SUBSTANCE EVALUATION REPORT

Public Name: 2-Ethylhexanoic acid (2-EHA)

EC Number(s): 205-743-6

CAS Number(s): 149-57-5

Submitting Member State Competent Authority:

Spain

Ministry of Health, Social Services and Equality

Paseo del Prado, 18-20

28071 - Madrid

Year of evaluation (as given in the CoRAP): 2012

VERSION NUMBER: 3 **DATE: 20/06/2017**

Conclusions of the most recent evaluation step*	Tick relevant box(es)
Concern not clarified; Need to request further information from the Registrant(s) with the draft decision	
Concern clarified; No need of further risk management measures	X
Concern clarified; Need for risk management measures; RMO analysis to be performed	
Other: [*please specify*]	

**Include details in the executive summary.*

Executive summary

Grounds for concern

Initial concern was described in the justification document for inclusion of 2-ethylhexanoic acid (2-EHA) in the CoRAP as follows:

"*2-Ethylhexanoic acid is classified as Repro. Category 2 for developmental effects. Apart from developmental toxicity data, in the registration dossiers only data from a scientific publication, of a one-generation study is available. This test is not following properly the test methods laid down in the Test Method Regulation or other international tests recognised by the Commission or the Agency as being appropriate. However, in this study effects on spermatozoa and epididymis, time to complete the mating and mean litter size were observed (the relative epididymal weights in high-dose males were significantly increased but no histological changes were found, the mean litter size in high-dose pregnant females was significantly reduced and a slight but not statistically significant increase in the number of abnormal sperm was noted). There is concern that the substance would need to be classified also for fertility but available data are not conclusive. For this reason it would be necessary to clarify the potential effect of the substance on fertility.*

The substance is used by workers and it is present in some consumer products. Furthermore, for some exposure scenarios the RCR for workers (industrial and professional) is close to 1 (>0.8, worker short-term exposure). The substance is used in consumer products as binder in concentrations < 1%. The registrants do not expect consumer exposure, so no consumer exposure assessment is provided in the CSR except for two small scenarios (exposure scenarios 25/26) in which the substance is used as a binder (fillers, putties, plasters, modelling clay and non-metal-surface treatment products)."

Apart from these initial grounds for concern relating to fertility, concern on postnatal development, related to potential neurodevelopmental toxicity, was also revealed during the evaluation. Also in the one-generation reproductive toxicity study, 2-EHA caused delayed postnatal development that was evidenced in the reflex and physical parameters evaluated.

Procedure

Pursuant to Article 44(2) of the REACH Regulation, 2-EHA was included on the Community rolling action plan (CoRAP) for evaluation in 2012. The Competent Authority of Spain was appointed to carry out the evaluation.

The evaluation was first based on the data contained in the IUCLID dataset that was compiled on 1 March 2012, including the Chemical Safety Reports. Furthermore, a literature search was also carried out by the Spanish evaluating MSCA (eMSCA) at the beginning of the evaluation procedure in February 2012. Additional updates of registration dossiers were also taken into account.

The evaluation of 2-EHA was targeted at human health endpoints and focused on the grounds for concern that were included in the justification document for the inclusion of the substance in the CoRAP. In addition to these initial concerns, an additional concern on postnatal development, related to potential developmental neurotoxicity, was also identified during the evaluation.

However, all human health hazard endpoints were reviewed.

During the process, fluent communication was established between the Spanish Ministry of Health, Social Services and Equality (eMSCA) and the lead registrant, as representative of all registrants. After a meeting held with the lead registrant to clarify the main preliminary issues, he considered the need of updating the registration dossier. Consequently, updated dossiers were submitted by all

registrants. Within these updated registration dossiers, 9 uses that only involve workers were identified. In addition, they declared the consumer use as advised against. Furthermore, the registrants corrected the DNEL derivation to be in accordance with ECHA Guidance. Finally, they included a justification for waiving the 2-generation reproductive toxicity study that was a standard information requirement at that tonnage level, based on its confidence that no additional information on fertility was necessary.

However, after evaluating all available information, the need to clarify the concern on reproduction remained and therefore, further information was required in a substance evaluation decision.

The draft decision pursuant to Article 46(1) of the REACH Regulation was submitted to ECHA on 28 February 2013.

Comments from the registrants and several proposals for amendment to the draft decision were received from other MSCAs. The eMSCA reviewed them and amended the draft decision accordingly.

On 21 October 2013 ECHA referred the draft decision to the Member State Committee.

By 11 November 2013 the Registrant provided comments on the proposed amendments. The MSC took the comments of the Registrant into account.

After discussion in the Member State Committee meeting on 10-13 December 2013, a unanimous agreement of the Member State Committee on the draft decision as modified at the meeting was reached on 12 December 2013. ECHA took the decision pursuant to Article 51(6) of the REACH Regulation.

On 26 February 2014 ECHA sent the final decision to the registrants. An extended one-generation reproductive toxicity study in rats, oral route, including the cohorts 2 and 3 to assess developmental neurotoxicity (DNT) and immunotoxicity (DIT) (test method: OECD 443) was required. The need for the extension of cohort 1B to mate the F1 animals to produce the F2 generation should be considered in case results obtained during the study did not allow drawing a clear conclusion on this endpoint.

On 24 May 2016 the Registrant submitted to ECHA an update of the registration dossier containing the information required. This new information has been assessed by the eMSCA.

Finally, on 15 March 2017 the eMSCA has concluded that the new information submitted by the registrants clarifies the concerns.

Evaluation of new information submitted by the Registrant(s) in response to the ECHA decision on substance evaluation

An extended one-generation reproductive toxicity study (EOGRTS) was requested in a substance evaluation decision issued on 26 February 2014[1], since concerns regarding fertility and neurodevelopmental toxicity had been identified. These concerns were based in 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, an apparent reduction in sperm motility and a delay in fertilization were observed in parental animals. In addition, delay in the development of the grip and cliff avoidance reflex observed in pups of the mid

[1]https://echa.europa.eu/documents/10162/598f7b4e-ad01-4541-a7b4-cde1be062942

and high-doses was considered as a potential neurodevelopmental toxicity effect. Furthermore, 2-EHA is an analogue of the anticonvulsant drug valproic acid.

Following the substance evaluation decision, both an oral combined repeated dose toxicity study with the reproduction/developmental toxicity screening test (OECD TG 422) as a range finder study for the OECD TG 443, and an extended one-generation reproductive toxicity study (OECD TG 443) were conducted according to GLP with 2-EHA in Wistar rats (Unnamed reports, 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).

No fertility or reproductive effects were observed in male and female rats in the oral combined repeated dose toxicity study with the reproduction/developmental toxicity screening test. The results obtained in the EOGRTS performed in Wistar rats dosed at 0, 80, 250 and 800 mg/kg bw/d 2-EHA did not show any treatment-related effects in fertility and reproductive parameters at any dose levels both in F0 and F1 generations. Additionally, neither treatment-related effects regarding neurodevelopmental toxicity nor neurodevelopmental effects associated with treatment with 2-EHA were reported for cohorts 2A and 2B. As a result of this study, a 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. The NOAEL for fertility and reproductive effects, developmental neuro and immunotoxicity effects was established at 800 mg/kg bw/d, due to the lack of effects.

This new information from good quality studies does not confirm any of the findings related to fertility and neurodevelopmental toxicity that were observed in the previous non-guideline and non-GLP studies. 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. No alterations in the neurodevelopmental parameters have been observed. In addition, no immunotoxic developmental effects were observed in cohort 3.

Final conclusions

The new studies results provide sufficient and reliable information for the eMSCA to conclude that 2-EHA does not show a specific effect on fertility and developmental neurotoxicity. For this reason, it is considered that the concerns have been clarified and neither further information nor additional classification is required based on this substance evaluation.

CONTENTS

TABLES

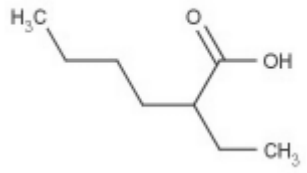
1 IDENTITY OF THE SUBSTANCE AND PHYSICAL AND CHEMICAL PROPERTIES

1.1 Name and other identifiers of the substance

Table 1: Substance identity

Public Name:	2-ethylhexanoic acid
EC number:	205-743-6
EC name:	2-ethylhexanoic acid
CAS number (in the EC inventory):	149-57-5
CAS number:	149-57-5
CAS name:	Hexanoic acid, 2-ethyl-
IUPAC name:	2-ethylhexanoic acid
Index number in Annex VI of the CLP Regulation	607-230-00-6
Molecular formula:	C8H16O2
Molecular weight range:	144.2114
Synonyms:	2-EHA; Hexanoic acid, 2-ethyl-(8CI, 9CI); Alpha-Ethylcaproic acid; 2-Ethylhexanoic acid; 2-Ethylcaproic acid; Butylhethylacetic acid; Ethylhexanoic acid; 2-Ethyl-1-hexanoic acid; 2-Butylbutanoic acid; Alpha-Ethylhexanoic acid; 2-Ethylhexoic acid; 3-Heptanecarboxylic acid

Structural formula:



1.2 Composition of the substance

Name: 2-ethylhexanoic acid

Description: mono-constituent substance (origin: organic)

Substance concentration details are described in the confidential annex.

1.3 Physico-chemical properties

An assessment of the physico-chemical properties has not been carried out. Results included in the table below correspond to those values reported by the registrant in the CSR.

Table 2: Overview of physicochemical properties

Property	Value
Physical state at 20 °C and 101.3 kPa	Colourless to slightly yellow liquid
Melting/freezing point	-57 ºC at 1013.25 hPa
Boiling point	226-229 °C at 1013.25 hPa
Vapour pressure	0.04 hPa at 20 °C
Surface tension	Not applicable
Water solubility	1.4 g/L at 20 °C
Partition coefficient n-octanol/water (log value)	2.7 at 25 °C / pH = 4.7
Flash point	118 ºC at 1013.25 hPa
Flammability	Not applicable
Explosive properties	Not applicable
Auto flammability	310 ºC at 1013 hPa
Oxidising properties	Not applicable
Granulometry	Not applicable
Stability in organic solvents and identity of relevant degradation products	Not applicable
Dissociation constant	4.76 at 25 ºC
Viscosity	8.4 mPa·s at 20.3 ºC

2 MANUFACTURE AND USES

2.1 Quantities

Table 3: Aggregated tonnage (per year)

1 – 10 t	10 – 100 t	100 – 1000 t	1000 - 10,000 t	10,000 - 50,000 t
50,000 – 100,000 t	x100,000 – 500,000 t	500,000 – 1000,000 t	> 1000,000 t	Confidential

During pre-registration period, several companies pre-registered the substance. Even though not all of the pre-registrations will result in a registration, some of these companies will register the substance in the following deadlines for registration, leading to a higher aggregate tonnage.

2.1.1 Manufacturing processes

2-EHA is commercially available as a racemate with purity higher than 99% (w/w).

The manufacturing process of the substance consists, basically, of an oxidation reaction of 2-ethylhexanal (Environment and Health Canada, 2011).

2.2 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%).

2.2.1 Uses by workers in industrial settings

Identified uses in industrial settings include: use as an intermediate in the manufacture of other substances, formulation of mixtures, use in laboratories and use as functional fluids (max. 15%).

2.2.2 Use by professional workers

Identified uses in professional settings include use of 2-EHA in laboratories and use as functional fluids (max. 15%).

2.2.3 Uses by consumers

No consumer use is described by the registrants in the updated registration dossiers.

2.3 Uses advised against

In the updated registration dossiers, there is a declaration of uses advice against for consumers.

Product category (PC): PC 0: Other: Not recommended for use in any consumer products.

2.3.1 Uses by workers in industrial settings advised against

None.

2.3.2 Use by professional workers advised against

None.

2.3.3 Uses by consumers advised against

Not recommended for use in any consumer products.

3 CLASSIFICATION AND LABELLING

3.1 Harmonised Classification in Annex VI of the CLP Regulation

The substance is listed in Annex VI of CLP Regulation. The harmonised classification included in tables 3.1 and 3.2 for 2-EHA is, respectively, the following:

Index No	International Chemical Identification	EC No	CAS No	Classification	
				Hazard Class and Category Code(s)	Hazard statement Code (s)
607-230-00-6	2-ethylhexanoic acid	205-743-6	149-57-5	Repr. 2	H361d

Index No	International Chemical Identification	EC No	CAS No	Classification
607-230-00-6	2-ethylhexanoic acid	205-743-6	149-57-5	Repr. Cat. 3; R63

3.2 Self classification

2-Ethylhexanoic acid has not been self-classified by the registrants.

4 ENVIRONMENTAL FATE PROPERTIES

The 2-EHA evaluation was targeted at human health and therefore, no environmental risk assessment has been carried out.

5 HUMAN HEALTH HAZARD ASSESSMENT

5.1 Toxicokinetics (absorption, metabolism, distribution and elimination)

5.1.1 Non-human information

The toxicokinetics of 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 IUCLID dataset. 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 (Unnamed report, 1987; English *et al*., 1998):

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

b. By gavage for 14 days with 100 mg unlabelled 2-EHA/kg 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 by occlusive application for 96 hours.

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

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 (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. 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 (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. 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 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) 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.

5.1.2 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).

5.1.3 Summary and discussion on toxicokinetics

Results from a 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.

5.2 Acute toxicity

5.2.1 Non-human information

Several acute toxicity studies have been reported in the IUCLID dataset. These assays have been carried out in different strains of rats and guinea pigs. The results are summarized in table 4:

Table 4: Summary of acute toxicity of 2-ethylhexanoic acid in rodents

Route	Species/strain	Dose	LD_{50} (mg/kg bw) LC_{50} (mg/L)	Remarks	References
Oral (Gavage)	Rats Fischer 4 (female) per dose	0, 90, 722, 1445, 2890 mg/kg bw	2043	Purity = 99,6% Vehicle: corn oil	Unnamed report (1987a)
	Rats US 5 per sex and dose	0.2, 1.6, 3.2, 4 mL/kg bw	4 mL/kg (reported to be 3640 mg/kg)	Purity = 99% Vehicle: aqueous emulsion containing 0.5% Traganth	Unnamed report (1967)
	Rats Wistar 6 (male) per dose	1000 y 10000 mg/kg bw	3000	Purity not reported	Unnamed report (1942) Smyth and Carpenter (1944)
Dermal	Rats Wistar 5 per sex and dose	2000 mg/kg bw	> 2000	Purity = 99% Limit test	Unnamed report (1986)
	Guinea pigs (strain and sex not reported) 6 animals per dose	1000, 10000 mg/kg bw	6300	Purity not reported	Unnamed report (1942) Smyth and Carpenter (1944)
Inhalation	Rats (strain not reported) 6 animals per sex and dose	0.11 mg/L (110 mg/m^3)	LC0 (8h): 0.11 mg/L	Purity = 99% No vehicle	Unnamed report (1967)
	Rats	-	-	-	Unnamed report (1942) Smyth and Carpenter (1944)

5.2.1.1 Acute toxicity: oral

In a GLP study equivalent to OECD 401 (1981), single doses of 0, 90, 722, 1445 or 2890 mg/kg bw of 2-EHA were administered, by gavage, to four female Fischer rats per dose using corn oil as a vehicle. This study has been flagged as the key study in the substance dataset. The animals were observed for 14 days for mortality and toxic signs. The test material caused mortality in all rats at 2890 mg/kg bw. The cause of these deaths has not been determined and no more deceases occurred at any other dose level tested. Observed clinical signs included weakness at lower doses (90, 722 and 1445 mg/kg bw) and prostration at the highest dose (2890 mg/kg bw). Gross pathology findings in the deceased animals included faecal discoloration, wetness of the inguinal hair and residues of 2-EHA in duodenum, jejunum, ileum, cecum and colon. A histopathological examination was not

conducted. The surviving animals showed a minor decrease in body weight during the first 24 hours after dosing, but they recovered and even increased weight within 7 days post-exposure. The acute oral LD_{50} (95% confidence limits) was calculated as 2043 (1445-2890) mg/kg bw (Unnamed report, 1987a).

In a non-GLP acute toxicity test, comparable to OECD 401 (1981) with acceptable restrictions and reported as supporting study, groups of five US-rats per sex were exposed, by oral gavage, to 0.2, 1.6, 3.2 and 4 mL/kg bw of 2-EHA dissolved in an aqueous emulsion containing 0.5% Traganth. No control groups were used and weighing for dose calculation was only at the beginning of the study. Post-exposure observation period was 7 days. Mortality was observed in five animals at 4 mL/kg, in three animals at 3.2 mL/kg bw and in one animal at 1.6 mL/kg bw. Clinical signs reported at the two highest doses included apathy, dyspnea, abdominal position, re-crusted eyes and snouts, disappearing by day 5. In the gross pathology observation no substance related findings were noted. No body weight was recorded. The LD_{50} was 4mL/kg bw, reported to be equivalent to 3640 mg/kg bw (Unnamed report, 1967).

In addition, in a poorly reported range finding test cited in the IUCLID dataset, single doses of 1000 and 10000 mg/kg bw of 2-EHA (no data on purity) were administered by oral gavage to six male Wistar rats per dose. No control animals were used. All animals died at 10 mg/kg bw. No information was given regarding the presence or absence of other signs of toxicity. The LD_{50} in male rats was established as 3000 mg/kg bw (Unnamed report, 1942; Smyth and Carpenter, 1944).

5.2.1.2 Acute toxicity: inhalation

In a non-GLP study conducted according to a protocol comparable to OECD 403 (1981) with some restrictions, groups of six rats per sex (strain not reported) were exposed for 8 hours, via whole body exposure method, to a nominal vapour concentration of 0.11 mg/L (400 ppm) 2-EHA. This study has been considered as the key study in the IUCLID dataset. No control animals were used. The observation period was 7 days. Body weight data have not been reported. No clinical signs were noted and no substance related findings were mentioned in the gross pathology. No deaths occurred and, therefore, the LC_0 of 0.11mg/L was established (Unnamed report, 1967).

In a poorly reported non-GLP range finding study, six rats (no data on sex and strain) were exposed for 8 hours to saturated vapours of 2-EHA. No mortality was reported (Unnamed report, 1942; Smyth and Carpenter, 1944).

5.2.1.3 Acute toxicity: dermal

A single dose of 2000 mg/kg bw 2-EHA (purity > 99%) was applied, semi occluded, to the clipped skin of five Wistar rats per sex for a period of 24 hours, in a GLP well-reported acute dermal toxicity study conducted according to OECD 402 (1981), which has been considered as the key study in the IUCLID dataset. The animals were observed for 14 days for toxic signs. No mortality, no clinical signs and no gross pathology were observed. The acute dermal LD_{50} (without confidence limits) was determined as greater than 2000 mg/kg bw (Unnamed report, 1986).

A LD_{50} of 6300 mg/kg bw was determined in a non-GLP poorly documented range finding study in guinea pigs (six animals per group, sex and strain not reported). The observation period was of 14 days following application of single occluded dose levels of 1000 and 10000 mg/kg bw 2-EHA for 4 days. There were no deaths at the lower dose group. The only clinical sign reported in surviving

animals was a dry and slightly cracked skin that returned to normal after 14 days (Unnamed report, 1942; Smyth and Carpenter, 1944).

5.2.1.4 Acute toxicity: other routes

5.2.2 Human information

No information is available on the effects of single exposure in humans.

5.2.3 Summary and discussion of acute toxicity

Acute toxicity studies have been carried out in different strains of rats and guinea pigs. No information is available on the effects of 2-EHA in humans.

In an acceptable oral toxicity study, cited as key study in the registration dataset, an oral LD_{50} value of 2043 mg/kg bw has been calculated. Death only occurred at the highest dose tested (2890 mg/kg bw). The main clinical signs observed were weakness and prostration. A supporting acute toxicity test established an oral LD_{50} of 3640 mg/kg bw. In addition, in a poorly reported range finding test, the oral LD_{50} was estimated in 3000 mg/kg bw.

A LD_{50} greater than 2000 mg/kg bw was obtained in a well-conducted acute dermal toxicity study in rats. All animals survived over the 14 days post-exposure period. In a poor- quality range finding study, a dermal LD_{50} was established in 6300 mg/kg bw.

Regarding the inhalation route, an 8-hour exposure to 0.11 mg/L 2-EHA produced no deaths and no signs of toxicity in rats. Related to these findings, a LC_0 of 0.11 mg/L was established.

No toxicologically significant effects were found following acute exposure via any route. Overall, it can be concluded that 2-EHA is of low acute toxicity by the three routes of exposure.

5.3 Irritation

5.3.1 Skin

The available information regarding skin irritancy yields divergent results ranging from not irritating to corrosive. Further, it should be taken into account that, in most cases, the information is scarce and of limited reliability.

In a GLP study performed according to OECD Test Guideline 404 and selected as the key study, 2-EHA was assessed as slightly irritating (Unnamed report, 1985a). A single dose of 0.5 ml of the undiluted test substance was applied to the shaved skin of three New Zealand White rabbits for 4 hours under semiocclusive tape. The average erythema/oedema scores calculated over 24-72 hours were 0.66 and 0.33, respectively. Erythema persisted through the 14 days post-exposure period (eschar formation was still visible). Due to oedema score of 0 at day 7 which raised to grade 1 in one animal and grade 2 in another animal at day 14, the authors concluded that scratching occurred. Although inflammation persisted at the end of the observation period, the grade of the reactions and the observed effects do not guarantee the classification of the substance as irritant to the skin.

In a briefly reported GLP study according to the US Department of Transportation corrosivity test, 0.5 ml of undiluted 2-EHA was applied to the skin of six New Zealand White rabbits for 4 hours. A slight necrosis was observed after 4 hours post-application with subsequent slight to moderate eschar formation after 48 hours, in five out of six rabbits. The authors noted that a necrosis which disappear within 48 hours has to be very slightly and superficial. However, data of any effect beyond 48 hours and erythema and oedema were not reported/evaluated by the authors (Unnamed report, 1986).

A non-GLP test using an internal standard method (Unnamed report, 1967) was conducted with 0.5 ml of undiluted 2-EHA. Two Vienna White rabbits were treated for 1, 5 and 15 minutes and two other animals for 20 hours, using occlusive conditions. Nevertheless, only the results for 20 hours and 15 minutes of exposure were included in this study. An average score (average of the two animals over 24, 48 and 72 hours) of 2.5 for erythema and of 0.5 for oedema was calculated for 20 hours exposure period. At the end of the 8 days observation period, one animal showed an erythema score of 2 and the other an erythema score of 1, with eschar formation. In the same way, the average scores for 15 minutes exposure period were 1.1 and 0 for erythema and oedema, respectively. It was concluded that 2-EHA was not irritating.

In a poorly reported non-GLP test, 2-EHA was applied occlusively to the belly of rabbits. The authors reported erythema, but there is a lack of information on number of animals used, duration of exposure, scores and a clear statement on the results (Smyth and Carpenter, 1944; Unnamed report, 1942).

As part of a GLP toxicokinetic study equivalent or similar to US EPA TSCA Health Effects Testing Guideline (CFR 40 798.7100), a test to evaluate the skin condition was conducted (Unnamed report, 1987; English *et al*., 1998). Only minimal gross pathologic changes were observed in the skin, despite the long exposure periods (96 hours) used in this test in comparison to the standard methods (typically of 4 hours). Authors concluded that the minimal effects caused in the skin, make it difficult to assume that 2-EHA will produce an irritating effect with any contact.

5.3.2 Eye

2-EHA was tested in three New Zealand White rabbits by a GLP procedure according to OECD 405 that was selected as the key study (Unnamed report, 1985b). The substance was applied in one of the eyes of each animal, using the other eye as control. The average score (24 to 72 hours), calculated after application of 0.1 ml of 99% pure 2-EHA for 24 hours, was 0.44 for corneal opacity, 0.56 for iritis, 1.2 for conjunctival redness and 0.89 for chemosis. Corneal clouding was reversible after 72 hours and all other ocular effects were fully reversible within 7 days after instillation. Considering the described results, 2-EHA was evaluated as not irritating to the eyes.

The IUCLID data include a non-GLP study conducted in rabbits (Smyth and Carpenter, 1944). This study lacks information on the number of animals used, duration and conditions of the test. The volumes applied were 0.001, 0.005, 0.02, 0.1 and 0.5 ml of 2-EHA and the duration of exposure was 24 hours. Corneal necrosis was reported at 0.001 ml test substance, graded 5 by the authors in their eye burn rating scale.

5.3.3 Respiratory tract

No data are available.

5.3.4 Summary and discussion of irritation

The available data indicate that 2-EHA produces only minimal dermal and eye irritation in animals following single exposure. Any mild effects observed were fully reversible. From these data it can be concluded that the substance is not skin or eye irritant.

5.4 Corrosivity

The data presented on skin and eye irritation suggest that 2-ethylhexanoic acid has no corrosive potential.

5.5 Sensitisation

5.5.1 Skin

The skin sensitisation potential of 2-EHA has been investigated in a non-GLP guinea pig maximization test equivalent or similar to OECD Guideline 406, considered the key study (Unnamed report, 1979). Ten females Durkin-Hartley guinea pigs were used in the treated group and five animals in the control group. A positive control was not included in the study. In the induction phase, 3 intradermal injections of a) 0.1 ml Freund's complete adjuvant (FCA) alone; b) 0.1 ml 2-EHA (1% w/w) alone; and c) 0.05 ml 2-EHA (1% w/w) emulsified with 0.05 ml FCA were administered to the treated group. The control group was only administered with 0.1 ml FCA alone. Six days afterwards the test area was treated with 10% w/w sodium lauryl sulphate solution and, following 24 hours, a filter paper saturated with 2-EHA (5% w/w) was applied to the test area for 48 hours and held in contact by an overlapping patch of impermeable plastic adhesive tape. Two weeks after the topical induction, the challenge phase was conducted by applying 2-EHA (2% w/w) on a 2 × 2 cm piece of filter paper to the test site. The patch was held in contact and removed 24 hours later.

No response was elicited at either 48 hours after induction or 48 hours post-challenge with 5% (w/w) and 2% (w/w) aqueous 2-EHA solution, respectively. Therefore, there is no evidence of sensitisation potential of 2-EHA.

5.5.2 Respiratory system

No data are available.

5.5.3 Summary and discussion on sensitisation

The only information available for 2-EHA is a non-GLP study in guinea pigs that indicates that 2-EHA has no sensitizing properties.

No information is available on the respiratory sensitisation potential of 2-EHA.

The available information in humans is based on a survey conducted on sawmills workers, which reported an allergic reaction in two out of 114 workers. However, the substance does not contain structural alerts for sensitisation.

5.6 Repeated dose toxicity

5.6.1 Non-human information

Several repeated dose toxicity studies have been reported in the IUCLID dataset. These studies were carried out in two different species, rats and mice. The results are summarized in table 5.

5.6.1.1 Repeated dose toxicity: oral

Existing information before ECHA decision on substance evaluation

In two studies conducted in accordance with GLP and EPA Guidelines (subchronic oral toxicity test), the systemic effect of subchronic oral administration of 2-EHA was evaluated in rats and mice (Unnamed report, 1988a; 1988b). These assays have been flagged as key studies in the IUCLID file. The same studies were published later by Juberg *et al.* (1998).

Groups of ten Fischer 344 rats per sex and dose level were fed diets containing 0, 0.1, 0.5 and 1.5% 2-EHA (purity 99.9%) in corn oil, reported to be equivalent to an average daily intake of 0, 61, 303 and 917 mg/kg bw/d in males and 0, 71, 360 and 1068 mg/kg bw/d in females, for a period of 91 to 93 days. An additional number of ten animals per sex were added to the high dose and control groups to evaluate recovery from any observed toxic effects for another 27-28 days. Doses were based on a 14-day feeding study. Body weight measurements were collected twice in the first week and, at least, once weekly from there on. Feed consumption was measured with the same frequency. Clinical signs of toxicity were monitored twice by day. Mortality was checked daily. Ophthalmological examinations were conducted prior to the beginning of the study, followed by an examination of five animals per sex and dose during the last week. Recovery animals suffered the same clinical observations to those performed on animals of the test phase. Animals were examined for clinical effects, and changes in haematology, clinical chemistry, urinalysis, gross pathology and histopathology.

During the study, no mortality or treatment-related signs of toxicity occurred. No ophthalmological abnormalities were seen either.

A reduced mean body weight in conjunction with reduced feed consumption was observed in males and females of the high-dose group. A mean body weight reduction of 8% and 10%, at necropsy, was seen in males and females of the 1.5% group, compared with the controls. No effects on body weight were observed in the other groups. During the recovery phase, the rate of body weight gain for the high-dose animals increased, but was still slightly below control levels at study termination.

Minor changes in haematology occurred (lower mean corpuscular haemoglobin (MCH) and mean corpuscular volume (MCV)) in mid-dose males and high-dose males and females. Slight poikilocytosis in treatment and control groups was observed. These differences were minimal and they were considered clinically insignificant by the authors.

Serum cholesterol, serum albumin, urea and nitrogen levels were increased in a dose-dependent manner in males of the high dose. All these findings returned to control levels within the post-exposure observation period. The authors suggested that elevated serum cholesterol levels only seen in males were consequence of sex-specific differences in fatty acid metabolism.

Urinalysis examination did not show any treatment-related findings.

Statistically significantly higher absolute and relative liver weights were observed in males and females of the mid- and high-dose groups compared to the controls. Following 28 days of recovery, all absolute liver weights were comparable to control values. For the high–dose males, the relative liver (to body) weight remained elevated after the recovery period.

No changes were seen in absolute kidney weights. Relative kidney weights were slightly increased for both the 0.5% and 1.5% diet groups. Authors suggested that these increases in relative kidney weights were related to decreases in body weights, rather than indicative of any particular effect on this organ. In addition, a minor but statistically significant decrease in absolute brain weight was not considered biologically significant. Slight increases in relative brain and testes weights were related to reduced body weights and not considered as an indicative of organ specific toxicity.

Hepatocyte hypertrophy with dose-dependent severity, primarily located in the portal area, was observed in all animals of the high-dose group and in males of the mid-dose group. In addition, a decreased incidence of small cytoplasmic vacuoles was observed in a dose-dependent manner. No treatment-related effects were seen in the recovery group.

The only persistent effects by treatment were slightly lower body weights and increased liver to body weights in the high-dose male rats. Most histological changes reversed after the recovery period. The liver enlargement was considered to be primarily an adaptive change rather than a toxic effect. The hepatocyte hypertrophy was judged to be consistent with increased liver weight and enzymatic induction.

A NOAEL of approximately 300 mg/kg bw/d was reported, based on reduced feed consumption and decreased rate of body weight gain.

A parallel study was carried out in mice with identical design and purpose. Groups of ten B6C3F1 mice per sex and dose level were offered feed containing 0, 0.1, 0.5 and 1.5% 2-EHA (purity 99.9%) reported to be equivalent to an average daily intake of 0, 180, 885 and 2728 mg/kg bw/d in males and 0, 205, 1038 and 3139 mg/kg bw/d in females, during 91 to 93 days. No vehicle was used. Additionally, ten animals per sex were added to the high-dose and control groups to evaluate the recovery from possible observed toxic effects, for another 28-29 days.

Similarly to rats, no mortality or clinical signs of toxicity were seen throughout the study. In addition, no treatment-related changes were obtained in the ophthalmological examination.

A reduced mean body weight was observed in males and females of the 1.5% group and in females of the 0.5% group. A reduction of 5.2%, 13.8% and 5.6% in mean body weight was seen in males and females of the high-dose group and in females of the mid-dose group, respectively, compared to the controls. At the end of the recovery phase, body weights were still below controls in males and females of the high-dose group. During the study, feed consumption was slightly less than or equal to that of the control group in mice of the highest dose, without any alteration in the other groups.

No haematological changes were observed in any of the treated groups.

Serum cholesterol in males and females of the mid- and high-dose groups showed statistically significant increases, returning to control levels after 28 days of recovery. Statistically significant increases in alanine aminotransferase (ALT) levels were seen in males of the high-dose group. On the contrary, in males and females of the 1.5% group and in females of the 0.5% group, triglyceride and bilirubin levels were statistically significantly lower, compared with the controls. After the recovery period, these values reached control levels in female mice but remained below controls in male mice. Authors did not consider hypobilirubinaemia as clinically significant since no signs of anaemia were seen throughout the study. However, this does not preclude the possibility that the

decrease in bilirubin, coupled with increased cholesterol, might reflect an impaired conversion of cholesterol to bilirubin.

No clinically significant treatment-related urinalysis changes were observed.

For males and females of the high dose and for males of the mid dose, absolute liver weights were statistically significantly greater than controls. Statistically significant higher relative liver weights were observed in animals of the mid- and high-dose groups compared to the controls. At the end of the recovery period, liver weight differences had largely disappeared.

No changes were seen in absolute kidney weights. Relative kidney weights for female mice consuming diets containing 0.5% or 1.5% 2-EHA were greater than those observed in the control groups. Authors suggested that these increases in relative kidney weights were related to decreases in body weights. Besides, minor but statistically significant decreases in absolute brain and adrenal weights in female mice were not considered biologically significant. Slight increases in relative brain and testes were also related to reduced body weights and not considered to be indicative of organ specific toxicity.

A dose-dependent hepatocyte hypertrophy, mainly located in the portal area, was seen in all animals of the high-dose group and in male mice of the mid-dose group. Moderate eosinophilia was observed in the affected hepatocytes in the high dose animals. A decreased incidence of small cytoplasmic vacuoles was observed in a dose-dependent manner. Minimal to minor increases in cytoplasmic basophilia of the proximal convoluted tubules were observed in the kidneys of the high-dose mice group. At the end of the recovery period all these findings returned to control levels.

Persistent effects after treatment were reduced body weight gain and increased relative liver to body weights in animals of the mid- and high-dose groups. Nearly all the histological changes observed throughout the study disappeared at the end of the recovery period. Hepatomegaly was considered to be an adaptive change rather than a toxic effect. Histopatology in high-dose mice exhibited hepatocyte hypertrophy coherent with increased liver weight and enzymatic induction.

A NOAEL of approximately 200 mg/kg bw/d based on reduced feed consumption and decreased rate of body weight gain was established.

Four GLP-compliant range finding studies in rats and mice have also been reported in the IUCLID dataset as supporting studies (Unnamed reports, 1987b; 1987c; 1987d; 1987e). The method of administration was either gavage or feeding, for 2 weeks. Control animals were included. Animals were observed each workday for clinical signs of toxicity. Mortality was checked daily. Body weights and feed consumption were measured twice weekly. No haematological and clinical chemistry examinations were performed. Histopathological examination was restricted to the liver, kidneys and any gross organ lesions.

In the gavage study in rats, groups of five Fischer 344 rats per sex and dose received doses of 0, 200, 800 and 1600 mg/kg bw/d 2-EHA (nominal in corn oil) five days per week for two weeks. The highest dose was lethal to most rats. Mid-dose animals showed weakness, lethargy, sialorrhea and unkempt haircoats generally less severe than in the high-dose animals. Lower body weights were seen in surviving high-dose and mid-dose male rats, compared to the control group. Feed consumption decreased in surviving rats receiving the highest test dose. Liver weight was increased in a dose-dependent manner in the males of the high and mid-dose groups and in all females that received the test substance. At the end of the study, high-dose animals which survived had hepatocyte hypertrophy, while necropsy in death animals showed minimal hepatocyte degeneration. No treatment-related changes were observed in the kidneys. The NOAEL was established in 200 mg/kg bw/d based on overall effects.

In addition, groups of five B6C3F1 mice per sex and dose received, in another gavage study, doses of 0, 200, 800 and 1600 mg/kg bw/d 2-EHA (nominal in corn oil), five days per week along two weeks. During the study, only one male from the mid-dose group was found dead, not being determined the cause of this decease. One female of the high-dose group showed gait disturbance, weakness and weight loss for the first two days of the study. A minimal decrease in feed consumption, not considered of biological significance, was seen in females from the low-dose group. Liver weight increased in males of the high-dose group. All males at the highest dose showed hepatocyte hypertrophy. A NOEL of 800 mg/kg bw/d was established for males, based on liver effects and a NOEL of 1600 mg/kg bw/d was established for females, based on overall effects.

In a feeding study, groups of five Fischer 344 rats per sex and dose received daily dietary doses containing 0, 0.75, 1.5 and 3% 2-EHA, reported to be equivalent to an average daily intake of 0, 706, 1351 and 2276 mg/kg bw/d in males and 0, 756, 1411 and 2658 mg/kg bw/d in females, for 14 days. No mortality occurred during the study. High-dose animals had slightly reduced amounts of faeces. The female rats at the high-dose level had urine soaked, discoloured and unkempt hair coat. A dose-dependent decrease in body weight and in feed consumption was observed in the high- and mid-dose groups. Absolute and relative liver weights increased at all dose levels. Hepatocyte hypertrophy affecting the portal area and a slight and multifocal coagulative necrosis affecting individual hepatocytes were observed in the high- and mid -dose animals. No changes in the kidneys were observed. A NOAEL could not be determined in this study. LOAELs of 706 and 756 mg/kg bw/d were established for male and female rats, respectively, based on increased liver weight.

In the corresponding study in mice, doses of 0, 0.75, 1.5 and 3%, reported to be equivalent to an average daily intake of 0, 1608, 3084 and 5794 mg/kg bw/d (males) and 0, 1965, 3986 and 9229 mg/kg bw/d (females) were administered to groups of five B6C3F1 mice per sex and dose in the same period specified for rats. Only one male from the mid-dose group was found dead throughout the study, although the cause of death was not apparent. A decrease in body weight was observed in males and females of the high-dose groups and in males of the mid-dose group. These body weight decreases persisted at the end of the study. No significant changes were observed in feed consumption. Absolute and relative liver weights were significantly increased in males and females of the mid- and high-dose groups. Hepatocyte hypertrophy, primarily in the portal region, was observed at all dose levels, except a few low dose animals. Minimal and multifocal coagulative necrosis was seen in all male groups and in females of the high-dose group. No changes in kidneys were observed. A NOAEL was not determined but a LOAEL was established in 1608 mg/kg bw/d for males and in 1965 mg/kg bw/d for females, mainly based on liver effects.

Some old studies cited in BG Chemie (2000) have reported hepatic peroxisome proliferation and related enzymatic changes in mice and rats after oral administration of 2-EHA over periods ranging from 4 days to 3 weeks. This effect has been associated with hepatomegaly caused by both cellular hypertrophy and cellular hyperplasia. According to *in vitro* studies, this effect is not observed in the guinea pig or monkeys.

New information after ECHA decision on substance evaluation

A GLP oral combined repeated dose toxicity study with the reproduction/developmental toxicity screening test **(OECD TG 422)** was conducted with 2-EHA (Unnamed report, 2015). This study was used as a dose-range finder for the planned OECD TG 443 required as a result of the substance evaluation process.

Groups of ten Wistar rats per sex and dose level were fed with diets containing 0, 1538, 4615 and 15385 ppm (corresponding to 82-86, 248-253, 761-797 mg/kg bw/d for males and to 107-116, 308-351, 809-1146 mg/kg bw/d for females) 2–EHA (purity 99.8%). Animals were treated during a premating period of 2 weeks, a mating period of one week, during gestation and lactation and until postnatal days 4 to 7 for females, and up to and including day 30 for males. In addition, satellite groups of 6 extra animals/sex were added and pregnant females were sacrificed on gestation day 20 to compare the analytical results to those obtained from animals sacrificed on PND 4 to 7 and to gain knowledge on the possible mechanism of toxicity.

Animals were daily observed for clinical signs of toxicity. Functional Observation Battery (FOB) tests and spontaneous Motor Activity Assessment (MAA) were performed shortly prior to sacrifice in 5 males per group on day 28 and in 5 females with a litter per group on PND 4.

Body weights were recorded at the beginning of the treatment in both sexes, in males weekly until sacrifice and in females once per week during premating, on days 0, 7, 14, 21 during gestation and on days 0 and 4 of lactation. Mated females that were sacrificed on GD 20 were weighed on GD 0, 7 and 14. Food consumption was measured over the same periods.

Hematology and clinical chemistry were conducted in 5 rats per sex and group. Full histopathology was performed in all parental animals.

Zinc was measured in liver, kidney and blood of non-fasted parental animals (including extra satellite animals) that were not used for hematology, clinical chemistry and possible hormone determinations; in liver, kidney, blood and homogenate of one pup per sex and litter and in homogenate of one fetus/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.

Sings of parturition were examined twice daily at the end of the gestation period. In addition, total litter size, sex ratio, stillbirths, live and dead pups, weights and behaviour of pups and gross malformations were recorded on days 0 and 4 of lactation.

No mortalities or clinical signs of toxicity were observed. No effects were reported in FOB and 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.

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.

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.

A NOAEL for general toxicity of 4615 ppm (corresponding to at least 248 mg/kg bw/d for males and 308 mg/kg bw/d for females) was established, based on the effects on body weights, food consumption, organ weights, haematology, clinical chemistry and zinc and metallothionein concentrations observed at the highest dose.

The results of this study related to reproductive toxicity are described in detail under 5.9 Toxicity to reproduction.

5.6.1.2 Repeated dose toxicity: inhalation

No studies are available.

5.6.1.3 Repeated dose toxicity: dermal

No studies are available.

5.6.1.4 Repeated dose toxicity: other routes

No information is available.

5.6.2 Human information

No information is available on the effects of repeated exposure in humans.

5.6.3 Summary and discussion of repeated dose toxicity

Information on the effects of repeated exposure to 2-EHA has been obtained from subacute and subchronic repeated oral exposure studies in rats and mice. Observed clinical signs of toxicity were highly similar in both species and throughout all the studies.

In two subchronic (90 days) toxicity studies, NOAELs of 300 and 200 mg/kg bw/d were established for rats and mice, respectively. The main observed effects were associated with growth retardation, decreases in body weight, increases in absolute and relative liver weights and hepatocyte hypertrophy. A NOAEL for general toxicity of 4615 ppm (corresponding to at least 248 mg/kg bw/d for males and 308 mg/kg bw/d for females) was established in a recent OECD 422 study, based on the effects on body weights, food consumption, organ weights, haematology, clinical chemistry and zinc and metallothionein concentrations observed at the highest dose.

No concern regarding repeated exposure to 2-EHA is identified by the eMSCA.

Table 5: Repeated dose toxicity of 2-ethylhexanoic acid in rats and mice

Study design	Mortality Clinical signs	Body weight Food consumption	Organ weights	Histopathology	Clinical chemistry/ Haemotoxicity	NO(A)EL LOAEL (mg/kg bw/d)
Oral: rats						
Oral combined repeated dose toxicity study with reproduction/developmental toxicity screening test (OECD 422, GLP)(1) Rat/Wistar 10 rats/sex/dose 0, 82-86, 248-253, 761-797 mg/kg bw/d Unnamed report, 2015 Key study	-	Body weight ↓feed consumption ↓	↑ relative liver (males and females) and relative kidneys (males). ↓ absolute and relative thymus (females)	Increased incidence of prteinaceous droplets in the kidney renal tubuli (males)	↓ MCV and MCHC. ↑ total white blood cells, monocytes and absolute number of neutrophils. ↓ total protein and albumin concentrations and ↑ albumin/globulin ratio (females). Changes in zinc (females) and MT concentrations in liver and kidneys	248-253 mg/kg bw/d (NOAEL)
91-93 day study Fischer 344 rats 10 rats/sex/dose 0, 0.1, 0.5, 1.5% Unnamed report, 1988a Key study	-	1.5% Body weight gain ↓ Feed consumption ↓	0.5% and 1.5% ↑ liver, relative kidney, relative brain and testis weights	0.5% (males) 1.5% (males/females) Hepatocyte hypertrophy	1.5% (males): ↑cholesterol, urea nitrogen and serum albumin (returned to normal - post-exposure observation) 0.5% (males) 1.5% (males/females) slightly ↓MCV ↓MCH (clinically insignificant)	300 mg/kg bw/d (NOAEL)
2-week gavage study Fischer 344 rats 5 animals/sex/dose 0, 200, 800, 1600 mg/kg bw/d Unnamed report, 1987b Supporting study	1600 mg/kg bw/d ≥ 800 mg/kg bw/d weakness lethargy sialorrhea	≥ 800 mg/kg bw/d (males): Body weight ↓ 1600 mg/kg bw/d (males/females): Feed consumption ↓	≥ 800 mg/kg bw/d (males) All doses (females) ↑ liver weight	1600 mg/kg bw/d hepatocyte hypertrophy	-	200 mg/kg bw/d (NOAEL)
2-week feeding study Fischer 344 rats 5 rats/sex/dose 0, 0.75, 1.5 and 3% Unnamed report, 1987d Supporting study	- 3% (females) urine soaked, disoloured and unkempt hair coat	1.5%, 3% Body weight ↓feed consumption ↓	↑ liver weight (All dose levels)	1.5%, 3% Hepatocyte hypertrophy slight/multifocal coagulative necrosis	-	706 mg/kg bw/d (males) 756 mg/kg bw/d (females) (LOAEL)
Oral: mice						

91-93d study B6C3F1 mice 10 animals/sex/dose 0, 0.1, 0.5, 1.5% Unnamed report, 1988b Key study	-	0.5% (females) 1.5% (males/females) Mean body weight ↓	0.5%, 1.5 % ↑liver weight (absolute/relative) 0.5%, 1.5% (females) ↑ relative kidney, relative brain and testes weights	0.5% (males) 1.5% (males/females) Hepatocyte hypertrophy 1.5%: eosinophilia 1.5% Cytoplasmic basophilia	0.5% (females) 1.5% (males/females): ↓ bilirubin / trygliceride levels 0.5%, 1.5% : ↑ serum cholesterol levels 1.5% (males): ↑ ALT	200 mg/kg bw/d (NOAEL)
2-week gavage study B6C3F1 mice 5 animals/sex/dose 0, 200, 800, 1600 mg/kg bw/d Unnamed report, 1987c Supporting study	800 mg/kg bw/d 1 dead male 1600 mg/kg bw/d 1 female Gait disturbance, weakness	1600 mg/kg bw/d 1 female Body weight ↓	1600 mg/kg bw/d (males) ↑liver weight	1600 mg/kg bw/d (males) Hepatocyte hypertrophy	-	800 mg/kg bw/d (males) 1600 mg/kg bw/d (females) (NOEL)
2-week feeding study B6C3F1 mice 0, 0.75, 1.5, 3% Unnamed report, 1987e Supporting study	1.5% 1 dead male	1.5% (males) 3% (males/females) Body weight ↓	1.5%, 3% ↑liver weights	All dose groups: Hepatocyte hypertrophy All male groups 3% (females): Minimal/multifocal coagulative necrosis	-	1068 mg/kg bw/d (males) 1965 mg/kg bw/d (females) (LOAEL)

(1) The unnamed report from 2015 was included upon receipt of the new information after ECHA decision on substance evaluation

5.7.1 Non-human information

The mutagenicity of 2-EHA has been investigated in several *in vitro* and *in vivo* test systems.

5.7.1.1 In vitro data

Bacterial systems

Reverse mutation assays

In a GLP study, according to OECD 471 (Bacterial Reverse Mutation Assay), mutagenicity of 2-EHA was tested using *Salmonella typhimurium* strains TA98, TA100 TA1535, TA1537 and *Escherichia coli* strain WP2 uvrA, with and without metabolic activation (S9 mix) and following the plate incorporation method. This study has been flagged as key study in the IUCLID dataset. Five dose levels of 50, 150, 500, 1500 and 5000 µg/plate, in triplicate, were used. The dose range to be used was determined in a preliminary toxicity assay. Dimethyl sulfoxide (DMSO), the solvent used to dissolve the test material, was considered as the negative control treatment. Positive control groups, with and without metabolic activation, were used as well. Cytotoxicity occurred at concentrations of 5000 µg/plate and above. Control plates gave counts of revertant colonies within the normal range and appropriate responses were observed with positive controls. In this assay 2-EHA gave a negative mutagenic response (Unnamed report, 1998a).

In addition, in a reported non-GLP bacterial reverse mutation assay, similar to OECD 471 with minor restrictions, 2-EHA was examined for mutagenic activity in an incubation test using

Salmonella typhimurium strains TA98, TA1535, TA1537 and TA100 in the presence and absence of metabolic activation (S9 fraction). Concurrent strain-specific positive and negative (solvent) controls were included. No cytotoxicity was seen at any dose level tested. 2-EHA did not induce a mutagenic response at concentrations of 100, 333, 1000, 3333 and 10000 µg/plate in ethanol (95%). In the dataset, this study has also been considered as a key study (Zeiger *et al*., 1988).

Mammalian cells

In vitro mammalian chromosome aberration test

2-EHA has been tested in a GLP *in vitro* mammalian chromosome aberration test using rat lymphocytes with and without S9 metabolic activation system. This study was conducted according to OECD 473 (1997) and has been considered as key study in the IUCLID dataset (Unnamed report, 1998b). DMSO was used as the negative control treatment. Positive control groups (mitomycin C in the absence of S9 and cyclophosphamide in the presence of S9) were used as well. Two independent tests were conducted.

In a first assay, cells were exposed to dose ranges between 0 and 5000 µg/ml for 4h in the presence and absence of S9 metabolic activation system. Cultures were harvested approximately 24h after treatment. The following doses were employed for determining the incidence of chromosomal aberrations: 0, 167, 500 and 1667 µg/ml without metabolic activation and 0, 500, 1667 and 5000 µg/ml with metabolic activation. There were no significant increases in the incidence of aberrant cells in 2-EHA treated cultures.

In a second experiment, rat lymphocytes cultures were treated in a continuous way for 24h with dose ranges between 0 and 5000 µg/ml in the presence and absence of S9 metabolic activation system. The doses of 0, 5, 16.7 and 50 µg/ml 2-EHA in the absence of S9 and 0, 1000, 2000 and 3000 µg/ml 2-EHA in the presence of S9 activation, were selected for determining the chromosomal aberration frequency. At any concentration of 2-EHA the percentage of metaphases with structural and numerical aberrations was statistically significant higher than those observed in the solvent controls. Based on the results, 2-EHA did not exhibit any potential to induce chromosome aberrations.

In a chromosome aberration test in Chinese hamster ovary (CHO) cells, not included in the dataset but cited in the literature (BG Chemie, 2000), 2-EHA was examined for mutagenic activity with and without metabolic activation system (S9 mix). Negative results were obtained without metabolic activation. Nevertheless, weak but statistically significant positive results were obtained with metabolic activation at concentrations of 3000 and 3500 µg/ml.

In vitro gene mutation test

A GLP mouse lymphoma L5178Y assay, conducted according to OECD 476, examined mutations at the thymidine kinase (*tk* +/-) locus. This study has been flagged as key study in the dataset. DMSO was used to dissolve the test material and in negative control treatments. Experimental design involved a pre-incubation period of 24 to 48 hours, an exposure time of 12 days and a subsequent expression time of 24 to 48 hours. A preliminary toxicity test was conducted in order to establish the concentration range to be used in the main assays. Per dish, 200 cells were scored. Cytotoxicity was determined by cloning efficiency and relative total growth. Two independent assays without S9 metabolic activation system were performed to evaluate the genotoxic potential of the test material using range concentrations, in duplicate, from 1.7 to 1500 µg/ml and from 10 to 5000 µg/ml, respectively. The mutant frequencies observed in the test material treated cultures were no significantly different from the negative control values. Solvent and positive controls gave the expected responses. Another two independent assays were performed in the presence of S9

metabolic activation system with range concentrations, in duplicate, from 10 to 3000 µg/ml and from 50 to 2500 µg/ml. No significant differences in the mutant frequencies were observed in treated cultures either. In all four cases described above, certain doses were removed due to excessive toxicity. It can be concluded that 2-EHA did not induce a mutagenic response in this test system (Unnamed report, 1998c).

Additionally, in a poorly reported GLP study, considered as key study in the registration dataset and performed according to OECD 476, Chinese hamster ovary (CHO) cells (*hprt* locus assay) were exposed to 1, 10, 50, 100, 250, 500, 750, 1000 and 1500 µg/ml 2-EHA in the presence and absence of Aroclor 1254 metabolic activation system. DMSO was selected as vehicle due to the limited solubility of the test substance in water. Positive controls were used. Experimental design involved an incubation period of 20-24 hours and an exposure period of 4 and 24 hours. Cells were washed, dried, fixed, stained and counted from day 16. Cytotoxicity was determined by cloning efficiency. With and without metabolic activation, no precipitation of the test substance was observed up to the highest applied test substance concentration. Negative results were obtained in this assay (Unnamed report, 2007).

Other in vitro studies

A sister chromatid exchange (SCE) assay performed in Chinese hamster ovary cells (CHO) has been cited in other publications (BG Chemie, 2000; Environment and Health Canada, 2011). Concentration ranges from 199 to 2500 µg/ml 2-EHA were used in absence of metabolic activation and from 9.38 to 313 µg/ml in the presence of metabolic activation (Aroclor 1254-induced rat liver). A buffer was added to prevent the drop of pH when 2-EHA was present. In these cases, concentration ranges from 400 to 1000 µg/ml without metabolic activation and from 505 to 5200 µg/ml with metabolic activation were used. Statistically significant dose-dependent increases in the sister chromatid exchange rates were seen from low to high doses, with and without metabolic activation.

A further sister chromatid exchange assay (SCE) has been carried out in human lymphocytes (Sipi *et al*., 1992; cited in BG Chemie (2000)). This study was not reported in the IUCLID dataset. Lymphocytes were taken from the blood of a 34-year-old man. Cultures were incubated with 2-EHA for 48 hours at concentration levels between 0.63 and 20 mM, reported to be equivalent to doses from 91 µg/ml to 2884 µg/ml (99% purity). Cytotoxicity was seen at doses of 0.63 mM and higher. Results showed that 2-EHA induced dose-dependent increases of sister chromatid exchanges at lower concentration ranges (0.63 - 2.5 mM) in the absence of metabolic activation, being less than twice the control rate.

5.7.1.2 In vivo data

Somatic cells

Micronucleus test

In a GLP mammalian erythrocyte micronucleus test, conducted according to OECD Guideline 474, doses of 400, 800 and 1600 mg/kg bw/d 2-EHA were administered, by gavage, to CD-1 male and female mice (five animals per sex at 400 and 800 mg/kg bw/d and eight animals per sex at 1600 mg/kg bw/d) using corn oil as a vehicle. One control group received the positive control agent, 50 mg cyclophosphamide/kg bw/d. 1000 polychromatic erythrocytes (PCE) per animal were scored for micronulei. The frequency of micronucleated cells was recorded. In the high dose male group, a slight increase in micronucleated PCE (0.28%) was noted. This value was outside the established control range of 0-0.25%. Because of this equivocal result, it was considered necessary to repeat the

analysis in the higher dose males with a larger sample size of cells (2000 PCE). The additional assessment was performed on re-randomized slides from the high, vehicle and positive control groups. In the high-dose group, the observed frequency of micronucleated PCE was within the normal negative response rate (0.10%). Vehicle and positive control groups had similar frequencies to those observed in the first assessment. No bone-marrow toxicity was noted. Negative results obtained indicated that, under these test conditions, 2-EHA did not produce micronuclei in the immature erythrocytes of the test species. This study has been considered as a key study in the IUCLID dossier (Holmstrom and Innes, 1994).

5.7.2 Human information

No information available.

5.7.3 Summary and discussion of mutagenicity

The mutagenicity of 2-EHA has been investigated in several experimental test systems reported in the IUCLID and in the literature.

The results obtained in bacteria assays indicated that 2-EHA is not mutagenic in any of the tested strains, with or without metabolic activation system (S9 mix).

Clastogenicity was negative in a chromosome aberration test using rat lymphocytes, with and without metabolic activation. Negative results were also obtained in two gene mutation tests (mouse lymphoma assay and *hprt* test) in the presence and absence of metabolic activation system.

Some papers reviewed included positive data for several *in vitro* studies. However, this activity is not expressed *in vivo* in somatic cells. 2-EHA was not clastogenic in a mouse micronucleus assay.

No studies in germ cells were reported.

Overall, based on the collective evidence on genotoxicity, especially in the negative *in vivo* results, the eMSCA considers that 2-EHA has not mutagenic activity.

5.8 Carcinogenicity

There are no studies available on the carcinogenicity of 2-EHA. However, neither the subchronic toxicity studies in rats and mice nor the reproductive toxicity studies in rats did provide any indication of carcinogenic activity of the substance.

5.9 Toxicity for reproduction

Table 6: Toxicity for reproduction of 2-ethylhexanoic acid

Type of study Species/strain	**Dose (mg/kg bw/d)**	**Findings**	**Reference**
Oral extended one-generation reproductive toxicity study (OECD 443, GLP) (1)	0		Unnamed report, 2016
	80		
	250	Increase in the absolute and relative weights of the testes (cohort 1B males).	

Design includes F2 and DNT and DIT cohorts. Two weeks premating period. Rat/Wistar	800	Reduction in body weight, body weight changes and food consumption in F0 and F1 animals. Increase in the absolute and relative weights of the liver (F0 males) along with microscopic findings. Increase of the relative weights of kidneys and thyroid (F0 males). No changes in TSH and T4 levels. Increase in the relative weights of heart, kidneys, liver and testes (cohort 1A males). Increase in the absolute and relative weights of the liver and relative weight of kidneys (cohort 1A females). Increase in the absolute weight of the kidneys and in the relative weights of the kidneys, liver, testes and cauda epididymis (cohort 1B males). Increase in the relative weights of liver and kidneys (cohort 1B females). Increase in the AGD on PND 4 (F1 male pups).	
Oral combined repeated dose toxicity study with the reproduction/developmental toxicity screening test (OECD 422, GLP) [(2)] Rat/Wistar	0		Unnamed report, 2015
	82-86		
	248-253		
	761-797	Decrease in body weight and food consumption. Lower values of MCV and MCHC. Increases in total white blood cells, monocytes and in the absolute number of neutrophils. Lower total protein and albumin concentrations and higher albumin/globulin ratio (females). Increases in the relative weight of the liver (males and females) and in the relative weight of the kidneys (males). Decrease in the absolute and relative weight of the thymus (females). Increased incidence of proteinaceous droplets in the kidney renal tubuli (males). Changes in zinc (females) and MT concentrations in liver and kidneys. Reduction of 14% in the weight of the pups on PND 4.	
One-generation study (non-GLP, non-guideline). Oral gavage in drinking water. Rat/Wistar	0		Pennanen *et al*., 1993
	100	Reduction of motile spermatozoa.	
	300	Epithelial hyperplasia in the vagina and slight dilation of the lumen in uterus. Pups: Increase in kinky tail. Delayed physical development. Delayed development of the grip and cliff avoidance reflexes.	
	600	Maternal body weight reduction. Epithelial hyperplasia in the vagina and slight dilation of the lumen in uterus. Delay in fertilization. Increase of relative weights of epididymides. Reduction of motile spermatozoa. Reduction of the average litter size. Pups: Increase in kinky tail. Delayed physical development. Delayed development of the grip and cliff avoidance reflexes.	

Oral prenatal developmental toxicity study (EPA Guideline). Oral gavage in corn oil from GD 6 to 15. Rat/Fischer 344	0		Unnamed report, 1988c; Hendrickx *et al*., 1993
	100		
	250	Reduced skeletal ossification.	
	500	Hypoactivity, ataxia, audible respiration, ocular discharge and periocular encrustations. Increase in absolute and relative liver weight. Growth retardation (reduction in ossification). Dilated lateral ventricles of the brain with no tissue compression.	
Oral prenatal developmental toxicity study (EPA Guideline). Oral gavage in corn oil from GD 6 to 18. Rabbit/New Zealand white	0		Unnamed report, 1988d; Hendrickx *et al*., 1993
	25		
	125	Mortality (1 female). One abortion (GD 27).	
	250	Mortality (1 female). Reduction in body weight and food consumption.	
Developmental toxicity study, (non GLP, similar to OECD 414). Oral gavage in drinking water from GD 6 to 19. Rat/Wistar	0		Pennanen *et al*., 1992
	100	Skeletal anomalies.	
	300	Non-significant decrease in the pregnancy rate. Reduction of placental weight. Skeletal anomalies. Clubfoot. Dilation of brain ventricles.	
	600	Non-significant decrease in the pregnancy rate. Reduction of mean body weight. Decrease in mean foetal body weight. Skeletal anomalies. Clubfoot. Dilation of brain ventricles.	
Developmental toxicity study with 2-ethylhexyl-2-ethylhexanoate (GLP, OECD 414). Oral gavage from GD 6 to 15. 2-EHA used as positive control. Rat/Wistar	600 (2-EHA positive control group)	Non influence on the gestational parameters (conception rate, implantation rates, postimplantation losses, resorptions and viable foetuses). Clear signs of selective developmental toxicity and teratogenicity (skeletal and overall variations and retardations).	Unnamed report, 1997
Chernoff/Kavlock screening developmental study. Administered by gavage from GD 6 to 15. Rat/SD	0		Narotsky *et al*., 1994
	900	Mortality. Maternal toxicity: weight loss, motor depression and respiratory effects. Developmental toxicity: increases in the gestation length. Decrease in the number of live pups and increase in perinatal loss. Variations of the skeleton.	
	1200		

(1, 2) The unnamed reports from 2015 and 2016 were included upon receipt of the new information after ECHA decision on substance evaluation

5.9.1 Effects on fertility

The effects of 2-EHA on fertility have been investigated in rats.

5.9.1.1 Non-human information

Existing information before ECHA decision on substance evaluation

In a non-GLP and non-guideline one-generation reproduction study, reported as the key one in the IUCLID dossier, reproductive toxicity of 2-EHA was evaluated in Wistar rats (Pennanen *et al*., 1993).

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. Plain water was administered to control groups. Males were exposed for 10 weeks and females for 2 weeks prior to mating, both sexes during mating and females during gestation and lactation. Clinical signs of toxicity were checked daily. Individual body weights and food consumption were collected weekly. Water consumption was measured, but the interval was not stated.

At the end of the mating period (maximum 21 days) males and non-pregnant females were sacrificed. In all non-gravid females, ovaries, uterus, cervix uteri and vagina were dissected for histopathological examination. Ovaries were weighed before fixation. In all males, testes and the right epididymis were weighed. Testes, the right epididymis, prostate, seminal vesicle and coagulating gland of five randomly selected males per group were fixed for histopathological assessment. The left cauda epididymis was dissected, weighed and minced for the evaluation of morphology, motility and sperm density.

During the period of expected parturition, the females were observed twice daily for newborn litters, considering as postnatal day 0 the day the litter was complete. Litter size, including stillbirths, and any gross anomalies were examined. On postnatal day 4, the number of pups per litter was reduced to eight and discarded pups were examined for external signs of toxicity and sacrificed.

Litters were kept with their dams for three weeks following birth. The weight of pups was recorded on postnatal days 0, 4, 7, 14 and 21. From postnatal day 1 onwards, pups were examined on alternate days. The appearance of pinna reflex, placing reaction, righting reflex in 5 sec, cliff avoidance in 5 sec, approach/avoidance, ipsilateral flexor reflex (hind toe), grip reflex in 5 sec, air righting, opening of eyes, teeth eruption and hair growth were recorded. All pups were externally examined and sacrificed on postnatal day 21.

Dams were also sacrificed and examined macroscopically on postnatal day 21. Examination of reproductive organs was made as for the non-gravid females. Histopathology was done in five randomly selected dams per group.

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 or during the mating period. Nevertheless, slightly but statistically significant reduction in water consumption of 14%, compared with controls, was seen in pregnant females of the high-dose group.

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

A statistically significant increase of 17% in the relative weights of the right epididymides 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.

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%, compared to the control group. Statistically significant reduction of motile spermatozoa of 37% and 22% was seen at 100 and 600 mg/kg bw/d, respectively. 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.

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.

Related to offspring parameters, a statistically significant reduction of 16% 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.

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.

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, hair growth or eruption of teeth 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. The development of the grip and cliff avoidance reflexes was delayed, being more pronounced in males than in females. 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.

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.

In an additional experiment, performed to evaluate the effects of 2-EHA on implantation, four groups (A to D) of five to 12 dams were administered by gavage a single dose of 600 mg/kg bw/d 2-EHA as a sodium salt, on gestational days 4 (group A), 5 (group B), 6 (Group C) and 7 (group D). Dams were sacrificed and examined on day 10 of gestation. Uterus was weighed and observed for hemorrhagic alterations in implantations sites of very early resorptions.

Gestational Day 6 appears to be the most critical and sensitive period for 2-EHA embryotoxicity. On day 6 of gestation, the number of implantations was lowest after administration of 2-EHA and the incidence of resorptions increased in 80% of the animals, with total resorptions in 40% of them. The authors suggested that 2-EHA affected implantation, since implantation in rats takes place around gestational day 6.

On the contrary, 2-EHA did not cause effects on implantation when used as positive control in the investigation of the prenatal toxicity of 2-ethylhexyl-2-ethylhexanoate in a well-reported GLP prenatal developmental toxicity study (OECD Guideline 414) (Unnamed report, 1997). This study was not considered by the Registrant(s) in the registration dossier. In this assay, a dose of 600 mg/kg bw/d 2-EHA was administered by gavage to the positive control group of 25 pregnant Wistar rats on day 6 through day 15 post coitum.

Results showed that 2-EHA induced some maternal toxicity related to impairment in maternal body weight gain (statistically significant on days 15 – 17 p.c.) and increases in liver weights. According to authors, 2-EHA did not have an influence on the gestational parameters as non-statistically significant changes in conception rate, implantation rates, postimplantation losses, resorptions and dead foetuses were observed.

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. At the same dose level, 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. In addition, the substance caused effects on male sex organs related to sperm quality and an increase in the relative weights of the epididymides.

In the IUCLID dossier, a NOAEL of 100 mg/kg bw/d was established for the offspring based on lower litter size, lower body weights and delayed physical development. For parental animals, a NOAEL of 300 mg/kg bw/d was reported, based on the delay in fertility.

New information after ECHA decision on substance evaluation

A GLP oral combined repeated dose toxicity study with the reproduction/developmental toxicity screening test **(OECD TG 422)** was conducted with 2-EHA (Unnamed report, 2015). This study was used as a dose-range finder for the planned OECD TG 443 required as a result of the substance evaluation process.

Groups of ten Wistar rats per sex and dose level were fed with diets containing 0, 1538, 4615 and 15385 ppm (corresponding to 82-86, 248-253, 761-797 mg/kg bw/d for males and to 107-116, 308-351, 809-1146 mg/kg bw/d for females) 2–EHA (purity 99.8%). Animals were treated during a premating period of 2 weeks, a mating period of one week, during gestation and lactation and until postnatal days 4 to 7 for females, and up to and including day 30 for males. In addition, satellite groups of 6 extra animals/sex were added and pregnant females were sacrificed on gestation day 20 to compare the analytical results to those obtained from animals sacrificed on PND 4 to 7 and to gain knowledge on the possible mechanism of toxicity.

Animals were daily observed for clinical signs of toxicity. Functional Observation Battery (FOB) tests and spontaneous Motor Activity Assessment (MAA) were performed shortly prior to sacrifice in 5 males per group on day 28 and in 5 females with a litter per group on PND 4.

Body weights were recorded at the beginning of the treatment in both sexes, in males weekly until sacrifice and in females once per week during premating, on days 0, 7, 14, 21 during gestation and on days 0 and 4 of lactation. Mated females that were sacrificed on GD 20 were weighed on GD 0, 7 and 14. Food consumption was measured over the same periods.

Hematology and clinical chemistry were conducted in 5 rats per sex and group. Full histopathology was performed in all parental animals.

Zinc was measured in liver, kidney and blood of non-fasted parental animals (including extra satellite animals) that were not used for hematology, clinical chemistry and possible hormone determinations; in liver, kidney, blood and homogenate of one pup per sex and litter and in homogenate of one fetus/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.

Sings of parturition were examined twice daily at the end of the gestation period. In addition, total litter size, sex ratio, stillbirths, live and dead pups, weights and behaviour of pups and grossly malformations were recorded on days 0 and 4 of lactation.

No mortalities or clinical signs of toxicity were observed. No effects were reported in FOB and 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.

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

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.

A NOAEL for general toxicity of 4615 ppm (corresponding to at least 248 mg/kg bw/d for males and 308 mg/kg bw/d for females) was established, based on the effects on body weights, food consumption, organ weights, haematology, clinical chemistry and zinc and metallothionein concentrations observed at the highest dose. The NOAEL for developmental effects was also

established at 4615 ppm, taking into account the pup weight reduction at the high-dose group. Due to the lack of reproductive effects the NOAEL for fertility was established at 15385 ppm (corresponding to at least 761 mg/kg bw/d for males and 809 mg/kg bw/d for females), the highest dose tested.

A GLP extended one-generation reproductive toxicity study performed according to **OECD guideline 443** and flagged in the IUCLID dataset as key study, was conducted with 2-EHA in Wistar rats (Unnamed report, 2016) following the information requirement included in the substance evaluation final decision under REACH. 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.

The substance was administered orally in the diet to 28 Wistar rats per sex and dose level at concentrations of 0, 80, 250 and 800 mg/kg bw/d during a premating period of 2 weeks and during mating and gestation. During the lactation period, concentration in the diet of females was halved. This dietary adjustment was made to maintain the dams at the desired target doses during this period of increased food intake. Animals of the cohort 1B received the diets from weaning until the day of sacrifice.

On postnatal day 4 (PND 4), litters of more than 10 pups were adjusted to obtained 5 pups per sex and litter. At weaning of the F1 generation (PND 21) 75 pups per sex and group were placed into different cohorts. The first cohort (cohort 1A with 20 animals/sex/group and cohort 1B with 25 animals/sex/group) was assessed for reproductive performance. The second cohort (cohorts 2A and 2B with 10 pups/sex/group) was evaluated for neurodevelopmental effects. The third cohort (cohort 3 with 10 pups/sex/group) was focused on developmental immunotoxicity. Additionally to this cohort, 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. Additionally, after at least 13 weeks of age, animals of cohort 1B were mated to produce the F2 generation.

The following parameters were determined: clinical signs of toxicity, body weights, food consumption and compound intake, sexual maturation, gross necropsy and histopathology, oestrus cycle, hematology, clinical biochemistry, hormone determinations, urinalysis, epididymal sperm motility, count and morphology, testicular sperm count, litter evaluation, anogenital distance and neonatal pup pathology. All observations and examinations were conducted following the OECD guideline 443.

Regarding the assessment of potential developmental neurotoxicity, cohort 2A animals were subjected to auditory startle response, functional observational battery (FOB) and motor activity. Neuropathology assessments were carried out in cohorts 2A and 2B animals.

The evaluation of the potential developmental immunotoxicity by determining the titer of KLH-specific IgM antibody were performed in the serum of cohort 3 animals by ELISA.

Parental (F0), cohort 1 (1A and 1B) and F2 animals

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 changes 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 changes were considered most probably related to lower food intake by the animals of the highest-dose group.

Males and females of the F0 generation showed statistically significant increases in the absolute and relative weights of the liver at the highest dose. Additionally, in males of this dose group statistically significant decreases of terminal body weights and increases of the relative weights of kidneys and thyroid were observed. In cohort 1A, statistically significant increases in the relative weights of the 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. 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 the 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 the liver and kidneys at the highest dose tested.

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. 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. 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.

Regarding fertility and reproductive 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. 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. These findings were not considered as adverse effects as they were within historical control ranges. No treatment-related effects on epididymal and testicular sperm parameters were observed. In addition, neither treatment-related effects were observed on fertility and reproductive performance of animals of the F0 generation and of cohort 1B of the F1 generation.

Duration of gestation was slightly, but statistically significant longer in the high-dose group of the F0 generation. However it was not considered biologically relevant by the authors being in the range of historical control data. Gestation index was 100% and perinatal loss was 0% for all groups. 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.

Regarding general and sexual developmental parameters, no treatment-related effects on the 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 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. 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.

Cohort 2 (2A and 2B) animals

No mortalities or clinical observations were recorded. Neither statistically significant effects were observed on body weights, body weight changes and food consumption of animals of cohort 2A.

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. In cohort 2A, 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. 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. In addition, no differences were observed in the brain length and brain width measurements of cohorts 2A and 2B. 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.

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. Final 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. Macroscopic observations did not reveal any treatment-related abnormalities.

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.

Taking into account this information, a 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. The NOAEL for fertility and reproductive effects, developmental neurotoxicity and immunotoxicity effects was established at 800 mg/kg bw/d, due to the lack of effects.

5.9.1.2 Human information

No studies are available.

5.9.2 Developmental toxicity

Several developmental toxicity studies, carried out in rats and rabbits, have been reported in the IUCLID dataset.

5.9.2.1 Non-human information

In two oral prenatal developmental toxicity studies in Fischer 344 rats and New Zealand white rabbits, conducted in accordance with GLP and EPA Guidelines, the developmental toxicity of 2-EHA was evaluated (Unnamed reports, 1988c; 1988d; Hendrickx *et al*., 1993). These assays were considered in the registration dataset as key studies. For both of them, doses had been previously determined in preliminary range-finding studies.

In the range-finding study in rats, eight plug-positive females per dose level received 0, 125, 250, 500 and 1000 mg/kg bw/d 2-EHA (nominal in corn oil) orally, by gavage, from gestational day 6 to 15. The maternal and foetal observations at sacrifice were the same as those employed in the definitive study, except for the sections of livers, which were not examined in this preliminary study. Results of the range-finding study showed significant maternal toxicity (death in seven of eight dams) and statistically significant reduction of maternal weight gain at 1000 mg/kg bw/d for GD 6-9. Indications of maternal toxicity were also observed at 500 mg/kg bw/d, including not statistically significant weight gain reduction and clinical sings of toxicity. Significantly increased incidence of non-viable implants per litter and, therefore, reduction of the percentage of live foetuses per litter were observed at the same dose. Furthermore, significantly reduced foetal body weights per litter were noted but there were no external malformations and variations.

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. Clinical signs of toxicity, morbidity and mortality were recorded in females once daily. Maternal body weight measures were collected on gestational days 0, 6 (prior to dosing), 12, 15, 18 and 21. Food consumption was measured for three-day intervals, from gestational day 0 to 21.

After sacrifice of dams on gestational day 21, gross examination of reproductive organs and abdominal and thoracic cavities were carried out. Gravid uteri were weighed, the number of live/dead foetuses and resorptions were recorded and corpora lutea were counted. Non-gravid uteri were used for the detection of early resorptions. All maternal livers were weighed and fixed for examination.

All viable foetuses were weighed, sexed and observed for external malformations and variations. One-half of the foetuses per litter were observed for visceral abnormalities. Remaining foetuses were inspected for skeletal malformations and variations.

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.

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.

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.

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.

From the results obtained, a NOAEL of 250 mg/kg bw/d for maternal toxicity was obtained, based on clinical signs of toxicity and increased liver weights. For developmental toxicity, a NOAEL of 100 mg/kg bw/d was established, based on reduced skeletal ossification.

An equivalent developmental toxicity study was carried out in New Zealand white rabbits. In the range-finding study, groups of eight pregnant rabbits were daily treated by gavage with doses of 0, 125, 250, 500 or 1000 mg/kg bw/d 2-EHA in corn oil, from gestational day 6 to 18. The evaluations at sacrifice were the same as those employed in the main study, except that maternal liver sections were not retained in fixative and the visceral and skeletal examinations on the foetuses were not performed, either.

The range-finding study showed high toxicity in pregnant rabbits treated with 500 and 1000 mg/kg bw/d 2-EHA. Mortality was observed at the high and mid doses. No changes in resorptions, deaths or malformed foetuses occurred. No external malformations were observed in foetuses at any of the treated groups.

In the definitive study, groups of 15 pregnant rabbits per dose level were administered, by gavage, daily doses of 0, 25, 125 and 250 mg/kg bw/d 2-EHA in corn oil on gestational days 6 to 18. Clinical signs of toxicity, morbidity and mortality were observed twice daily. Body weights were measured on gestational days 0, 6 (for dose calculations), 9 (only in the definitive study), 12, 15, 18 and 29. Food consumption was recorded daily between days 0 to 29 of gestation.

Dams were sacrificed on gestational day 29 and the examinations were carried out in the same way to that described for rats, with the exception of skeletal examinations that were performed in all foetuses.

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.

A maternal NOAEL of 25 mg/kg bw/d was established, based on deaths, abortions and decreased body weights. For developmental toxicity, a NOAEL of 250 mg/kg bw/d was established, since no effects were observed at any dose tested.

In addition, a non-GLP developmental toxicity study, equivalent or similar to OECD 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. Control animals received deionized water.

Animals were observed daily for clinical signs of toxicity. Water consumption was recorded throughout the exposure period and doses corrected according to the most recent body weights recorded on days 0, 6, 13 and 20. Food consumption was recorded between gestation days 13 to 16.

Dams were sacrificed on day 20 of gestation. Number of corpora lutea from ovaries and the uterine contents were examined. Weight of uterus with its contents, number of implantation sites, number and sex of live and dead foetuses, early and late resorptions, individual foetal weights, external abnormalities and placental weights were also recorded. Every second living foetus was fixed to detect skeletal anomalies. All foetuses with clubfoot were analyzed for skeletal anomalies. Other foetuses were observed for visceral anomalies.

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 onward. 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.

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. Dose-dependent 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.

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.

A NOAEL of 300 mg/kg bw/d was established for maternal toxicity based on reduced body weights. For teratogenicity, a NOAEL of 100 mg/kg bw/d has been established based on the reduction of foetal weight and skeletal variations at doses which did not cause visible maternal toxicity. In addition, clubfoot was observed at all dose levels, although the incidence was not statistically significant at 100 mg/kg bw/d. Because of this, that dose has been considered as a NOAEL in the absence of statistical significance.

The effects upon development observed in rats are the basis for the current classification of 2-EHA as toxic for reproduction, category 2 (H361d: suspected of damaging the unborn child).

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 also observed in the unnamed report from1997 study on the prenatal toxicity of 2-ethylhexyl-2-ethylhexanoate where 2-EHA was used as positive control at a dose of 600 mg/kg bw/d in rats from GD 6 to 15. These results fit well to the above findings described by Pennanen *et al.* (1992), Similar teratogenic effects have also been described in other studies using maternal lethal dose of 2-EHA and therefore with limited relevance for evaluation (Narotsky *et al*., 1994; Ritter *et al*.,1987).

In addition, other studies that attempt to explain the mechanisms of the 2-EHA-induced teratogenicity are summarized below.

A mechanistic study was conducted to investigate the role of maternal hepatic metallothionein (MT) induced in response to administration of 2-EHA on plasma zinc levels and zinc delivery to the conceptus. In this non-GLP and non-guideline study, carried out by Bui *et al*. (1998), three different experiments, two *in vivo* and one *in vitro*, were reported in order to investigate the effects of developmentally toxic doses of 2-EHA on zinc metabolism in pregnant rats. In this study the teratogenicity of 2-EHA was enhanced by dietary Zn deficiency. The results 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.

On the other hand, Taubeneck *et al*. (1994) stressed that the reduction of embryonic Zn uptake secondary to changes in maternal liver MT synthesis is not the only, or even the major, mechanism by which these insults induce developmental toxicity; rather they suggest that the effect of these insults on maternal-embryonic Zn metabolism contributes to their developmental toxicity.

In addition, a direct effect of 2-EHA on developing embryo was suggested by Pennanen *et al*. (1992) based on the experiment by Brown *et al*. (1987) where the substance induced growth retardation and dysmorphogenesis in rat whole-embryo culture *in vitro*. Therefore, even developmental toxicity of 2-EHA may be modulated, in part, by its influence on maternal-embryonic Zn metabolism, other mechanisms cannot be excluded.

Finally, an additional study investigated the possible connection between specific stereoselectivity of 2-EHA and teratogenicity. (R)-2-EHA enantiomer seems to be a more potent teratogen than (S)-2-EHA enantiomer (Collins *et al*., 1992; Hauck *et al*., 1990).

5.9.2.2 Human information

No studies are available.

5.9.3 Summary and discussion of reproductive toxicity

No data are available on the potential reproductive effects of 2-EHA in humans. The reproductive toxicity of the substance has been studied in rats and rabbits.

Fertility

An extended one-generation reproductive toxicity study was requested during the substance evaluation process of 2-EHA since concerns regarding fertility and developmental neurotoxicity had been identified. These concerns were based on 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, where an apparent reduction in sperm motility and a delay in fertilization were observed in parental animals. In addition, delay in the development of the grip and cliff avoidance reflex observed in pups of the mid and high-doses was considered as a potential neurodevelopmental toxicity effect. Furthermore, 2-EHA is an analogue of the anticonvulsant drug valproic acid.

Following the substance evaluation decision, both 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 (OECD TG 443) were conducted according to GLP with 2-EHA in Wistar rats (Unnamed reports, 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).

No fertility or reproductive effects were observed in male and female rats in the oral combined repeated dose toxicity study with the reproduction/developmental toxicity screening test. This study was performed as a dose-range finder for the planned OECD 443 study.

The results obtained in the EOGRTS performed in Wistar rats dosed at 0, 80, 250 and 800 mg/kg bw/d 2-EHA did not show any treatment-related effects in fertility and reproductive parameters at any dose levels both in F0 and F1 generations. . As a result of this study, a 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. The NOAEL for fertility and reproductive effects was established at 800 mg/kg bw/d, due to the lack of effects.

Therefore, taking into account the lack of reproductive effects, the initial concern regarding fertility is clarified.

Development

In 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 (Unnamed reports, 1988c; 1988d; Hendrickx *et al.*, 1993). In rats, findings indicating maternal toxicity and increased absolute and relative liver weights were observed at 500 mg/kg bw/d. 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. No teratogenic effects were observed up to the highest dose tested.

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.

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 first species was observed even at doses which did not cause maternal toxicity, while these effects did not occur in rabbits.

From the study in rats, a NOAEL of 250 mg/kg bw/d for maternal toxicity was obtained, based on clinical signs of toxicity and increased liver weights. For developmental toxicity a NOAEL of 100 mg/kg bw/d was established, based on reduced skeletal ossification.

In addition, in another developmental toxicity study in Wistar rats, 2-EHA proved to be teratogenic to rat foetuses at dose levels 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.

The results obtained in the EOGRTS performed in Wistar rats dosed at 0, 80, 250 and 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. Thus the NOAEL for development was established at 800 mg/kg bw/d, due to the lack of effects.

Finally, according to mechanistic studies, developmental toxicity of 2-EHA may be modulated, in part, by its influence on maternal-embryonic Zn metabolism. However, a direct effect of 2-EHA on developing embryo cannot be excluded.

In summary, taking into account the results obtained from reproductive and developmental studies, it seems that there is enough evidence to indicate that 2-EHA is harmful to the embryos and/or foetuses at dose levels without maternal toxicity. Those results justified the EU harmonised classification of 2-EHA as toxic for reproduction, category 2 (H361d: suspected of damaging the unborn child) based on observed developmental effects, such as skeletal variations (wavy ribs, reduced ossification) and skeletal malformations (clubfoot) in rat following oral doses given on days 6-19 of gestation.

On the other hand, the new data from the DNT cohorts do not confirm a neurodevelopmental toxic effect of 2-EHA. Thus, the eMSCA concludes that, taking into account the results from the EOGRTS, the additional concern related to developmental neurotoxicity has not been confirmed,

5.10 Endocrine disrupting properties

Not relevant.

5.11 Other effects

Not relevant.

5.11.1 Non-human information

5.11.1.1 Neurotoxicity

Existing information before ECHA decision on substance evaluation

No studies with specific investigations on the neurotoxicity of 2-EHA were available. However, the results from the available one-generation reproductive study (Pennanen *et al*., 1993) included in the registration dossiers showed that in the offspring, the substance caused a delay in the development of the grip and cliff avoidance reflexes, being more pronounced in males than in females.

In addition, 2-EHA is an analogue of valproic acid, a drug used for the treatment of epilepsy and bipolar disorder. In an attempt to find out anticonvulsant drugs with reduced sedative/hypnotic side effects, 2-EHA, among other metabolites and analogues of valproic acid, was tested in mice (Löscher and Nau, 1985). 2-EHA showed a relative anticonvulsant potency of 40% on molar base in comparison to valproic acid. Due to the reduced anticonvulsant potency of 2-EHA relative to valproic acid, this substance was not selected by the authors of the study for further evaluation including neurotoxicity investigation. However, sedative/hypnotic side effects can not be excluded for this substance. Keane *et al.* (1983) have reported sedative and/or toxic properties measured by loss of righting reflex and acute lethality respectively with several close analogues of valproic acid. These properties increased as a function of chain length and were observed for 2-EHA (8 carbons) and other analogues with 9 or 10 carbons.

Furthermore, valproic acid have been associated with neurodevelopmental disorders. Clinical studies show that exposure to valproic acid *in utero* is associated with higher incidence of autism and other behavioral changes in the offspring (Roullet *et al.,* 2013).

Overall, an investigation on the potential neurodevelopmental toxic effect of this substance is justified.

New information after ECHA decision on substance evaluation

Taking into account the above information, an investigation on the potential neurodevelopmental toxic effect of this substance was included in the EOGRTS design in the ECHA decision on substance evaluation where cohorts 2A and 2B (DNT cohorts) were included. More detailed information is reported under section 5.9 Toxicity to reproduction.

The new data from the DNT cohorts do not confirm a neurodevelopmental toxic effect of 2-EHA. Thus, the eMSCA concludes that, taking into account the results from the EOGRTS, the additional concern related to developmental neurotoxicity has not been confirmed.

5.11.1.2 Immunotoxicity

Existing information before ECHA decision on substance evaluation

The potential of 2-EHA to produce an immunosuppressive effect has been investigated *in vitro* (Pennanen *et al.,* 2000) in one study included in the registration dossiers. Human polymorphonuclear leukocytes (PMNL) were exposed to 2-EHA to measure the production of reactive oxygen species (ROS) and to explore the associated cellular mechanisms. This study did not detect effects on ROS production, intracellular calcium, nor PKC (protein kinase C) activation. 2-EHA inhibited PMA (phorbol myristate acetate) and DiC8 (dioctanoyl-s,n-glycerol) stimulated ROS production in PMNLs at mM concentrations, an effect of unknown functional significance in PMNL dependent defensive mechanisms.

Furthermore, 2-EHA is a biotransformation product of bis(2-ethylhexyl) phthalate (DEHP). The properties of phthalates in general, and DEHP in particular, as modulators of the immune system have been suggested based on several experimental and immunological studies (Larsen and Nielsen, 2007; Larsen *et al*., 2001; Jaakkola and Knight, 2008). In this regard, Tonk *et al.* (2012) have investigated the relative sensitivity of developmental and immune parameters in juvenile versus adult male rats after exposure to DEHP. The results of this study show the age-dependency of DEHP toxicity and the relative sensitivity of the developing immune system in juvenile animals as compared to general toxicity and developmental parameters. Therefore, the authors suggest that DEHP immunotoxicity during maturation of immune system may play a role in the expression of allergic symptoms observed in epidemiological studies.

Overall, without further testing, immunotoxic effect of 2-EHA cannot be excluded. Due to this substance is a known metabolite of DEHP, further investigation of these properties in the developing immune system would be of relevance.

New information after ECHA decision on substance evaluation

Further investigations of these effects in the developing immune system were included as part of the EOGRTS requested by ECHA decision after substance evaluation. The cohort 3 (DIT cohort) was included.

The results from this third cohort addressing developmental immunotoxicity (DIT cohort) do not show any effects. More detailed information is reported under section 5.9 Toxicity to reproduction.

Following the evaluation of the new information available on 2-EHA after substance evaluation, it is considered that the new study results provide sufficient and reliable information to conclude that 2-EHA does not show a specific effect on developmental immunotoxicity.

5.11.2 Human information

Not available.

5.12 Combined effects

5.13 Derivation of DNEL(s) / DMEL(s)

5.13.1 Overview of typical dose descriptors for all endpoints

A toxicokinetic study in rats showed that 2-EHA was rapidly and extensively absorbed after oral administration and more slowly absorbed from the skin. Dermal absorption was estimated to be 70% relative to i.v. administration. Oral absorption was assumed to be 100%.

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

The toxicokinetic study also showed that 2-EHA undergoes rapid and extensive oxidative metabolism and glucuronidation. The substance was primarily excreted via the urine, mainly as the glucuronide form.

The vast majority of 2-EHA is excreted within 24 hours of administration. Therefore, there is no evidence to suggest that it has the potential to bioaccumulate.

Acute toxicity studies have been carried out in different strains of rats and guinea pigs. No toxicologically significant signs were found following acute exposure via any route. Overall, it can be concluded that 2-EHA is of low acute toxicity by the three routes of exposure.

In an oral toxicity study a LD_{50} value of 2043 mg/kg bw has been established. A LD_{50} greater than 2000 mg/kg bw was obtained in a well-conducted acute dermal toxicity study in rats. Regarding the inhalation route, a LC_0 of 0.11 mg/L was established.

According to the available animal studies, 2-EHA is considered not irritant for skin and eye, and, consequently not corrosive.

No dermal sensitisation was observed in a Guinea-Pig Maximisation Test.

In two subchronic (90 days) toxicity studies, NOAELs of 300 and 200 mg/kg bw/d were established for rats and mice, respectively. The main observed effects were associated with growth retardation, decreases in body weight, increases in absolute and relative liver weights and hepatocyte hypertrophy. A NOAEL for general toxicity of 4615 ppm (corresponding to at least 248 mg/kg bw/d for males and 308 mg/kg bw/d for females) was established in an OECD 422 study, based on the effects on body weights, food consumption, organ weights, haematology, clinical chemistry and zinc and metallothionein concentrations observed at the highest dose.

The mutagenicity of 2-EHA has been investigated in several experimental test systems reported in the registration dossier and in the literature. Overall, based on the collective evidence on genotoxicity, especially in the negative *in vivo* results, it would be considered that 2-EHA has not mutagenic activity.

In an EOGRTS required by ECHA decision on substance evaluation, a 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. The NOAEL for fertility and reproductive effects, developmental neuro and immunotoxicity effects was established at 800 mg/kg bw/d, due to the lack of effects.

In a GLP-compliant oral developmental toxicity in rats (Unnamed report, 1988c; Hendrickx *et al.*, 1993), foetotoxic alterations related to reduced foetal body weights, visceral and skeletal variations were observed from 250 mg/kg bw/d onwards, where no maternal toxicity occurs. On the contrary, the same study carried out in New Zealand rabbits showed maternal toxicity in dams treated with 2-EHA at doses up to 250 mg/kg bw/d, but no findings related to embryotoxic, foetotoxic or teratogenic effects were observed up to the highest dose tested.

To summarize, an overall NOAEL of 100 mg/kg bw/d for developmental effects and a NOAEL of 250 mg/kg bw/d for maternal toxicity can be derived from the reported studies.

Considering all the information above, the NOAEL of 100 mg/kg bw/d from the developmental toxicity studies is selected for the derivation of DNELs, related to systemic effects.

5.13.2 Selection of the critical DNEL(s)/DMEL(s) and/or qualitative/semi-quantitative descriptor for critical health effect

DNELs for workers and the general population have been derived and they are shown below. However, consumer exposure is not expected and the exposure of humans via the environment is considered negligible.

An overall oral NOAEL of 100 mg/kg bw/d from the developmental toxicity studies is selected for the derivation of DNELs, related to systemic effects.

Specific short-term exposure DNELs have not been derived since 2-EHA is of low acute toxicity by the three routes of exposure. On the other hand, the overall DNEL established for systemic effects is considered sufficient to ensure that any acute effect does not occur.

The substance does not fulfil the CLP Regulation criteria for classification as skin or eye irritant. Furthermore, it has not shown sensitizing properties. In addition no local effects have been observed in acute or long-term toxicity studies.

Therefore, only systemic DNELs for long-term exposure are derived.

Workers

Occupational exposure to 2-EHA may occur via inhalation and dermal route. Oral exposure would not be a significant route of exposure under normal working practices. Therefore, only dermal and inhalatory DNELs have been derived.

CRITICAL DNELS/DMELS - WORKERS					
Endpoint of concern	**Type of effect**	**Critical study(ies)**	**Corrected dose descriptor(s) (e.g. NOAEL, NOAEC)**	**DNEL/ DMEL**	**Justification/ Remarks**
Toxicity for reproduction	Systemic effects, long term, dermal	Developmental study	NOAEL of 100 mg/kg bw/d	DNEL of 2 mg/kg bw/d	AF of 50 (interspecies: 4; intraspecies: 5; dose-reponse: 1; other interspecies: 2.5)
Toxicity for reproduction	Systemic effects, long term, inhalation	Developmental study	NOAEC of 176.21 mg/m^3	DNEL of 14 mg/m^3	AF of 12.5 (intraspecies: 5; dose-reponse: 1; other interspecies: 2.5)

General population

Even though consumer exposure is not expected and the exposure of humans via the environment is considered negligible, DNELs for the three routes of exposure have been estimated for the general population.

The NOAEL of 100 mg/kg bw/d from the reproductive and developmental toxicity studies has been selected as the relevant dose-descriptor.

CRITICAL DNELS/DMELS – GENERAL POPULATION					
Endpoint of concern	**Type of effect**	**Critical study(ies)**	**Corrected dose descriptor(s) (e.g. NOAEL, NOAEC)**	**DNEL/ DMEL**	**Justification/ Remarks**
Toxicity for reproduction	Systemic effects, long term, dermal	Developmental study	NOAEL of 100 mg/kg bw/d	DNEL of 1 mg/kg bw/d	AF of 100 (interspecies: 4; intraspecies: 10; dose-reponse: 1; other interspecies: 2.5)
Toxicity for reproduction	Systemic effects, long term, inhalation	Developmental study	NOAEC of 86.95 mg/m^3	DNEL of 3.5 mg/m^3	AF of 25 (intraspecies: 10; dose-reponse: 1; other interspecies: 2.5)
Toxicity for reproduction	Systemic effects, long term, oral	Developmental study	NOAEL of 100 mg/kg bw/d	DNEL of 1 mg/kg bw/d	AF of 100 (interspecies: 4; intraspecies: 10; dose-reponse: 1; other interspecies: 2.5)

5.14 Conclusions of the human health hazard assessment and related classification and labelling

2-EHA has 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.

Following the evaluation of the new information available on 2-EHA after substance evaluation, it is 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. For this reason, it is considered that the concerns have been clarified and neither further information nor additional classification is required.

6 HUMAN HEALTH HAZARD ASSESSMENT OF PHYSICO CHEMICAL PROPERTIES

There are no indications for classification of 2-EHA with regard to physico-chemical properties. The substance is considered of no concern for human health concerning physico-chemical properties.

7 ENVIRONMENTAL HAZARD ASSESSMENT

The 2-EHA evaluation was targeted at human health and therefore no environmental risk assessment has been carried out.

8 PBT AND VPVB ASSESSMENT

The 2-EHA evaluation was targeted at human health and therefore no PBT/vPvB assessment has been carried out.

9 EXPOSURE ASSESSMENT

This is a non-confidential summary of the exposure assessment section performed by the eMSCA. The complete section is included in the confidential annex.

9.1 Human Health

2-EHA is a colourless to slightly yellow liquid carboxylic acid with mild odour and low volatility (vapour pressure of 0.04 hPa at 20 ºC). It is commercially available as a racemate with purity higher than 99% (w/w).

Human exposure to the evaluated substance seems to occur mostly through occupational sources. 2-EHA is mainly used by workers as an intermediate for the manufacture of other substances. These processes take place generally in closed systems.

Consumer exposure to 2-EHA is not expected. The substance is readily biodegradable and it will rapidly disappear from water and soil via mineralization. Furthermore, the low n-octanol/water partition coefficient (log Kow) implies that an exposure via the food is not likely. Indirect exposure through the environment is considered negligible.

2-EHA can affect the body if it is inhaled, swallowed or if it is readily absorbed through the skin. Due to its physico-chemical properties and uses described, the relevant exposure routes for the substance are the inhalation and dermal routes.

According to the literature, the substance is mainly used as an intermediate for the synthesis of other substances such as metal carboxylates, synthetic esters and modified alkyd resins.

In the updated registration dossiers nine exposure scenarios have been described by the registrants. These exposure scenarios relate to occupational exposure and include industrial and professional uses. Described uses included manufacture of 2-EHA or its use as an intermediate for the production of other substances, formulation of mixtures, use in laboratories and use as functional fluids.

In a first instance, the registration dossiers from 2010 included 26 exposure scenarios. Several identified uses were wide dispersive and included industrial, professional and consumer uses. 2-EHA was additionally described to be used in cleaning agents, polymerisation and oligomerisation, coatings, as processing aid or as binders. However, Industry reported afterwards that some of the identified uses included in the registration dossiers from 2010 did not correspond to real uses of the substance itself, but of their derivatives, mainly the salts or esters of 2-EHA. Furthermore, other identified uses of 2-EHA had been inappropriately included in the dossier due to a mistaken identity of the substance (2-ethylhexanoic acrylate has the same acronym than 2-ethylhexanoic acid, i.e. 2-EHA). This misinterpretation with the 2-EHA acronym includes the consumer uses, which had been exclusively reported by only one registrant in the registration dossier from 2010.

Finally, updated dossiers have been submitted by all the registrants. These updated registration dossiers will be considered for substance evaluation purposes. It is important to highlight that a declaration of uses advice against for consumers has been reported by the registrants in the updated dossiers.

Regarding current information, consumer exposure is unlikely due to uses of 2-EHA as such (CAS 149-57-5). However, it is important to mention that there is a relevant source of exposure to 2-EHA from contact with products containing metal salts of this substance. These 2-EHA derivatives are described in the literature to be used in many different industrial applications, for example as PVC stabilizers, lubricants, drying additives for paints, inks, varnishes, lacquers and wood preservatives.

Information from the literature shows that 2-EHA is present after exposure to 2-EHA derivatives. The substance has been detected in workers exposed to a wood preservative containing 26% sodium 2-ethylhexanoate (Kröger *et al*., 1990). In addition, the presence of 2-EHA released from PVC plastic sealers has been demonstrated in baby food and fruit juices packed in glass jars closed with metal lids and sealed with plastic gaskets. As suggested by the authors, the acid is probably released from 2-EHA salts used as stabilizers in plastics (Elss *et al*., 2004).

Identifiers of metal salts of 2-EHA are different from the substance itself. Therefore, an assessment of exposure of the mentioned derivatives has not been carried out within this evaluation.

9.1.1 Exposure assessment for worker

Occupational exposure to 2-EHA may occur through inhalation and dermal contact in industries where it is produced, formulated or used. Oral exposure is assumed to be prevented by good hygiene practices. The production and further process of 2-EHA takes place in closed plants. The substance is mainly used by workers as an intermediate of other substances in closed installations.

There is no EU defined occupational exposure limits. In the majority of the EU countries an exposure value of 5 mg/m^3 for 8h-TWA is established, with a mention to the reprotoxic effects of this substance.

9.1.1.1 Overview of uses and exposure scenarios

Nine exposure scenarios are described for workers: three of them are related to manufacture and further formulation, four correspond to end-use in industrial settings and two to professional end-use. The following exposure scenarios are described in the updated dossiers:

- ES 1: “Manufacture and distribution of substance”
- ES 2: “Formulation and (re)packing of substance and mixtures (Registrant use)”
- ES 3: “Formulation and (re)packing of substance and mixtures”
- ES 4: “Use as an intermediate (Registrant use)”
- ES 5: “Use as an intermediate (Customer use)”
- ES 6: “Industrial use in laboratories”
- ES 7: “Professional use in laboratories”
- ES 8: “Industrial use as functional fluids (max. 15%)”
- ES 9: “Professional use as functional fluids (max. 15%)”

These scenarios include the following tasks/activities/processes as described by the registrants:

- Use in closed process, no likelihood of exposure (PROC 1).
- Use in closed, continuous process with occasional controlled exposure (PROC 2).
- Use in closed batch process (synthesis or formulation) (PROC 3).
- Use in batch and other process (synthesis) where opportunity for exposure arises (PROC 4).
- Mixing or blending in batch processes for formulation of mixtures and articles (multistage and/or significant contact) (PROC 5).
- Transfer of liquids by means of a (charging/discharging) from/to vessels/large containers at non-dedicated (PROC 8a) or dedicated facilities (PROC 8b).
- Transfer of substance or mixture into small containers (dedicated filling line, including weighing) (PROC 9).
- Use as laboratory reagent (PROC 15).
- Heat and pressure transfer fluids (closed systems) in dispersive use (PROC 20).

9.1.1.2 Scope and type of exposure

External exposure by inhalation and dermal routes has been reassessed by the eMSCA in all scenarios. It is assumed that oral exposure is prevented by personal hygienic measures. According to the low vapour pressure of 2-EHA, dermal exposure may contribute significantly to overall exposure.

9.1.1.2.1 Monitoring data

There are no monitoring data of occupational exposure to 2-EHA available.

9.1.1.2.2 Modelled data

ECETOC TRA v3 model (90th percentile of the percentile distribution) has been applied for the occupational exposure assessment of 2-EHA. When running the model, each activity within its exposure scenario has been reassessed by the eMSCA.

No peak exposure has been assessed due to the low acute toxicity of this substance.

9.1.1.2.3 Comparison of monitoring and modelled data

No comparison is possible due to the lack of monitoring data for occupational exposure.

9.1.2 Exposure assessment for consumer

No consumer use is described by the registrants in the updated registration dossiers. Furthermore, there is a declaration of uses advice against for consumers. Therefore, no consumer exposure to 2-EHA is expected.

9.2 Environmental exposure assessment

The 2-EHA evaluation was targeted at human health and therefore no environmental risk assessment has been carried out.

9.3 Combined exposure assessment

Not applicable.

10 RISK CHARACTERISATION

This is a non-confidential summary of the risk characterization section performed by the eMSCA. The complete section is included in the confidential annex.

10.1 Human Health

Only workers are assumed to be exposed to 2-EHA. The relevant exposure routes are the inhalation and dermal routes. Oral exposure is assumed to be prevented by good hygiene practices.

10.1.1 Workers

ECETOC TRA v3 model has been used for Risk Characterization purposes. For RCR calculation of each activity, the tool computes the quotient between external exposure estimates and the corresponding derived DNELs for the inhalation and dermal routes. In this context, the derived DNELs in section 5.13.2 have been used:

- DNEL dermal, systemic = 2 mg/kg bw/d
- DNEL long-term, inhalatory, systemic = 14 mg/m^3

It is important to highlight that all calculated RCR values are below 1 for the inhalation and dermal routes. Furthermore, a conservative tool (ECETOC TRA v3) has been used for an appropriate estimation of exposure. Consequently, the risk for human health is considered controlled for each route of exposure.

10.1.2 Consumers

No consumer use is described by the registrants in the updated registration dossiers. Furthermore, there is a declaration of uses advice against for consumers. Therefore, no consumer exposure of 2-EHA is expected.

10.1.3 Indirect exposure of humans via the environment

Indirect exposure through the environment is considered negligible.

10.2 Environment

The 2-EHA evaluation was targeted at human health and therefore no environmental risk assessment has been carried out.

10.3 Overall risk characterisation

10.3.1 Human health (combined for all exposure routes)

The combined values for inhalation and dermal routes have been considered for characterization of overall systemic health risks of 2-EHA in workers. Oral exposure is assumed to be prevented by good hygiene practices.

It is important to highlight that all calculated RCR combined values are below 1. Furthermore, a conservative tool (ECETOC TRA v3) has been used for an appropriate estimation of exposure. Consequently, the risk for human health is considered controlled for the combined routes of exposure. Hence, the initial concern for human exposure can be removed.

10.3.2 Environment (combined for all exposure routes)

Not evaluated.

11 OTHER INFORMATION

29 February 2012: Date of literature search.

11 April 2012: First contact of the lead registrant.

12 June 2012: Communication from the MSCA to all registrants on the intention to celebrate a scientific meeting on 2-EHA.

12 June 2012: Communication from the lead registrant declaring himself as the point of contact and coordinator for the 2-EHA consortium.

12 July 2012: Scientific meeting on 2-EHA held in the Ministry of Health. Industry reports about an update of registration dossiers concerning mainly uses and exposure scenarios.

25 September 2012: The lead registrant communicates the intention to update the registration dossier in October.

25 October 2012: Update of the lead registrant registration dossier.

31 October 2012: All registrants declare their intention to update their dossier.

23 November 2012: All registration dossiers updated.

5 December 2012: Draft decision sent to ECHA for legal screening.

28 February 2013: Draft decision submitted to ECHA.

21 October 2013: ECHA referred the draft decision to the MSC.

12 December 2013: Unanimous agreement of the MSC on the draft decision was reached.

26 February 2014: ECHA sent the final decision to the registrants.

24 May 2016: Registrant submitted to ECHA an update of the registration dossier with the information required.

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13 ABBREVIATIONS

2-EHA	2-Ethylhexanoic acid
AF	Assessment Factor
AGD	Anogenital Distance
bw	body weight / *Bw*, *b.w.*
CAS	Chemical Abstracts Service
CLP	Classification, Labelling and Packaging
CoRAP	Community Rolling Action Plan
CSA	Chemical Safety Assessment
CSR	Chemical Safety Report
DIT	Developmental Immunotoxicity
DMEL	Derived Minimal Effect Level
DMSO	Dimethyl sulfoxide
DNEL	Derived No Effect Level
DNT	Developmental Neurotoxicity
EC	European Communities
ECETOC	European Centre for Ecotoxicology and Toxicology of Chemicals
ECHA	European Chemicals Agency
EE	Exposure estimation
EN	European Norm
EOGRTS	Extended One-Generation Reproductive Toxicity Study
ERC	Environmental Release Category
ES	Exposure Scenario
EU	European Union
FOB	Functional Observation Battery
GC/MS	Gas chromatography / Mass spectrometry
GD	Gestational day
GLP	Good Laboratory Practice
HPLC	High Performance Liquid Chromatography
IUCLID	International Uniform Chemical Information Database
IUPAC	International Union for Pure and Applied Chemistry
i.v.	intravenous
LC_0	Lethal Concentration zero
LC_{50}	median Lethal Concentration
LD_{50}	median Lethal Dose
LEV	Local Exhaust Ventilation
LOAEL	Lowest Observed Adverse Effect Level
MAA	Motor Activity Assessment
MCHC	Mean Corpuscular Haemoglobin Concentration
MCV	Mean Corpuscular Volume
MSCA	Member State Competent Authority
MT	Metallothionein
NOAEC	No Observed Adverse Effect Concentration
NOAEL	No Observed Adverse Effect Level
NOEL	No Observed Effect Level
OECD	Organisation for Economic Cooperation and Development
PBT	Persistent, Bioaccumulative and Toxic
PNEC	Predicted No Effect Concentration

PPE	Personal Protective Equipment
PROC	Process Category
RCR	Risk Characterisation Ratio
REACH	Registration, Evaluation, Authorisation and Restriction of Chemicals
RMO	Risk Management Options
ROS	Reactive oxygen species
SCE	Sister Chromatid Exchange
sRV	standard Respiratory Volume
TGD	Technical Guidance Document
TRA	Targeted Risk Assessment
TSCA	Toxic Substances Control Act
TWA	Time-weighted average
US EPA	Environmental Protection Agency, USA
vPvB	very Persistent and very Bioaccumulative
w/w	weight per weight ratio
wRV	worker Respiratory Volume