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The Nordic Expert Group for Criteria Documentation of Health Risks from Chemicals

112. 2-Ethylhexanoic acid

Vesa Riihimäki

ARBETS MILJÖ INSTITUTET
NATIONAL INSTITUTE OF OCCUPATIONAL HEALTH

A Center for Research on Occupational Health

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Redaktör: Anders Kjellberg
Redaktionskommitté: Åsa Kilbom, Elisabeth Lagerlöf, Anders Colmsjö, och Nils Stjernberg.
Grafisk produktion: Eva Nilsson

Arbetsmiljöinstitutet,
171 84 Solna, Sverige

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Preface

The Nordic Council is an intergovernmental collaborative body for the five countries, Denmark, Finland, Iceland, Norway and Sweden. One of the committees, the Nordic Senior Executive Committee for Occupational Environmental Matters, initiated a project in order to produce criteria documents to be used by the regulatory authorities in the Nordic countries as a scientific basis for the setting of national occupational exposure limits.

The management of the project is given to an expert group. At present the Nordic Expert Group consists of the following member:

Helgi Gudbergsson	Municipal Institute of Public Health, Iceland
Petter Kristensen	National Institute of Occupational Health, Norway
Per Lundberg (chairman)	National Institute of Occupational Health, Sweden
Vesa Riihimäki	Institute of Occupational Health, Finland
Adolf Schaich Fries	National Institute of Occupational Health, Denmark

For each document an author is appointed by the Expert Group and the national member acts as a referent. The author searches for literature in different data bases such as Toxline, Medline, Cancerlit and Nioshtic. Information from other sources such as WHO, NIOSH and the Dutch Expert Committee is also used as are handbooks such as Patty's Industrial Hygiene and Toxicology. Evaluation is made of all relevant scientific original literature found. In exceptional cases information from documents difficult to access are used. The draft document is discussed within the Expert Group and is finally accepted as the Group's document.

An editorial work is performed by the Group's Scientific Secretary, Brita Beije, at the National Institute of Occupational Health in Sweden.

Only literature judged as reliable and relevant for the discussion is referred to in this document. Concentrations in air are given in mg/m^3 and in biological media in mol/l. In case they are otherwise given in the original papers they are if possible recalculated and the original values are given within brackets.

The documents aim at establishing a dose-response / dose-effect relationship and defining a critical effect based only on the scientific literature. The task is not to give a proposal for a numerical occupational exposure limit value.

The evaluation of the literature and the drafting of this document on industrial enzymes was made by Dr. Vesa Riihimäki, Department of Industrial Hygiene and Toxicology, Institute of Occupational Health, Topeliuksenkatu 41 a A, FIN-00250 Helsinki, Finland. The final version was accepted by the Nordic Expert Group June 13-15, 1994 as its document.

Brita Beije
Scientific Secretary

Per Lundberg
Chairman

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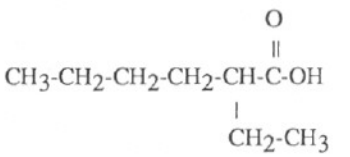
1. Introduction

2-Ethylhexanoic acid (2-EHA) has a limited number of known industrial applications, mainly in closed processes. In recent years the substance has found more widespread open uses as a component of wood preservatives (in the form of sodium salt). Moreover, 2-ethylhexanoic acid is formed in the mammalian metabolism of di(2-ethylhexyl)phthalate (1) and di(2-ethylhexyl)adipate (9, 56), which are extensively used as plasticizers in PVC plastics, resulting in some environmental and consumer exposure (27).

So far only few reports have been published dealing with the toxicology of 2-EHA in humans. However, in the open literature there are several peer-reviewed studies which elucidate the metabolic, hepatic and teratogenic effects of 2-EHA under experimental conditions, and some aspects of its basic experimental toxicology are covered in publicly available toxicity testing reports produced by the industry. Because the experimental findings show that 2-EHA has significant intrinsic toxic characteristics, a human health hazard assessment is warranted, particularly concerning the occupational exposure.

2. Substance identification

CAS number:	149-57-5
Name of substance:	2-Ethylhexanoic acid
Common synonyms:	α-Ethylhexanoic acid 2-Ethylcaproic acid α-Ethylcaproic acid 2-Ethylhexoic acid 2-Butylbutanoic acid Butylethylacetic acid 3-Heptanecarboxylic acid
Empirical formula:	$C_8H_{16}O_2$
Structural formula :	

Purity of industrial product: 99 % by weight

3. Physical and Chemical Properties

Molecular weight:	144.22
Melting point:	-118.4°C
Boiling point:	227.6°C
Vapour pressure:	$1.33 \cdot 10^{-3}$ kPa at 20°C
Saturation vapour concentration:	78 mg/m^3 (13.3 ppm)
Partition coefficient n-octanol/water:	log P_{ow} = 3 at 25°C
Water solubility:	25 mg/l at 25°C
pKa	4.0
Relative density:	0.90 at 25°C
Conversion factors:	1 ppm = 5.89 mg/m^3 1 mg/m^3 = 0.170 ppm at 25°C

2-Ethylhexanoic acid (2-EHA) is a colourless liquid with a mild characteristic odour (CHEMINFO). The substance is a weak acid with an estimated pKa of 4.0 (47). It is relatively soluble in lipids but only slightly soluble in water (Eastman Kodak Company). At room temperature the substance has a low vapour pressure (Eastman Kodak Company) and thus its ability to evaporate into the air is limited. Because the α-carbon atom of 2-EHA is asymmetric, the compound exists as two enantiomers; the industrial chemical is a racemic mixture.

4. Occurrence, Production and Use

According to estimates by the US-EPA about 9 000 - 14 000 tonnes of 2-ethylhexanoic acid was produced and imported per annum in the United States alone (58). The primary use of the compound is for chemical conversion to metallic salts, which are used as driers for paints and varnishes and as gelling agents for hydrocarbons (18). The substance may also be used as an intermediate in the manufacture of catalysts, plasticizers, inks, dyestuffs, flame retardants, surfactants and lubricants.

More recently, the sodium salt of 2-ethylhexanoic acid has found a new use (in mixture with other fungicides) as a wood preservative. In Finland, several hundred tonnes of sodium ethylhexanoate are annually consumed for the surface treatment of freshly sawn timber to prevent the growth of molds (23). This application of a derivative of 2-EHA has shown an increasing trend over the recent years. It is estimated that in Finland about 200 - 300 workers are potentially exposed in wood preservation.

5. Occupational Exposure Data

The US-EPA has estimated that workers could be exposed to 2-EHA at a daily rate of 60 mg/kg from dermal contact (58).

Only one report was found that contained quantitative data of occupational exposure to 2-EHA. The study involved four Finnish sawmills where the workers were exposed to ionized 2-ethylhexanoate from an alkaline wood preservative solution containing 1.3 % 2-EHA (23). The concentrations of 2-EHA in individual breathing zone air samples, collected over 1 - 3 h, ranged 0.01 - 1.3 mg/m^3, and the mean levels in air at different places of work ranged from 0.17 mg/m^3 (straightening) to 0.62 mg/m^3 (place near the dipping pool). The air concentrations of 2-EHA were higher when the treatment was done with the spray-irrigation method (about 0.5 mg/m^3) as compared to trough-dipping (about 0.2 mg/m^3). Furthermore, urine samples voided at the end of the workshift were found to contain 0.01 - 1.4 mmol of 2-EHA per mol of creatinine (after acid hydrolysis), and there was a significant correlation between the concentrations of 2-EHA in air and urine (23).

6. Measurement of Workplace Exposure

2-Ethylhexanoate was measured in the breathing zone air samples of workers employed in sawmills but the methods are poorly described (23). For the sampling the authors used conventional impinger flasks (containing ethanol) and millipore filters; the latter were extracted with ethanol. It seemed that, compared to the liquid absorption method, almost half of the sample was lost when using filters.

2-Ethylhexanoate containing ethanol samples were evaporated to a volume of 1-2 ml, and the analyte was derivatized to the pentafluorobenzyl ester. The chemical analysis was performed with a capillary gas chromatograph equipped with a ^{63}Ni electron capture detector (23, 24). The limit of detection for 2-EHA in the impinger absorbant was 0.1 mol/l.

Because it can be expected that evaporation of 2-ethylhexanoate from an alkaline solution is very low, the airborne substance was probably in the form of a liquid aerosol. This aspect of exposure has not, however, been further investigated, and neither has potential exposure from skin contact to

contaminated surfaces been explored. Therefore the potential and routes of human exposure to 2-ethylhexanoate in sawmills cannot be assessed conclusively.

7. Toxicokinetics

7.1. Human Observations

No quantitative data were found concerning absorption of 2-EHA via inhalation, gastro-intestinal tract and the skin in humans. It may be noted that the compound is relatively lipid soluble (in an octanol/water distribution system it attains a 10^3 times higher concentration in the hydrophobic phase), and hence it is likely to be well absorbed. However, when 2-EHA becomes dissolved in body fluids it is almost completely ionized, which probably limits its rate of diffusion through biological membranes.

The study on Finnish sawmill workers demonstrated that some 2-ethylhexanoate was absorbed because it could be detected in the urine and, since 2-EHA concentrations in the urine correlated to the substance concentrations in the air, inhalation seemed to be the primary route of uptake (23). Unfortunately, the study did not provide any important kinetic data, such as the time course of 2-EHA excretion, excepting the note that the highest concentrations were found immediately after the workshift. Likewise, it is not known in what form 2-EHA was excreted in the urine (probably mainly as a glucuronide), and no conclusions can be drawn concerning the magnitude of its excretion relative to the daily uptake of 2-EHA.

7.2 Animal studies

7.2.1. Overall kinetics

In an unpublished study by the chemical industry submitted to the US-EPA (12) the salient features of 2-EHA toxicokinetics in the rat have been reported. Female F344 rats were administered [2-^{14}C-hexyl] EHA (1) by a single oral gavage dose of 100 mg/kg bw or 1000 mg/kg bw, (2) after fourteen daily oral gavage doses (100 mg/kg bw) of unlabeled 2-EHA, (3) by dermal application under occlusion for 96 h (100 mg/kg bw or 1000 mg/kg bw) and (4) by intravenous injection (1 mg/kg bw). Urine, faeces and blood (from selected animals) were collected at intervals for 96 hours.

After oral administration of 100 mg/kg ^{14}C-EHA, the mean peak blood level of 85.1 g equivalents EHA/g was detected at 15 or 30 min. After dermal application of the same dosage, the mean peak blood level of 8.5 g equivalents EHA/g was attained at 5.7 h. Approximately 80 % of the dose was recovered in the urine and about 10 % in the faeces within 96 h after a single oral administration of both doses (100 mg/kg and 1000 mg/kg). However, the elimination processes

appeared to become saturated at the higher dose level because, in the first 8-h period after administration, only about 20 % of the dose was excreted renally in the high-dose group compared to about 50 % by the low-dose group. Furthermore, a greater share of the dose was recovered from the faeces of the low-dose group (12.5 %) compared to the high-dose group (6.7 %) suggesting that biliary excretion to the gastrointestinal tract took place, and that it also was saturated at the high dose level. Biliary excretion was confirmed by the presence of faecal radioactivity after intravenous administration of ^{14}C-EHA (3.6 % of the dose versus 66.6 % in the urine). Repeated oral gavage administration of 100 mg/kg 2-EHA resulted in a lower recovery of the dose from the urine (60.6 %) but not in faeces (14.9 %). After dermal application, approximately 45 % of the dose was found in the urine and 7.5 % in the faeces at both dose levels.

It may be concluded that 2-EHA was rapidly and nearly completely absorbed from the gastrointestinal tract of the rat, and more slowly but ultimately extensively absorbed from the occluded rat skin. In the latter case, however, the epidermis and hence the penetration barrier were severely damaged.

A separate study was performed with rats to determine the efficiency of removal of dermally applied 2-EHA by washing the skin with soapy water. The application site was washed 5 - 10 min after the dermal exposure to ^{14}C-EHA of 1000 mg/kg bw. Virtually all of the label was recovered during the washing procedure showing that (1) penetration of 2-EHA into the skin is slow and (2) conventional soap and water mixture is an efficient medium for decontamination.

After administration of ^{14}C-EHA intravenously and orally the label in blood declined triexponentially. Since most of the dose was excreted within 24 h, the elimination of 2-EHA was mainly associated with the intermediate half-time corresponding to about 6 - 7 h. The terminal half-times of ^{14}C after intravenous, oral and dermal administration were 117 h, 98 h and 251 h, respectively. The organ distribution of ^{14}C -EHA was not determined.

7.2.2. Biotransformation

The previous study with female F344 rats (12) also attempted to characterize the urinary metabolites of 2-EHA by using HPLC separation techniques and desorption chemical ionization mass spectrometry or GC-MS for identification. 2-EHA-glucuronide was the predominant urinary metabolite (12 - 45 % of the dose), and the extent of glucuronidation increased with increasing dose. Smaller amounts of unchanged 2-EHA were also detected; hydrolysis of 2-EHA-glucuronide is a possibility. Several other metabolites were tentatively identified or postulated, compatible with both microsomal cytochrome P450 oxygenation and mitochondrial and/or peroxisomal β-oxidation pathways. Tentatively identified major metabolites were 2-ethyl-6-hydroxyhexanoic acid and 2-ethyl-1,6-hexanedioic acid, which are probably formed by cytochrome P450 catalyzed ω-oxidation. Minor amounts of 2-ethyl-5-hydroxyhexanoic acid, ethylketohexanoic acid and two postulated lactones were found, which were likely to have arisen from cytochrome P450 catalyzed ω-1 oxidation. The detection of

Δ^5-2-heptenone in small amounts may be evidence of biotransformation via β-oxidation.

The role of β-oxidation in the metabolism of 2-ethylhexanol (a metabolic precursor of 2-EHA) has earlier been proposed by Albro (2) after he found labeled CO_2 (6 - 7 % of the 8.8 g dose) in the expired air and corresponding amounts of heptanone in the urine of rats given 2-ethyl(1-^{14}C)hexanol by gavage. He identified 2-EHA, 2-ethyl-1,6-hexanedioic acid and 2-ethyl-5-hydroxyhexanoic acid as the major urinary metabolites, and 2-ethyl-5-ketohexanoic acid as a minor metabolite.

Male Wistar rats were given 2-EHA in drinking water at 5 or 10 g/l corresponding to daily doses of 130 and 200 mg (370 and 570 mg/kg bw/day), respectively, for 20 days (31). During the last week of exposure, relatively stable levels of 2-EHA (free and conjugated forms were not separated) were found in the urine. The diurnally excreted amounts of 2-EHA were approximated for this review from the published data, based on the mean urinary creatinine concentration (9.4 mmol/l) and a mean daily urine volume of 15 ml given by the authors, and related to the daily intakes of 2-EHA. According to these calculations about 31 % of the lower dose and about 51 % of the higher dose were excreted as 2-EHA and conjugates. These figures are about 2 times higher than those found for the corresponding labeled metabolites in F344 rats that were repeatedly given 2-EHA by gavage at 100 mg/kg daily (12). On the other hand, the result would be consistent with the earlier observation that the share of 2-EHA glucuronide excretion in urine increases at higher doses (12).

The same study group has later investigated the urinary metabolites of 2-EHA in male Wistar rats given 2-EHA in drinking water (600 mg/kg bw daily) for nine weeks (44). The compounds were identified by GC-MS both in the electron-impact and chemical ionization mode. 2-EHA and ten different EHA metabolites were found. The main peak was 2-ethyl-1,6-hexanedioic acid. Six hydroxylated metabolites (one was identified as 2-ethyl-6-hydroxyhexanoic acid) and two lactones were detected but their detailed structures could not be defined. The origin of the lactones is uncertain as they may have been produced during the analysis (2). A small peak conformed to 2-ethyl-5-hexenoic acid which is presumably produced by cytochrome P450 catalyzed δ-dehydrogenation.

When human volunteers were given an oral dose of deuterated di-(2-ethylhexyl)adipate (which is transformed in mammalian metabolism to 2-EHA), 2-EHA (as a glucuronide), 2-ethyl-1,6-hexanedioic acid, 2-ethyl-5-hydroxyhexanoic acid and 2-ethyl-5-ketohexanoic acid were identified in the urine (26). Thus human metabolism of 2-EHA seems to show much the same profile as that of the rat.

A schematic presentation of the proposed metabolic pathways for 2-ethylhexanoic acid is given in figure 1.

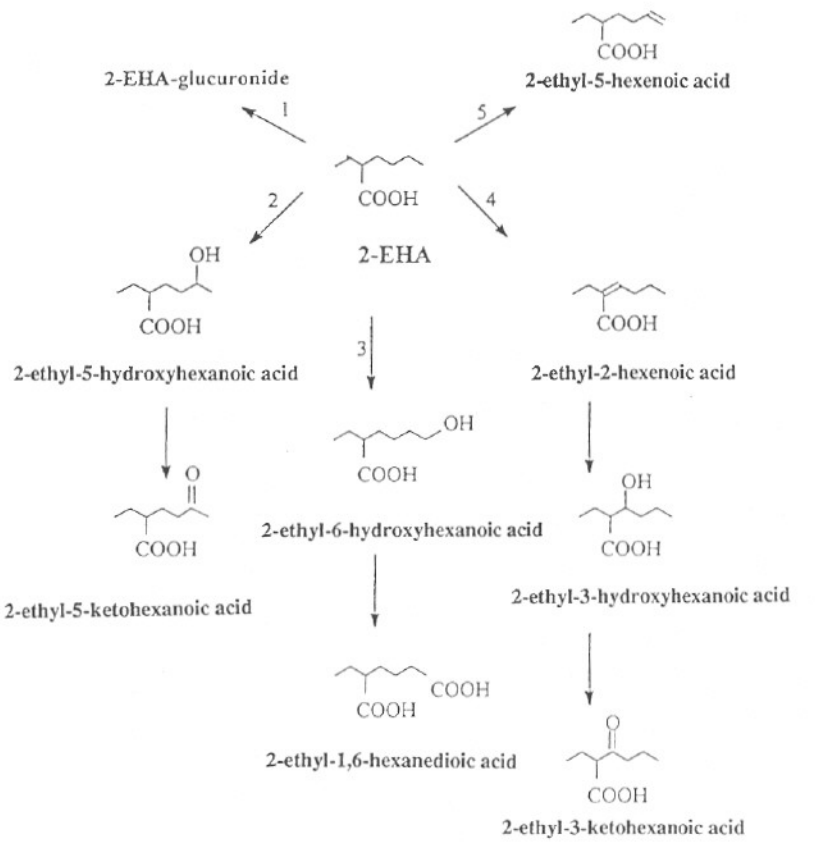


Figure 1. Metabolic pathways for 2-ethylhexanoic acid in the rat as proposed by Pennanen (43): 1 = glucuronidation; 2 = (ω-1)-oxidation; 3 = (ω)-oxidation; 4 = β-oxidation and 5 = (δ)-dehydrogenation.

7.2.3. Distribution

Organ distribution of 2-^{14}C-EHA was tentatively studied in mice and rats after intraperitoneal administration by analysis of the radioactivity in blood, liver, kidney and brain and by whole-body autoradiography in mice (46). 2-EHA was rapidly distributed to tissues and the label attained highest concentrations in the liver and kidney. Very little was found in the brain. The radioactivity was also rapidly excreted, and the detection limit was reached in all tissues by 24 hours.

8. Methods of Biological Monitoring

In view of the previous data on 2-EHA metabolism, methods can be envisaged for the assessment of 2-EHA uptake in humans, once the pertinent kinetic and metabolic data are available. This is not the case for the time being. Therefore, although analytical methods have been devised for the sensitive detection of 2-EHA in different matrices, including urine (17, 24), they are not applicable for biological monitoring.

An interesting finding among a limited number of sawmill workers was the apparently dose dependent increase in the urinary excretion of arginine and ornithine (45). The mechanism of this effect is not quite clear, but it probably relates to disturbances of the urea cycle which was demonstrated in rats already at low dosages of 2-EHA (31). Thus, urinary excretion of arginine and ornithine may provide an opportunity for the biological effect monitoring of 2-EHA, but the preliminary findings await verification and validation.

9. Mechanisms of Toxicity

Three types of effects by 2-EHA are known from animal experiments that may lead to significant toxicity: induction of peroxisome proliferation (22,28,35), derangements in embryonal/foetal development (8, 47, 49) and perturbations in endogenous metabolism of the liver (31, 36, 59).

Peroxisomes are subcellular organelles which major role is to break down long-chain fatty acids, thereby complementing mitochondrial fatty acid metabolism. Proliferation (increase in number) of peroxisomes in liver cells is associated with marked liver enlargement caused by both cellular hyperplasia and cellular hypertrophy (for review, see 3, 25). The hyperplasia is due to stimulation of replicative DNA synthesis and subsequent cell division. The hypertrophic cells show an increase of endoplasmic reticulum and the specific induction of cytochrome P450IVA, which exhibits high specificity for ω-oxidation of long-chain fatty acids (e.g. lauric acid). The long-chain dicarboxylic acids formed, which are mainly metabolized by the peroxisomal β-oxidation system, are thought to trigger peroxisomal proliferation. Peroxisome proliferation is accompanied by selective increases in the specific activities of certain peroxisomal enzymes, particularly those involved in β-oxidation of fatty acids (e.g. cyanide-insensitive palmitoyl-CoA oxidation and carnitine acetyltransferase), whereas other enzymes such as urate oxidase and catalase are hardly affected. This imbalance of peroxisomal enzymes may imply increased production of active oxygen and hence cellular effects from oxygen stress, such as deposition of lipofuscin and indirect DNA damage. Also the finding that cytosolic epoxide hydrolase is induced in the mouse liver after dietary administration of 2-EHA is in support of the hypothesis that active oxygen (hydrogen peroxide) may be formed thereby producing lipid peroxides in the cell (28). Many peroxisome proliferators induce

mice and rats, among them di(ethylhexyl)phthalate (DEHP) and di(ethylhexyl)adipate (DEHA) which both yield 2-EHA in mammalian metabolism. Moreover, 2-EHA was identified as the proximate peroxisome proliferator of DEHA in primary hepatocyte cultures from mice and rats (9). There are no animal carcinogenicity study data on 2-EHA itself.

The chain of events that leads to peroxisome proliferation and liver growth is a complex one, and it is thought to be initiated by the inhibition of mitochondrial fatty acid metabolism by the proliferator compound through CoA sequestration (25). A large number of different organic compounds with acid functions may cause peroxisome proliferation, among them alkylcarboxylic acids. In the latter group all of the active compounds have substituents at C-2 which renders them resistant to β-oxidation (3). Within a series of hexanoic acids the most effective peroxisomal inducer had an ethyl group at C-2 position (29). Apparently 2-EHA itself is the active compound, and the phenomenon is stereoselective. The (S)-enantiomer was a more potent inducer than the (R)-form suggesting that the molecular mechanism involves a chiral endogenous receptor (30).

It is important to note that species other than rodents such as guinea pigs, primates and humans are insensitive or non-responsive to peroxisome proliferators (including DEHP and DEHA) at dose levels that produce a marked response in mice and rats. A study with primary hepatocyte cultures showed that while mouse and rat hepatocytes were sensitive to peroxisome proliferation due to 2-EHA, guinea pig and marmoset hepatocytes were not (9). It may thus be argued that the relevance of the peroxisome proliferation by 2-EHA is highly doubtful for man.

Many carboxylic acids are teratogenic and the structural characteristics important for activity have been reviewed (11, 39). The critical structural components are (a) a free carboxylic group, (b) a hydrogen atom at C-2, (c) branching of carbon chains, (d) no double bond at C-2 or C-3, and (e) an alkyl substituent larger than methyl at C-2.

Most of the research work has concerned the antiepileptic drug valproic acid (2-propylpentanoic acid) which is an isomer of 2-EHA. Valproic acid is a known human teratogen (50). Although valproic acid is a more potent teratogen than 2-EHA (racemic mixture), the types of effect (neural tube and skeletal defects) seem identical (39, 49). However, the teratogenic activity of 2-EHA is stereoselective. The (R)-enantiomer was more potent than the (S)-form (8, 19) and, since there were no marked kinetic differences between the two antipodes in the embryo, the mechanism of effect presumably involves an interaction of the teratogen with chiral molecules in the embryo.

The activity of 2-EHA as a teratogen is presumed to depend on its metabolic stability (resistance to β-oxidation) and binding to receptors (11). Moreover, because the compound is a weak acid (pKa 4.0), it is expected to be trapped in the embryo during early organogenesis when the intracellular pH is transiently higher (47). This mechanism was demonstrated to increase the embryonal valproic acid concentration (40, 55).

2-EHA inhibited oxidations of several endogenous substrates (palmitoylcarnitine, glutamate and α-ketoglutarate) in rat liver mitochondria in vitro similarly to valproic acid (59). The underlying mechanism of inhibition may be sequesteration of CoA. Apart from the perturbed lipid metabolism described in the context of peroxisome proliferation, resulting in hypolipidaemia (36), 2-EHA inhibited urea synthesis (citrulline synthesis) in the rat (31). Workers exposed to 2-EHA showed exposure dependently increased urinary excretion of arginine and ornithine; the excretion of amino acids was thought to be compensatory to urea cycle inhibition (45). Hyperammonaemia without evidence of hepatotoxicity is not an uncommon finding among patients on valproic acid medication. Impaired hepatic mitochondrial citrullinogenesis was found primarily responsible for the hyperammonaemia after amino acid loading in rats (32).

It may be noted that valproic acid has different multiple metabolic effects in the liver (for review, see 10). Many of them might be shared by 2-EHA, but relevant study data are lacking.

10. Effects in Animal and *in Vitro* Studies

10.1. Irritation and sensitization

Undiluted 2-EHA in uncovered contact with rabbit skin for 24 h was a moderate to severe irritant to the skin (52, 54). In a more recent study, female F344 rats were exposed dermally to undiluted 2-EHA (approximately 1 g/kg bw) for 24, 48, 72 or 96 h, using an occlusive containment device (12). There was little visible damage in the skin, but histopathological examination showed patchy areas of necrosis throughout the entire epidermis, and the underlying dermis and hypodermis showed clear evidence of inflammation. After the first 24 h there was continued regeneration of the epidermis.

A 15 % solution of 2-EHA (presumably in propylene glycol) was severely irritating to the rabbit eye when applied in one 0.5 ml dose (53, 54).

No studies on the sensitizing potential by 2-EHA were found.

10.2. Acute toxicity

The acutely lethal dose (LD_{50}) of 2-EHA by gavage for (male Wistar and female Sprague-Dawley) rats was 1600 - 3200 mg/kg bw (54, 57). Female Sprague-Dawley rats given 3200 mg/kg were prostrate 4 h after the treatment and all rats died within 24 h; no distinct gross pathology was noted at autopsy. Rats given 800 or 1600 mg/kg bw 2-EHA were weak immediately after dosing, but they gained weight normally during the 14-d observation period. No clinical abnormalities were detected in animals that received 90 mg/kg bw 2-EHA (57).

A dermal LD_{50} value of 6.5 ml/kg bw was derived from a test with guinea pigs (54). The animals were treated with 2-EHA in an occluded patch for 4 d and observed for a total of 14 d.

No deaths were observed in rats exposed for 8 h to an atmosphere saturated with 2-EHA vapour (concentration was not given but it was presumable less than 100 mg/m^3) (54).

10.3. Short-term toxicity

Repeated dose studies with 2-EHA for two weeks have been conducted in mice and rats utilizing either gavage or feeding as the method of administration (5, 6, 15, 16). These complete reports by the industry have been submitted to the US-EPA, and have been made publicly available. They have also been evaluated for the OECD HPV-Programme (to be published).

10.3.1. Gavage studies

Groups of five male and five female B6C3F1 mice were given 0, 200, 800 or 1600 mg/kg bw of 2-EHA in corn oil as 11 doses over 15 days. The behaviour of the animals was observed and the development of weight was monitored. No haematological or clinical chemical examinations were performed. The liver and kidneys as well as any distinct macroscopic lesions were histologically examined. Gait disturbances, weakness and weight loss were noted in a single high-dose female during the first two days of the study. No differences were noted in body weight gain or feed consumption among the groups of either sex. Absolute and relative (to body weight) liver weights were increased in the high-dose males (but not in other groups), and all those animals showed hypertrophy of the hepatocytes in histological examination. No treatment-related pathological changes were detected in kidney histology (16).

F344 rats, five male and five female animals per dose group, were treated and examined in the same fashion as above. The highest dose (1600 mg/kg bw) was lethal to most rats after only two doses. Even 800 mg/kg caused lethargy and weakness after dosing. The animals receiving 200 mg/kg bw did not exhibit abnormal clinical signs. The body weight gains of the sole surviving male and female at the highest dose were depressed, and the 800 mg/kg males had slightly decreased body weight gains too (although feed consumption was comparable to controls). Absolute and relative liver weights were increased dose-dependently in animals of both sexes at the high and middle-dose levels; relative (to bodyweight) liver weight in females was increased at 200 mg/kg bw/day. The high-dose animals which died after one or two doses showed hepatocyte degeneration and vacuolization of cytoplasm. In the two surviving high-dose animals and in one 800 mg/kg male there was minimal hepatocyte hypertrophy, but no clear evidence of hepatotoxicity (6).

10.3.2. Feeding studies

Groups of five male and five female *B6C3F1 mice* were given 0, 0.75, 1.5 or 3 % of 2-EHA in feed for two weeks. The corresponding measured doses were approximately 0, 1600 - 2000, 3100 - 4000 and 5800 - 9200 mg/kg bw/day for both males and females. The examinations performed were as above. Terminal body weights were lower than in controls for both sexes at the highest dietary level (feed consumption in males which did not spill feed was reduced at all dose levels). The absolute and relative (to body weight) liver weights of males at the high- and middle-dose level and of females at all dose levels were increased dose-dependently. In histopathological examination, hepatocyte hypertrophy was found at all dose levels (but not in all animals at 0.75 %), affecting mainly cells in the periportal area. Multifocal coagulative necrosis of hepatocytes, which was rated as minor, was seen at all dose levels but with greater frequency at the two higher levels. Additionally, cortical atrophy of the thymus (in two males and one female) and atrophy of the red pulp of spleen (four males and two females) were noted at the high-dose level. There were no treatment-related effects in kidneys (15).

Groups of five male and five female *F344 rats* were given 0, 0.75, 1.5 or 3 % of 2-EHA in the feed for two weeks. The corresponding doses were approximately 0, 710 - 760, 1350 - 1400 and 2300 - 2700 mg/kg bw/day for both males and females. The female animals at the high-dose level had urine-soaked, discoloured and unkempt haircoat and piloerection. Even most of the middle-dose females showed the same signs. Terminal body weights were reduced at the top-dose and middle-dose levels for both sexes. Feed consumption was decreased in a dose-dependent manner in animals of both sexes. The absolute and relative (to body weight) liver weights were increased dose-dependently at all dose levels in both sexes. Hepatocyte hypertrophy in the periportal area was observed in all animals receiving the 3 % and 1.5 % diets. Multifocal coagulative necrosis affecting individual hepatocytes were noted in all high-dose males and females, and at lower frequencies in the middle-dose animals. No treatment-related effects were found in the kidneys (5).

10.4. Long-term toxicity/carcinogenicity

No reports concerning long-term toxicity or carcinogenicity studies with 2-EHA could be found. Subchronic 90-day dietary administration toxicity studies have, however, been conducted with 2-EHA in mice and rats (4, 14). These complete reports by the industry have been submitted to the US-EPA, and have been made publicly available. They have also been evaluated for the OECD HPV-Programme (to be published).

Groups of ten *B6C3F1 mice* of both sexes were given 0, 0.1, 0.5 or 1.5 % of 2-EHA in feed for 91-93 days. The corresponding measured mean doses were about 180, 890 or 2730 mg/kg bw/day for the males and 200, 1040 or 3140 mg/kg bw/day for the females. Separate groups of control and high-dose animals were

maintained on standard diet after the 90-day period for one further month to examine the reversibility of effects.

The high-dose groups and the middle-dose females had lower terminal body weights. Food consumption was at times reduced, especially among the male mice at the highest dose level. No abnormal clinical signs attributable to 2-EHA were noted. The main effects of 2-EHA involved the liver. The liver weights were increased at the high-dose and middle-dose levels; the histopathological examination showed hepatocyte hypertrophy and increased eosinophilia primarily in the portal areas. In the low-dose animals no liver changes were found. Serum triglyceride concentrations were decreased in the 1.5 and 0.5 % dose groups, but the cholesterol concentrations were increased in the same groups dose-dependently. Even serum bilirubin was slightly lower in the high-dose males and females and middle-dose females. Alanine aminotransferase (ALAT) was slightly but significantly elevated in the male high-dose mice and in one high-dose female mouse. Concerning the kidneys, the high-dose animals showed increased cytoplasmic basophilia and occasionally other changes in cellular organelles of the proximal tubular cells. Most of the high-dose male mice were found to have acanthosis and hyperkeratosis of the non-glandular forestomach.

At the end of the recovery period, the changes observed at 90 days were largely reversed. Single mice of both sexes still showed minor hepatocyte hypertrophy; no renal or gastric changes were noted.

Groups of ten *Fischer F-344 rats* of both sexes were given 0, 0.1, 0.5 or 1.5 % of 2-EHA in feed for 90 days. The corresponding mean doses were about 60, 300 or 920 mg/kg bw/day for the males and 70, 360 or 1070 mg/kg bw/day for the females. Separate groups of control and high-dose animals were maintained on standard diet after the 90-day period for further 28 days to examine the reversibility of effects.

The high-dose groups exhibited reduced body weight gain and the terminal body weights were lower; food consumption was also reduced. Slight haematological effects concerning the red blood cells were observed: MCH was lower in the 0.5 and 1.5 % groups and the occasionally found poikilocytosis was more frequent in the treated groups. Cholesterol levels increased dose-dependently in all dose groups in the males; triglyceride levels were significantly lower in the high-dose groups and in the middle-dose females. In the high-dose males urea nitrogen and albumin concentrations were slightly raised. The liver weights were increased dose-dependently in the high-dose and middle-dose groups; the histopathological examination showed hepatocyte hypertrophy and decreased cytoplasmic vacuolization (eosinophilia). The relative (to body/brain weight) kidney weights were slightly increased in the high-dose groups and in the middle-dose females; the histological picture of renal tissue was within normal limits. After the recovery period, the only persistent effects by treatment were slightly lower body weights and increased liver to body weights in the high-dose male rats.

10.5. Mutagenicity and genotoxicity

The mutagenicity of 2-EHA was studied with Salmonella typhimurium tester strains TA98 and TA100 with and without metabolic activation (60), and in another experiment with S. typhimurium TA97, TA98, TA100 and TA1535 with and without metabolic activation (61); both tests gave negative results.

In human lymphocytes, incubated with 2-EHA (0.63 - 2.5 mM) for 48 hours, a slight dose-dependent increase of sister-chromatid exchanges (SCE) was found (51). Since the maximum SCE-response was less than twice the control level, the authors proposed that the result should be interpreted with caution; however the response could not be attributed to a pH change in the medium.

10.6. Reproductive and developmental toxicity

A one-generation reproductive toxicity study with 2-EHA has been conducted in rats (48), and several studies have explored the developmental toxicity and teratogenicity of 2-EHA in mice (8, 19), rats (20, 37, 38, 47, 49) and rabbits (20).

10.6.1. Reproductive toxicity

In the study by Pennanen et al. (48) Wistar rats (24 animals per sex and dose group) were given 100, 300 or 600 mg/kg bw/day of 2-EHA as a sodium salt in drinking water; the control animals received plain water. Male rats were treated with 2-EHA for 10 weeks and females for 2 weeks prior to mating, both sexes were treated during the mating period and females during the entire gestation and lactation period. 2-EHA caused a slight, dose-dependent delay in fertilization: control animals conceived in the course of two estrous cycles while for several 2-EHA treated animals, particularly in the high-dose group, three or four cycles were needed, and all non-pregnant animals belonged to the 2-EHA treated groups. The spermatozoa were significantly less motile in the males receiving 100 and 600 mg/kg bw of 2-EHA and abnormal sperm occurred more frequently in the two highest dose levels. The average litter size was reduced by 16 % in the 600 mg/kg bw group. The body weights of the pups were unaffected but the body weight gain was transiently slower during lactation at 600 mg/kg bw. Several pups appeared abnormal (kinky tail, lethargic, slightly paralyzed legs) and the physical development (opening of eyes, eruption of teeth, hair growth, grip reflex, cliff avoidance) was delayed at 300 and 600 mg/kg bw.

In a separate pilot experiment, a single dose of 600 mg/kg 2-EHA was given to pregnant rats by gavage on day 4,5,6 or 7 of gestation, and the number of implantations were counted on day 10. The number of implantations was lowest after administration of 2-EHA on day 6; there were resorptions in 80 % (4/5) of the pregnant dams exceeding the historical control value of 0.6 resorptions per litter for this strain of rats and this laboratory. The authors speculated that the reduced fertility caused by 2-EHA in rats might result from disturbed implantation in uterus and that sperm changes in males played a minor role. The retarded

development of pups was attributed to teratogenicity and pre- and postnatal toxicity by 2-EHA.

10.6.2. Developmental toxicity

Since the discovery that the anticonvulsant drug valproic acid (2-propylpentanoic acid) is teratogenic to experimental animals and humans (for review, see 39), there has been an active interest to investigate the teratogenic potential of simple organic acids including 2-EHA, which is an isomer of valproic acid.

Ritter et al. (49) found with Wistar rats that a single gavage administration of 900 mg/kg bw (6.25 mmol/kg bw) of undiluted 2-EHA on day 12 of gestation caused 0.8 % of the living pups to be malformed, while 1800 mg/kg bw (12.5 mmol/kg bw) gave 67.8 % malformed. Thus there seemed to be a dose response and, when the lower dose was accompanied by intraperitoneal (i.p.) injection of 150 mg/kg bw of caffeine, the proportion malformed pups rose to 31.5 %, the effect being potentiated. Additionally, the high dose produced embryo- and foetotoxicity based on the 30 % decrease in foetal weight and a 30 % increase in the share of dead and resorbed foetuses. The types of defects found were limb malformations, hydronephrosis, short and kinky tail and cardiovascular malformations. The malformations were similar to those caused by valproic acid in the same study, but the latter compound appeared roughly two times more potent.

In NMRI mice four i.p. injections of 500 mg/kg bw of 2-EHA as the sodium salt in the morning and evening of days 7 and 8 of gestation caused embryotoxicity and teratogenicity in a stereoselective way (19). (S)-2-EHA did not elicit any teratogenic or embryotoxic response above control rates, whereas (R)-2-EHA induced a potent teratogenic response with 59 % of the living foetuses exhibiting exencephaly; there was also a significant reduction of foetal weight and the higher number of resorptions and dead foetuses suggested embryotoxicity. The exencephaly rate induced by the racemic 2-EHA mixture was between those of the two enantiomers.

The previous observations have later been confirmed and extended by Collins et al. (8) in SWV and C57BL/6NCr1BR mice with approximately the same dosing regimens. The SWV strain was more sensitive to the induction of exencephaly than the C57BL/6NCr1BR strain. The (R)-enantiomer of 2-EHA was a more potent teratogen than the (S)-enantiomer for the induction of exencephaly and also for other malformations: alteration of the axial skeleton and ablepharon. There were no major pharmacokinetic differences between the antipodes and in particular no difference in the peak embryonic 2-EHA concentration, which is regarded critical for the teratogenic response. Since valproic acid does not have an assymmetric carbon like 2-EHA, it seemed that there was not much difference in potency between (R)-2-EHA and valproic acid.

Pennanen et al. (47) conducted a developmental toxicity study with 2-EHA in Wistar rats. Pregnant animals (20 - 21 per dose group) were treated with 2-EHA in their drinking water at doses of 100, 300 or 600 mg/kg bw/day on days 6 - 19 of gestation. Control animals received vehicle water. 2-EHA was marginally

toxic to the dams at the high-dose level since the terminal body weight was reduced by 11 %. Similarly, the mean foetal body weights were slightly lower indicating foetotoxicity at 600 mg/kg bw/day. The treatments did not affect the number of implantations or live foetuses. Clubfoot was the most severe skeletal malformation induced by the treatment. It occurred dose-dependently at all dose levels, more notably at 300 and 600 mg/kg bw/day. Flabby legs, mild scoliosis and lordosis occurred even at 100 mg/kg. The incidences of several skeletal variations were also increased in 2-EHA treated animals, including the 100 mg/kg dose group. Only a few visceral malformations were found. The brain ventricles were slightly and dose-dependently dilated in 300 and 600 mg/kg groups; this was probably related to the delayed foetal development. When all the skeletal and visceral malformations were combined, the impact of 2-EHA was dose-dependent, reaching statistical significance at the middle- and high-dose levels. The proportions of affected foetuses per litter were 4.9, 8.9 and 15.3 % in the 2-EHA treatment groups versus 2.4 % in the control group.

Developmental toxicity tests in Fischer 344 rats and New Zealand white rabbits were conducted with 2-EHA by a group of investigators (20). The results of both the range-finding studies and the main studies were reported.

Studies in rats: In the range-finding study, pregnant rats (8 animals per treatment group) received 2-EHA by gavage 125, 250, 500 or 1000 mg/kg bw/day in corn oil on days 6 - 15 of gestation. Control animals were given corn oil. In the main study, the corresponding doses were 100, 250 or 500 mg/kg bw/day, and the group size was 25 animals. Severe maternal toxicity was noted in the 1000 mg/kg bw/day dose group: the dams exhibited ataxia, urogenital wetness, audible respiration and red periocular encrustation, and seven of eight animals died between days 7 and 9. The one surviving dam had a fully resorbed litter. The total level of postimplantation loss was increased and the corresponding percentage of living foetuses was decreased at 500 mg/kg bw/day; the foetal body weights were also reduced. In the main study, the animals receiving 500 mg 2-EHA/kg bw/day showed some clinical signs of toxicity: ocular discharge, periocular encrustation, but there was no impact on the weight gain during treatment or the terminal body weight; however, liver weight was increased. There was no difference between treated and control groups in the overall incidence of postimplantation loss or percentage of living foetuses. Foetal body weights were reduced in both sexes at the high-dose level. In the high-dose group there was an increase in the number of litters with foetuses exhibiting reduced ossification in the vertebral column, proximal phalanges of the forelimb and hindlimb, hindlimb metatarsals and sternebrae. Less extensive reduction of ossification was noted in foetuses in the 250 mg/kg bw/day group. Although not statistically significant, dilatation of lateral ventricles of the brain with tissue compression (a visceral malformation) was observed in one foetus each at 100 and 250 mg/kg bw/day and in six foetuses (in six litters) at 500 mg/kg bw/day compared to two foetuses (in two litters) in the control group. Dilatation of lateral

ventricles without tissue compression (a visceral variation) was increased dose dependently, reaching statistical significance at the high-dose level.

Studies in rabbits: In the range finding-study, pregnant rabbits (8 animals per treatment group) received 2-EHA by gavage 125, 250, 500 or 1000 mg/kg bw/day in corn oil on days 6 - 18 of gestation. Control animals were given corn oil. In the main study, the corresponding doses were 25, 125 or 250 mg/kg bw/day, and the group size was 15 animals. Treatment with 500 and 1000 mg/kg bw/day was highly toxic to pregnant rabbits; seven of eight and all eight does died during the experiment at these two dose levels, respectively. One female each in the 125 and 250 mg/kg bw/day groups aborted. Rabbits receiving 1000 mg/kg bw/day showed hypoactivity, laboured respiration and ataxia, and hypoactivity was also seen in the 250 and 500 mg/kg bw/day groups. There was no clear indication of developmental toxicity in the offspring in the range-finding study. In the main study, one pregnant female each died in the 125 and 250 mg/kg bw/day groups, and one doe aborted in the 125 mg/kg bw/day group. A reduction of maternal body weight gain and food consumption was found in the high-dose group during the post-treatment period. The treatment had no effect on prenatal mortality or the percentage of living foetuses. Foetal body weights were not significantly changed, although they tended to be lower in the high-dose group. There were no differences in the incidences of external, visceral or skeletal malformations or variations among all groups.

In *in vivo* screening studies for developmental toxicity by Narotsky et al. (38, 39), cited in more detail in Hendrickx et al. (20), Sprague-Dawley rats were administered 900 or 1200 mg 2-EHA/kg bw/day by gavage on days 6 - 15 of gestation, and the surviving offspring were examined on postnatal day 6. The treatments caused maternal respiratory toxicity and pronounced motor depression. The developmental effects observed were delayed parturition, decreased progeny viability, reduced foetal weights and malformations of the vertebrae and ribs.

10.7. Other studies

It was demonstrated in mice that large doses of 2-EHA (about 1000 mg/kg bw/day in diet for four days) caused peroxisomal proliferation and related enzymatic changes (28). Keith et al. (22) found that the induction of peroxisomal β-oxidation (measured as cyanide-insensitive palmitoyl CoA oxidation) was essentially linearly dose-dependent in the dose range 390 - 1900 mg/kg bw/day (by gavage for 20 days) in B6C3F1 mice and Fischer 344 rats. The relative liver weights were significantly and dose-dependently increased at 770 mg/kg bw/day and higher dose levels. Manninen et al. (31) exposed Wistar rats to 2-EHA via drinking water for 20 days at about 8.6, 94.3, 370 or 570 mg/kg bw/day and found a dose-dependent increase (with statistical significance even at the lowest level) of carnitine acetyltransferase in rat liver mitochondria (it should be noted that most of the enzyme activity found in the mitochondrial fraction originates from peroxisomes according to Lundgren et al. (28)). In the same study, inhibition of

citrulline synthesis (urea synthesis) was found in the rat liver mitochondria even at the lowest (8.6 mg 2-EHA/kg bw/day) dose level.

11. Observations in Man

There are only few published observations of adverse effects by 2-EHA in humans.

11.1. Acute effects

One case of corneal injury with healing within two days has been reported; no further details of the incident were given (33).

11.2. Effects of repeated exposure on the skin

A survey of skin effects was conducted on 114 sawmill workers exposed to Sinesto B® wood preservative containing 14 % (w/w) trimethylcocosammonium chloride, 26 % 2-ethylhexanoate-sodium and 5.6 % borax, and 84 control workers (21). Epicutaneous skin testing according to the recommended method by the International Contact Dermatitis Research Group with 0.05 % 2-ethylhexanoate in water gave a positive allergic reaction in two persons (last reading after three days). One of the two had a hand exzema; he also had an allergic reaction to trimethylcocosammonium chloride. There were no positive reactions to 2-EHA among the controls.

11.3. Effects on liver metabolism

Pennanen et al. (45) found two to three times higher concentrations of urinary arginine and ornithine among sawmill workers moderately exposed to 2-EHA (mean urinary 2-EHA concentration 1.8 mol/mmol creatinine, range 0.5 - 5.4; n = 5) as compared to only slightly exposed workers (mean 2-EHA concentration 0.03 mol/mmol creatinine, range 0.01 - 0.04; n = 4) and nonexposed controls; the difference was statistically significant. The authors proposed that the finding might be a reflection of a disturbance in the hepatic urea cycle. This inference was based on their prior finding that rather low doses of 2-EHA (8.6 mg/kg bw/day from drinking water for 20 days) inhibited citrulline synthesis in rat liver mitochondria (31), and in analogy to corresponding effects in rats and human patients receiving valproic acid (2-propylpentanoic acid) (32).

12. Dose-Effect and Dose-Response Relationships

Dose-effect and to a lesser extent dose-response relationships for 2-EHA can be examined on the basis of experimental animal data involving various repeated

Table 1. Dose-effect relationships for 2-ethylhexanoic acid in repeated dosing animal studies; M = males, F = females

Species/strain	Administration	Lowest dose level causing an effect (mg/kg bw/day)	Effect	Reference
Mouse/B6C3F1	Dietary for 2 weeks	1600 (M), 2000 (F)	Lower terminal body weight (M) Increased liver weight (F) Multifocal coagulative necrosis of hepatocytes	15
Rat/F344	By gavage for 2 weeks	1600 800 200	Lethal to most rats after only 2 doses Slightly decreased body weight gain Lethargy and weakness after dosing Increased liver weight Increased relative liver weight (F)	6
Rat/F344	Dietary for 2 weeks	1350 (M), 1400 (F) 710 (M), 760 (F)	Decreased terminal body weight Multifocal coagulative necrosis of individual hepatocytes Increased liver weight	5
Mouse/B6C3F1	Dietary for 90 days	890 (M), 1040 (F)	Lower terminal body weight (F) Increased liver weight Hepatocyte hypertrophy Decreased S-triglycerides Increased S-cholesterol	14
Rat/F344	Dietary for 90 days	920 (M), 1070 (F) 300 (M), 360 (F) 60	Lower terminal body weight Increased liver weight Hepatocyte hypertrophy Increased relative kidney weight (F) Lower MCH in RBC's Decreased S-triglycerides (F) Increased S-cholesterol (M)	4
Rat/Wistar	In drinking water for 20 days	8.6	Induction of carnitine acetyltransferase in liver "mitochondria" Inhibition of citrulline biosynthesis in liver mitochondria	31

Table 2. Dose-effect/response relationships for the reproductive toxicity of 2-ethylhexanoic acid.

Species/strain	Administration	Lowest dose level causing an effect (mg/kg bw/day)	Effect/response	Reference
Rat/Wistar	In drinking water, males for 10 weeks prior to mating, females for 2 weeks prior to mating, during mating period, gestation and lactation	600	Average litter size reduced	48
		300	Delayed postnatal physical development	
		100	Slight delay in fertilization	
Rat/Wistar	Single gavage dose on day 12 of gestation	900	0.8 % of living pups malformed	49
	Single gavage dose on day 12 of gestation + 150 mg/kg caffeine i.p.	900	31.5 % of living pups malformed	
Mouse/NMRI	Four i.p. injections in the mornings of day 7 and 8 of gestation	500 (racemic)	32 % of live foetuses with exencephaly	19
		500 (R)-enantiomer	59 % of live foetuses with exencephaly	
Rat/Wistar	In drinking water on days 6 - 19 of gestation	600	Reduced maternal terminal body weight lower mean foetal body weight	47
		300	Statistically significant increase of malformations (8.9 % vs 2.4 % among controls)	
		100	Occurrence of clubfoot with a dose-response (none among controls)	
Rat/Fischer	By gavage in corn oil on days 6-15 of gestation	1000	Seven of eight dams died	20
		500	Slight maternal toxicity Foetal body weights reduced	
		250	Reduced foetal ossification in several sites	
Rabbit/New Zealand White	By gavage in corn oil on days 6-18 of gestation	500	Seven of eight does died	20
		125	One doe died and one aborted	

dose toxicity studies and reproductive toxicity studies with mice, rats and rabbits. The main observations are summarized in Tables 1 and 2. It is customary to make an assessment of the no-observed-adverse-effect-level (NOAEL) and the no-observed-effect-level (NOEL) for each study. This was not considered relevant for the present purpose; however, NOAEL's and NOEL's can be deduced from the column "Lowest dose level causing an effect" and the doses used in any particular study.

For endpoints other than those determined in conventional toxicity studies, such as changes in hepatic biochemistry, the effective doses are remarkably lower than the directly toxic ones. In rats, 8.6 mg 2-EHA/kg bw/day from drinking water for 20 days inhibited citrulline synthesis in rat liver mitochondria (31). There is no evidence at the present time suggesting that this *per se* should lead to hepatic (or other systemic) toxicity.

13. Evaluation of Human Health Risks

13.1. Groups at extra risk

Because 2-EHA has in several animal studies shown potential for reproductive and especially developmental toxicity, women in fertile age should be regarded as a group at extra risk.

13.2. Assessment of health risks

Exposure: 2-EHA has a low vapour pressure and hence it will not evaporate markedly in the normal workroom temperature; liquid aerosols may, however, be generated depending on the process used. Exposure from skin contact concerning both the magnitude of skin contamination and uptake from the skin has not been well characterized for workers. 2-EHA penetrated extensively through the skin of the rat, but the application site was under occlusion for 96 hours, and the epidermis was severely damaged. The uptake (rate) from skin contamination of the worker is probably much less, but in the absence of any authentic measurements it cannot be estimated. The skin hazard is reduced by the fact that the substance was readily removed from the skin surface by washing.

In an evaluation of the workers' exposure to 2-EHA at sawmills, where personal samples of the inhaled air as well as urine samples were analyzed for 2-EHA, it seemed that the inhaled concentrations were reasonably correlated to the concentrations of 2-EHA in urine. Even the estimated amounts taken up via inhalation were of the same order of magnitude as the estimates of the day's internal load, calculated from the diurnal urinary excretion of 2-EHA (Table 3). However, the study identified one individual worker, a crane operator, whose urinary 2-EHA concentration was as high as 6.9 mg/g creatinine, corresponding to an internal load of 31 mg/day. The measured 2-EHA air concentration *under* the

Table 3. Estimates of the day's 2-ethylhexanoic acid (2-EHA) uptake via inhalation and internal load assessed from urinary excretion of 2-EHA and conjugates (n = 19) (23).

2-EHA in air (mg/m^3)	2-EHA conjugates in urine (mg/g creatinine)	Uptake via lungs[a] (mg/day)	Internal load[b] (mg/day)
0.3 (mean)	0.37	1.5	1.7
0.7 (max)	1.8	3.5	8.0

[a]Based on 10 m^3 respiratory volume and 50 % retention.
[b]Based on the following assumptions: (1) Subjects are at steady state (daily excretion equals daily absorption), (2) diurnal 2-EHA excretion can be estimated from a simple correction to the 24-h creatinine excretion of 1.5 g/day, (3) one third of absorbed 2-EHA is excreted in urine as 2-EHA/conjugates.

crane was unremarkable (mean 0.2 mg/m^3). Thus it would appear that uptake routes other than inhalation had been operative.

According to Kröger et al. (23) and Pennanen et al. (45) the exposure of the sawmill workers engaged in wood preservation with a solution containing 1.3 % 2-EHA can be roughly characterized on the body weight basis (70 kg) as follows:

Daily uptake	mean	24 µg/kg bw/day
	maximum	114 µg/kg bw/day
	exceptional	442 µg/kg bw/day

Effects: Animal studies demonstrate that the main targets of 2-EHA toxicity are the liver and reproduction. Increased liver weight and hepatocyte hypertrophy, an obvious consequence of a disturbance in lipid metabolism and peroxisomal proliferation, was found at the daily dose level of 200 - 300 mg 2-EHA/kg bw/day. Severe hepatocellular damage (multifocal coagulative necrosis) was found at a considerably higher (1400 mg/kg bw/day) dose level. The earliest effect in a 90-day study, probably attributable to a derangement of hepatic metabolism, was increased serum cholesterol in male rats at 60 mg/kg bw/day (4).

Increased carnitine acetyltransferase in the rat liver (a marker of peroxisomal proliferation, although the authors analyzed the activity in the mitochondrial fraction) was found at as low a level as about 8.6 mg/kg bw/day (3 mg per rat per day) (31). Moreover, in the same study, 2-EHA inhibited citrulline synthesis (urea synthesis) in the liver mitochondria even at the lowest dose level used. Valproic acid has a similar metabolic effect in mitochondria, resulting at an i.p. injection dose of 50 mg/kg bw in a moderate arterial hyperammonemia after amino acid loading (32). (No corresponding increase in blood ammonia was detected when rats were given 150 mg 2-EHA/kg bw in an i.p. injection according to an unpublished report to the Finnish Work Environment Fund.)

In a developmental toxicity study with rats, 300 mg 2-EHA/kg bw/day caused a significantly increased incidence of clubfoot, and all malformations combined, while 100 mg/kg bw/day caused a lower (non-significantly) increased incidence of clubfoot, scoliosis, lordosis and flabby legs (none occurred among the controls) and skeletal variations. However, the number of foetuses having skeletal or visceral malformations increased in a dose-dependent manner ($p < 0.001$) being 4.9, 8.9 and 15.3 % per litter in the 100, 300 and 600 mg/kg bw/day groups versus 2.4 % in the control group (47). Thus, it seems that developmental toxicity may occur, in a sensitive strain of rats, even below 100 mg/kg bw/day. In other studies with rats and mice, larger 2-EHA doses have been used (often with specific protocols involving sensitive periods of development) to induce teratogenicity or foetotoxicity. Teratogenicity was not found in a rabbit study which, however, demonstrated that the does were exceptionally susceptible to 2-EHA toxicity.

Apart from developmental toxicity, 2-EHA may affect the reproductive process adversely by other mechanisms, such as by interference with implantation. This was demonstrated at 600 mg/kg bw/day; a slight delay in fertilization was observed even at 100 mg/kg bw/day (48).

The consequences of hepatic peroxisome proliferation by 2-EHA in terms of the risk of liver tumour development can also be raised because, although carcinogenicity studies with 2-EHA are lacking, (1) 2-EHA is the proximate proliferator derived from DEHA and (2) DEHA is a liver carcinogen in the B6C3F1 mouse (42). The aspects of hepatic peroxisome proliferation in rodent and human cancer risk have been extensively reviewed recently (3 ,34). Although the question is a matter of contention, current knowledge suggests that the hepatocarcinogenic risks to humans by low levels of exposure to peroxisome proliferators are not significant.

Assessment: Developmental toxicity by 2-EHA was found in the most sensitive species/strain at 100 mg/kg bw/day, and there was some indication of a reproductive disturbance at the same dose level. The NOEL is not known. Limited measurements of occupational exposure to 2-EHA suggest that the daily absorbed doses are probably about 100 times lower than the effective dose in animals. Although developmental toxicity is a serious effect, it is believed that (1) teratogenicity caused by simple organic acids has a threshold, and (2) single high doses during the vulnerable period are more hazardous than low level exposures over a longer period of time (8, 41). Thus, prevention of high accidental exposures should be the primary concern.

With regard to alterations in liver (and possibly kidney) metabolism, a range of events may be caused by variable doses of 2-EHA. At the low dose end, less than 10 mg/kg bw/day was associated with enzymatic changes heralding peroxisome proliferation and disturbance of ammonia metabolism. The more heavily exposed sawmill workers, whose estimated 2-EHA doses were approximately 100 µg/kg bw/day, excreted higher amounts of arginine and ornithine in the urine possibly as a compensatory mechanism to changes in the urea cycle. Whether these effects are essentially adaptive, or slight shifts in the balance of normal biochemistry

which the healthy human easily can compensate for, remains to be seen in further studies. There is no evidence at the present time that these effects indicate organ or systemic toxicity.

Positive allergic reactions to 2-EHA in epicutaneous testing were reported among two sawmill workers exposed to Sinesto B® in 1989. Despite continued use, no new reports of positive reactions or cases of allergic contact dermatitis have emerged. Neat 2-EHA and concentrated solutions are expected to be moderate to severe irritants to the mucous membranes and the eye, and less irritating to the skin.

13.3. Scientific basis for an occupational exposure limit

The critical effect of 2-EHA for female workers is developmental toxicity. For male workers the critical effects are probably alterations in lipid and ammonia metabolism (of the liver).

In view of the positive evidence for the skin penetrating capability of 2-EHA in rats (albeit exaggerated because of artificial conditions), a note concerning potential skin absorption should be given.

14. Research Needs

There is a very limited amount of data concerning human exposure to 2-EHA, human toxicokinetics and biological effects. Especially the potential for percutaneous exposure should be investigated, and further studies are needed to verify and extend the preliminary observations concerning metabolic effects in the liver (and kidney). Toxicokinetic studies with 2-EHA should be applied for method development in biological monitoring, and further toxicodynamic studies on endogenous metabolism are needed to explore the possibility of biological effect monitoring.

15. Summary

Riihimäki V. 112. 2-Ethylhexanoic acid. The Nordic Expert Group for Criteria Documentation of Health Risks form Chemicals. *Arbete och Hälsa* 1994;31:1-30.

There are few data concerning the toxicity of 2-ethylhexanoic acid (2-EHA) in humans. Animal studies demonstrate that the main target organs of 2-EHA toxicity are the liver and the reproduction. Dose dependent liver effects ranging from metabolic disturbances to cytotoxicity have been observed in repeated dosing studies with rats and mice. Less than 10 mg/kg bw/day was associated with cellular enzymatic changes heralding peroxisome proliferation and disturbance of ammonia metabolism in rats. Developmental toxicity by 2-EHA was found in the most sensitive species/strain at 100 mg/kg bw/day, and there was some indication of a reproductive disturbance at the same dose level. The no observed effect level (NOEL) is not known.

The critical effect of 2-EHA for female workers is developmental toxicity. For male workers, the critical effects are probably alterations in lipid and ammonia metabolism (of the liver).

Key words: Ammonia metabolism, developmental effects, 2-ethylhexanoic acid, lipid metabolism, liver toxicity, occupational exposure limits, peroxisomal proliferation, reproductive toxicity.

16. Summary in Swedish

Riihimäki V. 112. 2-Etylhexansyra. Nordiska expertgruppen för kriteriedokumentation av kemiska hälsorisker. *Arbete och Hälsa* 1994;31:1-30.

Det finns endast begränsat med data om toxiciteten av 2-etylhexansyra (2-EHS) hos människa. Studier med försöksdjur har visat att 2-EHS orsakar skadliga effekter främst i levern och i samband med fortplantningen. Olika slag av dosrelaterade effekter i levern, från metaboliska störningar till cytotoxicitet, har observerats vid upprepad dosering i råtta och mus. Halter på <10 mg/kg kroppsvikt/dag var förknippade med enzymatiska förändringar i cellerna, vilket förebådar peroxisom proliferering och störningar i metabolismen av ammonium i råtta. Utvecklingstoxikologiska effekter av 2-EHS upptäcktes hos de mest känsliga arterna/stammarna vid 100 mg/kg kroppsvikt/dag, tillsammans med indikationer på reproduktiva störningar vid samma dosnivå. Nivån för ingen observerad effekt (NOEL) är inte känd.

Den kritiska effekten vid exponering för 2-EHS bland kvinnliga arbetare gäller utvecklingstoxikologiska effekter. För manliga arbetare är störningar i metabolismen av lipider och ammonium (i levern) de kritiska effekterna.

Nyckelord: Ammoniummetabolism, arbetshygieniska gränsvärden, 2-etylhexansyra, levertoxicitet, lipidmetabolism, peroxisom proliferering, reproduktionstoxikologi, utvecklingstoxikologiska effekter.

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Appendix

Permitted or recommended maximum levels of 2-ethylhexanoic acid in air

Country	ppm	mg/m^3	Comments	Year	Ref.
Denmark	-	-		1988	1
Finland	-	-		1993	2
Iceland	-	-		1989	3
Netherlands	-	-		1994	4
Norway	-	-		1989	5
Sweden	- -	-		1993	6
USA (ACGIH)	-	-		1994-95	7
(NIOSH)	-	-		1990-91	8

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