Committee for Risk Assessment

RAC

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

Adopted

11 June 2020

11 June 2020

CLH-O-0000006817-63-01/F

OPINION OF THE COMMITTEE FOR RISK ASSESSMENT ON A DOSSIER PROPOSING HARMONISED CLASSIFICATION AND LABELLING AT EU LEVEL

In accordance with Article 37 (4) of Regulation (EC) No 1272/2008, the Classification, Labelling and Packaging (CLP) Regulation, the Committee for Risk Assessment (RAC) has adopted an opinion on the proposal for harmonised classification and labelling (CLH) of:

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

EC Number: **-**

CAS Number: **-**

The proposal was submitted by **Spain** and received by RAC on **16 April 2019.**

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

PROCESS FOR ADOPTION OF THE OPINION

Spain has submitted a CLH dossier containing a proposal together with the justification and background information documented in a CLH report. The CLH report was made publicly available in accordance with the requirements of the CLP Regulation at ***http://echa.europa.eu/harmonised-classification-and-labelling-consultation/*** on **27 May 2019**. Concerned parties and Member State Competent Authorities (MSCA) were invited to submit comments and contributions by **26 July 2019**.

ADOPTION OF THE OPINION OF RAC

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

The opinion takes into account the comments provided by MSCAs and concerned parties in accordance with Article 37(4) of the CLP Regulation and the comments received are compiled in Annex 2.

The RAC opinion on the proposed harmonised classification and labelling was adopted on **11 June 2020** by **consensus**.

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

	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.
RAC opinion	607-230-00-6	**Retain:** 2-Ethylhexanoic acid **Add:**	**Delete:** 205-743-6	**Delete:** 149-57-5	**Modify:** Repr. 1B	**Modify:** H360D	**Retain:** GHS08 **Modify:** Dgr	**Modify:** H360D			**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

		and its salts, with the exception of those specified elsewhere in this Annex									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.
Resulting Annex VI entry if agreed by COM	607-230-00-6	2- Ethylhexanoic acid and its salts, with the exception of those specified elsewhere in this Annex			Repr. 1B	H360D	GHS08 Dgr	H360D			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.

GROUNDS FOR ADOPTION OF THE OPINION

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.

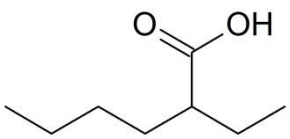
Substance evaluation

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.

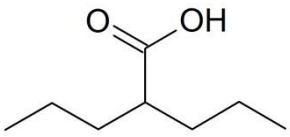
In 2017, the eMSCA concluded that the new EOGRTS (Anonymous, 2016) removed the initial concerns regarding fertility and developmental neurotoxicity and no follow-up action was needed.

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).

HUMAN HEALTH HAZARD EVALUATION

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.

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 Bui *et al.* (1998)	500 mg/kg bw/d, 2-EHA in corn oil, Sprague-Dawley	↑ resorptions, rib and tail anomalies, ↓ foetal weight (9%)	Reduced corrected bw gain (ca. 25 g)
Developmental, single dose Ritter *et al.* (1987)	1800 mg/kg bw, 2-EHA undiluted, Wistar	Limb, tail and cardiovascular defects, hydronephrosis	No information available

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.

Formation of a common compound

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.

Additional references

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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

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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

ANNEXES

Annex 1 The Background Document (BD) gives the detailed scientific grounds for the opinion. The BD is based on the CLH report prepared by the Dossier Submitter; the evaluation performed by RAC is contained in 'RAC boxes'.

Annex 2 Comments received on the CLH report, response to comments provided by the Dossier Submitter and RAC (excluding confidential information).