DRAFT

For Review Only

Public Health Goal for
Di-(2-ethylhexyl)adipate
in Drinking Water

NOT A REGULATORY STANDARD

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PREFACE

Drinking Water Public Health Goals

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California Environmental Protection Agency

This Public Health Goal (PHG) technical support document provides information on health effects from contaminants in drinking water. PHGs are developed for chemical contaminants based on the best available toxicological data in the scientific literature. These documents and the analyses contained in them provide estimates of the levels of contaminants in drinking water that would pose no significant health risk to individuals consuming the water on a daily basis over a lifetime.

The California Safe Drinking Water Act of 1996 (amended Health and Safety Code, Section 116365), amended 1999, requires the Office of Environmental Health Hazard Assessment (OEHHA) to perform risk assessments and publish PHGs for contaminants in drinking water based exclusively on public health considerations. Section 116365 specifies that the PHG is to be based exclusively on public health considerations without regard to cost impacts. The Act requires that PHGs be set in accordance with the following criteria:

1. PHGs for acutely toxic substances shall be set at levels at which no known or anticipated adverse effects on health will occur, with an adequate margin of safety.
2. PHGs for carcinogens or other substances which can cause chronic disease shall be based upon currently available data and shall be set at levels which OEHHA has determined do not pose any significant risk to health.
3. To the extent the information is available, OEHHA shall consider possible synergistic effects resulting from exposure to two or more contaminants.
4. OEHHA shall consider the existence of groups in the population that are more susceptible to adverse effects of the contaminants than a normal healthy adult.
5. OEHHA shall consider the contaminant exposure and body burden levels that alter physiological function or structure in a manner that may significantly increase the risk of illness.
6. In cases of insufficient data to determine a level of no anticipated risk, OEHHA shall set the PHG at a level that is protective of public health with an adequate margin of safety.
7. In cases where scientific evidence demonstrates that a safe dose-response threshold for a contaminant exists, then the PHG should be set at that threshold.
8. The PHG may be set at zero if necessary to satisfy the requirements listed above.

9. OEHHA shall consider exposure to contaminants in media other than drinking water, including food and air and the resulting body burden.

10. PHGs published by OEHHA shall be reviewed every five years and revised as necessary based on the availability of new scientific data.

PHGs published by OEHHA are for use by the California Department of Health Services (DHS) in establishing primary drinking water standards (State Maximum Contaminant Levels, or MCLs). Whereas PHGs are to be based solely on scientific and public health considerations without regard to economic cost considerations, drinking water standards adopted by DHS are to consider economic factors and technical feasibility. Each standard adopted shall be set at a level that is as close as feasible to the corresponding PHG, placing emphasis on the protection of public health. PHGs established by OEHHA are not regulatory in nature and represent only non-mandatory goals. By federal law, MCLs established by DHS must be at least as stringent as the federal MCL if one exists.

PHG documents are used to provide technical assistance to DHS, and they are also informative reference materials for federal, state and local public health officials and the public. While the PHGs are calculated for single chemicals only, they may, if the information is available, address hazards associated with the interactions of contaminants in mixtures. Further, PHGs are derived for drinking water only and are not to be utilized as target levels for the contamination of other environmental media.

Additional information on PHGs can be obtained at the OEHHA Web site at www.oehha.ca.gov.

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PUBLIC HEALTH GOAL FOR DI-(2-ETHYLHEXYL)ADIPATE IN DRINKING WATER

SUMMARY

The Office of Environmental Health Hazard Assessment (OEHHA) proposes a public health goal (PHG) of 0.6 mg/L (0.6 ppm) for di-(2-ethylhexyl)adipate (DEHA) in drinking water. The PHG is based on adverse reproductive and developmental effects observed in rats. Two reproduction and developmental toxicity studies conducted by Imperial Chemical Industries (ICI) (1988a,b, as cited in U.S. EPA, 1999) reported a reduction in weight gain and food consumption during gestation in female Wistar-derived rats exposed to a high concentration (12,000 ppm or 1.2 percent) of DEHA in the diet. At this concentration, a reduction in total litter weight and litter size, abnormal ossification, and a reduction in weight gain in the offspring were also observed. These adverse health effects were not observed in the dams or offspring at a lower concentration of 1,800 ppm (equivalent to 170 mg/kg-day). The lower dose was identified as the no-observed-adverse-effect level (NOAEL) and was used in the derivation of the proposed PHG.

DEHA belongs to a group of chemicals known as peroxisome proliferators. One of the main health concerns for this group of chemicals is their potential to cause hepatic peroxisome proliferation and liver tumors in rodents. There are no human cancer data on the chemical. The genotoxicity data on DEHA are largely negative. In a series of cancer bioassays, NTP (1982) administered DEHA in the diet to groups of $B6C3F_1$ mice and F344 rats of both sexes for 103 weeks. NTP (1982) found that DEHA produced hepatocellular carcinomas in female mice and hepatocellular adenomas in male mice but found no evidence of carcinogenicity in rats. Based on a 1991 evaluation, U.S. Environmental Protection Agency (U.S. EPA, 1999) classified DEHA as a possible human carcinogen (Group C). More recently, IARC (2000a) determined that there is only limited evidence in experimental animals for the carcinogenicity of DEHA and concluded that DEHA was not classifiable as to its carcinogenicity to humans.

U.S. EPA (1998) used the ICI data in the development of a maximum contaminant level goal (MCLG) and maximum contaminant level (MCL) for DEHA. They calculated an MCLG of 0.4 mg/L (0.4 ppm) based on a NOAEL of 170 mg/kg-day, a water consumption rate of 2 L/day, a relative source contribution of 20 percent, and an overall uncertainty factor of 3,000. The overall uncertainty factor includes a factor of 100 for the uncertainties in the intra- and inter-species extrapolation, a factor of 3 for the lack of a multi-generation reproductive study, and an additional factor of 10 for possible human carcinogenicity.

The proposed PHG of 0.6 mg/L is calculated by assuming a water consumption rate of 2 L/day, a relative source contribution of 10 percent, and an overall uncertainty factor of 1,000. The uncertainty factor includes a factor of 100 for the uncertainties in the intra- and inter-species extrapolation and a factor of 10 for the lack of a multi-generation reproductive study and some uncertainty as to potential carcinogenicity.

California's current drinking water standard for DEHA is 0.4 mg/L (0.4 ppm) (22 CCR Section 64444, Organic Chemicals). This standard, referred to as the California MCL, is identical to the federal MCL of 0.4 mg/L.

INTRODUCTION

The purpose of this document is to review the toxicological and exposure data for di-(2-ethylhexyl)adipate (DEHA) with the goal of developing a proposed PHG for DEHA in drinking water. DEHA is used as a plasticizer in a wide variety of consumer and industrial products, including plastics used for food wrapping and cosmetics. Its physical properties and uses are similar to those of the phthalate esters such as di-(2-ethylhexyl)phthalate.

California's current drinking water standard for DEHA is 0.4 mg/L (0.4 ppm) (22 CCR Section 64444, Organic Chemicals). This California MCL is identical to the federal MCL of 0.4 mg/L (U.S. EPA, 1998).

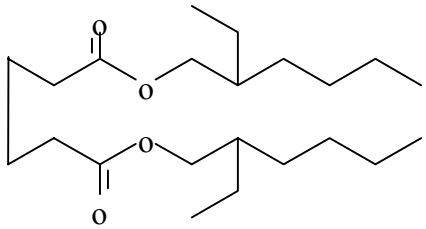
In developing the proposed PHG for DEHA, OEHHA staff evaluated the basis for the U.S. EPA MCL and MCLG and researched the scientific literature for new information and evaluations of tumor mechanisms. In the analysis described in this document, the primary focus was on oral exposure as it is the most relevant exposure route for the development of a drinking water standard for DEHA. Other exposure sources such as food were also considered.

CHEMICAL PROFILE

DEHA is a light-colored, oily liquid. It has low solubility in water and relatively low vapor pressure at room temperature. Some common names of DEHA and its molecular formula and structure are provided in Table 1. Table 2 lists some of the important physical properties of DEHA.

Chemical Identity

Table 1. Chemical Identity of DEHA (from HSDB, 1999)

Parameter	Value or information
CAS No.	103-23-1
Synonyms	DEHA; bis(2-ethylhexyl) adipate; BEHA; di-2-ethylhexyl adipate; dioctyl adipate; hexanedioic acid, dioctyl ester
Molecular formula	$C_{22}H_{42}O_4$
Molecular structure	

Physical and Chemical Properties

Table 2. Physical Properties of DEHA (from HSDB, 1999; Felder *et al.*, 1986)

Parameter	Value or information
Molecular weight	370.58 g/mol
Vapor pressure	8.5×10^{-7} mm Hg at 20 °C, 2.3 mm Hg at 200 °C
Henry's Law constant	0.33 torr-L/mol
Melting point	-67.8 °C
Boiling point	214 °C at 5 mm Hg
Color/Form	light colored liquid
Odor	slightly aromatic
Specific gravity	0.922 at 25 °C
Soil partition coefficient	$K_{oc} = 1.5\times10^4$
Octanol/Water partition coefficient	$K_{ow} > 1.3\times10^6$
Solubility	0.78 mg/L in water at 22 °C; soluble in alcohol, ether, acetone, acetic acid, and most organic solvents

Production and Uses

DEHA is not known to occur in nature; it is manufactured by the reaction of adipic acid and 2-ethylhexanol in the presence of an esterification catalyst such as sulfuric acid or p-toluenesulfonic acid (IARC, 1982). Estimated production of DEHA in the United States in 1984 and 1975 was 13,000 and 140,000 metric tons, respectively (HSDB, 1999).

DEHA is principally used as a plasticizer in polyvinyl chloride (PVC, "vinyl") plastics. DEHA is not chemically bound to the plastic, but it is dispersed in the matrix of polymer chains. Plastic films and containers with DEHA as the plasticizer (up to 24 percent by weight of the polymer) are often used for storing and protecting food (U.S. FDA, 2000).

DEHA can be found in many consumer products such as bath oils, eye shadow, cologne, cosmetic foundations, rouge, blusher, nail polish remover, moisturizers, and indoor tanning preparations (IARC, 1982). DEHA is also used in many industrial products such as electric wire insulator, vinyl-coated fabrics, synthetic rubber, and hydraulic fluid.

ENVIRONMENTAL OCCURRENCE AND HUMAN EXPOSURE

Air

DEHA is released into the atmosphere from manufacturing facilities that make or use the chemical. Vaporization from contaminated water or soil is not considered a significant source of airborne DEHA because of the strong adsorption to soil particles and the low Henry's Law constant (0.33 torr-L/mol) (HSDB, 1999). A small amount of DEHA may be released into the air by plastics containing it. However, this release is believed to be small due to the relatively low volatility of the chemical at ambient temperatures. Low concentrations of DEHA (about 2 ng/m^3) have been detected in indoor air of office buildings (HSDB, 1999).

In the ambient atmosphere, DEHA may exist in both the vapor phase and particulate phase. Vapor phase DEHA has an estimated half-life of about 16 hours due to reactions with photochemically produced hydroxyl radicals (HSDB, 1999). Particulate phase DEHA is removed from air physically by dry and wet deposition processes.

Soil

One of the major routes by which DEHA enters the general environment is via disposal of municipal solid wastes and industrial wastes. DEHA is relatively immobile in soil and is subjected to microbial degradation. Because of its low water solubility and estimated high soil partition coefficient (K_{oc}) of 1.5×10^4 (Felder *et al*., 1986), leaching of DEHA from contaminated soil to groundwater is generally not a serious concern.

Background levels of DEHA in soil have not been quantified. High local concentrations are regularly observed near sources of contamination such as incinerators, waste disposal sites and discharge points of contaminated water (HSDB, 1999).

Water

Main sources of water contamination are industrial effluents and runoff from waste disposal sites. Because of DEHA's low water solubility and high soil partition coefficient, once in an aquatic environment, it is expected to partition to biota, sediment, and soil. DEHA has been shown to be rapidly biodegraded in activated sludge; the first order half-life estimated for the degradation is less than one day (Felder *et al*., 1986).

DEHA is frequently detected in surface water, groundwater, and drinking water in the United States, at levels of up to a few parts per billion (ppb). It was detected in water samples collected in August 1982 from 5 of 23 U.S. sites at levels of 0.2-1 ppb (detection limit of 0.2 ppb). The samples positive for DEHA were from the Ohio River, Lake Ontario, Mississippi River below St. Louis, Mississippi River at Memphis, TN, and San Francisco Bay (Felder *et al*., 1986).

Between 1984 and 1999, approximately 2,000 samples from various water sources in California were analyzed for DEHA. With the exception of two samples, all of them were below the detection limit. The concentrations of DEHA in the two samples (taken from the same well) were 11.0 and 6.1 µg/L (ppb) (DHS, 1999).

A whole-fish bioconcentration factor of 27 was observed for blue-gill fish exposed to DEHA levels of 250 ppb for a 28-day period (Felder *et al*., 1986). The equilibrium level of DEHA in fish appeared to have been reached after 7 days of exposure. Based on a log K_{ow} of >6.1, the estimated fish bioconcentration factor of DEHA exceeds 2,700. The measured bioconcentration factor is far less than the estimated value due primarily to metabolism of DEHA by the blue-gill.

Food

The principal route of exposure of the general population to DEHA is through food consumption. The European Scientific Committee for Food recommended a maximum tolerable daily intake of 0.3 mg/kg (approximately 21 mg/person) for DEHA (EEC Commission, 1997; as cited in Petersen and Breindahl, 1998).

The migration of DEHA from polyvinyl chloride film has been investigated in a variety of foods (Loftus *et al*., 1994). It has been shown that DEHA migration is highest when plasticized polyvinyl films come in direct contact with fatty food (Petersen and Breindahl, 1998). Page and Lacroix (1995) reported a Canadian study that analyzed DEHA in 98 food samples collected between 1985 and 1989. They evaluated DEHA in food-contacting film and as a migrant in store-wrapped meat, poultry, fish, cheese, and ready to eat foods. The investigators reported that on a whole food basis, DEHA at levels up to 9.5, 14, 220, and 310 µg/g was found in chicken breast, regular ground beef, smoked salmon fillet, and cheese, respectively. DEHA in non-contacting or "core" samples obtained from several of the meat and chicken samples was below the detection limit (<0.4 µg/g). The DEHA levels found in the interior "core" of the cheese samples were about 1-7 percent of the levels found in the whole food. These results demonstrated that the retail wrap is the source of DEHA contamination.

Petersen and Breindahl (2000) analyzed 29 adult diet samples, 11 baby food samples and 11 samples of infant formula. They found DEHA in 18 adult diet samples, none of the baby food samples, and 2 of the infant formula samples. The average DEHA concentrations in the adult diet and infant formula samples were 140 and 35 μg/kg, respectively.

In 1987, a survey of DEHA levels in food indicated that the maximum intake of DEHA was 16 mg/person-day (MAFF, 1987; as cited in Loftus *et al*., 1994). A few years later, the estimated maximum daily intake dose was reduced to 8.2 mg/person, following reductions in the levels of DEHA used in plastic films (MAFF, 1990; as cited in Loftus *et al*., 1994). In another study where DEHA intake was determined by measurement of urinary levels of 2-ethylhexanoic acid, the median human intake was estimated to be 2.7 mg/person-day (Loftus *et al*., 1994). However, it should be noted that 2-ethylhexanoic acid is also a metabolite of the ubiquitous di(2-ethylhexyl)phthalate and not all the measured urinary 2-ethylhexanoic acid can be attributed to DEHA.

Tsumura *et al*. (2001) monitored DEHA in diet samples to estimate daily intake in Japan. Daily diet samples consisting of breakfast, lunch and supper were obtained from three hospitals over a period of one week and analyzed for DEHA and eleven phthalate esters. Daily intake of DEHA was found to vary from hospital to hospital and from day to day. The estimated intake level ranged from non-detect (<0.1 μg/day) to 429 μg/day, with an average of 86 μg/day.

METABOLISM AND PHARMACOKINETICS

Absorption

A majority of the orally administrated DEHA is absorbed by rodents. Guest *et al*. (1985; as cited in U.S. EPA, 1990) showed that at a single oral dose of 50 or 500 mg/kg ^{14}C-labeled DEHA, B6C3F$_1$ mice excreted 91, 7, and 1 to 2 percent of the radioactive dose in the urine, feces, and expired air in 24 hours, respectively. El-hawari *et al*. (1985; as cited in U.S. EPA, 1990) administered 500 mg/kg of ^{14}C-labeled DEHA to test animals and in 24 hours observed over 91 percent and 74 percent of the administered dose in the urine of mice and rats, respectively.

Using ^{14}C-carbonyl-labeled DEHA, Takahashi *et al*. (1981) administered a single oral dose of 500 mg/kg to two male Wistar rats. Eighty-six percent of the administered dose was excreted within 24 hours and over 90 percent of the dose in 48 hours. Roughly equal amounts of radioactivity were excreted in breath and urine. The authors concluded that DEHA was almost completely absorbed from the gastrointestinal tract of rats. However, Bergman and Albanus (1987) pointed out that Takahashi *et al*. (1981) used dimethyl sulfoxide as the solvent vehicle, and it might have enhanced the gastrointestinal absorption of DEHA. Bergman and Albanus (1987) investigated the effect of solvent vehicle on the absorption of DEHA and reported that absorption was faster and the blood levels of DEHA were two to three times greater with dimethyl sulfoxide than with corn oil as the vehicle. The authors interpreted the results with corn oil as more reflective of normal absorption.

DEHA is also absorbed from the gastrointestinal tract by humans and monkeys. Loftus *et al*. (1993) orally administered 46 mg DEHA (deuterium-labeled on the ethyl side-chains) to six male volunteers and recovered 8.7 to 16 percent of the administered dose in urine within 24 hours. Less than 1 percent of the deuterium-labeled dose was detected in fecal samples. The relatively low recovery of the administered dose was probably due to further systemic metabolism of the metabolites of DEHA. The fraction of the dose absorbed is likely to be much higher than 16 percent. Loftus *et al*. (1993) analyzed for DEHA and its metabolites in blood samples taken from the volunteers at 0.5, 1, 2, 3, 4, 5, 6, 8, and 12 hours after dosing. They reported that unconjugated 2-ethylhexanoic acid was the only DEHA-related compound measurable in plasma; traces of 2-ethylhexanol (EH) were also detected but could not be quantified. El-Hawari *et al*. (1985; as cited in U.S. EPA, 1990) administered radiolabeled DEHA to Cynomolgus monkeys via the oral route and found that 49-69 percent of the radioactivity was excreted in the urine and 23-40 percent in the feces by 48 hours after administration. These studies indicate that gastrointestinal absorption efficiency of DEHA in primates ranged from 50 to 100 percent.

Takahashi *et al*. (1981) demonstrated that tissue preparations of liver, pancreas, and small intestine of rat contained enzymes that can quickly hydrolyze DEHA to EH, adipic acid (AA) and mono-(2-ethylhexyl)adipate (MEHA) *in vitro* (Table 3). They also reported that MEHA was more rapidly hydrolyzed to AA than DEHA by these preparations, and the intestinal preparation was the most active among them. For comparison purposes, Takahashi *et al*. (1981) also studied hydrolysis of di(2-ethylhexyl)phthalate, and found that the total rates of hydrolysis of DEHA by enzymes extracted from liver, pancreas, and intestine were about 10 times that of di(2-ethylhexyl)phthalate. Based on these results, Takahashi *et al*. (1981) suggested that when DEHA is orally administered, a significant amount of hydrolysis occurs prior to absorption.

Table 3. Hydrolysis of DEHA and MEHA by Tissue Enzymes of Rats *In Vitro* (Takahashi et al., 1981)*

Substrate	Enzyme source	Product (nmol/mg protein/min)[Ψ]		
		AA	MEHA	Total
DEHA	Liver	16.7	1.0	17.7
	Pancreas	17.1	41.7	58.8
	Intestine	14.8	trace	14.8
MEHA	Liver	86		86
	Pancreas	57.9		57.9
	Intestine	251.4		251.4

*In each experiment, 0.4 ml of 0.1 M DEHA or MEHA was mixed with 0.2 M buffer and 0.5 ml of enzyme preparation in a total volume of 2 ml. The mixture was incubated at 37 ºC for 60 min or 90 min, the reaction was stopped by addition of hydrochloric acid and the reaction mixture was extracted with diethyl ether.

[Ψ]The figures are the mean values of two rats.

Fox *et al*. (1984; as cited in U.S. EPA, 1990) studied the hydrolysis of DEHA, di(2-ethylhexyl)phthalate, di(2-ethylhexyl)terephthalate, and tri(2-ethylhexyl)trimillitate by gut homogenates from Sprague-Dawley rats, *in vitro*. Formation of EH increased with time for DEHA, di(2-ethylhexyl)phthalate, and di(2-ethylhexyl)terephthalate. Tri(2-ethylhexyl)trimillitate was not hydrolyzed by the homogenate. The stoichiometry of the hydrolysis reaction indicated that DEHA and di(2-ethylhexyl)terephthalate were converted to EH and their respective diacids, whereas di(2-ethylhexyl)phthalate was largely converted to EH and mono(2-ethylhexyl)phthalate. The *in vitro* disappearance half-life for DEHA was only 6 minutes.

Deisinger *et al*. (1994) reported that EH was also readily orally absorbed in rats. They administered a 50 mg/kg neat oral dose of ^{14}C-labeled EH to female F344 rats and recovered 67.4 percent, 11.6 percent, and 10.4 percent of the dose from urine, feces, and sodium hydroxide breath traps, respectively. The breath traps were used to trap $^{14}CO_2$. These data indicated that over 75 percent of the oral dose of EH was absorbed in rats. This finding is consistent with an earlier study reported by Albro (1975) who showed that when ^{14}C-labeled EH was administered to rats by gavage, about 6-7 percent of the radioactivity was excreted in respiratory CO_2 and 80-82 percent in urine.

Distribution

The distribution of DEHA is similar in rats and mice (Takahashi *et al*., 1981; Bergman and Albanus, 1987). High levels of radioactivity were detected in fat, liver, bone marrow, brown fat, adrenal cortex, corpora lutea of the ovary, salivary gland, kidney, stomach, and small intestine of the animals 12-24 hours after an oral administration of ^{14}C-labeled DEHA. Bergman and Albanus (1987) reported that DEHA might have been excreted in the bile, as they found some radioactivity in the intestinal contents after intravenous injection.

Based on the results of animal studies, DEHA and its metabolites do not seem to be extensively retained or stored in tissues. *In vivo* studies showed that approximately 90 percent of an oral dose of DEHA was excreted in mice and rats within 48 hr (Guest *et al*., 1985 and El-hawari *et al*., 1985; both cited in U.S. EPA, 1990; Takahashi *et al*., 1981). Bergman and Albanus (1987) found no retention of ^{14}C-labeled-DEHA and/or its metabolites in the tissues of mice 4 days after oral administration. In pregnant mice administered ^{14}C-labeled DEHA by either gavage or intravenous injection, Bergman and Albanus (1987) found radioactivity in the fetal liver, intestine, and bone marrow during the first 24 hours after the treatment.

Metabolism

Limited information is available regarding the metabolism and excretion of DEHA in humans. Loftus *et al*. (1993) studied metabolism and pharmacokinetics of deuterium-labeled DEHA in humans by administrating 46 mg DEHA formulated in corn oil to six male volunteers. Blood and urine samples were collected from the subjects at regular intervals. Following the administration, 2-ethylhexanoic acid was the only DEHA-related compound measurable in plasma. The formation of 2-ethylhexanoic acid was

rapid as peak concentrations were reached between 1 and 2 hours. The plasma elimination rate of 2-ethylhexanoic acid was also rapid, with an elimination half-life of approximately 1.7 hours. Besides 2-ethylhexanoic acid, other metabolites of DEHA were also detected in urine of the exposed subjects, including 5-hydroxy-2-ethylhexanoic acid, 2-ethylhexanedioic acid, and 2-ethyl-5-keto-hexanoic acid.

Takahashi *et al*. (1981) studied DEHA metabolism both *in vitro* and *in vivo*. For the *in vitro* studies, they exposed DEHA to extracts of tissue preparations from rat liver, pancreas, and small intestine. These tissue preparations hydrolyzed DEHA to mono-(2-ethylhexyl)adipate, EH, and adipic acid. For the *in vivo* studies, DEHA was administered by gavage to five male Wistar rats (500 mg/kg, assuming body weights of 200 g). DEHA and its metabolites were detected in urine, blood, stomach, small intestine, and liver. Based on the data shown in Table 4, Takahashi *et al*. (1981) suggested that DEHA was hydrolyzed to mono-(2-ethylhexyl)adipate and adipic acid in the stomach. The concentration of DEHA in the stomach declined with time, accompanied by the appearance of mono-(2-ethylhexyl)adipate and adipic acid. Peak values of these hydrolysis products occurred at 3 hours after dosing.

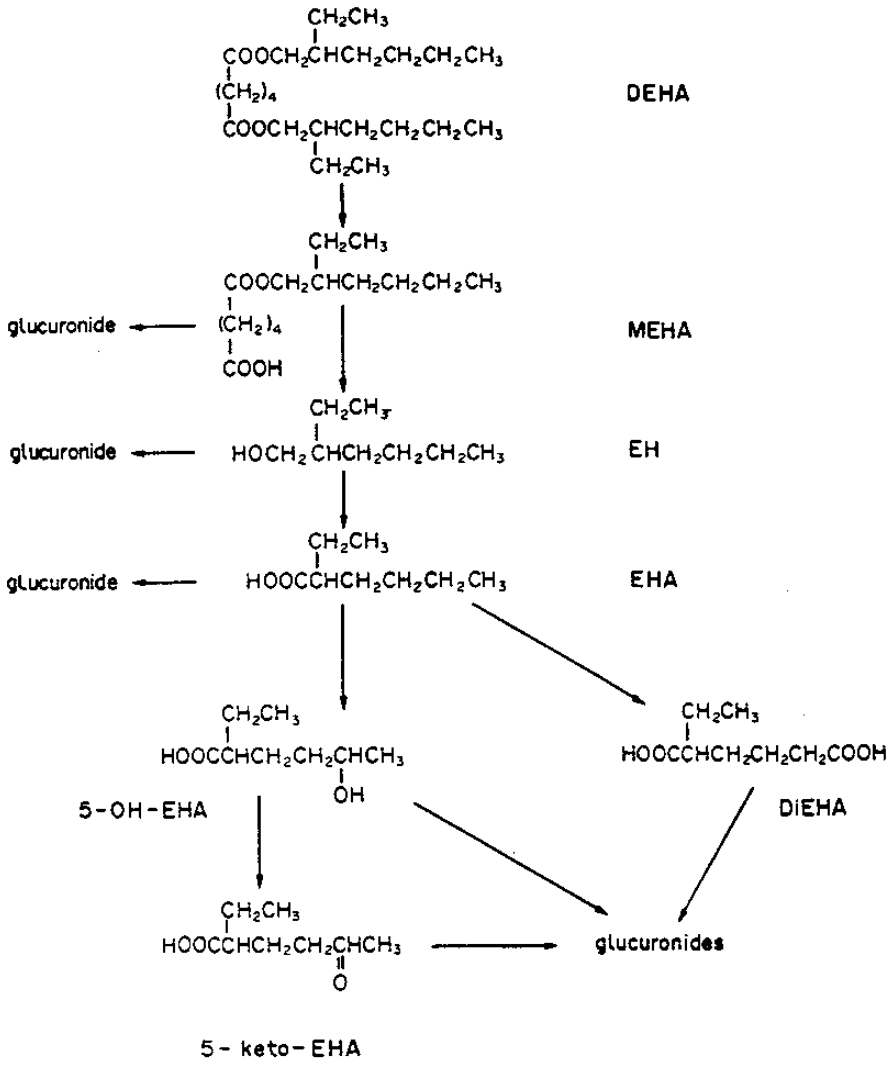
Keith *et al*. (1992) suggested that in contrast to the metabolism of di(2-ethylhexyl)phthalate, monoester metabolites of DEHA were not produced in rodents. Instead, hydrolysis of DEHA to EH and adipic acid appeared to be rapid and complete.

Table 4. Appearance of Metabolites of DEHA After Oral Administration to Rats (from Takahashi et al., 1981)

Rat	Time (hr)	Percent of administered dose					
		Urine adipic acid	Blood adipic acid	Stomach adipic acid	Stomach MEHA	Stomach DEHA	Intestine adipic acid
1	1	5.7	-	3.9	8.7	57.4	2.0
2	3	5.6	0.5	8.9	6.3	43.2	4.4
3	3	1.5	0.5	9.8	11.6	45.6	13.8
4	6	23.0	0.7	3.3	4.5	5.6	19.7
5	6	29.5	0.6	3.6	1.0	2.9	19.1

El-Hawari *et al*. (1985; as cited in U.S. EPA, 1990) studied DEHA metabolism in mice, rats, and Cynomolgus monkeys. They reported that after orally dosing mice with DEHA, the urine had metabolites of EH including 2-ethylhexanoic acid, its glucuronide, 5-hydroxy-2-ethylhexanoic acid, and 2-ethylhexanedioic acid. By contrast, rat urine contained more oxidized metabolites and less 2-ethylhexanoic acid glucuronide. Monkey urine contained mono-(2-ethylhexyl)adipate, its glucuronide, and small amounts of EH and DEHA. No oxidized metabolites of mono-(2-ethylhexyl)adipate were detected. A schematic diagram of the metabolism of DEHA is presented in Figure 1.

Figure 1. A proposed metabolic pathway of DEHA in rats (from Cornu *et al.*, 1992)



MEHA: mono-(2-ethylhexyl) adipate
EH: 2-ethylhexanol
EHA: 2-ethylhexanoic acid
5-OH-EHA: 5-hydroxy-2-ethylhexanoic acid
DiEHA: 2-ethylhexanedioic acid
5-keto-EHA: 2-ethyl-5-keto-hexanoic acid

Since it has been shown that a significant portion of the DEHA dose may be hydrolyzed to adipic acid and EH in the gastrointestinal tract of rats, metabolic pathways of these two compounds are discussed below.

Metabolism of adipic acid

Metabolism of adipic acid, a key metabolite of DEHA, has been studied by Rusoff *et al*. (1960). The investigators administered approximately 50 mg ^{14}C-labeled adipic acid to rats by gavage and within 24 hours recovered up to 70 percent of the radioactivity in the breath as carbon dioxide. In addition to adipic acid, Rusoff *et al*. (1960) also detected five radioactive metabolites including urea, glutamic, lactic, β-ketoadipic, and citric acids in the urine. Due to the presence of β-ketoadipic acid in the urine, they suggested that some of the administered adipic acid underwent β-oxidation to β-ketoadipic acid which in turn could be further metabolized to succinic acid.

Metabolism of 2-ethylhexanol (EH)

Deisinger *et al*. (1994) administered a 50 mg/kg neat oral dose of ^{14}C-labeled EH to female F344 rats and recovered approximately 67.4 percent of the dose from urine. They found the major urinary metabolites were 2-ethyladipic acid (2-ethylhexanedioic acid), 2-ethylhexanoic acid, and 6-hydroxy-2-ethylhexanoic acid. Most of the compounds were detected as glucuronide conjugates. Minor metabolites detected also included 5-hydroxy-2-ethylhexanoic acid, 2-ethyl-5-hexenoic acid, and EH, the parent compound.

English *et al*. (1998) suggested that most of the EH administered to rats was metabolized through the formation of 2-ethylhexanoic acid. They administered ^{14}C-labeled 2-ethylhexanoic acid at 1 g/kg to female F344 rats by gavage and detected the glucuronic acid conjugate of 2-ethylhexanoic acid (45 percent), 6-hydroxy-2-ethylhexanoic acid and 2-ethyl-adipate (7.3 percent), and the parent compound (2.4 percent) in the urine. Several other minor metabolites also detected in the urine included 5-hydroxy-2-ethylhexanoic acid and ethylketohexanoic acid.

Excretion

Takahashi *et al*. (1981) administered ^{14}C-labeled DEHA (500 mg/kg) in dimethyl sulfoxide to rats by gavage and found that 86 percent of the administered dose was excreted in 24 hours. They found that almost all (98 percent) the dose was excreted in 48 hours. Approximately equal amounts of the administered dose were recovered from breath and urine (45 percent each), with about 1-5 percent from feces. Guest *et al*. (1985; as cited in U.S. EPA, 1990) found that $B6C3F_1$ mice receiving oral doses of 50 or 500 mg/kg DEHA excreted within 24 hours, 91, 7, and 1 to 2 percent of the radioactive dose in urine, feces, and expired air, respectively.

El-Hawari *et al*. (1985; as cited in U.S. EPA, 1990) found the excretion of radioactivity 24 hours following an oral dose of DEHA in the urine, feces, and expired air was 91, 6-8, and 1-2 percent of the dose, respectively, in mice; and 74-78, 15-20, and 1-2 percent, respectively, in rats. They also showed that Cynomolgus monkeys excreted 49-69

percent of the radioactivity in urine and 23-40 percent in the feces 48 hours after the oral administration of DEHA.

The large discrepancies in the percentage of the administered dose excreted in the exhaled air reported by the three studies described above may be explained by the difference in the location of the label. Takahashi *et al*. (1981) labeled the carbonyls of the DEHA molecule whereas Guest *et al*. (1985; as cited in U.S. EPA, 1990) and El-Hawari *et al*. (1985; as cited in U.S. EPA, 1990) labeled the hexyl carbon chains of the molecule.

TOXICOLOGY

Toxicological Effects in Animals

The adverse health effects of DEHA in animals have been reviewed by U.S. EPA (1990), IARC (1982, 2000a), NTP (1982), and Kluwe (1986). Much of the information provided below was obtained from these sources.

Acute Toxicity

DEHA is only slightly toxic to rodents through the oral route. NTP (1982) has summarized the reported LD_{50}s and the results are reproduced in Table 5.

Table 5. Acute Toxicity Values of DEHA in Rodents (adapted from NTP, 1982)

Species	Sex	Route	LD_{50} (mg/kg)
Rats	Unspecified	Intravenous	830
Rats	Unspecified	Oral	5600
Rats	Unspecified	Intraperitoneal	43,300
Rats (F344)	Male	Gavage	45,000
Rats (F344)	Female	Gavage	25,000
Mice (Harlan/ICR Swiss)	Male/female	Intraperitoneal	43,300
Mice ($B6C3F_1$)	Male	Gavage	15,000
Mice ($B6C3F_1$)	Female	Gavage	25,000

Acute toxicity of some structural analogs of DEHA has also been reported. Oral LD_{50}s of >1,600 mg/kg, 12,900 mg/kg, and 5,6 mg/kg in rats were estimated for diethyl adipate, dibutyl adipate, and diethylbutyl adipate, respectively (reviewed by Sandmeyer and Kirwin, 1981).

LD_{50} values of EH in rats resulting from single oral doses have been reported to be greater than 2,000 mg/kg (Rowe and McCollister, 1982; as cited in Deisinger *et al*., 1994).

Subchronic Toxicity

The British Industrial Biological Research Association (1986; as cited in HSDB, 1999) fed nominal concentrations of 0, 0.1, 0.6, 1.2, or 2.5 percent DEHA to groups of male and female F344 rats (5/sex/dose) in diet for 21 days. Compared with the controls, absolute and relative liver weights were increased in the 0.6, 1.2, and 2.5 percent group for females and in the 1.2 and 2.5 percent group for males. Relative kidney weights were also increased in the male and female groups exposed to 1.2 and 2.5 percent DEHA. There was a dose-related increase in peroxisome proliferation at doses above 0.1 percent (1,000 ppm).

Smyth *et al*. (1951; as cited in U.S. EPA, 1990) fed DEHA in diet to rats (five/sex/group) at doses from 160 to 4,740 mg/kg-day for 90 days. At 4,740 mg/kg-day, increased mortality was observed. Animals receiving 2,920 mg/kg-day exhibited reduced growth, reduced appetite, altered liver or kidney weights, and microscopic lesions in the liver, kidney, spleen, or testes. No adverse effects were observed at doses of 610 mg/kg-day or below. Based on this study, a no-observed-adverse-effect level (NOAEL) of 610 mg/kg-day can be estimated for rats.

NTP (1982) exposed groups of 10 F344 rats and $B6C3F_1$ mice of each sex to 0, 1,600, 3,100, 6,300, 12,500, or 25,000 ppm DEHA in diet for 13 weeks. Weight gain depression was 11 percent or more for male rats fed 12,500 or 25,000 ppm and weight gain depression was 6 percent or more for female rats fed 12,500 or 25,000 ppm. No compound-related histopathologic effects or reduction in feed consumption were observed. Assuming a feed ingestion rate of 0.018 kg/day (U.S. EPA, 1988) and a final body weight of 320 g for male rats (NTP, 1982), a NOAEL for rats was estimated to be 6,300 ppm or 354 mg/kg-day for weight gain depression. Weight gain depression was 10 percent or more for male mice fed 3,100 ppm or more and weight gain depression was 10 percent or more for female rats fed 6,300 or 25,000 ppm. No compound-related histopathologic effects or reductions in feed consumption were observed. Assuming a feed ingestion rate of 0.0057 kg/day (U.S. EPA, 1988) and a final body weight of 32 g for male mice (NTP, 1982), a NOAEL for mice was estimated to be 1,600 ppm or 285 mg/kg-day for weight gain depression.

Many researchers have shown that when high doses of DEHA were orally administered to mice or rats, the treatment induced peroxisomal and microsomal fatty acid oxidizing enzymes in the liver. Keith *et al*. (1992) administered DEHA, EH, or 2-ethylhexanoic acid by gavage to rats and mice for 14-consecutive days. Five doses were used in the study, ranging from 0.54 to 6.74 mmol/kg-day (200 mg/kg-day to 2,500 mg/kg-day) for DEHA and 1.1 to 13.5 mmol/kg-day for EH and 2-ethylhexanoic acid. At doses above 8 mmol/kg-day, EH was toxic to male and female rats, leading to death of the animals. Similarly, 2-ethylhexanoic acid at 13.5 mmol/kg-day was toxic to female rats. Administration of all compounds increased relative liver weight (Table 6) and induced hepatic peroxisome proliferation (as measured by electron microscopy) as well as

cyanide-insensitive palmitoyl CoA oxidase[1] in both species. Furthermore, a linear dose-response relationship was observed for all three end-points. On a molar basis, they found that DEHA was approximately twice as potent as EH or 2-ethylhexanoic acid in increasing relative liver weights. Based on the relative liver weight data, a NOAEL of 500 mg/kg-day can be identified for both species (Table 6).

Table 6. Effect of DEHA on Relative Liver Weight of Male and Female Rats and Mice (Keith et al., 1992)

DEHA dose (mg/kg-day)	Liver weight / body weight × 100			
	Male rat	Female rat	Male mouse	Female mouse
0	5.24 ± 0.57	4.92 ± 0.49	5.43 ± 0.35	5.09 ± 0.42
200	5.32 ± 0.18	4.55 ± 0.27	5.55 ± 0.24	5.02 ± 0.42
500	5.78 ± 0.47	4.94 ± 0.35	5.70 ± 0.30	5.44 ± 0.23
1000	6.38 ± 0.17 *	5.56 ± 0.16 *	6.28 ± 0.46 *	6.01 ± 0.29 *
1500	6.86 ± 0.34 *	5.44 ± 0.40	7.01 ± 0.45 *	6.40 ± 0.58 *
2500	7.39 ± 0.40 *	6.35 ± 0.53 *	7.85 ± 0.57 *	6.86 ± 0.57 *

Values are mean ± standard deviation (n=5).
* Values significantly different from controls, $p<0.05$.

Lake *et al*. (1997) fed diets containing different levels of DEHA to female rats and female mice for periods of 1, 4, and 13 weeks. Six doses (144, 282, 577, 1,135, 2,095, or 3,140 mg/kg-day) were used in the rat study and five doses (343, 808, 1,495, 3,075, or 5,330 mg/kg-day) were used in the mouse study. Treatment of rats and mice with DEHA produced a dose-related increase of liver cyanide-insensitive palmitoyl-CoA oxidase as well as liver microsomal lauric acid 11- and 12- hydroxylase activities at all time points. A significant increase in relative liver weight was observed in rats treated with DEHA at 1,135 mg/kg-day or above for 1 or 4 weeks. At 13 weeks, a significant increase in relative liver weight was observed in rats treated at 577 mg/kg-day and above. Similar results were observed in mice. A significant increase in relative liver weight was observed in mice treated with DEHA at 1,495 mg/kg-day and above for 1 or 4 weeks. At 13 weeks, a significant increase in relative liver weight was observed at 3,075 and 5,330 mg/kg-day. Based on the relative liver weight data in rats, a NOAEL of 282 mg/kg-day (13 weeks of exposure) can be identified.

DEHA has also been found to cause a decrease in serum cholesterol and triglycerides in rats. Moody and Reddy (1982) fed nine male F344 rats diets containing 2 percent DEHA for 3 weeks. At the end of the experiment, they found serum cholesterol levels in treated

[1] Cyanide-insensitive palmitoyl-CoA oxidation is often used to measure the overall activity of the peroxisomal fatty acid β-oxidation. Another method is to measure the level of acyl-CoA oxidase which is the first rate-limiting enzyme of the peroxisomal fatty acid β-oxidation (Lake, 1995).

animals decreased by about 15 percent ($p<0.05$) compared with the control, and serum triglyceride levels decreased by >66 percent ($p<0.05$).

Chronic Toxicity

F344 rats and $B6C3F_1$ mice (50/sex/group) were fed diet containing 12,000 or 25,000 ppm DEHA for 103 weeks (NTP, 1982). Groups of 50 unexposed rats and mice of each sex were used as controls. NTP (1982) reported that mean body weights of high-dose rats of either sex were lower than those of the controls throughout the study. No other clinical signs were observed. Mean body weights of dosed mice of either sex were also lower than those of the corresponding controls throughout the bioassay, and the decrease in weight gain was dose-related. NTP (1982) reported increased hepatocellular adenomas or carcinomas in the dosed male and female mice (see carcinogenicity section).

A chronic NOAEL was derived from the rat data in the NTP study. Using a figure in the NTP report (1982), it was estimated that near the end of the study the average body weights of male and female F344 rats were 400 and 300 g, respectively. In the "Recommendations for and Documentation of Biological Values for Use in Risk Assessment," U.S. EPA (1988) estimated that average food consumption rates of male and female F344 rats are 0.03 and 0.021 kg/day, respectively. Using these estimates, the calculated DEHA doses administered to male and female rats in the low-dose groups were 900 and 840 mg/kg-day, respectively. Based on the results of this study, a NOAEL of 840 mg/kg-day can be estimated for rats.

Developmental and Reproductive Toxicity

Di(2-ethylhexyl)adipate (DEHA)

Results are available from two reproductive and developmental studies performed by ICI Central Toxicology Laboratory (ICI, 1988a, unpublished data; as cited in U.S. EPA, 1999). In the developmental toxicity study, Wistar-derived pregnant rats (24/dose) were fed diets containing 0, 300, 1,800 or 12,000 ppm DEHA (corresponding to doses of 0, 28, 170 or 1,080 mg/kg-day) on gestation days 1-22. At the high dose, slight reductions in maternal body weight gain and food consumption were observed, and reduced ossification and kinked or dilated ureters were found in the fetuses.

In a companion one-generation reproductive study (ICI, 1988b, unpublished data; as cited in U.S. EPA, 1999), groups of Wistar-derived rats (15 males/dose, 30 females/dose) were administered DEHA in their diets at the same levels, again yielding approximate doses of 0, 28, 170, or 1,080 mg/kg-day. After 10 weeks on the diet, the animals were mated to produce one generation of offspring that was reared to day 36 postpartum. Test diets were fed continuously throughout the study (approximately 18-19 weeks of exposure). No effects were seen on male or female fertility. However, at the highest dose, there was a reduction in the body weight gain of the dams during gestation, an increase in liver weight in both male and female parents, and reductions in offspring weight gain, total litter weight, and litter size. The NOAEL and lowest-observed-adverse-effect level (LOAEL) for these two studies were identified as 170 mg/kg-day and 1,080 mg/kg-day,

respectively. U.S. EPA (1999) applied an uncertainty factor of 300 to the NOAEL to derive an oral reference dose (RfD) of 0.6 mg/kg-day.

Singh *et al*. (1973; as cited in U.S. EPA, 1990) administered DEHA by intraperitoneal injection to groups of five Sprague-Dawley rats on days 5, 10, and 15 of gestation. DEHA was given at dose levels of 900, 4,600, or 9,200 mg/kg. No increase in embryolethality occurred, but reduced fetal weight ($p<0.05$) was seen at the two highest dose levels.

Singh *et al*. (1975; as cited in U.S. EPA, 1990) also studied the effects of DEHA on fertility in male mice. Harlan/ICR albino Swiss strain mice were administered a single interperitoneal dose (10 animals/dose) of 0, 450, 900, 4,600, or 9,200 mg/kg. The mice were then mated with two virgin females per week for 8 weeks. The mean incidence of pregnancies for the 160 control mice was 82 ± 1.9 percent. The investigators found that DEHA treatment significantly reduced the number of pregnancies in the highest dosed group to 67±4.0 percent ($P<0.05$). Pregnancy rates of the three lower dosed groups were not statistically different from that of the controls. Based on the stages of spermatogenesis (postmeiotic or premeiotic) at the time of injection, the data were analyzed separately. When DEHA was injected at the postmeiotic stage, a time-dependent ($P<0.01$) and dose-dependent ($P<0.05$) reduction in implants was observed. The investigators concluded that the injection of DEHA to male mice before mating elicited some dose-related antifertility activity and dominant lethal mutations, as manifested by a decrease in the incidence of pregnancies and an increase in the number of early fetal deaths.

As DEHA can be readily hydrolyzed into EH and adipic acid, the developmental and reproductive toxicity data of EH and the oxidized product of EH, 2-ethylhexanoic acid, are described below. While these data are not suitable for quantitative dose-response evaluation of DEHA, they clearly indicate the potential of EH and 2-ethylhexanoic acid, two key metabolites of DEHA, for causing adverse reproductive and developmental effects in rodents.

2-Ethylhexanol (EH)

Tyl *et al*. (1992) administered EH by occluded dermal application to pregnant F344 rats on gestation days 6 through 15. Animals were treated at 0, 252, 840, or 2,520 mg/kg-day. Maternal weight gain was reduced at 2,520 mg/kg-day. Maternal organ weights and gestational and fetal parameters were unaffected by treatment with EH. There were no treatment-related increases in the incidence of individual or pooled external, visceral, and skeletal malformations or variations following the application. Tyl *et al*. (1992) suggested a NOAEL of 252 mg/kg-day based on skin irritation and a NOAEL of 840 mg/kg-day based on systemic toxicity for the maternal toxicity. They reported that the developmental toxicity NOAEL was at least 2,520 mg/kg-day, with no teratogenicity.

Ritter *et al*. (1987) administered a single gavage dose of undiluted EH to seven pregnant female Wistar rats on gestation day 12. Two dose levels were used, 810 or 1,620 mg/kg. The rats were killed on day 20 of gestation. In the low- and high-dosed group, 2.0 ± 1.3 percent and 22.2 ± 14.7 percent of the living fetuses were found to be

malformed, respectively. By comparison, none of the living fetuses in the control group was malformed.

U.S. EPA (1990; as cited in Tyl *et al*., 1992) administered gavage doses of EH at 130, 650, and 1,300 mg/kg-day on gestation days 6 through 15 to groups of 10 pregnant female Wistar rats per dose level. Marked maternal toxicity was seen at the highest dose together with increased numbers of fetal resorptions, marked post-implantation loss, and reduced fetal body weight. An increased incidence of skeletal malformations and variations and dilated renal pelvis and hydroureter were also seen at the top dose. There were only marginal indications of these effects at the intermediate dose and there were no adverse effects at the lowest dose.

Hellwig and Jäckh (1997) administered gavage doses of 144, 720, or 1,440 mg/kg-day of EH to groups of female Wistar rats between gestation day 6 and 15. There were two control groups in the study. Control group one was fed with distilled water and control group two was fed with distilled water with approximately 0.005 percent Cremophor EL. There were nine or ten animals in each group. In the highest dose group, the investigators found severe maternal toxicity, an increased number of early resorptions, and a high postimplantation loss. Reduced body weight and an increased frequency of skeletal malformation were also observed in the fetuses of this group. At 720 mg/kg-day, slight maternal toxicity was visible. Fetal body weights were slightly reduced and an increased number of fetuses with skeletal variations and retardations was observed. Six fetuses in three litters of this group showed asymmetric dumbbell-shaped thoracic vertebral bodies. This effect was significant at the $p<0.05$ level and was not observed in the low-dose group and in control group one, but twice in control group two. Price *et al*. (1991) exposed timed-pregnant Swiss (CD-1) mice to EH at 0, 17, 60, or 194 mg/kg-day in feed from gestation days 0 through 17. On gestation day 17, implant viability, fetal weight, sex and morphological development (external, visceral, and skeletal) were examined. EH did not produce maternal or developmental toxicity at the levels tested.

Hardin *et al*. (1987; as cited in Tyl *et al*., 1992) administered gavage doses of 1,525 mg/kg-day of EH to 49 female CD-1 mice on gestation days 6 through 13. They observed a 30 percent mortality rate in the dams, and maternal body weights, numbers of viable litters, and litter sizes were all reduced at delivery.

2-Ethylhexanoic acid

Ritter *et al*. (1987) administered a single gavage dose of undiluted 2-ethylhexanoic acid to groups of pregnant female Wistar rats on gestation day 12. Two dose levels were used, 900 mg/kg (7 rats) and 1,800 mg/kg (10 rats). The rats were killed on day 20 of gestation. In the low- and high-dosed group, 0.8 ± 0.8 percent and 67.8 ± 10.9 percent of the living fetuses were found to be malformed, respectively. By comparison, none of the living fetuses in the control group was malformed.

Pennanen *et al*. (1992 and 1993) studied reproductive and developmental toxicity of 2-ethylhexanoic acid in Wistar rats. Pennanen *et al*. (1992) exposed pregnant rats to 2-ethylhexanoic acid in drinking water at doses of 0, 100, 300, or 600 mg/kg-day on days 6-19 of gestation. They reported that the chemical was toxic to the dams and the fetuses at the highest dose. At doses of 100 mg/kg-day and above, 2-ethylhexanoic acid caused

skeletal malformations (clubfoot, absence of fibula, polydactyly). The number of affected fetuses increased in a dose-dependent way (4.9, 8.9, and 15.3 percent of treated offspring at 100, 300, and 600 mg/kg-day, respectively, compared with 2.4 percent in the controls). In another study, Pennanen *et al*. (1993) exposed male and female Wistar rats to 2-ethylhexanoic acid in drinking water at 0, 100, 300, or 600 mg/kg-day for at least 2 weeks prior to mating and during the mating period. Female rats were also exposed during the entire gestation and lactation period. 2-Ethylhexanoic acid caused a slight but dose-dependent decrease in fertility at 300 and 600 mg/kg-day. 2-Ethylhexanoic acid reduced sperm mobility at 100 and 600 mg/kg-day and abnormal sperm occurred more frequently at the two highest dose levels. The average litter size was reduced by 16 percent in the 600 mg/kg-day dose group and some developmental abnormalities (kinky tail, lethargic, slightly paralyzed legs) were observed in offspring of rats dosed at 300 and 600 mg/kg-day.

Hendrickx *et al*. (1993) studied developmental toxicity of 2-ethylhexanoic acid in F344 rats and New Zealand white rabbits. They gave pregnant rats gavage doses of 100 to 1,000 mg/kg-day of EH on gestation days 6-15. They observed increased maternal mortality at 1,000 mg/kg-day and maternal toxicity (increased liver weight, increased resorptions, dead fetuses, and growth retardation, but no malformations) at 500 mg/kg-day. Slight developmental toxicity such as a reduction in skeletal ossification was observed in fetuses exposed to 250 mg/kg-day. No adverse effects of treatment were associated with the two lower doses (100 and 125 mg/kg-day).

Hendrickx *et al*. (1993) gave pregnant rabbits gavage doses of 25 to 1,000 mg/kg-day of EH on gestation days 6-18 and observed excessive maternal mortality at 500 and 1,000 mg/kg-day. A low incidence of maternal death as well as abortion occurred following treatment with 125 and 250 mg/kg-day. There were no adverse effects on fetal viability, growth, or morphology at any dose level.

Genetic Toxicity

DEHA was not mutagenic in *Salmonella typhimurium* TA1535, TA1537, TA1538, TA98, and TA100, with and without metabolic activation (Simmon *et al*., 1979; Zeiger *et al*., 1982). Dirven *et al*. (1991) tested DEHA and its metabolites, mono-(2-ethylhexyl)adipate, mono-(2-ethyl-5-hydroxyhexyl)adipate, and mono-(2-ethyl-5-oxohexyl)adipate, for mutagenicity in the Ames assay using TA97, TA98, TA100, and TA102, with and without metabolic activation. They showed that concentrations of these compounds up to 1,000 µg/plate were negative. DEHA was also negative in the forward mutation at the TK locus in the L5178Y mouse lymphoma cells, with or without metabolic activation (McGregor *et al*., 1988).

Singh *et al*. (1975) studied dominant-lethal mutation by administering a single intraperitoneal injection of DEHA to male Harlan/ICR albino Swiss strain mice (10 animals/dose) at doses of 0, 450, 900, 4,600, or 9,200 mg/kg. The mice were then mated with two virgin females per week for 8 weeks. DEHA was associated with a statistically significant dose-related increase in dominant-lethal mutations as measured by early fetal deaths.

DEHA was not clastogenic in a number of *in vitro* and *in vivo* tests. Reisenbichler and Eckl (1993) exposed rat hepatocytes to DEHA *in vitro* for 3 hr and 51 hr. In both experiments, DEHA did not induce micronuclei or chromosomal aberrations at concentrations up to 200 µM. The clastogenic potential of DEHA *in vivo* was evaluated by Litton Bionetics Inc. (1982). A single or multiple intraperitoneal injections were given to two groups of mice at 5,000 mg/kg-day for either one (single) or two (multiple) days. There were no significant differences in the percent of micronucleated polychromatic erythrocytes between treated animals and controls. Woodruff *et al*. (1985; as cited in IARC, 2000a) evaluated DEHA for the induction of sex-linked recessive lethal mutations in *Drosophila melanogaster* and found that DEHA was negative in these tests at 5,000 ppm by injection and 20,000 ppm by feeding.

Von Däniken *et al*. (1984) investigated the potential binding of DEHA to liver DNA *in vivo*. They orally administered DEHA radiolabeled in different parts of the molecule to female mice and found only a minute amount of radioactivity was associated with liver DNA. Nucleoside and base analyses as well as correlations obtained between $^{14}CO_2$ expired and specific activity of DNA indicated that the radioactivity associated with the DNA was largely due to biosynthetic incorporation of radiolabeled breakdown products. Von Däniken *et al*. (1984) suggested that covalent interaction with DNA is unlikely to be the mode of tumorigenic action of DEHA in rodents.

At high doses, DEHA has been shown to cause DNA damage *in vivo*. Takagi *et al*. (1990) fed DEHA in the diet at 2.5 percent to rats for one or two weeks and observed a significant increase of 8-hydroxydeoxyguanosine (8-OH-dG) in liver DNA of rats. It is hypothesized that DEHA induces peroxisome proliferation, increases production of H_2O_2, and causes the formation of 8-OH-dG in the liver cells. An increased level of 8-OH-dG is often used as an indicator of oxidant-induced DNA damage. From the same study, Takagi *et al*. (1990) also reported that the treatment significantly increased liver weights of rats exposed to DEHA compared to the controls. It is important to note that liver is also the target organ of DEHA carcinogenesis in mice.

DEHA has also been shown to induce DNA synthesis *in vivo*. Büsser and Lutz (1987) administered a single oral dose of DEHA at 3.78 mmol/kg (1400 mg/kg) to male Fischer F344 rats and observed a significant increase of liver DNA synthesis 24 hours following the administration. Lake *et al*. (1997) fed 0.15 to 4 percent DEHA in diet to female F344 rats and female $B6C3F_1$ mice for 1, 4, or 13 weeks. Seven-day osmotic pumps (containing 15 mg/ml 5-bromo-2'-deoxyuridine) were implanted in the rats subcutaneously at the beginning of the last week of the exposure period to study replicative DNA synthesis. The investigators found that after 1 week of exposure, replicative DNA synthesis assessed as the hepatocyte labeling index was significantly increased to 395 and 345 percent of control in rats given 2,095 and 3,140 mg/kg-day of DEHA, respectively (Figure 2A). Replicative DNA synthesis was increased to 245, 245, and 445 percent of control in mice given 1,495, 3,075, and 5,330 mg/kg-day of DEHA, respectively (Figure 2B). While DEHA treatment for 4 and 13 weeks did not increase labeling index values in the rat, a sustained stimulation of replicative liver DNA synthesis was observed in mice given DEHA at 3,075 and 5,330 mg/kg-day (Figure 2). The investigators suggested that sustained liver DNA synthesis may be better correlated with

the observed formation of liver tumors in chronic studies with DEHA in female mice, but not in female rats, than the magnitude of stimulation of hepatic peroxisome proliferation.

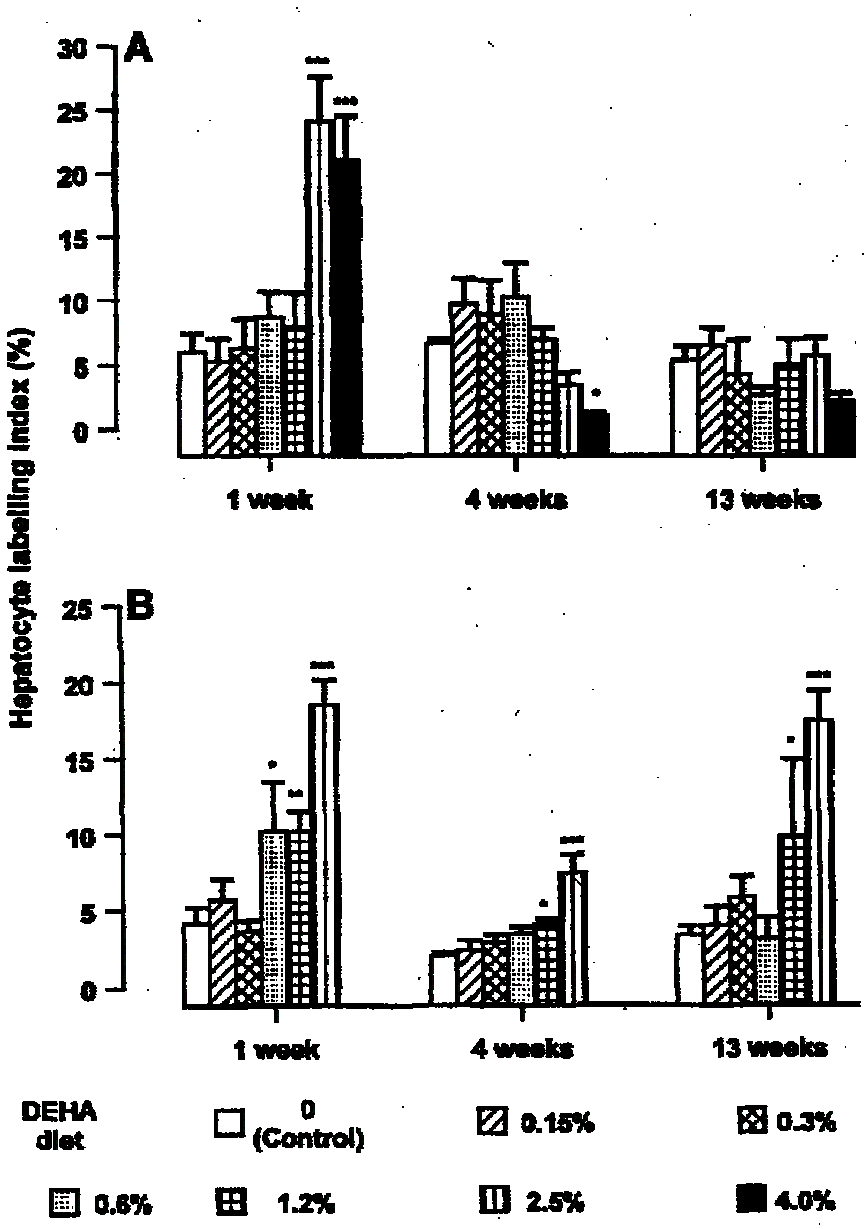


Figure 2. Effect of feeding diets containing 0-4 percent DEHA for periods of 1, 4, and 13 weeks to rats (A) and mice (B) on the hepatocyte labeling index.

Results are expressed as means ± SEM of five to eight animals. Values significantly different from control are: * $p<0.05$; ** $p<0.01$; *** $p<0.001$ (from Lake *et al*., 1997).

DEHA was negative in a cell transformation assay using the BALB/3T3 mouse cell line. DEHA was tested at 3.38, 6.75, 13.5, and 27 nL/mL levels, with cell survival ranging from 32 to 89 percent of the solvent control. It did not induce a significant number of transformed foci (Litton Bionetics, 1982). A similar test was conducted by Microbiological Associates (1984; as cited in HSDB, 1999) also showing negative results.

EH, a key metabolite of DEHA, has also been shown to be not mutagenic in *Salmonella typhimurium* TA1535, TA1537, TA98, and TA100, with and without metabolic activation (Zeiger *et al*., 1982). Phillips *et al*. (1982) tested clastogenicity of EH in Chinese hamster ovary cells and found that it did not induce chromosomal aberration up to 2.2 mM. They observed a very slight effect at 2.4 mM, the maximum dose consistent with continued cell division.

Carcinogenicity

The DEHA carcinogenicity data have been reviewed by IARC (1982, 1987, 2000a) and U.S. EPA (1999). IARC (2000a) found that there is limited evidence that DEHA is carcinogenic in mice, and there was no data on the carcinogenicity of the compound to humans. IARC (2000a) identified DEHA as a Group 3 chemical, not classifiable as to carcinogenicity in humans.

Based on a 1991 evaluation, U.S. EPA (1999) classified DEHA as a possible human carcinogen, Group C. The classification was based on an absence of human data and increased incidence of liver tumors in female mice. U.S. EPA (1999) also noted that there was no evidence of genotoxicity, except for a positive dominant lethal assay, and the chemical is structurally related to other nongenotoxic compounds classified as probable and possible human carcinogens. In the same evaluation, U.S. EPA applied the linearized multistage high-to-low dose extrapolation procedure to the female mouse data reported by NTP (1982) and developed an oral slope factor of 1.2×10^{-3} $(mg/kg\text{-}day)^{-1}$ for DEHA (U.S. EPA, 1999).

Animal bioassay

Only one series of cancer studies on DEHA has been reported in the literature. NTP (1982) administered 12,000 or 25,000 ppm of DEHA in the diet to groups of 50 male and 50 female F344 rats and 50 male and 50 female $B6C3F_1$ mice for 103 weeks. Groups of 50 unexposed rats and mice of each sex were used as controls. All surviving animals were killed at 104 to 107 weeks. Mean body weights of dosed mice and high-dose rats of either sex were lower than those of the corresponding controls throughout the study. No other clinical signs were observed.

Survival in the female control rats declined relative to the dose groups after 80 weeks on study. The survival between the dosed groups in female rats and among all three groups in male rats was comparable. NTP (1982) found that DEHA administration was not associated with tumor formation in F344 rats of either sex.

While survival among all three groups of female mice was comparable, survival in the low-dose group of male mice was less than that in the control from week 15 to the end of the study. Survival of the high-dose male mice was comparable to that of the controls.

A significantly higher incidence of hepatocellular adenomas or carcinomas was observed in high-dose mice of both sexes and in low-dose female mice. The time to observation of hepatocellular adenomas or carcinomas in the dosed female mice, but not in dosed male mice, was significantly shorter than the time to observation of these tumors in the controls (Tables 7 and 8). Assuming a food consumption rate of 0.0062 kg/day (U.S. EPA, 1988) and an average body weight of 40 g (NTP, 1982) for mice, the estimated low and high doses administered to mice were 1,860 and 3,880 mg/kg-day, respectively.

NTP (1982) did not consider the association of liver tumors in the male mice with administration of DEHA conclusive because:

1. the incidence of hepatocellular adenomas or carcinomas in the male high-dose group was not greatly increased over that in the male $B6C3F_1$ historical control mice in the same laboratory, and
2. the time to observation of tumors in dosed groups was not significantly different than in the control group.

Table 7. Summary of the Incidence of Primary Liver Tumors in Male Mice Fed Diets Containing DEHA (from NTP, 1982)

Morphology	Control	Low dose (12,000 ppm in feed)	High dose (25,000 ppm in feed)
Hepatocellular adenoma [a]	6/50	8/49	15/49
P values [b]	P=0.013	NS	P=0.021
Weeks to first observed tumor	46	37	101
Hepatocellular carcinoma	7/50	12/49	12/49
P values	NS	NS	NS
Weeks to first observed tumor	86	68	65
Hepatocellular adenoma or carcinoma	13/50	20/49	27/49
P values	P=0.002	NS	P=0.003
Weeks to first observed tumor	46	37	65

[a] Number of tumor-bearing animals/number of animals examined at site.
[b] Beneath the tumor incidence in the control group is the probability level for the Cochran-Armitage test when P is less than 0.05; otherwise, not significant (NS). Beneath the tumor incidence in a dosed group is the probability level for the Fisher exact test for comparison of that dosed group with the control group when P is less than 0.05; otherwise, NS is indicated.

Table 8. Summary of the Incidence of Primary Liver Tumors in Female Mice Fed Diets Containing DEHA (from NTP, 1982)

Morphology	Control	Low dose (12,000 ppm in feed)	High dose (25,000 ppm in feed)
Hepatocellular adenoma [a]	2/50	5/50	6/49
P values [b]	NS	NS	NS
Weeks to first observed tumor	106	103	84
Hepatocellular carcinoma	1/50	14/50	12/49
P values	P=0.003	P<0.001	P=0.001
Weeks to first observed tumor	106	85	79
Hepatocellular adenoma or carcinoma	3/50	19/50	18/49
P values	P=0.001	P<0.001	P<0.001
Weeks to first observed tumor	106	85	79

[a] Number of tumor-bearing animals/number of animals examined at site.
[b] Beneath the incidence of tumors in the control group is the probability level for the Cochran-Armitage test when P is less than 0.05; otherwise, not significant (NS). Beneath the incidence of tumors in a dosed group is the probability level for the Fisher exact test for the comparison of that dosed group with the control group when P is less than 0.05; otherwise, not significant (NS) is indicated.

NTP (1982) concluded that "under the condition of this bioassay, di(2-ethylhexyl)adipate was not carcinogenic for F344 rats. Di(2-ethylhexyl)adipate was carcinogenic for female $B6C3F_1$ mice, causing increased incidences of hepatocellular carcinomas, and was probably carcinogenic for male $B6C3F_1$, causing hepatocellular adenomas."

Structure-activity relationship (carcinogenicity)

Kluwe (1986) and Kluwe *et al*. (1985) studied liver carcinogenicity of four compounds containing the 2-ethylhexyl moiety (DEHA, di(2-ethylhexyl)phthalate, tris(2-ethylhexyl) phosphate, and 2-ethylhexyl sulfate) in male and female Fischer 344 rats and $B6C3F_1$ mice. Groups of 50 animals of each species and sex were exposed through diet for approximately 104 weeks to one of two chemical doses or control. Doses used in the studies are shown in Table 9, and the carcinogenic results are presented in Tables 10 and 11; the data for DEHA are from the NTP bioassay (NTP, 1982). Kluwe (1986) found that all four compounds possessed some hepatocarcinogenic activity, indicating the 2-ethylhexyl moiety may have a propensity for causing hepatocarcinogenesis in mice, particularly among females. The compound that caused the greatest hepatocarcinogenic

response in mice, di(2-ethylhexyl)phthalate, was also hepatocarcinogenic in rats. Similarly, those with a relatively greater effect in female mice were also active in male mice. Kluwe (1986) concluded that sex and species differences in 2-ethyhexyl-induced hepatocarcinogenesis in rodents are probably quantitative rather than qualitative in nature.

Table 9. Doses Used for the Carcinogenicity Studies (from Kluwe, 1986)

Compound	Species	Sex	Control	Low dose	High dose
DEHA*	Rat	M, F	Untreated	12,000 ppm	25,000 ppm
	Mouse	M, F	Untreated	12,000 ppm	25,000 ppm
Di(2-ethylhexyl)phthalate	Rat	M, F	Untreated	6,000 ppm	12,000 ppm
	Mouse	M, F	Untreated	3,000 ppm	6,000 ppm
Tris(2-ethylhexyl)phosphate	Rat	M	Vehicle control	2,000 mg/kg	4,000 mg/kg
		F	Vehicle control	1,000 mg/kg	2,000 mg/kg
	Mouse	M, F	Vehicle control	500 mg/kg	1,000 mg/kg
2-Ethylhexyl sulfate	Rat	M	Untreated	10,000 ppm	20,000 ppm
		M	Untreated	5,000 ppm	10,000 ppm
		F	Untreated	10,000 ppm	20,000 ppm

* The cancer bioassay data for DEHA described by Kluwe (1986) were obtained from the NTP report (1982).

Table 10. Comparative Effects of Compounds with a 2-Ethylhexyl Moiety on Occurrence of Hepatocellular Tumors in Rats (from Kluwe, 1986)[a]

Liver tumor type	Test compound	Males, low dose	Males, high dose	Females, low dose	Females, high dose
All combined[b]	Di(2-ethylhexyl) phthalate	-	+	+	+++
	DEHA	-	-	-	-
	Tris(2-ethylhexyl) phosphate	-	-	-	-
	2-Ethylhexyl sulfate	-	-	-	-
Carcinomas	Di(2-ethylhexyl) phthalate	-	±	-	++
	DEHA	-	-	-	-
	Tris(2-ethylhexyl) phosphate	-	-	-	-
	2-Ethylhexyl sulfate	-	-	-	-

[a] Legend (level of statistical significance): -, $P \geq 0.05$; ±, $p \approx 0.05$; +, $0.01<P<0.05$; ++, $0.001<P<0.01$; +++, $0.0001<P<0.001$; ++++, $P<0.0001$.
[b] Combined = hepatocellular carcinomas and neoplastic nodules.

Table 11. Comparative Effects of Compounds with a 2-Ethylhexyl Moiety on Occurrence of Hepatocellular Tumors in Mice (from Kluwe, 1986)[a]

Liver tumor type	Test compound	Males, low dose	Males, high dose	Females, low dose	Females, high dose
All combined[b]	Di(2-ethylhexyl) phthalate	+	++	+++	++++
	DEHA	-	++	+++	+++
	Tris(2-ethylhexyl) phosphate	-	-	-	+
	2-Ethylhexyl sulfate	-	-	-	+
Carcinomas	Di(2-ethylhexyl) phthalate	-	+	++	++++
	DEHA	-	-	+++	++
	Tris(2-ethylhexyl) phosphate	-	-	-	+
	2-Ethylhexyl sulfate	-	-	-	±

[a] Legend (level of statistical significance): -, $P \geq 0.05$; ±, $p \approx 0.05$; +, $0.01<P<0.05$; ++, $0.001<P<0.01$; +++, $0.0001<P<0.001$; ++++, $P<0.0001$.
[b] Combined = hepatocellular carcinomas and adenomas.

A study by Astill *et al*. (1996) also indicated that the 2-ethylhexyl moiety was carcinogenic in mice. Astill *et al*. (1996) administered EH to groups of 50 male and 50 female Fischer 344 rats or $B6C3F_1$ mice. Oral gavage doses of EH were given five

times a week to rats: 0 (water), 0 (vehicle, an emulsion with 0.005 percent Cremophor in water), 50, 150, and 500 mg/kg for 24 months, and to mice: 0 (water), 0 (vehicle, an emulsion with 0.005 percent Cremophor in water), 50, 200, and 750 mg/kg for 18 months.

In rats, body weight gain was reduced at 150 and 500 mg/kg. Astill *et al*. (1996) reported increased early mortality in female rats at 500 mg/kg, but did not observe an increase in cancer incidence in any dosed group.

In mice, Astill *et al*. (1996) reported increased mortality and reduced body weight gain in both sexes at 750 mg/kg. There was an 18 percent incidence of hepatocellular carcinomas in male mice at 750 mg/kg, though it was not statistically significant compared with either control group (Table 12). There was also a 10 percent incidence of hepatocellular carcinomas in female mice at 750 mg/kg, which was statistically significant (P<0.05) compared with the vehicle but not the water control group (Table 12). The time-adjusted incidence of hepatocellular carcinomas in male mice (18.8 percent) was within the historical normal range at the testing facility (0-22 percent), but that in females (13.1 percent) lay outside the normal range (0-2 percent). Astill *et al*. (1996) reported that EH was not carcinogenic in rats, but there were weak trends toward higher incidences of hepatocellular carcinoma in mice at high dose levels under the conditions of these studies.

Table 12. Liver Abnormalities Found in Male and Female Mice Receiving 2-ethylhexanol (EH) by Gavage for 78 Weeks (from Astill et al., 1996)

Sex	Finding	Number of animals with finding (%)				
		Water control	Vehicle control	50 mg/kg	200 mg/kg	750 mg/kg
Male	Peripheral fatty infiltration	0	0	0	1 (2)	31 (62)**
	Basophilic foci	4 (8)	4 (8)	5 (10)	12 (24)*	6 (12)
	Focal hyperplasia	2 (4)	7 (14)	4 (8)	9 (18)	10 (20)
	Adenoma	0	0	0	0	1 (2)
	Carcinoma	4 (8)	6 (12)	6 (6)	7 (14)	9 (18)
Female	Peripheral fatty infiltration	0	1 (2)	0	3 (6)	22 (44)**
	Basophilic foci	2 (4)	1 (2)	2 (4)	4 (8)	6 (12)*
	Focal hyperplasia	1 (2)	0	3 (6)	4 (8)*	1 (2)
	Carcinoma §	1 (2)	0	1 (2)	3 (6)	5 (10)*

* P<0.05, ** P<0.001, compared with vehicle controls.
§ Adenoma data were not reported.

Structure-activity relationship (peroxisome proliferation)

Moody and Reddy (1978) studied the effect of DEHA and its metabolites on hepatic peroxisome proliferation in rats. They fed DEHA and related compounds to male F344 rats at the 2 percent level in the diet for 3 weeks and noted hepatic peroxisome proliferation and hepatomegaly in some of the treated animals (Table 13). They noted that the changes in the liver induced by EH and 2-ethylhexanoic acid were comparable to those induced by DEHA, and suggested that EH is the active part of the molecule responsible for the peroxisome proliferation.

Table 13. Effect of Dietary DEHA and Related Compounds on Liver Weight and Hepatic Peroxisome Proliferation in Male Rats (from Moody and Reddy, 1978)

Group	Number of animals	Liver weight (% body weight)	Peroxisome proliferation [a]
Control	13	3.8±0.05	-
Di(2-ethylhexyl)phthalate	5	7.9±0.13 [b]	+ + + +
Di(2-ethylhexyl)adipate	8	5.6±0.08 [b]	+ + + +
Adipic acid	5	3.9±0.02	-
2-Ethylhexyl alcohol (EH)	5	4.9±0.1 [b]	+ + + +
2-Ethylhexanoic acid	5	5.9±0.41 [b]	+ + + +
2-Ethylhexyl aldehyde	7	5.0±0.14 [b]	+ +
Hexyl alcohol	5	3.8±0.07	-
Hexanoic acid	5	3.9±0.18	-

[a] Peroxisome proliferation assessed semi-quantitatively from the mitochondria:peroxisome ratio: -, 5:1 (normal); +, 5:2; + +, 5:3; + + +, 5:4; and + + + +, 1:1 or more.
[b] $p<0.001$ (Student's t test).

The findings of Moody and Reddy (1978) were supported by a more recent study by Keith *et al*. (1992). Keith *et al*. (1992) administered DEHA, EH, or 2-ethylhexanoic acid to groups of F344 rats or $B6C3F_1$ mice of both sexes by gavage for 14 consecutive days. Using cyanide-insensitive palmitoyl CoA oxidation as an enzyme marker of peroxisome proliferation, they obtained linear dose-response relationships for all three compounds in the range of 0.54 to 13.5 mmol/kg-day. Relative liver weights were also increased in a dose-related manner. They reported that on a molar basis, DEHA was twice as potent as EH or 2-ethylhexanoic acid, which were approximately equipotent. Keith *et al*. (1992) also suggested that EH is the proximate peroxisome proliferator derived from DEHA.

Cornu *et al*. (1992) studied the proximate peroxisome proliferators derived from DEHA by using mouse and rat hepatocytes *in vitro*. They showed that DEHA had no effect on cyanide-insensitive fatty acyl CoA oxidase. However, the primary metabolites of DEHA, mono (2-ethylhexyl)adipate and EH, induced cyanide-insensitive fatty acyl CoA oxidase activity in rat and mice hepatocytes. The secondary metabolite of DEHA, 2-ethylhexanoic acid, was the most potent peroxisome proliferator in the systems tested.

A tertiary metabolite of DEHA, 5-hydroxy-2-ethylhexanoic acid, was less effective than EH and 2-ethylhexanoic acid in inducing fatty acyl CoA oxidase in mouse and rat hepatocytes. Other tertiary metabolites such as 2-ethyl-5-oxohexan-1-oic acid and 2-ethylhexandioic acid were negative in the system tested (Cornu *et al*., 1992).

Mode of action

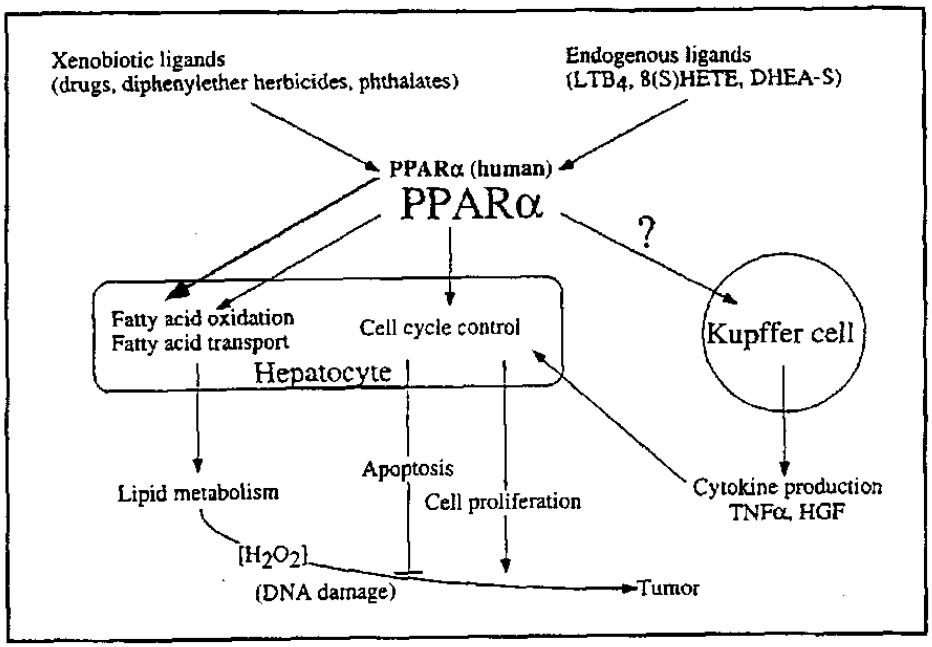
As discussed in the section on genotoxicity, DEHA and its metabolites are generally considered to be neither mutagenic nor clastogenic. It has been suggested that DEHA induced liver tumors through a nongenotoxic pathway, such as indirect oxidative damage to hepatic DNA or increased hepatic DNA synthesis and cell replication. DEHA belongs to a group of chemicals called peroxisome proliferators. Many synthetic chemicals are peroxisome proliferators including plasticizers (e.g., di-(2-ethylhexyl)phthalate and dibutyl phthalate), chlorinated solvents (e.g., trichloroethylene), and hypolipidemic drugs (e.g., clofibrate, ciprofibrate, gemfibrozil, Wy-14,643, and nafenopin). When administered to rodents, these compounds have been shown to induce liver enlargement due to hypertrophy and hyperplasia, increase the size and number of peroxisomes in liver cells, alter transcription of target genes, and cause liver tumors (Takagi *et al*., 1992; Reddy *et al*., 1986; Vanden Heuvel, 1999a,b).

It has been suggested that peroxisome proliferators induce liver tumor in rodents through one or more of the following mechanisms (also shown in Figure 3:

- Increased peroxisomal β-oxidation of fatty acids and hydrogen peroxide production in liver. The resulting reactive oxygen species may cause DNA damage or alter gene transcription (Tomaszewski *et al*., 1986; Rao *et al*., 1984; Gonzalez *et al*., 1998).
- Increased frequency of replicative DNA synthesis in liver (Lake *et al*., 1997). The increased hepatocellular proliferation may result in increased mutation as well as the number of hepatocytes at risk. Furthermore, hepatocellular proliferation is likely to be involved in growth promotion of preneoplastic hepatocytes (IARC, 2000b).
- Induction of oncogenes. It has been shown that Wy-14,643, clofibrate, ciprofibrate and di-(2-ethylhexyl)phthalate are inducers of c-myc, c-fos, c-jun, junB egr-1 and NUP475 *in vivo* (Ledwith *et al*., 1996; Belury *et al*., 1998).
- Alteration of the rate of apoptosis and replication. It has been shown that di(2-ethylhexyl)phthalate and Wy-14,643 induce a persistent increase in replicative DNA synthesis in rat liver (Marsman *et al*., 1988). Also, peroxisome proliferators decrease the rate of programmed cell death, thereby altering the balance between mitosis and apoptosis (Roberts *et al*., 1997, as cited in Vanden Heuvel, 1999b). Gonzalez *et al*. (1998) and Vanden Heuvel (1999b) suggested that together with growth factors, peroxisome proliferators regulate gene expression, cell differentiation, and mitogenesis.

Figure 3. Schematic representation of the role of peroxisome proliferation-activated receptor α (PPARα) in lipid metabolism and carcinogenesis.

PPARα can be activated by xenobiotics and endogenous agents, and the response achieved might be due to its expression level in liver. Low expression, as found in human liver, may be sufficient to alter the pathway leading to lipid catabolism, which is the basis for the therapeutic value of hypolipidemic drugs such as clofibrate and gemfibrozil. Higher expression of PPARα, as observed in rats and mice, leads to a stimulation of mitogenesis and an inhibition of apoptosis that may fix gene mutations generated by endogenous metabolites such as H_2O_2. The question mark denotes uncertainty as to whether PPARα directly controls the genes encoding cell cycle control proteins and cytokines. LTB4 = leukotriene B4; 8(S)HETE = 8(S)-hydroxy-6,8,11,14-eicosatetraenoic acid; DHEA-S = dehydroepiandrosterone-3β-sulfate; H_2O_2 = hydrogen peroxide; TNFα = tumor necrosis factor-α; and HGF = hepatocyte growth factor (from Gonzalez *et al.*, 1998).



Recently, it has been suggested that peroxisome proliferators produce the cellular effects listed above through activation of a class of hormone nuclear receptors, also known as peroxisome proliferator activated receptors (PPARs). There are at least three known PPAR isoforms found in vertebrates termed alpha, beta, and gamma (PPARα, PPARβ/δ, and PPARγ). PPARα predominates in the liver, kidney, and intestine. PPARβ/δ is expressed ubiquitously and often at higher levels than PPARα and PPARγ. PPARγ is expressed predominantly in adipose tissue and the immune system. The distinct tissue distribution suggests that PPAR subtypes play different biological roles. It has been suggested that PPARα predominates in hepatic lipid metabolism and PPARγ plays a

pivotal role in adipogenesis and immune responses (Green, 1995; Kliewer and Willson, 1998; Bass, 1999; Vanden Heuvel, 1999b; Kliewer *et al*., 1999).

When activated, PPAR forms a heterodimer with the 9 cis-retinoic acid receptor that binds to specific DNA sequences located upstream of responsive genes. Many studies have demonstrated that PPARs regulate genes encoding enzymes involved in fatty acid metabolic pathways, such as acyl-CoA oxidase, peroxisomal bifunctional enzyme, fatty acid-binding protein, microsomal CYP4A, and cytochrome P450 fatty acid ω-hydroxylase (reviewed by Peters *et al*., 1998; Vanden Heuvel, 1999a,b; Lee *et al*., 1995). In the liver, PPARα modulates oxidation of fatty acids and detoxification of xenobiotic compounds.

Natural occurring activators of PPARα receptor

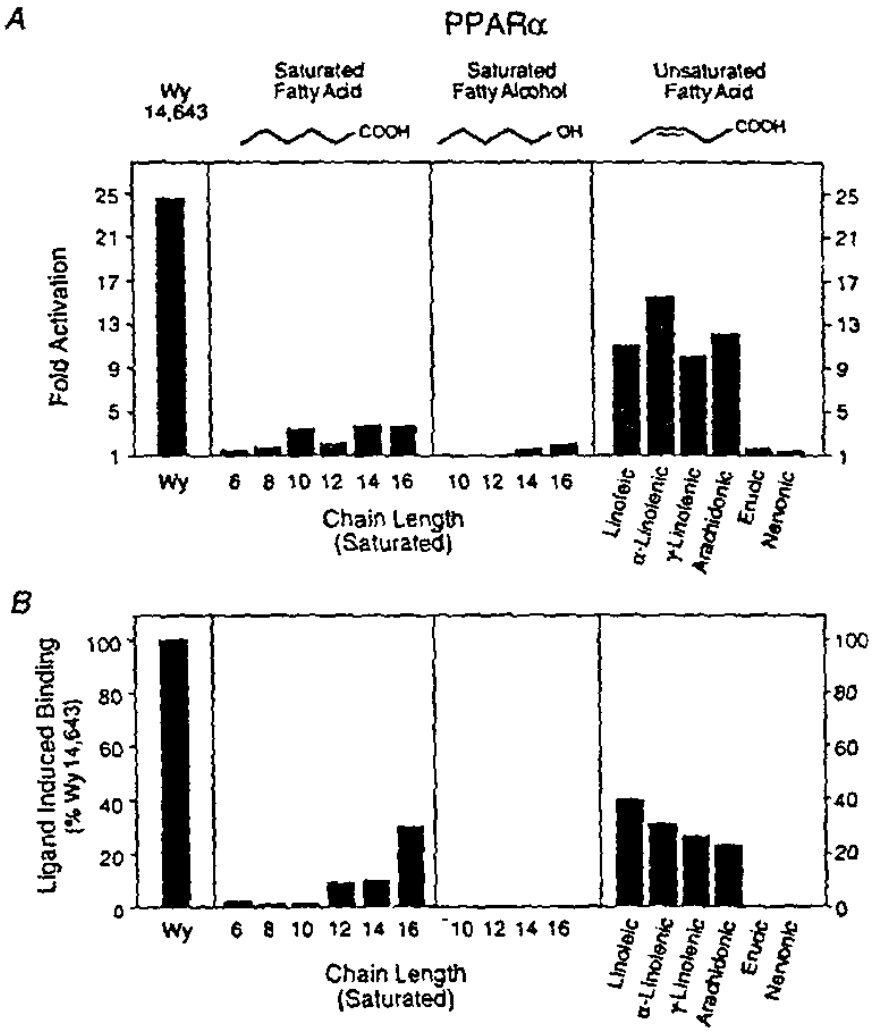
Due to the involvement of PPARs in lipid homeostasis, it is not surprising to find that many poly- and mono-unsaturated fatty acids are activators of PPARα (Forman *et al*., 1997; Issemann *et al*., 1993; Krey *et al*., 1997; Latruffe and Vamecq, 1997). Forman *et al*. (1997) demonstrated that at 30 μM, saturated short-chain fatty acids (<C10) were poor activators of PPARα while longer-chain fatty acids (C10-C16) possessed weak activity. At the same concentration (30 μM) *in vitro*, unsaturated fatty acids such as linoleic, arachidonic, docosahexaenoic, and eicosapentaenoic acids all bound to and activated PPARα. In this study, optimal binding activity was observed with compounds containing a 16-20 carbon chain length with several double bonds in the chain (see Figure 4).

Certain high fat diets (Neat *et al*., 1980; Willumsen *et al*., 1993; Flatmark *et al*., 1988; de Craemer *et al*., 1993 and 1994) have been shown to induce peroxisomal β-oxidation as well as peroxisome proliferation in rats and mice. Flatmark *et al*. (1988) reported a study that fed groups of male Wistar rats with semi-synthetic diets containing 0, 5, or 20 percent (by weight) partially hydrogenated fish oil. Feeding of partially hydrogenated fish oil (20 percent) for up to 20 days increased the number of peroxisomes per liver cell by approximately 1.4 fold. The investigators also found a significant increase in peroxisomal β-oxidation activity during feeding of partially hydrogenated fish oil (1 through 20 days). They also observed that feeding male rats with a diet containing 20 percent of partially hydrogenated fish oil increased the levels of peroxisomal β-oxidation enzymes and their mRNA in liver cells. The increased level of mRNA indicates that partially hydrogenated fish oil contains components that act, directly or indirectly, on gene expression.

De Craemer and van den Branden (1993) fed groups of adult male NMRI mice with a diet supplemented with 10 percent Beromegan® by weight, a commercial fish oil preparation, for up to three days. Fish oil is rich in docosahexaenoic acid (C22:6 (n-3) and in eicosapentanoic acid (C20:5 (n-3)). The investigators observed an increase in peroxisomal staining and peroxisomal proliferation in livers of the treated mice treated for three days. Peroxisomal proliferation was identified by both light microscopy and electron microscopy. The number, volume density and surface density of the peroxisomes were more than doubled after a three day diet containing fish oil, compared with the controls. In a similar study, groups of male NMRI mice were fed a diet with ten

Figure 4. PPARα ligand activity of saturated and unsaturated long-chain fatty acids.

(A) Activation of PPARα by fatty acids and fatty alcohols. (B) Enhancement of PPARα - RXRα heterodimer formation by fatty acids and fatty alcohols. For both figures, all compounds were added to a final concentration of 30 μM except for Wy-14,643, which was used at 5 μM. Saturated fatty acids and fatty alcohols are indicated by their chain length. Unsaturated fatty acids are as follows: linolenic (cis-$\Delta^{9,12}$ – C18:2), α-linolenic (cis-$\Delta^{9,12,15}$ – C18:3), γ-linolenic (cis-$\Delta^{6,9,12}$ – C18:3), arachidonic (cis-$\Delta^{5,8,11,14}$ – C20:4), erucic (cis-Δ^{13} – C22:1), and nervonic (cis-Δ^{15} – C24:1) acids (from Forman *et al.*, 1997).



percent by weight Beromegan® for up to three weeks (de Craemer *et al*., 1994). The investigators reported hepatomegaly in the treated animals: liver weight expressed as percentage of body weight was increased 25 percent after 3, 14, and 21 days of feeding, when compared to animals fed the control diet. They also found a significant increase in peroxisomal β-oxidation, catalase activity, and peroxisomal number after 3 days of dietary treatment. These changes were more pronounced after 3 weeks. Ultrastructural morphometry of the hepatic peroxisomes confirmed the light microscopic impression: the number of peroxisomes was doubled in fish oil-fed mice for 3 days (+98 percent) and for 21 days (+124 percent). De Craemer *et al*. (1994) also found that peroxisomal β-oxidation induced by natural fatty acids was accompanied by a corresponding increase in hepatic catalase activities, whereas a coordinated increase of catalase and β-oxidation capacity was not found when xenobiotic peroxisome proliferators such as clofibrate were administered.

In addition to the fish oil, many arachidonic acid metabolites such as prostaglandins, thromboxanes, and leukotrienes have been shown to be powerful activators of PPARα (Kliewer *et al*., 1995; Krey *et al*., 1997). Lin *et al*. (1999) showed that some of the chemicals such as linoleic acid (18:2 and 18:3) and arachidonic acid (20:4) can bind and activate PPARα at physiological concentrations (5-60 nM range) *in vitro*.

Using reporter assays, Vanden Heuvel (1999b) also demonstrated that a variety of naturally occurring fatty acids and dietary fatty acids are as efficacious as the xenobiotic peroxisome proliferators in activating PPAR (see Figure 5).

Toxicity studies using genetically altered mice

Recent studies (Lee *et al*., 1995; Ward *et al*., 1998; Peters *et al*., 1997) with PPARα knockout mice demonstrated that increase in liver weight, peroxisome proliferation, increase in replicative DNA synthesis and induction of peroxisomal and microsomal fatty acid-oxidizing enzyme as a result of exposure to peroxisome proliferators require the expression of functional PPARα (IARC, 2000b).

Lee *et al*. (1995) produced PPARα knockout mice and found that the homozygous mice were viable, fertile, and healthy and lacked any observable gross defects. They fed 4-5 PPARα (-/-) male Sv/129 mice with either 0.5 percent (wt/wt) clofibrate or 0.1 percent (wt/wt) Wy-14,643 rodent chow diet for 2 weeks. The knockout mice did not respond to the prototypical peroxisome proliferators clofibrate and Wy-14,643, and lacked detectable hepatomegaly, peroxisome proliferation, or induction of the mRNA encoding the peroxisomal and microsomal lipid metabolizing enzymes.

Ward *et al*. (1998) examined the toxic effects of di(2-ethylhexyl)phthalate in PPARα knockout mice. They exposed PPARα (-/-) or wild-type (+/+) male Sv/129 mice to either a control diet or one containing 12,000 ppm di(2-ethylhexyl)phthalate for up to 24 weeks. Ward *et al*. (1998) found that mean liver weight of di(2-ethylhexyl)phthalate-treated PPARα (+/+) mice was significantly greater compared to control PPARα (+/+) mice at all time periods, while mean liver weight in treated PPARα (-/-) mice was not different from untreated controls at any time period. The data are shown in Table 14.

Figure 5. Activation of peroxisome proliferator-activated receptor (PPAR) by peroxisome proliferators and fatty acids (from Vanden Heuvel, 1999b).

A rat hepatoma cell line, stably transfected with a peroxisome proliferator response element-luciferase reporter gene, was treated with 100 µmol/L of the different chemicals for 4 hours before harvesting and luciferase measurement. Data are normalized for protein concentration and expressed relative to DMSO vehicle-treated controls as a fold induction (DMSO control = 1).

Abbreviations: DMSO, dimethylsulfoxide; Wy, Wy-14,643; Bz, bezafibrate; TZD, troglitazone; PFDA, perfluorodecanoic acid; ETYA, 5,8,11,14-eicosatetraynoic acid; CLA, conjugated linoleic acid (e, trans; z, cis); DHA, docosahexaenoic acid.

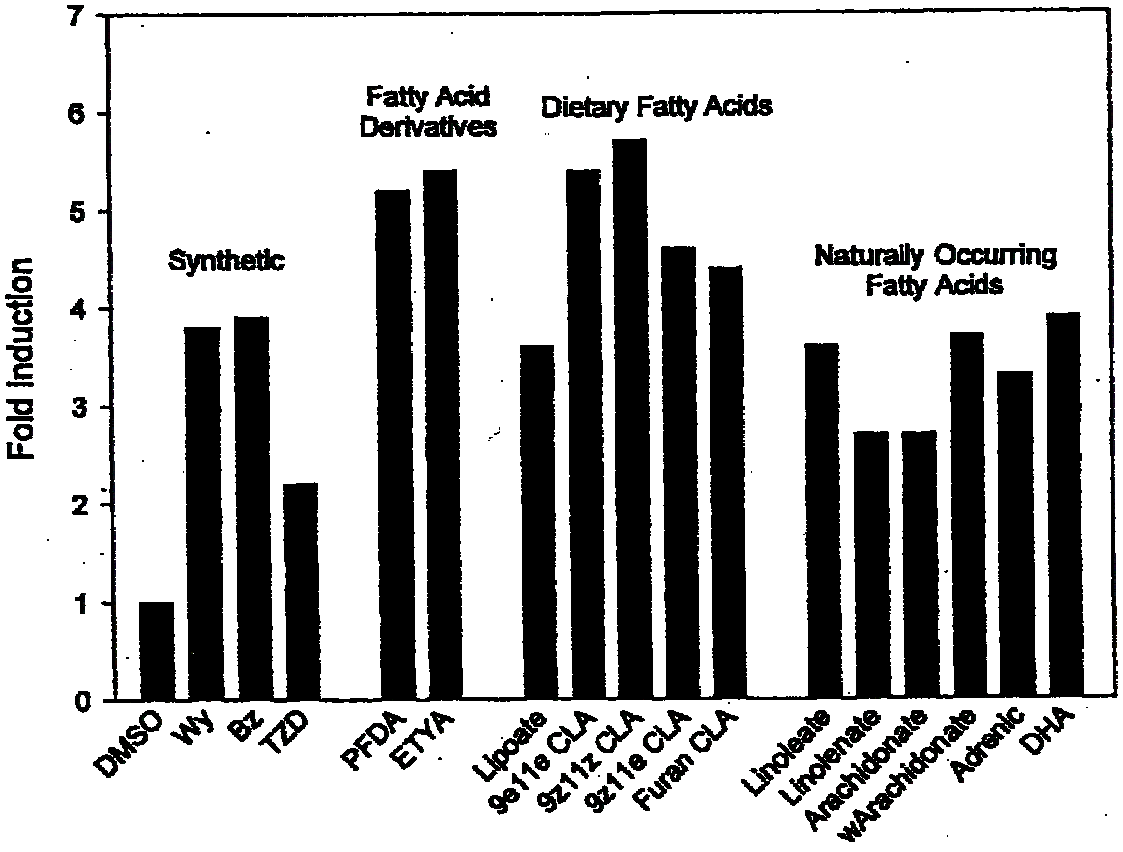


Table 14. Effect of di(2-ethylhexyl)phthalate on Mice, Relative Liver Weights After 4 to 24 Weeks of Continuous Feeding [a] (from Ward et al., 1998)

PPARα genotype/treatment	Weeks	Liver weight/body weight
+/+ Control	4 8 24	4.2±0.2 4.1±0.6 4.2±0.8
+/+ DEHP	4 8 12-16 [c]	6.3±0.4 [b] 7.4±0.8 [b] 8.7±0.8 [b]
-/- Control	4 8 24	4.4±1.2 4.6±0.7 4.4±0.5
-/- DEHP	4 8 24	4.6±0.3 4.8±0.2 5.3±1.3

[a] Values expressed as g tissue/g body weight × 100, mean ± SD. Five mice/group.
[b] $p \leq 0.05$ vs control.
[c] Represents 15 dead or moribund animals sacrificed between 3 and 4 months of continuous feeding.

Ward *et al*. (1998) demonstrated that increased liver weights and induction of peroxisomal and microsomal enzymes induced by di(2-ethylhexyl)phthalate in mice were mediated by PPARα. Ward *et al*. (1998) reported marked diffuse hepatocytomegaly and cytoplasmic granular hepatocyte eosinophilia (due to peroxisome proliferation) in livers of the PPARα (+/+) mice fed di(2-ethylhexyl)phthalate, the severity of which was time-related. These lesions were not present in di(2-ethylhexyl)phthalate-treated PPARα (-/-) mice at any time period. They also detected higher levels of peroxisomal acyl CoA oxidase, peroxisomal bifunctional enzyme, peroxisomal-3-ketoacyl-CoA thiolase, cytochrome P-450 4A1, and cytochrome P-450 4A3 mRNA in the liver from treated PPARα (+/+) mice compared to the controls. This effect was also absent in liver from di(2-ethylhexyl)phthalate-treated PPARα (-/-) compared to controls.

Also using PPARα knockout mice, Peters *et al*. (1997) studied the mechanism of the hepatocarcinogenicity of Wy-14,643. They fed PPARα (-/-) or wild-type (+/+) male Sv/129 mice to either a control diet or one containing 0.1 percent Wy-14,643 for either 1 week, 5 weeks, or 11 months. Wild type mice fed the Wy-14,643 diet for 1 or 5 weeks showed increased liver weight and increased hepatic labeling by bromodeoxyuridine compared to untreated controls. In contrast, there was no increase in liver weight and hepatic bromodeoxyuridine labeling index in PPARα (-/-) mice fed the Wy-14,643 diet for the same time periods compared to controls, as shown in Table 15.

Table 15. Effect of Wy-14,643 on Relative Liver Weight in Wild-Type and PPARα Knockout Mice (from Peters et al., 1997)

Group	Number of mice	Diet	Treatment period	Relative liver weight [a] (g/kg)
PPARα (+/+)	5	Control	1 week	48.3±1.1
PPARα (+/+)	5	0.1% Wy	1 week	93.4±1.1 *
PPARα (-/-)	5	Control	1 week	44.2±2.0
PPARα (-/-)	5	0.1% Wy	1 week	45.0±1.2
PPARα (+/+)	7	Control	5 weeks	44.5±1.0
PPARα (+/+)	5	0.1% Wy	5 weeks	153.5±6.9 *
PPARα (-/-)	4	Control	5 weeks	42.2±0.3
PPARα (-/-)	5	0.1% Wy	5 weeks	43.1±0.9
PPARα (+/+)	9	Control	11 months	47±2.3
PPARα (+/+)	5	0.1% Wy	11 months	214.4±12.9 *
PPARα (-/-)	9	Control	11 months	45.3±2.1
PPARα (-/-)	9	0.1% Wy	11 months	47.9±1.6

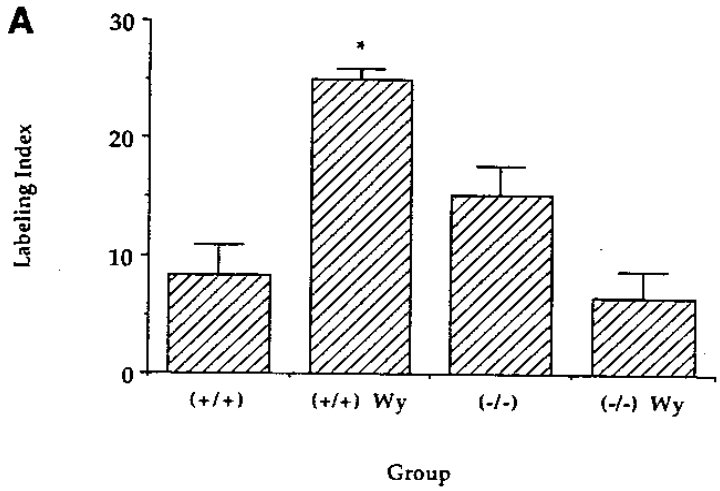
[a] Values represent the mean ± SD. Comparisons made between groups for either the 1 week, 5 week, or 11 month experiment.
* Significantly different from values within the same column at $P \leq 0.05$, 2-way ANOVA.

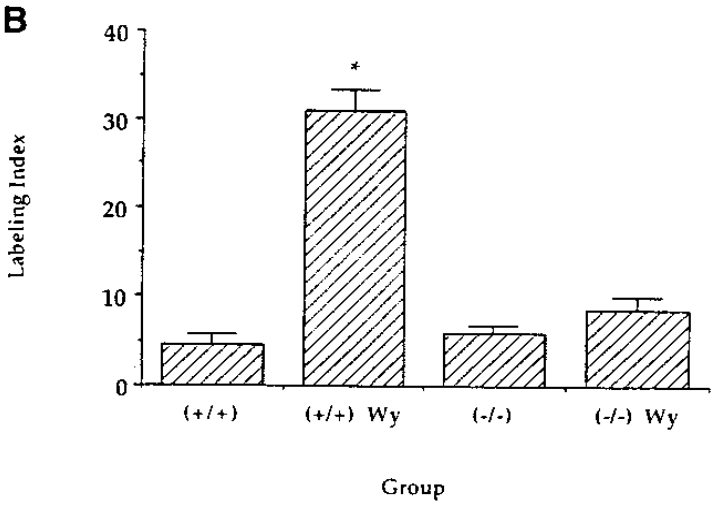
It is important to note that in an earlier study, it has been shown that peroxisome proliferation correlated poorly with the relative hepatocarcinogenicity of peroxisome proliferators. Marsman *et al*. (1988) exposed groups of male Fischer 344 rats to control diet, 1.2 percent di(2-ethylhexyl)phthalate in diet, or 0.1 percent Wy-14,643 in diet for up to 52 weeks. They found the relative hepatocarcinogenicity of di(2-ethylhexyl)phthalate and Wy-14,643 correlated poorly with the degree of peroxisome proliferation but was strongly correlated with their ability to induce a persistent increase in replicative DNA synthesis. In the study reported by Peters *et al*. (1997), they observed no increase in hepatic cell proliferation in the PPARα (-/-) mice fed with Wy-14,643, after 1 and 5 weeks. This is in stark contrast to the wild type mice fed with Wy-14,643 (Figure 6).

After 11 months of treatment, 100 percent (5/5) of the PPARα (+/+) mice fed the Wy-14,643 diet had multiple hepatocellular neoplasms, including adenomas and carcinomas, while none (0/9) of the PPARα (-/-) mice fed the Wy-14,643 diet were affected. Peters *et al*. (1997) suggested that since hepatocellular neoplasms, hepatomegaly, and the increase in hepatic nuclear bromodeoxyuridine labeling due to Wy-14,643-feeding were not observed in the PPARα (-/-) mice, these adverse effects induced by Wy-14,643 were mediated by PPARα.

Figure 6. Quantification of BrDU labeling of hepatic cells.

Wild-type (+/+) or PPARα-(-/-) mice were treated for either 1 week (5A) or 5 weeks (5B) with or without dietary Wy-14,643, and tissues were collected and processed for immunohistochemistry (from Peters *et al*., 1997). The relatively higher BrDU labeling index in the control (-/-) mice in Figure 6A is likely due to the fact that these mice were 7 weeks of age versus 8 weeks for the other groups at this time point and the higher growth rate of the livers might reflect normal postnatal development (Peters *et al*., 1997).





Values represent mean±S.E.M.
* Significantly different at $P \leq 0.05$, 2-way ANOVA.

Fan *et al*. (1998) and Bass (1999) demonstrated that long-chain fatty acids were natural biological ligands for PPARα. These authors theorized that when the normal metabolism of these chemicals was blocked, they activated PPARα, induced peroxisome proliferation in the liver, and ultimately caused hepatocarcinogenesis.

Peroxisomal β-oxidation consists of four consecutive reactions to preferentially metabolize very long chain fatty acids. The first and rate-limiting step of this pathway is catalyzed by acyl-CoA oxidase (AOX). Bass (1999) suggested that PPARα can be perceived as a new kind of oncogene, and proposed that AOX gene functions as a tumor suppressor gene under normal physiological conditions by metabolizing naturally occurring PPARα ligands.

Fan *et al*. (1998) showed that mice with the AOX gene knocked out AOX (-/-) exhibited steatohepatitis, increased hepatic hydrogen peroxide levels, and hepatocellular regeneration. By 3 to 4 months of age, high levels of long-chain fatty acids ($>C_{22}$) appeared in the serum as well as hepatomegaly with microvesicular fat accumulation. This evolved into hepatocellular necrosis and over subsequent months the steatotic hepatocytes, which lack discernible peroxisomes, were replaced by a new population of hepatocytes devoid of fat but which display an abundance of peroxisomes. The magnitude of peroxisome proliferation observed in these AOX-deficient mice was comparable with that induced in wild-type mice by exogenous peroxisome proliferators such as Wy-14,643, clofibrate, and ciprofibrate. This transition was also associated with increased mRNA levels of genes that are regulated by PPARα. The authors suggested that PPARα was transcriptionally activated in the AOX-deficient mice due to increases in the levels of biological (natural) ligands. Hepatocellular adenomas and carcinomas were observed in AOX (-/-) mice between 10 and 15 months of age. By 14-16 months of age, all AOX (-/-) mice (>30 mice) had developed hepatocellular carcinomas (Reddy, 1999).

Data presented above indicate that activation of PPARα by peroxisome proliferators is a critical step in the induction of hepatomegaly, liver peroxisome proliferation, hepatic DNA synthesis and hepatocellular neoplasms in mice. Mice without a functional PPARα were not responsive to a variety of peroxisome proliferators including di(2-ethylhexyl)phthalate, Wy-14,643, and clofibrate. Fan *et al*. (1998) and Bass (1999) suggested that PPARα can be perceived as an oncogene, and that AOX acts as a tumor suppressor gene in mice. When the AOX gene is disabled, long-chain fatty acids metabolism is impaired. The accumulation of the long-chain fatty acids activates the PPARα receptor of the liver and it in turn causes hepatomegaly, peroxisome proliferation, and ultimately hepatocellular neoplasms.

Species difference

There seems to be a significant difference in the susceptibility of hepatocyte cultures of difference species to peroxisome proliferation (Lhuguenot, 1988; IARC, 2000b). Cornu *et al*. (1992) used cyanide-insensitive fatty acyl CoA oxidase as a marker enzyme for peroxisome proliferation, and showed that mono-(2-ethylhexyl)adipate, EH, and 2-ethylhexanoic acid induced oxidase activity in rat and mice hepatocytes *in vitro* (over 5-fold at 0.5 mM concentration). The parent compound DEHA had no effect on

peroxisomal β-oxidation. By contrast, DEHA and its metabolites did not stimulate peroxisomal β-oxidation in guinea pig and marmoset primary hepatocyte cultures up to a final concentration of 2 mM. Higher concentrations led to cytotoxicity. This finding is similar to those reported previously using di(2-ethylhexyl)phthalate metabolites. Elcombe and Mitchell (1986) observed that there was little or no induction of peroxisome proliferation by mono (2-ethylhexyl)phthalate, mono (2-ethyl-5-oxyhexyl)phthalate, and mono(2-ethyl-5-hydroxyhexyl)phthalate in guinea pig, marmoset, or human hepatocytes *in vitro*.

De La Iglesia *et al*. (1982) studied liver biopsies of 9 patients exposed to gemfibrozil for 17-27 months and did not find significant changes in the overall peroxisome population in the liver samples. However, they observed fat accumulation, vesiculation, and changes of the rough-surfaced endoplasmic reticulum in some of the samples examined. Hanefeld *et al*. (1983) studied liver biopsies in 16 patients treated with clofibric acid for periods from about 3 to over 80 months. They observed a 50 percent rise in numerical density of peroxisomes, which was correlated with an increase in mitochondria and a decrease in triglyceride and cholesterol levels. There was a smaller, non-significant increase in peroxisomal volume density, which they interpret as a trend toward smaller peroxisomes. The results are said to be similar to their earlier paper (Hanefeld *et al.*, 1980) in which no overall increase in peroxisomes was observed in a qualitative study in 67 patients. The authors note that these results are vastly different from rats, which show a similar increase in mitochondria but a much greater increase in peroxisomes after clofibrate. They suggest that the mitochondrial changes appear more related to the hypolipemic actions of these chemicals than do the peroxisomal changes.

IARC (2000b) suggested that the species differences in peroxisomal proliferation, particularly with respect to humans compared to rats and mice, could be potentially attributed to low expression of PPARα in human liver, a truncated version of PPARα in human liver, or other gene-specific factors (Palmer *et al.*, 1994, 1998; Tugwood *et al.*, 1998; Gervois *et al.*, 1999; Lambe *et al.*, 1999; Woodyatt *et al.*, 1999).

However, it has recently become apparent that Kupffer cells, non-parenchymal cells of the liver, are required for peroxisome proliferators to exhibit hepatoproliferative effects (Peters *et al.,* 2000). It is therefore difficult to ascertain species differences from *in vitro* studies in the absence of information regarding Kupffer cells in such cultures (Peters *et al.*, 2000). Also, the time elapsing between exposure to the peroxisome proliferator and biopsy, as well as between biopsy and cell culture preparation, may differ considerably between rodents and humans. These factors would tend to diminish the responsiveness of human cells compared to the rodent cells.

Summary

Based on the carcinogenicity studies reported by NTP (1982), there is limited evidence of potential carcinogenicity of DEHA in male and female $B6C3F_1$ mice. Some concern over the carcinogenic potential of DEHA is supported by structure-activity relationship studies showing EH and di(2-ethylhexyl)phthalate also caused liver tumors in rodents (OEHHA, 2001). It has been suggested that EH and 2-ethylhexanoic acid might be the causal agents in the liver tumorigenesis of DEHA in mice (Moody and Reddy, 1978;

Keith *et al*., 1992). However, the available DEHA carcinogenicity data are not adequate for a direct extrapolation to humans.

Toxicological Effects in Humans

No studies were found in the available literature on the effects of oral ingestion of DEHA in humans.

DOSE-RESPONSE ASSESSMENT

Noncarcinogenic Effects

Based on the OEHHA review of the literature, a number of candidate studies were identified for the derivation of a noncarcinogenic PHG for DEHA. Details of these studies are listed in Table 16. As shown in the table, the estimated NOAELs of the studies ranged from 170 to 610 mg/kg-day. The most common effects were reduction in body weight gain and induction of liver enzymes, commonly resulting in peroxisomal proliferation and increased liver weight. However, the most sensitive noncarcinogenic endpoint appears to be that for reproductive and developmental toxicity, as reported by ICI (1988a,b). These two reports are unpublished toxicity studies submitted to the U.S. EPA, cited in U.S. EPA, 1999. For the purpose of this evaluation, the NOAEL of 170 mg/kg-day for reproductive and developmental effects from these studies was selected for the derivation of a health-protective level for noncarcinogenic effects of DEHA.

U.S. EPA (1999) selected the same study for the development of an oral reference dose for DEHA. By applying an uncertainty factor of 300 to the NOAEL of 170 mg/kg-day, U.S. EPA (1999) derived an oral reference dose of 0.6 mg/kg-day.

Table 16. Summary of Candidate Studies for Derivation of a Non-Carcinogenic Health-Protective Level for DEHA

Chemical/ animal species	Exposure route and duration	Toxic end-point	Animal NOAEL (mg/kg-d)	Reference
DEHA/rat	Diet, 90 days	Reduced growth, toxicity in specific organs	610 [a]	Smyth *et al*. (1951; as cited in U.S. EPA, 1990)
DEHA/rat	Diet, 91 days	Weight gain depression	354	NTP (1982)
DEHA/mouse	Diet, 91 days	Weight gain depression	285	NTP (1982)
DEHA/rat	Diet, 103 weeks	Weight gain depression	840	NTP (1982)
DEHA/rat	Diet, 28 weeks	Reduction in body weight gain of the dams; reductions in offspring weight gain, total litter weight, and litter size	170 [b]	ICI (1988a,b; as cited in U.S. EPA, 1999)
DEHA/rat	Gavage, gestation days 6 through 15	Reduced fetal body weight and increased skeletal variations and retardations	370	Hellwig and Jäckh (1997)
DEHA/rat	Diet, 13 weeks	Increase in relative liver weight	282	Lake *et al*. (1997)
EH/mouse	Diet, gestation days 0 through 17	No observed maternal or developmental toxicity at the highest dose tested	276 [c]	Price *et al*. (1991)

[a] The NOAEL was used by U.S. EPA (1990) for the development of a longer-term health advisory (drinking water) for children and adults.
[b] The NOAEL was used by U.S. EPA (1999) for the development of an oral reference dose for DEHA.
[c] The NOAEL was derived from a NOAEL of 1.49 mmol EH/kg-day. Assuming complete hydrolysis of DEHA took place in the gastrointestinal tract of mouse, this dose level is equivalent to 0.745 mmol DEHA/kg-day, or 276 mg DEHA/kg-day.

Carcinogenic Effects

As reviewed above, there is limited evidence for carcinogenicity of DEHA in animals, and no evidence in humans. IARC (1987; 2000a) reached a similar conclusion, and identified DEHA as a Group 3 chemical, not classifiable as to its carcinogenicity to humans. Based on a 1991 evaluation, U.S. EPA classified DEHA as Group C, a possible human carcinogen. They developed an oral slope factor of 1.2×10^{-3} $(\text{mg/kg-day})^{-1}$ for the compound (U.S. EPA, 1999; the carcinogenicity assessment was conducted in 1991). However, OEHHA concludes that it is more appropriate to derive a PHG value from a non-carcinogenic endpoint with this limited data set, while acknowledging the argument for potential carcinogenic effects of this chemical.

CALCULATION OF PHG

Calculations of concentrations of chemical contaminants in drinking water associated with negligible risks as carcinogens or noncarcinogens must take into account the toxicity of the chemical itself, as well as the potential exposure of individuals using the water. Tap water is used directly as drinking water, and for preparing foods and beverages. It is also used for bathing or showering, and in washing, flushing toilets and other household uses that may result in dermal and inhalation exposures.

Based on two developmental and reproductive studies of DEHA in rats reported by ICI (1988a,b, unpublished data cited in U.S. EPA, 1999), a NOAEL of 170 mg/kg-day was selected for the derivation of a proposed PHG. Calculation of a public health-protective concentration (C, in mg/L) for DEHA in drinking water for noncarcinogenic endpoints follows the general equation:

$$C = \frac{NOAEL \times BW \times RSC}{UF \times L_{eq}/day}$$

$$= \frac{170\ mg/kg\text{-}day \times 70\ kg \times 0.1}{1{,}000 \times 2\ L/day} = 0.6\ mg/L \text{ or } 0.6\ ppm$$

where:

NOAEL	=	no-observed-adverse-effect level of 170 mg/kg-day, for reproductive and developmental effects observed in rats;
BW	=	adult body weight, a default of 70 kg for adults;
RSC	=	relative source contribution; 10 percent was used due to the low water solubility of DEHA and known dietary sources of DEHA[2].
UF	=	combined uncertainty factor (10 each for intra- and inter-species variability, plus 10 for lack of a multi-generation reproductive study and some positive carcinogenicity data;
L_{eq}/day	=	adult daily water consumption rate; the 2 L/day default was used.

[2] A drinking water DEHA concentration of 0.6 mg/L and a water consumption rate of 2 L/day represents a DEHA intake of 1.2 mg/day. A maximum daily dietary intake of DEHA of about 8.2 mg/person has been estimated (MAFF, 1990, as cited in Loftus *et al*., 1993). Under this exposure scenario, drinking water would contribute about 13 percent to the total oral dose. Average intake levels as low as 86 µg/person have been reported in a Japanese study (Tsumura *et al*., 2001). Differences in use of plastic food containers and wraps in various countries and over different time periods may explain the differences.

Based on the two reproductive and developmental studies reported by ICI (1988a,b, unpublished data cited in U.S. EPA, 1999), OEHHA developed a proposed PHG of 0.6 mg/L or (0.6 ppm) for DEHA in drinking water.

RISK CHARACTERIZATION

DEHA has low acute toxicity in test animals. It has been estimated that the oral LD_{50} of DEHA in rodents is in the range of 5-45 g/kg. Rats and mice repeatedly exposed to high levels of DEHA (>3,000 ppm or >0.3 percent) in the diet showed depression in body weight gain, increased liver and kidney weights, and hepatic peroxisome proliferation.

DEHA has been shown to cause reproductive and developmental effects in rats and mice. When rats were exposed to DEHA at 12,000 ppm in the diet (1,080 mg/kg-day) prenatally, the chemical caused reduced total litter weight and litter size, abnormal ossification, and lower body weight gain in the offspring. No adverse health effects were observed in the dams or offspring at a lower dose of 1,800 ppm (170 mg/kg-day) (ICI, 1988a,b, unpublished data cited in U.S. EPA, 1999), and this dose was identified as the NOAEL for the derivation of a proposed PHG for DEHA.

The carcinogenic potential of DEHA has been investigated. While there are no human data, there are two animal cancer studies on the compound conducted by NTP (1982). NTP (1982) concluded that "under the condition of this bioassay, di(2-ethylhexyl)adipate was not carcinogenic for F344 rats. Di(2-ethylhexyl)adipate was carcinogenic for female $B6C3F_1$ mice, causing increased incidences of hepatocellular carcinomas, and was probably carcinogenic for male $B6C3F_1$, causing hepatocellular adenomas."

DEHA is generally not considered to be genotoxic. It was negative in Ames tests, negative in the mouse lymphoma assay, and negative in micronuclei tests *in vitro* as well as *in vivo*. When DEHA was administered to rats at high doses, it caused oxidative DNA damage and induced DNA synthesis in the liver.

DEHA belongs to a group of chemicals called peroxisome proliferators, which are characterized by their ability to induce hepatic peroxisome proliferation, especially in rodents. Peroxisome proliferation is visible microscopically as a massive increase in the number of peroxisomes, small membranous organelles that contain various oxidative enzymes. Many synthetic chemicals are peroxisome proliferators including plasticizers (e.g., di-(2-ethylhexyl)phthalate and dibutyl phthalate), chlorinated solvents (e.g., trichloroethylene), and hypolipidemic drugs (e.g., clofibrate, ciprofibrate, gemfibrozil, Wy-14,643, and nafenopin). Some of the peroxisome proliferators have been shown to cause liver tumors as well as pancreatic tumors, testicular tumors and tumors of the hematopoietic system in rats and mice. The evidence of cancer in DEHA-treated rodents is limited.

Inter-species comparisons with other peroxisome proliferators, along with the role of peroxisome proliferator-activated receptors in this response, indicate that humans may be less sensitive than rodents to induction of peroxisome proliferation and hepatocellular proliferation by DEHA (IARC, 2000a, OEHHA, 2001).

Based on the genotoxicity and carcinogenicity data available, IARC (2000a) determined that DEHA was not classifiable as to its carcinogenicity to humans (Group 3). Using a

1991 evaluation, U.S. EPA (1999) classified DEHA as a Group C, possible human carcinogen, and developed an oral slope factor of 1.2×10^{-3} (mg/kg-day)$^{-1}$ for the compound.

U.S. EPA identified a NOAEL of 170 mg/kg-day from the two reproductive and developmental studies reported by ICI (1988a,b, unpublished data cited in U.S. EPA, 1999). An uncertainty factor of 100 was used to account for intra- and inter-species variability. An uncertainty factor of 3 was used for lack of a multi-generation reproductive study, and an additional uncertainty factor of 10 was introduced for a Group C possible human carcinogen. A MCLG of 0.4 mg/L (U.S. EPA, 1999) was calculated from the NOAEL using a 3,000-fold uncertainty factor, a 20 percent relative source contribution, 70 kg body weight, and 2 L/day drinking water consumption. The MCL for DEHA was also set at 0.4 mg/L (U.S. EPA, 1999).

OEHHA proposes a PHG of 0.6 mg/L (0.6 ppm) for DEHA in drinking water. This value is based on a NOAEL of 170 mg/kg-day derived from two toxicity studies reported by ICI (1988a,b; unpublished data cited in U.S. EPA, 1999). The proposed PHG is calculated by assuming a water consumption rate of 2 L/day, a relative source contribution of 10 percent, and an overall uncertainty factor of 1,000. A factor of 100 is applied to account for the uncertainties in the inter-species and intra-species extrapolations, and an additional factor of 10 is used to account for the lack of a multi-generation reproductive study and some positive carcinogenicity data. Considering the large uncertainty factor, the resulting level is considered to be adequately protective of potentially sensitive subpopulations, including infants, children, and the elderly.

OTHER REGULATORY STANDARDS

U.S. EPA identified a NOAEL of 170 mg/kg-day from a one-generation reproductive toxicity and teratogenicity study reported by ICI (1988a,b; unpublished data cited in U.S. EPA, 1999). An uncertainty factor of 100 was used to account for intra- and inter-species variability. An uncertainty factor of 3 was used for lack of a multi-generation reproductive study, and an additional uncertainty factor of 10 was introduced for a Group C carcinogen. An MCLG of 0.4 mg/L (U.S. EPA, 1999) was calculated from the NOAEL using a 3,000-fold uncertainty factor, a 20 percent relative source contribution, 70 kg body weight, and 2 L/day drinking water consumption. The MCL for DEHA is also 0.4 mg/L (U.S. EPA, 1999).

The European Scientific Committee for Food recommended a maximum tolerable daily intake of 0.3 mg/kg (approximately 21 mg in a 70 kg adult) for DEHA (EEC Commission, 1997; as cited in Petersen and Breindahl, 1998).

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