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TOXICOLOGICAL EVALUATIONS

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TOXICOLOGICAL EVALUATION

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2-Ethyl-hexanoic acid

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CAS No. 149-57-5

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2-Ethylhexanoic acid

In addition to 2-ethylhexanoic acid, Toxicological Evaluations on 2-ethylhexanal (volume 2) and 2-ethylhexanol (volume 14) are available and can be consulted for comparison. In particular, there is a large body of data on 2-ethylhexanol. Biotransformation studies demonstrate that 2-ethylhexanol is rapidly and quantitatively metabolised to 2-ethylhexanoic acid. In view of such metabolisation, both chemicals may be assumed to have virtually identical toxicity profiles.

1 Summary and assessment

2-Ethylhexanoic acid is absorbed via the gastrointestinal tract, the skin and, as human studies demonstrate, the airways. On administration by oral gavage, maximum plasma levels are attained after approx. 19 minutes. Following occlusive dermal application, 63% of the dose is absorbed. The maximum plasma levels following dermal application, which are reached after 5.7 hours and have a half-life of 3.2 hours, are approx. ten times lower than those seen after oral administration. In the mouse and rat, 2-ethylhexanoic acid exhibits preferential distribution in the kidneys, liver and blood. In pregnant mice, 2-ethylhexanoic acid is able to cross the placenta and can be detected in the embryos. The concentrations found in the embryos are similar to those in the dams. Irrespective of the route of administration, in rats the predominant portion of the orally, dermally or intravenously dose of 2-ethylhexanoic acid is excreted essentially in the urine and to a lesser extent in the faeces, the half-lives ranging between 4.2 and 6.8 hours. In the rat, complete elimination from the blood after oral and intravenous administration takes place in three phases with half-lives of elimination of 19 minutes, 6.8 hours and 92.2 hours, and 11.1 minutes, 6.6 hours and 117 hours, respectively. Following dermal application, complete elimination is biphasic, the mean half-lives of the phases being 4.2 and 251 hours. Total excretion in urine and faeces to take place within 96 hours of administration amounts to approx. 90% following a single oral dose, approx. 75% upon repeated oral administration, approx. 77% of the radioactivity absorbed after single dermal exposure and approx. 71% after intravenous injection. Excretion via the airways has not been investigated.

2-Ethylhexanoic acid is metabolised by conjugation with glucuronic acid as well as by cytochrome P-450-dependent ω-oxidation and ω-1-oxidation. In addition, 2-ethylhexanoic acid, similarly to fatty acids, is likely to be broken down by β-oxidation, with mitochondrial and, in particular, peroxisomal catabolism ultimately leading to acetyl-CoA. The major metabolites to be found in urine include the glucuronide conjugate of 2-ethylhexanoic acid as well as 2-ethyl-1,6-hexanedioic acid and 6-hydroxy-2-ethylhexanoic acid and their glucuronide conjugates. Unmetabolised 2-ethylhexanoic acid accounts for only a small percentage of the amounts excreted in urine. With increasing doses, the portion of glucuronidated 2-ethylhexanoic acid increases, while the portion of cytochrome P-450-dependent more strongly oxidised metabolites decreases. Following repeated administration, there is a decrease in total elimination and in the amounts of glucuronidated 2-ethylhexanoic acid and cytochrome P-450-dependent metabolites, compared with single-dose administration. It has been suggested that repeated administration induces β-oxidation of 2-ethylhexanoic acid and results in the compound being incorporated into normal cellular metabolism to a greater extent.

2-Ethylhexanoic acid is of low acute oral toxicity (LD_{50} rat oral 2043 to 3640 mg/kg body weight). In the rat and guinea pig, LD_{50} values (> 2000 and 6300 mg/kg body weight, respectively) also indicate low dermal toxicity, whereas the LD_{50} value of 1260 mg/kg body weight reported for dermal application in the rabbit indicates that the chemical is harmful. A 6-hour inhalation exposure of rats to approx. 2356 mg/m³ and an 8-hour exposure of rats or guinea pigs to an atmosphere enriched or saturated at room temperature is tolerated without findings. On oral administration, signs of toxicity have been reported to include dyspnoea, apathy, lying on the abdomen, weakness and prostration, while at necropsy of the deceased animals the liver has been found to be mottled and the stomach and upper gastrointestinal tract have been observed to have an opaque white, cooked appearance. Terminal necropsy of the animals surviving to the end of the study has been without findings.

As regards skin irritancy, the evaluation of 2-ethylhexanoic acid varies considerably from nonirritating to corrosive. Thus, the chemical has been assessed as corrosive to the skin in a study conducted in accordance with a guideline (21 CFR § 191.11) issued by the U.S. Department of Transportation. Reversibility of the findings was not addressed in the study. In a study

carried out in accordance with EC directive No. 84/449/EEC, 2-ethylhexanoic acid must be evaluated as nonirritating or irritating to the skin, depending on whether judgement is based on EC directive No. 83/467/EEC or EC directive No. 93/21/EEC.

In the rabbit eye, 2-ethylhexanoic acid causes severe irritation with clouding of the cornea, marked redness and oedema formation, iritis and ocular discharge. 2-Ethylhexanoic acid has been assessed as nonirritating to the eye, based on a study which was carried out and evaluated in accordance with EC directives Nos. 84/449/EEC and 83/467/EEC, respectively, and showed all findings to be reversible 7 days after treatment. 2-Ethylhexanoic acid was evaluated as severely irritating to the eye in a study in which the findings were not reversible within an observation period of 8 days after exposure.

Subchronic administration to rats and mice of up to 1.5% 2-ethylhexanoic acid in the diet results in liver changes and retardation of body weight gain in conjunction with reduced feed consumption. The liver changes characteristically consist in dose-dependently increased organ weights, histopathological changes in the form of dose-dependent slight to moderate hepatocyte hypertrophy and reduction in the number of cytoplasmic vacuoles as well as in altered clinical chemistry parameters (elevated serum cholesterol levels and alanine aminotransferase activity). In addition, mice have shown reversible low-grade histopathological changes in the proximal renal tubules without impairment of renal function as well as changes of the forestomach. All findings are reversible within a 28-day observation period or exhibit a tendency towards reversibility. The results in the rat and the mouse are in good agreement, the *no effect level* (NOEL) for subchronic administration being 0.1% 2-ethylhexanoic acid in the diet (equivalent to 61 and 71 mg/kg body weight per day for male and female rats, respectively, and 180 and 205 mg/kg body weight per day for male and female mice, respectively). Preliminary studies with 14-day oral treatment administered in the diet or by gavage (rat and mouse) have produced qualitatively identical results. For the 14-day administration by oral gavage, the *no effect level* (NOEL) for mice is 800 mg/kg body weight. The *lowest observed effect level* (LOEL) for rats receiving 14-day administration by oral gavage has been established as 200 mg/kg body weight, and it has been found to be 0.75% for rats and mice receiving 2-ethylhexanoic acid in the feed (equivalent to daily doses of 706 and 756 mg/kg body weight for male and female rats, respectively, and 1608 and 1965 mg/kg body weight for male

and female mice, respectively). At these dose levels, liver changes have been found to be low-grade.

In vitro and in vivo, 2-ethylhexanoic acid causes peroxisome proliferation or an increase in activity of the marker enzymes for peroxisome proliferation, cyanide-insensitive palmitoyl-CoA oxidase, lauroyl-CoA oxidase and carnitine acetyltransferase in the liver of the rat and the mouse. According to in-vitro studies, 2-ethylhexanoic acid does not induce peroxisome proliferation in hepatocytes of guinea pigs and monkeys.

2-Ethylhexanoic acid exhibits no mutagenic potential in the Salmonella/microsome assay, either with or without metabolic activation. In the chromosome aberration test in Chinese hamster ovary cells, the result is negative in the absence of metabolic activation but weakly positive in the presence of metabolic activation. The sister chromatid exchange test in Chinese hamster ovary cells gives a positive result for 2-ethylhexanoic acid, both with and without metabolic activation. In human lymphocytes, 2-ethylhexanoic acid induces a slight increase in the sister chromatid exchange rate in the absence of metabolic activation.

2-Ethylhexanol (cf. Toxicological Evaluations, volume 14), which undergoes quantitative metabolisation via 2-ethylhexanoic acid, has been comprehensively studied in vitro with respect to its genotoxic potential in the Salmonella/microsome assay, mouse lymphoma test, HPRT test, DNA repair test, UDS test, chromosome aberration test as well as in vivo in the micronucleus test and the dominant lethal test. The studies of 2-ethylhexanol employing these test systems have not produced any relevant indications of a genotoxic potential for that chemical. Combined evaluation of the genotoxicity results obtained for 2-ethylhexanoic acid and 2-ethylhexanol under the aspect of their metabolic pathways also makes it seem unlikely that 2-ethylhexanoic acid possesses a genotoxic potential.

In the Fischer-344 rat, administration by oral gavage of 250 mg/kg body weight 2-ethylhexanoic acid, a dose which does not cause maternal toxicity, exhibits foetotoxic activity (reduced ossification). No teratogenic alterations have been found in Fischer-344 rats up to 500 mg/kg body weight, the dose which causes maternal toxicity. In contradiction with these findings, sodium 2-ethylhexanoate was evaluated as teratogenic at dose levels devoid of maternal toxicity by the authors of a study in the Han:Wistar rat.

The overall number of skeletal malformations (club foot) exhibited a dose-dependent increase which was numerically evident at 100 mg/kg body weight and statistically significant at doses of 300 mg/kg body weight and higher. Other findings which were evaluated as malformations by the authors occurred dose-dependently but were not statistically significant, included flabby legs as well as slight scoliosis and lordosis in all dose groups, abnormal cartilage in the ankles at 300 mg/kg body weight and higher, and the absence of a fibula and supernumerary thoracic ribs in the top dose group of 600 mg/kg body weight. In addition, the authors reported significantly increased frequencies of dose-independent visceral malformations including dilatation of the urinary tract in the low and mid dose groups as well as dilated ventricles of the brain in the top dose group. Foetotoxic effects in the form of skeletal variations were observed at 100 mg/kg body weight and higher, while foetal body weights were reduced at dose levels of 300 mg/kg body weight and higher. Maternal toxicity was seen in the form of reduced placental weights at 300 mg/kg body weight and higher, and in the form of lower body weights and reduction in drinking water consumption in the top dose group receiving 600 mg/kg body weight. A fertility study by the same team of authors which included monitoring of postnatal development found that in the Han:Wistar rat, 600 mg/kg body weight administered in the drinking water, a dose mildly toxic to the parent rats, tended to reduce fertility, increase time to mating, inhibit implantation and cause delay in physical development of the pups during lactation. Retardation of physical development was also observed in the mid dose group receiving 300 mg/kg body weight, for which it is not absolutely clear whether or not the parents in this group exhibited signs of toxicity. Without giving details as to maternal toxicity, it has been reported that administration by gavage of a single oral dose of approx. 1820 mg 2-ethylhexanoic acid/kg body weight on day 12 of gestation causes teratogenic alterations in Wistar and Sprague-Dawley rats. In NMRI and SWV mice, but not C57BL/6NCrlBR mice, receiving doses of ≥ 500 mg/kg body weight (3 or 4 times at 12-hour intervals) by invasive routes of administration, teratogenic alterations in the form of exencephaly have been induced. These studies show that the *R*-(–)-enantiomer of 2-ethylhexanoic acid appears to be highly teratogenic, while the *S*-(+)-enantiomer is only a weak teratogen, or lacks teratogenicity altogether. In the New Zealand rabbit, there are no findings to indicate that 2-ethylhexanoic acid might have an embryotoxic, foetotoxic or teratogenic potential, even at 250 mg/kg body weight, the highest tested dose causing

maternal toxicity. In summary, even though the overall results are not consistent, there is sufficient evidence to indicate that 2-ethylhexanoic acid is harmful to the foetus at dose levels devoid of marked maternal toxicity.

Studies in Finnish sawmill workers exposed to Sinesto B, a wood preservative containing 26% sodium 2-ethylhexanoate, have demonstrated that the main route of exposure to 2-ethylhexanoic acid is by inhalation and that the chemical is excreted in the urine. As urinary ornithine and arginine levels were found to be elevated in the workers, it was suggested that 2-ethylhexanoic acid inhibits citrulline synthesis in the urea cycle. In a plant manufacturing 2-ethylhexanoic acid, two cases each of irritation of the skin, the eyes and the respiratory tract occurred after acute local or inhalation exposure during the 1989–1996 period. Furthermore, in one case 2-ethylhexanoic acid is reported to have caused corrosive injury to the cornea which healed up after treatment. From the experience in humans, there are no indications that 2-ethylhexanoic acid has a sensitising potential.

2-Ethylhexanoic acid has been legally classified in the TRGS 905 and placed into category R_D3 of substances toxic to reproduction (i.e. “substances which cause concern for humans owing to possible developmental toxic effects”) in accordance with the EU classification criteria.

2 Name of substance

2.1	Usual name	2-Ethylhexanoic acid
2.2	IUPAC name	2-Ethylhexanoic acid
2.3	CAS No.	149-57-5
2.4	EINECS No.	205-743-6

3 Synonyms, common and trade names

2-Aethylcapronsäure
α-Aethylhexansäure
2-Butylbutanoic acid
Butylethylacetic acid
α-Ethylcaproic acid

2-Ethylcaproic acid
2-Ethylcapronsäure
α-Ethylhexanoic acid
2-Ethyl-1-hexanoic acid
α-Ethylhexansäure
2-Ethylhexansäure
2-Ethylhexoic acid
3-Heptanecarboxylic acid
Hexanoic acid, 2-ethyl- (8CI, 9CI)
Sinesto B

4 Structural and molecular formulae

4.1 Structural formula $CH_3—(CH_2)_3—CH(CH_2—CH_3)—COOH$

4.2 Molecular formula $C_8H_{16}O_2$

5 Physical and chemical properties

5.1	Molecular mass, g/mol	144.21	
5.2	Melting point, °C	–35	(Merck, 1987)
		ca. –59	(BASF, 1981 a)
		< –60	(EC, 1996)
5.3	Boiling point, °C	218–222	(Merck, 1987)
		220	(Riemenschneider, 1986)
		ca. 226–229	(BASF, 1981 a, b)
		226–230 (commercial product)	(Riemenschneider, 1986)
		228	(EC, 1996; Katz and Guest, 1994; Lide and Frederikse, 1996)
5.4	Vapour pressure, hPa	0.03 (at 20 °C)	(Katz and Guest, 1994)
		0.04 (at 20 °C)	(BASF, 1981 a; Merck, 1987)
		< 0.1 (at 20 °C)	
		0.3 (at 50 °C)	(EC, 1996)

5.5	Density, g/cm^3	0.91 (at 20 °C) (BASF, 1981 a; Merck, 1987) 0.908 (at 20 °C) (Riemenschneider, 1986) 0.906 (EC, 1996) 0.905–0.907 (commercial product) (Riemenschneider, 1986) 0.904–0.909 (at 20 °C) (BASF, 1981 b) 0.9031 (at 25 °C) (Lide and Frederikse, 1996) 0.902 (at 25 °C) (Katz and Guest, 1994)
5.6	Solubility in water	1.4 g/l (at 20 °C) (BASF, 1981 a; Merck, 1987) 2 g/l (Fassett, 1963; EC, 1996; Riemenschneider, 1986) absorbs 1.2% (v/v) water at 20 °C (Riemenschneider, 1986)
5.7	Solubility in organic solvents	soluble in ether and tetrachloromethane, low solubility in alcohol (Katz and Guest, 1994; Lide and Frederikse, 1996) soluble in polar solvents (Hoechst, 1986)
5.8	Solubility in fat	log P_{ow}: 2.7 (experimental, in accordance with OECD guideline No. 107) (BASF, 1988) log P_{ow}: 2.64 (experimental, in accordance with OECD guideline No. 107) (EC, 1996) log P_{ow}: 2.81 (calculated) (BASF, 1988)
5.9	pH value	ca. 3 (1.4 g/l water) (BASF, 1981 a) ca. 3.3 (2 g/l water) (EC, 1996)
5.10	Conversion factor	1 ml/m^3 (ppm) ≙ 5.89 mg/m^3 1 mg/m^3 ≙ 0.170 ml/m^3 (ppm) (at 1013 hPa and 25 °C)

6 Uses

Intermediate for use in producing driers for varnishes and surface coatings, enamels, catalysts for oxidising hydrocarbons, thickeners for mineral oils

and mineral spirit, wetting agents, emulsifiers and plasticisers, stabilisers for silicones, corrosion inhibitors, fungicides, 2-ethylhexanoate metal soaps and peroxy esters (BASF, 1981 b; Eastman Kodak, 1987 f; Riemenschneider, 1986).

Sodium 2-ethylhexanoate is used as a replacement for pentachlorophenol in the lumber industry (Katz and Guest, 1994; Kröger et al., 1990).

7 Experimental results

7.1 Toxicokinetics and metabolism

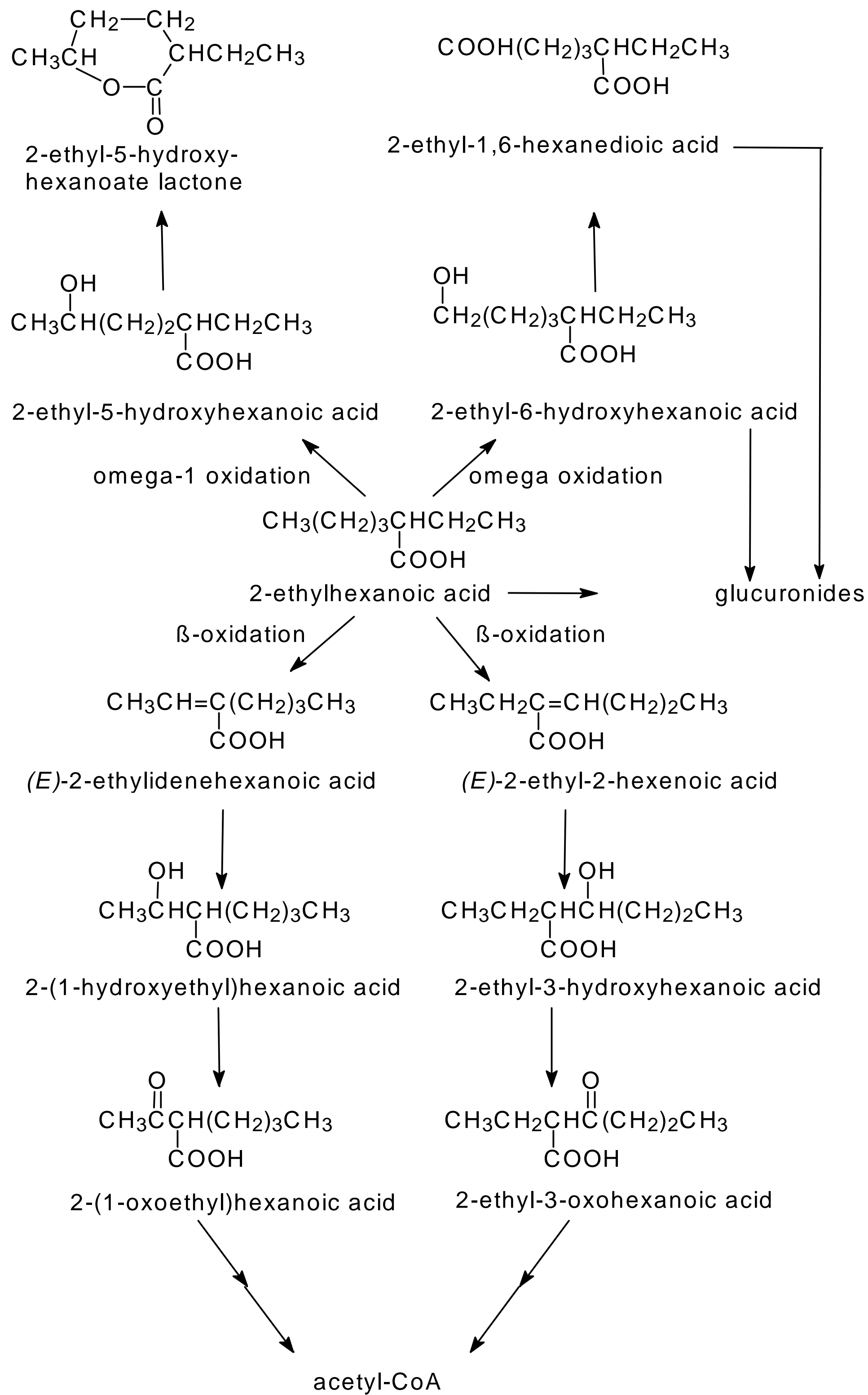
The toxicokinetics and metabolism of 2-ethylhexanoic acid were studied in the female Fischer-344 rat in accordance with the "2-Ethylhexanoic Acid, Final Test Rule" promulgated by the Environmental Protection Agency (EPA, 1986). In a series of individual studies, groups of 8 animals per dose received 100 and 1000 mg 2-ethylhexanoic acid/kg body weight once by oral gavage, 100 and 1000 mg as occlusive dermal applications to the depilated dorsal skin for 96 hours and 1 mg 2-ethylhexanoic acid/kg body weight as a single intravenous injection. Irrespective of total dose, a mixture of [2-^{14}C-hexyl]-2-ethylhexanoic acid (radiochemical purity: 97.6%, specific radioactivity: 25 mCi/mmol, no radiolabelled impurities) and unlabelled 2-ethylhexanoic acid (purity: 99.6%) was administered to each rat at 10 µCi/animal. In a further study, 4 rats were given 100 mg unlabelled 2-ethylhexanoic acid/kg body weight once daily by oral gavage for 14 days and an equivalent dose of the radiolabelled compound on day 15. The levels of radioactivity were determined in urine, faeces and plasma at intervals over a total period of 96 hours; elimination in the exhaled air and residual radioactivity in the body were not investigated. The metabolites in the urine collected within the first 24 and 48 hours were analysed both quantitatively and qualitatively by HPLC and GC/MS; metabolites in faeces were not determined. A comparative summary of the toxicokinetic data thus obtained is given in Table 1 in the appendix. 2-Ethylhexanoic acid was absorbed well both via the gastrointestinal tract and the skin. Peak plasma concentrations of 85.1 µg 2-ethylhexanoic acid equivalents per gram blood were reached after 18.8 minutes following oral administration of 100 mg/kg body weight. Absorption via the skin was detectable after 7.9 minutes following dermal application of an equivalent dose. With a half-life of absorption of 3.2

hours, dermal application, as compared with oral administration, resulted in a 10-fold lower peak plasma level of 8.5 µg 2-ethylhexanoic acid equivalents per gram blood, which was reached after 5.7 hours. When the exposed area of skin was thoroughly washed 5 minutes after dermal application of 1000 mg/kg body weight, all of the applied radioactivity (101.9%) was recovered during the washing procedure, with less than 0.2% of the applied radioactivity being found in the urine and faeces over 96 hours. Bioavailability of the administered radioactivity after dermal application was 63 to 70% relative to that after intravenous administration. Distribution in the individual organs or tissues was not monitored. Following oral administration of 100 mg/kg body weight and intravenous administration of 1 mg/kg body weight, complete elimination from the blood was found to follow a triphasic course with half-lives of elimination of 19 minutes, 6.8 hours and 92.2 hours, and 11.1 minutes, 6.6 hours and 117 hours, respectively. After dermal application of 100 mg/kg body weight, complete elimination from the blood was biphasic, the mean half-lives of elimination being 4.2 and 251 hours. In all of the individual studies, irrespective of the route of administration employed, the radioactivity was predominantly excreted in the urine and faeces within 24 hours, with half-lives of elimination ranging from 4.2 to 6.8 hours. Following single oral administration, repeated oral administration, dermal application and intravenous administration, total elimination in the urine and faeces over 96 hours was approx. 90%, approx. 75%, approx. 77% of the absorbed radioactivity (approx. 51% of the radioactivity applied to the skin) and 71%, respectively. 2-Ethylhexanoic acid metabolisation was found to take place via conjugation with glucuronic acid as well as cytochrome P-450-dependent ω-oxidation and ω-1-oxidation. In addition, the chemical probably enters the oxidative pathway by which nutrients are degraded and, similarly to fatty acids, is ultimately catabolised to acetyl-CoA by β-oxidation, which in particular takes place in the mitochondria and peroxisomes (cf. Figure 1). The major urinary metabolites identified were the glucuronide of 2-ethylhexanoic acid as well as 2-ethyl-1,6-hexanedioic acid and 6-hydroxy-2-ethylhexanoic acid and their respective glucuronides. Unmetabolised 2-ethylhexanoic acid – together with its metabolites 5-hydroxy-2-ethylhexanoic acid, the latter's lactones, 2-(1-oxoethyl)hexanoic acid, a presumably additional lactone of a dihydroxylated diacid of molecular weight 188, a compound of molecular weight 112 (presumably 5-hepten-2-one) as well as a compound of molecular weight 136 – accounted for only a small percentage of the radioactivity ex-

creted in the urine. With increasing single dose the fraction of glucuronidated 2-ethylhexanoic acid increased while the percentage of cytochrome P-450-dependent, more highly oxidised metabolites (2-ethyl-1,6-hexanedioic acid, 6-hydroxy-2-ethylhexanoic acid) decreased. Compared with single-dose administration, repeated administration resulted in a decrease in total elimination and in the amounts of glucuronidated 2-ethylhexanoic acid and cytochrome P-450-dependent metabolites. To explain their findings, the authors proposed that repeated administration induced β-oxidation of 2-ethylhexanoic acid, practically a short-chain branched fatty acid, which resulted in the compound being incorporated into normal cellular metabolism to a greater extent (Eastman Kodak, 1987 f; English et al., 1989).

The 48-hour urine samples of Sprague-Dawley rats receiving a single intraperitoneal administration of 400 mg sodium 2-ethylhexanoate/kg body weight were also analysed by gas chromatography and mass spectrometry for the products of ω-, ω-1- and β-oxidation of the compound. In addition to the previously mentioned metabolites (cf. Eastman Kodak, 1987 f; English et al., 1989; see above) and subsequent to positive identification by means of appropriate reference substances, the structures of a number of, in particular, β-oxidation products were elucidated. Analysis revealed that *(E)*-2-ethyl-2-hexeneoic acid, 2-ethyl-3-hydroxyhexanoic acid and 2-ethyl-3-oxohexanoic acid were products of β-oxidation at position 3 of the molecule whilst *(E)*-2-ethylidenehexanoic acid, 2-(1-hydroxyethyl)hexanoic acid and 2-(1-oxoethyl)hexanoic acid were found to be products of β-oxidation at the 1' position of the molecule. Furthermore, the authors also identified 2-ethyl-5-oxohexanoic acid, a derivative of ω-1-oxidation product 2-ethyl-5-hydroxyhexanoic acid, as well as 2-ethyl-5-hexeneoic acid, a metabolite which is possibly formed as a secondary product of either ω-1-oxidation product 2-ethyl-5-hydroxyhexanoic acid or ω-oxidation product 2-ethyl-6-hydroxyhexanoic acid and was also found and analysed by Pennanen et al. (1991 a; see below) but referred to by them as 5,6-dehydro-2-ethylhexanoic acid (Rettenmeier, 1997).

Figure 1. Biotransformation of 2-ethylhexanoic acid in the rat (after Eastman Kodak, 1987 f; English et al., 1989; Rettenmeier, 1997)



Specific aspects of the toxicokinetics and metabolism of 2-ethylhexanoic acid have been investigated in the following series of additional studies.

The distribution of 2-^{14}C-ethylhexanoic acid in the body was monitored following intraperitoneal administration to male Balb/C mice and male Han:Wistar rats. In the mouse studies, whole-body autoradiography was carried out in 4 animals per time point of examination at 0.5, 1 and 6 hours after single-dose administration of 2-^{14}C-ethylhexanoic acid (5 µCi (specific activity 6.7 mCi/mmol) per mouse). Most of the radioactivity was detected in the kidneys, the liver and the gastrointestinal tract 30 minutes after administration, and small amounts were also found in the lungs. At one hour, concentrations in the kidneys and the liver reached peak levels. Salivary gland and skin were found to contain low levels. Small amounts were detectable in the olfactory bulb but not in the brain. Six hours after administration, residual radioactivity was still visible in the liver and kidney. Groups of 4 rats per time point had the levels of radioactivity in blood, brain, liver and kidneys measured 2, 6 and 24 hours following administration of 2-^{14}C-ethylhexanoic acid (2 µCi (specific activity 6.7 mCi/mmol) per rat). The highest levels were found in the blood, liver and kidneys (0.3, 0.2 and 0.1% of the administered radioactivity per gram of tissue, respectively) 2 hours after administration. In the brain, only 0.02% of the radioactivity administered per gram tissue was detected. After 6 hours, the blood, liver and kidney levels had dropped to approx. one fifth of the levels seen after 2 hours; the brain no longer contained any detectable radioactivity. Twenty-four hours after administration, the blood no longer contained any measurable radioactivity, either, and the levels in the liver and lungs had dropped to 0.01% of the radioactivity administered per gram tissue (Pennanen and Manninen, 1991).

Pregnant SWV mice were treated intraperitoneally, on each of days 8.5, 9.0 and 9.5 of gestation, with 576 mg/kg body weight of the sodium salt of either the *S*- or the *R*-enantiomer of 2-ethylhexanoic acid (equivalent to approx. 500 mg of the anion of the acid, purity of the enantiomers > 99%). The concentrations of 2-ethylhexanoic acid in maternal plasma, maternal muscle tissue as well as embryo were determined 0.25, 0.5, 1, 2 and 4 hours after administration of the last dose. For both enantiomers, peak concentrations (C_{max}) in plasma (free and protein-bound 2-ethylhexanoic acid), maternal muscle and embryo were reached 15 minutes after the last dose had been administered, with the highest and lowest levels being

found in maternal plasma (predominantly not protein-bound 2-ethylhexanoic acid) and maternal muscle, respectively. The highest concentration found in the embryos was only slightly lower than that detected in maternal plasma. While the peak concentrations of the two enantiomers showed no significant differences in the four compartments investigated, the areas under the concentration versus time curves (AUC) calculated for the *S*-(+)-enantiomer were 10% smaller on average, due to slightly faster elimination, than those obtained for the *R*-(–)-enantiomer. No metabolic conversion of the *R*- to the *S*-enantiomer, or vice versa, was detectable (Collins et al., 1992).

In pregnant Sprague-Dawley rats, the distribution of 2-ethylhexanoic acid in maternal plasma and the embryos was also monitored for a period of 24 hours after a single oral dose of approx. 1800 mg (12.5 mmol) undiluted 2-ethylhexanoic acid/kg body weight, administered by gavage on day 12 of gestation. Graphical representation of the results showed the levels measured in the embryos to be closely correlated with, but markedly lower than, the maternal plasma concentrations (no precise details; Scott et al., 1994).

In 4 anaesthetised male Wistar rats with surgically implanted portal vein and thoracic duct cannulas which were given 150 mg 2-^{14}C-ethylhexanoic acid (3.2 µCi) intragastrically, approx. 50%, less than 3%, and 1% of the administered radioactivity was detected in portal vein blood, thoracic duct lymph and the intestinal wall, respectively, within or after 8 hours. Peak levels were measured in lymph after 2 to 4 hours, while maximum concentrations in portal vein blood were reached 30 minutes after administration (Hyun et al., 1967).

In male Wistar rats (4 animals) given a single intraperitoneal dose of 150 mg 2-ethylhexanoic acid/kg body weight, renal excretion of the free acid was reported to be biphasic with an initial half-life of 3 hours (no further details). Following 20-day administration in drinking water (5 and 10 g/l water, which according to the authors was equivalent to approx. 130 and 200 mg/day (not specified whether per kilogram body weight or per animal)), the male Wistar rats (5 animals/dose) of the 5 g/l dose group displayed constant urinary excretion of 2-ethylhexanoic acid (approx. 500 mmol/mol creatinine) 2 days after initiation of treatment. After 11 days, 2-ethylhexanoic acid excretion showed a marked increase but from day 14 onwards remained at plateau values which were, however, approx. 4 times

higher than the values seen from days 2 to 11 of the study (approx. 2000 mmol/mol creatinine). In the top dose group, there was a slight progressive increase in excretion to values of approx. 2000 mmol/mol creatinine up to day 11 of the study. Thereafter, as of day 13, excretion also displayed a sudden increase to constant values of approx. 5000 mmol/mol creatinine (no further details; Manninen et al., 1989).

A further biotransformation study confirmed several of the metabolites discussed above (cf. Eastman Kodak, 1987 f; English et al., 1989; above). The 24-hour urine samples from male Han:Wistar rats having previously received 600 mg sodium 2-ethylhexanoate/kg body weight per day in their drinking water over a period of 9 weeks (purity: 99.5%, not radioactively labelled) were also found to contain the glucuronic acid conjugate of 2-ethylhexanoic acid, free 2-ethylhexanoic acid, 6-hydroxy-2-ethylhexanoic acid and 2-ethyl-1,6-hexanedioic acid, as unequivocally detected by qualitative GC/MS. The distribution of the metabolites was not quantified any further. However, gas chromatograms recorded after acid hydrolysis showed 2-ethyl-1,6-hexanedioic acid to be the main metabolite. A further metabolite, which occurred only in traces, was identified as 5,6-dehydro-2-ethylhexanoic acid. That compound had not been detected in the studies by Eastman Kodak (1987 f) and English et al. (1989). In their discussion, the authors suggested that 5,6-dehydro-2-ethylhexanoic acid may have been formed by cytochrome P-450-catalysed ω-1-oxidation. In addition, the authors succeeded in identifying 2 lactones of molecular weight 142 as well as 5 hydroxylated compounds of molecular weight 160 but did not elucidate their structures (Pennanen et al., 1991 a).

Note: Comparison with the study discussed above (Eastman Kodak, 1987 f; English et al., 1989) suggests that at least part of the compounds of molecular weight 160 presumably are enantiomers of 5-hydroxy-2-ethylhexanoic acid and that the lactones are those derived from 5-hydroxy-2-ethylhexanoic acid. The animals used were a subgroup of those which were investigated in the fertility study described in Section 7.8 (cf. Pennanen et al., 1991 b, 1992 a, 1993).

In a subsequent study, the authors were able to confirm in vivo in the male Han:Wistar rat and in vitro in microsomes from rat, mouse and human liver that the cytochrome P-450 isoenzymes are involved in the biotransformation of 2-ethylhexanoic acid. In particular, they demonstrated the involve-

ment of isoenzymes CYP2A, CYP3A, CYP2B and CYP2D in the formation of small amounts of the unsaturated metabolite 5,6-dehydro-2-ethylhexanoic acid (referred to as 2-ethyl-5-hexenoic acid in this study; Pennanen et al., 1996).

As early as 1949, oral and subcutaneous administration to rabbits of sodium 2-ethylhexanoate (1 g per animal, equivalent to 867 mg of the free acid) was demonstrated to increase urinary excretion of glucuronic acid. Based on the assumption that the compounds in question were exclusively conjugates of 2-ethylhexanoic acid, 83 to 86% (4 rabbits) and 87% (one rabbit) of the administered oral and subcutaneous dose, respectively, were excreted as conjugates of glucuronic acid. Keto derivatives of 2-ethylhexanoic acid were not detectable in urine (period of urine sample collection not specified; Dziewiatkowski et al., 1949).

In an even earlier study, no ketone bodies had been detectable, either, in the blood of rabbits receiving intravenous injections of 6 mmol (approx. 865 mg) sodium 2-ethylhexanoate/kg body weight (Wick, 1941).

In vitro studies were conducted to investigate the glucuronidation of 2-ethylhexanoic acid in greater detail. The acid was found to be glucuronidated by liver microsomes from all investigated species (rat, rabbit, dog, guinea pig, rhesus monkey, man). UDP-glucuronyltransferase activities were highest in microsomes from dog liver and lowest in microsomes from human and rabbit liver. In enantioselectivity studies, no differences were observed between the enantiomers with respect to glucuronidation in liver microsomes originating from humans, rats, rhesus monkeys and dogs, while guinea pig and rabbit microsomes glucuronidated the *R*-enantiomer to a greater extent (*R/S* glucuronidation ratios being 3.2 and 2, respectively). In the rat, it was possible to induce glucuronidation of either enantiomer by pretreating the liver donors with phenobarbital or, to a lesser extent, with 3-methylcholanthrene, while clofibrate had no inductive effect. Primarily, 2-ethylhexanoic acid was converted by the isoenzyme, UDP-glucuronyltransferase-2B1, not by bilirubin-UDP-glucuronyltransferase. In their discussion, the authors suggest that in addition to UDP-glucuronyltransferase-2B1 other isoenzymes of UDP-glucuronyltransferase may be involved in the glucuronidation of 2-ethylhexanoic acid (Hamdoune et al., 1995).

Metabolisation of 2-ethylhexanol to 2-ethylhexanoic acid

The primary step in the metabolisation of 2-ethylhexanol is its oxidation to 2-ethylhexanoic acid. Subsequently, 2-ethylhexanol undergoes biotransformation via the same metabolic pathway as 2-ethylhexanoic acid (cf. BG Chemie, 1999; Deisinger et al., 1994).

7.2 Acute and subacute toxicity

Acute toxicity

The results of the studies investigating the acute toxicity of 2-ethylhexanoic acid following oral, dermal and invasive administration and inhalation exposure are compiled in Table 2 in the appendix.

In the rat, acute oral toxicity of 2-ethylhexanoic acid was found to be low, with LD_{50} values ranging from 2043 to approx. 3640 mg/kg body weight. Dyspnoea, apathy, lying on the abdomen, weakness and prostration were described as clinical signs of toxicity. Gross pathology findings in the deceased animals included mottled liver, an opaque white, cooked appearance of the stomach and upper gastrointestinal tract, residues of the test compound in the gastrointestinal tract as well as faecal discoloration. Terminal necropsy of the animals surviving to the end of the study was without findings (BASF, 1953, 1967; Eastman Kodak, 1987 e; Mellon Institute, 1942; Smyth and Carpenter, 1944). In an exploratory study in the guinea pig, oral administration of a single 1600 mg/kg body weight dose was lethal, while a dose of 800 mg/kg body weight was survived (Eastman Kodak, 1966, 1982).

In an acute dermal toxicity study conducted in rats in accordance with the EC and OECD guidelines, the LD_{50} was determined as > 2000 mg/kg body weight. At that dose, none of the 10 animals in the study died. Except for slight temporary eschar formation on the skin of 2 of the animals, no symptoms were observed, and necropsy at study termination was without findings (Hoechst, 1986). In contradiction with the results of this study, which indicate that acute dermal toxicity in the rat is low, the results of an older study in rats show that immersion of the abdominal skin in 2 ml neat 2-ethylhexanoic acid, even for only 10 minutes, or in a 50-percent solution for 4 hours was lethal to several of the animals (BASF, 1953). For the guinea

pig, the dermal LD_{50} was found to be 6300 mg/kg body weight, and for the rabbit it has been reported to be 1260 mg/kg body weight (Mellon Institute, 1942; Smyth and Carpenter, 1944; BASF, 1981 a; Union Carbide, 1971).

Six-hour inhalation exposure to approx. 2356 mg/m³ (400 ppm) and, in inhalation hazard tests, 8-hour exposure to an atmosphere enriched or saturated at 20 °C or room temperature was not lethal to rats or guinea pigs. The animals showed no clinical signs, either. Terminal necropsy performed after one inhalation hazard test in the rat was without findings (BASF, 1967; Eastman Kodak, 1966, 1982; Mellon Institute, 1943; Smyth and Carpenter, 1944).

In the mouse, parenteral LD_{50} values of approx. 910 mg (1 ml)/kg body weight and approx. 273 mg (0.3 ml)/kg body weight were reported for subcutaneous and intraperitoneal administration, respectively. Single intraperitoneal doses of 1600 and 400 mg/kg body weight were lethal in the rat and the guinea pig, respectively, while 800 and 200 mg/kg body weight, respectively, were survived (Eastman Kodak, 1966, 1982).

Subacute toxicity

The toxic effects of 2-ethylhexanoic acid following subacute administration was assessed in preliminary studies to the subchronic toxicity studies described in detail in Section 7.5 (Eastman Kodak, 1988 a, b; Topping et al., 1989; Juberg et al., 1998). In these studies which were conducted in F344 rats and B6C3F1 mice in accordance with OECD guideline No. 407, groups of 5 animals per dose level and sex received 14-day treatment with 0 (controls), 200, 800 and 1600 mg 2-ethylhexanoic acid/kg body weight by oral gavage (rats) or 0.75, 1.5 and 3% 2-ethylhexanoic acid in feed (mice). Based on feed consumption, daily doses received by dietary administration were as follows: 706, 1351 and 2276 mg/kg body weight in male rats, 756, 1411 and 2658 mg/kg body weight in female rats, 1608, 3084 and 5794 mg/kg body weight in male mice, and 1965, 3986 and 9229 mg/kg body weight in female mice. The 2-ethylhexanoic acid administered was 99.8 to 99.9% pure. No clinical chemistry and haematology parameters were determined and histopathological examination as stipulated in the study protocols was restricted to the liver and kidneys and any gross organ lesions. A comparative summary of the studies is presented in Table 4 in the ap-

pendix. In the rats, both dietary administration and dosing by oral gavage, and in the mice dietary administration led to dose-dependent liver changes including increased organ weight and hypertrophy as well as degenerative changes in the hepatocytes in all dose groups. Reductions in body weight gain and/or feed consumption were found to start at the mid doses of 800 mg 2-ethylhexanoic acid/kg body weight given to the rats by gavage, and of 1.5% 2-ethylhexanoic acid administered to the rats and mice in the diet. In addition, the rats displayed unspecific clinical signs, such as weakness, lethargy, sialorrhoea and urine-soiled haircoats. In the top dose group receiving 1600 mg/kg body weight by oral gavage, 8 of 10 rats died or had to be killed in moribund condition. There were no findings in the mice receiving 200 or 800 mg/kg body weight by oral gavage. Of the mice dosed with 1600 mg/kg body weight by gavage there was only one male with increased liver weight and hepatocyte hypertrophy and only one female which periodically showed clinical signs (see Table 4; Eastman Kodak, 1987 a, b, c, d).

The studies investigating hepatic peroxisome proliferation by 2-ethylhexanoic acid are summarised in Section 7.11. In the rat and mouse, oral administration of 2-ethylhexanoic acid over periods ranging from 4 days to 3 weeks led to an increase in hepatic peroxisome proliferation, or an increase in the marker enzymes for peroxisome proliferation, cyanide-insensitive palmitoyl-CoA oxidase, lauroyl-CoA oxidase and carnitine acetyltransferase. According to in-vitro studies, this effect is not observed in the guinea pig or monkey (see also appendix, Table 8).

The systemic effects of subacute administration of 2-ethylhexanoic acid have been investigated in further studies which must be considered to be exploratory because only single parameters were measured, no more than 1 or 2 animals were used per dose and no controls were included. The results of these studies are compiled in Table 3, see appendix.

7.3 Skin and mucous membrane effects

The skin irritancy studies carried out with 2-ethylhexanoic acid are summarised in Table 5 in the appendix. Application of 2-ethylhexanoic acid to the skin of rabbits resulted in severe irritation with erythema, oedema, and necrotic changes which were not reversible within the observation period or healed up only very slowly. Evaluation of skin irritancy by the various authors

is extremely divergent, spanning all assessments from not irritating, mildly irritating, moderately irritating and severely irritating to corrosive (cf. Table 5). 2-Ethylhexanoic acid was evaluated as corrosive on the basis of the results of a study conducted in accordance with a guideline (21 CFR § 191.11) issued by the U.S. Department of Transportation, in which 4-hour occlusive application resulted in 2 of the 4 animals developing necrosis after treatment. Reversibility of the findings was not investigated in that study (Mellon Institute, 1972 b). 2-Ethylhexanoic acid was assessed as not irritating in a study carried out in accordance with EC directive No. 84/449/EEC and evaluated in accordance with EC directive No. 83/467/EEC. Evaluation of irritancy was accomplished by rating the individual findings for erythema and eschar formation as well as oedema formation on a numerical scale 24, 48 and 72 hours after the end of application, with the mean value for all animals being 0.7 for erythema and eschar formation and 0.3 for oedema formation (Hoechst, 1985 a). Note: According to the current classification directive 93/21/EEC and based on the results of this study with 4-hour semi-occlusive application to the depilated dorsal skin, 2-ethylhexanoic acid should, however, be evaluated as irritating to the skin, as 2 of the 3 animals still showed clear signs of irritation at the end of the 14-day observation period. One animal was found to have very mild erythema and very slight oedema, while the second one was found to have clearly circumscribed erythema and very minor oedema. In addition, both rabbits had dry, chapped, parchment-like skin and in one animal, the skin displayed large areas with light-brown discoloration.

In the rabbit eye, 2-ethylhexanoic acid caused severe irritation with clouding of the cornea, severe reddening and oedema formation, iritis and ocular discharge. In a study conducted in 3 rabbits in accordance with EC directive No. 84/449/EEC, instillation of 99-percent pure test substance was observed to result in slight to definite swelling as well as clearly visible hyperaemia to diffuse crimson discoloration of the conjunctivae, with 2 of the 3 animals displaying redness of the iris and slight clouding of the cornea as well as production of initially clear discharge followed by white muciform ocular discharge. The conjunctiva and nictitating membrane showed whitish discoloration one hour after instillation. Corneal clouding was reversible after 72 hours and all other findings cleared up within 7 days after instillation. In accordance with EC directive No. 83/467/EEC, 2-ethylhexanoic acid was evaluated as not irritating to the eye on the basis of the mean scores

for the individual study findings, which were 0.44, 0.56, 1.2 and 0.89 for clouding of the cornea, iritis, reddening of the conjunctiva and swelling of the conjunctiva, respectively (Hoechst, 1985 b). 2-Ethylhexanoic acid (no details of purity) was evaluated as severely irritating to the eye and capable of causing injury to the cornea, based on the results of studies in which the clinical signs were not reversible or not reversible in all animals within the 8- or 6-day observation periods (BASF, 1953, 1967, 1978 a, b; cf. Table 6).

7.4 Sensitisation

No information available.

7.5 Subchronic and chronic toxicity

The systemic effect of subchronic oral administration of 2-ethylhexanoic acid was investigated in the Fischer-344 rat in a GLP study conducted in accordance with the “2-Ethylhexanoic Acid, Final Test Rule” promulgated by the EPA (1986). Groups of 10 animals per dose level and sex were given feed containing 0.1, 0.5 and 1.5% 2-ethylhexanoic acid (purity: 99.9%) for a period of 91 to 93 days. Together with the highest dose group, an additional satellite group of 10 rats per sex was treated with 1.5% 2-ethylhexanoic acid in feed for 93 days and then placed under observation for another 27 or 28 days. Control groups were offered feed which did not contain the test substance. The average daily intake of test substance was 61, 303 and 917 mg/kg body weight in the male rats and 71, 360 and 1068 mg/kg body weight/day in the females. The animals were comprehensively examined for clinical effects and changes in haematology, clinical chemistry, gross pathology and histopathology. Significant, toxicologically relevant effects were limited to depressed body weight gain in conjunction with reduced feed consumption in the rats of the top dose group and dose-dependent liver changes. The latter included dose-dependent increases in absolute and relative organ weights in the males and females of the mid and top dose groups, histopathological abnormalities in the form of slight to moderate hepatocyte hypertrophy and a reduction in the number of small cytoplasmic vacuoles in the males and females of the top dose group and the males of the mid dose group. In addition, serum cholesterol levels were found to be dose-dependently elevated by 25 to 78% over the controls in

all of the treated males, and increased serum albumin levels were observed in the males of the top dose group. Serum triglycerides were not elevated. As it is conceivable that 2-ethylhexanoic acid, on account of its structural similarity to fatty acids, could serve as a nutrient, the authors suggested that the elevated serum cholesterol levels seen only in the males were the consequence of normal, sex-specific differences in fatty acid metabolism. All of the findings were reversible within the post-exposure observation period, or showed a tendency towards reversibility. While feed consumption during the observation period was at control levels (females) or higher (males), body weight gain was found to be partially depressed in the males but fully restored in the females. Only the males were found still to have increased relative liver weights; absolute liver weights, histopathological liver findings and serum cholesterol and albumin levels no longer differed significantly from the controls. The liver findings in the females were fully reversible. All other findings were negative or were assessed as being of no toxicological relevance. There was no increase in mortality. No treatment-related, significant clinical signs and no ophthalmological abnormalities were seen. Minor changes in red blood cell count were considered clinically insignificant by the authors (minor decreases in mean corpuscular volume and mean corpuscular haemoglobin concentration in the two top dose groups, mild poikilocytosis in all treatment groups and in the control group, spherocytosis in 2 of 5 males and microcytosis in 1 of 5 females, respectively, of the top dose group). Equally, no clinical significance was attached to the marginal increase, compared with the controls, in serum concentrations of urea nitrogen seen in the males of the top dose group. Only the females had reduced urine volume in all dose groups, and in the top dose group, specific gravity was slightly increased. As urine osmolality was comparable with the controls in all dose groups, the authors concluded that the animals had normal urine concentrating ability. They suggested that the changes in urine volume and specific gravity were incidental findings and/or the consequence of reduced feed consumption. The slight changes observed in the top dose group, and to some extent in the mid dose group, with regard to relative and/or absolute kidney, brain and testis weights, none of them having macroscopic or histopathological correlates, were interpreted by the authors as the consequence of impaired body weight gain rather than as indicators of the target organs for the toxic effects of 2-ethylhexanoic acid. In summary, the no effect level was found to be 0.1% in feed, equivalent to a daily dose of 61

mg/kg body weight and 71 mg/kg body weight for male and female rats, respectively. This level is determined by the liver changes, which in the authors' assessment were not signs of toxicity but rather were adaptive changes (Eastman Kodak, 1988 a; Topping et al., 1989; Juberg et al., 1998).

In B6C3F1 mice, a parallel study was carried out which was identical in design and scope. Similarly, the animals were also offered feed containing 0 (controls), 0.1, 0.5 and 1.5% 2-ethylhexanoic acid (purity: 99.9%) for a period of 91 to 93 days (the equivalent daily dose being 0, 180, 885 and 2728 mg/kg body weight, and 0, 205, 1038 and 3139 mg/kg body weight/day for the male and female mice, respectively). Together with the highest dose group, an additional satellite group of 10 mice per sex received dietary treatment with 1.5% 2-ethylhexanoic acid for 93 days followed by an observation period of 28 or 29 days. Similarly to the rats, the mice also displayed treatment-related impairment of body weight gain and dose-dependent liver changes. The male and female mice of the top dose group and the females of the mid dose group showed depressed body weight gain. On the whole, feed consumption data showed high variability due to spillage, but was none the less reported as reduced for the top dose group. The relative liver weights were dose-dependently increased in the top and mid dose groups in the animals of both sexes, and absolute liver weights were dose-dependently increased in both sexes of the top dose group as well as in the males of the mid dose group. Both the males and females of the top dose group and the males of the mid dose group displayed mild to moderate dose-dependent hepatocyte hypertrophy. Affected hepatocytes were also more eosinophilic in the animals of the top dose group. A dose-dependent decrease in the incidence of small cytoplasmic vacuoles in hepatocytes was observed in the males and females of the two highest dose groups. In the top dose group, alanine aminotransferase levels were increased. Dose-dependent elevations in serum cholesterol levels were seen in both the males and females of the mid and high dose groups, the authors suggesting that the elevations were probably indicative of incorporation of 2-ethylhexanoic acid into normal intermediary fatty acid metabolism. The serum triglyceride levels were significantly reduced in the males and females of the top dose group and in the females of the mid dose group. Histopathological findings, which had not occurred in the parallel study conducted in rats, additionally included marginal changes in the epithelial cells of the proximal convoluted renal tubules without impairment

of renal function in 4 of 10 males and 4 of 10 females of the top dose group as well as minimal acanthosis and hyperkeratosis of the forestomach in 6 of 10 males of the top dose group. As in the rat study, all findings in the mouse were reversible, or showed a tendency towards to being reversible, within the post-exposure observation period. With feed consumption not differing significantly from that of the controls during the observation period, body weight at the end of recovery was found to be statistically lower than controls only in the females. Absolute and relative liver weights in the males were comparable with controls. In the females, relative liver weights were still increased at the end of the observation period, but the authors attributed the increase to lower body weight rather than heavier livers. Absolute liver weights seen in the females at the end of the recovery period was lower than in the control group. The histopathological effects on the liver and the alterations in hepatic clinical chemistry parameters were found to be largely reversible. Only a few males and females were observed to have minor hepatocyte hypertrophy. The serum cholesterol levels in the males and females as well as the triglyceride levels in the females were comparable with those observed in the controls. Triglyceride levels in the males were nearly completely reversible. Increased alanine aminotransferase activity, a sign of altered hepatic function, seen at the end of the treatment period was slightly lower in the males compared with the controls at the end of the recovery period; in the females alanine aminotransferase activity was comparable with the controls. Kidneys and stomach were without histopathological findings in the animals of the recovery group. As in the rat study, all other results were insignificant, or were evaluated as toxicologically irrelevant. There was no increase in mortality. No treatment-related clinically significant signs of toxicity, ophthalmological abnormalities or altered haematology parameters were observed. Reductions in serum bilirubin levels were very minor in both males and females of the top dose group and in the females of the mid dose group, and were considered by the authors to be of no clinical significance. Due to control animals having very high serum urea nitrogen levels, all treated males displayed lowered urea nitrogen levels compared with controls. Serum urea nitrogen levels in the females of the top dose group were elevated relative to controls. For the females of all dose groups, urinalysis revealed increased ketone levels which were explained by the authors as resulting from excretion of metabolic ketone bodies. The slight changes in relative and/or absolute organ weights of the kidneys, adrenal glands, brain and testes seen in the top

dose group, and partly in the mid dose group, were interpreted by the authors as being consequences of impaired body weight gain rather than being an indication of the target organs for the toxic effects of 2-ethylhexanoic acid. In summary, it was observed, as in the rat study, that body weight retardation and liver changes determined the no effect level, which was found to be 0.1% in feed, equivalent to daily doses of 180 and 205 mg/kg body weight for male and female mice, respectively (Eastman Kodak, 1988 b; Topping et al., 1989; Juberg et al., 1998).

7.6 Genotoxicity

7.6.1 In vitro

2-Ethylhexanoic acid showed no mutagenic potential in the Salmonella/microsome assay performed in *Salmonella typhimurium* strains TA 98, TA 100, TA 1535, TA 1537 and TA 1538 as well as *Escherichia coli* WP2uvrA both in the presence and absence of metabolic activation (S-9 mix from Aroclor 1254-induced rat liver). The test was carried out as a plate incorporation test with concentrations ranging from 4 to 2500 µg/plate (purity of the test substance not specified). Concentrations ≥ 500 µg/plate were toxic to the bacteria (Hoechst, 1982).

A further Salmonella/microsome assay was carried out as an incubation test in *Salmonella typhimurium* strains TA 97, TA 98, TA 100 and TA 1535, again both with and without metabolic activation (S-9 mix from Aroclor 1254-induced rat and hamster liver). In the test concentration range from 0 to 3333 µg/plate, and 6666 µg/plate, 2-ethylhexanoic acid (purity: > 99%) also displayed no mutagenic potential in this assay, neither with nor without metabolic activation. The highest concentrations were toxic to the bacteria (NTP, 1985; Zeiger et al., 1988).

In a Salmonella/microsome assay which was performed only in *Salmonella typhimurium* strains TA 98 and TA 100, 2-ethylhexanoic acid again showed no mutagenicity in the 10^{-6} to 10^{-2} M concentration range (approx. 0.14 to 1442 µg/ml) both in the presence and absence of metabolic activation (S-9 mix from rat liver, no details of what chemical was used for induction; no further details; Warren et al., 1982).

The chromosome aberration test in Chinese hamster ovary cells was negative for 2-ethylhexanoic acid in the absence of metabolic activation. In

the presence of metabolic activation (S-9 mix from Aroclor 1254-induced rat liver), the authors considered the test result to be weakly positive because at the highest evaluable concentration levels of 3000 and 3500 µg/ml in the repeat study there was a statistically significant increase in aberration rate. In the absence of metabolic activation, concentrations ranged from 1030 to 2530 µg/ml while in the presence of activation the range was from 2250 to 3470 µg/ml and in a repeat test from 2700 to 4000 µg/ml, the highest concentrations all being toxic and not evaluable (purity of the test substance not specified). Per concentration, 100 cells were scored, with the exception of the 3500 µg/ml group in the repeat test, for which only 25 cells were scored, probably on account of toxicity (NTP, 1986, 1991).

2-Ethylhexanoic acid gave a positive response in the sister chromatid exchange (SCE) assay performed in Chinese hamster ovary cells both with and without metabolic activation (S-9 mix from Aroclor 1254-induced rat liver). The result was also positive if a buffer was added which prevented the pH of the medium from dropping in the presence of the test chemical. In the absence of metabolic activation, concentration ranges tested were from 199 to 2500 µg/ml (no buffer added) and from 400 to 1000 µg/ml (with buffer), and in the presence metabolic activation they were from 9.38 to 313 µg/ml (no buffer added) and from 505 to 5200 µg/ml (with buffer), with the top concentrations all being toxic and not evaluable. The sister chromatid exchange rates exhibited statistically significant concentration-dependent increases at 199 and 400 µg/ml and higher (with/without buffer, without metabolic activation) and at 93.8 and 1000 µg/ml and higher (with metabolic activation). Fifty cells per concentration were scored (NTP, 1986, 1991).

A further SCE assay was carried out in human lymphocytes. The lymphocyte cultures were obtained from the blood of a healthy 34-year-old male donor and incubated with 2-ethylhexanoic acid at concentration levels ranging from 0.63 to 20 mM (approx. 91 to 2884 µg/ml, purity: 99%) for 48 hours. The results as presented in the text of the report, describe a concentration-dependent positive effect in the 0.63 to 2.5 mM range. Based on the graphical presentation of the findings, there was a slight concentration-dependent increase in sister chromatid exchange rate, but not twice the control rate. At each concentration level, 50 to 60 metaphases from two parallel cultures were scored in total. Dose-dependent cytotoxic effects (reduction in replication index) were seen at 0.63 mM and higher in the

graphical presentation of the results; according to the authors, the 5 mM concentration was clearly toxic (Sipi et al., 1992).

In summary, 2-ethylhexanoic acid did not exhibit any mutagenic potential in the Salmonella/microsome assay or in *Escherichia coli* either with or without metabolic activation. In the chromosome aberration test performed in Chinese hamster ovary cells, response was negative and weakly positive in the absence and presence of metabolic activation, respectively, with regard to induction of chromosomal damage. In the sister chromatid exchange assay carried out in Chinese hamster ovary cells, 2-ethylhexanoic acid gave a positive result both in the presence and absence of metabolic activation. In human lymphocytes, 2-ethylhexanoic acid induced a slight increase in sister chromatid exchange rate in this test system in the absence of metabolic activation.

7.6.2 In vivo

No information available.

Genotoxicity of 2-ethylhexanol

2-Ethylhexanol, which undergoes biotransformation via quantitative metabolism to 2-ethylhexanoic acid, has been comprehensively studied with regard to its genotoxic potential both in vitro in the Salmonella/microsome and mouse lymphoma assays and the HPRT, DNA repair, UDS and chromosome aberration tests as well as in vivo in the micronucleus assay and the dominant-lethal test. In these test systems, there have been no relevant findings to indicate that 2-ethylhexanol has a genotoxic potential (cf. BG Chemie, 1999).

Combining the results of genotoxicity studies on 2-ethylhexanoic acid and 2-ethylhexanol and bearing in mind the metabolic relationship between the two compounds, it appears very likely that 2-ethylhexanoic acid is also devoid of any genotoxic potential.

7.7 Carcinogenicity

No information available.

7.8 Reproductive toxicity

A detailed summary of the available teratogenicity/embryotoxicity studies and screening tests investigating the reproductive toxicity of 2-ethylhexanoic acid in vivo can be found in Table 7 in the appendix.

The findings vary and do not present a consistent picture. In order to estimate the risk associated with 2-ethylhexanoic acid in terms of the chemical's teratogenic/embryotoxic potential, a study was conducted in the Fischer-344 rat in accordance with the "2-Ethylhexanoic Acid, Final Test Rule" promulgated by the EPA (1986). Up to the highest test dose of 500 mg/kg body weight, which showed maternal toxicity, there were no teratogenic abnormalities. Foetotoxic effects were seen in the form of reduced ossification upon administration of 250 mg/kg body weight and, in particular, in the form of reduced foetal weights, visceral variations (dilatation of the lateral ventricles of the brain without tissue compression) as well as skeletal variations (reduced ossification, variations at vertebra 14) following administration of the maternally toxic dose of 500 mg/kg body weight. Findings indicating maternal toxicity in the top dose group of 500 mg/kg body weight were reported to include hypoactivity, ataxia, audible respiration, ocular discharge and periocular encrustation as well as increased liver weights. In a parallel study in the New Zealand rabbit, there were no findings, up to the highest, maternally toxic test dose of 250 mg/kg body weight, to indicate that 2-ethylhexanoic acid had an embryotoxic, foetotoxic or teratogenic potential. In the 125 and the 250 mg/kg body weight dose groups one of 15 dams died; in addition, one female of the 125 mg/kg body weight dose group aborted and the dams of the top dose group exhibited depressed body weight gain in conjunction with reduced feed consumption and clinical signs of toxicity. In these two studies, daily 2-ethylhexanoic acid doses of 100, 250 as well as 500 mg/kg body weight (rat) and 25, 125 as well as 250 mg/kg body weight (rabbit), which had previously been determined in preliminary range-finding studies, were administered by oral gavage as solutions in maize germ oil (corn oil) during organogenesis from days 6 to 15 and on day 18 of gestation, respectively. In the preliminary studies employing dose ranges from 100 to 1000 mg/kg body weight (rat) and 25 to 1000 mg/kg body weight (rabbit), the 1000 mg/kg body weight dose level and the dose levels of and above 500 mg/kg body weight resulted in maternal lethality in the rat and the rabbit, respectively. The *no effect levels* for reproductive toxicity in the rat and the rabbit were found to

be 100 mg/kg body weight and 250 mg/kg body weight, respectively, and for maternal toxicity the *no effect levels* in the rat and the rabbit were 250 mg/kg body weight and 25 mg/kg body weight, respectively (Bushy Run, 1988 a, b; Fisher et al., 1989; Hendrickx et al., 1993; see Table 7). In contradiction with these findings, according to which 2-ethylhexanoic acid induces no damage in the embryo or foetus even at maternally lethal doses, oral administration to Han:Wistar rats in a drinking water study was found to result in foetal abnormalities due to foetotoxic and teratogenic effects. In the latter study, in which 2-ethylhexanoic acid was administered in the form of its sodium salt on days 6 to 19 of gestation, increases in frequencies of skeletal malformations were seen numerically at and above 100 mg/kg body weight, and statistically significantly at 300 mg/kg body weight and above, with club foot being the single malformation to occur most frequently. In addition, the following, largely dose-dependent but not statistically significant changes were observed which were evaluated by the authors as malformations: flabby legs and slight scoliosis and lordosis in all dose groups, abnormal cartilage in the ankle at 300 mg/kg body weight and higher, and absence of fibula as well as extra thoracic ribs in the top dose group treated with 600 mg/kg body weight. Visceral malformations reported as showing significant, dose-independent increases in frequency included dilatation of the urinary tract in the lower and mid dose groups as well as dilatation of the brain ventricles (no precise details to specify the type of dilatation, but according to the authors' discussion the finding correlated with decreased foetal weight) in the top dose group. In the control group, extra ribs, dilatation of the urinary tract and dilatation of the brain ventricles were seen, but none of the other above-mentioned malformations occurred. Foetotoxic effects in the form of skeletal variations were observed at and above 100 mg/kg body weight, and reduced foetal weight started to occur at 300 mg/kg body weight. Maternal toxicity in the form of decreased placental weights was seen at 300 mg/kg body weight and higher, and in the form of reduced body weight and lower consumption of drinking water in the top dose group receiving 600 mg/kg body weight. In summary, 2-ethylhexanoic acid was evaluated by the authors as teratogenic at dose levels which did not cause maternal toxicity (Pennanen et al., 1992 b, c; see Table 7). In further studies in the Wistar rat and the Sprague-Dawley rat, single-dose administration by oral gavage of approx. 1820 mg undiluted 2-ethylhexanoic acid/kg body weight on day 12 of gestation was observed to cause teratogenic abnormalities of the limbs, the tail and the

heart as well as hydronephrosis. The authors of this study also evaluated 2-ethylhexanoic acid as teratogenic (Ritter et al., 1985, 1987; Scott et al., 1994; University of Cincinnati, 1985). However, in the publications based on these studies, no detailed data are reported as to the maternal toxicity of this, by comparison with other rat studies, very high dose, and no or no definitive information is supplied with regard to statistical analysis. Therefore, these studies are of limited use in evaluating the reproductive toxicity of 2-ethylhexanoic acid. None the less, there is further evidence of the teratogenic potential of 2-ethylhexanoic acid from another group of researchers whose studies demonstrate that 2-ethylhexanoic acid is capable of inducing teratogenic abnormalities in the form of exencephaly after high-dosage parenteral administration (≥ 500 mg/kg body weight 3 or 4 times at 12-hour intervals). These authors were also able to demonstrate that the teratogenic potential of 2-ethylhexanoic acid depends on the enantiomer administered. At position 2, the 2-ethylhexanoic acid molecule contains an asymmetric carbon atom. Therefore, 2-ethylhexanoic acid exists as two enantiomers, *(R)*-2-ethylhexanoic acid and *(S)*-2-ethylhexanoic acid. Only the mixture of the two enantiomers, racemic (+/-)-2-ethylhexanoic acid, is of technical importance. It has been demonstrated that the *S*-(+)-enantiomer has no, or no definite, teratogenic potential, while the *R*-(–)-enantiomer is a potent teratogen and the teratogenic potential of the racemate is in between that of the two enantiomers. On the basis of the results obtained from a pharmacokinetic study conducted in parallel with one of the studies, it was possible to preclude that the differences between the enantiomers with respect to teratogenicity reflect differences in pharmacokinetics. The authors suggested that the marked differences in teratogenic activity are due to stereoselective interactions of the enantiomers with proteins (enzymes, receptors) or other chiral cellular constituents (Collins et al., 1992; Hauck et al., 1990; see Table 7). However, for the following reasons these studies are also only of very limited suitability for assessing the potential of 2-ethylhexanoic with regard to reproductive toxicity acid under working-place conditions: largely, no details of maternal toxicity results were given, mostly no controls were included and frequently statistical analysis was lacking; moreover, 2-ethylhexanoic acid was administered by subcutaneous or intraperitoneal injection. In a Chernoff/Kavlock screening assay employing the Sprague-Dawley rat, administration of maternally lethal doses of 900 or 1200 mg 2-ethylhexanoic acid/kg body weight on days 6 to 15 of gestation resulted in increased incidences of postimplantation losses and

skeletal variations and in adverse effects on postnatal physical development of the pups (Narotsky et al., 1989, 1991, 1994).

The effect of 2-ethylhexanoic acid on fertility and postnatal development was investigated in the Han:Wistar rat. Groups of 24 animals/dose and sex (females aged 9 to 10 weeks, males 5 to 6 weeks) received 2-ethylhexanoic acid (purity: 99.5%) in a solution with equimolar NaOH, and thus in the form of its sodium salt, by oral administration in the drinking water for 10 weeks prior to mating and throughout a 3-week breeding period (males) or for 2 weeks prior to mating, during the 3-week breeding period, throughout pregnancy and for 3 weeks postpartum (females). Daily doses of 100, 300 and 600 mg 2-ethylhexanoic acid/kg body weight were administered, with the controls receiving ordinary tap water. Up to the end of the breeding period, treatment had no effect on the clinical picture, body weight development or consumption of feed and drinking water. During pregnancy, the females receiving the 600 mg/kg body weight treatment displayed reduced drinking water consumption and retardation of body weight gain as of day 7 of gestation. By the end of lactation (3 weeks postpartum), the retardation of body weight had completely reversed. The males of the top dose group which were examined at the end of the breeding period showed a nonsignificant increase in absolute epididymal weight compared with controls, while relative epididymal weight was statistically significantly increased by 12% and the number of spermatozoa in the epididymis was statistically nonsignificantly reduced by 14%. Relative to controls, there was a statistically significant reduction in sperm motility by 37% and 22% in the low dose group and the top dose group, respectively. In the mid dose group, sperm motility was not affected. The number of morphologically abnormal spermatozoa (agglutinated spermatozoa and sperm cells with abnormal heads) exhibited a statistically nonsignificant increase in the mid and top dose groups. In the authors' interpretation, there was a dose-dependent effect on fertility, since the controls conceived in the course of one or two oestrous cycles while 1/25, 2/24 and 4/24 of the animals receiving 100, 300 and 600 mg 2-ethylhexanoic acid/kg per day did not conceive until the third or fourth oestrus cycle; 3 males of the mid dose group and 6 males of the top dose group also copulated in dioestrus; and all females failing to become pregnant during the 21-day breeding period (4 oestrus cycles) belonged to the 2-ethylhexanoic acid-treated groups (2/23 and 1/23 receiving daily doses of 100 mg and 600 mg/kg body weight, respectively). No infor-

mation is given on the extent to which the effect on fertility was statistically significant. In their discussion, the authors pointed out that the effect on mating efficiency did not correlate with the abnormalities observed in the sperm cells (reduction in number and motility as well as abnormal morphology), since the alterations were found to be equally frequent in successfully and unsuccessfully mating males. Histopathological examination of the reproductive organs from the nonpregnant females and the males with which they were mated was without findings. In the top dose group receiving 600 mg/kg body weight, there was a statistically significant reduction in litter size by 16% relative to controls. The authors pointed out that pups with severe anomalia (severe flabby legs) were cannibalised by the dams, which may have had an effect on recorded litter size (during expected parturition dams were observed twice daily). In all dose groups, the body weights of the pups at birth and the number of stillbirths was comparable with the controls. There was a statistically significant increase in external anomalies in the pups of the mid dose group (kinky tails, lethargy) and the top dose group (kinky tails). In addition, the authors pointed out that an increased numbers of animals had haematomas, abnormally thin hair and leg anomalies (long thin legs, twisted hind legs, flabby legs); however, the incidences of these changes were not statistically significantly increased. No instances of club foot were reported, which had been observed to occur as a statistically significant dose-dependent malformation in a teratogenicity study conducted by the same group of authors under comparable treatment conditions (administration in the drinking water on days 6 to 19 of gestation and identical doses of 100, 300 and 600 mg sodium 2-ethylhexanoate/kg body weight; see above and appendix, Table 7; Pennanen et al., 1992 b). Effects of 2-ethylhexanoic acid treatment on postnatal development were seen in the pups of the top dose group (body weight retardation, less hair growth and delays in eruption of teeth, opening of the eyes, raising of the ears and detectable grip and cliff avoidance reflexes) and to a lesser extent in the pups of the mid dose group (detectability of the grip reflex and raising of the ears delayed). At macroscopic examination 21 days after birth, one male pup of the top dose group was found to have a not further characterised mass in the left testis while the left epididymis was missing; no examination for internal teratogenic changes was performed. Histopathological examination of the dams' reproductive organs revealed a slight dilatation of the lumen in uterus and epithelial hyperplasia in vagina in 2 of 5 examined females of both the mid

dose group and the top dose group; cervix uteri and ovaries were normal. In an additional study on the effect of 2-ethylhexanoic acid on implantation, 5 to 12 females which had been mated with untreated males received by oral gavage a single dose of 600 mg 2-ethylhexanoic acid/kg body weight as the sodium salt on days 4, 5, 6 or 7 of gestation and were examined on day 10 of gestation. At the time of mating, the females were 15 to 16 weeks old and the males were 16 weeks old. No information was given with regard to possible maternal toxicity. Treatment on days 4 or 5 of gestation did not affect the numbers of implantations and resorptions. In 4 of the 5 pregnant females treated on day 6 of gestation, the mean number of implantations per dam was increased, with high variability being observed in the individual number of implantations per animal, and there was an increase in the number of resorptions; in 2 of the 5 pregnant females the litters were completely resorbed. Treatment on day 7 of gestation led to resorptions in 3 of the 10 pregnant females, but did not affect implantation rate. From these results, the authors concluded that 2-ethylhexanoic acid has an embryotoxic effect which is due to interference with implantation. Resorption rate was found to be increased, which is in contradiction with an earlier teratogenicity study by the same authors (see Table 7, daily doses of up to 600 mg 2-ethylhexanoic acid/kg body weight dissolved equimolarly with NaOH and administered orally in the drinking water on days 6 to 19 of gestation; number of corpora lutea, sex ratio, pre- and postimplantation losses comparable with controls; Pennanen et al., 1992 b), but the authors explained this observation with the marked differences in peak plasma levels obtained with continual administration in the drinking water as compared with single-dose administration by gavage. They argued that 2-ethylhexanoic acid may well be potentially embryotoxic, but that such embryotoxicity might not manifest itself unless relatively high plasma levels are exceeded, as is the case with the structural isomer, valproic acid (2-propylpentanoic acid). With 2-ethylhexanoic acid having a plasma half-life of only approx. 3 hours (cf. Section 7.1; Pennanen and Manninen, 1991; study with intraperitoneal administration), single oral administration by gavage could be expected to lead to markedly higher plasma levels than continual administration of the same daily dose in the drinking water. Summarising their results, the authors concluded that treatment with 600 mg/kg body weight 2-ethylhexanoic acid tended to reduce fertility, increase time to mating, inhibit implantation and delay physical development of pups during lactation, and that delayed physical development was also observed in the

mid dose group receiving 300 mg/kg body weight (Pennanen et al., 1991 b, 1992 a, 1993). Note: The results of this study have been reported in a total of three publications which contain partly inconsistent information on study conduct and findings. The data reviewed in the present Toxicological Evaluation was largely taken from the detailed publication by Pennanen et al., 1993. Contradictory information is found with respect to the exposure period for the males; Pennanen et al., 1991 b, 1992 a, stated it as 12 weeks prior to mating, while according to Pennanen et al., 1993, it was 10 weeks. In addition to the above-mentioned results, a short paper by the authors (Pennanen et al., 1991 b) reported that the males showed a dose-dependent increase in relative kidney and liver weights as well as a reduction in serum triglyceride, cholesterol and bilirubin levels; it was not stated whether these findings pertained to the parental animals or the pups, but based on the comprehensive description of the scope of the study given by Pennanen et al. (1993) the findings must relate to the parents. The animals treated with 600 mg/kg body weight had their urinary metabolite excretion determined prior to mating (cf. Section 7.1; Pennanen et al., 1991 a).

According to a mechanistic study, the reproductive toxicity of 2-ethylhexanoic acid may be attributable to alterations in endogenous zinc distribution. It was demonstrated that 2-ethylhexanoic acid induces the biosynthesis of the zinc-binding enzyme metallothionein in the livers of the dams, causing more zinc to remain bound in the dams' livers and thus reducing the supply of zinc to the developing embryos and foetuses. In their discussion, the authors suggest that zinc deficiency leads to retardation of embryonal/foetal development and malformations. Pregnant Sprague-Dawley rats (6 to 8 animals per dose group) were offered feed containing sufficient zinc to meet normal dietary requirements (25 µg zinc/g feed). On day 11.5 of gestation (day of positive proof of copulation = day 0 of gestation), the females received single doses of 0 (controls), 451, 902, 1355 and 1804 mg 2-ethylhexanoic acid (no indication of purity) per kilogram body weight as a solution in maize germ oil (corn oil) by oral gavage. After 8 hours the rats were given 32 µCi ^{65}Zn (purity: 99%, specific activity 2.47 mCi/mg) by gavage and 10 hours later terminal necropsy was performed. None of the dose groups displayed any differences in food intake and numbers of resorptions compared with controls. In all dams which had received 2-ethylhexanoic acid, liver zinc content was numerically elevated relative to controls, but this alteration was statistically significant only in the three highest

dose groups. The increased liver zinc concentrations correlated with elevated concentrations of the zinc-binding protein metallothionein in maternal livers. Plasma zinc concentrations in all toxicant-treated dose groups were comparable with controls. The amounts of zinc recovered in the embryos of the two highest dose groups were significantly reduced relative to controls. In a subsequent experiment, groups of 7 to 10 animals were fed diets containing different zinc concentrations (1.15, 25.44 or 97.46 µg zinc/g diet (low, adequate or supplemental zinc, respectively)), treated by gavage with 0 (controls) and 3.5 mmol (approx. 505 mg) 2-ethylhexanoic acid/kg body weight as a solution in maize germ oil (corn oil) on days 8 to 15 of gestation, and killed and examined on day 16 or 19 of gestation. In the low-zinc control group and the groups treated with 2-ethylhexanoic acid, food intake was reduced. As expected, the low-zinc controls displayed decreases in maternal liver metallothionein as well as liver and plasma zinc concentrations, maternal body weight gain and foetal body weight and length, and increases in percentages of resorptions and foetuses with morphological changes (encephalocele and tail anomalies). Supplemental zinc led to an increase in liver metallothionein levels as well as liver and plasma zinc concentrations but did not affect maternal or foetal development. When 2-ethylhexanoic acid was administered concurrently, increased frequencies of morphological changes were seen among the low-zinc animals, and animals receiving adequate dietary zinc also were found to have higher incidences of foetuses with hydrocephaly, encephalocele and tail anomalies. Only under supplemental zinc conditions did administration of 2-ethylhexanoic acid not affect pup development or result in an incidence of malformed foetuses different from that of the adequate zinc controls. In dams fed the low-zinc diet, concurrent administration of 2-ethylhexanoic acid resulted in further reduction of plasma zinc concentrations; liver metallothionein concentrations remained unchanged. The dams treated with 2-ethylhexanoic acid while being fed adequate and supplemental zinc diets showed no significant changes in plasma zinc concentrations compared with those fed the adequate zinc diet; their liver metallothionein concentrations were increased, but not as markedly as after single-dose administration (see above). In a subsequent in-vitro experiment, embryos were explanted on day 10.5 of gestation and cultured in plasma containing different concentrations of zinc for 48 hours. The plasma used was obtained from males which had been fed marginal zinc or adequate zinc diets (4.5 or 25.0 µg zinc/g diet, respectively) for a period of 3 weeks and had either received

by gavage a single dose of 9.38 mmol (approx. 1353 mg) 2-ethylhexanoic acid/kg body weight 48 hours prior to plasma preparation or had remained untreated. The embryos cultured in the serum obtained from adequate-zinc males which had not been conditioned with 2-ethylhexanoic acid showed normal development. The sera from the marginal-zinc males and those conditioned with 2-ethylhexanoic acid contained lower zinc concentrations, and the embryos cultured in these sera displayed retarded development. When the low-zinc sera were repleted to normal zinc levels by adding zinc chloride, embryos developed normally (Bui et al., 1998).

In summary, although findings are not generally consistent, there is enough experimental evidence to indicate that 2-ethylhexanoic acid is harmful to the embryo and/or foetus at dose levels without marked maternal toxicity.

In an in-vitro study, 48-hour incubation of rat embryos explanted on gestation day 9.5 with 0.5 to 2 mM 2-ethylhexanoic acid (approx. 72 to 288 µg/ml) induced morphological changes in the embryos. Incubation with 1 mM (approx. 144 µg/ml) caused minor morphological changes, reduction in protein content of the embryos and yolk sac changes in 25% of the embryos. Following incubation with 2 mM 2-ethylhexanoic acid, all recorded parameters were altered compared with controls (higher incidences of morphological malformations and retardation phenomena, decrease in crown-rump length, number of somite pairs and protein content of the embryos as well as abnormal morphology in all yolk sacs, and reduction in yolk-sac diameter and protein content; Brown and Coakley, 1984; Brown et al., 1988).

7.9 Effects on the immune system

No information available.

7.10 Neurotoxicity

The anticonvulsant effect of 2-ethylhexanoic acid was investigated in male NMRI mice following intraperitoneal administration in comparison with its structural isomer, the antiepileptic drug valproic acid (2-propylpentanoic acid). Upon administration of equimolar doses, the anticonvulsant potency of 2-ethylhexanoic acid was 40% relative to valproic acid when electroshocks were delivered via eye electrodes (Löscher and Nau, 1985).

Groups of 10 male IFFA-CREDO-OF1 mice were given intraperitoneal injections of 2-ethylhexanoic acid dissolved in olive oil 30 minutes prior to injection of pentylenetetrazol at a dose level which induced tonic convulsions in 95% of the mice (130 mg/kg body weight). At 1.14 mmol (approx. 164 mg) per kilogram body weight, 2-ethylhexanoic acid showed anticonvulsant activity in 50% of the mice (ED_{50}). Under the same experimental conditions, the ED_{50} for the antiepileptic drug, valproic acid, was found to be 0.81 mmol/kg body weight. Additional groups of 6 mice were killed 30 minutes subsequent to injection of 0.5, 1, 2 and 4 mmol (approx. 72 to 576 mg) 2-ethylhexanoic acid/kg body weight and their brain tissue was analysed for content of inhibitory neurotransmitter, γ-aminobutyric acid (GABA). Treatment with 0.5 and 1 mmol 2-ethylhexanoic acid had no effect on brain GABA levels; 2 and 4 mmol resulted in less significant elevation of GABA levels by approx. 22 and 50%, respectively, relative to untreated controls. The authors reported that 2-ethylhexanoic acid treatment also resulted in sedative effects (loss of righting reflex) and/or toxic effects (acute lethality; no further details; Keane et al., 1983).

7.11 Other effects

The studies on hepatic peroxisome proliferation induction by 2-ethylhexanoic acid are summarised in Table 8, see appendix.

In the rat and mouse, 2-ethylhexanoic acid causes dose-dependent increase in relative liver weights upon short-term oral administration (Hamdoune et al., 1995; Keith et al., 1988, 1992; Lundgren et al., 1987 a, 1988 a, b; Moody and Reddy, 1978, 1982; Sundberg et al., 1994; see also Table 8). The increases in hepatic cyanide-insensitive palmitoyl-CoA oxidase, lauroyl-CoA oxidase and/or carnitine acetyltransferase which are characteristic of peroxisome proliferation has been demonstrated in the rat and the mouse in numerous individual studies with oral administration ranging from 4 to 14 days (see Table 8). In the rat, confirmation was obtained by electron microscopy that 3-week oral administration of approx. 1333 mg 2-ethylhexanoic acid/kg body weight (2% in feed) has a proliferative effect on peroxisomes (Moody and Reddy, 1978, 1982). Elevated cytosolic and microsomal epoxide hydrolase activity seen in the liver of the mouse was evaluated as a secondary effect of hepatic peroxisome proliferation (Lundgren et al., 1987 a, 1988 a, b; Sundberg et al., 1994). Cata-

lase activity was increased in murine cytosolic and mitochondrial hepatocyte fractions, whereas no increase in activity levels was observed in the microsomal (peroxisomal) fractions (Lundgren et al., 1987 a, 1988 a, b; Sundberg et al., 1994). In the mouse, peroxisome proliferation was fully reversible 4 days after the end of administration; epoxide hydrolase and carnitine acetyltransferase activity had returned to control levels (Lundgren et al., 1987 a, 1988 a, b). In in-vitro studies, increased cyanide-insensitive palmitoyl-CoA oxidation, characteristic of peroxisome proliferation, was also demonstrated in rat and mouse hepatocytes (Cornu et al., 1992; ICI, 1985; Macherey et al., 1992, 1993). In contrast, guinea pig and marmoset hepatocytes were not found to have increased cyanide-insensitive palmitoyl-CoA oxidation following incubation with up to 2 mM 2-ethylhexanoic acid (approx. 288 µg/ml; higher concentrations were cytotoxic; Cornu et al., 1992; ICI, 1985). Studies on the differential peroxisome-proliferative effects of the 2-ethylhexanoic acid enantiomers carried out in vivo in the mouse and in vitro in rat hepatocytes demonstrated that cyanide-insensitive palmitoyl-CoA oxidation and lauroyl-CoA oxidase activity were induced slightly more strongly by the *S*-(+)-enantiomer than by the *R*-(–)-enantiomer (Macherey et al., 1992, 1993; Sundberg et al., 1994). In longer in-vivo administration, this effect was not detectable (Sundberg et al., 1994).

8 Experience in humans

A study conducted in 19 workers (4 women and 15 men) at 4 Finnish sawmills investigated the exposure to the wood preservative, Sinesto B, which contains 26% sodium 2-ethylhexanoate. Samples of air were taken at the breathing zone of each worker (by means of an impinger flask worn on one of the shoulders, and millipore filters), and patch samples (filter papers worn on and under the clothing) were analysed in order to characterise skin exposure. Workers' urine samples were collected during the period from the end of the work shift until the beginning of work on the following morning and analysed for 2-ethylhexanoic acid content. The concentrations detected in urine ranged from 0.01 to 1.4 mmol 2-ethylhexanoic acid/mol creatinine and correlated with measured levels of 0.01 to 0.7 mg 2-ethylhexanoic acid/m³ air. There was no correlation between the concentrations measured on and under the clothes and excretion in the urine. The urine samples collected immediately after work were found to contain the highest

2-ethylhexanoic acid concentrations. From their results, the authors concluded that the predominant route by which 2-ethylhexanoic acid entered the body was inhalation and that in order to characterise 2-ethylhexanoic acid exposure, excretion of the chemical should be determined in the urine collected immediately after the work shift (Kröger et al., 1990).

In a subsequent study in 9 healthy sawmill workers (aged 20 to 30 years), of whom 5 were reported as being exposed to higher and 4 to lower levels of 2-ethylhexanoic acid (no details of absolute exposure given), urinary excretion of 2-ethylhexanoic acid, arginine, ornithine and glutamine as well as serum aminotransferase activity were determined. Twenty individuals without exposure served as controls. Excretion of 2-ethylhexanoic acid in urine samples collected after an 8-hour work shift was analytically determined to be 1.8 ± 1.6 and 0.03 ± 0.01 µmol/mmol creatinine in workers classed as high-exposure and low-exposure individuals, respectively. Ornithine and arginine excretion per millimole creatinine was increased in the high-exposure workers relative to the controls and the low-exposure workers (ornithine: controls 1.5 ± 0.8 µmol, low-exposure workers 1.4 ± 0.4 µmol, high-exposure workers 4.5 ± 2.5 µmol; arginine: controls and low-exposure workers 1.5 ± 0.8 µmol, high-exposure workers 3.2 ± 1.5 µmol). With regard to glutamine excretion and serum aminotransferase activity, there was no significant change in the groups with exposure relative to controls. In the discussion of their results, the authors point out that 2-ethylhexanoic acid inhibits citrulline synthesis in the urea cycle by affecting carbamyl phosphate synthase and/or ornithine transcarbamoylase in the hepatic mitochondria and that the formation of arginine and ornithine is increased as a corrective mechanism to prevent hyperammonaemia, a procedure which has been observed in selected inborn errors of metabolism affecting the urea cycle. The authors proposed that assessment of occupational exposure to 2-ethylhexanoic acid should also include determining urinary amino acid excretion in addition to measuring the urinary concentrations of the chemical itself (Pennanen et al., 1990).

According to the occupational medicine department of a company manufacturing 2-ethylhexanoic acid, two cases each of irritation of the skin, eyes and respiratory tract following local and inhalation exposure, respectively, were registered in the department's outpatient database during the 1989–1996 period (BASF, 1997).

In addition, one reported case of corrosive injury to the cornea caused by 2-ethylhexanoic acid was completely reversible within 48 hours following medical treatment (surgical denudation technique; no further details; McLaughlin, 1946).

According to the Informationsverbund Dermatologischer Kliniken (IVDK, Information Network of Departments of Dermatology for the surveillance and scientific evaluation of contact allergies in Germany), there are no data on the sensitising potential of 2-ethylhexanoic acid. On the basis of structure-activity relationships and in view of the fact that the chemical lacks salient structural features typical of allergens, it was deemed unlikely that 2-ethylhexanoic acid has an allergenic potential (IVDK, 1997).

The occupational medicine and healthcare protection departments of two companies manufacturing and processing 2-ethylhexanoic acid report that after many years of experience, there is no evidence to indicate that the chemical has a sensitising potential (BASF, 1997; Hoechst, 1997).

9 Classifications and threshold limit values

2-Ethylhexanoic acid has been legally classified in the TRGS 905 and placed into category R_D3 of substances toxic to reproduction (i.e. “substances which cause concern for humans owing to possible developmental toxic effects”) in accordance with the EU classification criteria (TRGS 905, 2000).

Appendix

Table 1 Toxicokinetics and metabolism of 2-ethylhexanoic acid in the female Fischer-344 rat (after Eastman Kodak, 1987 f, and English et al., 1989)
Table 2 Acute toxicity studies with 2-ethylhexanoic acid
Table 3 Effects of 2-ethylhexanoic acid in exploratory subacute studies
Table 4 Subacute toxicity studies with 2-ethylhexanoic acid
Table 5 Skin irritancy of 2-ethylhexanoic acid
Table 6 Mucous membrane effects of 2-ethylhexanoic acid
Table 7 Reproductive toxicity of 2-ethylhexanoic acid
Table 8 Studies on the induction of peroxisome proliferation by 2-ethylhexanoic acid

Table 1. Toxicokinetics and metabolism of 2-ethylhexanoic acid in the female Fischer-344 rat (after Eastman Kodak, 1987 f, and English et al., 1989)

	100 mg/kg b.w. single oral dose	1000 mg/kg b.w. single oral dose	100 mg/kg b.w. repeated oral dose (15 days)	100 mg/kg b.w. single dermal application	1000 mg/kg b.w. single dermal application	1 mg/kg b.w. single intravenous dose
Bioavailability (% of radioactivity administered)	not determined	not determined	not determined	63%	70%	not determined
C_{max} (µg EHS equivalents/g blood)	85.1 µg/g	not determined	not determined	8.5 µg/g	not determined	not determined
t_{max}	18.8 minutes	not determined	not determined	5.7 hours	not determined	not determined
Half-lives of elimination for tri- and biphasic total elimination from the blood	19.0 minutes 6.8 hours 98.2 hours	not determined	not determined	4.2 hours 251 hours	not determined	11.1 minutes 6.6 hours 117.0 hours
Excretion in urine and faeces within 8 hours (% of radioactivity administered)	50.4% (urine) 0.02% (faeces)	19.8% (urine) 0.01% (faeces)	37.9% (urine) 0.03% (faeces)	10.3% (urine) 0.16% (faeces)	4.0% (urine) 0.05% (faeces)	47.9% (urine) 0.03% (faeces)
Excretion in urine and faeces within 24 hours (% of radioactivity administered)	75.7% (urine) 11.2% (faeces)	72.4% (urine) 3.8% (faeces)	57.3% (urine) 13.8% (faeces)	33.2% (urine) 5.8% (faeces)	29.7% (urine) 3.3% (faeces)	64.2% (urine) 2.9% (faeces)
Excretion in urine and faeces within 96 hours (% of radioactivity administered)	79.3% (urine) 12.4% (faeces)	82.3% (urine) 6.7% (faeces)	60.6% (urine) 14.9% (faeces)	41.7% (urine) 7.5% (faeces)	46.6% (urine) 7.1% (faeces)	66.6% (urine) 3.6% (faeces)
Total excretion in urine and faeces within 96 hours (% of radioactivity administered)	91.7%	88.9%	75.5%	49.2%	53.7%	70.2%
Total excretion in urine and faeces within 96 hours (% of radioactivity absorbed)	not determined	not determined	not determined	78.1%	76.7%	–
Distribution of metabolites in urine collected for 24 or 48 hours:	(24-hour urine)	(48-hour urine)	(24-hour urine)	(24-hour urine)	(48-hour urine)	
- 2-ethylhexanoic acid glucuronide	20.11%	45.03%	12.36%	3.95%	16.92%	not determined
- 2-ethyl-1,6-hexanedioic acid and 6-hydroxy-2-ethylhexanoic acid	14.26%	7.26%	11.56%	8.6%	3.44%	not determined
- 2-ethylhexanoic acid (% of radioactivity administered)	6.69%	2.39%	4.50%	1.56%	2.86%	not determined

b.w. body weight; C_{max} maximum plasma concentration; t_{max} time of maximum plasma concentration

Beginning of Table 2

Table 2. Acute toxicity studies with 2-ethylhexanoic acid

Species, strain, sex[1)]	Route of administration	Dose (mg/kg b.w. or mg/m³)	Purity	Effect	Observation period	Reference
Rat	oral	ca. 3640 (4 ml)	no information	LD_{50}; dyspnoea, apathy, lying on the abdomen; necropsy: no findings	7 days	BASF, 1967
Rat	oral	ca. 3276 (3.6 ml)	no information	LD_{50}; no characteristic signs of toxicity	8 days	BASF, 1953
Rat, Wistar, male	oral	3000	commercial grade	LD_{50}; 10000 mg/kg body weight lethal, 1000 mg/kg body weight not lethal; necropsy of deceased animals: liver mottled, stomach and upper intestine had an opaque white, cooked appearance	14 days	Mellon Institute, 1942; Smyth and Carpenter, 1944
Rat, F344, female	oral	2043	99.6%	LD_{50}; weakness, prostration, death within 24 hours after administration; necropsy of deceased animals: test compound in the gastrointestinal tract, faecal discoloration, wetness of the inguinal hair; terminal necropsy: no findings	14 days	Eastman Kodak, 1987 e
Rat	oral	1600–3200	no information	LD_{50}*	no information	Eastman Kodak, 1966, 1982
* Referred to by the authors as the LD_{50}, which is actually > 1600 but < 3200, as 3200 mg/kg b.w. were lethal while 1600 mg/kg b.w. were not.						
Guinea pig	oral	800–1600	no information	LD_{50}*	no information	Eastman Kodak, 1966, 1982
* Referred to by the authors as the LD_{50}, which is actually > 800 but < 1600, as 1600 mg/kg b.w. were lethal while 800 mg/kg b.w. were not.						
Rat, Wistar, male, female	dermal (24-hour occlusive application to the depilated dorsal skin)	> 2000	> 99%	LD_{50}; mortality: 0/10; 2/10 rats showed eschar formation from days 5 to 8 of the study, no other symptoms; terminal necropsy: no findings	14 days	Hoechst, 1986
Study conducted in accordance with EC directives and OECD guidelines.						

Table 2. Acute toxicity studies with 2-ethylhexanoic acid

Species, strain, sex[1)]	Route of administration	Dose (mg/kg b.w. or mg/m³)	Purity	Effect	Observation period	Reference
Rat	dermal	immersion of the depilated abdominal skin in 2 ml of neat substance, or 50% or 20% solutions in oil for 10 minutes up to 4 hours	no information	Exposure to concentrated product for more than 30 minutes caused drowsiness, lying on the side, and ultimately death within only a few hours. After a 10-minute exposure, 4/6 rats died. Exposure to the 50% oily solution for 4 hours caused death in 1 of 3 rats. Application of the 20% formulation was tolerated without findings except mild irritation.	no information	BASF, 1953
Rabbit	dermal	1260	no information	LD_{50}	no information	BASF, 1981 a; Union Carbide, 1971
Guinea pig	dermal	6300* (single 4-day application to the intact abdominal skin)	commercial grade	LD_{50}; 10000 mg/kg body weight were lethal in ca. 66% of the animals, 1000 mg/kg body weight were not lethal; deceased animals: skin was dry and cracked, survivors: no skin findings	14 days	Mellon Institute, 1942; Smyth and Carpenter, 1944
* According to the Mellon Institute's test report (1942), the LD_{50} is 6.3 g/kg body weight, while the publication by Smyth and Carpenter (1944) gives it as 6.3 ml/kg body weight (approx. 5733 mg/kg body weight).						
Guinea pig	dermal	ca. 5700	no information	LD_{50}	no information	BASF, 1978 a, b
Note: It is not clear whether this information in safety data sheets issued by BASF (1978 a, b) was taken from the publication by Smyth and Carpenter (1944), but in view of the similarity of the data, this seems likely.						
Guinea pig	dermal	ca. 4550 (5 ml neat substance)	no information	lethal within 2 days	no information	Eastman Kodak, 1966, 1982
Guinea pig	dermal	ca. 3640 (20 ml of a 20% solution in acetone and maize germ oil (corn oil))	no information	not lethal; body weight loss, mild irritation	no information	Eastman Kodak, 1982
Rat	inhalation	ca. 2356 (400 ppm, calculated, 6 hours)	no information	not lethal; no clinical signs of toxicity	no information	Eastman Kodak, 1966
Rat	inhalation	atmosphere enriched or saturated at 20 °C (8 hours)	no information	mortality: 0/12; no clinical signs of toxicity; necropsy: no findings	no information	BASF, 1967

Table 2. Acute toxicity studies with 2-ethylhexanoic acid

Species, strain, sex[1)]	Route of administration	Dose (mg/kg b.w. or mg/m³)	Purity	Effect	Observation period	Reference
Rat, albino, male	inhalation	atmosphere enriched or saturated at room temperature, (8 hours)	no information	mortality: 0/6	no information	Smyth and Carpenter, 1944
Guinea pig	inhalation	> ca. 2356 (400 ppm, 6 hours)	no information	LC_{50}	no information	Eastman Kodak, 1982
Guinea pig	inhalation	atmosphere enriched or saturated at room temperature, (8 hours)	no information	mortality: 0/6	no information	Mellon Institute, 1943
Mouse	subcutaneous	ca. 910 (1 ml)	no information	LD_{50}; no characteristic signs of toxicity	8 days	BASF, 1953
Rat	intraperitoneal	800–1600	no information	LD_{50}*	no information	Eastman Kodak, 1966, 1982
* Referred to by the authors as the LD_{50}, which is actually > 800 but < 1600, as 1600 mg/kg b.w. were lethal while lower doses (presumably only the 800 mg/kg b.w. dose was tested) were not.						
Mouse	intraperitoneal	ca. 273 (0.3 ml)	no information	LD_{50}; dyspnoea, apathy, lying on the abdomen, reeling, narcosis, late deaths; necropsy: isolated cases of adhesions in the abdominal cavity	7 days	BASF, 1967
Guinea pig	intraperitoneal	200–400	no information	LD_{50}*	no information	Eastman Kodak, 1966, 1982
* Referred to by the authors as the LD_{50}, which is actually > 200 but < 400, as 400 mg/kg b.w. were lethal while lower doses (presumably only the 200 mg/kg b.w. dose was tested) were not.						

1) If stated in the reference
b.w. body weight

End of Table 2

Table 3. Effects of 2-ethylhexanoic acid in exploratory subacute toxicity studies

Species, sex, number/group, parameters studied[1)]	Route of administration, duration of treatment/regimen	Dose (mg/kg b.w. or mg/m³)	Findings	Reference
Rabbit, 2 animals, mortality, clinical signs of toxicity	administered by oral gavage, 5 times within 5 days, and 10 times within 12 days	no controls 1000	 lethal, body weight loss, drowsiness, lying on the side, urinary cylinders and proteinuria; necropsy: mild pulmonary oedema in one rabbit, no other macroscopic findings	BASF, 1953
Administered as an aqueous solution adjusted to pH 7, containing 5% of the sodium salt (no details of purity).				
Rabbit, 2 animals, mortality, clinical signs of toxicity	administered by oral gavage, 2 times within 2 days, and 4 times within 4 days	no controls 1000	 lethal, body weight loss, drowsiness, lying on the side, urinary cylinders, urine sediment containing erythrocytes and leukocytes, uric acid crystals, diarrhoea; necropsy: haemorrhages in the gastrointestinal tract, much fluid in the lung of one rabbit	BASF, 1953
Administered as a 10% formulation in oil (no details of purity).				
Cat, 1 animal per dose level, mortality, clinical signs of toxicity	administered by oral gavage, a maximum of 7 times within 9 days	no controls 1000 100 10	 lethal after a single dose, imbalance, proteinuria; necropsy without findings lethal after the second dose, lying on the side, unconsciousness; necropsy without findings lethal one day after the last dose, vomiting after the last dose, poor general condition, lying on the side, differential blood count shifted to the left; necropsy: circumscribed, fibrinopurulent pleuritis, microscopically visible slight bronchopneumonia	BASF, 1953
Administered as a 20% and a 1% formulation in oil (no details of purity).				
Cat, 1, and 2 cats per dose, mortality, clinical signs of toxicity	administered by oral gavage, a maximum of 40 times within 58 days	no controls 1000 100 (10 times as a 5% solution) 100 (17 times as a 10% solution) 10 (25, and 40 times as a 1% solution)	 lethal after the 2nd or the 3rd dose, imbalance, lying on the side; necropsy without findings not lethal, initial imbalance, elevated erythrocyte and leukocyte counts, proteinuria not lethal, no clinical findings, increase in blood eosinophils and lymphocytes, decrease in neutrophil counts, no urine findings; necropsy: no macroscopic or microscopic findings not lethal, vomiting, diarrhoea, blood count without findings, one cat with proteinuria; necropsy: no macroscopic or microscopic findings (necropsy carried out only in one cat)	BASF, 1953
Administered as aqueous solutions containing 10%, 5% or 1% of the sodium salt (no details of purity) and adjusted to pH 7.				

[1)] If stated in the reference

Beginning of Table 4

Table 4. Subacute toxicity studies with 2-ethylhexanoic acid

Species, number/group, regimen (reference)	Dose (mg/kg b.w.)	Mortality	Clinical signs	Macroscopic/histopathological changes	Clinical chemistry/ haematology findings
F344 rat, 5 males and 5 females per dose, oral gavage, 5 days per week, 15 days (11 doses)	controls	0/10	no findings	no findings	not investigated
	200	0/10	no findings	increased relative liver weight (females)	not investigated
	800	0/10	weakness, lethargy, sialorrhoea, unkempt fur (females only), depressed body weight gain (males only)	increased relative and absolute liver weights, minimal hepatocyte hypertrophy (one male)	not investigated
	1600	8/10*	weakness, lethargy, sialorrhoea, hypothermia, urine-soiled fur, porphyrin nasal discharge, tremor, depressed body weight gain, reduced feed consumption	increased relative and absolute liver weights; deceased and sacrificed rats exhibited minor cytoplasmic vacuolisation and degeneration of hepatocytes; minimal hepatocyte hyper-trophy in the 2 rats killed at end of study	not investigated
* The animals died spontaneously or were killed *in extremis* on the first or second day of the study. GLP Study conducted in accordance with OECD guideline No. 407 as promulgated by the EPA (1986), histopathological examination was restricted to the liver, kidneys and any gross organ lesions; purity of the 2-ethylhexanoic acid administered was 99.8 to 99.9%. (Eastman Kodak, 1987 a)					
F344 rat, 5 males and 5 females per dose, given orally in the feed*, daily for 15 days	controls	0/10	no findings	no findings	not investigated
	706 (males) 756 (females)	0/10	no findings	dose-dependent increase in relative and absolute liver weights in all dose groups, and hepatocyte hypertrophy and coagulation necrosis of the liver in the two higher dose groups	not investigated
	1351 (males) 1411 (females)	0/10	depressed body weight gain (males) in conjunction with decreased feed consumption (males and females), urine-discoloured hair in the inguinal region (females)		not investigated
	2276 (males) 2658 (females)*	0/10	depressed body weight gain in conjunction with reduced feed consumption, urine-discoloured hair in the inguinal region (females); periodically poor general condition in one female		not investigated
* 0.75, 1.5 and 3% were administered in the feed, converted into mg/kg body weight on the basis of the authors' information; purity of the 2-ethylhexanoic acid used was 99.9%. GLP Study conducted in accordance with OECD guideline No. 407 as promulgated by the EPA (1986), histopathological examination was restricted to the liver, kidneys and any gross organ lesions. (Eastman Kodak, 1987 c)					

Table 4. Subacute toxicity studies with 2-ethylhexanoic acid

Species, number/group, regimen (reference)	Dose (mg/kg b.w.)	Mortality	Clinical signs	Macroscopic/histopathological changes	Clinical chemistry/ haematology findings
B6C3F1 mouse, 5 males and 5 females per dose, oral gavage, 5 days per week, 15 days (11 doses)	controls	1/10*	no findings	no findings	not determined
	200	0/10	no findings	no findings	not determined
	800	1/10	no findings	no findings	not determined
	1600	0/10	one female with gait disturbance, weakness and weight loss during the first two days of the study	only the males had increased relative and absolute liver weights and hepatocyte hypertrophy	not determined
* One male was killed in moribund condition resulting from a gavage error. GLP Study conducted according to OECD guideline No. 407 as promulgated by the EPA (1986); histopathological examination was restricted to the liver, kidneys and any gross organ lesions; purity of the 2-ethylhexanoic acid administered was 99.8 to 99.9%. (Eastman Kodak, 1987 b)					
B6C3F1 mouse, 5 males and 5 females per dose, oral in the feed*, daily for 15 days	controls	0/10	no findings	no findings	not determined
	1608 (males) 1965 (females)	1/10	reduced feed consumption (males)	in all dose groups: dose-dependently increased absolute and relative liver weights, hepatocyte hypertrophy and coagulation necrosis of the liver; top dose group: additionally, small spleens and thymuses with thymic cortex atrophy and splenic red pulp atrophy (considered by the authors to be stress-related rather than substance-related)	not determined
	3084 (males) 3986 (females)	0/10	slightly depressed body weight gain (males) in conjunction with reduced feed consumption		not determined
	5794 (males) 9229 (females)*	0/10	depressed body weight gain in conjunction with reduced feed consumption (males, females)		not determined
* 0.75, 1.5 and 3% were administered in the feed, converted into mg/kg body weight on the basis of the authors' information; they point out, however, that the data for the females offered the 3% diet may be inaccurate due to small sample size and variability of the data; purity of the 2-ethylhexanoic acid used was 99.9%. GLP Study conducted in accordance with OECD guideline No. 407 as promulgated by the EPA (1986); histopathological examination was restricted to the liver, kidneys and any gross organ lesions. (Eastman Kodak, 1987 d)					

End of Table 4

Beginning of Table 5

Table 5. Skin irritancy of 2-ethylhexanoic acid					
Species	Guideline and/or dose, route, duration[1)]	Findings	Reversibility	Assessment (by the authors)	Reference
Rabbit	EC directive 84/449/EEC (sample purity: 99%)	up to 14 days after application mild to clearly circumscribed erythema and very slight to slight oedema, accompanied by dry, chapped, hardened, parchment-like skin with light-brown discoloration; numerical evaluation (mean of 3 animals) for erythema and eschar formation was 0.7, for oedema formation 0.3	Irritation not reversible within 14 days, no further examinations carried out after day 14	not irritating (according to EC directive 83/467/EEC)	Hoechst, 1985 a
Note: According to the current classification directive 93/21/EEC and based on the results of this study, 2-ethylhexanoic acid should, however, be evaluated as irritating to the skin, as 2 of the 3 animals still showed clear signs of irritation at the end of the observation period. One animal was found to have very mild erythema and very slight oedema, while the second one had clearly circumscribed erythema and very minor oedema. In addition, both rabbits had dry, chapped, parchment-like skin and in one animal, the skin displayed large areas with light-brown discoloration.					
Rabbit	Test in accordance with a U.S. Department of Transportation guideline (21 CFR § 191.11), neat substance, 4-hour occlusive application (no details of sample purity)	necrosis in 2 of 4 animals	no information	corrosive	Mellon Institute, 1972 b
Rabbit	undiluted substance (no details of sample purity)	moderate to marked erythema	no information	mildly irritating grade 4 (maximum grade not given)	Mellon Institute, 1972 a
Rabbit	undiluted substance, dorsal skin, 1, 5, 15 minutes and 20 hours, and skin of the ear, 20 hours (BASF test, no details of sample purity)	applied for 1 or 5 minutes: no findings; applied for more than 15 minutes: questionable reddening after 24 hours, slight desquamation after 8 days; applied for more than 20 hours: dorsal skin: marked reddening and minor oedema after 24 hours, questionable reddening and heavy desquamation after 8 days; applied for more than 20 hours: skin of the ear: slight reddening after 24 hours, slight reddening after 8 days and minor macular necrosis, heavy desquamation	irritating when duration of exposure > 5 minutes, not reversible within 8 days, no further examinations carried out after day 8	moderately irritating	BASF, 1967, 1978 a, b
Rabbit	undiluted substance, 20 hours, dorsal skin (no details of sample purity)	severe necrotic inflammation	after 23 days no findings, and coarse scaly desquamation	severely irritating	BASF, 1953

Table 5. Skin irritancy of 2-ethylhexanoic acid

Species	Guideline and/or dose, route, duration[1)]	Findings	Reversibility	Assessment (by the authors)	Reference
Rabbit	undiluted substance, 20 hours, skin of the ear (no details of sample purity)	severe necrotic inflammation	after 23 and 30 days: perforation of the ear, and hard transauricular scab	severely irritating	BASF, 1953
Rabbit	50%, 20%, 10% and 5% solutions in oil, 20 hours, dorsal skin (no details of sample purity)	mild irritation 2 days after application, 5% formulation was without findings	no findings after 3 days	no information	BASF, 1953
Rabbit	50%, 20% and 10% solutions in oil, 20 hours, skin of the ear (no details of sample purity)	concentration-dependent, more severe skin injury with oedema formation and necrotic changes	no information	no information	BASF, 1953
Rabbit	undiluted substance, 4 hours (no details of sample purity)	in 5 of 6 animals: minor necrosis, later followed by slight to moderate eschar formation	no information	no information	Eastman Kodak, 1986
Rabbit	undiluted substance and 10% or 1% solutions in acetone, abdominal skin (“vesicant test”, “commercial grade” given as indication of purity, no further details)	undiluted substance and 10% solution: erythema formation; 1% solution: no findings	no information	no information	Mellon Institute, 1942
Rabbit	0.01 ml undiluted substance, depilated ab-dominal skin (no details of sample purity)	irritation corresponded to that caused by morpholine	no information	no information	Smyth and Carpenter, 1944
Rat	1000 mg 99.6% undiluted substance/kg body weight, occlusive application for 24, 48, 72, 96 hours, depilated dorsal skin (preliminary study to a dermal toxicokinetic study; sample purity > 99%)	macroscopic examination: minimal changes in shape of epidermal scales and slight reddening; histopathology: marked epidermal injury with necrotic and inflammatory changes, complete absorption of the applied dose within the first 24 hours	signs of continued epidermal regener-ation 48 to 96 hours after application; no further examination after 96 hours	no information	Eastman Kodak, 1987 g

Table 5. Skin irritancy of 2-ethylhexanoic acid

Species	Guideline and/or dose, route, duration[1)]	Findings	Reversibility	Assessment (by the authors)	Reference
Guinea pig	5, 10 and 20 ml undiluted substance or a 20% solution in acetone/maize germ oil (corn oil; 90/10), 24-hour occlusive application (no details of sample purity)	undiluted substance: minor oedema, erythema and necrosis; 20% formulation: no or very minor oedema with slight to moderate erythema formation	no information	no information	Eastman Kodak, 1955
No information	undiluted substance, 24 hours, occlusive (no details of sample purity)	mild irritation	no information	no information	Eastman Kodak, 1966, 1982

[1)] If stated in the reference

End of Table 5

Beginning of Table 6

Table 6. Mucous membrane effects of 2-ethylhexanoic acid

Species	Guideline and/or dose, route, duration[1)]	Findings	Reversibility	Assessment (by the authors)	Reference
Rabbit	EC directive 84/449/EEC (sample purity 99%)	slight to marked swelling as well as marked hyperaemia to diffusely crimson-coloured conjunctivae; in 2 of 3 animals: reddening of the iris and slight clouding of the cornea, initially, clear, colourless ocular discharge followed by white muciform ocular discharge; one hour after instillation: whitish discoloration of the conjunctiva and nictitating membrane, numerical evaluation (mean of 3 animals): clouding of the cornea 0.44, iritis 0.56, reddening 1.2 and conjunctival swelling 0.89	reddening and swelling of the conjunctiva as well as iritis 7 days upon instillation, clouding of the cornea 72 hours upon instillation was reversible in all animals	not irritating (according to EC directive 83/467/EEC)	Hoechst, 1985 b
Rabbit	50 µl undiluted substance, single application (BASF test, no details of sample purity)	severe reddening and oedema formation as well as slight clouding after one hour; severe reddening, slight oedema, slight clouding and mucous membrane haemorrhage after 24 hours; slight reddening and questionable clouding after 8 days	not reversible within 8 days, no further examination after day 8	severely irritating, may cause damage to the cornea	BASF, 1967, 1978 a, b
Rabbit	undiluted substance (no details of sample purity)	severe reddening and swelling, ocular discharge, clouding of the cornea, iritis	persistent clouding of the cornea and ocular discharge in 1 of 2 animals after 6 days; 2nd animal was without findings	severely irritating	BASF, 1953
Rabbit	50 and 20% solutions in oil (no details of sample purity)	concentration-dependent reddening, swelling, lacrimation and clouding of the cornea	no findings after 6 and 3 days, depending on concentration	no information	BASF, 1953
Rabbit	0.005 ml undiluted substance as well as 0.5 ml of 40%, 15%, 5% and 1% solutions in propylene glycol (no details of sample purity)	undiluted substance as well as 40%, 15%, 5% solutions: marked corneal damage, mild iritis; 1% solution: mild irritation	no information	grade 9 (maximum grade not given)	Mellon Institute, 1972 a

Table 6. Mucous membrane effects of 2-ethylhexanoic acid

Species	Guideline and/or dose, route, duration[1)]	Findings	Reversibility	Assessment (by the authors)	Reference
Rabbit	0.001 ml undiluted substance (“commercial grade” as indication of purity, no further details)	corneal necrosis	no information	grade 5 (maximum grade not given)	Mellon Institute, 1942
Rabbit	0.02 and 0.05 ml undiluted substance (no details of sample purity)	no information	no information	grade 5 (the maximum grade being 10)	Carpenter and Smyth, 1946
Rabbit	undiluted substance (“commercial grade” as indication of purity)	irritation corresponded to that caused by acetic anhydride	no information	no information	Smyth and Carpenter, 1944

[1)] If stated in the reference

End of Table 6

Beginning of Table 7

Table 7. Reproductive toxicity of 2-ethylhexanoic acid

Type of study, test system (reference)	Dose (mg/kg b.w. per day)	Parental toxicity	Embryotoxicity/foetotoxicity	Teratogenicity	Development of offspring
Teratogenicity/embryotoxicity study, Fischer-344 rat, 25 animals/dose level, oral administration by gavage on days 6 to 15 of gestation, sacrifice on day 21 of gestation, purity of the 2-ethylhexanoic acid used: 99.4%, given in maize germ oil (corn oil; GLP study as promulgated by the EPA (1986)) (Bushy Run, 1988 a; Fisher et al., 1989; Hendrickx et al., 1993)	controls	no findings	no findings	no findings	not determined
	100	no findings	no findings	no findings	not determined
	250	no findings	number of skeletal variations (reduced ossification) increased	no findings	not determined
	500	hypoactivity, ataxia, audible respiration, ocular discharge and periocular encrustation, absolute and relative liver weight increased	reduced foetal weights, number of visceral variations (dilatation of the lateral ventricle of the brain with no tissue compression) and skeletal variations (reduced ossification, variations at vertebra 14) increased	no findings	not determined
Teratogenicity/embryotoxicity study, Fischer-344 rat, 8 animals/dose level, oral administration by gavage on days 6 to 15 of gestation, sacrifice on day 21 of gestation, purity of the 2-ethylhexanoic acid used: 99.4%, given in maize germ oil (corn oil; dose-finding study for the above-cited study Bushy Run, 1988 a; Fisher et al., 1989; Hendrickx et al., 1993; foetuses inspected for external malformations only) (Hendrickx et al., 1993)	controls	no findings	no findings	no findings	not determined
	125	no findings	no findings	no findings	not determined
	250	no findings	no findings	no findings	not determined
	500	no findings	increased level of postimplantation loss (early and late resorptions, dead foetuses), decrease in percentage of live foetuses and foetal weights	no findings	not determined
	1000	mortality: 7/8, ataxia, audible respiration, read periocular encrustation, unkempt fur	litter completely resorbed in the one surviving female	no findings	not determined

Table 7. Reproductive toxicity of 2-ethylhexanoic acid

Type of study, test system (reference)	Dose (mg/kg b.w. per day)	Parental toxicity	Embryotoxicity/foetotoxicity	Teratogenicity	Development of offspring
Teratogenicity/embryotoxicity stuy, Han:Wistar rat, 20 and 21 animals/dose level, oral administration in the drinking water on gestation days 6 to 19, sacrifice on day 20 of gestation, purity of the 2-ethylhexanoic acid used: 99.5%, given as the sodium salt in the form of an equimolar NaOH solution	controls	no findings	no findings	malformations in 2.4% of the foetuses*	not determined
	100	no findings	skeletal variations (wavy ribs, reduced ossification)	malformations in 4.9% of the foetuses (not statistically significant)*	not determined
	300	placental weight slightly reduced	weight of female foetuses slightly reduced, skeletal variations (wavy ribs, twisted hind legs), slight but not statistically significant reduction in pregnancy rate	8.9% of the foetuses (statistically significant)*	not determined
	600	reduced body weight at end of study (11%), reduced consumption of drinking water, placental weight slightly reduced	foetal weight slightly reduced, skeletal variations (wavy ribs, reduced or no ossification), slight but not statistically significant reduction in pregnancy rate	malformations in 15.3% of the foetuses (statistically significant)*	not determined

* Dose-dependent increases were seen numerically at and above 100 mg/kg body weight and were shown to be statistically significant at and above 300 mg/kg body weight for the overall number of malformations and club foot as a single malformation. In addition, the authors observed the following alterations to which they referred as malformations and which were mostly dose-dependent but not statistically significant: flabby legs and slight scoliosis and lordosis in all dose groups; abnormal cartilage in the ankle at 300 mg/kg body weight and above, and absence of the fibula as well as extra thoracic ribs in the top dose group; further significant visceral malformations included non-dose-dependent dilatation of the urinary tract in the low and mid dose groups, and of the brain ventricle in the top dose group (type not specified, but according to the authors' discussion of their results this malformation correlated with reduced foetal weight, implying that this change might more appropriately be considered a foetotoxic rather than a teratogenic effect; cf. above-mentioned study Bushy Run, 1988 a; Fisher et al., 1989; Hendrickx et al., 1993)). No such changes occurred in the control group, with the exception of extra ribs, urinary tract dilatation and ventricular dilatation. In summary, 2-ethylhexanoic acid was evaluated by the authors as teratogenic at doses which are not maternally toxic.
(Pennanen et al., 1992 b, c)
Note: The publications by Pennanen et al., 1992 b, c, report slightly different incidence figures for what is obviously the same study. The data cited above are those from the detailed publication by Pennanen et al., 1992 b.

Table 7. Reproductive toxicity of 2-ethylhexanoic acid

Type of study, test system (reference)	Dose (mg/kg b.w. per day)	Parental toxicity	Embryotoxicity/foetotoxicity	Teratogenicity	Development of offspring
Teratogenicity/embryotoxicity study, Wistar rat, 7 to 10 animals/dose level, single dose by oral gavage on day 12 of gestation, sacrifice on day 20 of gestation, 2-ethylhexanoic acid administered in undiluted form, no details of purity of the 2-ethylhexanoic acid used	controls	no information	9.6 ± 4.1% of implantations were resorbed or died, mean foetal weight: 4.1 g	no malformations	not determined
	ca. 910 (1 ml)	no information	5.9 ± 2.4% of implantations were resorbed or died, mean foetal weight: 4.0 g*	0.8 ± 0.8% of foetuses (presumably 1 animal) with hydronephrosis*	not determined
	ca. 1820 (2 ml)	no information	12.9 ± 3.3% of implantations were resorbed or died, mean foetal weight: 2.9 g*	67.8 ± 10.9% of foetuses had malformations, of which 51.2% afflicted the limbs, 15.5% the tail, 20.9% hydronephrosis, 10.1% heart defects and 10.9% unspecified malformations*	not determined
	ca. 910 (1 ml) plus 150 mg caffeine/kg b.w. by intraperitoneal injection	no information	15.2 ± 6.0% of implantations were resorbed or died, mean foetal weight: 3.6 g*	31.5 ± 15.4% of foetuses had malformations*	not determined

* Despite statistical analysis, no precise details are given as to the extent to which these data differ from those of the control group. Overall, the authors evaluated 2-ethylhexanoic acid as teratogenic. A parallel study with additional administration of caffeine showed, in the authors' opinion, that caffeine enhances the teratogenic effect of 2-ethylhexanoic acid.

(Ritter et al., 1985, 1987; University of Cincinnati, 1985)

Note: Lack of information on maternal toxicity and clear indications as to the significance of the reported findings (particularly with respect to the low dose group) limit the suitability of the study for evaluating the reproductive toxicity of 2-ethylhexanoic acid. The publications by Ritter et al., 1985, 1987 and the University of Cincinnati, 1985, report slightly different incidence figures for what is obviously the same study. The data cited above were taken from the detailed publication by Ritter et al., 1987.

Table 7. Reproductive toxicity of 2-ethylhexanoic acid

Type of study, test system (reference)	Dose (mg/kg b.w. per day)	Parental toxicity	Embryotoxicity/foetotoxicity	Teratogenicity	Development of offspring
Teratogenicity/embryotoxicity study, Sprague-Dawley rat, 7 to 10 animals/group, single dose by oral gavage on day 12 of gestation, sacrifice on day 20 of gestation, 2-ethylhexanoic acid was given in undiluted form, no details of purity of the 2-ethylhexanoic acid used	controls	no precise details (no increase in maternal mortality)	6% of implantations were resorbed or died, mean foetal weights: 3.90 (male foetuses) and 3.71 g (female foetuses)	no information	not determined
	ca. 1803 (12.5 mmol/kg)	no precise details (no increase in maternal mortality)	14% of implantations were resorbed or died, mean foetal weights: 2.82 (male foetuses) and 2.68 g (female foetuses)	examination of 97 foetuses in total, describing 41 individual malformations *	not determined
	ca. 2253 (15.625 mmol per kg)	no precise details (no increase in maternal mortality)	60% of implantations were resorbed or died, mean foetal weights: 2.06 (male foetuses) and 1.93 g (female foetuses)	no information	not determined
* Of a total of 97 examined foetuses, all were inspected for external, 65 for visceral and 32 for skeletal malformations. Malformations reported afflicted the limbs (20/97 foetuses with ectrodactyly or polydactyly and 5 additional changes of radius or fibula in 32 examined foetuses), the cardiovascular system (8 individual malformations in 65 examined foetuses), the urogenital system (hydronephrosis in 6 of 65 examined foetuses) and the tail (2 of 97 examined foetuses). No statistical analysis was performed. Note: Lack of information on maternal toxicity (except maternal lethality) and indications as to the significance of the reported findings limit the suitability of the study for evaluating the reproductive toxicity of 2-ethylhexanoic acid. (Scott et al., 1994)					
Teratogenicity study, Sprague-Dawley rat, oral administration on day 12 of gestation (no further details)	no information on controls which may have been included				
	ca. 2048 (2.25 ml)	no information	no information	teratogenic (no further details)	no information
According to the authors, the teratogenic effect correlated with an increase in intracellular pH in the embryos one hour after administration (no precise details given in the available abstract). (Collins et al., 1986)					

Table 7. Reproductive toxicity of 2-ethylhexanoic acid

Type of study, test system (reference)	Dose (mg/kg b.w. per day)	Parental toxicity	Embryotoxicity/foetotoxicity	Teratogenicity	Development of offspring
Teratogenicity/embryotoxicity study, New Zealand rabbit, 15 animals/dose level, oral administration by gavage on days 6 to 18 of gestation, sacrifice on day 29 of gestation, purity of the 2-ethylhexanoic acid used: 99.4%, given in maize germ oil (corn oil; GLP study as promulgated by EPA (1986)) (Bushy Run, 1988 b; Fisher et al., 1989; Hendrickx et al., 1993)	controls	no findings	no findings	no findings	not determined
	25	no findings	no findings	no findings	not determined
	125	mortality 1/15, 1 female aborted	no findings	no findings	not determined
	250	mortality 1/15, depressed body weight gain in conjunction with reduced feed consumption (days 18 to 29 of gestation), signs that were not statistically significant but occurred only in this dose group (e. g. ataxia, hypoactivity, breathing difficulties)	no findings	no findings	not determined
Teratogenicity/embryotoxicity study, New Zealand rabbit, 8 animals/dose level, oral administration by gavage on days 6 to 18 of gestation, sacrifice on day 29 of gestation, purity of the 2-ethylhexanoic acid: 99.4%, given in maize germ oil (corn oil; dose-finding study for the above-cited study Bushy Run, 1988 b; Fisher et al., 1989; Hendrickx et al., 1993, foetuses inspected for external malformations only)	controls	mortality: 1/8	no findings	no findings	not determined
	125	mortality: 1/8; 1 female aborted	mean foetal weight reduced*	no findings	not determined
	250	mortality: 1/8; 1 female aborted, hypoactivity	no findings	no findings	not determined
	500	mortality: 7/8, hypoactivity	no findings	no findings	not determined
	1000	mortality: 8/8, hypoactivity, laboured breathing, ataxia	not determined	not determined	not determined

* Attributed by the authors to larger litter size (11.3 foetuses/litter) relative to the controls (7.0 foetuses/litter). (Hendrickx et al., 1993)

Table 7. Reproductive toxicity of 2-ethylhexanoic acid

Type of study, test system (reference)	Dose (mg/kg b.w. per day)	Parental toxicity	Embryotoxicity/foetotoxicity	Teratogenicity	Development of offspring
Teratogenicity/embryolethality study, NMRI mouse, 9 to 20 litters/dose evaluated, i.p. administration once on day 8 of gestation or b.i.d. at 12-hour intervals on days 7 and 8 of gestation (as the sodium salt), sacrifice on day 18 of gestation, examination only with respect to embryolethality, foetal weight and exencephaly	controls	no information	6% of implantations were resorbed or died	no exencephaly	not determined
	4 x 500 (*R*-(–)-enantiomer)	no information	11% of implantations were resorbed or died, foetal weight slightly reduced	exencephaly in 59% of foetuses	not determined
	4 x 500 (*S*-(+)-enantiomer)	no information	1% of implantations were resorbed or died	exencephaly in 1% of foetuses	not determined
	4 x 500 (racemate)	no information	10% of implantations were resorbed or died	exencephaly in 32% of foetuses	not determined
	1 x 500 (racemate)	no information	7% of implantations were resorbed or died	exencephaly in 5% of foetuses	not determined

Note: No information regarding statistical analysis. The carbon atom at position 2 of 2-ethylhexanoic acid is asymmetrical. Hence, 2-ethylhexanoic acid exists as two enantiomers, *R*-(–)- and *S*-(+)-2-ethylhexanoic acid. However, only the mixture of the two enantiomers, racemic (+/–)-2-ethylhexanoic acid, is of technical importance. The objective of the cited study was to compare the two enantiomers for potential differences in teratogenic potency (induction of neural tube defects in the form of exencephaly). As can be seen from the table above, the result was that the *S*-(+)-enantiomer had no teratogenic effect while the *R*-(–)-enantiomer was a potent teratogen (exencephaly in 59% of foetuses), and that the teratogenic potential of the racemate was in between that of the two enantiomers (exencephaly in 32% of foetuses). In the discussion of their results, the authors suggested that the marked differences in teratogenic effect could be due to stereoselective interactions of the enantiomers with proteins (enzymes, receptors) or other chiral cellular constituents. Optical purity of the *R*-(–)- and *S*-(+)-enantiomers was 93% and 90%, respectively (cf. Collins et al., 1992).
(Hauck et al., 1990)

Table 7. Reproductive toxicity of 2-ethylhexanoic acid

Type of study, test system (reference)	Dose (mg/kg b.w. per day)	Parental toxicity	Embryotoxicity/foetotoxicity	Teratogenicity	Development of offspring
Teratogenicity/embryolethality study, SWV mouse, 6 to 10 litters/dose evaluated, i.p. administration t.i.d. at 12-hour intervals on days 8, 8.5 and 9 of gestation, sacrifice on day 17.5 or 18 of gestation, examination for embryolethality and skeletal and visceral malformations, particularly exencephaly	no controls, no information on statistical analysis				
	3 x 576* (*S*-(+)-enantiomer)	no information	12.5% (13/104) of implantations died or were resorbed	no exencephaly or marked increase in other teratogenic alterations	not determined
	3 x 864* (*S*-(+)-enantiomer)	mortality 4/10	18% (12/68) of implantations died or were resorbed	exencephaly in 18% (10/56) of foetuses	not determined
	3 x 576* (racemate)	no information	14% (17/122) of implantations died or were resorbed	exencephaly in 43.8% (46/105) of foetuses	not determined
	3 x 403* (*R*-(–)-enantiomer)	no information	9% (6/67) of implantations died or were resorbed	no exencephaly	not determined
	3 x 518* (*R*-(–)-enantiomer)	no information	20% (21/105) of implantations died or were resorbed	exencephaly in 33.3% (28/84) of foetuses	not determined
	3 x 576* (*R*-(–)-enantiomer)	no information	11.8% (14/119) of implantations died or were resorbed	exencephaly in 50% (53/105) of foetuses and additional mal-formations, particularly of the vertebrae and ribs, as well as absence of eye lids and/or open eyes	not determined

* The racemate, (+/–)-2-ethylhexanoic acid, and the separate enantiomers were administered as the sodium salts (403, 518, 576 and 864 mg of which correspond to ca. 350, 450, 500 and 750 mg of the hexanoate anion). The two enantiomers had an optical purity of > 99%.

Note: The carbon atom at position 2 of 2-ethylhexanoic acid is asymmetrical. Hence, 2-ethylhexanoic acid exists as two enantiomers, *R*-(–)- and *S*-(+)-2-ethylhexanoic acid. However, only the mixture of the two enantiomers, racemic (+/–)-2-ethylhexanoic acid, is of technical importance. The objective of the cited study was to compare the two enantiomers for potential differences in teratogenic potency. As shown in the table above and reported in a previous publication by the same team of researchers (Hauck et al., 1990; see above), the result was that the *S*-(+)-enantiomer showed no or no clear teratogenic effect while the *R*-(–)-enantiomer was a potent teratogen, and that the teratogenic potential of the racemate was in between that of the two enantiomers. In a pharmacokinetic study carried out in parallel, these differences in activity were not found to be attributable to any differences in the pharmacokinetics of the two enantiomers.

(Collins et al., 1992)

Table 7. Reproductive toxicity of 2-ethylhexanoic acid

Type of study, test system (reference)	Dose (mg/kg b.w. per day)	Parental toxicity	Embryotoxicity/foetotoxicity	Teratogenicity	Development of offspring
Teratogenicity/embryolethality study, SWV mouse, small, varying number of animals per dose level, i.p. or s.c.), administration once daily on day 8 or 8.5 of gestation, sacrifice on day 17.5 or 18 of gestation, examination only with respect to embryolethality and exencephaly	no controls, no statistical analysis				
	doses ± 864 i.p.*	maternally lethal	no information	no information	not determined
	doses < 864 i.p.*	not lethal	no information	relatively low incidence of exencephaly (no precise details)	not determined
	807 s.c.* (day 8 of gestation)	mortality: 0/3	12.8% (5/39) of implantations were resorbed	exencephaly in 8.8% (3/34) of foetuses	not determined
	864 s.c.* (day 8 of gestation)	mortality: 1/7	22% (13/59) of implantations were resorbed	exencephaly in 10.9% (5/46) of foetuses	not determined
	864 s.c.* (day 8.5 of gestation)	mortality: 0/3	39.5% (15/38) of implantations were resorbed	no exencephaly	not determined
	1037 s.c.* (day 8 of gestation)	mortality: 5/6	27.3% (3/11) of implantations were resorbed	no exencephaly	not determined
* The racemate, (+/–)-2-ethylhexanoic acid, was administered as the sodium salt (807, 864 and 1037 mg of which correspond to ca. 700, 750 and 900 mg of the hexanoate anion, respectively). The objective of the study was to establish the dose of 2-ethylhexanoic acid which causes exencephaly in SWV mice. The fact that no control group was included and no statistical analysis was performed render the study unsuitable for evaluating the potential of 2-ethylhexanoic acid in terms of its reproductive toxicity. (Collins et al., 1992)					
Teratogenicity/embryolethality study, SWV and C57BL/6NCrlBR mouse, 19 and 22 litters in the controls, 10 and 11 litters treated with 2-ethylhexanoic acid were evaluated, 4 i.p. doses at 12-hour intervals on days 7.5, 8, 8.5 and 9 of gestation, sacrifice on day 17.5 or 18 of gestation, examination only with respect to embryo-lethality and exencephaly	controls (SWV mouse)	no information	8.6% (20/232) of implantations died or were resorbed	no exencephaly	not determined
	4 x 576* (SWV mouse)	no information	21.2% (28/132) of implantations died or were resorbed (statistically significant)	exencephaly in 49% (51/104) of foetuses (statistically significant)	not determined
	controls (C57 mouse)	no information	12.5% (22/176) of implantations died or were resorbed	no exencephaly	not determined
	4 x 576* (C57 mouse)	no information	17.2% (17/99) of implantations died or were resorbed (not statistically significant)	exencephaly in 7.3% (6/82) of foetuses (not statistically significant)	not determined
* The racemate, (+/–)-2-ethylhexanoic acid, was administered as the sodium salt (576 mg of which correspond to ca. 500 mg of the hexanoate anion). The objective of the study was to select the mouse strain which was more sensitive to 2-ethylhexanoic acid (SWV mouse). (Collins et al., 1992)					

Table 7. Reproductive toxicity of 2-ethylhexanoic acid

Type of study, test system (reference)	Dose (mg/kg b.w. per day)	Parental toxicity	Embryotoxicity/foetotoxicity	Teratogenicity	Development of offspring
Teratogenicity/embryolethality study, SWV mouse, 7 to 10 litters/interval of administration were evaluated, 3 i.p. doses at 12-hour intervals on days 7 to 10 of gestation, sacrifice on day 17.5 or 18 of gestation, examination only with respect to embryolethality and exencephaly	no controls 3 x 576* (on days 7, 7.5 and 8 of gestation)	no information	12% (13/110) of implantations died or were resorbed	exencephaly in 30% (29/97) of foetuses	not determined
	3 x 576* (on days 7.5, 8 and 9.5 of gestation)	no information	6.3% (6/95) of implantations died or were resorbed	exencephaly in 24% (21/89) of foetuses	not determined
	3 x 576* (on days 8, 8.5 and 9 of gestation)	no information	14% (17/122) of implantations died or were resorbed	exencephaly in 44% (46/105) of foetuses (significantly different from the other intervals of administration)	not determined
	3 x 576* (on days 8.5, 9 and 9.5 of gestation)	no information	3.5% (4/115) of implantations died or were resorbed	exencephaly in 12% (13/111) of foetuses	not determined
	3 x 576* (on days 9, 9.5 and 10 of gestation)	no information	25% (22/88) of implantations died or were resorbed	no exencephaly	not determined

* The racemate, (+/–)-2-ethylhexanoic acid, was administered as the sodium salt (576 mg of which correspond to ca. 500 mg of the hexanoate anion). The objective of the study was to identify the time of gestation at which sensitivity to induction of exencephaly was highest (days 8 to 9 of gestation). (Collins et al., 1992)

Table 7. Reproductive toxicity of 2-ethylhexanoic acid

Type of study, test system (reference)	Dose (mg/kg b.w. per day)	Parental toxicity	Embryotoxicity/foetotoxicity	Teratogenicity	Development of offspring
Screening test according to Chernoff and Kavlock (including examination of the dead foetuses for visceral alterations and examination of the foetuses killed at the end of the study for skeletal alterations), Sprague-Dawley rat, 15 to 20 animals per dose level, oral administration by gavage on days 6 to 15 of gestation, natural delivery, pups sacrificed on postnatal day 6, purity of the 2-ethylhexanoic acid used: > 99% (Narotsky et al., 1989, 1991, 1994)	controls	no findings	no findings	no findings	no findings
	900	mortality 4/15, ataxia, decreased motor activity, dyspnoea, initially body weight loss, later depressed body weight gain	gestation length increased by one day, increased postimplantation loss, reduced foetal weight, extra presacral vertebrae and lumbar ribs	no findings	retarded body weight gain, increased pup mortality up to postnatal day 6
	1200	mortality 6/15, ataxia, decreased motor activity, dyspnoea, body weight loss	gestation length increased by one day, pups examined on postnatal day 6, extra sacral vertebrae and lumbar ribs	no findings	pups not examined at birth, losses up to postnatal day 6 were ca. 76% relative to the implanted foetuses, as compared with 9% losses in the controls

End of Table 7

Beginning of Table 8

Table 8. Studies on the induction of peroxisome proliferation by 2-ethylhexanoic acid

Species, number/group, regimen (reference)	Dose (mg/kg b.w. per day)	Liver enzyme alterations	Macroscopic/histopathological liver changes	Other findings
C57BL/6 mouse, 3 males/dose or group, daily oral dietary administration for 4 days, purity of the 2-ethylhexanoic acid used: > 98% (Lundgren et al., 1987 a)	controls 0.25, 0.5, 1.0, 2.0 and 4% in feed	maximum increase in cytosolic epoxide hydrolase in the 1% dose group, maximum increase in microsomal epoxide hydrolase in the 2% dose group (no further increase in activity in the higher dose groups)	no information	no clinical signs
C57BL/6 mouse, 3 males/dose or group, daily oral dietary administration for 4 days, purity of the 2-ethylhexanoic acid used: > 98%	controls 1% in feed*	significant increase in cytosolic epoxide hydrolase (activity and amount), microsomal epoxide hydrolase (activity and amount), carnitine acetyltransferase activity, cyanide-insensitive palmitoyl-CoA oxidation, cytochrome oxidase; no significant increase in cytosolic glutathione transferase activity, mitochondrial epoxide hydrolase activity, catalase activity and microsomal cytochrome P-450 content; cytosolic and microsomal epoxide hydrolase activity and carnitine acetyltransferase activity were back to control levels 4 days after the end of treatment	relative liver weight increased, mitochondrial, microsomal and cytosolic protein content as in the controls	no clinical signs, body weight and feed consumption as in the controls
The authors discussed induction of epoxide hydrolase in terms of a secondary effect of peroxisome proliferation. * Equivalent to 30 mg/animal/day, according to the authors. (Lundgren et al., 1987 a, 1988 a, b)				
C57BL/6 mouse, 3 males, daily oral dietary administration for 4 days (Lundgren et al., 1987 b)	controls 1% in feed	significant increases in carnitine acetyltransferase activity, cyanide-insensitive palmitoyl-CoA oxidation, cytochrome oxidase activity and amount of enoyl-CoA hydratase (also referred to as protein PPA 80); no significant increase in catalase activity (activities determined in the mitochondrial cell fraction)	relative liver weight increased, mitochondrial protein content as in the controls	no clinical signs, no effect on body weight gain

Table 8. Studies on the induction of peroxisome proliferation by 2-ethylhexanoic acid

Species, number/group, regimen (reference)	Dose (mg/kg b.w. per day)	Liver enzyme alterations	Macroscopic/histopathological liver changes	Other findings
C57BL/6 mouse, 10 males/group, daily oral dietary administration for 4 days	controls 1% in feed*	significant increases in peroxisomal cyanide-insensitive palmitoyl-CoA oxidation (2- and 3.5-fold over control values) and peroxisomal lauroyl-CoA oxidase activity (2 and 3 times the control value), microsomal catalase activity (2 to 3 times the control value), cytosolic catalase activity (6 times the control value), microsomal ω-oxidation of lauric acid (7 times the control value), mitochondrial, microsomal and cytosolic epoxide hydrolase activity (slight increase); no significant increase in mitochondrial (peroxisomal) catalase activity	absolute and relative liver weights were increased, protein content in mitochondrial, microsomal and cytosolic hepatocellular fractions was comparable with the controls	depressed body weight gain
* The *R*-(–)- and *S*-(+)-enantiomers of 2-ethylhexanoic acid were tested. An enantiomer-dependent effect was seen only with respect to cyanide-insensitive palmitoyl-CoA oxidation and lauroyl-CoA oxidase activity, both of which showed 1.5-fold higher induction by the *S*-(+)-enantiomer relative to the *R*-(–)-enantiomer. (Sundberg et al., 1994)				
C57BL/6 mouse, 10 males/group, daily oral dietary administration for 10 days	controls (pair fed) 1% in feed*	significant increase in peroxisomal cyanide-insensitive palmitoyl-CoA oxidation (4 to 5 times the control value) and peroxisomal lauroyl-CoA oxidase activity (9 to 12 times the control value), microsomal catalase activity (2 to 3 times the control value), cytosolic catalase activity (12 to 16 times the control value), microsomal ω-oxidation of lauric acid (7 times the control value), mitochondrial, microsomal and cytosolic epoxide hydrolase activity (slight increase); no significant increase in mitochondrial (peroxisomal) catalase activity	absolute and relative liver weights increased following administration of the enantiomers, protein content was significantly increased in mitochondrial and microsomal hepatocellular fractions but in the cytosolic hepatocellular fractions it was comparable with the controls	depressed body weight gain
* The *R*-(–)- and *S*-(+)-enantiomers and the racemate (> 98% pure) of 2-ethylhexanoic acid were tested. In contrast with the 4-day administration, there were no clear differences in efficacy between the two enantiomers, which according to the authors' discussion of their results suggests that the proteins involved are only stereoselective but not stereospecific. (Sundberg et al., 1994)				

Table 8. Studies on the induction of peroxisome proliferation by 2-ethylhexanoic acid

Species, number/group, regimen (reference)	Dose (mg/kg b.w. per day)	Liver enzyme alterations	Macroscopic/histopathological liver changes	Other findings
C57BL/6 mouse, 3 males/dose or group, daily oral dietary administration for 14 days, purity of the 2-ethylhexanoic acid used: > 98% (Lundgren et al., 1987 a)	controls 1% in feed	cytosolic and microsomal epoxide hydrolase activity peaked after 3 days, carnitine acetyltransferase activity peaked after 7 days, slight decrease in cytosolic glutathione transferase activity after 11 days	no information	one mouse died after 11 days, another 3 animals after 14 days, no effect on body weight gain, no clinical signs
Swiss mouse, 5 males and 5 females/dose, daily oral administration by gavage for 14 days (Keith et al., 1988, 1992)	controls 150–1900	dose-dependent increase in cyanide-insensitive palmitoyl-CoA oxidation by maximum factors of ca. 8.75 and ca. 5 in males and females, respectively	dose-dependent increase in relative liver weight at dose levels of and above 770 mg/kg body weight	mortality not increased
Fischer-344 rat, 5 males, daily oral dietary administration for 3 weeks (Moody and Reddy, 1978, 1982)	controls 2% in feed (ca. 1333 mg/kg)	significant increases in carnitine acetyltransferase activity and catalase activity	relative liver weight increased, peroxisome proliferation (ratio of mitochondria to peroxisomes significantly reduced to 1 : 1 from 5 : 1 in the controls)	serum cholesterol and triglycerides significantly elevated, body weight gain as in the controls
Wistar rat, 5 to 7 males/group, daily oral administration by gavage for 3 days (Dirven et al., 1992)	controls 550	no significant changes in lauric acid-ω-hydrolase and ω-1-hydroxylase activity, ca. 2-fold increase in palmitoyl-CoA oxidase activity	absolute increase and relative increase in total amounts of cytochrome P-450 4A1 compared with total amount of cytochrome P-450, no increase in total amount of cytochrome P-450 and relative liver weight	no information
Wistar rat, 5 males/dose, daily oral administration by gavage for 3 days (Hamdoune et al., 1995)	controls 4 mmol (ca. 577 mg/kg) and 7 mmol (ca. 1010 mg/kg)	no significant change in cyanide-insensitive palmitoyl-CoA oxidase, ca. 5.5-fold increase in cyanide-insensitive palmitoyl-CoA oxidase activity	relative liver weight and cytochrome P-450 content unchanged, relative liver weight increased by 17% and 1.3-fold increase in cytochrome P-450 content	no information

Table 8. Studies on the induction of peroxisome proliferation by 2-ethylhexanoic acid

Species, number/group, regimen (reference)	Dose (mg/kg b.w. per day)	Liver enzyme alterations	Macroscopic/histopathological liver changes	Other findings
Wistar rat, males, daily oral administration by gavage for 3 days (Macherey et al., 1993)	controls 4 mmol (ca. 577 mg/kg) and 6.9 mmol (ca. 996 mg/kg)	dose-dependent increase in cyanide-insensitive palmitoyl-CoA oxidation and 12-lauric acid-ω-hydroxylation (cyanide-insensitive palmitoyl-CoA oxidation also determined following administration of 1.8 mmol (ca. 260 mg)/kg body weight (tendency towards an increase))	no information	no information
Wistar rat, 5 males and 5 females/dose, daily oral administration by gavage for 14 days (Keith et al., 1988, 1992)	controls 150–1900	dose-dependent increase in cyanide-insensitive palmitoyl-CoA oxidation by a maximum factor of 8 in the males of the 1900 mg/kg body weight dose group and a factor of 3.5 in the females of the 1160 mg/kg body weight dose group (higher dose lethal)	dose-dependent increase in relative liver weight at dose levels of 770 mg/kg body weight and above	1900 mg/kg body weight lethal to the females
Wistar rat, 5 males/dose, daily oral administration in the drinking water for 20 days	controls 0.1, 1, 5 and 10 g/l water*	dose-dependent increase in mitochondrial carnitine acetyltransferase activity by a maximum factor of ca. 7.7, citrulline synthesis reduced by ca. 50% in all dose groups	no information	body weight gain as in the controls
* Equivalent to ca. 0.3, 33, 130 and 200 mg/day, according to the authors (no indication whether per kg body weight or per animal). (Manninen et al., 1989)				
In vitro, hepatocytes from male Swiss mice, Wistar rats, marmosets, Dunkin-Hartley guinea pig (Cornu et al., 1992; ICI, 1985)	controls 0.5–2 mM (ca. 72–288 µg/ml)	3-day incubation increased cyanide-insensitive palmitoyl-CoA oxidation in rat hepatocytes by a maximum factor of 10 (at 2 mM), in mouse hepatocytes by a maximum factor of 25 (at 1 mM, higher concentration levels were toxic) while hepatocytes from guinea pigs and marmosets exhibited no changes in cyanide-insensitive palmitoyl-CoA oxidation (concentration levels > 2 mM were toxic)	–	–

Table 8. Studies on the induction of peroxisome proliferation by 2-ethylhexanoic acid

Species, number/group, regimen (reference)	Dose (mg/kg b.w. per day)	Liver enzyme alterations	Macroscopic/histopathological liver changes	Other findings
In vitro, hepatocytes from male Wistar rats (Keith et al., 1988)	controls 0.1–1.0 mM (ca. 14–144 µg/ml)	 concentration-dependent 14-fold increase in cyanide-insensitive palmitoyl-CoA oxidation relative to control values	–	–
In vitro, hepatocytes male Wistar rats	controls 0.25–1 mM* (ca. 36–144 µg/ml)	 concentration-dependent increase in cyanide-insensitive palmitoyl-CoA oxidation, with the *S*-(+)-enantiomer exhibiting ca. 1.6-fold greater activity than the *R*-(–)-enantiomer	–	–
* The *R*-(–)- and *S*-(+)-enantiomers and the racemate of 2-ethylhexanoic acid were tested. (Macherey et al., 1992, 1993)				
In vitro, liver cytosol from male Sprague-Dawley rats and Swiss-Webster mice (Law and Moody, 1991)	–	IC_{50} (concentration at which cytosolic glutathione *S*-transferase activity is reduced by 50% relative to the control) was determined as 4.2 mM (ca. 605 µg/ml) and 10 mM (ca. 1440 µg/ml) for the rat and mouse, respectively	–	–
In vitro, mitochondria from hepatocytes of male Wistar rats (Veitch and Van Hoof, 1990)	1 mM (ca. 144 µg/ml)	reduction in oxidation rates of succinate, glutamate malate α-ketoglutarate and palmitoylcarnitine	–	–
In vitro, mitochondria from hepatocytes of male Sprague-Dawley rats (Chance and McIntosh, 1995)	0–200 µmol/l (ca. 0–29 µg/ml)	no changes in oxidation rates of succinate, pyruvate and malate (incubation with and without addition of ADP)	–	–

End of Table 8

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