Opinion of the Panel on Food Additives, Flavourings, Processing Aids, Materials in Contact with Food and Cosmetics of the Norwegian Scientific Committee for Food Safety

Date: 8 March 2005

Risk assessment of health hazards from 2-ethylhexanoic acid (2-EHA) migrated from lids used on glass containers of baby food

Summary

The Norwegian Food Safety Authority [*Mattilsynet*] asked The Norwegian Scientific Committee for Food Safety [*Vitenskapskomiteen for mattrygghet* (VKM)] to issue an opinion on the risk for infants linked to intake of 2-ethylhexanoic acid (2-EHA, CAS no. 149-57-5), based on values of 2-EHA found in baby foods on the Norwegian market in a survey conducted by the Norwegian Food Safety Authority in 2004. The case was evaluated by the Panel on Food Additives, Flavourings, Processing Aids, Materials in Contact with Food and Cosmetics.

2-EHA is used as a heat stabiliser in polyvinyl chloride (PVC) gaskets of metal lids used to seal jars and bottles of baby foods. The levels of 2-EHA determined in baby foods on the Norwegian market were 0.08-0.60 mg/kg. No value for tolerable daily intake (TDI) for 2-EHA has been established. 2-EHA has toxic effects mainly on the liver, being a peroxisome proliferator, and is teratogenic in laboratory animals. The lowest NOAEL value for 2-EHA found in the literature was 25 mg/kg bw/day calculated on the basis of maternal toxicity in New Zealand white rabbits found in a developmental toxicity study. By using an uncertainty factor of 100 (10 for extrapolation from rabbits to humans, and 10 for intraindividual variation), we derived a TDI value for 2-EHA of 0.25 mg/kg bw. The exposure assessments show that intakes of the levels of 2-EHA found in the survey did not result in intakes in infants of 6-12 months that exceeded the estimated TDI of 0.25 mg/kg bw/day, but were 12- to 125-fold below the TDI. 2-EHA was negative for mutagenicity, but positive for clastogenicity *in vitro*. However, *in vivo* genotoxicity studies are lacking.

Terms of reference

In its letter of 8 October 2004 the Norwegian Food Safety Authority asked the Norwegian Scientific Committee for Food Safety to issue an opinion on the risk for infants linked to intake of 2-ethylhexanoic acid (2-EHA, CAS no. 149-57-5), based on values of 2-EHA found in baby foods on the Norwegian market in a survey conducted by the Norwegian Food Safety Authority in 2004 (1). The case was evaluated by the Panel on Food Additives, Flavourings, Processing Aids, Materials in Contact with Food and Cosmetics.

Background

2-EHA is used as a heat stabiliser in polyvinyl chloride (PVC) gaskets of metal lids used to seal jars and bottles (2). The gasket forms an airtight seal preventing microbiological and other contamination. This type of lids is commonly used for glass jars containing baby foods. This evaluation was conducted in the context of the amounts of 2-EHA determined in a survey of baby foods on the Norwegian market, which were found to be 0.08-0.60 mg/kg (1). No tolerable daily intake (TDI) or acceptable daily intake (ADI) for 2-EHA is established by either the EC Scientific Committee for Food (SCF), European Food Safety Authority (EFSA), Joint FAO/WHO Expert Committee on Food Additives (JECFA) or the U.S. Food and Drug Administration (FDA). 2-EHA is listed in the Synoptic document on SCF list 6B (substances suspected to have toxic properties (other than carcinogenic) with the SCF opinion "Needed: toxicological data depending on migration level (see SCF guidelines) and, if migration exceeds 0.05 mg/kg of food, peroxisome proliferation studies too"), without a specific migration limit (SML) (3). 2-EHA is not listed on the positive list for plastics (Directive 2002/72/EC with amendments) (4), and is therefore regulated by the general requirements in Article 3 in the Framework Regulation (EC) No 1935/2004 (5), limited upwards by the overall migration limit of 60 mg/kg food.

Toxicology

Many of the studies cited below were reported in the HSDB data base (2). Those studies for which the original paper could not be obtained are referred in the following as stated by HSDB.

Pharmacokinetics
2-EHA has been shown to be excreted dose-dependently in the urine of occupationally exposed sawmill workers (6).

The distribution of 2-EHA was studied in rats and mice given a single i.p. injection of 2-^{14}C-EHA, and the animals were sacrificed after 30 minutes, 2 hours and 6 hours (7). In rats, the highest concentration was in the blood, and it was also relatively high in the liver and kidney, but low in the brain, after 2 hours. By 6 hours the radioactivity had decreased rapidly and it was hardly measurable at 24 hours, suggesting that 2-EHA was rapidly cleared from the tissues. In mice, the highest uptake was in the liver, kidney and blood, whereas low uptake was seen in the brain. Uptake was also well detectable in the olfactory bulb and the salivary gland.

Excretion balance studies were conducted with 2-EHA in female Fischer 344 rats (4-8/group) following single high (1000 mg/kg bw) and low (100 mg/kg bw) oral doses of 2-^{14}C-EHA, following repeated oral dosing with unlabeled 2-EHA and a final 2-^{14}C-EHA oral dose at the low dose level, following dermal exposure with a high (1000 mg/kg bw) and low (100 mg/kg bw) dose of 2-^{14}C-EHA, and following a 1 mg/kg bw i.v. dose of 2-^{14}C-EHA (8). After a single oral dose, the majority of the administered doses (approximately 90%) was eliminated within 24 hours. The rats excreted 79.3 and 82.3 % of the low and high dose, respectively, in the urine, and 12.5 and 6.7%, respectively, in the feces, during 96 hours. After oral dosing of 100 mg/kg, the maximum blood concentrations were detected after 15-30 minutes, with a terminal half-life of 60 hours. After dermal dosing, about 50% of the dose was excreted during 96 hours. The rats excreted 41.7 and 46.6 % of the low and high dose, respectively, in

the urine, and 7.5 and 7.1%, respectively, in the feces, during 96 hours. After dermal application of 100 mg/kg, the peak blood level was attained at 8 hours, with a terminal half-life of 112 hours. After i.v. dosing of 1 mg/kg, 70.2% was excreted over 96 hours. 64.2% was excreted in the urine during 24 hours, and another 2.4% between 24 and 96 hours. Fecal elimination was 3.6% of the dose during 96 hours. The total amount in the blood declined >60% the initial hour after dosing, with a terminal half-life of 85 hours. Occlusive dermal exposure caused damage to the epidermis in the first 24 hours after application and resulted in dermal absorption of 70% relative to i.v. dosing, based on the ratio of percent dose in excreta. Dermal application followed by prompt washing of the skin resulted in recovery of 101.9% from the skin surface and <0.2% in the excreta. The major urinary metabolites were the glucuronide of 2-EHA, 2-ethyl-1,6-hexanedioic acid, 2-ethyl-5-hydroxyhexanoic acid, 2-ethyl-6-hydroxyhexanoic acid and ethylketohexanoic acid. Evidence for metabolism via β-oxidation was also found, consistent with the incorporation of 2-EHA into normal cellular intermediary metabolism.

Male Wistar rats receiving 600 mg/kg bw daily of 2-EHA in drinking water for 9 weeks eliminated 10 different metabolites in the urine (9). The main metabolite was 2-ethyl-1,6-hexanedioic acid. The others were 2-ethyl-6-hydroxyhexanoic acid, 5 other hydroxylated metabolites and 2 lactones, for which the detailed structures were not determined. The unsaturated 5,6-dehydro-EHA was also identified, and the parent compound partly as the glucuronic acid conjugate.

2-EHA metabolism was studied *in vitro* using liver microsomes from male Han:Wistar rats, male DBA/2N/Kuo mice and humans (10). The main metabolite produced by all three microsomes was 2-ethyl-1,6-hexanedioic acid. Unsaturated 2-ethyl-5-hexenoic acid, a terminal olefin, was produced only in human and phenobarbital-induced rat liver microsomes. Production of this metabolite was inhibited in the presence of various cytochrome P450 inhibitors. Both of these metabolites were also detected in human and rat urine. It was concluded that several cytochrome P450 enzyme families may contribute to 2-EHA metabolism in the liver, that the same metabolites were formed in rats and humans, and that 2-EHA resembled the hepatotoxicant valproic acid, since 2-ethyl-5-hexenoic acid is structurally similar to a terminal olefin metabolite of valproic acid.

Irritation and sensitization
2-EHA is severely irritating for eyes, mucous membranes and upper respiratory tract, and showed corrosive dermal irritation in rats (2,11). No data was found on contact sensitization or allergy.

Acute/subacute toxicity
Acute oral LD50 values were reported to be 1600-3000 mg/kg bw for rats, and 1300 mg/kg bw for rabbits (2,11).

Female Fischer 344 rats (4/dose group) were administered single doses of 0 (vehicle control), 90, 722, 1445 or 2890 mg 2-EHA/kg bw/day by gavage in corn oil (12, cited in 2). All rats treated with 2890 mg/kg died on day 1 of undetermined causes. The remaining rats survived the 14-day observation period. Rats given 722 mg/kg or higher exhibited weakness on the day of dosing. Weight loss was observed in 14 of 16 rats during the first 24 hours after dosing, but by day 7, all had regained and exceeded their original weight. Absolute and relative liver weight of the surviving rats did not differ from controls. A histopathological examination was not performed. A LD50-value of 2043 mg/kg bw/day was calculated.

Male and female Fischer 344 rats were treated with 0, 200, 800 or 1600 mg/kg by gavage 5 days/week for 2 weeks (13, cited in 2). Weakness, lethargy, hypothermia, sialorrhea, tremors, poor body condition and substantial mortality were observed in both sexes of the high dose group. Mid-dose rats showed generally less severe signs than the high-dose rats, and no mortality. Body weights in high-dose rats and mid-dose male rats were lower than in the controls, and feed consumption in high-dose rats was decreased. Liver weights (absolute and relative to body weight) were increased in a dose-related manner in the high- and mid-dose rats. Hepatocyte hypertrophy was observed in surviving high-dose rats. The no observable effect level (NOEL) was 143 mg/kg bw/day for males and <143 mg/kg bw/day for females.

Male and female B6C3F1 mice were treated with 0, 200, 800 or 1600 mg/kg by gavage 5 days/week for 2 weeks (13, cited in 2). Body weights and feed consumption were unaffected. Male mice in the high-dose group only had increased liver weight (absolute and relative to body weight) and hepatocyte hypertrophy. NOEL was 571 mg/kg bw/day for males and 1143 mg/kg bw/day for females.

Male and female B6C3F1 mice received 0, 0.75, 1.5 or 3.0 % 2-EHA in the diet (equivalent to approximately 0, 1800, 3500 and 7500 mg/kg bw/day, respectively) for 2 weeks (13, cited in 2). Body weights in the high-dose group were reduced. Feed consumption was initially reduced in the treated groups, but was eventually comparable to in the controls. Absolute and relative (to body weight) liver weights of mice in the high- and mid-dose groups were increased. Hepatocyte hypertrophy was observed in a dose-related manner in all treated mice except a few low-dose mice, ranging from moderate to minor.

Rats fed 2-EHA for 2 or 3 weeks showed decreased serum triglycerides, hepatomegaly and a large increase in hepatic peroxisomes (13, cited in 2). Similar hepatic changes occurred in hepatocytes *in vitro*. 2-EHA also affected lipid metabolism *in vivo* and *in vitro*, and caused inhibition of triglyceride biosynthesis in intestinal mucosa, leading to changes in absorption of fatty acids and cholesterol.

Male Fischer 344 rats fed 2% 2-EHA in the diet for 3 weeks showed 15% less body weight gain, hepatomegaly, 55% increased liver-to-body weight ratio, a substantial increase in hepatic peroxisomes, 17% lower cholesterol levels and 68% lower triglyceride levels compared with controls, but no increased mortality (13, cited in 2).

2-EHA given to Sprague-Dawley rats *ad libitum* in 2% in the diet for 4 weeks increased the ubiquinone levels in the liver and blood, increased the cholesterol level in the liver and decreased the dolichol level in the liver (14).

Subchronic toxicity

Groups of 10 male and female Fischer 344 rats received 0, 0.1, 0.5 or 1.5 % 2-EHA in the diet (equivalent to approximately 0, 61, 303 and 917 mg/kg bw/day, respectively, for males, and 0, 71, 360 and 1068 mg/kg bw/day, respectively, for females) for 13 weeks, followed by a 4-week recovery period without treatment (15). No mortality or significant clinical signs of toxicity were observed. Body weights and food consumption of rats fed 1.5% were lower beginning after the first week of treatment. Other groups were unaffected by treatment. Cholesterol levels were higher in all male test groups and in females fed either 0.5 or 1.5% 2-EHA, although this effect was reversible following the recovery period. The principal effects of 2-EHA involved the liver or metabolic processes associated with the liver. The 0.5 and

1.5% diets were associated with increased relative liver weight and histological changes in hepatocytes, specially hypertrophy and reduced cytoplasmic vacuolisation, along with some slight hematologic differences. The observed histopathological and clinical pathological changes were reversible after recovery. These results indicate that 2-EHA does not produce persistent, overt toxicity in rats following subchronic dietary exposure at concentrations up to 1.5% in feed. The no observed adverse effect level (NOAEL) for males was 0.1 % in the diet (61 mg/kg bw/day) and the NOEL for females was 0.1% (71 mg/kg bw/day). The lowest observed adverse effect level (LOAEL) was approximately 300 mg/kg bw/day in the rats.

Groups of 10 male and female $B6C3F_1$ mice received 0, 0.1, 0.5 or 1.5 % 2-EHA in the diet (equivalent to approximately 0, 180, 885 and 2728 mg/kg bw/day for males, respectively, and 0, 205, 1038 and 3139 mg/kg bw/day for females, respectively), for 13 weeks, followed by a 4-week recovery period without treatment (15). No mortality or significant clinical signs of toxicity were observed. Body weights and food consumption of mice fed 1.5% were lower beginning after the first week of treatment. Other groups were unaffected by treatment. After 13 weeks, lower triglyceride levels occurred in males fed 1.5% 2-EHA and females fed 0.5 and 1.5% 2-EHA. Cholesterol levels were higher in males and females fed either 0.5 or 1.5% 2-EHA, although this effect was reversible following the recovery period. The principal effects of 2-EHA involved the liver or metabolic processes associated with the liver. The 0.5 and 1.5% diets were associated with increased relative liver weight and histological changes in hepatocytes, specially hypertrophy and reduced cytoplasmic vacuolisation. The observed histopathological and clinical pathological changes were reversible after recovery. These results indicate that 2-EHA does not produce persistent, overt toxicity in mice following subchronic dietary exposure at concentrations up to 1.5% in feed. NOEL for males was 0.1 % in the diet (180 mg/kg bw/day) and NOEL for females was 0.1% (205 mg/kg bw/day). LOAEL was approximately 1000 mg/kg bw/day in the mice.

Chronic toxicity
No chronic toxicity studies were found.

Developmental and reproductive toxicity
Pregnant female Wistar rats (20-21/dose) were exposed to 2-EHA in their drinking water at doses of 100, 300 or 600 mg/kg bw/day on days 6 to 19 of gestation (16). 2-EHA was marginally toxic to the dams at 600 mg/kg since the mean near term body weight was reduced by 11%, but not at lower doses. This dose level was also slightly fetotoxic as indicated by a 5-8% decrease in the mean fetal body weight both in males and females. No treatment-related effects were observed in the number of implantations or live fetuses. At doses of 100 mg/kg and above, 2-EHA caused skeletal malformations (clubfoot, absence of fibula, polydactyly), while the development of visceral tissues was less affected. The number of affected fetuses increased in a dose-dependent way (4.9, 8.9 and 15.3% of treated offspring at 100, 300 and 600 mg/kg bw/day, respectively, versus 2.4% in controls). These results indicate that 2-EHA is teratogenic in rats already at doses which are not maternally toxic. The skeleton appears to be the main target of 2-EHA in developing rats. LOAEL for malformations in the offspring was 100 mg/kg bw/day.

Male Wistar rats (24/group) were treated for 10 weeks with daily doses of 100, 300 or 600 mg/kg bw 2-EHA in drinking water and mated to females (24/group) treated with similar doses of 2-EHA for 2 weeks prior to mating, to study the reproductive toxicity of 2-EHA (17). Both sexes were treated throughout the mating, and females also throughout gestation and lactation. A decrease in maternal body weight was seen after 7 days of gestation in the group

treated with 600 mg/kg. A nonuniform dose-dependent effect on sperm quality was seen following the treatment, along with a dose-dependent delay in fertilization. Decreases in average litter size were seen in treated rats along with increases in the frequency of lethargy, abnormally thin hair, kinky tails and abnormal legs. For these effects on offspring NOAEL was 100 mg/kg bw/day. A delay in physical development was seen as well. Few histological changes in the reproductive organs and tissues of adult males, non-gravid females and dams were seen. In another experiment, pregnant Wistar rats were treated with 600 mg/kg 2-EHA on day 4, 5, 6 or 7 of gestation, and the uterine contents examined on day 10 of gestation (17). Exposure on day 6 resulted in the lowest number of implantations and the highest number of resorptions. It was concluded that 2-EHA impaired fertility and delayed postnatal development in rats.

Pregnant Fischer 344 rats (25/group) were gavaged with 0, 100, 250, 500 or 1000 mg/kg bw/day 2-EHA on days 6 to 15 of gestation, and killed on day 21 and necropsied (18). Seven of the eight dams given 1000 mg/kg bw died. The one surviving rat had a completely resorbed litter. Rats receiving 500 mg/kg bw exhibited ocular discharge and periocular encrustation as well as significant increases in absolute and relative liver weight and decreased fetal body weight. No effects were seen with respect to body weight, feed consumption, number of resorptions or fetal viability in rats exposed to 500 mg/kg bw. The 250 and 500 mg/kg doses caused a reduction in fetal skeletal ossification. The results suggested that 2-EHA induces developmental toxicity in rats only at doses that cause maternal toxicity. No evidence of teratogenicity of 2-EHA was seen. NOAEL for maternal toxicity was 250 mg/kg bw/day, and NOAEL for developmental toxicity was 100 mg/kg bw/day.

Sprague-Dawley rats were gavaged with 2-EHA in corn oil in doses of 900 or 1200 mg/kg bw/day on days 6 to 15 of gestation (13, cited in 2). The dams were allowed to deliver, and their litters were examined through postnatal day 6. Effects on development included delayed parturition (day 22 instead of day 21), decreased progeny viability, reduced pup weights and induced malformations of the vertebrae and ribs. These effects, however, occurred at highly maternally toxic doses. Maternal effects included mortality (27% and 40% at the two doses, respectively), decreased body weight and body weight gain, maternal respiratory toxicity (rales and dyspnea), and transient signs of depressed motor activity (ataxia and lethargy).

Pregnant Sprague-Dawley rats (6/group) were intubated with 2-EHA in corn oil in doses of 451, 902, 1355 and 1804 mg/kg bw on gestational day (GD) 11.5, and with ^{65}Zn later on day 11.5, and terminated on GD 12.5 (19). At the higher dose levels of 2-EHA, the percentage of ^{65}Zn retained in maternal liver was higher, while that in the embryos was lower, than in controls. Chemical-associated changes in ^{65}Zn distribution were associated with increased maternal liver metallothionein concentrations. In the next experiment, dams (7-10/group) were fed diets of 1, 25 or 97 μg Zn/g (low, adequate or supplemental Zn, respectively) from GD 0-16, and intubated with 3.5 mmol (505 mg) 2-EHA or 1.0 ml corn oil per kg bw/day from GD 8-15, and terminated on GD 16 or 19. High incidences of encephalocele and tail defects were noted in the GD 16 fetuses of 2-EHA-treated dams fed either the low or adequate Zn diets, the highest incidences being in the low Zn group. On GD 19 the incidence of tail defects tended to be higher in the 2-EHA groups than in the corn oil-treated groups, the highest incidence occurring in the low Zn 2-EHA group. Encephalocele was only observed in the low Zn 2-EHA-treated group. Fetal weight and crown-rump lengths were decreased by 2-EHA treatment and low Zn. The incidence of rib anomalies was higher in the 2-EHA-treated groups than in the controls. In the next experiment, GD 10.5 embryos (18-44/group) collected from control dams were cultured for 48 hours in serum from control or 2-EHA-treated male

rats fed 4.5 or 25 μg Zn/g diets. Embryos cultured in either 2-EHA or low Zn sera exhibited delayed development. The addition of Zn to these sera eliminated their developmental toxicity. The results support the hypothesis that chemicals such as 2-EHA, which induce maternal toxicity, act in part to influence embryonic Zn metabolism and trigger abnormal development. The teratogenic effects of these chemicals can be modulated by dietary Zn intake.

The stereoselectivity of the teratogenic activity of 2-EHA was studied in pregnant NMRI mice that were injected i.p. each morning and evening on days 7 and 8 of gestation with 500 mg/kg bw of aqueous solutions of the sodium salts of either (R)- or (S)-2-EHA or the racemic 2-EHA ((±)-2-EHA) (20). (S)-2-EHA did not yield any teratogenic or embryotoxic response, while (R)-2-EHA was highly teratogenic, as 59% of living fetuses exhibited exencephaly, and embryotoxic, as indicated by embryo lethality and fetal weight retardation. The rate of exencephaly induced by racemic 2-EHA was between those of the two enantiomers (32%).

In another study where exencephaly was induced by three consecutive administrations of (±)-2-EHA in one-half-day intervals on days 8 and 9 of gestation, the SWV mouse strain was more sensitive than the C57BL/6NCrlBR strain (21). The (R)-enantiomer was a more potent teratogen than the (S)-enantiomer for induction of exencephaly as well as malformations of other organs. Based on pharmacokinetic analyses, it was concluded that the teratologic differences in the enantiomers were not due to differences in the concentrations of these antipodes in the embryo, but more likely result from the specific interaction of the enantiomers with chiral molecules in the embryo.

Pregnant New Zealand white rabbits (15/group) were gavaged with 0, 25, 125, 250, 500 or 1000 mg/kg bw 2-EHA on days 6 to 18 of gestation, and killed on day 29 and necropsied (18). The 500 and 1000 mg/kg bw doses caused 87.5% and 100% mortality, respectively. A few dams in the 125 and 250 mg/kg groups aborted or died. Hypoactivity, labored breathing and ataxia were frequently seen in all treated rabbits. Maternal body weight gain and feed consumption were decreased by the 250 mg/kg dose. The 25, 125 and 250 mg/kg bw doses did not increase the number of resorptions, affect fetal viability or induce fetal malformations. The results suggested that 2-EHA causes maternal toxicity in rabbits without affecting fetal development. NOAEL for maternal toxicity was 25 mg/kg bw/day, and NOAEL for developmental toxicity was ≥250 mg/kg bw/day.

2-EHA is on the Norwegian environmental authorities' list of substances with adverse effects on health or environment that may represent particular problems in Norway ("Obs-listen"), because of its developmental and reproductive toxicity (22).

Carcinogenicity
No carcinogenicity studies were found.

Genotoxicity
2-EHA was evaluated to be overall negative when tested for mutagenicity in Salmonella strains TA100, TA1535, TA97 and TA98 with and without metabolic activation, but overall positive for *in vitro* chromosome aberrations and sister chromatid exchanges in CHO cells (23). 2-EHA also induced sister chromatid exchanges in human lymphocytes *in vitro* (24). No *in vivo* genotoxicity studies were found.

Immunotoxicity

2-EHA (10-2000 μM) inhibited dose-dependently formyl-methionyl-leucyl-phenylalanine (FMLP)-induced respiratory burst in human polymorphonuclear leukocytes (PMNL) (25). 2-EHA also decreased oxidative burst evoked by the protein kinase C (PKC) activators, phorbol myristate acetate and dioctanoyl-s,n-glycerol, without affecting the levels of free intracellular calcium or inhibiting PKC. The results indicate that 2-EHA inhibits activation of PMNL to produce reactive oxygen species (ROS), i.e. has an immunosuppressive effect *in vitro*.

Table 1. NOAEL/NOEL and LOAEL values for 2-EHA

Type of study	Sex/Species/Strain	Exposure	*NOAEL/NOEL/LOAEL (mg/kg bw/day)	End points	References
Subacute	male Fischer 344 rat	gavage, 5 days/week, 2 weeks	143	body weight, liver weight	13, cited in 2
	female Fischer 344 rat	gavage, 5 days/week, 2 weeks	<143	body weight, liver weight	13, cited in 2
Subacute	male B6C3F1 mouse	gavage, 5 days/week, 2 weeks	571	liver weight, hepatocyte hypertrophy	13, cited in 2
	female B6C3F1 mouse	gavage, 5 days/week, 2 weeks	1143	no effects observed at highest dose	13, cited in 2
Subchronic	male Fischer 344 rat	diet, 13 weeks (90 days)	61	liver weight, hepatocyte hypertrophy	15
	female Fischer 344 rat	diet, 13 weeks (90 days)	71	cholesterol, liver weight, hepatocyte hypertrophy	15
			300 (LOAEL)		15
Subchronic	male B6C3F$_1$ mouse	diet, 13 weeks (90 days)	180	triglycerides, cholesterol, liver weight, hepatocyte hypertrophy	15
	female B6C3F$_1$ mouse	diet, 13 weeks (90 days)	205	cholesterol, liver weight, hepatocyte hypertrophy	15
			1000 (LOAEL)		15
Developmental	Wistar rat	drinking water, gestational days 6-19	100 (LOAEL)	offspring (skeletal and visceral malformations)	16
Developmental	Wistar rat	drinking water, from 2 weeks prior to mating, through gestation and lactation	100	offspring (kinky tails, paralyzed legs etc.)	17
Developmental	Fischer 344 rat	gavage, gestational days 6-15	250	maternal toxicity (liver weight, fetal b.w.)	18
			100	offspring (skeletal ossification)	18
Developmental	New Zealand white rabbit	gavage, gestational days 6-18	25	maternal toxicity (death, abortion)	18

*Unless marked LOAEL, the values are NOAEL/NOEL.

Derivation of a tolerable daily intake (TDI) value based on NOAEL/NOEL values

A TDI/ADI value has not been set for 2-EHA either by SCF, EFSA, JECFA or FDA. However, a provisional TDI can be derived from the NOAEL values determined in animal experiments found in the literature (Table 1).

2-EHA has toxic effects mainly on the liver, being a peroxisome proliferator, and is teratogenic in laboratory animals. It was negative for mutagenicity, but positive for clastogenicity *in vitro*. No *in vivo* genotoxicity studies were found. The lowest NOAEL value was 25 mg/kg bw/day calculated on the basis of maternal toxicity in New Zealand white rabbits found in a developmental toxicity study (18). By using an uncertainty factor of 100 (10 for extrapolation from rabbits to humans, and 10 for intraindividual variation), a TDI value can be derived as follows:

TDI = NOAEL/100 = 25 mg/kg bw/day : 100 = 0.25 mg/kg bw/day

Analyses of migration

In 2004, the Norwegian Food Safety Authority conducted a survey on 2-EHA in commercial baby foods on the Norwegian Market (1). Three parallel samples of each type of baby food were analyzed for 2-EHA at the laboratory of the Official Food Control Authority of the Canton of Zürich, Switzerland. Values ranging from 0.08-0.60 mg/kg 2-EHA were determined in the examined baby foods in the survey, therefore, none of the samples contained 2-EHA in amounts exceeding the overall migration limit of 60 mg/kg food.

Exposure assessment of 2-EHA from baby foods

2-EHA was found in concentrations of 0.08 - 0.60 mg/kg baby food in the Norwegian survey (1). In the following, intake of 2-EHA from baby foods has been estimated by the Norwegian Food Safety Authority (Christina Bergsten, personal communication) based on data for baby food intake determined in a Norwegian survey, SPEDKOST (26), calculated for a 12-month infant weighing 10 kg. Intake of 2-EHA from other potential sources than commercial baby foods in glass jars was not taken into consideration due to lack of data.

Based on data of consumption from all the study participants and using the mean values for 2-EHA in meat, fish and vegetable containing baby foods, respectively, an estimated **mean intake** of 0.002 mg/kg bw/day (0.02 mg 2-EHA/day) was found. This intake value is approximately 125-fold below the TDI of 0.25 mg/kg bw/day (2.5 mg/day).

Assuming a **worst case scenario** where an infant has a high consumption (90 percentile) of the specific baby food product in which the highest level of 2-EHA was found in the survey will give an estimated intake of 0.017 mg/kg bw/day (0.167 mg 2-EHA/day). This intake value is approximately 15-fold below the TDI of 0.25 mg/kg bw/day (2.5 mg/day).

In the Norwegian survey the 6-month infants weighed 8 kg (26). Therefore, the two scenarios above will give intakes of 2-EHA for 6-month infants of 0.0025 and 0.021 mg/kg bw/day, respectively, being approximately 100-fold and 12-fold below the TDI of 0.25 mg/kg bw/day.

Conclusions

2-EHA was found in amounts of 0.08-0.60 mg/kg baby food in Norway (1). The exposure assessments show that intake of these levels of 2-EHA did not result in intakes in infants of 6-12 months that exceed the estimated TDI of 0.25 mg/kg bw/day, but were 12- to 125-fold below the TDI. 2-EHA was negative for mutagenicity, but positive for clastogenicity *in vitro*. However, *in vivo* genotoxicity studies are lacking.

Assessed by

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