-15	12.72	10.90	13.33	10.44
	D 15-22	9.73	10.38	9.13	8.84
	D 22-29	11.89	12.09	12.80	**8.90* (-2.51%)**
	D 29-36	6.11	7.28	6.31	6.00
	D 36-43	3.37	2.79	4.83	5.60
	D1-43	57.10	55.66	59.07	50.13
F0 - Mean food consumption (pre-mating)	D 0-7	22.80	22.79	22.47	**20.70** (-9.21%)**
	D 7-14	21.89	21.52	21.87	**20.65** (-5.66%)**
F0 - Mean food consumption (post-mating)	D 1-8	21.58	20.52	21.85	19.27
	D 8-15	21.20	21.16	21.82	20.85
	D 15-22	21.17	20.90	21.31	20.24
	D 22-29	21.24	21.60	21.77	20.38
	D 29-36	20.40	20.44	20.84	19.95
	D 36-43	19.84	19.87	20.36	19.79
	D 43-48	20.58	20.69	21.06	19.97

*: $p < 0.05$; **: $p < 0.01$

Table 13: Body weight (in grams) and food consumption data for F0 female animals from the EOGRTS (Anonymous, 2016)

		0 mg/kg bw/d	80 mg/kg bw/d	250 mg/kg bw/d	800 mg/kg bw/d
F0 - Mean body weight (pre-mating)	Day 0	202.15	203.12	203.17	200.68
	Day 7	212.21	212.74	215.61	207.79
	Day 14	221.59	221.57	223.73	215.68
F0 - Mean body weight (gestation)	Day 0	221.77	221.40	226.92	217.93
	Day 7	245.29	242.09	244.40	**232.01** (-5.41%)**
	Day 14	269.41	264.92	267.66	**253.02** (-6.08%)**
	Day 21	343.50	333.98	343.46	**318.02** (-7.42%)**
F0 - Mean body weight (lactation)	Day 0	254.72	253.34	255.94	244.10
	Day 4	268.53	264.55	269.93	**256.25* (-4.57%)**
	Day 7	273.00	270.73	280.20	264.62
	Day 14	288.49	280.38	292.36	279.04
	Day 21	284.23	278.57	282.26	**269.57** (-5.16%)**
F0 - Mean body weight gain (pre-mating)	D 0-7	10.06	9.62	12.44	7.11
	D 7-14	9.38	8.83	8.12	7.89
	D 0-14	19.44	18.45	20.56	**15.01* (-22.79%)**
F0 - Mean body weight gain (gestation)	D 0-7	23.53	20.69	**17.48** (-25.71%)**	**14.08** (-40.16%)**
	D 7-14	24.12	22.83	23.26	21.02
	D 14-21	74.09	69.06	75.80	**64.99* (-12.28%)**
	D 0-21	121.74	112.58	116.54	**100.09** (-17.78%)**
F0 - Mean body weight gain (lactation)	D 0-4	13.80	11.20	13.99	12.15
	D 4-7	4.47	6.18	**10.28* (+129.98%)**	8.37
	D 7-14	15.49	9.65	12.16	14.42
	D 14-21	-4.26	-1.82	-10.10	-9.47
	D 0-21	29.51	25.22	26.33	25.47
F0 - Mean food consumption (pre-mating)	D 0-7	15.52	15.21	15.08	**13.00** (-16.24%)**
	D 7-14	14.76	14.66	14.62	**13.94* (-5.55%)**
F0 - Mean food consumption (gestation)	D 0-7	18.00	**16.76* (-6.89%)**	**16.84* (-6.44%)**	**15.07** (-16.28%)**
	D 7-14	18.98	18.47	18.15	**17.24** (-9.17%)**
	D 14-21	20.47	20.94	20.50	**19.03* (-7.03%)**
F0 - Mean food consumption (lactation)	D 0-4	30.39	28.45	32.01	30.13
	D 4-7	44.04	**39.29* (-10.78%)**	45.60	**38.63* (-12.28%)**
	D 7-14	51.70	48.00	54.11	49.18

	D14-21	69.28	64.17	69.40	**60.94** (-12.04%)**

*: p < 0.05 **: p < 0.01

Table 14: Body weight (in grams) and food consumption data for cohort 1A male animals from the EOGRTS (Anonymous, 2016)

		0 mg/kg bw/d	80 mg/kg bw/d	250 mg/kg bw/d	800 mg/kg bw/d
Cohort 1A - Mean body weight	Day 0	70.32	69.85	65.93	**61.30** (-12.83%)**
	Day 7	114.68	117.30	110.13	**102.57** (-10.56%)**
	Day 14	162.06	164.75	154.76	**145.66** (-10.12%)**
	Day 21	206.34	212.56	201.89	**187.40** (-9.18%)**
	Day 28	242.27	252.53	244.93	**226.50* (-6.51%)**
	Day 35	285.48	300.35	284.49	**264.26** (-7.43%)**
	Day 42	311.03	**330.91* (+6.39%)**	311.38	**290.30* (-6.66%)**
	Day 49	330.50	353.19	330.80	**308.18* (-6.75%)**
	Day 56	347.58	371.96	348.09	326.39
Cohort 1A - Mean body weight gain	D 0-7	44.36	**47.45* (+6.96%)**	44.20	**41.27* (-6.96%)**
	D 7-14	47.38	47.46	44.63	**43.10** (-9.03%)**
	D 14-21	44.28	47.81	47.14	41.74
	D 21-28	35.94	**39.97* (+11.21%)**	**43.04** (+19.75%)**	39.10
	D 28-35	43.21	47.83	39.57	37.77
	D 35-42	25.55	**30.56** (+19.61%)**	26.89	26.04
	D 42-49	19.47	22.29	19.42	17.88
	D 49-56	17.08	18.77	17.29	18.21
Cohort 1A - Mean food consumption	D 0-7	13.68	13.68	12.76	**11.49* (-16.01%)**
	D 7-14	18.55	19.45	18.21	**16.63* (-10.35%)**
	D 14-21	18.87	**20.48** (+8.53%)**	19.67	**17.60* (-6.73%)**
	D 21-28	20.97	21.62	21.65	19.76
	D 28-35	22.54	24.27	22.53	**20.18* (-10.47%)**
	D 35-42	21.65	23.22	21.83	19.89
	D 42-49	20.86	22.71	20.98	19.25
	D 49-56	20.62	22.23	20.88	19.23

*: p < 0.05 **: p < 0.01

Table 15: Body weight (in grams) and food consumption data for cohort 1A female animals from theEOGRTS (Anonymous, 2016)

		0 mg/kg bw/d	80 mg/kg bw/d	250 mg/kg bw/d	800 mg/kg bw/d
Cohort 1A - Mean body weight	Day 0	62.71	65.13	64.57	61.25
	Day 7	98.50	102.88	102.84	97.36
	Day 14	131.89	133.95	133.03	128.00
	Day 21	152.00	155.14	153.61	148.91
	Day 28	172.06	173.27	170.88	167.02
	Day 35	186.36	188.46	184.77	181.70
	Day 42	198.87	197.29	195.61	190.13

	Day 49	205.61	203.28	204.35	195.87
	Day 56	214.41	213.39	212.77	204.36
Cohort 1A - Mean body weight gain	D 0-7	35.79	37.75	**38.27* (+6.93%)**	36.11
	D 7-14	33.39	31.08	30.20	30.64
	D 14-21	20.11	21.18	20.58	20.92
	D 21-28	20.06	18.13	17.28	18.11
	D 28-35	14.30	15.19	13.89	14.69
	D 35-42	12.52	**8.83* (-29.47%)**	10.85	**8.43** (-32.67%)**
	D 42-49	6.74	5.98	8.74	5.75
	D 49-56	8.80	10.12	8.42	8.49
Cohort 1A - Mean food consumption	D 0-7	11.25	11.63	11.46	10.66
	D 7-14	14.57	15.16	14.79	14.14
	D 14-21	14.19	15.15	14.02	13.80
	D 21-28	14.81	15.13	14.37	14.19
	D 28-35	14.57	15.34	15.04	14.15
	D 35-42	15.35	14.99	14.91	**13.82* (-9.97%)**
	D 42-49	14.70	15.11	14.93	13.68
	D49-56	14.54	14.77	14.39	13.46

*: $p < 0.05$ **: $p < 0.01$

Table 16: Body weight (in grams) and food consumption data for cohort 1B male animals from the EOGRTS (Anonymous, 2016)

		0 mg/kg bw/d	80 mg/kg bw/d	250 mg/kg bw/d	800 mg/kg bw/d
Cohort 1B - Mean body weight (pre-mating)	Day 0	76.50	81.75	78.33	71.21
	Day 7	123.26	129.32	125.76	115.62
	Day 14	172.91	174.72	170.82	**159.00** (-8.04%)**
	Day 21	217.08	218.54	215.40	**198.54** (-8.54%)**
	Day 28	262.43	264.45	262.05	**239.29** (-8.82%)**
	Day 35	296.21	297.66	297.28	**271.78** (-8.25%)**
	Day 42	324.40	327.14	324.87	**295.13** (-9.02%)**
	Day 49	345.28	348.02	348.02	**315.46** (-8.63%)**
	Day 56	362.78	366.82	366.52	**331.70** (-8.57%)**
	Day 63	380.04	383.67	382.51	**348.76** (-8.23%)**
	Day 70	390.62	394.58	396.53	**360.45** (-7.72%)**
Cohort 1B - Mean body weight (post-mating)	Day 82	407.15	410.82	411.74	**375.78** (-7.70%)**
	Day 89	417.08	421.25	423.82	**387.67* (-7.05%)**
	Day 96	431.24	433.47	436.96	**397.98** (-7.71%)**
	Day 103	441.93	440.19	444.98	**401.54** (-9.14%)**
Cohort 1B - Mean body weight gain (pre-mating)	D 0-7	46.76	47.57	47.43	44.40
	D 7-14	49.64	**45.40** (-8.54%)**	**45.06** (-9.22%)**	**43.39** (-12.59%)**
	D 14-21	44.17	43.82	44.58	**39.54** (-10.48%)**
	D 21-28	45.35	45.91	46.64	**40.75** (-10.14%)**
	D 28-35	33.78	33.21	35.23	32.48
	D 35-42	28.19	29.47	27.59	**23.36** (-17.13%)**
	D 42-49	20.88	20.89	23.14	20.32
	D 49-56	17.50	18.80	18.50	16.24
	D 56-63	17.26	16.84	15.99	17.06
	D 63-70	10.58	10.91	**14.02** (-32.51%)**	11.69
Cohort 1B - Mean body weight gain (post-mating)	D 82-89	9.93	10.43	12.08	11.89
	D 89-96	14.17	12.22	13.14	**10.31** (-27.24%)**
	D 96-103	8.87	9.43	10.65	8.76
Cohort 1B - Mean food consumption (pre-mating)	D 0-7	14.59	14.98	14.56	13.09
	D 7-14	18.30	19.24	18.64	**17.05* (-6.83%)**
	D 14-21	19.25	19.81	19.54	18.18
	D 21-28	21.83	22.23	22.54	**19.88** (-8.93%)**
	D 28-35	23.07	23.15	23.05	**20.68** (-10.36%)**
	D 35-42	22.73	22.40	21.95	**19.64** (-13.59%)**
	D 42-49	21.68	21.44	21.15	**19.03** (-12.22%)**
	D 49-56	21.55	21.48	21.37	**19.27** (-10.58%)**
	D 56-63	21.17	20.90	20.77	**19.08** (-9.87%)**
	D 63-70	20.77	20.78	20.75	**19.11* (-7.99%)**

Cohort 1B - Mean food consumption (post-mating)	D 82-89	18.59	18.35	18.50	17.68
	D 89-96	20.71	20.47	20.37	**18.77** (-9.37%)**
	D 96-103	20.36	20.39	20.70	**18.64* (-8.45%)**

*: $p < 0.05$ **: $p < 0.01$

Table 17: Body weight (in grams) and food consumption data for cohort 1B female animals from the EOGRTS (Anonymous, 2016)

		0 mg/kg bw/d	80 mg/kg bw/d	250 mg/kg bw/d	800 mg/kg bw/d
Cohort 1B - Mean body weight (pre-mating)	Day 0	73.68	74.36	72.30	68.80
	Day 7	111.05	111.29	109.88	105.42
	Day 14	138.86	139.49	137.06	133.83
	Day 21	156.68	158.11	156.16	154.10
	Day 28	176.71	177.66	178.63	171.92
	Day 35	189.28	191.01	191.25	185.52
	Day 42	199.15	200.55	197.61	194.10
	Day 49	205.69	206.60	208.76	202.38
	Day 56	211.47	213.78	214.55	208.62
	Day 63	221.09	221.84	222.61	215.94
	Day 70	223.59	225.03	227.42	220.98
Cohort 1B - Mean body weight (gestation)	Day 0	228.63	229.98	230.58	223.23
	Day 7	244.30	246.60	245.52	237.58
	Day 14	268.10	270.29	270.34	258.27
	Day 21	340.40	342.26	339.76	327.48
Cohort 1B - Mean body weight (lactation)	Day 0	254.39	257.87	258.20	249.69
	Day 4	267.41	272.82	272.99	264.59
	Day 7	278.77	279.38	280.82	272.47
	Day 14	290.43	288.92	291.15	285.02
	Day 21	288.21	283.81	286.18	279.63
Cohort 1B - Mean body weight gain (pre-mating)	D 0-7	37.36	36.94	37.58	36.62
	D 7-14	27.81	28.20	27.19	28.41
	D 14-21	17.82	18.62	19.10	20.27
	D 21-28	20.03	19.56	22.47	17.82
	D 28-35	12.56	13.35	12.62	13.60
	D 35-42	9.87	9.54	**6.36* (-35.56%)**	8.58
	D 42-49	6.54	6.06	**11.16 (+70.64%)**	8.28
	D 49-56	5.78	7.18	5.79	6.23
	D 56-63	9.62	8.06	8.06	7.33
	D 63-70	2.50	3.18	4.82	5.04
Cohort 1B - Mean body weight gain (gestation)	D 0-7	15.67	16.62	14.94	14.35
	D 7-14	23.80	23.69	24.83	**20.68* (-13.11%)**
	D 14-21	72.31	71.97	69.42	69.21
	D 0-21	111.77	112.28	109.18	104.24
Cohort 1B - Mean body weight gain (lactation)	D 0-4	13.02	14.94	14.15	14.42
	D 4-7	11.35	6.57	7.55	7.95
	D 7-14	11.67	9.53	10.33	12.55
	D 14-21	-2.22	-5.10	-4.97	-5.39
	D 0-21	33.82	25.94	27.99	29.94
Cohort 1B - Mean food consumption (pre-mating)	D 0-7	12.77	12.58	12.49	11.66
	D 7-14	14.13	14.78	14.40	13.99
	D 14-21	13.72	14.11	14.00	13.97
	D 21-28	14.93	14.87	15.29	14.34
	D 28-35	15.12	15.24	15.27	14.70
	D 35-42	14.90	14.54	14.62	**13.88* (-6.84%)**
	D 42-49	14.06	13.81	14.04	13.42
	D 49-56	14.27	15.60	14.10	13.60
	D 56-63	14.38	13.97	14.10	13.67
	D 63-70	14.28	14.03	14.42	13.60
Cohort 1B - Mean food consumption	D 0-7	15.28	15.01	**13.78* (-9.82%)**	**13.65* (-10.67%)**
	D 7-14	18.20	16.72	16.64	**14.98** (-17.69%)**

(gestation)	D 14-21	19.01	18.36	18.19	17.54
Cohort 1B - Mean food consumption (lactation)	D 0-4	28.71	30.44	28.99	29.69
	D 4-7	43.60	42.87	41.46	40.59
	D 7-14	53.29	50.25	51.38	49.30
	D 14-21	64.72	63.67	61.28	65.07

*: $p < 0.05$ **: $p < 0.01$

Males and females of the F0 parental generation showed statistically significant increases in the absolute and relative weights of the liver at the highest dose. Additionally, statistically significant decreases of terminal body weights and increases of the relative weights of kidneys and thyroid were observed in males of this dose group (Table 18). In cohort 1A, statistically significant increases in the relative weights of heart, kidneys, liver and testes were observed in male animals at the highest dose. Females of this dose group showed statistically significant increases in the absolute and relative weight of the liver and in the relative weight of the kidneys (Table 19). In cohort 1B, significant increases in the absolute and relative weights of the testes in the mid-dose group, in the absolute weight of the kidneys, and in the relative weights of kidneys, liver, testes and cauda epididymis in the high-dose group were observed in male rats. At this dose, male animals showed significant decreases in terminal body weights. Changes in females were related to statistically significant increases in the relative weights of liver and kidneys at the highest dose tested (Table 20).

The statistically significant slight increases in the weight of the kidneys observed in both generations were considered to be treatment-related in males as they were accompanied by microscopic observations. These microscopic examinations showed minimal to moderate accumulation of proteinaceous droplets in the tubuli of the male animals at the highest dose in the F0 generation (Table 21). In the mid and high-dose groups in cohort 1A, increase in the incidence and severity of proteinaceous accumulation in the kidneys of the male animals were observed. In addition, minimal to mild basophilic tubuli formation was also observed in high-dose male animals in cohort 1A (Table 22). No microscopic effects were observed in other tissues and organs. Results of this histopathological examination in animals of cohort 1A did not indicate a need for additional histopathological examination of the tissues and organs of the animals of cohort 1B.

No changes in TSH and T4 levels were reported for F0 and F1 (cohort 1A) generations.

Table 18: Absolute and relative organ weights (in grams) for F0 parental animals from the EOGRTS (Anonymous, 2016)

			0 mg/kg bw/d	80 mg/kg bw/d	250 mg/kg bw/d	800 mg/kg bw/d
♂	Terminal body weight		462.85	461.69	462.46	**442.13* (-4.47%)**
	Liver	Absolute weight	10.3383	10.1550	10.6792	**11.3121** (+9.42%)**
		Relative weight	2.2351	2.2003	2.3085	**2.5556** (+14.34%)**
	Kidneys	Absolute weight	2.3781	2.3437	2.4356	2.4974
		Relative weight	0.5150	0.5076	0.5272	**0.5653** (+9.77%)**
	Thyroid	Absolute weight	0.0175	0.0174	0.0190	0.0196
		Relative weight	0.0038	0.0038	0.0041	**0.0044* (+15.79%)**
♀	Terminal body weight		233.47	232.90	237.74	226.20
	Liver	Asolute weight	7.8792	7.9530	8.6417	**9.7769** (+24.08%)**
		Relative weight	3.3770	3.4108	3.6319	**4.3159** (+27.80%)**
	Kidneys	Asolute weight	1.6963	1.6629	1.7493	1.6798
		Relative weight	0.7270	0.7148	0.7365	0.7434

	Thyroid	Asolute weight	0.0150	0.0154	0.0170	0.0150
		Relative weight	0.0064	0.0066	0.0072	0.0067

*: p < 0.05; **: p< 0.01

Table 19: Absolute and relative organ weights (in grams) for cohort 1A animals from the EOGRTS (Anonymous, 2016)

			0 mg/kg bw/d	80 mg/kg bw/d	250 mg/kg bw/d	800 mg/kg bw/d
♂	Terminal body weight		338.53	363.13	339.75	318.19
	Liver	Absolute weight	8.949	9.976	9.103	9.667
		Relative weight	26.39	27.37	26.79	**30.31**(+14.85%)**
	Heart	Absolute weight	0.913	**0.978*(+7.12%)**	0.911	0.905
		Relative weight	2.700	2.696	2.683	**2.851**(+5.59%)**
	Kidneys	Absolute weight	2.059	2.177	2.091	2.176
		Relative weight	6.083	6.003	6.157	**6.842**(+12.48%)**
	Testes	Absolute weight	3.652	3.693	3.763	3.760
		Relative weight	10.804	10.225	11.126	**11.921** (+10.34%)**
	Cauda epididymis	Absolute weight	0.446	0.441	0.432	0.442
		Relative weight	1.320	**1.219* (-7.65%)**	1.278	1.402
♀	Terminal body weight		209.05	209.08	208.40	200.13
	Liver	Asolute weight	5.759	5.818	5.734	**6.318* (+9.71%)**
		Relative weight	27.55	27.81	27.54	**31.57** (+14.59%)**
	Heart	Asolute weight	0.636	0.642	0.637	0.625
		Relative weight	3.048	3.072	3.057	3.124
	Kidneys	Absolute weight	1.343	1.380	1.359	1.376
		Relative weight	6.428	6.603	6.530	**6.873* (+6.92%)**

*: p < 0.05; **: p< 0.01

Table 20: Absolute and relative organ weights (in grams) for cohort 1B animals from the EOGRTS (Anonymous, 2016)

			0 mg/kg bw/d	80 mg/kg bw/d	250 mg/kg bw/d	800 mg/kg bw/d
♂	Terminal body weight		441.59	445.27	450.72	**409.51* (-7.26%)**
	Liver	Absolute weight	13.534	14.123	14.154	14.386
		Relative weight	30.62	31.67	31.42	**35.08** (+14.56%)**
	Kidneys	Absolute weight	2.146	2.183	2.288	**2.345* (+9.27%)**
		Relative weight	4.864	4.914	5.080	**5.727** (+17.74%)**
	Testes	Absolute weight	3.805	3.899	**4.146** (+8.96%)**	3.970
		Relative weight	8.653	8.860	**9.231* (+6.68%)**	**9.717** (+12.29%)**
	Cauda epididymis	Absolute weight	0.514	0.522	0.548	0.525
		Relative weight	1.170	1.185	1.219	**1.286* (+9.91%)**
♀	Terminal body weight		288.00	285.49	283.85	280.75
	Liver	Asolute weight	13.9934	14.4514	14.4536	15.0041
		Relative weight	4.8501	5.0624	5.0609	**5.3429** (+10.16%)**
	Kidneys	Absolute weight	1.7685	1.8166	1.7862	1.8506
		Relative weight	0.6140	0.6357	0.6299	**0.6602** (+7.52%)**

*: p < 0.05; **: p< 0.01

Table 21: Microscopic observations for F0 parental animals from the EOGRTS (Anonymous, 2016)

Removal Reason(s): ALL	Male				Female			
	0 mg/kg	80 mg/kg	250 mg/kg	800 mg/kg	0 mg/kg	80 mg/kg	250 mg/kg	800 mg/kg
Number of Animals:	28	28	28	26	28	28	28	28
kidneys (Continued...)								
transitional epithelium; hyperplasia; focal	1	0	0	0	1	-	0	1
.... mild	1	0	0	0	0	-	0	1
.... moderate	0	0	0	0	1	-	0	0
inflammation; mononuclear, focal	0	0	2	1	1	-	0	0
.... minimal	0	0	1	0	1	-	0	0
.... mild	0	0	1	1	0	-	0	0
pelvic; inflammation; epithelial, focal	0	0	0	1	0	-	0	0
.... mild	0	0	0	1	0	-	0	0
mineralization; cortical, focal	1	0	0	0	1	-	0	0
.... minimal	1	0	0	0	1	-	0	0
mineralization; corticomedullary	0	0	0	0	6	-	0	7
.... minimal	0	0	0	0	2	-	0	6
.... mild	0	0	0	0	4	-	0	1
basophilic tubules	3	5	4	4	1	-	0	5
.... minimal	0	4	4	1	0	-	0	3
.... mild	3	1	0	3	1	-	0	2
proteinaceous droplets; tubular	4	3	3	19***	0	-	0	0
.... minimal	3	2	2	2	0	-	0	0
.... mild	1	1	1	14	0	-	0	0
.... moderate	0	0	0	3	0	-	0	0
liver								
Examined	28	0	0	26	28	1	0	28
No Visible Lesions	15	-	-	14	23	0	-	26
degeneration; focal	0	-	-	1	0	0	-	0
.... minimal	0	-	-	1	0	0	-	0
haematopoiesis; extramedullary	0	-	-	0	0	0	-	1
.... minimal	0	-	-	0	0	0	-	1
inflammation; mononuclear	12	-	-	11	5	0	-	1
.... minimal	1	-	-	5	3	0	-	0
.... mild	11	-	-	6	2	0	-	1
gross finding not confirmed	0	-	-	0	0	1	-	1
accumulation, pigment, brown; focal	0	-	-	0	0	1	-	0
.... mild	0	-	-	0	0	1	-	0
periportal; accumulation, pigment, brown	0	-	-	1	0	0	-	0
.... mild	0	-	-	1	0	0	-	0

Fisher's Exact: * = p < 0.05; *** = p < 0.001

Table 22: Microscopic observations for cohort 1A animals from the EOGRTS (Anonymous, 2016)

Removal Reason(s): ALL	Male				Female			
	0 mg/kg	80 mg/kg	250 mg/kg	800 mg/kg	0 mg/kg	80 mg/kg	250 mg/kg	800 mg/kg
Number of Animals:	20	20	20	20	20	19	20	20
intestine, duodenum								
Examined	20	0	0	20	20	0	0	20
No Visible Lesions	20	-	-	20	20	-	-	20
intestine, ileum								
Examined	20	0	0	20	20	0	0	20
No Visible Lesions	20	-	-	20	20	-	-	20
intestine, jejunum								
Examined	20	0	0	20	20	0	0	20
No Visible Lesions	20	-	-	20	20	-	-	20
intestine, rectum								
Examined	20	0	0	20	20	0	0	20
No Visible Lesions	20	-	-	20	20	-	-	20
kidneys								
Examined	20	20	20	20	20	1	0	20
No Visible Lesions	15	14	4	2	19	0	-	17
basophilic tubules	2	0	6	9[#]	0	0	-	1
.... minimal	2	0	6	4	0	0	-	1
.... mild	0	0	0	5	0	0	-	0
hyperplasia; transitional epithelium, focal	1	0	0	0	0	0	-	0
.... mild	1	0	0	0	0	0	-	0
pelvic; dilatation	0	1	0	0	1	1	-	1
.... mild	0	1	0	0	0	1	-	1
.... moderate	0	0	0	0	1	0	-	0
pyelonephritis	0	0	0	0	0	0	-	1
.... mild	0	0	0	0	0	0	-	1
proteinaceous droplets; tubular	3	6	14[##]	15[###]	0	0	-	0
.... minimal	2	5	7	3	0	0	-	0
.... mild	1	1	7	10	0	0	-	0
.... moderate	0	0	0	2	0	0	-	0
proteinaceous droplets	0	0	0	2	0	0	-	0
.... mild	0	0	0	2	0	0	-	0
cyst(s)	0	1	0	0	0	0	-	0
inflammation; mononuclear, focal	0	0	1	0	0	0	-	0
.... minimal	0	0	1	0	0	0	-	0
liver								
Examined	20	0	0	20	20	0	0	20
No Visible Lesions	5	-	-	12	10	-	-	11

Fisher's Exact: * = p < 0.05; ** = p < 0.01; *** = p < 0.001

Regarding fertility and sexual function parameters, the mean length of the longest oestrus cycles in the high-dose group in the F0 generation was statistically higher as compared to the control group (Table 23). Nevertheless, this was considered a fortuitous finding, due to a low value in the control group that was out of the historical control data. On the other hand, high-dosed females of the F1 generation (cohort 1A) showed a significantly higher mean cycle length and 4 animals showed a longer oestrus period (Table 24). These findings were not considered as adverse effects as they were within historical control ranges (Appendix 1). No treatment-related effects on epididymal and testicular sperm parameters were observed.

Table 23: Oestrus cycle evaluation of F0 parental females from the EOGRTS (Anonymous, 2016)

Table F0 - 7: Estrus cycle evaluation

			Control		Low dose 80 mg/kg	Mid dose 250 mg/kg	High dose 800 mg/kg	
Number of females evaluated		n	28		28	28	28	
Number of acyclic females		n	0	f	0	0	1	
		%	0		0	0	3.6	
Length of the longest cycle								
	4	n	28		28	28	19	
	5	n	0		0	0	8	
	>5	n	0		0	0	0	
Mean length of the longest cycle (days)		mean	4	kw	4	4	4.3	***
		sd	0		0	0	0.47	
		n	28		28	28	27	
Number of animals with prolonged estrus period		n	0	f	0	0	0	
		%	0		0	0	0	
Number of complete cycles per animal in 15 days		mean	2.6	kw	2.5	2.8	2.6	
		sd	0.50		0.51	0.42	0.50	
		n	28		28	28	27	

Statistics:
f = Fisher's exact test
k/w = Kruskall-Wallis/Mann Whitney U test

*** = P < 0.001

Table 24: Oestrus cycle evaluation of cohort 1A females from the EOGRTS (Anonymous, 2016)

Table 1A - 8: Estrus cycle evaluation

			Control 0 mg/kg		Low dose 80 mg/kg	Mid dose 250 mg/kg	High dose 800 mg/kg	
Number of females evaluated		n	19		19	20	20	
Number of acyclic females		n	0	f	0	0	0	
		%	0		0	0	0	
Length of the longest cycle								
	4	n	16		17	14	10	
	5	n	3		1	5	6	
	6	n	0		1	1	4	
Mean length of the longest cycle (days)		mean	4.16	kw	4.16	4.35	4.70	*
		sd	0.37		0.5	0.59	0.82	
		n	19		19	20	19	
Number of animals with prolonged estrus period		n	0	f	0	0	4	
		%	0		0	0	20	
Number of complete cycles per animal in 15 days		mean	2.89	kw	2.89	2.79	2.55	*
		sd	0.32		0.32	0.42	0.61	
		n	19		19	20	20	

Statistics:
f = Fisher's exact test
k/w = Kruskall-Wallis/Mann Whitney U test

* = P < 0.05

In addition, neither biologically relevant treatment-related effects were observed on fertility and reproductive performance of animals of the F0 generation and of cohort 1B of the F1 generation.

In the F0 generation, 28 females were placed in each group with 28 males for mating. Within 2 weeks, 28, 27, 28 and 27 females of the control, low-, mid- and high-dose groups, respectively, were mated. In the control and low-dose groups, 2 females were not pregnant and in the mid- and high-dose groups 1 female (per group) was not pregnant. This resulted in 26, 25, 27 and 26 pregnant females (in the control, low-, mid- and high-dose groups, respectively). There were no differences in pre-coital time, male and female mating indices and male and female fertility indices (Table 25).

Duration of gestation was slightly, but statistically significant longer in the high-dose group of the F0 generation, compared to the control group (mean length of the gestation period in the control group was 22.5 days versus 22.9 days in the high-dose group). However it was not considered biologically relevant since it is in the range of historical control data (Appendix). All pregnant females gave birth to a litter and all pups were born alive, consequently, the gestation index was 100% (Table 26). The mean number of implantations sites was slightly, not statistically significant lower in the low and high-dose groups as compared to the control group. In addition, also the number of lost implantations and the mean number of post-implantation losses were higher but not statistically significant in the low- and high-dose groups than in the control group. These findings were not considered as adverse effects of treatment since no dose-relationship was observed (effect on low-dose group was more pronounced than in high-dose group and no effects in mid-dose group were observed) and the values in the high-dose group were within the range of historical control data. Consequently, the mean number of pups per litter was lower in the low- and high-dose groups, being statistically significant only in the high-dose group (mean number of pups delivered in the control- and high-dose groups was 12 and 10, respectively). Since no dose-relationship was observed and since the lower number of pups observed was well within the range of historical control data (Appendix) , this finding was considered as fortuitous and not related to treatment. Additionally, a non-statistically significant increase in the mean number of prenatal loss was also observed in the low- and high-dose groups, compared to controls. Perinatal loss was 0% for all groups (Table 27).

Table 25: Mating and pregnancy performance F0 parental generation: Mating, from the EOGRTS (Anonymous, 2016)

		Control F 0 mg/kg BW		Low-dose F 80 mg/kg BW	Mid-dose F 250 mg/kg BW	High-dose F 800 mg/kg BW
Mating						
No. of females placed with males	N	28		28	28	28
- Inseminated	N	28		27	28	27
- Non mated females	N	0		1	0	1
Female mating index	%	100.0		96.4	100.0	96.4
- Pregnant	N	26	cx	25	27	26
- Not Pregnant	N	2		2	1	1
Female fertility index	%	92.9		92.6	96.4	96.3
No. of males placed with females	N	28		28	28	28
- With inseminated females	N	28	cx	27	28	27
Male mating index	%	100.0		96.4	100.0	96.4
- with pregnant females	N	26	cx	25	27	26
Male fertility index	%	92.9		89.3	96.4	92.9
Females with defined day 0 pc	N	28		27	28	27
Pre-coïtal time	Mean	2.1	k	2.2	2.5	2.5
	S.d.	1.2		1.5	1.1	1.2

Statistic Profile = DecisionTree, * = p < 0.05, ** = p < 0.01, X = Group excluded from statistics k=KRUSKAL-WALLIS; a=ANOVA cx=CHI-SQUARE-EXACT

Female mating index : number of females mated * 100 / number of females placed with males
Female fertility index : number of females pregnant * 100 / number of females placed with males
Male mating index : number of males mated * 100 / number of males placed with females
Male fertility index : number of males that became sire * 100 / number of males placed with females

Table 26: Mating and pregnancy performance F0 parental generation: Delivery, from the EOGRTS (Anonymous, 2016)

		Control F 0 mg/kg BW		Low-dose F 80 mg/kg BW	Mid-dose F 250 mg/kg BW	High-dose F 800 mg/kg BW	
Delivery							
Females delivering	N	26	cx	25	27	26	
- With liveborn pups	N	26	cx	25	27	26	
	%	100		100	100	100	
- With stillborn pups	N	0	cx	0	0	0	
	%	0		0	0	0	
- With all pups stillborn	N	0	cx	0	0	0	
	%	0		0	0	0	
Gestation index	%	100		100	100	100	
Gestation days	Mean	22.5	u	22.7	22.5	22.9	**
	s.d.	0.5		0.7	0.5	0.3	
	N	26		25	27	26	

Statistic Profile = DecisionTree, * = p < 0.05, ** = p < 0.01, X = Group excluded from statistics k=KRUSKAL-WALLIS; a=ANOVA cx=CHI-SQUARE-EXACT; u=KRUKSAL-WALLIS-DUNN

Gestation index : number of females with live pups * 100 / number of pregnant females

Table 27: Mating and pregnancy performance F0 parental generation: Fertility, from the EOGRTS (Anonymous, 2016)

		Control F 0 mg/kg BW		Low-dose F 80 mg/kg BW	Mid-dose F 250 mg/kg BW	High-dose F 800 mg/kg BW	
Fertility							
Implantation sites	Total	331		297	339	292	
	Mean	12.7	k	11.9	12.6	11.2	
	s.d.	1.3		2.4	2.1	2.6	
	N	26		25	27	26	
Number of lost implantations	N	18		41	16	31	
Post-implantation lost	Mean%	6.6	g	15.1	5.2	11.3	
	s.d.	8.4		22.3	7.6	11.5	
Pups delivered	Total	313		256	323	261	
	Mean	12.0	u	10.2	12.0	10.0	*
	s.d.	2.0		3.4	2.0	2.7	
Prenatal loss	Mean %	6.59		15.06	5.22	11.35	
- Live born	Total	313		256	323	261	
	%	100		100	100	100	
- Stillborn	Total	0		0	0	0	
	%	0.0		0.0	0.0	0.0	
Perinatal loss	Mean %	0.00		0.00	0.00	0.00	

Statistic Profile = DecisionTree, * = p < 0.05; ** = p < 0.01; X = Group excluded from statistics; k=KRUSKAL-WALLIS; a=ANOVA; cx=CHI-SQUARE-EXACT; u=KRUKSAL-WALLIS-DUNN g=Generalised linear model using the binomial distribution

Prenatal loss : mean of number of implantation sites per litter – total number of pups delivered per litter * 100 / number of implantation sites per litter
Perinatal loss: mean of number of pups delivered per litter - number of live pups per litter at day 0 * 100 / number of pups delivered per litter
Post-implantation loss: mean of number of implantation sites per litter – number of live pups per litter *100 / number of implantation sites per litter

In the F1 generation (cohort 1 B) 25 females were placed with 25 males for mating. Within 2 weeks, 25, 25, 24 and 25 females of the control, low-, mid- and high-dose groups, respectively, were mated. In the control and high-dose group, one female (per group) was not pregnant and in the mid-dose group 2 females were not pregnant. All females were pregnant in the low-dose group. This resulted in 24, 25, 23 and 24 pregnant females (in the control, low-, mid- and high-dose groups, respectively). There were no differences in pre-coital time, male and female mating indices, male and female fertility indices and duration of gestation (Table 28).

All pregnant females gave birth to a litter. In the control, low-, mid- and high-dose groups, 0, 2, 1 and 2 females delivered stillborn pups but no female delivered only stillborn pups. Consequently, the gestation index was 100% for all groups and the mean perinatal loss did not suffer statistically significant changes (Tables 29 and 30).

Table 28: Mating and pregnancy performance cohort 1B: Mating, from the EOGRTS (Anonymous, 2016)

Mating		Control F 0 mg/kg BW		Low-dose F 80 mg/kg BW	Mid-dose F 250 mg/kg BW	High-dose F 800 mg/kg BW
No. of females placed with males	N	25		25	25	25
- Inseminated	N	25		25[1]	24	25
- Non mated females	N	0		0	1	0
Female mating index	%	100.0		100.0	96.0	100
- Pregnant	N	24	cx	25	23	24
- Not Pregnant	N	1		0	2	1
Female fertility index	%	96.0		100.0	95.8	96.0
No. of males placed with females	N	25		25	25	25
- With inseminated females	N	25	cx	25	24	25
Male mating index	%	100.0		100.0	96.0	100.0
- with pregnant females	N	24	cx	25	23	24
Male fertility index	%	96.0		100.0	92.0	96.0
Females with defined day 0 pc	N	25		24	24	25
Pre-coïtal time	Mean	3.4	k	3.1	3.2	2.6
	S.d.	2.7		2.3	2.4	2.4

Statistic Profile = DecisionTree, * = p < 0.05, ** = p < 0.01, X = Group excluded from statistics k=KRUSKAL-WALLIS; a=ANOVA cx=CHI-SQUARE-EXACT

[1] Female 107-06 was misjudged to be not mated
Female mating index : number of females mated * 100 / number of females placed with males
Female fertility index : number of females pregnant * 100 / number of females placed with males
Male mating index : number of males mated * 100 / number of males placed with females
Male fertility index : number of males that became sire * 100 / number of males placed with females

Table 29: Mating and pregnancy performance cohort 1B: Delivery, from the EOGRTS (Anonymous, 2016)

Delivery		Control F 0 mg/kg BW		Low-dose F 80 mg/kg BW	Mid-dose F 250 mg/kg BW	High-dose F 800 mg/kg BW
Females delivering	N	24	cx	25	23	24
- With liveborn pups	N	24	cx	25	23	24
	%	100		100	100	100
- With stillborn pups	N	0	cx	2	1	2
	%	0		8.0	4.3	8.3
- With all pups stillborn	N	0	cx	0	0	0
	%	0		0	0	0
Gestation index	%	100		100	100	100
Gestation days	Mean	22.6	k	22.7	22.7	22.8
	s.d.	0.5		0.5	0.5	0.5
	N	24		24[1]	23	24

Statistic Profile = DecisionTree, * = p < 0.05, ** = p < 0.01, X = Group excluded from statistics k=KRUSKAL-WALLIS; a=ANOVA cx=CHI-SQUARE-EXACT; u=KRUKSAL-WALLIS-DUNN

[1] Female 107-06 was misjudged to be not mated
Gestation index : number of females with live pups * 100 / number of pregnant females

Table 30: Mating and pregnancy performance cohort 1B: Fertility, from the EOGRTS (Anonymous, 2016)

Fertility		Control F 0 mg/kg BW	Low-dose F 80 mg/kg BW	Mid-dose F 250 mg/kg BW	High-dose F 800 mg/kg BW
Pups delivered	Total	271	287	249	251
	Mean	11.3 k	11.5	10.8	10.5
	s.d.	1.7	2.1	2.0	2.3
- Live born	Total	271	285	246	248
	%	100.0	99.3	98.8	98.8
- Stillborn	Total	0	2	3	3
	%	0.0	0.7	1.2	1.2
Perinatal loss	Mean %	0.00	0.62	2.61	1.25

Statistic Profile = DecisionTree, * = p < 0.05; ** = p < 0.01; X = Group excluded from statistics; k=KRUSKAL-WALLIS; a=ANOVA; cx=CHI-SQUARE-EXACT; u=KRUKSAL-WALLIS-DUNN

Perinatal loss: mean of number of pups delivered per litter - number of live pups per litter at day 0 * 100 / number of pups delivered per litter

Additionally, in the cohort 1A, ovarian follicle counts were performed. The absolute number of follicles in the high-dose group was lower than in the control group, however, the relative distribution of the follicles in each phase (small, growing, antral and corpora lutea) was comparable in the control and high-dose groups. Even though a statistically significant decrease in the development of small follicles into growing follicles was observed at the high dose, no effects were observed in the development of this small follicles to antral follicles an corpora lutea, indicating that the substance has no effect on the development of these cells (Table 31).

Table 31: Differential ovarian follicle count for cohort 1A animals from the EOGRTS (Anonymous, 2016)

Absolute

Animal	Small follicles	Growing follicles	Antral Follicles	Corpora lutea	Total
1-001-06	93	131	75	170	469
1-009-07	100	126	69	130	425
1-011-06	89	219	129	200	637
1-013-04	173	124	55	109	461
1-019-06	135	186	113	152	586
1-021-08	161	190	54	240	645
1-025-06	108	183	79	193	563
1-027-07	74	187	60	141	462
1-029-05	100	178	106	248	632
1-033-07	75	206	83	139	503
Average	111	173	82	172	
SD	34	34	26	47	
Animal	Small follicles	Growing follicles	Antral Follicles	Corpora lutea	Total
4-171-06	70	167	78	246	561
4-173-07	87	117	54	136	394
4-179-07	78	154	88	171	491
4-183-08	86	84	59	132	361
4-197-08	128	106	36	164	434
4-199-08	178	152	57	163	550
4-205-07	71	116	106	133	426
4-213-07	113	148	98	133	492
4-215-09	104	96	47	116	363
4-217-04	56	179	106	156	497
Average	97	132 *	73	155	
SD	36	32	26	37	

Percentage

Animal	Small follicles	Growing follicles	Antral Follicles	Corpora lutea	Total
1-001-06	20	28	16	36	100
1-009-07	24	30	16	31	100
1-011-06	14	34	20	31	100
1-013-04	38	27	12	24	100
1-019-06	23	32	19	26	100
1-021-08	25	29	8	37	100
1-025-06	19	33	14	34	100
1-027-07	16	40	13	31	100
1-029-05	16	28	17	39	100
1-033-07	15	41	17	28	100
Average	21	32	15	32	
SD	7	5	4	5	
Animal	Small follicles	Growing follicles	Antral Follicles	Corpora lutea	Total
4-171-06	12	30	14	44	100
4-173-07	22	30	14	35	100
4-179-08	16	31	18	35	100
4-183-08	24	23	16	37	100
4-197-08	29	24	8	38	100
4-199-08	32	28	10	30	100
4-205-07	17	27	25	31	100
4-213-07	23	30	20	27	100
4-215-09	29	26	13	32	100
4-217-04	11	36	21	31	100
Average	22	29 *	16	34	
SD	7	4	5	5	

Statistical Test: Generalised linear mixed model analysis

* - The development of small follicles into growing follicles was statistically significantly slower in high-dose females than in control females (P=0.0174)

Oral combined repeated dose toxicity study with the reproduction/developmental toxicity screening test (OECD TG 422) (Anonymous, 2015)

A GLP oral combined repeated dose toxicity study with the reproduction/developmental toxicity screening test (OECD TG 422) was conducted with 2-EHA at doses of 82-86, 248-253, 761-797 mg/kg bw/d in males and 107-116, 308-351, 809-1146 mg/kg bw/d in females. This study was used as a dose-range finder for the OECD TG 443 required as a result of the substance evaluation process under REACH Regulation.

Apart from the usual observations and examinations, zinc was measured in liver, kidney and blood of non-fasted parental animals (including extra satellite animals) that were not used for haematology, clinical chemistry and possible hormone determinations; in liver, kidney, blood and homogenate of one pup per sex and litter and in homogenate of one foetus/sex/litter. In addition, metallothionein determinations were performed in liver and kidneys of non-fasted animals as used for zinc determination.

To determine peroxisome proliferation in the liver, analysis of the activity of palmitoyl-CoA oxidase was carried out in the same animals as used for zinc and metallothioneins determinations.

Sperm parameters were analyzed. No information on oestrous cyclicity was included.

No mortalities or clinical signs of toxicity were observed. No effects were reported in Functional Observation Battery (FOB) and spontaneous Motor Activity Assessment (MAA) tests.

Decreases in body weight and food consumption were observed in animals of the high-dose group (up to 10% decreased body weight in females at the end of the gestation period) throughout the major part of the study. These changes were considered to be related to treatment.

Hematological observations related to lower values of mean corpuscular volume (MCV), mean corpuscular haemoglobin concentration (MCHC) and reticulocytes were observed in the females of the high-dose group. In addition, these females also showed increases in total white blood cells, monocytes and in the absolute number of neutrophils.

Clinical chemistry showed an increase in bile acids in high-dose males on day 30 of the study. Lower total protein and albumin concentrations and higher albumin/globulin ratio were observed in high-dose females.

At necropsy, decreases in terminal body weights were observed in both sexes of the high-dose group. At this dose level, increases in the relative weight of the liver for both sexes and in the relative weight of the kidneys in male rats were reported. In addition, female rats showed a decrease in the absolute and relative weights of the thymus.

Concerning histopathological findings, no macroscopic effects related to treatment were observed. Microscopic examination showed an increased incidence of proteinaceous droplets in the kidney renal tubuli of males in the control and high-dose groups. Reduction in the incidence of extramedullary hematopoiesis in the spleen was observed in females at the same dose level. No evidence of peroxisome proliferation in the liver was reported.

No effects on fertility or reproductive performance were observed in male and female rats.

Female rats of the high-dose group showed an increase in the mean zinc concentration in liver (satellite group) and kidneys (all F0-generation females and pups). No effects were observed in male rats. Concentrations of metallothionein-1 (MT-1) and metallothionein-2 (MT-2) in kidneys and livers of high-dose females were increased, with the exception of MT-1 in kidneys of high-dose group which was not affected. In males, only higher concentrations of MT-1 in liver of the high-dose group were observed.

<u>One-generation reproductive toxicity study (Pennanen *et al*., 1993)</u>

The reproductive toxicity of 2-EHA was investigated in a non-GLP and non-guideline one-generation reproductive toxicity study in Wistar rats. Daily average doses of 100, 300 or 600 mg/kg bw/d 2-EHA as a sodium salt in drinking water were administered to groups of 24 Wistar rats per sex and dose level.

During the study, no mortality or visible clinical signs of toxicity occurred at any dose group after 2-EHA exposure. No changes in food or liquid consumption were observed in any of the treatment groups prior to or during the mating period. Nevertheless, slightly but statistically significant reduction in water consumption of 14% was seen in pregnant females of the high-dose group.

A significant maternal body weight reduction of 9 to 12%, was observed in females at 600 mg/kg bw/d from gestational day 7 onwards, compared to control group. At the same dose, the gestational weight gain was statistically significantly lower ($p<0.01$). All these differences disappeared during lactation. On the other hand, the body weights of male rats were unaffected (Tables 32 and 33).

Table 32: Maternal parameters in pregnant Wistar rats from the one-generation reproductive toxicity study (Pennanen *et al.*, 1993)

	2-Ethylhexanoic acid (mg/kg body wt)			
	Control	100	300	600
Subjects (dams)				
Total in study	23	23	24	24
Nonpregnant at termination	0	2	0	1
Pregnant (%) at termination	23 (100)	21 (91.3)	24 (100)	23 (95.8)
Maternal body weight (g)[a]				
Gestational Day 0	229.0 ± 21.7	228.0 ± 24.1	233.0 ± 19.3	217.7 ± 19.2
Gestational Day 7	250.0 ± 24.1	246.5 ± 23.1	249.3 ± 20.8	228.5 ± 19.1**
Gestational Day 14	280.9 ± 32.5	271.3 ± 24.6	278.3 ± 31.1	245.8 ± 20.2**
Gestational Day 21	337.9 ± 32.2	337.3 ± 32.4	341.8 ± 27.6	303.3 ± 31.8**
Gestational weight gain (g)[a]	108.9 ± 18.6	109.3 ± 20.8	108.8 ± 18.4	85.6 ± 20.3**
Body weight on Postnatal Day 21 (g)[a]	268.0 ± 28.9	265.8 ± 30.6	263.9 ± 20.2	253.4 ± 26.8
Food consumption[b]	8.2 ± 1.5	8.2 ± 1.5	8.5 ± 1.9	8.1 ± 1.5
Water consumption[b]	12.8 ± 3.5	13.6 ± 3.5	12.7 ± 3.1	11.0 ± 3.0*
Relative[c] ovary weight (right)[a]	0.056 ± 0.08	0.041 ± 0.01	0.039 ± 0.01	0.040 ± 0.01
Relative[c] ovary weight (left)[a]	0.058 ± 0.08	0.041 ± 0.01	0.041 ± 0.01	0.042 ± 0.01

* $p < 0.05$, Fisher PLSD.
** $p < 0.01$, Fisher PLSD.
[a] Means ± SD.
[b] g/100 g of body weight/day.
[c] Organ weight/body weight.

Table 33: Body weight and relative reproductive organ weights of male rats from the one-generation reproductive toxicity study (Pennanen *et al.*, 1993)

	2-Ethylhexanoic acid (mg/kg/day)			
	Control	100	300	600
Body weight[a] (g)	391.0 ± 47.2	385.5 ± 35.1	378.3 ± 45.7	363.3 ± 42.8
Testis (right)	0.47 ± 0.05[b]	0.48 ± 0.05	0.49 ± 0.06	0.51 ± 0.07
Testis (left)	0.48 ± 0.06	0.48 ± 0.05	0.48 ± 0.05	0.51 ± 0.06
Epididymis (right)	0.18 ± 0.03	0.18 ± 0.03	0.20 ± 0.04	0.21 ± 0.03*

* $p < 0.05$, Scheffe's test.
[a] Body weight after mating at the time of sacrifice.
[b] ×0.01.

An increase of 12% in the relative weights of the right epididymides ($p<0.05$) was seen in high-dose males. Absolute weights were also increased but not statistically significantly. No changes were observed in the relative weights of ovaries and testes (Tables 32 and 33).

A slight but not uniformly dose-dependent decrease on the sperm quality occurred in males. In the high-dose group, the total number of spermatozoa in the cauda epididymis showed a non-statistically significant reduction of 14%. Reduction of motile spermatozoa of 37% and 22% was seen at 100 and 600 mg/kg bw/d ($p<0.05$), respectively (Table 34). The increase of morphologically abnormal spermatozoa at 300 and 600 mg/kg bw/d was not statistically significant. The most common abnormalities were agglutination and abnormal heads of spermatozoa. In the mid- and high-dose groups, amorphous heads (short and straight heads) were observed in 13% and 21% of the male rats, respectively (Table 35).

In connection with fertility parameters, a dose-dependent delay in fertilization was observed. 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 (Table 36).

In the histological evaluation of sex organs, epithelial hyperplasia in the vagina and slight dilation of the lumen in uterus were seen in two of five dams at the two highest doses. In dams, no other histological changes were seen. All sex organs of non-gravid females and males appeared normal at all treatment doses.

Table 34: Epididymal sperm density and motility from the one-generation reproductive toxicity study (Pennanen *et al.*, 1993)

	2-Ethylhexanoic acid (mg/kg body wt/day)			
	Control	100	300	600
Total cells ($\times 10^6$/g cauda epididymis)	666.0 ± 347.0	683.5 ± 443.3	616.9 ± 295.5	574.5 ± 302.9
Motile cells ($\times 10^6$/g cauda epididymis)	249.4 ± 194.6	170.2 ± 168.6	197.3 ± 172.5	173.8 ± 133.9
Motility (%)	34.8 ± 12.6	21.9 ± 13.1*	28.0 ± 13.9	27.0 ± 12.3*
Rapid (%)	18.6 ± 10.8	8.6 ± 8.4*	13.0 ± 10.1	14.7 ± 10.7
Moderate (%)	14.7 ± 9.0	12.3 ± 7.8	14.2 ± 6.6	12.0 ± 4.4
Slow (%)	1.2 ± 1.9	0.8 ± 0.9	0.9 ± 1.1	0.25 ± 0.5*
Static (%)	68.1 ± 15.2	78.1 ± 13.1*	71.9 ± 13.9	73.0 ± 12.3

Note. The figures are means ± SD of 24 animals per group.
* $p < 0.05$, Fisher PLSD.

Table 35: Epididymal sperm morphology from the one-generation reproductive toxicity study (Pennanen *et al.*, 1993)

	2-Ethylhexanoic acid (mg/kg body wt/day)			
	Control	100	300	600
Normal	21[a] (87.5)[b]	22 (91.6)	16 (66.7)	17 (70.9)
Agglutinated sperm	2 (8.3)	1 (4.2)	6 (25.0)	2 (8.3)
Abnormal heads	1 (4.2)	1 (4.2)	3 (12.5)	5 (20.8)

[a] Number of rats with the observation.
[b] Percentage of the examined rats.

Table 36: Fertility parameters of female rats from the one-generation reproductive toxicity study (Pennanen *et al.*, 1993)

	2-Ethylhexanoic acid (mg/kg/day)			
	Control	100	300	600
Pregnancy index[a]:	23/23 (100%)	21/23 (91.3%)	24/24 (100%)	23/24 (95.8%)
Estrous cycle	Females pregnant			
1[b]	21 (91.3%)	20 (87%)	22 (91.7%)	17 (70.8%)
2	2 (9.6%)	0	0	2 (8.3%)
3	0	1 (4.3%)	1 (4.2%)	2 (8.3%)
4	0	0	1 (4.2%)	2 (8.3%)
Nonpregnant	0	2 (8.7%)	0	1 (4.2%)

[a] Number of pregnant females/number of mated females.
[b] The number of consecutive estrous cycles.

In summary, it has been observed that 2-EHA increased time to mating, and tended to decrease fertility in Wistar rats at 600 mg/kg bw/d. In addition, the substance caused effects on male sex organs related to sperm quality and an increase in the relative weights of the epididymides.

Summary of the available studies

An extended one-generation reproductive toxicity study (OECD TG 443) was conducted according to GLP with 2-EHA in Wistar rats (Anonymous, 2015; 2016). The EOGRTS design included the extension of cohort 1B to mate the F1 animals to produce the F2 generation and cohorts 2 and 3 to assess developmental neurotoxicity (DNT) and immunotoxicity (DIT).

None the results obtained in the EOGRTS at doses up to 800 mg/kg bw/d 2-EHA did show any treatment-related effects in fertility and sexual function parameters in F0 or F1 generations. Neither effects on sexual function or fertility were observed in male and female rats in a OECD TG 422 study conducted as a range-finding study for the EOGRTS.

Both studies have been recently conducted due to the uncertainties arose from a one-generation reproductive toxicity study (Pennanen *et al.*, 1993) neither carried out in accordance with any internationally recognized test method nor in compliance with GLP. In this study, some adverse effects regarding sexual function and fertility were noted. Furthermore, an apparent reduction in sperm motility and a delay in fertilization were observed in parental animals. These adverse effects on sexual function and fertility were not reproduced in the new studies previously described.

Therefore, taking into account the available old and new information and the quality of data, there are no indications of fertility or reproductive effects for 2-EHA.

10.10.3 Comparison with the CLP criteria

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

  on development;

- effects on or via lactation.

CLP define adverse effects on sexual function and fertility as: *"Any effect of substances that has the potential to interfere with sexual function and fertility. This includes, but is not limited to, alterations to the female and male reproductive system, adverse effects on onset of puberty, gamete production and transport, reproductive cycle normality, sexual behaviour, fertility, parturition, pregnancy outcomes, premature reproductive senescence, or modifications in other functions that are dependent on the integrity of the reproductive systems".*

The CLP regulation criteria for classification as reproductive toxicants are as follows:

The classification in Category 1A (Known human reproductive toxicant) *"is largely based on evidence from humans"*.

The classification of a substance in Category 1B (Presumed human reproductive toxicant) *"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".*

Further, substances are classified in Category 2 (Suspected human reproductive toxicant), *"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".

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. Therefore, no classification is proposed for 2-EHA and its salts for this endpoint.

10.10.4 Adverse effects on development

Table 37: 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
Oral prenatal developmental toxicity study (EPA OTS 798.4900). Rat/Fischer 344 Range-finding study: 8 females/group Main study: 25 females/group	2-EHA (purity 99.4%) Range-finding study: 125, 500 and 1000 mg/kg bw/d Main study: 0, 100, 250 and 500 mg/kg bw/d Oral gavage in corn oil from GD 6 to 15.	**<u>Range-finding study</u>** **Maternal toxicity** <u>Mortality</u> 87.5% at 1000 mg/kg bw/d (GD 7-9). <u>Clinical signs</u> Ataxia, urogenital wetness, audible respiration and red periocular encrustation at 1000 mg/kg bw/d. **Developmental toxicity** *At 500 mg/kg bw/d:* ↑ Pos-timplantation loss (early and late resorptions). ↓ Percentage of live foetuses. ↓ Fetal body weights. **<u>Main study</u>** **Maternal toxicity** <u>Clinical signs</u> Hypoactivity, ataxia, audible respiration, ocular discharge and periocular encrustations at 500 mg/kg bw/d. <u>Organ weights and histopathology</u> (Table 38) ↑ Absolute ($p<0.01$) and relative ($p<0.001$) liver weight at 500 mg/kg bw/d. **Developmental toxicity** (Tables 39-41) *250 mg/kg bw/d*	Anonymous, 1988c; Hendrickx *et al.*, 1993

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels, duration of exposure	Results	Reference
		Increase in the number of litters with foetuses with reduced skeletal ossification. *500 mg/kg bw/d* ↓ Fetal body weight ($p<0.001$). Growth retardation, increase in the number of litters with foetuses with reduced skeletal ossification. Skeletal anomalies: extra (14^{th}) thoracic centrum and arches (16 foetuses from 195 examined in 21 litters) ($p<0.01$) Dilated lateral ventricles of the brain with no tissue compression (21 foetuses from 195 examined in 21 litters) ($p<0.01$). **NOAEL for maternal toxicity of 250 mg/kg bw/d, based on clinical signs of toxicity and increased liver weights.** **NOAEL for developmental toxicity of 100 mg/kg bw/d, based on reduced skeletal ossification.**	
Oral prenatal developmental toxicity study (EPA OTS 798.4900). Rabbit/New Zealand white Range-finding study: 8 females/group Main study: 15 females/group	2-EHA (purity 99.4%) Range-finding study: 125, 250, 500 and 1000 mg/kg bw/d Main study: 0, 25, 125 and 250 mg/kg bw/d Oral gavage in corn oil from GD 6 to 18.	**<u>Range-finding study</u>** **Maternal toxicity** <u>Mortality</u> 100% at 1000 mg/kg bw/d. 87.5% at 500 mg/kg bw/d. One dead animal each at 250 and 125 mg/kg bw/d. <u>Clinical signs</u> Hypoactivity, labored respiration and ataxia at 1000 mg/kg bw/d. Hypoactivity at 500 and 250 mg/kg bw/d. **Developmental toxicity** One abortion on GD 25 each at 250 and 125 mg/kg bw/d. **<u>Main study</u>** **Maternal toxicity** <u>Mortality</u> One pregnant female each died at 125 (GD 15) and 250 mg/kg bw/d and (GD 16). <u>Body weight and food consumption</u> ↓ Maternal body weight change and food consumption (GD 18-29) ($p<0.01$) at 250 mg/kg bw/d. **Developmental toxicity** One abortion (GD 27) at 125 mg/kg bw/d. No effects on the pups. **NOAEL for maternal toxicity at 25 mg/kg bw/d, based on deaths, abortions and decreased body weights.**	Anonymous, 1988d; Hendrickx *et al*., 1993

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels, duration of exposure	Results	Reference
		NOAEL for developmental toxicity at 250 mg/kg bw/d based on the lack of effects.	
Developmental toxicity study (similar to OECD TG 414). GLP: No. Rat/Wistar 20-21 dams/group	2-EHA (purity 99.5%) 0, 100, 300 and 600 mg/kg bw/d (administered as sodium salt). Oral in drinking water from GD 6 to 19.	**Maternal toxicity** Body weight (Table 42) ↓ Mean body weight (11%, p<0.001) at termination and ↓ corrected maternal body weight gain (53.8%, p<0.001) at 600 mg/kg bw/d. **Developmental toxicity** Body weight (Table 42) ↓ Mean body weight in females (5.7%, p<0.001) at 300 mg/kg bw/d. ↓ Mean foetal body weight/litter in males (5.6%, p<0.001) and in females (8.6%, p<0.001) at 600 mg/kg bw/d. Placental weight ↓ 10.2% (p<0.001) in both 300 and 600 mg/kg bw/d. Skeletal or visceral malformations (Tables 43 and 44) *100 mg/kg bw/d* 4.9% per litter (p<0.001) Skeletal malformations/variations: Clubfoot (0.8%, not statistically significant) Wavy ribs (19.8%, p<0.001) Reduced cranial ossification (42.4%, p<0.05) Visceral anomalies: pelvic dilatation (33.9%, p<0.005) *300 mg/kg bw/d* 8.9% per litter (p<0.001) Skeletal malformations/variations: Clubfoot (5.6%, p<0.05) Wavy ribs (14.1%, p<0.001) Twisted hind legs (7%, p<0.005) Visceral anomalies: pelvic dilatation (41.8%, p<0.001) *600 mg/kg bw/d* 15.3% per litter (p<0.001) Skeletal malformations/variations: Clubfoot (6.7%, p<0.05) Wavy ribs (22.4%, p<0.001) Nonossified sternebrae (19.7%, p<0.05) Bipartite vertebral centra (34.5%, p<0.05) Reduced cranial ossification (66.7%, p<0.001) Reduced lumbar ossification (5%, p<0.05) Visceral anomalies: dilation of brain ventricles (24%, p<0.05). **NOAEL for maternal toxicity at 300 mg/kg bw/d based on reduced body weights.** **NOAEL for developmental toxicity at 100 mg/kg bw/d based on the reduction of foetal weight and**	Pennanen *et al.*, 1992

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels, duration of exposure	Results	Reference
		skeletal variations at doses which did not cause visible maternal toxicity.	
Developmental toxicity study with 2-ethylhexyl-2-ethylhexanoate (OECD TG 414) GLP: Yes Rat/Wistar 25 pregnant females	In this study, 2-EHA was administered at 600 mg/kg bw/d as a positive control. Oral gavage from GD 6 to 15. The test substance was 2-ethylhexyl-2-ethylhexanoate.	**Maternal toxicity** ↓ Mean body weight (9%). ↑ Relative liver weight (6%). **Developmental toxicity** ↓ Mean body weight. ↓ Placental weight. External malformations: adactyly, tail malformations. Malformations of the fetal skeletons: vertebral column, the sternum, ribs, femur, os ilium. Skeletal variations: accessory vertebra, rudimentary cervical, accessory 14th and wavy rib(s). Skeletal retardations: incomplete or missing ossification of skull bones, vertebral column and sternebra.	Anonymous, 1997
One-generation reproductive toxicity study (no guideline) GLP: No Rat/Wistar 24 animals/sex/dose	2-EHA (purity 99.5%) (administered as sodium salt) Oral in drinking water. Doses. 0, 100, 300 and 600 mg/kg bw/d Exposure: Males 10 weeks and females for 2 weeks prior to mating, both sexes during mating period and females during gestation and lactation.	See general toxicity and effects on fertility in Table 11 (Section 10.10.1). **F1 generation** <u>Developmental parameters</u> (Table 45, Fig. 4 and 5) *100 mg/kg bw/d* Delayed physical development: hair growth. Delayed development of the grip and cliff avoidance reflexes. *300 mg/kg bw/d* ↑ Frequency of lethargy, hematomas and abnormally thin hair (not statistically significant). Delayed physical development of ears. ↑ Kinky tail ($p<0.05$). *600 mg/kg bw/d* ↓ Average litter size (16%) ($p<0.05$). ↑ Frequency of lethargy, hematomas and abnormally thin hair but not statistically significant. Delayed physical development: eye opening and teeth eruption, hair growth and ears. Delayed development of the grip and cliff avoidance reflexes. ↑ Kinky tail ($p<0.05$). ↓ Body weights transiently during lactation. **NOAEL for maternal toxicity at 600 mg/kg bw/d based on reduced body weights.** **NOAEL for developmental toxicity at 100 mg/kg bw/d based on delayed physical development and the presence of kinky tail.**	Pennanen *et al.*, 1993
Oral combined repeated dose toxicity study with the reproduction/developmental toxicity screening test (OECD TG 422)	2-EHA (purity 99.8%) Oral feed.	See parental general toxicity in Table 11 (Section 10.10.1). **Developmental toxicity** ↓ Weight on PND 4 (14%) at doses of 761-797	Anonymous, 2015

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels, duration of exposure	Results	Reference
GLP: Yes Rat/Wistar 10 animals/sex/dose. Satellite groups of 6 extra animals/sex were added and pregnant females were sacrificed on gestation day 20 to gain knowledge on the possible mechanism of toxicity.	Doses males: 82-86, 248-253, 761-797 mg/kg bw/d females: 107-116, 308-351, 809-1146 mg/kg bw/d; PND 0-4: 190, 530 and 1371 mg/kg bw/d Exposure: 2-week premating period, mating and up to and including day 30 (males) and 2-week premating period, mating, gestation and lactation and up to and including the day of sacrifice (day 4 to 7 of lactation).	(males) and 809-1146 (females). **NOAEL for general toxicity of at least 248 mg/kg bw/d for males and 308 mg/kg bw/d for females, based on the effects on body weights, food consumption, organ weights, haematology, clinical chemistry and zinc and metallothionein concentrations observed at the highest dose.** **NOAEL for development was established at 248 mg/kg bw/d for males and 308 mg/kg bw/d for females, taking into account the pup weight reduction at the high-dose group.**	
Oral extended one-generation reproductive toxicity study (OECD TG 443). Design includes the extension of cohort 1B to mate the F1 animals to produce the F2 generation and cohorts 2 (DNT) and 3 (DIT). GLP: Yes Rat/Wistar F0: 28 animals/sex/dose F1: 75 pups/sex/group Cohort 1A: 20 pups/sex/group Cohort 1B: 25 pups/sex/group Cohort 2A: 10 pups/sex/group Cohort 2B: 10 pups/sex/group Cohort 3: 10 pups/sex/group (an extra group of 6 male and female pups treated with cyclosporine A were included as positive control group for the determination of the KLH-specific IgM response). The evaluation of the	2-EHA (purity 99.6%) Oral feed. Doses: 0, 80, 250, 800 mg/kg bw/d. Exposure: 2-week premating period, mating, gestation and lactation (females) and up to and including the day of sacrifice.	See parental general toxicity in Table 11 (Section 10.10.1). **Developmental toxicity** Perinatal loss was 0% for all groups. **F1 generation** (Tables 46-49) *250 mg/kg bw/d and 800 mg/kg bw/d* ↑ Anogenital distance (AGD) after correction for pup weight on PND 4 in male pups (3% and 7% respectively at 250 and 800 mg/kg bw/d). This effect was not observed in the second generation pups and was considered as a fortuitous finding by the study director. ***F2 generation of cohort 1B*** *800 mg/kg bw/d* ↑ Mean weights of male and female pups on PN day 21. Not considered adverse and probably due to the slightly lower number of pups. ***Cohort 2A*** (Tables 50-52) No effects on neurodevelopment (FOB, spontaneous motor activity, auditory startle response) were observed. *800 mg/kg bw/d* ↓ Mean body weight in male animals during the entire period, statistically significant on days 14, 28 and 35. ↓ Mean body weight gain in male animals from days 7-14. ↓ Food consumption in female animals from days 28-35 and 42-49. ↓ Mean absolute brain weight in males (not related to	Anonymous, 2016

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels, duration of exposure	Results	Reference
potential developmental immunotoxicity by determining the titer of KLH-specific IgM antibody was performed in the serum of cohort 3 animals by ELISA. After at least 13 weeks of age, animals of cohort 1B were mated to produce the F2 generation.		treatment). ***Cohort 2B*** (Table 53) *250 mg/kg bw/d and 800 mg/kg bw/d* ↑ Mean absolute brain weight in males (not related to treatment). ***Cohort 3*** (Tables 54-57) No effects on developmental immunotoxicity (KLH-inmunization) were observed. <u>Mortality and general clinical observations</u> One male animal of the positive control group was found dead on day 24 (considered not to be treatment-related). <u>Body weight and food consumption</u> *80 mg/kg bw/d* ↓ Mean body weight gain in males from days 7-14. ↑ Food consumption in females from days 7-14. *800 mg/kg bw/d* ↓ Mean body weight in male animals during the entire period, statistically significant on days 14, 21, 28 and 35. ↓ Mean body weight gain in males for the entire period. *Cyclosporine A positive control group* ↓ Mean body weight in male animals on day 35. ↓ Mean body weight gain in males from days 21-28 and 28-35. ↑ Mean body weight and body weight gain in females on day 35 and from days 28-35, respectively. ↓ Food consumption in male animals from days 28-35. ↑ Food consumption in female animals from days 7-14 and 28-35, respectively. <u>Organ weights and histopathology</u> *800 mg/kg bw/d* ↓ Absolute weight of the spleen in males . *Cyclosporine A positive control group* ↓ Absolute weight of the spleen in males . ↓ Absolute weight of the thymus in males . **NOAEL for developmental and developmental neurotoxicity and immunotoxicity effects was established at 800 mg/kg bw/d, due to the lack of effects.**	

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

Seven studies with 2-EHA are available for examination of adverse effects on development for the substances covered by this CLH proposal.

Oral prenatal developmental toxicity study in Fischer 344 rats (EPA Guideline) (Anonymous, 1988c; Hendrickx *et al*., 1993)

In the main developmental study, groups of 25 pregnant Fischer 344 rats per dose level received daily doses of 0, 100, 250 and 500 mg/kg bw/d 2-EHA (nominal in corn oil) by oral gavage from gestational day 6 to 15.

Maternal clinical signs were only observed at the high-dose level and included hypoactivity, ataxia, audible respiration, ocular discharge and periocular encrustations. No mortality and no effects on body weight were observed. Liver weight (absolute and relative) was significantly increased in the high-dose group (Table 38).

There were no changes in the incidence of resorptions and dead foetuses or in the percentage of viable foetuses. Foetal body weights (males and females) per litter were significantly reduced at 500 mg/kg bw/d, but these findings may be confounded by the slightly larger mean litter size. There was a growth retardation related to a reduction in ossification of the axial and appendicular skeletons at 500 mg/kg bw/d. An increase in the number of foetuses with unossified anterior arch of the atlas and proximal phalanges of the forelimb and hindlimb was also observed at 250 mg/kg bw/d (Table 39).

Although several foetal skeletal variations were observed, only the variation concerning extra 14th thoracic centrum and arches at the high dose was statistically significant. Related to visceral variations, statistically significant increases of dilated lateral ventricles of the brain with no tissue compression were seen at 500 mg/kg bw/d (Table 40).

Table 38: Maternal parameters (*Hendrickx et al*., 1993)

	2-Ethylhexanoic acid (mg/kg/day) by gavage, GD 6–15			
	0	100	250	500
No. of females	25	25	25	25
Females that delivered[a]	0	0	1	0
Nonpregnant at termination	2	1	2	4
Pregnant at termination	23	24	22	21
Females with viable fetuses	23	24	22	21
Maternal weight gain (g)[b]				
Pretreatment (GD 0–6)	12.94 ± 2.78	13.02 ± 3.48	12.89 ± 3.54	14.35 ± 2.87
Treatment (GD 6–15)	24.71 ± 3.93	24.08 ± 4.84	24.62 ± 4.07	22.29 ± 5.40
Post-treatment (GD 15–21)	43.99 ± 8.83	41.72 ± 12.65	43.81 ± 11.12	45.98 ± 10.06
Gestation (GD 0–21)	81.64 ± 12.12	78.82 ± 16.76	81.33 ± 15.38	82.62 ± 13.72
Corrected gestation (minus gravid uterus)	30.76 ± 8.06	32.94 ± 8.97	30.94 ± 7.38	29.48 ± 7.53
Maternal liver weight (g)[a]	9.42 ± 0.78	9.79 ± 0.94	9.65 ± 0.76	10.30 ± 0.78*
Relative maternal liver weight (% body wt)	4.93 ± 0.36	5.07 ± 0.44	5.04 ± 0.32	5.41 ± 0.37**

[a] The data for one animal which delivered on GD 20 are not included in statistical analyses.
[b] Includes all females pregnant at termination; mean ± SD.
* $p < 0.01$.
** $p < 0.001$.

Table 39: Developmental parameters (*Hendrickx et al*., 1993)

	2-Ethylhexanoic acid (mg/kg/day) by gavage, GD 6–15)			
	0	100	250	500
Total postimplantation loss/litter[a]	0.5 ± 0.7	0.2 ± 0.5	0.3 ± 0.6	0.4 ± 0.7
Early resorptions	0.5 ± 0.7	0.2 ± 0.4	0.3 ± 0.6	0.4 ± 0.7
Late resorptions	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Dead fetuses	0.0 ± 0.0	0.1 ± 0.3	0.0 ± 0.0	0.0 ± 0.0
Percentage live fetuses/litter[a]	94.8 ± 7.5	97.4 ± 5.4	96.4 ± 6.5	95.4 ± 9.4
Live fetuses/litter[a]	8.4 ± 2.9	7.5 ± 3.8	8.4 ± 3.3	9.3 ± 2.9
Percentage males/litter[a]	49.2 ± 17.9	53.3 ± 21.7	43.4 ± 16.9	55.2 ± 19.0
Fetal weights/litter (g)[a]				
All fetuses	4.41 ± 0.24	4.50 ± 0.38	4.36 ± 0.28	4.06 ± 0.18*
Male fetuses	4.54 ± 0.18	4.62 ± 0.40	4.49 ± 0.23	4.18 ± 0.16*
Female fetuses	4.25 ± 0.25	4.24 ± 0.28	4.24 ± 0.31	3.91 ± 0.17*

[a] Includes all females pregnant at termination; mean of litter values ± SD.
* $p < 0.001$.

Table 40: Summary of malformations and variations (*Hendrickx et al.*, 1993)

	2-Ethylhexanoic acid (mg/kg/day) by gavage, GD 6–15			
	0	*100*	*250*	*500*
Fetuses examined[a]	193	179	184	195
Litters examined	23	24	22	21
Malformations				
External[b]				
No. (%) of fetuses with malformations[c]	1 (0.5)	0 (0.0)	0 (0.0)	0 (0.0)
No. (%) of litters with malformations[d]	1 (4.3)	0 (0.0)	0 (0.0)	0 (0.0)
Visceral[e]				
No. (%) of fetuses with malformations[c]	2 (2.0)	3 (3.2)	2 (2.1)	6 (5.8)
No. (%) of litters with malformations[d]	2 (8.7)	2 (8.3)	2 (9.1)	6 (28.6)
Skeletal[f]				
No. (%) of fetuses with malformations[c]	0 (0.0)	2 (2.4)	2 (2.3)	0 (0.0)
No. (%) of litters with malformations[d]	0 (0.0)	2 (9.1)	2 (9.5)	0 (0.0)
Total				
No. (%) of fetuses with malformations[c]	2 (1.0)	5 (2.8)	4 (2.2)	6 (3.1)
No. (%) of litters with malformations[d]	2 (8.7)	4 (16.7)	4 (18.2)	6 (28.6)
Variations				
External[b]				
No. (%) of fetuses with variations[c]	24 (12.4)	21 (11.7)	27 (14.7)	34 (17.4)
No. (%) of litters with variations[d]	17 (73.9)	14 (58.3)	16 (72.7)	16 (76.2)
Visceral[e]				
No. (%) of fetuses with variations[c]	52 (52.0)	53 (55.8)	57 (58.8)	80 (76.9)
No. (%) of litters with variations[d]	20 (87.0)	20 (83.3)	20 (90.9)	21 (100.0)
Skeletal[f]				
No. (%) of fetuses with variations[c]	93 (100.0)	84 (100.0)	87 (100.0)	91 (100.0)
No. (%) of litters with variations[d]	22 (100.0)	22 (100.0)	21 (100.0)	21 (100.0)
Total				
No. (%) of fetuses with variations[c]	150 (77.7)	141 (78.8)	152 (82.6)	176 (90.3)
No. (%) of litters with variations[d]	23 (100.0)	22 (91.7)	22 (100.0)	21 (100.0)

[a] A single fetus may be represented more than once in listing specific types of malformations or variations; only live fetuses were examined.
[b] All fetuses were examined externally.
[c] Fetuses with one or more malformations or variations.
[d] Litters with one or more malformed or variant fetuses.
[e] Approximately 50% of each litter was examined for visceral and soft tissue craniofacial malformations/variations.
[f] Approximately 50% of each litter was examined for skeletal malformations/variations after staining with alizarin red S.

No significant differences in the incidence of external, skeletal or visceral malformations were observed among all groups. Nevertheless, a non-statistically significant dilation of lateral ventricles of the brain with tissue compression was observed in all treatment-groups (Table 41).

Table 41: Specific malformations and variations (*Hendrickx et al.*, 1993)

	2-Ethylhexanoic acid (mg/kg/day) by gavage, GD 6–15			
	0	100	250	500
Fetuses examined[a]	193	179	184	195
Litters examined	23	24	22	21
Malformations				
External[b]				
Cleft palate	1	0	0	0
Visceral[c]				
Lateral ventricle dilated, tissue compressed	2	1	1	6
Hydroureter, unilateral	0	0	1	0
Epididymis absent, bilateral	0	1	0	0
Epididymis absent, unilateral	0	1	0	0
Skeletal[d]				
Lumbar arch 3 missing	0	1	0	0
13th rib forked	0	1	0	0
Proximal phalanges (hindlimb) missing	0	1	2	0
Distal phalanges (hindlimb) missing	0	1	2	0
Variations				
External[b]				
Ecchymosis, trunk	23	20	23	34
Ecchymosis, head	1	1	4	0
Ecchymosis, extremities	0	1	0	0
Visceral[c]				
Nasal passages constricted, bilateral	0	0	0	1
Lateral ventricle dilated, no compression	3	7	10	21*
Third ventricle dilated, no compression	0	0	0	1
Fetal atelectasis	21	20	17	39
Partial fetal atelectasis	28	28	34	39
Liver nodule	0	1	2	0
Stomach nodule	0	0	1	0
Stomach empty	2	0	0	0
Dilated renal pelvis, unilateral	0	0	3	3
Dilated renal pelvis, bilateral	2	1	1	0
Dilated ureter, unilateral	2	0	0	2
Dilated ureter, bilateral	0	1	0	1
Skeletal[d]				
Extra No. 14 thoracic centrum and arches	0	0	0	16*
Extra rib, No. 14 thoracic, unilateral	0	0	0	1
Rudimentary rib, No. 14 thoracic, unilateral	0	0	0	2
Rudimentary rib, No. 14 thoracic, bilateral	0	0	0	1
Extra rib, No. 1 lumbar, unilateral	0	0	0	1
Rudimentary rib, No. 1 lumbar, unilateral	0	0	0	1
Bone island, No. 14 thoracic arch, unilateral	0	0	0	5
Bone island, No. 14 thoracic arch, bilateral	0	0	0	6
Bone island, No. 1 lumbar arch, unilateral	1	0	2	9
Bone island, No. 1 lumbar arch, bilateral	0	0	0	4

[a] A single fetus may be represented more than once in listing individual malformations or variations; only live fetuses were examined.
[b] All fetuses were examined externally.
[c] Approximately 50% of each litter was examined for visceral and soft tissue craniofacial defects.
[d] Approximately 50% of each litter was examined for skeletal defects after staining with alizarin red S.
* $p < 0.01$ (when calculated on a litter basis; i.e., No. of affected litters/total litters).

Oral prenatal developmental toxicity study in New Zealand white rabbits (EPA Guideline) (Anonymous, 1988d; Hendrickx *et al*., 1993)

A developmental toxicity study was carried out in New Zealand white rabbits. In this study, mortality was recorded at 125 and 250 mg/kg bw/d (one female each) on days 15 and 16 of gestation, respectively. One

abortion was observed on gestational day 27 at 125 mg/kg bw/d. A significant reduction in body weight gain and food consumption was observed in the high-dose group during the post-treatment period (gestational days 18 to 29). At necropsy, no gross pathology, no changes in corrected body or gestational weights or in absolute and relative liver weights were observed.

There was no increase of resorptions and dead foetuses or changes in the percentage of viable foetuses. No effects on foetal body weights and sex ratios were observed and no differences in malformations or variations were seen either.

Developmental toxicity study in Wistar rats (similar to OECD TG 414) (Pennanen *et al*., 1992)

A non-GLP developmental toxicity study, equivalent or similar to OECD TG 414, has been reported in the IUCLID dataset (Pennanen *et al.*, 1992). Groups of 20 or 21 female Wistar rats per dose level received daily doses of 100, 300 and 600 mg/kg bw/d 2-EHA as sodium salt via drinking water, during gestational days 6 to 19.

A non-statistically significant decrease in the pregnancy rate was seen in the mid- and high-dose groups, but these differences were unrelated to treatment, which was limited to gestational days 6-19. Body weight of dams suffered a slight decrease at the high-dose level from day 13 onwards. At termination, statistically significant reductions in mean body weight and corrected maternal body weight gain were observed. In the same dose group, a decrease of 20% in the consumption of drinking water containing 2-EHA was seen from day 6, compared to the control group. No differences in food consumption were observed at any dose level. No maternal toxicity was noted at the low- and mid-dose groups.

In the mid- and high-dose groups the placental weight was also statistically significant reduced. No changes in gravid uterus weight were observed. At necropsy, no gross pathological changes in the organs of the dams occurred. The number of implantations, living foetuses or resorptions did not suffer any significant change (Table 42).

Related to developmental toxicity, no dead foetuses were seen either in treated or control groups. Significant decreases in mean foetal body weight per litter were observed at 600 mg/kg bw/d. At 300 mg/kg bw/d, the mean body weight of female foetuses was also decreased.

Results showed that 2-EHA affected normal development of foetuses at all dose levels. Increases in the number of foetuses with skeletal or visceral anomalies were observed at all dose levels, compared to controls. It has to be pointed out that the number of litters affected by these alterations has not been indicated. Clubfoot, the most severe skeletal malformation, occurred in all treatment groups, being only statistically significant at the two highest doses. The major skeletal variations were related to non-uniformly dose-dependent increases in the incidence of wavy ribs, observed in all treatment groups, and reduced cranial ossification, observed at 100 and 600 mg/kg bw/d. Unossified sternebrae, reduced ulna/lumbar ossification, bipartite vertebral centra and twisted hind legs were other variations observed, with lower incidence, at the highest dose (Table 43).

Only few visceral malformations were found. The degree of dilation of brain ventricles, which is inversely related to the developmental stage of conceptus, was increased in the dose groups of 300 and 600 mg/kg bw/d, being statistically significant at 600 mg/kg bw/d. Non-dose related but statistically significant increase of pelvic dilation of the urinary tract was observed at 100 and 300 mg/kg bw/d, although this variation was also common in control groups (Table 44).

Table 42: Summary table of maternal and fetal body weights (Pennanen *et al.*, 1992)

Parameter	2-Ethylhexanoic acid dose (mg/kg/day)			
	Control	100	300	600
Initial maternal body weight (g)	219 ± 19 (21)[a]	217 ± 21 (21)	220 ± 25 (20)	212 ± 21 (20)
Terminal maternal body weight (g)	314 ± 23 (21)	310 ± 37 (21)	310 ± 32 (20)	280 ± 23* (20)
Weight of uterus with fetuses	56 ± 12 (21)	57 ± 15 (21)	56 ± 19 (20)	50 ± 9 (20)
Corrected maternal body weight gain (g)[b]	39 ± 3 (21)	36 ± 4 (21)	34 ± 3 (20)	18 ± 1* (20)
Mean fetal body weight (g)/litter	3.6 ± 0.5 (21)	3.6 ± 0.4 (21)	3.4 ± 0.3* (20)	3.3 ± 0.5* (20)
Mean male fetal body weight (g)/litter	3.6 ± 0.5 (21)	3.7 ± 0.5 (21)	3.6 ± 0.4 (20)	3.4 ± 0.5* (20)
Mean female fetal body weight (g)/litter	3.5 ± 0.4	3.5 ± 0.4	3.3 ± 0.3*	3.2 ± 0.4*
Mean placental weight (g)	0.49 ± 0.08	0.49 ± 0.07	0.44 ± 0.06*	0.44 ± 0.07*

[a] Number of dams.
[b] (Weight on Day 20 postconception − weight of uterus with fetuses) − weight on Day 0 postconception.
* $p < 0.001$.

Table 43: Summary table of reproduction and litter data (Pennanen *et al.*, 1992)

Parameter	2-Ethylhexanoic acid dose (mg/kg/day)			
	Control	100	300	600
Bred females	25	24	30	30
Litters	21 (84)[a]	21 (88)	20 (67)	20 (67)
Implantations	220	235	240	233
Living fetuses	202 (92)[b]	225 (96)	216 (90)	202 (87)
Resorptions	18 (8)[b]	10 (4)	24 (10)	31 (13)
Early	15	9	20	22
Late	3	1	4	9
Litters with resorptions	12 (57)[c]	5 (24)	9 (45)	14 (70)
Total resorptions	0 (0)	0 (0)	1 (5)	0 (0)
Skeletal malformations	4 (19)	8 (38)	12 (60)	12 (60)
Visceral malformations	0 (0)	1 (5)	2 (10)	2 (10)
Implantations/litter[d]	10.9 ± 2.1	11.6 ± 2.6	12.6 ± 2.4	11.7 ± 1.8
Living fetuses/litter[d]	9.6 ± 1.8 (88)[b]	10.7 ± 2.7 (92)	10.8 ± 2.8 (86)	10.1 ± 1.9 (86)
Sex ratio[e]	0.50	0.43	0.46	0.51
Preimplantation loss (%)[d]	5.7 ± 1.8	7.3 ± 2.2	8.8 ± 4.8	6.7 ± 2.3
Postimplantation loss (%)[d]	8.4 ± 2.1	3.2 ± 1.4	14.0 ± 5.5	11.9 ± 2.5
Affected fetuses[f]	4[g] 2.0[h] 2.4 ± 1.2[i]	11 4.9 4.9 ± 1.9	22 10.2*** 8.9 ± 1.9**	29 14.4*** 15.3 ± 3.8***
Fetuses with	4[g] 3.8[h]	10 9.1	20 18.3**	27 24.8***
Skeletal malformations	3.8 ± 1.8[i]	8.3 ± 2.4	15.9 ± 3.6**	26.3 ± 6.2**
Visceral malformations	0 0 0	1 0.9 0.8 ± 0.8	2 1.9 1.8 ± 1.8	2 2.0 1.8 ± 1.5

[a] Percentage of pregnant females.
[b] Percentage of implantations.
[c] Percentage of litters.
[d] Mean ± SEM.
[e] Males/all fetuses.
[f] Fetuses with all malformations.
[g] Number of affected fetuses.
[h] Percentage of examined fetuses.
[i] Group mean (±SEM) of litter percentages (affected fetuses in litter).
* $p \leq 0.05$.
** $p \leq 0.01$.
*** $p \leq 0.001$.

Table 44: Summary table of skeletal anomalies in foetuses (Pennanen *et al.*, 1992)

Observations	2-Ethylhexanoic acid dose (mg/kg/day)			
	Control	100	300	600
Number of examined fetuses	94	110	109	103
Number of litters	21	21	19	20
Malformations				
Clubfoot	0[a]	0.8 ± 0.8	5.6 ± 2.0*	6.7 ± 2.8*
Abnormal cartilage[b]	0	0	0.7 ± 0.7	1.7 ± 1.5
Absence of fibula	0	0	0	0.8 ± 0.8
Polydactyly	1.0 ± 1.0	0	0	0.8 ± 0.8
Scoliosis	0	3.6 ± 1.8	2.4 ± 1.8	3.8 ± 1.8
Lordosis	0	0.8 ± 0.8	0.7 ± 0.7	0.8 ± 0.8
Flabby legs[c]	0	0.8 ± 0.8	7.0 ± 3.0	8.8 ± 3.6
Extra thoracic ribs (>5 mm)	4.1 ± 2.6	3.7 ± 2.2	3.6 ± 2.0	13.3 ± 5.5
Variations				
Wavy ribs	1.0 ± 1.0	19.8 ± 4.9***	14.1 ± 3.8***	22.4 ± 5.4***
Narrowed frontal bones	0	0	0	1.7 ± 1.5
Nonossified sternebrae (at least one)	6.2 ± 2.3	8.3 ± 2.4	12.0 ± 3.1	19.7 ± 4.7*
Asymmetric sternebrae (at least one)	13.8 ± 4.0	31.7 ± 6.2	28.9 ± 5.9	26.2 ± 5.5
Bipartite vertebral centra (at least one)	14.1 ± 5.0	14.3 ± 6.4	13.2 ± 4.8	34.5 ± 7.2*
Reduced cranial ossification	22.1 ± 6.3	42.4 ± 6.4*	29.6 ± 6.4	66.7 ± 7.1***
Reduced lumbar ossification	0	0	1.8 ± 1.8	5.0 ± 2.0*
Reduced ulna ossification	0	0	0	1.7 ± 1.5
Nonossified sacral vertebra	0	0	0	0.8 ± 0.8
Twisted hind legs[d]	1.0 ± 1.0	3.3 ± 2.0	7.0 ± 3.0*	5.4 ± 2.5

[a] Group mean (±SEM) of litter percentages (affected fetuses in litter).
[b] Acampsia, strongly cartilagenous ankle, no flexure of the tarsal joints.
[c] External, slightly paralyzed.
[d] Inflexibility of a limb, abnormal flexure of the tarsal joints.
* $p \leq 0.05$.
** $p \leq 0.01$.
*** $p \leq 0.001$.

Developmental toxicity study with 2-ethylhexyl-2-ethylhexanoate in Wistar rats (OECD TG 414) (Anonymous, 1997)

A developmental toxicity study with 2-ethylhexyl-2-ethylhexanoate (OECD TG 414) was performed in Wistar rats. In this study on the prenatal toxicity of 2-ethylhexyl-2-ethylhexanoate, 2-EHA was used as positive control at a dose of 600 mg/kg bw/d in Wistar rats from GD 6 to 15 (Anonymous, 1997).

Clear signs of selective developmental toxicity and teratogenicity related to external (adactyly, tail malformations) and skeletal malformations (vertebral column, sternum, ribs, femur) and skeletal and overall variations and retardations were observed in animals of the positive control group treated with 2-EHA.

These results fit well with the above findings described by Pennanen *et al.* (1992).

One-generation reproductive toxicity study (Pennanen *et al.*, 1993)

In a non-GLP and non-guideline one-generation reproductive toxicity study, reproductive toxicity of 2-EHA was evaluated in Wistar rats. Daily average doses of 100, 300 or 600 mg/kg bw/d 2-EHA as a sodium salt in drinking water were administered to groups of 24 Wistar rats per sex and dose level.

Related to offspring parameters, a statistically significant reduction of 16% ($p<0.05$) in the average litter size was observed in the high-dose group. No changes in the number of stillbirths or in postnatal deaths were observed. Nevertheless, postnatal deaths tended to be more common in 2-EHA-treated animals but not dose-related (Table 45).

In the live 2-EHA-exposed pups, the frequency of lethargy, hematomas, abnormally thin hair and abnormal legs was higher at the two highest dose levels. Also at these doses, a statistically significant dose-dependent increase in kinky tail occurred in the pups (Table 45).

Table 45: Effects on litter size, development and survival of pups form the one-generation reproductive toxicity study (Pennanen *et al.*, 1993)

	2-Ethylhexanoic acid dose (mg/kg/day)			
	Control	100	300	600
Litters	23	21	24	23
Live pups	251	214	258	208
Sex ratio[a]	0.53	0.53	0.57	0.58
Stillbirths (M/F)	3/1	0/0	0/2	5/1
Mean litter size Postnatal Day 0	10.9 ± 2.2[c]	10.2 ± 1.9	10.8 ± 2.1	9.2 ± 2.4*
Postnatal deaths (M/F)	0/2 (0.7)[b]	4/7 (5.1)*	0/5 (1.9)	5/1 (2.9)
Lactation index[d]	175/177 (99%)	152/163 (93%)	203/208 (98%)	167/173 (96%)
	Observations on pups			
Kinky tail	13[e] (4.86)[f]	32 (14.99)	66 (24.48)*	54 (25.59)*
Hematomas	7 (2.91)	18 (7.59)	12 (4.56)	12 (7.60)
Hypothermic	0 (0.00)	0 (0.00)	1 (0.32)	9 (4.35)
Thin hair	1 (0.40)	0 (0.00)	12 (5.98)	4 (3.45)
Diarrhea	0 (0.00)	0 (0.00)	2 (0.64)	2 (0.79)
Lethargy	0 (0.00)	0 (0.00)	65 (26.74)*	29 (13.04)
Flabby legs[g]	0 (0.00)	3 (1.30)	7 (2.71)	5 (2.69)
Long, thin legs	0 (0.00)	0 (0.00)	8 (3.17)	2 (1.24)
Twisted hind legs	0 (0.00)	0 (0.00)	2 (0.68)	0 (0.00)

* $p \leq 0.05$.
[a] Males/all pups.
[b] Percentage of pups (males and females).
[c] Means ± SD.
[d] Number of pups live on Postnatal Day 21/Postnatal Day 4.
[e] Number of pups.
[f] Group mean of litter percentages (affected fetuses in litter).
[g] Slightly paralyzed.

Delayed physical development of pups occurred in animals exposed to 2-EHA. In the course of lactation, a transitional decrease in pup body weights was observed at 600 mg/kg bw/d. In addition, it was observed a statistically significant delay in eye opening ($p<0.01$ in males and $p<0.05$ in females), hair growth ($p<0.01$ in boh sexes) or eruption of teeth ($p<0.01$ in both sexes) at the high-dose level, compared to control. At the same time, in the mid- and high-dose groups, the raise of the ears occurred later on time ($p<0.05$). The development of the grip ($p<0.05$ in males of the low- and mid-dose groups and in females of the high-dose group; $p<0.001$ in males of the high-dose group) and cliff avoidance ($p<0.01$ in males and $p<0.05$ in females dosed 600 mg/kg bw/d) reflexes was delayed. A mass in the left testis and the missing of the left epididymis was observed in one male pup at 600 mg/kg bw/d at necropsy (Figures 4 and 5).

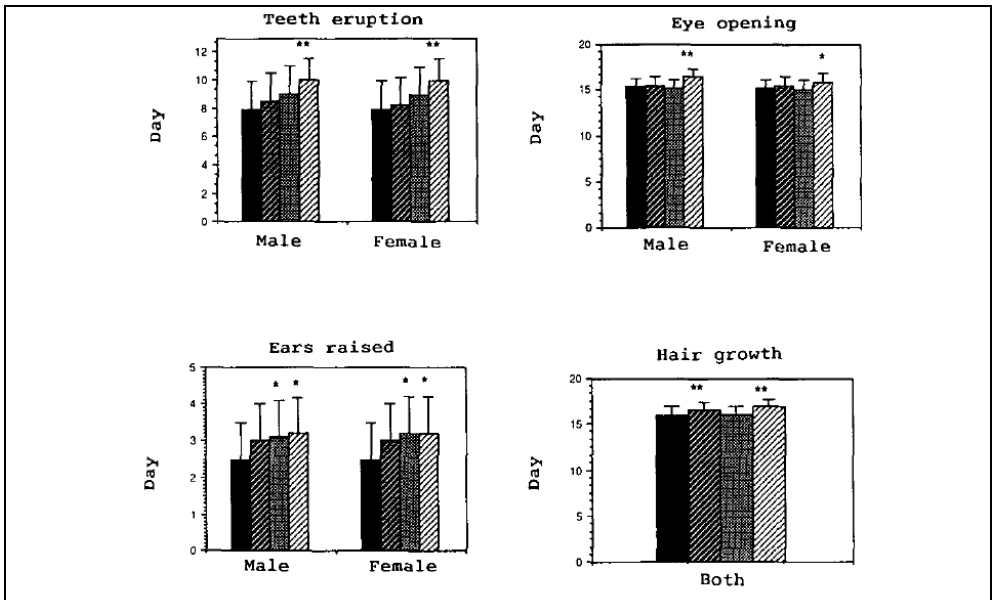


FIG. 4. Physical development of pups. The day when the landmark appeared (means ± SD of all pups in each group). ■, Control; ▨, 100 mg/kg; ▤, 300 mg/kg; ⊡, 600 mg/kg. *$p < 0.05$, **$p < 0.01$, significantly different from control values.

Figure 4. Physical development of pups (Pennanen *et al.*, 1993)

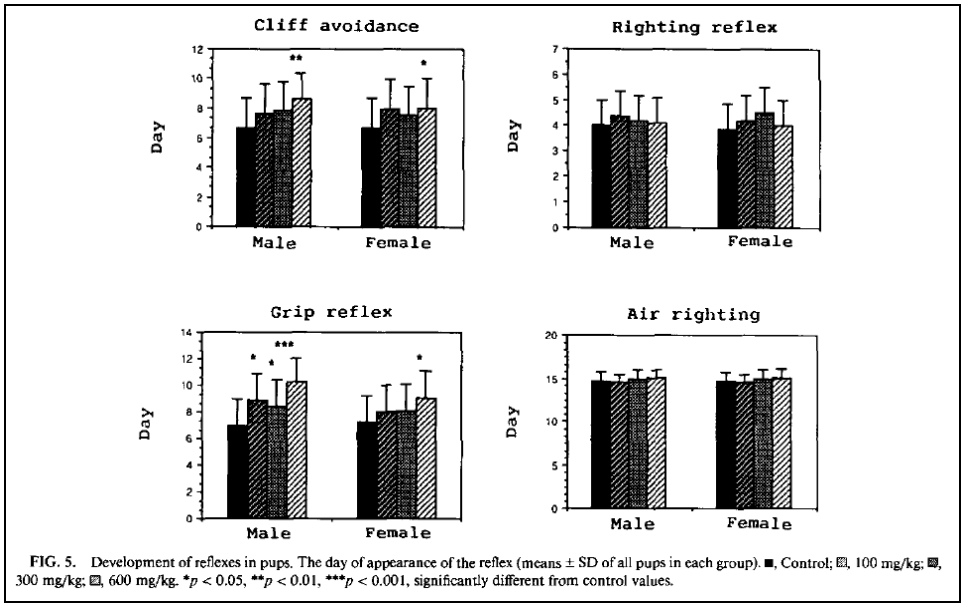


FIG. 5. Development of reflexes in pups. The day of appearance of the reflex (means ± SD of all pups in each group). ■, Control; ▨, 100 mg/kg; ▩, 300 mg/kg; ▧, 600 mg/kg. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, significantly different from control values.

Figure 5. Development of reflexes in pups (Pennanen *et al.*, 1993)

In summary, at 600 mg/kg bw/d, the substance decreased transiently pup weights during lactation. Delayed postnatal development of pups, as noted in the reflex and physical parameters evaluated, was observed at and above 300 mg/kg bw/d.

Oral combined repeated dose toxicity study with the reproduction/developmental toxicity screening test (OECD TG 422) (Anonymous, 2015)

A GLP oral combined repeated dose toxicity study with the reproduction/developmental toxicity screening test (OECD TG 422) was conducted with 2-EHA. This study was used as a dose-range finder for an OECD TG 443 required as a result of the substance evaluation process.

No changes in the incidences of liveborns and stillborns, viability indices and sex ratios of pups and fetuses were reported. In the females of the satellite group, no effects on fetal and placental weights were reported after the caesarian section performed on GD 20. Only a reduction of 14% in the weight of the pups at the highest dose on PND 4 was considered treatment-related.

Oral extended one-generation reproductive toxicity study (OECD TG 443) (Anonymous, 2016)

A GLP extended one-generation reproductive toxicity study performed according to OECD TG 443 was conducted with 2-EHA in Wistar rats following the information requirement included in the substance evaluation final decision under REACH Regulation. The initial study design included cohorts 2 and 3 to assess developmental neurotoxicity (DNT) and immunotoxicity (DIT). The extension of the cohort 1B to produce the second generation was left to the consideration of the Registrant who finally decided to produce the F2 generation to allow drawing a clear and reliable conclusion.

Cohort 1 (1A and 1B) and F2 animals

Details on the general toxicity caused by 2-EHA in the animals included in the different cohorts is described in Section 10.10.2.

In the F1 generation, The mean number of pups per litter was lower in the low- and high-dose groups, being statistically significant in the high-dose group, although no dose-relationship was observed and the number was well within the range of historical control data. No effects were observed on prenatal loss. (Tables 46 and 47).

No effects on the number of pups per litter were observed in the F2 generation pups.

Table 46: Number of pups per litter for F1 generation from the EOGRTS (Anonymous, 2016)

Fertility		Control F 0 mg/kg BW		Low-dose F 80 mg/kg BW		Mid-dose F 250 mg/kg BW	High-dose F 800 mg/kg BW	
Live pups at day 0	Total	313		256		323	261	
Live pups/litter at day 0	Mean	12.0	u	10.2		12.0	10.0	*
	S.d.	2.0		3.4		2.0	2.7	
	N	25		25		27	26	
Live pups at day 4	Total	313		253		322	260	
Live pups/litter at day 4	Mean	12.0	u	10.1		11.9	10.0	*
	S.d.	2.0		3.3		2.0	2.8	
	N	25		25		27	26	
Live pups at day 7	Total	257		222		264	233	
Live pups/litter at day 7	Mean	9.9	u	8.9	*	9.8	9.0	**
	S.d.	0.6		2.4		0.8	1.8	
	N	25		25		27	26	
Live pups at day 14	Total	257		222		264	233	
Live pups/litter at day 14	Mean	9.9	u	8.9	*	9.8	9.0	**
	S.d.	0.6		2.4		0.8	1.8	
	N	25		25		27	26	
Live pups at day 21	Total	257		222		264	233	
Live pups/litter at day 21	Mean	9.9	u	8.9	*	9.8	9.0	**
	S.d.	0.6		2.4		0.8	1.8	
	N	25		25		27	26	
Sex ratio male day 0	%	49.8		42.6		46.7	47.5	
Sex ratio female day 0	%	50.2		57.4		53.3	52.5	
Sex ratio male day 21	%	47.9		45.0		47.3	48.5	
Sex ratio female day 21	%	52.1		55.0		52.7	51.5	

Statistic Profile = DecisionTree, * = p < 0.05, ** = p < 0.01, X = Group excluded from statistics, u=KRUSKAL-WALLIS-DUNN

Sex ratio male day x: number of live male pups * 100 / total number of live pups
Sex ratio female day x: number of live female pups * 100 / total number of live pups

Table 47: Viability of pups of the F1 generation from the EOGRTS (Anonymous, 2016)

Pup fate		Control F 0 mg/kg BW		Low-dose F 80 mg/kg BW		Mid-dose F 250 mg/kg BW	High-dose F 800 mg/kg BW
Alive at day 0	Total	313		256		323	261
Found dead day 0-4	Total	0		0		0	0
	%	0		0		0	0
Killed interim day 0-4	Total	0		1		0	0
	%	0		0.4		0	0
Missing day 0-4	Total	0		2		1	1
	%	0		0.8		0.3	0.4
Missing day 5-7	Total	0		0		0	0
	%	0		0		0	0
Missing day 8-14	Total	0		0		0	0
	%	0		0		0	0
Missing day 15-21	Total	0		0		0	0
	%	0		0		0	0
Alive day 4	Total	313		253		322	260
Viability index 0-4	%	100	k	99.0		99.7	99.6
Culled day 4	Total	56		31		58	27
	%	17.9		12.1		18.0	10.3
Alive day 21	Total	257		222		264	233
Viability index 5-21	%	100.0		100.0		100.0	100.0

Statistic Profile = DecisionTree, * = p < 0.05; ** = p < 0.01; X = Group excluded from statistics; k=KRUSKAL-WALLIS

Viability index : number of live pups at day 0 - number of live pups at day 4 * 100 / number of live pups at day 0

Regarding developmental parameters, no treatment-related effects on the perinatal loss, incidences of liveborn and stillborn pups, viability indices, sex ratios, pup weights, pup organ weights, clinical signs or macroscopic observations, were observed in pups of the F1 and F2 generations. In the F1 generation pups, no effects were observed on nipple retention and on sexual maturation parameters (preputial separation and vaginal opening). In addition, no treatment-related effects were reported on the developmental of the follicles from primordial small follicles into corpora lutea.

Statistically significant increase (+9.15%) of the anogenital distance (AGD) after correction for pup weight was observed in F1-generation male pups of the high-dose group on PND 4 (Table 48). Nevertheless, these effects were considered fortuitous and no treatment-related since in case of an anti-androgenic activity a decrease in the AGD would be expected, but not an increase. The lack of treatment-related effects was confirmed in the F2 generation pups where no changes in this parameter were observed between PND 0 and PND 4 (Table 49).

Table 48: Anogenital distance on lactation day 4 in F1generation male pups from the EOGRTS (Anonymous, 2016)

Anogenital distance males per litter

	Control M 0 mg/kg	Low-dose M 80 mg/kg	Mid-dose M 250 mg/kg	High-dose M 800 mg/kg	
Litter mean	5,90	6,12	6,15	6,44	***
S.d.	0,42	0,40	0,44	0,43	
N	26	23	27	25	

AGD/cub root BW males per litter

	Control M 0 mg/kg	Low-dose M 80 mg/kg	Mid-dose M 250 mg/kg	High-dose M 800 mg/kg	
Litter mean	2,61	2,67	2,70 *	2,78	***
S.d.	0,13	0,14	0,11	0,14	
N	26	23	27	25	

* = P < 0.05; *** = P < 0.001

Table 49: Anogenital distance on lactation day 4 in F2 generation male pups from the EOGRTS (Anonymous, 2016)

Anogenital distance females per litter

Day 0	Control F 0 mg/kg	Low-dose F 80 mg/kg	Mid-dose F 250 mg/kg	High-dose F 800 mg/kg
Litter mean	2.00	1.99	2.06	2.05
S.d.	0.22	0.12	0.13	0.17
N	24	25	23	24

AGD/cub root BW females per litter

Day 0	Control F 0 mg/kg	Low-dose F 80 mg/kg	Mid-dose F 250 mg/kg	High-dose F 800 mg/kg
Litter mean	1.08	1.07	1.11	1.10
S.d.	0.10	0.06	0.05	0.09
N	24	25	23	24

Statistics: Anova + Dunnett

Cohort 2 (2A and 2B) animals

Male animals of the high-dose groups of cohort 2A showed.lower mean body weights than the control group during the entire period, reaching the level of statistically significance on days 14, 28 and 35. A statistically significant decrease in the mean body weight gain for this group was observed from days 7 to 14, compared to controls. No statistically significant effects were observed on food consumption (Table 50).

Female animals of cohort 2A did not showed differences on body weights and body weight gain. Nevertheless, a statistically significant decrease on food consumption from days 28-35 and 42-49 was observed at the high-dose group (Table 51).

Table 50: Body weight (in grams) and food consumption data for cohort 2A male animals from the EOGRTS (Anonymous, 2016)

		0 mg/kg bw/d	80 mg/kg bw/d	250 mg/kg bw/d	800 mg/kg bw/d
Cohort 2A - Mean body weight	Day 0	70.10	71.08	67.59	62.41
	Day 7	114.42	116.66	112.80	102.92
	Day 14	165.18	165.78	162.32	**147.77* (-10.64%)**
	Day 21	206.72	210.81	203.81	188.60
	Day 28	255.20	260.63	251.63	**232.23* (-9.00%)**
	Day 35	294.51	300.46	290.71	**269.32* (-8.55%)**
	Day 42	320.88	330.33	319.49	294.88
	Day 49	341.68	351.21	338.95	314.38
Cohort 2A - Mean body weight gain	D 0-7	44.32	45.58	45.21	40.51
	D 7-14	50.76	49.12	49.52	**44.85* (-11.64%)**
	D 14-21	41.54	45.03	41.49	40.83
	D 21-28	48.48	49.82	47.82	43.63
	D 28-35	39.31	39.83	39.08	37.09
	D 35-42	26.37	29.87	28.78	25.56
	D 42-49	20.80	20.88	19.46	19.50
Cohort 2A - Mean food consumption	D 0-7	13.75	13.44	12.83	12.24
	D 7-14	18.32	19.50	18.56	17.03
	D 14-21	19.31	20.48	19.43	18.45
	D 21-28	21.32	22.22	21.13	19.35
	D 28-35	23.26	23.52	23.20	20.84
	D 35-42	21.69	22.13	22.48	19.86
	D 42-49	21.99	22.02	22.23	19.85

*: $p < 0.05$

Table 51: Body weight (in grams) and food consumption data for cohort 2A female animals from the EOGRTS (Anonymous, 2016)

		0 mg/kg bw/d	80 mg/kg bw/d	250 mg/kg bw/d	800 mg/kg bw/d
Cohort 2A - Mean body weight	Day 0	64.85	61.46	60.97	58.01
	Day 7	101.15	98.80	97.87	92.65
	Day 14	135.37	131.85	129.53	124.79
	Day 21	153.38	151.39	148.86	142.99
	Day 28	174.16	169.93	170.17	161.20
	Day 35	190.76	185.93	184.97	174.70
	Day 42	200.53	196.37	197.28	184.30
	Day 49	211.51	207.89	203.46	195.25
Cohort 2A - Mean body weight gain	D 0-7	36.30	37.34	36.90	34.64
	D 7-14	34.22	33.05	31.66	32.14
	D 14-21	18.01	19.54	19.33	18.20
	D 21-28	20.78	18.54	21.31	18.21
	D 28-35	16.60	16.00	14.80	13.50
	D 35-42	9.77	10.44	12.31	9.60
	D 42-49	10.98	11.52	6.18	10.95
Cohort 2A - Mean food consumption	D 0-7	11.70	11.83	11.19	10.38
	D 7-14	14.59	14.98	14.45	13.60
	D 14-21	13.81	14.61	13.82	13.38
	D 21-28	14.31	14.73	14.06	13.14
	D 28-35	15.49	15.07	14.85	**13.74* (-11.29%)**
	D 35-42	14.68	14.85	14.28	12.92
	D 42-49	15.42	15.18	13.98	**13.16* (-14.65%)**

*: $p < 0.05$

Regarding neuro (developmental) parameters, no treatment-related effects were reported from functional observatory battery (FOB) and spontaneous motor activity analysis in cohort 2A of the F1 generation. The auditory startle response did not show a neurotoxic potential of the test substance. Mean absolute brain weight of the male animals of the high-dose group was slightly, but statistically significantly, lower as compared to the control group. No changes were observed in female animals (Table 52).

Table 52: Brain measurements for cohort 2A animals from the EOGRTS (Anonymous, 2016)

		0 mg/kg bw/d	80 mg/kg bw/d	250 mg/kg bw/d	800 mg/kg bw/d
♂	Terminal body weight (g)	343.01	353.32	341.95	315.90
	Absolute brain weight (g)	1.899	1.851	1.867	**1.797** (-5.37%)**
	Brain relative weight (g/kg bw)	5.564	5.260	5.490	5.709
	Brain length (mm)	21.763	21.686	21.610	21.492
	Brain width (mm)	16.145	16.054	16.266	16.086
♀	Terminal body weight	216.92	213.73	209.16	198.42
	Absolute brain weight (g)	1.673	1.683	1.635	1.624
	Brain relative weight (g/kg bw)	7.721	7.919	7.837	8.227
	Brain length (mm)	20.607	20.580	20.648	20.504
	Brain width (mm)	15.429	15.492	15.209	15.357

**: $p < 0.01$

In cohort 2B, mean absolute brain weight of the male animals of the mid- and high-dose groups was slightly, but statistically higher as compared to the control group. Nevertheless these findings were considered not to be related to treatment since no effects were observed on absolute brain weight in females and on the relative brain weights of male and female animals (Table 53). In addition, no differences were observed in the brain length and brain width measurements of cohorts 2A and 2B (Tables 52 and 53). Thicknesses of the 10 major brain regions measured did not show any variation in cohort 2A animals. No macroscopic or microscopic effects were reported in animals of cohorts 2A and 2B.

Table 53: Brain measurements for cohort 2B animals from the EOGRTS (Anonymous, 2016)

		0 mg/kg bw/d	80 mg/kg bw/d	250 mg/kg bw/d	800 mg/kg bw/d
♂	Terminal body weight (g)	50.84	47.84	51.38	52.52
	Absolute brain weight (g)	1.319	1.320	**1.380* (+4.62%)**	**1.398** (+5.99%)**
	Brain relative weight (g/kg bw)	26.102	27.873	27.039	26.813
	Brain length (mm)	18.382	18.452	18.531	18.506
	Brain width (mm)	14.504	14.545	14.775	14.746
♀	Terminal body weight	49.83	50.96	49.92	47.05
	Absolute brain weight (g)	1.289	1.304	1.277	1.331
	Brain relative weight (g/kg bw)	26.148	25.974	25.729	28.564
	Brain length (mm)	18.199	18.189	18.038	18.073
	Brain width (mm)	14.393	14.221	14.228	14.403

*: $p < 0.05$; **: $p < 0.01$

Cohort 3 animals

One dead male was reported for the cyclosporine A positive control group. Mean body weights of the male animals of the high-dose and of the positive control groups and mean body weight changes of the male animals of the low-dose, high-dose and control groups were statistically significantly decreased as compared to the control group. Food consumption was statistically significantly decreased from days 28 to 25 in the cyclosporine A positive control group (Table 54). For females, only the positive control group showed statistically significant increases in mean body weight and mean body weight gain. In addition, food consumption was increased in the low-dose form days 7-14 and in the positive control group from days 7-14 and 28-35 (Table 55).

Table 54: Body weight (in grams) and food consumption data for cohort 3 male animals from the EOGRTS (Anonymous, 2016)

		0 mg/kg bw/d	80 mg/kg bw/d	250 mg/kg bw/d	800 mg/kg bw/d	Positive control
Cohort 3-Mean body weight	Day 0	65.55	69.76	67.12	59.36	68.87
	Day 7	110.65	118.36	114.31	98.62	114.78
	Day 14	163.98	166.91	165.65	**142.71** (-12.97%)**	162.55

	Day 21	207.06	214.03	208.41	**180.58** (-12.79%)**	206.13
	Day 28	258.47	264.65	257.12	**223.36** (-13.58%)**	239.78
	Day 35	292.15	297.70	289.80	**252.22** (-13.67%)**	**260.82* (-10.72%)**
Cohort 3- Mean body weight gain	D 0-7	45.10	48.60	47.19	**39.26* (-12.94%)**	45.92
	D 7-14	53.33	**48.55* (-8.96%)**	51.34	**44.09** (-17.32%)**	47.77
	D 14-21	43.08	47.12	42.76	**37.87* (-12.09%)**	43.58
	D 21-28	51.41	50.62	48.71	**42.78** (-16.78%)**	**37.42** (-27.21%)**
	D 28-35	33.68	33.05	32.68	**28.86** (-14.31%)**	**21.04** (-37.52%)**
Cohort 3- Mean food consumption	D 0-7	12.96	13.72	13.37	11.55	14.22
	D 7-14	18.33	19.39	19.00	16.46	18.60
	D 14-21	19.24	20.30	19.37	17.64	18.92
	D 21-28	22.02	22.25	21.60	18.80	19.70
	D 28-35	23.64	24.46	23.74	20.60	**19.54* (-17.34%)**

*: p < 0.05; **: p< 0.01**Table 55: Body weight (in grams) and food consumption data for cohort 3 female animals from the EOGRTS (Anonymous, 2016)**

		0 mg/kg bw/d	80 mg/kg bw/d	250 mg/kg bw/d	800 mg/kg bw/d	Positive control
Cohort 3- Mean body weight	Day 0	61.97	62.56	62.37	58.24	66.72
	Day 7	98.95	99.85	100.23	94.44	105.92
	Day 14	131.10	133.13	131.65	125.85	136.63
	Day 21	154.28	151.76	152.40	145.96	158.58
	Day 28	171.27	172.50	173.13	166.30	179.70
	Day 35	182.46	186.05	184.32	178.92	**201.05* (+10.18%)**
Cohort 3- Mean body weight gain	D 0-7	36.98	37.29	37.86	36.20	39.20
	D 7-14	32.15	33.28	31.42	31.41	30.72
	D 14-21	23.18	18.63	20.75	20.11	21.95
	D 21-28	16.99	20.74	20.73	20.34	21.12
	D 28-35	11.19	13.55	11.19	12.62	**21.35** (+90.79%)**
Cohort 3- Mean food consumption	D 0-7	11.99	11.99	11.57	10.59	13.02
	D 7-14	14.58	**15.31* (+5%)**	14.67	13.99	**15.57* (+6.79%)**
	D 14-21	14.50	14.27	14.51	13.34	14.58
	D 21-28	14.90	14.72	14.75	14.30	14.60
	D 28-35	15.26	15.98	15.01	14.42	**17.75* (+16.31%)**

*: p < 0.05; **: p< 0.01Terminal body weight was statistically significantly decreased in male animals of the high-dose and of the positive control group. In these groups also the absolute weight of the spleen was decreased. In the male animals of the positive control group, the absolute weight of the thymus was statistically different as compared to the control group. Nevertheless, no effects were observed in relative weights of the spleen and thymus amongst the groups (Table 56). Macroscopic observations did not reveal any treatment-related abnormalities.

Table 56: Absolute and relative organ weights of cohort 3 animals from the EOGRTS (Anonymous, 2016)

			0 mg/kg bw/d	80 mg/kg bw/d	250 mg/kg bw/d	800 mg/kg bw/d	Positive control
♂	Terminal body weight		304.30	309.33	300.65	**261.25**(-14.15%)**	264.00
	Spleen	Absolute weight	0.6886	0.6986	0.6550	**0.5880*(-14.60%)**	**0.5392* (-21.69%)**
		Relative weight	2.270	2.262	2.179	2.260	2.038
	Thymus	Absolute weight	0.6517	06510	0.5904	0.6132	**0.470** (-27.88%)**
		Relative weight	2.149	2.109	1.969	2.339	1.779
♀	Terminal body weight		186.63	186.17	187.75	179.19	206.33
	Spleen	Asolute weight	0.4131	0.4606	0.4370	0.4389	**0.4298* (+10.55%)**
		Relative weight	2.217	2.474	2.327	2.456	2.090
	Thymus	Absolute weight	0.4345	0.4571	0.4525	0.4608	0.4192
		Relative weight	2.341	2.463	2.406	2.576	2.040

*: p < 0.05; **: p < 0.01

Regarding immune (developmental) parameters, no treatment-related effect was observed on the composition of the splenic lymphocyte subpopulation in animals of the cohort 3. In addition, the substance had no effect on the KLH specific IgM antibody levels in animals of the cohort 3, compared with positive control cyclosporine A group (Table 57).

Table 57: Group mean KLH-specific IgM antibody levels for cohort 3 animals from the EOGRTS (Anonymous, 2016)

Group	Name	sex	N	Mean (ng/mL)	SD	p-value[1]
1	Control	♂	10	850886	442634	n.s.
2	Low dose	♂	10	920849	527018	n.s.
3	Mid dose	♂	9*	901149*	405398*	n.s.
4	High dose	♂	10	567733	208474	n.s.
5	Positive control	♂	5#	158231	36672	P<0.0001
1	Control	♀	10	902531	704780	n.s.
2	Low dose	♀	10	707962	303970	n.s.
3	Mid dose	♀	10	1052771	371610	n.s.
4	High dose	♀	10	807232	440827	n.s.
5	Positive control	♀	6	226135	88427	P=0.0004

[1] See statistical analysis report; n.s. not significant

* Rat 113-04 was identified as a statistical outlier and excluded from the group mean calculation.

No data are available of rat 043-03

Specific investigations

A mechanistic study was conducted to determine the influence of 2-EHA on maternal zinc metabolism and its relation to the developmental effects (Bui *et al*., 1998). The results of this non-GLP and non-guideline study would support the hypothesis that the developmental toxicity of 2-EHA may be mediated, in part, by its influence on maternal zinc metabolism that causes embryonic zinc deficiency and trigger abnormal development. However, effects of 2-EHA on zinc metabolism were not confirmed in the OECD TG 422 study performed in 2015, where no zinc deficiency in the liver and kidney was observed.

Summary of the available studies

2-EHA has been shown to cause adverse effects on development in a non-GLP developmental toxicity study in Wistar rats at dose levels (up tp 600 mg/kg bw/d) that did not cause a clear maternal toxicity (Pennanen *et al.*, 1992). Increases in the frequency of skeletal malformations and variations, with clubfoot as the most frequently significantly anomaly, were observed at the two highest doses tested. Dose-dependent significant increases of visceral malformations were also observed at these doses. These results fit well to the findings in another prenatal developmental study with 2-ethylhexyl-2-ethylhexanoate where 2-EHA was used as the positive control substance at a dose of 600 mg/kg bw/d (Anonymous, 1997).

In another two developmental toxicity studies in Fischer 344 rats and New Zealand white rabbits, daily doses of 2-EHA up to 500 mg/kg bw/d (rat) and 250 mg/kg bw/d (rabbit) were administered by oral gavage as solutions in corn oil during organogenesis (Anonymous, 1988c; 1988d; Hendrickx *et al.*, 1993). In rats, foetotoxic alterations were seen in the form of reduced foetal body weights, visceral and skeletal variations. Although these variations began to be observed at 250 mg/kg bw/d, most of them only occurred at 500 mg/kg bw/d, the dose which did cause maternal toxicity (deaths and decreased body weights)

In the parallel developmental toxicity study carried out in rabbits, no findings related to embryotoxic, foetotoxic or teratogenic effects were observed up to the highest dose tested. Maternal toxicity was manifested by the incidence of death and abortion (abortion could be considered as a secondary non-specific effect due to maternal toxicity (deaths) at the same dose level).

Results obtained from these studies showed a relatively higher sensitivity to 2-EHA in rats compared to rabbits since foetotoxic activity (reduced ossification) in the rat was observed even at doses which did not cause maternal toxicity, while these effects did not occur in rabbits.

In addition, some information on the developmental effects of 2-EHA was obtained from a non-GLP and non-guideline one-generation reproductive toxicity study in Wistar rats where a statistically significant reduction in the average litter size was observed in the high-dose group. Furthermore, delayed physical

development of pups occurred in animals exposed to 2-EHA: delay in eye opening, hair growth or eruption of teeth at the high-dose level. At the same time, in the mid- and high-dose groups, the raise of the ears occurred later on time. The development of the grip and cliff avoidance reflexes was also delayed. The incidence of kinky tail was statistically significant at the mid- and high-dose groups. Even though in this study, a slight but statistically significant reduction in water consumption of 14%, a significant maternal body weight reduction of 9 to 12% from gestational day 7 onwards and a statistically significantly decrease in the gestational weight gain ($p<0.01$), were observed in females at 600 mg/kg bw/d, compared to control group, several developmental effects were observed at lower doses where this maternal toxicity was not reported. (Pennanen *et al.*, 1993).

The results obtained in the recently performed OECD TG 422 and OECD TG 443 studies performed in Wistar rats at doses up to 800 mg/kg bw/d 2-EHA did not show any treatment-related effects regarding developmental effects or developmental neurotoxicity and immunotoxicity in the corresponding cohorts (Anonymous, 2015; 2016). Nevertheless, these studies are not designed to provide information on substance-induced effects on growth and survival of the foetuses, and increased incidences in external, skeletal and soft tissue malformations and variations in foetuses.

10.10.6 Comparison with the CLP criteria

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

  on development;

- effects on or via lactation.

Concerning adverse effects on development of the offspring, the CLP regulation states as a basis of classification: *"Developmental toxicity includes, in its widest sense, any effect which interferes with normal development of the conceptus, either before or after birth, and resulting from exposure of either parent prior to conception, or exposure of the developing offspring during prenatal development, or postnatally, to the time of sexual maturation. However, it is considered that classification under the heading of developmental toxicity is primarily intended to provide a hazard warning for pregnant women, and for men and women of reproductive capacity. Therefore, for pragmatic purposes of classification, developmental toxicity essentially means adverse effects induced during pregnancy, or as a result of parental exposure. These effects can be manifested at any point in the life span of the organism. The major manifestations of developmental toxicity include (1) death of the developing organism, (2) structural abnormality, (3) altered growth, and (4) functional deficiency".*

The CLP regulation criteria for classification as reproductive toxicants have been previously mentioned in section 10.10.3.

Rationale for classification

2-Ethylhexanoic acid was found to cause developmental effects in a non-GLP developmental toxicity study (Pennanen *et al.*, 1992) in Wistar rats at doses of 100, 300 and 600 mg/kg bw/d 2-EHA as sodium salt via drinking water, during gestational days 6 to 19. Skeletal variations (wavy ribs, reduced ossification) and skeletal malformations (clubfoot) were observed at dose levels without maternal toxicity. These adverse effects were the basis for the classification of 2-EHA as toxic for reproduction, category 3, according to the criteria of Directive 67/548/EEC. Accordingly, the corresponding classification in Table 3.1 of Annex VI to CLP was Repr. 2 (H361d).

Results showed that 2-EHA affected normal development of foetuses at all dose levels. Dose-dependent increases in the number of foetuses with skeletal or visceral anomalies were observed at all dose levels. Clubfoot occurred in all treatment groups, being only statistically significant at the two highest doses. The

major skeletal variations were related to non-dose-dependent increases in the incidence of wavy ribs, observed in all treatment groups, and reduced cranial ossification, observed at 100 and 600 mg/kg bw/d. Unossified sternebrae, reduced ulna/lumbar ossification, bipartite vertebral centra and twisted hind legs were other variations observed, with lower incidence, at the highest dose.

Only few visceral malformations were found. The degree of dilation of brain ventricles, which is inversely related to the developmental stage of conceptus, was increased in the dose groups of 300 and 600 mg/kg bw/d, being statistically significant at 600 mg/kg bw/d. Non-dose related but statistically significant increase of pelvic dilation of the urinary tract was observed at 100 and 300 mg/kg bw/d, although this variation was also common in control groups.

These results fit well to the findings in another prenatal developmental study with 2-ethylhexyl-2-ethylhexanoate where 2-EHA was used as the positive control substance (Anonymous, 1997). Clear signs of selective developmental toxicity and teratogenicity related to external (adactyly, tail malformations) and skeletal malformations (vertebral column, sternum, ribs, femur) and skeletal and overall variations and retardations were reported.

In addition, in the one-generation reproductive toxicity study with 2-EHA in Wistar rats, delayed physical development of pups and delayed development of the grip and cliff avoidance reflexes was also noted. Furthermore, the incidence of kinky tail was statistically significant at the mid- and high-dose groups. These effects were observed at doses where maternal toxicity was not observed (only slight reductions in body weight and body weight gain were observed at the highest dose) (Pennanen *et al.*, 1993). Nevertheless, developmental neurotoxicity was not confirmed in the EOGRT study, where these effects were further evaluated with the inclusion of the DNT cohort.

There are no human reproductive data on 2-EHA or its salts, therefore they are not candidate for Category 1A.

As established in the CLP criteria, classification in Category 1B should be chosen if data 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. On the other hand, classification in Category 2 should be chosen 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.

In this case, even though clear developmental effects were observed in a non-GLP developmental toxicity study (Pennanen *et al.*, 1992), some of them were not dose-dependent (skeletal variations such as wavy ribs) or were non-dose related (visceral malformation of pelvic dilation of the urinary tract).

In a developmental toxicity study in Fischer 344 rats (Hendrickx *et al*., 1993), foetotoxic alterations began to be observed at 250 mg/kg but most of them were only seen at 500 mg/kg, the dose which did cause maternal toxicity (hypoactivity, ataxia, audible respiration, ocular discharge and periocular encrustation). Therefore, the influence of maternal toxicity on developmental effects cannot be excluded. The same study performed in New Zealand white rabbits did not show developmental effects.

In addition, results obtained in the recently performed OECD TG 422 and OECD TG 443 did not show any treatment-related developmental effects. Nevertheless, these studies are not designed to provide information on substance-induced effects on growth and survival of the foetuses, and increased incidences in external, skeletal and soft tissue malformations and variations in foetuses.

Finally, according to a mechanistic study (Bui *et al.,* 1998) it was suggested that developmental toxicity of 2-EHA may be modulated, in part, by its influence on maternal zinc metabolism that causes embryonic zinc deficiency and trigger abnormal development. Nevertheless, effects of 2-EHA on zinc metabolism were finally not confirmed in the OECD TG 422 study performed in 2015, where no zinc deficiency in the liver and kidney was observed.

In summary, taking into account the whole available data from the reproductive toxicity studies with 2-EHA, it has been considered that it is justified the current classification with respect to developmental toxicity as Repr. 2 (H361d) in accordance with the criteria for classification as defined in Annex I, Regulation (EC) No.

1272/2008 (CLP). This classification is appropriate as there is some evidence from experimental animals of adverse effects on development, but this evidence is not sufficiently convincing to place the substance in Category 1B.

This classification for reproductive toxicity of 2-EHA is made extensive to all its salts according to the category approach and the read-across hypothesis based on the formation and bioavailability of 2-EHA from all the salts (See "Justification for the grouping approach" in section 10).

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

    on 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-EHA adverse effect on or via lactation. Therefore, no classification is proposed.

10.10.8 Conclusion on classification and labelling for reproductive toxicity

All the salts of 2-EHA have the common feature of readily dissociation to the corresponding cation and 2-ethylhexanoate anions. Further protonation at acidic pH may allow bioavailability of 2-ethylhexanoic acid. Therefore, it is assumed that all category members share at least the same mode of action than the free acid, independently of the effects due to the cation moiety. Thus, provided that the cations do not merit a more severe classification for the toxicity for reproduction and/or additional hazards, the classification and labelling established for 2-EHA in the Annex VI to CLP (index no. 607-230-00-6) as Repr. 2 (H361d) shall be applied also to the salts of 2-EHA.

At this regard, in order to take into account the potential effects due to the cationic moiety, the following note is proposed as part of this proposal: "*The classification for the hazard class(es) in this entry is based only on the hazardous properties of the part of the substance which is common to all members in the entry. The hazardous properties of any member in the entry also depends on the properties of the part of the substance which is not common to all members of the group; they must be evaluated to assess whether (a) more severe classification(s) (e.g. a higher category) or (b) a broader scope of the classification (additional differentiation, target organs and/or hazard statements) might apply for the hazard class(es) in the entry*".

RAC evaluation of reproductive toxicity

Summary of the Dossier Submitter's proposal

Fertility

The dossier submitter (DS) discussed reduced sperm motility and delayed fertilisation in the non-guideline study by Pennanen *et al.* (1993). As these effects were not reproduced in the high-quality and GLP compliant EOGRTS (Anonymous, 2016) and both the EOGRTS and the range-finding study (Anonymous, 2015), the studies were considered negative with regard to sexual function and fertility, the DS proposed no classification.

Development

The DS presented guideline PNDT studies with 2-EHA in rats (Anonymous, 1988c) and rabbits (Anonymous, 1988d), a rat PNDT study from the literature (Pennanen *et al.*, 1992) and briefly also a rat PNDT study with another substance where 2-EHA was used as a positive control (Anonymous, 1997). Relevant findings from the generational studies were also discussed.

The DS considered the current classification in Category 2 justified mainly due to the following effects:

- Clubfoot, skeletal variations (wavy ribs, reduced ossification) and dilated brain ventricles in the absence of maternal toxicity in the rat PNDT study by Pennanen *et al.* (1992)
- Tail malformations, skeletal malformations and skeletal variations in the rat PNDT study by Anonymous (1997)
- Kinky tail and delayed development in the rat one-generation study by Pennanen *et al.* (1993); the DS noted that no developmental delay was observed in the EOGRTS by Anonymous (2016)
- Skeletal and visceral variations in the rat PNDT study by Anonymous (1988c) in presence of some maternal toxicity

The DS noted the absence of developmental effects in the generational studies by Anonymous (2015, 2016). However, they pointed out that these studies are not specifically designed for detection of post-implantation loss and malformations/variations.

Lactation

The DS proposed no classification due to lack of data.

Read-across from 2-EHA to its salts

While a number of studies investigating reproductive toxicity are available for 2-EHA or its sodium salt, the DS was not aware of reproductive toxicity studies with other salts. Read-across from 2-EHA has been proposed by the REACH registrants of 2-EHA metal salts for the vast majority of human health endpoints including reproductive toxicity. All registered salts of 2-EHA screened by the DS have been self-classified by the registrants as Repr. 2; H361d.

No bioavailability studies are available for any salt of 2-EHA. Still, the DS considered the read-across appropriate as they expected the salts to dissociate to a significant extent already in the

neutral pH range and then completely and rapidly at the low pH in the stomach.

The influence of the cation on overall toxicity of the specific salts should be evaluated independently. This is specified in a note that is proposed to be part of the entry.

Comments received during the Consultation

Comments were received from 2 MSCAs and 2 industry commenters.

One MSCA proposed Category 1B for development based on analogy with valproic acid. They proposed to take into account a non-guideline teratogenicity study by Ritter *et al.* (1987) where both substances (2-EHA and valproic acid) showed similar effects. The DS replied that during the substance evaluation process, the analogy with valproic acid was only used as one of the reasons for inclusion of a developmental neurotoxicity cohort in the EOGRTS, and the EOGRTS was negative regarding developmental neurotoxicity. As for the study by Ritter *et al.* (1987), the DS pointed out that 2-EHA was less potent than valproic acid in this study and hypothesized that 2-EHA may have a different mode of action; in addition, the study had a non-standard design (a single dose on GD 12). Overall, the DS considered Category 2 more appropriate than Category 1B because some of the effects were not dose-related (e.g. wavy ribs in Pennanen *et al.*, 1992) or occurred in the presence of maternal toxicity (Anonymous, 1988c) and no developmental toxicity was observed in the recent EOGRTS.

The other MSCA supported the read-across, but again recommended considering Repr. 1B; H360D mainly based on clubfoot, kinky tail and delayed development in the studies by Pennanen *et al.* (1992, 1993). Although these effects were not seen in the other rat PNDT study (Anonymous, 1988c) and in the EOGRTS (Anonymous, 2016), this may be due to the use of different strains and different administration forms. This MSCA also requested further details on the PNDT study by Anonymous (1997).

One of the industry commenters strongly opposed to the proposed read-across, arguing that mere theoretical considerations without actual *in vivo* toxicokinetic and repeat dose studies with the salts do not provide sufficient justification for such a read-across. They pointed out differences in physicochemical properties of the salts (water solubility, lipophilicity, metal basicity) that are likely to result in differences in toxicokinetic behaviour.

This industry commenter further disagreed with skeletal variations being used as a reason for classification and advised against inclusion of the non-guideline and non-GLP study Pennanen *et al.* (1992) in the assessment. The DS replied that the classification is based mainly on malformations and maintained that the studies by Pennanen *et al.* are valid. In addition, the DS pointed out the observed skeletal malformations in the GLP study by Anonymous (1997).

The other industry commenter provided a summary of a new OECD TG 422 study with iron tris(2-ethylhexanoate). The study was negative and the commenter proposed that no read-across from 2-EHA is needed for this particular salt as substance-specific data are available. The DS explained that a screening according to OECD TG 422 does not provide complete information on all aspects of reproductive toxicity and cannot be used to disregard positive PNDT studies with 2-EHA.

Additional key elements

Reprotoxicity screening in rats according to OECD TG 422 with iron tris(2-ethylhexanoate)

This study was submitted during the Consultation. Iron tris(2-ethylhexanoate) in corn oil was administered via gavage to Sprague-Dawley rats at 0, 75, 150 and 300 mg/kg bw/d. Both sexes (10 animals/sex/group) were treated for 14 days prior to mating and during mating, females throughout gestation and lactation until day 13 postpartum. Males were treated for a total 32-33 days, females for 42-63 days. Additional recovery groups sacrificed 4 weeks after the end of treatment were also included. No significant general or reproductive toxicity was observed in this study.

Assessment and comparison with the classification criteria

Adverse effects on fertility and sexual function

One-generation reproductive toxicity study in rats (Pennanen et al., 1993)

In this non-guideline study from literature, male and female Han:Wistar rats (23-24/sex/dose) were administered sodium salt of 2-EHA in drinking water. Males were exposed for 10 weeks and females for 2 weeks before mating, both sexes during mating, and females during gestation and lactation. The top dose of 600 mg/kg bw/d had a modest effect on body weight (generally reduction by <10% as compared to controls, limited information available in the publication).

Sperm analysis did not show any clear treatment-related effect, but the range of parameters investigated was rather limited and the validity of motility results is questionable (motility in the control was 35% while ≥70% is required by OECD GD 43). The substance seems to have caused a slight delay in fertilisation (see the table below).

1-generation study Pennanen *et al.* (1993): effects related to fertility and sexual function

Dose (mg/kg bw/d)	**0**	**100**	**300**	**600**
Total no. of females	23	23	24	24
Females pregnant in oestrous cycle:				
1	21	20	22	17
2	2	0	0	2
3	0	1	1	2
4	0	0	1	2
Non pregnant	0	2	0	1

Reprotoxicity screening in rats according to OECD TG 422 (Anonymous, 2015)

This study served as a dose-range finding study for the EOGRTS (Anonymous, 2016). 2-EHA was administered via diet to Wistar rats at dietary concentrations of ca. 1500, 4600 and 15000 ppm. The top dose corresponded to 760-800 mg/kg bw/d in males and 810-1370 mg/kg bw/d in females. Dams were sacrificed between lactation day 4 and 7. Additional satellite groups were included for investigation of zinc and metallothionein levels and peroxisome proliferation.

General toxicity at the top dose was manifested as reduced body weight (by up to 10% as compared to controls) and food consumption, increased liver weight and in males additionally increased kidney weight and proteinaceous droplets in renal tubules. Reduced pup weight on PND 4 (by 14%) was the only reproductive finding in this study.

EOGRTS in rats (Anonymous, 2016)

In this GLP and OECD TG 443 compliant study, 2-EHA was administered via diet to Wistar rats at dietary concentrations of ca. 0, 1200, 3800 and 12000 ppm; the dietary concentrations were reduced to 50% during lactation in order to adjust for the increased food intake of the dams during this period. The target doses were 0, 80, 250 and 800 mg/kg bw/d, the actual doses are shown in the following table.

EOGRTS Anonymous (2016): mean test item intakes (mg/kg bw/d) at the top dose

	Males	Females
F0 premating	660	800
F0 gestation	-	840
F0 lactation	-	1030
F1 – cohort 1A	1170	1150
F1 – cohort 1B premating	1040	1060
F1 – cohort 1B gestation	-	740
F1 – cohort 1B lactation	-	1030
F1 – cohort 2A	1250	1200
F1 – cohort 3	1420	1340

Body weight and food consumption were decreased but the decrease was not large (body weight reduction mostly by <10% as compared to controls), males were generally affected more than females. Both males and females at the top dose showed increased liver weight without histopathological correlates, males had additionally increased kidney weight with increased incidence of proteinaceous droplets in renal tubuli (not related to α2u-globulin). Two top dose F0 males were killed moribund; one of them had a fast-growing tumour, the other one showed changes in the respiratory tract and blood clots in the stomach.

There was no effect on mating index, fertility index, pre-coital time, gestation index, sperm parameters, weight and histopathology of reproductive organs, anogenital distance and time of preputial separation. Slight changes in several parameters related to female fertility were observed at the top dose: delayed vaginal opening (by 1 day), prolonged oestrous cycle, and slower development of small follicles into growing follicles (see the table below). Due to the

small magnitude of the changes and lack of effects on other parameters, these findings are not considered to warrant classification.

EOGRTS Anonymous (2016): effects related to fertility and sexual function					
Intended dose (mg/kg bw/d)	**0**	**80**	**250**	**800**	**HCD[a]**
F0					
Mean length of the longest cycle (d; ±SD)	4 (±0)	4 (±0)	4 (±0)	4.3* (±0.5)	Mean 4.9 Range 4.2-5.5
No. of complete cycles per animal in 15 days	2.6 (±0.5)	2.5 (±0.5)	2.8 (±0.4)	2.6 (±0.5)	
No. of animals with prolonged oestrus period	0	0	0	0	Mean 1.3 Range 0-4
F1 – cohort 1A					
Day of vaginal opening	32.9 (±1.6)	32.9 (±1.4)	33.0 (±1.1)	34.1 (±1.9)	Mean 37.5 Range 35.2-39.6
Body weight on the day of vaginal opening (g)	104 (±10)	104 (±10)	105 (±8)	107 (±10)	
Mean length of the longest cycle (d)	4.2 (±0.4)	4.2 (±0.5)	4.4 (±0.6)	4.7* (±0.8)	Mean 4.9 Range 4.2-5.5
No. of complete cycles per animal in 15 days	2.9 (±0.3)	2.9 (±0.3)	2.8 (±0.4)	2.6* (±0.6)	
No. of animals with prolonged oestrus period	0	0	0	4	Mean 1.3 Range 0-4
Ovarian follicle count: – small follicles	111 (±34)	n.d.	n.d.	97 (±36)	
– growing follicles	173 (±34)	n.d.	n.d.	132* (±32)	
– antral follicles	82 (±26)	n.d.	n.d.	73 (±26)	
– corpora lutea	172 (±47)	n.d.	n.d.	155 (±37)	

Statistically significant different from control: * p≤0.05

[a] HCD as provided in the study report; 6 studies for oestrous cycle, 8 studies for vaginal opening; no further details available

n.d. = not determined

Conclusion on classification for fertility and sexual function

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**.

Adverse effects on development

There is one guideline and GLP compliant PNDT study on 2-EHA in rats (Anonymous, 1988c) and another GLP compliant PNDT study in rats with an ester of 2-EHA where 2-EHA was used as a positive control (Anonymous, 1997). Published studies by Pennanen *et al.* (1992, 1993) on sodium salt of 2-EHA were conducted sufficiently in line with the guidelines but with less detailed reporting.

No developmental toxicity was observed in a guideline PNDT study on 2-EHA in rabbits (Anonymous, 1988d) nor in the EOGRTS on 2-EHA in rats (Anonymous, 2016).

Narotsky *et al.* (1994) investigated developmental structure-activity relationships of aliphatic acids including 2-EHA and valproic acid in rats. Nau and co-workers reported developmental structure-activity relationships of valproic acid analogues in the mouse (Nau *et al.*, 1991); results of experiments with 2-EHA have been published separately (Hauck *et al.*, 1990). Bui *et al.* (1998) investigated a possible MoA of developmental toxicity by 2-EHA in rats via metallothionein induction. Ritter *et al.* (1987) investigated developmental effects in rats after a single high dose of 2-EHA or valproic acid.

Rat PNDT study (Anonymous, 1988c; Hendrickx et al., 1993)

In this GLP and guideline compliant study, 2-EHA in corn oil was administered to Fischer 344 rats via gavage from GD 6 to 15. Since a dose level of 1000 mg/kg bw/d led to excessive mortality (7 out of 8 animals) in a range-finding study, 500 mg/kg bw/d was chosen as the top dose for the definitive study.

Maternal toxicity at 500 mg/kg bw/d in the main study was limited to clinical signs in several dams (hypoactivity or ataxia in 4 pregnant dams on one or two days, ocular discharge and/or periocular encrustation) and increased liver weight (by 10%). Food consumption and maternal body weight were not affected.

Foetal weight at the top dose was reduced by 8% compared to control. Part of the foetal weight reduction may be due to increased litter size. Reduced ossification at the top dose may be related to the decrease in foetal weight. Incidence of dilated lateral ventricles without tissue compression (considered a variation by the author of the study) and of extra 14th thoracic centrum and arches was increased at the top dose in the absence of marked maternal toxicity.

Rat PNDT study Anonymous (1988c)				
Dose (mg/kg bw/d)	**0**	**100**	**250**	**500**
Females on study	25	25	25	25
Early delivery	0	0	1[a]	0
Non pregnant females	2	1	2	4
Females with viable foetuses	23	24	22	21
Body weight gain GD 6-15 (g)	25	24	25	22
Food consumption GD 6-15 (g/animal/day)	14	14	14	14

Corrected body weight change (g)	31	33	31	29
Viable implants/litter	8.4	7.5	8.4	9.3
Non-viable implants/litter	0.5	0.2	0.3	0.4
Foetal weight (g)	4.41	4.50	4.36	4.06*
No. of foetuses (litters) examined viscerally	100 (23)	95 (24)	97 (22)	104 (21)
Brain: lateral ventricle dilated, tissue compressed; foetuses (litters), % affected per litter	2 (2) 5.2%	1 (1) 4.2%	1 (1) 4.5%	6 (6) 6.0%
Brain: lateral ventricle dilated, no tissue compression; foetuses (litters), % affected per litter	3 (3) 2.7%	7 (5) 6.7%	10 (8) 9.3%	21 (15)* 22.7%
No. of foetuses (litters) examined skeletally	93 (22)	84 (22)	87 (21)	91 (21)
Extra no. 14 thoracic centrum and arches; foetuses (litters), % affected per litter	0 0%	0 0%	0 0%	16 (10)* 20.6%
Thoracic centrum no. 1 poorly ossified; foetuses (litters)	5 (4)	8 (6)	9 (7)	24 (12)*
Some metatarsals unossified	0	1 (1)	2 (1)	69 (18)*
Sternebra no. 2 poorly ossified	1 (1)	4 (4)	2 (2)	15 (11)*

Statistically significant different from control: * p≤0.05; statistical significance for anomalies is based on litter incidence (no. of affected litters/total no. of litters)

[a] Delivery on GD 20; 9 implantations, 7 live pups; not included in the data summaries

Rabbit PNDT study (Anonymous, 1988d; Hendrickx et al., 1993)

In this guideline and GLP compliant study, 2-EHA in corn oil was administered to New Zealand rabbits from GD 6 to 18. In a range-finding study, 500 mg/kg bw/d was lethal to most animals (7 out of 8) and 1 animal per group died also at 250 and 125 mg/kg bw/d. The doses of 250 and 125 mg/kg bw/d caused mortality also in the main study but at a low incidence (1 out of 15 per group). In addition, 1 dam from the 125 mg/kg bw/d group aborted on GD 27. The study did not show any evidence of developmental toxicity.

Rat PNDT study (Anonymous, 1997)

In this GLP and OECD guideline compliant study with 2-ethylhexyl-2-ethylhexanoate, 2-EHA was used as a positive control. Only results for 2-EHA are presented here. 2-EHA in olive oil was administered via gavage to pregnant Wistar rats from GD 6 to 15 at 600 mg/kg bw/d. No maternal toxicity was present. Developmental effects included reduced foetal weight (by 21%) and increased incidence of skeletal malformations, variations and retardations. Absent caudal vertebra(e) was classified as a malformation in the study report. RAC notes that the adversity of this finding depends on the position of the missing vertebra(e) and may be considered a variation in certain cases (Solecki *et al.*, 2001). Absent caudal vertebra(e) might be related to tail anomalies in other studies.

Rat PNDT study Anonymous (1997); only data on 2-EHA presented here		
Dose (mg/kg bw/d)	**0**	**600**
No. of pregnant females	22	22
Food consumption GD 6-15 (g/day)	21	21
Body weight gain GD 6-15 (g)	42	38
Corrected body weight gain (vs GD 6) (g)	37	35
Post implantation loss (%)	8.0	10.4
Live foetuses (mean)	12.4	12.7
Foetal weight (g)	3.8	3.0**
External examination: total no. of foetuses (litters)	274 (22)	279 (22)
Tail filiformed; foetuses (litters)	0	3 (2)
Tail shortened	0	1 (1)
Tail absent (acaudia)	0	1 (1)
Skeletal examination: total no. of foetuses (litters)	142 (22)	149 (22)
For all effects listed below: foetuses (litters), % affected foetuses per litter		
Total skeletal malformations	1 (1) 1.5%	11 (7) 8.9%*
Sacral vertebra(e) absent	0 0%	2 (2) 1.1%
Caudal vertebra(e) absent	0 0%	4 (3) 2.2%*
Total skeletal variations	67 (21) 48.4%	107 (22) 73.2%**
Accessory thoracic vertebra	0 0%	8 (7) 5.4%**
Accessory lumbar vertebra	0 0%	13 (7) 7.6%**
Rudimentary cervical rib(s)	4 (4) 4.1%	45 (19) 31.7%**
Accessory 14th rib(s)	0 0%	33 (16) 21.9%**
Total skeletal retardations	84 (20) 61.2%	148 (22) 99.4%**

Statistically significant difference from control: *, p≤0.05; **, p≤0.01

Rat PNDT study (Pennanen et al., 1992)

In this published study, 2-EHA as a sodium salt was administered to Han:Wistar rats in drinking water from GD 6 to 19. Dams at the top dose of 600 mg/kg bw/d showed reduced body weight gain. A statistically significant and dose-related increase in the incidence of clubfoot (malformation) was observed already at the mid-dose in the absence of maternal

toxicity; it is noted that this malformation was not observed/reported in the 1-generation study from the same authors (Pennanen *et al.*, 1993). Incidence of dilated brain ventricles was increased at the top dose. Wavy ribs and reduced ossification were also increased, wavy ribs without a clear dose-response relationship.

Rat PNDT study Pennanen *et al.* (1992)				
Dose (mg/kg bw/d)	**0**	**100**	**300**	**600**
Pregnant females	21	21	20	20
Corrected maternal bw gain (g; ±SD)	39 (±3)	36 (±4)	34 (±3)	18* (±1)
Implantations/litter	10.9	11.6	12.6	11.7
Live foetuses/litter	9.6	10.7	10.8	10.1
Post implantation loss (%; ±SD)	8.4 (±2.1)	3.2 (±1.4)	14.0 (±5.5)	11.9 (±2.5)
Foetal weight (g; ±SD)	3.6 (±0.5)	3.6 (±0.4)	3.4* (±0.3)	3.3* (±0.5)
Clubfoot (%; ±SD)	0	0.8 (±0.8)	5.6* (±2.0)	6.7* (±2.8)
Scoliosis (%; ±SD)	0	3.6 (±1.8)	2.4 (±1.8)	3.8 (±1.8)
Wavy ribs (%; ±SD)	1.0 (±1.0)	19.8* (±4.9)	14.1* (±3.8)	22.4* (±5.4)
Nonossified sternebrae, at least one (%; ±SD)	6.2 (±2.3)	8.3 (±2.4)	12.0 (±3.1)	19.7* (±4.7)
Bipartite vertebral centra, at least one (%; ±SD)	14.1 (±5.0)	14.3 (±6.4)	13.2 (±4.8)	34.5* (±7.2)
Reduced cranial ossification (%; ±SD)	22.1 (±6.3)	42.4* (±6.4)	29.6 (±6.4)	66.7* (±7.1)
Reduced lumbar ossification (%; ±SD)	0	0	1.8 (±1.8)	5.0* (±2.0)
Dilatation of brain ventricles (%; ±SD)	3.8 (±1.8)	4.8 (±2.4)	13.7 (±5.9)	24.0* (±7.2)

Statistically significant different from control: *, p≤0.05

<u>One-generation reproductive toxicity study in rats (Pennanen et al., 1993)</u>

2-EHA as a sodium salt was administered to female Han:Wistar rats in drinking water from 2 weeks prior to mating until lactation day 21; males were treated as well. Body weight gain during gestation was slightly reduced at the top dose (limited data available, net body weight gain probably comparable to that in Pennanen *et al.*, 1992).

Litter size was slightly reduced at the top dose and incidence of kinky tail (on external examination) increased without a clear dose-response relationship. Dose-dependent delays in several developmental landmarks (ear unfolding, teeth eruption, eye opening) and development of reflexes (cliff avoidance, grip reflex) were observed, at least partly reflecting a generalized developmental delay (pup weight reduced by about 10% in the relevant period).

1-generation study Pennanen *et al.* (1993): effects related to development				
Dose (mg/kg bw/d)	**0**	**100**	**300**	**600**
Pregnant females	23	21	24	23
Live pups	251	214	258	208
Stillbirths	4	0	2	6
Mean litter size on PND 0 (±SD)	10.9 (±2.2)	10.2 (±1.9)	10.8 (±2.1)	9.2* (±2.4)
Postnatal deaths	2	11	5	6
Kinky tail: no. of pups (% affected foetuses per litter)	13 (4.9%)	32 (15.0%)	66* (24.5%)	54* (25.6%)

Statistically significant different from control: *, p≤0.05

Extended one-generation reproductive toxicity study in rats (Anonymous, 2016)

This guideline and GLP compliant study was extended to produce all cohorts, i.e. reproductive cohort F1-1A (terminated at the age of 13 weeks), reproductive cohort F1-1B (used to produce F2 generation), neurotoxicity cohort F1-2A (terminated at the age of 11 weeks), neurotoxicity cohort F1-2B (terminated at weaning) and immunotoxicity cohort F1-3. Test substance intake during gestation was ca. 840 mg/kg bw/d for F0 and 740 mg/kg bw/d for F1.

There was no treatment related effect on any of the development related parameters investigated, including pup survival, pup weight, gross abnormalities, FOB, brain weight and morphometry, histopathology of the nervous system and developmental immunotoxicity.

Developmental study in rats (Narotsky et al., 1994)

Narotsky, Francis and Kavlock examined developmental structure-activity relationships for a group of 15 aliphatic acids including 2-EHA and valproic acid using an assay developed by Chernoff and Kavlock. 2-EHA or valproic acid in corn oil were administered via gavage to pregnant Sprague-Dawley rats from GD 6 to 15. The dams were allowed to deliver and the study was terminated on PND 6 (PND 1 was defined as GD 22 irrespective of the actual time of parturition). Skeletal examination was conducted on 2 pups from each control litter and in all pups from the compound exposed litters. It is not clear from the article whether visceral examination was carried out or not.

Both 2-EHA (900 mg/kg bw/d) and valproic acid (500 mg/kg bw/d) caused significant maternal toxicity including clinical signs (motor depression, rales) and 2-EHA caused also mortality. Most maternal deaths in the study were attributed to respiratory effects, probably due to the irritant nature of the material (leading to gavage related reflux and aspiration).

As to developmental toxicity, both substances caused reduced pup weight, increased perinatal loss (possibly secondary to maternal toxicity), and increases in skeletal anomalies (extra presacral vertebrae, lumbar ribs, cervical ribs). The article also mentions syndactyly, vestigial tail and fused ribs for 2-EHA and oligodactyly and fused ribs for valproic acid (incidences not provided). Both substances showed a similar profile, 2-EHA appears to be less potent than valproic acid.

It is noted that 2-EHA caused high maternal mortality, probably in excess of 10% (CLP Regulation, Annex I, 3.7.2.4.4). Thus, the developmental effects in the 2-EHA-administered group are not directly relevant for classification. Still, the study demonstrates similarity

between developmental toxicity profiles of 2-EHA and valproic acid under similar experimental conditions (the same strain and source of animals, the same laboratory).

Narotsky *et al.* (1994): Chernoff/Kavlock assay with 2-EHA and valproic acid				
	2-EHA		**Valproic acid**	
Dose (mg/kg bw/d)	**0**	**900**	**0**	**500**
No. of females	20	15	20	15
Rales	0	5	0	6
Motor depression	0	15	0	13
Mortality	0	4	0	0
No. of dams	13	10	17	12
Body weight gain GD 6-20, adjusted for litter size (g; ±SD)	44 (±5)	19* (±11)	52 (±5)	21** (±5)
No. of implants per dam (±SD)	12.1 (±0.9)	13.1 (±1.3)	15.4 (±0.6)	14.9 (±1.0)
No. of live pups per litter PND 1 (±SD)	11.2 (±1.0)	9.3* (±1.5)	14.2 (±0.6)	13.3 (±0.7)
No. of live pups per litter PND 6 (±SD)	11.1 (±1.0)	7.4** (±1.6)	13.9 (±0.5)	7.8** (±1.9)
Perinatal loss (%; ±SD)	9.0 (±2.4)	41.2** (±10.8)	8.9 (±1.9)	48.7** (±12.0)
Pup weight PND 1 (g; ±SD)	6.9 (±0.2)	6.0** (±0.3)	6.6 (±0.1)	5.7** (±0.3)
Pup weight PND 6 (g; ±SD)	13.8 (±0.6)	11.3** (±0.6)	13.1 (±0.3)	9.7** (±0.9)
Skeletal examination PND 6: no. of foetuses (litters) examined	26 (13)	72 (8)	30 (15)	94 (9)
Extra presacral vertebrae: foetuses (litters), % affected foetuses per litter	0 0%	37 (7) 56.4%**	0 0%	48 (8) 50.1%**
Lumbar ribs: foetuses (litters), % affected foetuses per litter	6 (3) 23.1%	70 (8) 98.2%**	4 (4) 13.3%	66 (9) 74.7% **
Cervical ribs: foetuses (litters), % affected foetuses per litter	1 (1) 3.8%	11 (4) 19.4%	1 (1) 3.3%	12 (5) 21.1%

Statistically significant difference from control: *, p≤0.05; **, p≤0.01

In addition to the Chernoff/Kavlock assay, a standard rat PNDT study with valproic acid has been carried out. At the top dose 400 mg/kg bw/d, foetal weight was reduced by 29% and skeletal examination revealed increased incidence of extra presacral vertebrae, fused vertebrae, extra lumbar ribs, cervical ribs, fused ribs and reduced ossification. A table with results is provided under 'Supplemental information'.

Developmental studies in mice (Hauck et al., 1990; Nau et al., 1991)

Hauck *et al.* (1990) administered (R)-2-EHA, (S)-2-EHA or racemic mixture of 2-EHA to

pregnant Han:NMRI mice as 4 consecutive intraperitoneal injections of 500 mg/kg bw (3.0 mmol/kg bw) on GD 7 (morning and evening) and 8 (morning and evening). An additional group was given only a single i.p. injection of 500 mg/kg bw racemic 2-EHA on the morning of GD 8. The study was terminated on GD 18, the investigated parameters were the number of implantations, embryolethality (resorptions and dead foetuses), foetal weight and occurrence of exencephaly.

The reason for the choice of exencephaly in the mouse as a model for investigation of teratogenicity of valproate related compounds was explained by Nau *et al.* (1991): The main malformation associated with valproate exposure in humans is spina bifida aperta. Neural tube defects are very difficult to produce with valproic acid in rats and rabbits. Exencephaly is the dominant valproate-related malformation in the mouse, is reproducible and can be unambiguously determined by external inspection.

The results of the experiment with 2-EHA are presented in the table below. The teratogenic action of 2-EHA showed high stereospecificity: the (R)-enantiomer was highly teratogenic while the (S)-enantiomer was practically inactive. 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. Stereospecificity was also demonstrated for 4-yn-valproic acid and 4-en-valproic acid (Nau *et al.*, 1991).

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)

Valproic acid administered to mice as a single i.p. dose of 3.0 mmol/kg on the morning of GD 8 induced exencephaly of 44% of foetuses (Nau *et al.*, 1991), compared to 5% for racemic 2-EHA. Although 2-EHA was less potent than valproic acid and the study was used a non-standard route, the increased incidence of exencephaly still raises a concern about induction of neural tube defects in humans.

Developmental study in rats (Ritter et al., 1987)

Ritter *et al.* (1987) administered a single gavage dose of 2-EHA (undiluted) to pregnant Wistar rats (at least 7 per group) on GD 12. The animals were sacrificed on GD 20. Valproic acid was also tested in this study. Doses of 1800 mg/kg bw 2-EHA and 900 mg/kg bw valproic acid induced tail defects, cardiovascular defects and hydronephrosis; 2-EHA additionally induced limb defects.

Although malformations occurred only at a very high dose of 2-EHA, the study employed a non-standard design and no information on maternal toxicity is available in the publication, the fact that 2-EHA showed a similar developmental toxicity profile to that of valproic acid has to

be taken into account in the weight of evidence assessment.

Rat developmental study Ritter *et al.* (1987)				
Substance	**Control**	**2-EHA**		**Valproic acid**
Dose (mg/kg bw)	**0**	**900**	**1800**	**900**
Dose (mmol/kg bw)	0	6.25	12.5	6.25
No. of litters	7	7	10	8
No. of implants	91	112	149	124
Foetal weight (g)	4.1	4.0	2.9	3.5
% dead and resorbed (±SD)	9.6 (±4.1)	5.9 (±2.4)	12.9 (±3.3)	15.6 (±4.5)
% survivors malformed (±SD)	0.0 (±0.0)	0.8 (±0.8)	67.8 (±10.9)	48.3 (±1.0)
% survivors with:				
– hydronephrosis			20.9	14.4
– cardiovascular defects			10.1	8.7
– tail defects			15.5	19.2
– limb defects			51.2	2.9
– other defects			10.9	1.9

Developmental study in rats (Bui et al., 1998)

The aim of this published non-guideline study was to investigate the relationship between developmental toxicity of 2-EHA and maternal zinc status. Data on dams with adequate zinc intake exposed over main organogenesis are presented first, followed by data related to MoA.

2-EHA in corn oil was administered via gavage to pregnant Sprague-Dawley rats from GD 8 to 15. The animals were sacrificed either on GD 16 (10/group) or GD 19 (7/group). Only a single dose level of 3.5 mmol/kg bw/d (equivalent to ca. 500 mg/kg bw/d) was employed. Treatment led to reduced body weight gain in dams and increased resorptions, reduced foetal weight (by 9% on GD 19) and increased incidence of skeletal anomalies. Results from GD 16 are difficult to interpret as the incidence of anomalies was much higher than on GD 19. Lack of skeletal examination further limits the utility of results from GD 16 given that skeleton was the main target of developmental toxicity of 2-EHA in guideline studies.

Bui *et al.* (1998): developmental study with 2-EHA in dams with adequate zinc intake				
	Sacrifice GD 16		**Sacrifice GD 19**	
Dose (mg/kg bw/d)	**0**	**500**	**0**	**500**
Number of litters	10	10	7	7
Corrected bw gain (g)	57	42*	61	35*
Resorptions (%; ±SD)	4.1 (±1.8)	3.7 (±1.7)	4.5 (±2.3)	22.9* (±6.0)
Foetal weight (g)	0.46	0.42	1.96	1.78*

Foetuses with anomaly(ies) (%; ±SD)	4.9 (±1.8)	53.2* (±7.2)	0	7.9 (±3.4)
Encephalocele (%; ±SD)	0	14.1* (±3.5)	0	0
Rib anomalies (%; ±SD)	n.i.	n.i.	4.4 (±2.2)	20.8* (±4.6)
Tail anomalies, external examination (%; ±SD)	2.0 (±1.4)	26.0 (±7.1)	0	7.9 (±3.4)
Tail anomalies, skeletal examination (%; ±SD)	n.i.	n.i.	10.3 (±3.7)	23.1* (±4.4)

n.i. = not investigated

Statistically significant difference from control: *, p≤0.05

Results from parallel groups with low zinc intake (1.2 µg Zn/g diet; adequate zinc intake is 25 µg Zn/g diet) showed that reduced zinc intake by itself causes maternal toxicity (markedly reduced corrected bw gain) and induces tail anomalies in foetuses on GD 19 (20% in low Zn control vs 0% in adequate Zn control). Zn deficiency also enhanced developmental toxicity of 2-EHA as shown by increased incidence of tail anomalies (8% → 33%) and encephalocele (0% → 12%) on GD 19.

In another experiment, pregnant females (6/group, control 8/group) fed with adequate Zn diet were administered a single dose of 2-EHA in corn oil on GD 11.5, intubated with ^{65}Zn 8 hours later and sacrificed 10 hours after ^{65}Zn administration. The results showed that 2-EHA causes liver metallothionein induction in maternal animals, increases zinc uptake by maternal liver and slightly reduces zinc uptake by the embryos. Plasma Zn or ^{65}Zn were not affected. A concurrent experiment with valproic acid showed similar effects.

Bui et al. (1998): impact of single dose of 2-EHA on zinc distribution in pregnant dams with adequate zinc intake					
Dose of 2-EHA (mg/kg bw)	***0***	***450***	***900***	***1350***	***1800***
Liver metallothionein (nmol/g; ±SD)	*6.9 (±1.1)*	*11.8* (±1.8)*	*18.2* (±3.8)*	*19.3* (±2.4)*	*21.5* (±4.6)*
Liver Zn (nmol/g; ±SD)	*450 (±22)*	*512* (±29)*	*553* (±40)*	*619* (±17)*	*618* (±42)*
Liver ^{65}Zn (%; ±SD)	*24 (±1)*	*30* (±2)*	*33* (±3)*	*32* (±2)*	*33* (±4)*
Embryo ^{65}Zn (%; ±SD)	*0.22 (±0.02)*	*0.21* (±0.03)*	*0.18* (±0.02)*	*0.13* (±0.02)*	*0.15* (±0.02)*

*Statistically significant difference from control: *, p≤0.05*

The authors concluded that developmental toxicity of 2-EHA may be mediated, in part, by its influence on maternal-embryonic Zn distribution. RAC is of the view that the mere fact that severe zinc deficiency (a teratogenic regimen on its own) enhances developmental toxicity of 2-EHA is not a proof that interference with zinc distribution is the mode of action of, or a major

contributor to the developmental toxicity of 2-EHA observed under the conditions of adequate zinc intake. No supplemental Zn group (with zinc intake » 25 mg/kg feed) was included in the experiment terminated on GD 19 to show whether additional zinc is able to prevent the 2-EHA-related increase in skeletal anomalies.

Reprotoxicity screening in rats according to OECD TG 422 with iron tris(2-ethylhexanoate)

This study employed a top dose of 300 mg/kg bw/d (in corn oil via gavage), which did not induce any developmental nor maternal toxicity. Absence of developmental effects in this study does not negate the positive studies with 2-EHA for two reasons: (1) the OECD 422 does not cover the whole range of endpoints investigated in an OECD 414 study; (2) the top dose did not induce maternal toxicity, which indicates that a higher top dose should have been tested.

Summary and assessment of developmental effects in rodents

Developmental effects in the available rat studies are summarized in the following table.

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	500 mg/kg bw/d, 2-	↑ resorptions, rib and tail	Reduced corrected bw

Bui *et al.* (1998)	EHA in corn oil, Sprague-Dawley	anomalies, ↓ foetal weight (9%)	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

The most consistent developmental findings in the most reliable studies were extra thoracic and lumbar vertebrae and cervical and lumbar ribs (Anonymous, 1988c; Anonymous, 1997; Narotsky *et al.*, 1994, only in the presence of excessive maternal mortality). These anomalies are generally considered variations rather than malformations. Rather concerning, however, is the fact that these variations, together with vertebral and rib malformations at a lower incidence, were the only anomalies detected in a rat PNDT study with valproic acid, a human teratogen (Narotsky *et al.*, 1994; see 'Supplemental information').

It is further noted that these skeletal variations together with a low incidence of tail anomalies (absent caudal vertebrae, filiformed tail) occurred in the absence of significant maternal toxicity in the GLP study by Anonymous (1997).

Kinky tail observed in the one-generation study by Pennanen *et al.* (1993) appears to be consistent with results of several other studies (Anonymous, 1997; Bui *et al.*, 1998; Ritter *et al.*, 1987).

A dose-related increase in club-foot was observed in the published PNDT study by Pennanen *et al.* (1992). This external malformation was, however, not observed/reported in the subsequent one-generation study conducted by the same group and using similar experimental setup (Pennanen *et al.*, 1993), nor in other rat PNDT studies. Therefore this finding is given a lower weight in the assessment.

Dilated brain ventricles were observed not only by Pennanen *et al.* (1992) but also in the GLP study by Anonymous (1988c). They were classified as variations in the latter study.

Absence of any developmental effect in the high-quality EOGRTS (Anonymous, 2016) may be due to different toxicokinetics upon dietary vs gavage administration. Dietary administration generally leads to a lower C_{max} in the plasma than a single bolus dose in a vehicle facilitating absorption. If the developmental effects are driven by C_{max}, the threshold for developmental toxicity may not have been reached with 800 mg/kg bw/d 2-EHA via diet even though it was reached with 500 mg/kg bw/d via gavage in vegetable oil. In addition, subtle effects such as skeletal variations are unlikely to be detected without skeletal examination.

In the experiments reported by Ritter *et al.* (1987), Nau *et al.* (1991) and Narotsky *et al.* (1994), 2-EHA showed a similar developmental toxicity profile in rodents to that of valproic acid, although 2-EHA was less potent. Importantly, both substances induced exencephaly in mice after i.p. injection, which is an indication that 2-EHA might also induce neural tube defect in humans.

The mode of action of developmental toxicity of 2-EHA is not established. Hauck *et al.* (1990) reported that induction of exencephaly in mice by 2-EHA is highly stereospecific, with only the (R)-enantiomer being responsible for the observed teratogenic effect; this suggests interaction of 2-EHA with a chiral target in the embryo. Göttlicher *et al.* (2001) presented some evidence for a MoA via inhibition of histone deacetylases. They tested valproic acid, (R)- and (S)-2-EHA, (R)- and (S)-4-yn-valproic acid, and valpromide. Out of these substances, only those previously identified as teratogenic by Nau *et al.* (1991) in a mouse model (valproic acid, (R)-2-EHA, (S)-4-yn-valproic acid) showed inhibition of histone deacetylases *in vitro*.

Bui *et al.* (1998) showed that severe Zn deficiency (a teratogenic regimen on its own) enhances developmental toxicity of 2-EHA and that 2-EHA alters Zn distribution probably via metallothionein induction. However, this study failed to demonstrate (due to its design) that interference with zinc distribution is a major contributor to developmental toxicity of 2-EHA under conditions of adequate zinc intake.

Conclusion on classification for development

No human data are available for 2-EHA. In rats the substance caused foetal weight reduction, skeletal variations (supernumerary vertebrae, cervical and lumbar ribs), reduced ossification and, in two studies, also dilated brain ventricles without marked maternal toxicity. One study also reported tail malformations at a low incidence in the absence of maternal toxicity (Anonymous, 1997). A wide range of malformations at a high incidence was reported in a non-standard study (Ritter *et al.*, 1987) using a single high dose (1800 mg/kg bw) without information on maternal toxicity.

The DS proposed to base the classification on the data with 2-EHA alone. Nevertheless, reprotoxicity classification under CLP should be based on weight of evidence and information on chemically related substances may also be included in the assessment (CLP Regulation, Annex I, 3.7.2.3.1). Valproic acid is considered a related substance not only based on structural similarity but also on a similar developmental toxicity profile in animal studies (Narotsky *et al.*, 1994; Ritter *et al.*, 1987; Nau *et al.*, 1991), although potency of 2-EHA was lower than that of valproate. Valproic acid is an established human teratogen causing several types malformations in humans, most of which are not reproduced in standard rat PNDT studies; humans appear to be more sensitive than rodents to the teratogenicity of valproate.

In a weight of evidence assessment taking into account not only (1) animal studies with 2-EHA alone, but in addition also (2) animal and human data on its structural analogue and known human teratogen valproic acid and (3) comparative developmental toxicity studies with 2-EHA and valproic acid, **RAC concluded that 2-EHA should be classified in Category 1B for development.**

Adverse effects on or via lactation

The DS proposed no classification due to lack of data. However, some information is available from the generational studies. No treatment-related clinical signs and no effects on pup body weight or pup survival were observed in the EOGRTS (Anonymous, 2016). Pup weight reduction by 14% on PND 4 was reported at ca. 15000 ppm in the range-finding study (Anonymous, 2015), but the available information is limited (study report not available to RAC, pup weight difference at birth not known, further body weight development not known either as the pups were sacrificed soon after PND 4) and no effect on pup weight was observed at only a slightly lower dose (ca. 12000 ppm) in the main study.

The one-generation study by Pennanen *et al.* (1993) reported statistically significant pup weight reductions during lactation; the difference can be only roughly estimated from the figures in the article and seems to be slightly above 10% on PND 7 and 14. Pup weight at birth was not affected.

Conclusion on classification for effects on or via lactation

Moderate pup weight reductions during the period when maternal milk is the only nutrition source for the pups were observed in studies Pennanen *et al.* (1993) and Anonymous (2015) while no effect was reported in the EOGRTS (Anonymous, 2016). The reduction of about 10%

in the one-generation study by Pennanen *et al.* (1993) is not considered of a sufficient magnitude to warrant classification. The information from the OECD TG 422 compliant screening study (Anonymous, 2015) is considered too limited to be used as a basis for classification, especially given the availability of a full EOGRTS study not showing any weight reduction during lactation in F1 nor F2 pups. Therefore, RAC concludes that no classification for effects on or via lactation is warranted.

Overall conclusion on reproductive toxicity of 2-ethylhexanoic acid

RAC agrees with the DS that the available information does not warrant classification for sexual function and fertility or for effects on or via lactation. For development effects, RAC proposes classification **Repr. 1B; H360D** based on weight of evidence taking into account not only data for 2-ethylhexanoic acid alone but also information on the structural analogue and human teratogen valproic acid, which showed a qualitatively similar developmental toxicity profile in rodent studies.

Read-across from 2-EHA to metal 2-ethylhexanoates

The DS proposed read-across from 2-ethylhexanoic acid to its salts for reproductive toxicity based on transformation of the salts to 2-EHA. This corresponds to Scenario 1 of 'Read-Across Assessment Framework' (RAAF; ECHA, 2017). The read-across assessment proposed by the DS is limited to the oral exposure route; the dermal and inhalation routes have not been addressed in the CLH report. No dermal or inhalation reproductive toxicity studies are available for the source substance 2-EHA.

RAAF lists several key elements specific for the assessment according to Scenario 1:

1. Formation of a common compound
2. The biological targets for the common compound
3. Exposure of the biological targets for the common compound
4. The impact of parent compounds
5. Formation and impact of non-common compounds

The individual elements are discussed below.

<u>Formation of a common compound</u>

2-EHA is a weak carboxylic acid with a pK_a of 4.8. 2-Ethylhexanoates of highly electropositive metals such as sodium readily dissociate according to the following equation already at a neutral pH ('R' stands for hept-3-yl, 'M' for metal):

$$RCOOM \rightarrow RCOO^- + M^+$$

2-Ethylhexanoate anion (represented with $RCOO^-$), being an anion of a weak acid, readily accepts protons to form 2-ethylhexanoic acid (RCOOH). Both forms coexist in equilibrium:

$$RCOO^- + H_3O^+ \leftrightarrows RCOOH + H_2O$$

At a pH equal to pK_a (4.8), half of the molecules will occur in the form of RCOOH, the other half as $RCOO^-$; below the pH of 4.8 the free acid (RCOOH) will prevail. At the low pH in human stomach (ca. 1.5-3) the substance is expected to occur practically exclusively as free acid. Acid-base reactions are generally very rapid and this case is unlikely to be an exception. Thus, salts such as sodium 2-ethylhexanoate are expected to become indistinguishable from the free acid in the stomach. This behaviour is also predicted for 2-ethylhexanoates with an organic

cation.

Cations of many group 3-15 metals show a tendency to form coordination complexes. Dissociation of the salt in an acidic solution may be described as an exchange of ligands, which can be expressed with the following simplified equation (in reality, one metal ion is usually surrounded by six molecules of water, and the stoichiometries and structures of metal carboxylates are quite variable):

$$RCOO^{(-)}{-}M^{(+)} + H_2O \leftrightarrows RCOO^- + H_2O{-}M^{(+)}$$

The equilibrium is shifted to the right because there is an excess of water and the anion is removed by conversion to 2-ethylhexanoic acid in the acidic environment (Le Chatelier's principle).

The hydrogens in the aqua ion $H_2O{-}M^{(+)}$ are often acidic as the metal cation further polarizes the O−H bond. As a result, the hydrogens may be released, which results in formation of hydroxo-complexes:

$$H_2O{-}M^{(+)}\ (aq) \leftrightarrows HO^{(-)}{-}M^{(+)}\ (s) + H^+$$

Under acidic conditions the equilibrium is shifted to the left, but at a neutral or alkaline pH a significant proportion of the metal is in the form of hydroxides (or hydroxides-oxides). These hydroxides are often poorly soluble in water. Low water solubility of some 2-ethylhexanoates at a neutral pH, if the solubility is determined by measuring the concentration of dissolved metal, probably reflects formation of these hydroxides. The precipitates usually dissolve upon acidification.

Overall, although the speciation of 2-ethylhexanoates of group 3-15 metals in aqueous solutions may be complex at a neutral pH, conversion to 2-EHA at a low pH is expected also for these salts.

Biological targets for the common compound

The conversion of metal 2-ethylhexanoates to 2-EHA following oral exposure is expected to occur already in the stomach, i.e. before absorption. Therefore, the biological targets are expected to be the same for this exposure route.

Exposure of the biological targets for the common compound

The toxicokinetics after oral exposure is expected to be similar for the salts as for 2-EHA.

The impact of parent compounds

Most salts are expected to be rapidly and practically completely converted to 2-EHA in the stomach, leaving no parent compound. Absorption of small amounts of unchanged parent cannot be excluded for less ionic 2-ethylhexanoates if applied in a lipophilic vehicle. If this is the case, the parent would probably convert to 2-EHA, or 2-ethylhexanoates depending on the pH, relatively soon after absorption.

Formation and impact of non-common part of the salts

The most important non-common part of the salts is the metal cation. The need to separately assess reproductive toxicity of the cation is specifically stipulated in a Note that is part of the proposed entry: "The classification for the hazard class(es) in this entry is based only on the hazardous properties of the part of the substance which is common to all substances in the entry. The hazardous properties of any substance in the entry also depends on the properties

of the part of the substance which is not common to all substances of the group; they must be evaluated to assess whether (a) more severe classification(s) (e.g. a higher category) or (b) a broader scope of the classification (additional differentiation, target organs and/or hazard statements) might apply for the hazard class(es) in the entry." The wording of the Note is discussed under a separate heading below.

RAC notes that the toxicity of the cation might potentially also lead to a less severe classification if the developmental toxicity has a true threshold and the cation causes severe general toxicity below this threshold in humans. However, this would be difficult to reflect in a broad group entry. In addition, it could be argued that animal tests have limited sensitivity for detection of rare malformations due to the low number of animals used, and therefore high doses need to be tested to increase sensitivity. This is one of the reasons why also developmental effects at doses with general toxicity may be relevant for classification. Thus, RAC decides not to take into account the general toxicity of the cation and follow a worst-case approach.

Conclusion on read-across

As all individual elements of the read-across according to the RAAF are fulfilled, RAC agrees with the DS's conclusion that the read-across from 2-EHA to metal 2-ethylhexanoates and to salts of 2-EHA with an organic cation is **acceptable**.

The Note

The Note stipulates that hazardous properties of the counter-ion must be evaluated to assess whether (a) more severe classification(s) (e.g. a higher category) or (b) a broader scope of the classification (additional differentiation, target organs and/or hazard statements) might apply for the hazard class(es) in the entry.

As to part (b) of the proposed note, a broader scope of the classification is already covered by CLP, Article 4(3): "If a substance is subject to harmonised classification and labelling in accordance with Title V through an entry in Part 3 of Annex VI, that substance shall be classified in accordance with that entry, and a classification of that substance in accordance with Title II shall not be performed for the hazard classes or differentiations covered by that entry. However, where the substance also falls within one or more hazard classes or differentiations not covered by an entry in Part 3 of Annex VI, classification under Title II shall be carried out for those hazard classes or differentiations."

Thus, part (b) of the proposed Note could be omitted. Despite, RAC would prefer to retain it as a reminder of the obligation to self-classify.

Supplemental information - In depth analyses by RAC

Developmental toxicity of valproic acid in humans and animals

The anticonvulsant properties of valproic acid were discovered in 1960s. Valproic acid acts via multiple mechanisms (including GABA potentiation, blockage of voltage gated sodium channels, inhibition of histone deacetylase), which probably explains its broad-spectrum anti-seizure effects. The first reports of congenital abnormalities, including neural tube defects, date back to early 1980s. A facial phenotype combined with other birth defects was also reported, and the term foetal valproate syndrome was proposed. A recent large case control study (Jentink *et al.*, 2010) reported statistically significant associations between valproate monotherapy in the first trimester of pregnancy and several malformations including spina

bifida, cleft palate, hypospadias and atrial septal defect. An analysis of pooled data from 32 prospective cohort studies or national birth registers confirmed the increased risk associated with valproic acid exposure, particularly for neural tube defects and hypospadias compared with other antiepileptic drugs (Thomson *et al.*, 2016). Some studies also identified poorer cognitive and behavioural outcomes in prenatally exposed children.

In a standard PNDT study by Narotsky *et al.* (1994), valproic acid in corn oil was administered by gavage to Sprague-Dawley rats from GD 6 to 15. No significant maternal toxicity was observed at the top dose of 400 mg/kg bw/d. Significant reduction in foetal weight was observed at the top (-29%) and mid (-15%) dose. Non-significantly increased incidence of fused vertebrae and fused ribs was observed at the top dose as well as significantly increased incidence of several skeletal variations (extra presacral vertebrae, lumbar and cervical ribs) and reduced ossification. Soft tissue examination showed increased dilation of renal pelvis.

Narotsky *et al.* (1994): rat PNDT study with valproic acid				
Dose (mg/kg bw/d)	**0**	**100**	**200**	**400**
No. of dams	19	13	19	15
Total litter loss	0	0	0	1
Body weight gain GD 6-20 corrected for gravid uterine weight (g)	49	48	49	43
Post implantation loss (%; ±SD)	9.3 (±3.2)	3.7 (±1.5)	6.6 (±2.0)	15.2 (±6.6)
Foetal weight (g)	4.1	4.5	3.5**	2.9**
Vertebrae fused (%; ±SD)	0	0	0	9.2 (±5.1)
Vertebrae extra presacral (%; ±SD)	4.0 (±2.7)	1.0 (±1.0)	9.1 (±2.4)	74.1** (±9.3)
Ribs fused (%; ±SD)	0	0	0	13.3 (±7.7)
Ribs lumbar (%; ±SD)	12.9 (±5.6)	26.7 (±6.9)	36.0* (±6.2)	81.3** (±8.3)
Ribs cervical (%; ±SD)	0.7 (±0.7)	0	2.1 (±1.5)	10.7** (±3.6)
Calvaria reduced ossification (%; ±SD)	7.1 (±2.4)	16.3 (±5.5)	21.1* (±6.9)	31.9* (±6.4)
Sternebrae reduced ossification (%; ±SD)	61.2 (±8.0)	63.6 (±11.5)	89.7** (±4.6)	95.0** (±3.7)

Statistically significant difference from control: *, p≤0.05; **, p≤0.01

In a non-guideline PNDT study by Binkerd *et al.* (1988), sodium valproate in distilled water was administered via gavage to Sprague-Dawley rats (10/group) from GD 8 to 17. A dose of 600 mg/kg bw/d was associated with excessive maternal toxicity (mortality, clinical signs), increased resorptions and a high incidence of malformations (skeletal, craniofacial, urogenital, cardiovascular). The dose of 500 mg/kg bw/d appears to have been better tolerated by the dams; the developmental findings included reduced foetal weight (by 24%), reduced ossification, craniofacial anomalies (low-set and posteriorly rotated ears, upturned nose,

dome-shaped cranium), skeletal malformations (vertebrae, ribs) and variations (extra and rudimentary ribs).

Rat PNDT study with sodium valproate Binkerd *et al.* (1988)				
Dose (mg/kg bw/d)	**0**	**200**	**500**	**600**
Pregnant animals	10	10	10	12
Maternal deaths	0	0	0	2
Body weight gain GD 8-20 corrected for gravid uterus weight (g; ±SD)	25 (±6)	22 (±7)	21 (±8)	13 (±15)
Resorptions (%; ±SD)	18 (±24)	24 (±32)	7 (±7)	48 (±43)
Foetal weight males (g)	3.7	3.3*	2.8*	2.2*
Foetal weight females (g)	3.3	3.1	2.6*	2.0*
Reduced ossification (%; ±SD)	21 (±25)	29 (±20)	78* (±25)	98* (±6)
Malformations (%; ±SD)	0.8 (±2.4)	3.3 (±7.0)	22.5 (±22.8)	57.3* (±31.9)
– cardiovascular	0	0	2.2 (±3.5)	10.6* (±16.5)
– urogenital	0	0	0	23.5 (±38.4)
– craniofacial	0.8 (±2.4)	3.3 (±7.0)	17.0 (±25.6)	43.4* (±39.3)
– vertebral	0	0	3.4 (±6.3)	23.0 (±37.2)
– rib	0	0	8.5 (±11.3)	48.4* (±37.9)
Extra and rudimentary ribs (%; ±SD)	4.5 (±9.6%)	25.5 (±14.9)	52.9 (±27.4)	83.3 (±37.0%)

Statistically significant difference from control: *, p≤0.05

Although some special animal studies were able to reproduce, at least partly, some of the developmental effects associated with valproate exposure in humans (e.g. spina bifida in the rat, Ceylan *et al.*, 2001; spina bifida in the mouse, Nau *et al.*, 1991), standard rat studies showed mainly variations and malformations of ribs and vertebrae.

Repeated dose studies with 2-EHA

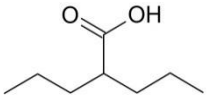
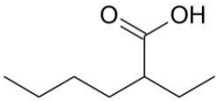
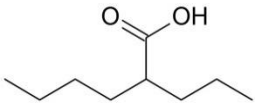
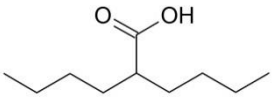
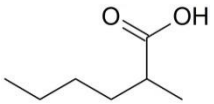
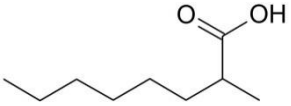
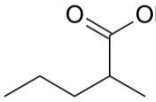
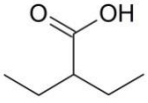
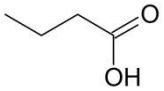
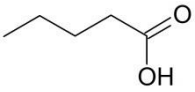
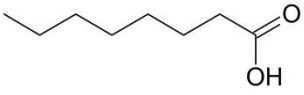
The table below has been compiled from the information available in the disseminated REACH registration dossier on the ECHA website (the OECD TG 422 compliant screening Anonymous, 2015, is not included in the table).

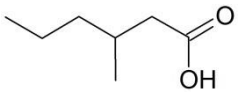
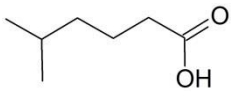
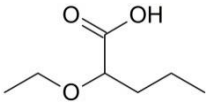
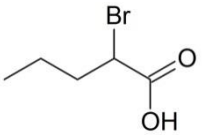
Overview of repeat dose studies with 2-EHA		
Study type, year of report	**Method**	**Observations**
Rat		
2-week, gavage; 1987	Strain: Fischer 344 Doses: 0, 200, 800, 1600 mg/kg bw/d Vehicle: corn oil No. of animals: 5/sex/dose	1600 mg/kg bw/d: mortality 8/10 800 mg/kg bw/d: clinical signs (weakness, lethargy, unkempt fur), ↓ bw, ↑ liver weight 200 mg/kg bw/d: ↑ liver weight (f)
2-week, dietary; 1987	Strain: Fischer 344 Doses: 0, 7500, 15000, 30000 ppm (0, 710/760, 1350/1410, 2280/2660 mg/kg bw/d m/f) No. of animals: 5/sex/dose	30000 ppm: clinical signs (piloerection, unkempt fur), ↓ bw by 24%/14% m/f, ↓ fc, ↑ liver weight, hepatocellular hypertrophy and necrosis 15000 ppm: ↓ bw by 8% (m), ↓ fc, ↑ liver weight, hepatocellular hypertrophy and necrosis 7500 ppm: ↑ liver weight
90-day, dietary; 1988	Strain: Fischer 344 Doses: 0, 1000, 5000, 15000 ppm (0, 61/71, 300/360, 920/1070 mg/kg bw/d m/f) No. of animals: 10/sex/dose	15000 ppm: ↓ bw by 8%/10% m/f, ↓ fc, ↑ cholesterol (m), ↑ urea (m), ↑ liver weight, hepatocellular hypertrophy 5000 ppm: ↑ liver weight, hepatocellular hypertrophy 1000 ppm: no effects
Mouse		
2-week, gavage; 1987	Strain: B6C3F1 Doses: 0, 200, 800, 1600 mg/kg bw/d Vehicle: corn oil No. of animals: 5/sex/dose	1600 mg/kg bw/d: ↑ liver weight (m), hepatocellular hypertrophy (m) 800 mg/kg bw/d: no effects
2-week, dietary; 1987	Strain: B6C3F1 Doses: 0, 7500, 15000, 30000 ppm (0, 1610/1970, 3080/3990, 5790/9230 mg/kg bw/d m/f) No. of animals: 5/sex/dose	30000 ppm: no bw gain, ↑ liver weight, hepatocellular hypertrophy and necrosis 15000 ppm: ↓ bw (m), ↑ liver weight, hepatocellular hypertrophy 7500 ppm: hepatocellular hypertrophy
90-day, gavage; 1988	Strain: B6C3F1 Doses: 0, 1000, 5000, 15000 ppm (0, 180/210,	15000 ppm: ↓ bw by 5%/14% m/f, ↑ cholesterol, ↑ ALT (m), ↑ urea (f), ↑ liver weight, hepatocellular hypertrophy, renal tubular change (basophilic tubules, enlarged

	890/1040, 2730/3140 mg/kg bw/d m/f) No. of animals: 10/sex/dose	nuclei), keratosis of non-glandular stomach (m) 5000 ppm: ↓ bw by 5% (f), ↑ liver weight, hepatocellular hypertrophy (m) 1000 ppm: no effects

Structure-activity relationships for developmental toxicity in rats

The following table shows a summary of structural alterations in developmental toxicity assays with short-chain aliphatic acids in rats (adapted from Narotsky *et al.*, 1994). Dosing by gavage on GD 6-15 (2-propylhexanoic acid GD 8-12), sacrifice on PND 6.

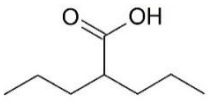
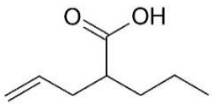
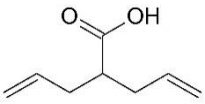
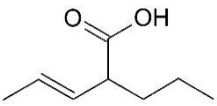
Substance	Structure	Lumbar ribs	Malformations
Valproic acid		+	+
2-Ethylhexanoic acid		+	+
2-Propylhexanoic acid		+	–
2-Butylhexanoic acid		+	–
2-Methylhexanoic acid		–	–
2-Methyloctanoic acid		–	–
2-Methylpentanoic acid		–	–
2-Ethylbutanoic acid		–	–
Butanoic acid		–	–
Pentanoic acid		–	–
Octanoic acid		–	–

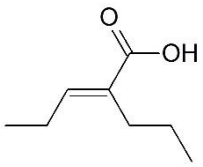
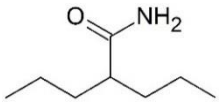
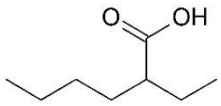
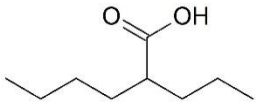
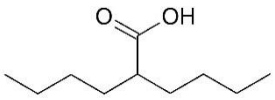
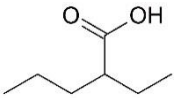
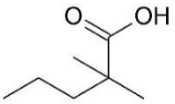
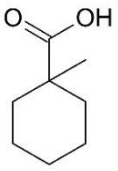
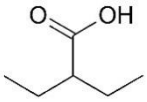
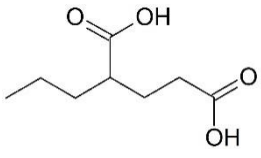
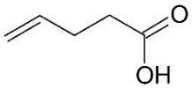
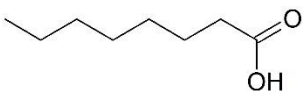
3-Methylhexanoic acid		–	–
5-Methylhexanoic acid		–	–
2-Ethoxypentanoic acid		–	–
2-Bromopentanoic acid		–	–

Structure activity relationships for teratogenicity and anticonvulsant activity in mice

Extensive investigations into structure activity relationships for anticonvulsant activity and teratogenicity of valproic acid metabolites and analogues using a mouse model have been conducted by Löscher, Nau and co-workers. The table below has been compiled from data in publications Löscher and Nau (1985) and Nau *et al.* (1991).

Both the teratogenic and anticonvulsant potency of 2-EHA was lower than that of valproic acid: 5% of foetuses from dams administered a single i.p. dose of 3.0 mmol/kg bw 2-EHA on GD 8 showed exencephaly, compared to 44% for valproic acid (i.p., 3.0 mmol/kg bw on GD 8). The anticonvulsant activity of 2-EHA was 40% of that of valproic acid in a screening test. This comparison might lead to a hypothesis that the MoA of anticonvulsant activity is the same as that of teratogenicity. However, examples of compounds showing high anticonvulsant potency without appreciable teratogenicity (valpromide, 2,2-dimethylpentanoic acid, 1-methylcyclohexanecarboxylic acid) suggest that the MoAs of teratogenicity and antiepileptic activity are unrelated.

Substance (achiral or racemic mixture)	**Structure**	**Anticonvulsant activity**	**Teratogenicity (exencephaly)**
Valproic acid (VPA)		++	++
4-en-VPA		++	++
4,4'-dien-VPA		±	0
3-en-VPA		+	0

(E)-2-en-VPA		+	0
Valpromide		++	0
2-Ethylhexanoic acid		+	+
2-Propylhexanoic acid		++	+
2-Butylhexanoic acid		++	+
2-Ethylpentanoic acid		±	+
2,2-Dimethylpentanoic acid		++	0
1-Methylcyclohexanecarbo-xylic acid		++	0
2-Ethylbutanoic acid		±	±
2-Propylglutaric acid		0	0
4-Pentenoic acid		0	0
Octanoic acid		0	0

Anticonvulsant activity (Löscher and Nau, 1985): ++, comparable to or higher than valproic acid; +, 30-70% of valproic acid; ±, 15-30% of valproic acid; 0, <15% of valproic acid

Exencephaly, foetal incidence after a single intraperitoneal or subcutaneous dose of 2.5-5.7 mmol/kg bw on GD 8 (Nau et al., 1991): ++, above 30%; +, 5-30%; ±, 2%; 0, 0-1%

10.11 Specific target organ toxicity-single exposure

Not evaluated in this dossier.

10.12 Specific target organ toxicity-repeated exposure

Not evaluated in this dossier.

10.13 Aspiration hazard

Not evaluated 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

Albro PW (1975). The metabolism of 2-ethylhexanol in rats. Xenobiotica **5**, 625-636.

Bui LM, Taubeneck MW, Commisso JF, Uriu-Hare JY, Faber WD, Keen CL (1998). Altered zinc metabolism contributes to the developmental toxicity of 2-ethylhexanoic acid, 2-ethylhexanol and valproic acid. Toxicology **126**, 9-21.

Collins MD, Scott WJ, Miller SJ, Evans DA, Nau H (1992). Murine teratology and pharmacokinetics of the enantiomers of sodium 2-ethylhexanoate. Toxicology and Applied Pharmacology **112**, 257-265.

Deisinger PJ, Boatman RJ, Guest D (1994). Metabolism of 2-ethyhexanol administered orally and dermally to the female Fisher 344 rat. Xenobiotica **24**, 429-440.

ECHA (European Chemicals Agency) (2008). Guidance Document for categories, Guidance on information requirements and chemical safety assessment, Chapter R.6: QSARs and grouping of chemicals. Available from ECHA website: http://echa.europa.eu/

ECHA (European Chemicals Agency) (2017). Read-Across Assessment Framework (RAAF). Available from ECHA website: http://echa.europa.eu/

English JC, Deisinger PJ, Guest D (1998). Metabolism of 2-ethylhexanoic acid administered orally or dermally to the female Fischer 344 rat. Xenobiotica **28**, 699-714.

Hamdoune M, Duclos S, Mounie J, Santona L, Lhuguenot JC, Magdalou J, Goudonnet H (1995). In vitro glucuronidation of peroxisomal proliferators: 2-ethylhexanoic acid enantiomers and their structural analogs. Toxicology and Applied Pharmacology **131**, 235-243.

Hendrickx AG, Peterson PE, Tyl RW, Fisher LC, Fosnight JJ, Kubena MF, Vrbanic MA, Katz GV (1993). Assessment of the developmental toxicity of 2-ethylhexanoic acid in rats and rabbits. Fundamental and Applied Toxicology **20**, 199-209.

Kröger S, Liesivuori J, Manninen A (1990). Evaluation of workers' exposure to 2-ethylhexanoic acid (2-EHA) in Finnish sawmills. International Archives of Occupational and Environmental Health **62**, 213-216.

Pennanen S, Manninen A (1991). Distribution of 2-ethylhexanoic acid in mice and rats after an intraperitoneal injection. Pharmacology and Toxicology **68**,57-59.

Pennanen S, Auriola S, Manninen A, Komulainen H (1991). Identification of the main metabolites of 2-ethylhexanoic acid in rat urine using gas chromatography-mass spectrometry. Journal of Chromatography **568**, 125-134.

Pennanen S, Tuovinen K, Huuskonen H, Komulainen H (1992). The developmental toxicity of 2-ethylhexanoic acid in Wistar rats. Fundamental and Applied Toxicology **19**, 505-511.

Pennanen S, Tuovinen K, Huuskonen H, Kosma V, Komulainen H (1993). Effects of 2-ethylhexanoic acid on reproduction and postnatal development in Wistar rats. Fundamental and Applied Toxicology **21**, 204-212.

Pennanen S, Kojo A, Pasanen M, Liesivuori J. Juvonen RO, Komulainen H (1996). CYP enzymes catalyze the formation of a terminal olefin from 2-ethylhexanoic acid in rat and human liver. Human and Experimental Toxicology **15**, 435-442.

Scott WJ Jr, Collins MD, Nau H (1994). Pharmacokinetic determinants of embryotoxicity in rats associated with organic acids. Environmental Health Perspectives **102**, 97-101.

US EPA (U.S. Environmental Protection Agency) (2002). Category development and justification, and proposed test plan for the metal carboxylates category.

Walker V, Mills GA (2001). Urine 4-heptanone: a beta-oxidation product of 2-ethylhexanoic acid from plasticisers. Clinica Chimica Acta **306**, 51-61.

Additional references

Binkerd *et al.* (1988) Evaluation of valproic acid (VPA) developmental toxicity and pharmacokinetics in Sprague-Dawley rats. Fundamental and Applied Toxicology 11:485-493

Ceylan *et al.* (2001) Valproic acid sodium-induced spina bifida occulta in the rat. Neurosurgical Review 24:31-34

Göttlicher et al. (2001) Valproic acid defines a novel class of HDAC inhibitors inducing differentiation of transformed cells. The EMBO Journal 20:6969-6978

Hauck *et al.* (1990) Assymetric synthesis and teratogenic activity of (R)- and (S)-2-ethylhexanoic acid, a metabolite of the plasticizer di-(2-ethylhexyl)phthalate. Life Sciences 46:513-518

Jentink *et al.* (2010) Valproic acid monotherapy in pregnancy and major congenital malformations. New England Journal of Medicine 362:2185-2193

Löscher and Nau (1985) Pharmacological evaluation of various metabolites and analogues of valproic acid. Anticonvulsant and toxic potencies in mice. Neuropharmacology 24:427-435

Narotsky *et al.* (1994) Developmental toxicity and structure-activity relationships of aliphatic acids, including dose-response assessment of valproic acid in mice and rats. Fundamental and Applied Toxicology 22:251-265

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

Ritter *et al.* (1987) Teratogenicity of di(2-ethylhexyl) phthalate, 2-ethylhexanol, 2-ethylhexanoic acid, and valproic acid, and potentiation by caffeine. Teratology 35:41-46

Solecki *et al.* (2001) Harmonisation of rat foetal skeletal terminology and classification. Report of the third workshop on the terminology in developmental toxicology. Berlin, 14-16 September 2000. Reproductive toxicology 15:713-721

Tomson *et al.* (2016) Valproic acid after five decades of use in epilepsy: time to reconsider the indications of a time-honoured drug. The Lancet Neurology 15:210-218

15 APPENDIX. HISTORICAL CONTROL DATA

Oestrus cycle data

	Mean length
Study nr	estrus cycle
20062 F0	5.3
20062 F1	5.5
7931F0	5.43
7931F1	5.41
8394F0	4.9
8394F1	4.8
6791F0	4.6
6791F1	4.2
6763F0	4.4
6763F1	4.7
5029F0	4.83
5029F1	4.68
Mean	**4.90**
SD	**0.43**
Range	**4.2 - 5.5**

	Number of animals
Study nr	with a prolonged estrus period
20062 F0	2
20062 F1	4
7931F0	0
7931F1	0
8394F0	0
8394F1	0
6791F0	1
6791F1	0
6763F0	0
6763F1	4
5029F0	1
5029F1	4
Mean	**1.33**
SD	**1.72**
Range	**0 - 4**

Duration of gestation

	Gestation days	
Study nr.	mean	Number of litters
20026	21.6	12
20063	21.5	12
20070	21.4	12
20098	21.4	12
20099	21.8	12
20114	21.9	12
20137	22.8	12
20138	22.6	8
20139	22.8	9
20277	23.0	7
20556	22.8	9
20062 F0	22.7	25
20062 F1	22.3	22
20521	22.9	12
20553	22.5	12
Mean	22.3	12.5
SD	0.6	4.8
Range	21.4 - 23.0	7.0 - 25.0

Number of lost implantation sites

	No. of lost implantation sites	
Study nr.	N	%
2849F0	18	5.85
2849F1	29	12.05
3280F0	34	9.76
3280F1	25	8.38
5029F0	16	6.79
5029F1	21	6.98
6763F0	34	10.98
6763F1	18	6.59
7098F0	33	11.07
7098F1	19	5.95
6791F0	23	7.71
6791F1	18	7.41
8394F0	34	11.75
8394F1	33	8.6
9127F0	22	8.6
9127F1	29	8.9
Mean	25.38	8.59
SD	6.80	2.03
Range	16 - 34	5.85 - 12.05

Mean number of pups delivered

	Litter size	
Study nr.	mean	Number of litters
20026	11.2	11
20063	11.2	12
20070	12.1	12
20098	12.0	7
20099	13.1	12
20114	10.6	11
20137	11.1	12
20138	12.6	9
20139	11.4	9
20193	12.2	20
20277	11.7	7
20347	10.5	22
20364	11.6	22
20365	11.8	26
20556	7.1	12
20062 F0	9.2	25
20062 F1	8.3	22
20521	12.0	12
20540	11.1	23
20553	12.5	12
Mean	11.0	14.8
SD	1.5	6.5
Range	7.1 - 13.1	7.0 - 26.0