DI(2-ETHYLHEXYL) ADIPATE

This substance was considered by previous working groups in October 1981 (IARC, 1982) and March 1987 (IARC, 1987). Since that time, new data have become available, and these have been incorporated in the monograph and taken into consideration in the evaluation.

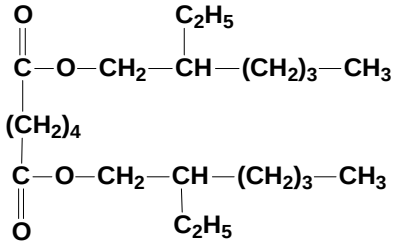
1. Exposure Data

1.1 Chemical and physical data

1.1.1 *Nomenclature*

Chem. Abstr. Serv. Reg. No.: 103-23-1
Deleted CAS Reg. Nos: 39393-67-4; 63637-48-9; 70147-21-6
Chem. Abstr. Name: Hexanedioic acid, bis(2-ethylhexyl) ester
IUPAC Systematic Names: Adipic acid bis(2-ethylhexyl) ester; bis(2-ethylhexyl) adipate
Synonyms: BEHA; DEHA; dioctyl adipate; DOA; hexanedioic acid, dioctyl ester; octyl adipate

1.1.2 *Structural and molecular formulae and relative molecular mass*



$C_{22}H_{42}O_4$ Relative molecular mass: 370.58

1.1.3 *Chemical and physical properties of the pure substance*

(*a*) *Description*: Light-coloured, oily liquid (Verschueren, 1996)
(*b*) *Boiling-point*: 417 °C (Lewis, 1993)
(*c*) *Melting-point*: –67.8 °C (Lide & Milne, 1996)
(*d*) *Density*: 0.922 g/cm^3 at 20 °C (Lide & Milne, 1996)
(*e*) *Spectroscopy data*: Infrared (grating [8003]), nuclear magnetic resonance [943C] and mass spectral data have been reported (Lide & Milne, 1996; Aldrich Chemical Co., 1998; National Institute for Standards and Technology, 1998)
(*f*) *Solubility*: Very slightly soluble in water (< 200 mg/L at 20 °C) (Verschueren, 1996); very soluble in acetone, diethyl ether and ethanol (Lide & Milne, 1996)
(*g*) *Volatility*: Vapour pressure, 346 Pa at 200 °C (Lewis, 1993; Verschueren, 1996); flash-point, 196 °C (Lewis, 1993)
(*h*) *Octanol/water partition coefficient (P)*: log P, 8.1 (Verschueren, 1996)
(*i*) *Conversion factor*[1]: mg/m^3 = 15.16 × ppm

1.1.4 *Technical products and impurities*

Di(2-ethylhexyl) adipate is commercially available with the following specifications: purity, 99–99.9%; acidity, 0.25 meq/100 g max.; moisture, 0.05–0.10% max. (C.P. Hall Co., undated; Solutia, Inc., 1995; Velsicol Chemical Corp., 1997; Aldrich Chemical Co., 1998; Eastman Chemical Co., 2000).

Trade names for di(2-ethylhexyl) adipate include Adimoll DO; Adipol 2EH; ADO; ADO (lubricating oil); Arlamol DOA; Bisoflex DOA; Crodamol DOA; Diacizer DOA; Eastman DOA Plasticizer; Effomoll DA; Effomoll DOA; Ergoplast AdDO; Flexol A 26; Hatcol 2908; Kodaflex DOA; Lankroflex DOA; Monoplex DOA; Plasthall DOA; Plastomoll DOA; Reomol DOA; Sansocizer DOA; Sicol 250; Truflex DOA; Vestinol OA; Wickenol 158; Witamol 320.

1.1.5 *Analysis*

Di(2-ethylhexyl) adipate can be extracted from a water sample by passing this water through a cartridge or disk containing a solid inorganic matrix coated with a chemically bonded C18 organic phase (liquid–solid extraction). Organic material eluted from the liquid–solid extraction cartridge or disk with dichloromethane is analysed for di(2-ethylhexyl) adipate by gas chromatography/mass spectrometry (Environmental Protection Agency, 1995).

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[1] Calculated from: mg/m^3 = (relative molecular mass/24.45) × ppm, assuming a temperature of 25 °C and a pressure of 101 kPa

1.2 Production

Di(2-ethylhexyl) adipate can be prepared by the reaction of adipic acid and 2-ethylhexanol in the presence of an esterification catalyst such as sulfuric acid or *para*-toluenesulfonic acid (National Library of Medicine, 1999).

Information available in 1999 indicated that di(2-ethylhexyl) adipate was manufactured by eleven companies in Japan, eight companies in the United States, five companies each in India and Taiwan, four companies each in Canada, France and Germany, three companies each in Brazil, Mexico and Spain, two companies each in Argentina, Australia, Chile, China, Italy and the United Kingdom and one company each in Belgium, Colombia, the Czech Republic, the Republic of Korea, the Netherlands, Peru, Romania, Russia, South Africa, Turkey and Venezuela (Chemical Information Services, 1999).

1.3 Use

Di(2-ethylhexyl) adipate is used primarily as a plasticizer in the flexible vinyl industry and is widely used in flexible poly(vinyl chloride) (PVC) food film (cling film). It is commonly blended with di(2-ethylhexyl) phthalate (see monograph in this volume) and di(isooctyl) phthalate in PVC and other polymers. It is used as a solvent and as a component of aircraft lubricants. It is important in the processing of nitrocellulose and synthetic rubber, in plasticizing polyvinyl butyral, cellulose acetate butyrate, polystyrene and dammar wax and in cosmetics (cellulose-based liquid lipsticks) (Cadogan & Howick, 1992, 1996; Verschueren, 1996; National Toxicology Program, 1999).

1.4 Occurrence

1.4.1 *Natural occurrence*

Di(2-ethylhexyl) adipate is not known to occur as a natural product.

1.4.2 *Occupational exposure*

According to the 1981–83 National Occupational Exposure Survey, as many as 15 600 workers in the United States were potentially exposed to di(2-ethylhexyl) adipate (NOES, 1999). Occupational exposure may occur through inhalation, mainly as an aerosol, during its manufacture and its use, particularly as a plasticizer of PVC films and in other materials used in food packaging such as adhesives, cellophane and hydroxyethyl cellulose films. Exposure may also occur during the manufacture of rubber products, nonferrous wire, cosmetics, lubricants and hydraulic fluids (Opresko, 1984). No measurements of di(2-ethylhexyl) adipate exposure in manufacturing and processing industries are available.

Workers wrapping meat are potentially exposed to particulate di(2-ethylhexyl) adipate while cutting PVC films by drawing them across a heated cutter (hot wire or cool rod process) (Smith *et al*., 1983). Exposure concentrations of 0.25 mg/m^3 and 0.14 mg/m^3 just above the hot wire of a PVC film cutting machine have been reported in tests simulating normal operating conditions when the wire was operated at 182 °C and 104 °C, respectively (Van Houten *et al*., 1974). Cook (1980) estimated from test emission data that maximum di(2-ethylhexyl) adipate concentrations of 0.2 mg/m^3 in workroom air could be reached in hot wire operations. In the United States, the National Institute for Occupational Safety and Health reported non-detectable levels of di(2-ethylhexyl) adipate (less than 0.08 mg/m^3) near a cool rod machine (operating temperature of 190 °C) used to cut PVC film in a meat cutting and wrapping department of a grocery store (Daniels *et al*., 1985).

1.4.3 *Environmental occurrence*

Di(2-ethylhexyl) adipate may be released into the environment during its manufacture and distribution, during PVC blending operations and cutting of PVC film, and from consumer use and disposal of finished products (IARC, 1982; Environmental Protection Agency, 1998).

(*a*) *Air*

According to the Toxics Release Inventory (Environmental Protection Agency, 1996), air emissions of di(2-ethylhexyl) adipate from 148 industrial facilities amounted to approximately 315 000 kg in 1994 in the United States.

(*b*) *Water*

Di(2-ethylhexyl) adipate has been detected infrequently in fresh water, generally at < 1 μg/L (Sheldon & Hites, 1979; IARC, 1982; Felder *et al*., 1986; WHO, 1996). Di(2-ethylhexyl) adipate is relatively insoluble in water and is likely to partition to sediment and biota in the aquatic environment. A survey of 23 natural surface water sites in 12 states showed that 7% of 82 samples contained di(2-ethylhexyl) adipate at levels ranging from 0.25 to 1.0 μg/L with an average of 0.46 μg/L (Felder *et al*., 1986).

Surface water discharges of di(2-ethylhexyl) adipate from 148 industrial facilities in the United States in 1994 amounted to 560 kg, as reported in the Toxics Release Inventory (Environmental Protection Agency, 1996).

Di(2-ethylhexyl) adipate was found at microgram-per-litre levels in two of five samples of finished water from a water-treatment plant in the United States (WHO, 1996). It was detected in ‘finished’ drinking-water in New Orleans, Louisiana, at an average concentration of 0.10 μg/L but not in drinking-water in two smaller nearby cities (IARC, 1982). Di(2-ethylhexyl) adipate was detected in the Delaware River at levels of 0.08–0.3 μg/L (Sheldon & Hites, 1979). It has also been identified in Europe

as a trace level contaminant of the River Rhine (WHO, 1996) and in the Great Lakes of North America at levels of 0.01–7.0 μg/L (Hrudey *et al*., 1976).

Di(2-ethylhexyl) adipate was found at levels of 2000 μg/L near a chemical plant source near the Delaware River, north of Philadelphia in 1977 and at levels of 90 and 10 μg/L at sampling sites at influent and effluent waste-treatment sites, respectively (Sheldon & Hites, 1979).

(*c*) *Soil*

Releases of di(2-ethylhexyl) adipate to land from 148 industrial facilities in the United States in 1994 amounted to 67 000 kg, as reported in the Toxic Release Inventory (Environmental Protection Agency, 1996).

(*d*) *Biodegradation and bioconcentration*

Model experiments with acclimated activated sludge systems have shown essentially complete biodegradation of relatively high concentrations (~ 20 mg/L) of di(2-ethylhexyl) adipate to carbon dioxide and water in 35 days (Saeger *et al*., 1976; Felder *et al*., 1986).

A bioconcentration study with bluegill showed that di(2-ethylhexyl) adipate is not an accumulative or persistent chemical in this species of fish (Felder *et al*., 1986).

(*e*) *Food*

Food is the major source of exposure of the general population to di(2-ethylhexyl) adipate because of its migration, particularly to fatty foods such as cheese and meat, from PVC films used for packaging that have been plasticized with di(2-ethylhexyl) adipate (IARC, 1982; Castle *et al*., 1987; Startin *et al*., 1987; Page & Lacroix, 1995; WHO, 1996).

Di(2-ethylhexyl) adipate has been found at generally low levels in a broad variety of foods including milk, cheese, margarine, butter, meat, cereals, poultry, baked goods and sandwiches, fruits and vegetables (Castle *et al*., 1987; Startin *et al*., 1987; Mercer *et al*., 1990; Gilbert *et al*., 1994; Page & Lacroix, 1995; Petersen *et al*., 1995).

A United Kingdom survey of di(2-ethylhexyl) adipate levels in 83 retail samples wrapped in plasticized PVC films was reported by Castle *et al*. (1987). Foodstuffs analysed (from both retail and take-away outlets) included fresh meat and poultry, ready-cooked poultry, cheese, fruit, vegetables and baked goods (cakes, bread rolls and sandwiches). Ranges of di(2-ethylhexyl) adipate levels were 1.0–72.8 mg/kg in uncooked meat and poultry, 9.4–48.6 mg/kg in cooked chicken portions, 27.8–135.0 mg/kg in cheese, 11.0–212 mg/kg in baked goods and sandwiches, and < 2.0 mg/kg in fruits and vegetables. The level of di(2-ethylhexyl) adipate in meat exposed to plasticized film was not reduced significantly by volatilization or chemical transformation on subsequent cooking by grilling or frying.

The highest levels of migration of di(2-ethylhexyl) adipate from PVC films during home-use and microwave cooking in the United Kingdom were observed for cheese, cooked meats, cakes and microwave-cooked foods, whilst lower levels were found for wrapping of unfilled buttered sandwiches, fruit and vegetables (except avocado). Levels of migration of di(2-ethylhexyl) adipate into purchased ready-cooked meats, rewrapped in the home in PVC film and kept for seven days at 5 °C or 30 days at –18 °C were: chicken, 75 and 29 mg/kg, respectively; salami, 181 and 109 mg/kg, respectively; ham, 107 and 25 mg/kg, respectively; and beef (minced), 78 and 23 mg/kg, respectively. Overall, migration of di(2-ethylhexyl) adipate increased with both the length of contact time and temperature of exposure, with the highest levels found where there was direct contact between the film and food and where the latter had a high fat content in the contact surface (Startin *et al*., 1987).

A survey of di(2-ethylhexyl) adipate in Canadian packaging and food sampled during the period 1985–89 was reported by Page and Lacroix (1995). Selected foods (260 samples) packaged in materials with potential to contribute plasticizers to the food and available food composites (98 samples) obtained from the Canadian Health Protection Branch Total Diet Program were analysed for plasticizers including di(2-ethylhexyl) adipate and di(2-ethylhexyl) phthalate. Di(2-ethylhexyl) adipate was found in food-contacting PVC film and as a migrant in store-wrapped meat, poultry, fish, cheese and ready-to-eat foods at levels as high as 310 mg/kg (cheese). Di(2-ethylhexyl) adipate levels in unheated film-wrapped ready-to-eat foods were increased by heating. Di(2-ethylhexyl) adipate residues found in fresh fruits and vegetables were typically < 4 mg/kg.

In a study in New South Wales, Australia, of 184 samples of food packaged in a range of plastics, only samples in contact with PVC film were found to contain a detectable amount of di(2-ethylhexyl) adipate. Of the 98 samples wrapped in PVC films, 44 (45%) showed levels of migration of di(2-ethylhexyl) adipate exceeding 30 mg/kg. Significant quantities of di(2-ethylhexyl) adipate were found in cheeses which had been wrapped at the point of sale. Di(2-ethylhexyl) adipate was detected in 36 of 38 samples of cheese wrapped in PVC film, at levels ranging from 31 to 429 mg/kg. Five out of 42 samples (12%) of fresh meat packaged in PVC film gave positive results, with levels ranging from 49 to 151 mg/kg. Migration of di(2-ethylhexyl) adipate at levels of 64 and 325 mg/kg was also found in other foods such as sandwiches wrapped in PVC (Kozyrod & Ziaziaris, 1989).

Badeka and Kontominas (1996) reported the effect of microwave heating on the migration of di(2-ethylhexyl) adipate from food-grade PVC into olive oil and water. Migration was dependent on heating time, microwave power setting, the nature of the food simulant and the initial concentration of the plasticizer in the film.

Petersen *et al.* (1997) reported that, compared with a specific migration limit of 3 mg di(2-ethylhexyl) adipate/dm^2 from PVC cling films used in Denmark, 77% of the films used for fatty foodstuffs sampled from importers, wholesalers and retail shops

were found to be unacceptable. The migration of di(2-ethylhexyl) adipate to non-fatty foods defined as the food simulant water was ≤ 0.1 mg/dm^2 for all PVC films.

The maximum daily intake of di(2-ethylhexyl) adipate through the diet in the United Kingdom was estimated in 1987 to be 16 mg (Anon., 1991; Loftus *et al*., 1993, 1994; WHO, 1996). Reformulation of PVC film reflecting the use of less di(2-ethylhexyl) adipate necessitated a more recent evaluation which suggested that the maximum daily intake of di(2-ethylhexyl) adipate in the United Kingdom was 8.2 mg (Loftus *et al*., 1993, 1994).

The major urinary metabolite of di(2-ethylhexyl) adipate, 2-ethylhexanoic acid, has been shown to be an appropriate marker for biological monitoring of dietary di(2-ethylhexyl) adipate intake (Loftus *et al*., 1993, 1994). A limited population study in the United Kingdom was undertaken to estimate the daily intake of di(2-ethylhexyl) adipate following intake of a mean dose of 5.4 mg di(2-ethylhexyl) adipate presented with food. The study involved the determination of the urinary metabolite, 2-ethylhexanoic acid (24-h urine sample) in 112 individuals from five geographical locations. A skewed distribution with a median value for the daily intake of 2.7 mg was determined (Loftus *et al*., 1994). This value is about one third of the indirectly estimated maximum intake of 8.2 mg per day. The probability of a daily intake in excess of 8.2 mg in the limited population (112 individuals) was calculated to be 3% (Loftus *et al*., 1994).

1.5 Regulations and guidelines

The World Health Organization has established an international drinking water guideline for di(2-ethylhexyl) adipate of 80 μg/L (WHO, 1996). The United States Environmental Protection Agency (1998) has set a maximum contaminant level (MCL) for di(2-ethylhexyl) adipate in drinking water of 0.4 mg/L.

In the United States, the Food and Drug Administration (1999) permits the use of di(2-ethylhexyl) adipate as a component of adhesives used in food packaging, as a component of cellophane, as a plasticizer in polymeric substances used in the manufacture of articles for the food industry, as a component of paper and paper-board in contact with aqueous and fatty foods, and as a component of closures with sealing gaskets for food containers.

2. Studies of Cancer in Humans

No data were available to the Working Group.

3. Studies of Cancer in Experimental Animals

3.1 Oral administration

3.1.1 *Mouse*

Groups of 50 male and 50 female B6C3F_1 mice, six weeks of age, were fed diets containing 0, 12 000 or 25 000 mg/kg diet (ppm) di(2-ethyhexyl) adipate (> 98% pure) for 103 weeks and were killed 105–107 weeks after the beginning of treatment. Mean body weights of treated mice of each sex were lower than those of the corresponding controls and the decrease in weight gain was dose-related. Survival in males was 36/50 (72%), 32/50 (64%) and 41/50 (82%) in control, low-dose and high-dose animals, respectively, and that in females was 42/50 (84%), 39/50 (78%) and 36/49 (73%) in the control, low-dose and high-dose animals, respectively. Di(2-ethylhexyl) adipate increased the incidence of hepatocellular adenomas in both males (6/50 control, 8/49 low-dose and 15/49 high-dose ($p < 0.025$) and females (2/50, 5/50 and 6/49 in control, low-dose and high-dose animals). Hepatocellular carcinomas were observed in 7/50 control, 12/49 low-dose and 12/49 high-dose males and 1/50 control, 14/50 low-dose ($p < 0.001$) and 12/49 high-dose ($p = 0.001$) females. The incidences of hepatocellular adenomas and carcinomas combined were also increased in males (control, 13/50; low-dose, 20/49 and high-dose, 27/49, $p = 0.003$, pairwise comparison; $p = 0.002$, trend test) and in females (control, 3/50, low-dose, 19/50 $p < 0.001$; and high-dose, 18/49 $p < 0.001$, pairwise comparisons). [The Working Group noted that negative trends were reported for certain tumour types (lymphomas, lung and subcutaneous tumours in males and pituitary adenomas in females)] (National Toxicology Program, 1982; Kluwe *et al*., 1985).

3.1.2 *Rat*

Groups of 50 male and 50 female Fischer 344 rats, five weeks of age, were fed diets containing 0, 12 000 or 25 000 ppm di(2-ethylhexyl) adipate (purity, > 98%) for 103 weeks and were killed 105–107 weeks after the beginning of treatment. Mean body weights of high-dose rats of each sex were lower than those of the controls throughout the study. Survival in males was 34/50 (68%) in the control and low-dose groups and 40/50 (80%) in the high-dose group and in females was 29/50 (58%), 39/50 (78%), and 44/50 (88%) in the control, low-dose and high-dose groups, respectively. There was no treatment-related increase in tumours. Neoplastic nodules or hepatocellular carcinomas were found in 2/49 control, 2/50 low-dose and 2/50 high-dose males and in 0/49, 3/50, and 1/50 females, respectively (National Toxicology Program, 1982; Kluwe *et al*., 1985).

4. Other Data Relevant to an Evaluation of Carcinogenicity and its Mechanisms

4.1 Absorption, distribution, metabolism and excretion

4.1.1 *Humans*

In six male volunteers given 46 mg deuterium-labelled di(2-ethylhexyl) adipate [approx. 0.5 mg/kg bw] in corn oil, 2-ethylhexanoic acid was the only metabolite that could be determined in the plasma. It had an elimination half-life of 1.65 h. In urine, the following metabolites were identified (percentage fraction of administered deuterium label): 2-ethylhexanoic acid (8.6%), 2-ethyl-5-hydroxyhexanoic acid (2.6%), 2-ethyl-1,6-hexanedioic acid (0.7%), 2-ethyl-5-ketohexanoic acid (0.2%) and 2-ethylhexanol (0.1%). The half-life for elimination of all metabolites excreted in the urine averaged 1.5 h, and none of the metabolites could be detected after 36 h (Loftus *et al.*, 1993).

4.1.2 *Experimental systems*

Di(2-ethylhexyl) adipate is rapidly and completely absorbed from the gastrointestinal tract of experimental animals. In rats, there is evidence for cleavage of the parent compound and subsequent absorption of the monoester and the acid (Takahashi *et al.*, 1981), whereas in cynomolgus monkeys unchanged di(2-ethylhexyl) adipate is also absorbed (BUA, 1996). Radiolabel from di(2-ethylhexyl) [*carbonyl*-^{14}C]adipate is distributed to a number of tissues, with maximum levels being reached after 6–12 h. While most radioactivity was found in the gastrointestinal tract, muscle, liver, fat, blood and kidney had relatively high levels of di(2-ethylhexyl) adipate-associated radiolabel (Takahashi *et al.*, 1981; Bergman & Albanus, 1987; BUA, 1996).

A half-life of 6 min for metabolism of di(2-ethylhexyl) adipate has been determined in rat small intestinal mucous membrane homogenates. The dominant urinary metabolite of di(2-ethylhexyl) adipate (500 mg/kg bw) in male Wistar rats is adipic acid, which accounts for 20–30% of the administered oral dose. The other major metabolite which was found only in the stomach is mono(2-ethylhexyl) adipate (Takahashi *et al.*, 1981). In cynomolgus monkeys, the glucuronide of mono(2-ethylhexyl) adipate and traces of unchanged di(2-ethylhexyl) adipate were found in the urine (BUA, 1996).

Di(2-ethylhexyl) adipate is rapidly eliminated, with most of the dose appearing in the urine after oral administration to Fischer 344 rats, B6C3F$_1$ mice and cynomolgus monkeys (rats, 34–78% of the dose after 24 h; mice, 75–92%; monkeys, 47–57%). In rats, total radioactivity in the body after 96 h was approximately 0.5%. Some of the biliary-secreted radioactivity (approximately 3% in rats) flows into the enterohepatic circulation. Passage of di(2-ethylhexyl) adipate (84.3 µg per animal) through the placenta of pregnant NMRI mice has been described (Bergman & Albanus, 1987; BUA, 1996).

4.2 Toxic effects

4.2.1 *Humans*

No data were available to the Working Group.

4.2.2 *Experimental systems*

The acute oral LD_{50} values for di(2-ethylhexyl) adipate in Fischer 344 rats were estimated to be 45 (males) and 25 (females) g/kg bw by gavage and in $B6C3F_1$ mice were estimated to be 15 (males) and 25 (females) g/kg bw by gavage (National Toxicology Program, 1982). Consumption of 2.5% di(2-ethylhexyl) adipate in the diet [5 g/kg bw per day] by female $B6C3F_1$ mice and 4.0% di(2-ethylhexyl) adipate in the diet [3 g/kg bw per day] by female Fischer 344 rats was not associated with lethality, although body weight gain was diminished (Lake *et al.*, 1997). Similar dietary administration of di(2-ethylhexyl) adipate (25 000 ppm [2.5%] in diet) did not affect survival in studies of two years' duration in mice and rats (National Toxicology Program, 1982).

The effects of di(2-ethylhexyl) adipate (1% of diet) on plasma lipids were evaluated in male Upjohn:TUC (SD) rats (Bell, 1984). After two weeks and four weeks (but not seven weeks) of feeding, plasma cholesterol levels were significantly decreased. After four weeks (but not two or seven weeks) of feeding, plasma triglyceride levels were significantly decreased. Hepatic cholesterol synthesis was diminished by di(2-ethylhexyl) adipate consumption (Bell, 1983, 1984).

A variety of studies have evaluated the effects of oral administration of di(2-ethylhexyl) adipate on rodent liver. In male and female Fischer 344 rats and $B6C3F_1$ mice, administration of di(2-ethylhexyl) adipate by gavage in corn oil at levels of 0.5–2.5 mg/kg bw per day for 14 days increased peroxisomal cyanide-insensitive palmitoyl-coenzyme A (CoA) oxidation activity from approximately twofold up to 15-fold in male rats and female mice, up to fourfold in female rats and ninefold in male mice. These effects were accompanied by slight but statistically significant increases in catalase activity in mice but not rats. Light microscopic evaluation of liver sections from rats revealed a di(2-ethylhexyl) adipate-dependent loss of glycogen in hepatocytes that progressed from centrilobular to panlobular with increasing dose. In both rats and mice, there was dose-related hypertrophy and increased eosinophilia of hepatocytes. No evidence of hepatotoxicity was observed by light microscopy. Morphometric analysis of liver ultrastructure demonstrated increases in peroxisomal volume density, as summarized in Table 1 (Keith *et al.*, 1992). Similar increases in peroxisomal volume density (by morphometric analysis) were reported following dietary administration of di(2-ethylhexyl) adipate (1.0 or 2.0 but not ≤ 0.5% of diet) to Fischer 344 rats [sex not specified] for 30 days (Reddy *et al.*, 1986).

The hepatic effects of di(2-ethylhexyl) adipate were evaluated in female $B6C3F_1$ mice and Fischer 344 rats fed diets containing 0–4.0% (up to 3140 mg/kg bw per day

Table 1. The effect of di(2-ethylhexyl) adipate administered daily by gavage for 14 days on peroxisomal volume density in Fischer 344 rats and B6C3F$_1$ mice

DEHA (mg/kg bw per day)		0.0	0.5	1.0	1.5	2.5
Species	Sex	Peroxisomal volume density (% of cytoplasmic volume)				
Fischer 344 rats	Male	1.4	2.8	4.0	6.2	10.4
	Female	1.4	2.5	7.1	7.2	Not measured
B6C3F$_1$ mice	Male	1.4	1.5	2.9	4.3	6.3
	Female	1.4	2.0	3.8	5.0	7.1

From Keith *et al*. (1992)

in rats and 5330 mg/kg bw per day in mice) di(2-ethylhexyl) adipate for one, four and 13 weeks (Lake *et al*., 1997). In mice, di(2-ethylhexyl) adipate at ≥ 0.6% (1495 mg/kg bw per day) induced dose-dependent increases in relative liver weight and hepatic peroxisome proliferation, as demonstrated by the induction of peroxisomal cyanide-insensitive palmitoyl-CoA oxidation (increases were statistically significant for at least one treatment interval). Microsomal lauric acid 11- and 12-hydroxylase activities (CYP4A) were similarly increased at the same or the next lowest dietary concentration (0.3%; 282 mg/kg bw per day in rats and 808 mg/kg bw per day in mice). Hepatocellular replication (measured as nuclear 5-bromo-2′-deoxyuridine [BrdU] labelling) was increased during week 1 of di(2-ethylhexyl) adipate treatment of mice at ≥ 0.6% (1495 mg/kg bw per day) and was still elevated at weeks 4 and 13 at doses of ≥ 1.2% (3075 mg/kg bw per day). In contrast to mice, rats fed di(2-ethylhexyl) adipate had much smaller increases in relative liver weight and peroxisomal palmitoyl-CoA oxidation at doses matching those used in a bioassay (National Toxicology Program, 1981), although at even higher dietary concentrations, rats were similarly responsive. This apparent difference in magnitude of response between mice and rats could in part be accounted for by the different rates of di(2-ethylhexyl) adipate intake (see Table 2). Therefore, the apparent difference in the results of carcinogenicity testing between mice and rats could be related to differences in intake of di(2-ethylhexyl) adipate and the resulting peroxisome proliferation and related responses in liver. In rats, there was similar induction of microsomal lauric acid 11- and 12-hydroxylase activity. While hepatocellular replication (measured as nuclear BrdU labelling) was increased during week 1 of administration in rats, this response was not sustained during weeks 4 or 13, although the magnitude of response during week 1 was similar to that in mice.

Increased liver weights (absolute, 32%; relative, 36% over respective control values) were observed in male Fischer 344 rats fed di(2-ethylhexyl) adipate (2.5% of

Table 2. Comparison of responses in liver of female mice and rats following four weeks of di(2-ethylhexyl) adipate treatment

Diet (%)	Intake (mg/kg bw per day)		Relative liver weight (% increase over controls)		Peroxisomal palmitoyl-CoA oxidation (increase over control)		Microsomal lauric acid hydroxylase (increase over control)			
							Mouse		Rat	
	Mouse	Rat	Mouse	Rat	Mouse	Rat	11-position	12-position	11-position	12-position
0.15	343	144	NC	NC	NC	NE	NC	NC	NC	NC
0.30	808	282	NC	NC	NC	NC	NC	2-fold	NC	NC
0.60	1495	577	10	NC	2-fold	NC	2-fold	4-fold	NC	NC
1.20[a]	3075	1135	50	10	7-fold	< 2-fold	3-fold	8-fold	NC	NC
2.50[a]	5330	2095	60	30	13-fold	8-fold	5-fold	16-fold	2-fold	2-fold
4.00	NE	3140	NE	80	NE	17-fold	NE	NE	3-fold	8-fold

Adapted from Lake *et al*. (1997)

[a] Dietary levels administered to mice and rats in a carcinogenesis bioassay, resulting in an increase in tumour incidence in mice but not in rats (National Toxicology Program, 1981).

NC, not different from controls; NE, not evaluated

diet) for one week. Slight but statistically significant increases in 8-hydroxydeoxyguanosine (8-OH-dG), an indicator of oxidative DNA damage, in liver but not kidney DNA were reported, and also after two weeks' administration (Takagi *et al.*, 1990).

Several studies have evaluated the effects of oral di(2-ethylhexyl) adipate on various aspects of hepatic lipid metabolism. Feeding di(2-ethylhexyl) adipate (2% of diet) to male Wistar rats for seven days resulted in increased hepatic fatty acid-binding protein as well as in increased microsomal stearoyl-CoA desaturation activity (Kawashima *et al*., 1983a,b). Feeding the compound at this dose for 14 days resulted in increased levels of hepatic phospholipids and a decline in phosphatidylcholine:phosphatidylethanolamine ratio (Yanagita *et al*., 1987). Feeding di(2-ethylhexyl) adipate (2% of diet) to male NZB mice for five days resulted in induction of fatty acid translocase, fatty acid transporter protein and fatty acid binding protein in the liver (Motojima *et al*., 1998).

Primary hepatocyte cultures may be employed to study species differences in hepatic peroxisome proliferation (IARC, 1995). The effects of di(2-ethylhexyl) adipate and its metabolites in cultured hepatocytes from rats, mice, guinea-pigs and marmosets have been studied (Cornu *et al*., 1992). In hepatocytes from each species, the parent compound di(2-ethylhexyl) adipate had no effect on peroxisomal cyanide-insensitive palmitoyl-CoA oxidation activity. However, in rat and mouse hepatocytes, the metabolites mono(2-ethylhexyl) adipate, 2-ethylhexanol, 2-ethylhexanoic acid and 2-ethyl-5-hydroxyhexanoic acid at concentrations ≤ 1 mM induced peroxisomal palmitoyl-CoA oxidation. No induction of peroxisomal palmitoyl-CoA oxidation was seen at concentrations ≤ 1 mM for mono(2-ethylhexyl) adipate or ≤ 2 mM for 2-ethylhexanol, 2-ethylhexanoic acid and 2-ethyl-5-hydroxyhexanoic acid in guinea-pig or marmoset hepatocytes (2-ethylhexanol was evaluated only at ≤ 1 mM in marmoset hepatocytes).

4.3 Reproductive and developmental effects

4.3.1 *Humans*

No data were available to the Working Group.

4.3.2 *Experimental systems*

(*a*) *Developmental toxicity studies*

Groups of five Sprague-Dawley rats were given intraperitoneal injections of 1, 5 or 10 mL/kg bw di(2-ethylhexyl) adipate on days 5, 10 and 15 of pregnancy. Fetal weight in the two high-dose groups showed a dose-dependent reduction. The incidence of externally visible malformations [no further details supplied] was significantly higher in the high-dose group. The rate of skeletal malformations lay in the same range as in the control groups; the rate of visceral malformations was reported

to be higher in the middle- and high-dose groups than in the control animals. Correlations with maternally toxic effects were not described (Singh *et al.*, 1973).

Groups of 24 Alpk:APF50 rats were given approximately 28, 170 and 1080 mg/kg bw via the feed from days 1 to 22 of gestation. In the high-dose group, there was a slight reduction of maternal body weight gain. The incidence of unilateral ureter kinking was slightly but significantly higher in the middle- and high-dose groups. In addition, in the high-dose group, there was a significantly higher incidence of skeletal variations/retardations (Hodge, 1991, cited in BUA, 1996).

(*b*) *Mechanistically oriented developmental toxicity studies*

No effects on three-day-old chick embryos were found after exposure by injection of 17 µmol di(2-ethylhexyl) adipate per egg into the air chamber of the egg (Korhonen *et al*., 1983a,b).

A single intraperitoneal injection of di(2-ethylhexyl) adipate (12.5 mL/kg bw) on day 15 of gestation increased the cytochrome P450 content in hepatic microsomes in pregnant and non-pregnant NP C57BL/6J mice, but increased the aminopyrine-*N*-demethylase activity only in pregnant mice. In the pregnant mice, di(2-ethylhexyl) adipate decreased levels of P450-gest, an isoenzyme induced in mouse pregnancy, but increased other P450 isoenzymes (Lamber *et al*., 1987, cited in BUA, 1996).

Radioactivity was observed in fetal liver, intestine and bone marrow during the first 24 h after intravenous or intragastric administration of di(2-ethylhexyl) [*carbonyl*-^{14}C]adipate to pregnant NMRI mice on gestational day 17 (Bergman & Albanus, 1987). When [*ethylhexyl*-^{14}C]di(2-ethylhexyl) adipate was administered, there was very little accumulation of radiolabel but some was found in the urinary bladder, liver and intestinal contents of the fetus as well as in the amniotic fluid. A remarkably strong uptake of radioactivity was observed in the corpora lutea of the ovary in the pregnant mice.

(*c*) *Reproductive toxicity studies*

In a dominant lethal study, a reduced percentage of pregnancies and an increased number of fetal deaths were observed in Harlan/ICR albino Swiss mice after a single intraperitoneal dose of 10 mL/kg bw was given to male mice before an eight-week mating period (Singh *et al*., 1975).

Groups of 30 female and 15 male Alpk:APF50 rats were fed diets containing 300, 1800 or 12 000 ppm di(2-ethylhexyl) adipate for a period of 10 weeks before mating and during the gestation and lactation periods. In the high-dose group, there was a significant reduction in body weight gain of the females during the last part of the gestation period. There was no effect on male and female fertility, the number of live births or the survival rate of the pups up to day 22 of life. In the high-dose group, the body weight gain of the pups was significantly reduced throughout the postnatal follow-up period (up to day 36 of life) (Tinston, 1988, cited in BUA, 1996).

In a multigeneration study, rats were given 100 mg/kg di(2-ethylhexyl) adipate per day via the feed. For four successive generations, no substance-specific influence on reproduction rate, lactation or growth was reported [no further details supplied] (Le Breton, 1962, cited in BUA, 1996).

4.4 Genetic and related effects

4.4.1 *Humans*

No data were available to the Working Group.

4.4.2 *Experimental systems* (see Table 3 for references)

Di(2-ethylhexyl) adipate was not mutagenic to *Salmonella typhimurium* strains TA100, TA1535,TA1537, TA1538 or TA98 in the presence or absence of exogenous metabolic activation in three studies. It was also not mutagenic to *Photobacterium phosphoreum* in a single study. A single study found no induction of sex-linked recessive lethal mutations after administration of di(2-ethylhexyl) adipate to adult *Drosophila melanogaster* by feeding or injection.

One study employing the mouse lymphoma gene mutation assay found no induction of mutations at the *Tk* locus in L5178Y mouse lymphoma cells following exposure to di(2-ethylhexyl) adipate in the absence of exogenous metabolic activity. With exogenous metabolic activation, one experiment similarly showed no induction of mutation, while a second experiment showed an effect but only at a concentration at which precipitation occurred (above 1000 μg/mL). A single in-vitro study using rat hepatocytes found no induction of sister chromatid exchanges, chromosomal aberrations or micronuclei after treatment with di(2-ethylhexyl) adipate for either 3 or 51 h. In bone marrow of mice treated *in vivo* with di(2-ethylhexyl) adipate, chromosomal aberrations were not induced in one study and no effect was seen in a single micronucleus assay.

A weak positive result has been reported in a dominant lethal assay in male mice.

Urine samples from rats treated by gavage with 15 daily doses of di(2-ethylhexyl) adipate were not mutagenic to *S. typhimurium* strains TA100, TA1535, TA1537, TA1538 or TA98. In one study, formation of 8-OH-dG was measured as an indicator of oxidative DNA damage in liver and kidney of rats exposed to di(2-ethylhexyl) adipate in the diet for two weeks. Increased levels of 8-OH-dG were found in the liver but not in the kidney. A separate study found no evidence of covalent binding of di(2-ethylhexyl) adipate to mouse liver DNA.

Three putative metabolites of di(2-ethylhexyl) adipate—(mono(2-ethylhexyl) adipate, mono(2-ethyl-5-hydroxyhexyl) adipate and mono(2-ethyl-5-oxohexyl) adipate)—were not mutagenic to *S. typhimurium* strains TA100, TA102, TA98 or TA97 in the presence or absence of exogenous metabolic activation.

Table 3. Genetic and related effects of di(2-ethylhexyl) adipate and some derivatives

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Salmonella typhimurium TA100, TA1535, TA1537, TA1538, TA98, reverse mutation	–	–	5000 µg/plate	Simmon *et al.* (1977)
Salmonella typhimurium TA100, reverse mutation	–	–	NR	Seed *et al.* (1982)
Salmonella typhimurium TA100, TA1535, TA1537, TA98, reverse mutation	–	–	10 000 µg/plate	Zeiger *et al.* (1985)
Photobacterium phosphoreum, bioluminescence assay Mutatox	–	NT	NR	Elmore & Fitzgerald (1990)
Drosophila melanogaster, sex-linked recessive lethal mutations	–		20 000 ppm in feed	Woodruff *et al.* (1985)
Drosophila melanogaster, sex-linked recessive lethal mutations	–		1.15 µg/animal; inj.	Woodruff *et al.* (1985)
Gene mutation, L5178Y cells, *Tk* locus *in vitro*, forward mutation	–	–	1000[c]	McGregor *et al.* (1988)
Sister chromatid exchange, primary female Fischer 344 rat hepatocytes *in vitro*	–	NT	74 (3 and 51 h incubation)	Reisenbichler & Eckl (1993)
Chromosomal aberrations, primary female Fischer 344 rat hepatocytes *in vitro*	–	NT	74 (3 and 51 h incubation)	Reisenbichler & Eckl (1993)
Micronucleus assay, primary female Fischer 344 rat hepatocytes *in vitro*	–	NT	74 (3 and 51 h incubation)	Reisenbichler & Eckl (1993)
Chromosomal aberrations and micronucleus formation, male $B6C3F_1$ mouse bone marrow *in vivo*	–		NR	Shelby & Witt (1995)
Micronucleus test, male $B6C3F_1$ mouse bone marrow *in vivo*	–		2000 ip × 3	Shelby *et al.* (1993)
Rat (Sprague-Dawley) urine/*Salmonella typhimurium* TA100, TA1535, TA1537, TA1538, TA98, reverse mutation	–	–	2000 po × 15	DiVincenzo *et al.* (1985)
Binding (covalent) to DNA, female NMR1 mouse liver *in vivo*	–		1100–1440 po × 1[d]	Däniken *et al.* (1984)
Oxidative DNA damage (8-OH-dG), male Fischer 344 rat liver DNA *in vivo*	+[e]		2.5% diet for 1 and 2 w	Takagi *et al.* (1990)

Table 3 (contd)

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Dominant lethal assay, male Harlan/ICR albino Swiss mice *in vivo*	(+)		9220 ip × 1	Singh *et al.* (1975)
Mono(2-ethylhexyl) adipate				
Salmonella typhimurium TA100, TA102, TA98, TA97, reverse mutation	–	–	1000 µg/plate	Dirven *et al.* (1991)
Mono(2-ethyl-5-hydroxyhexyl) adipate				
Salmonella typhimurium TA100, TA102, TA98, TA97, reverse mutation	–	–	1000 µg/plate	Dirven *et al.* (1991)
Mono(2-ethyl-5-oxohexyl) adipate				
Salmonella typhimurium TA100, TA102, TA98, TA97, reverse mutation	–	–	1000 µg/plate	Dirven *et al.* (1991)

[a] +, positive; –, negative; NT, not tested; NR, not reported
[b] LED, lowest effective dose; HID, highest ineffective dose; in-vitro tests, µg/mL; in-vivo tests, mg/kg bw/day; po, oral; d, day; w, week
[c] Precipitate formed at doses ≥ 1000 µg/mL
[d] There was no effect of pre-treatment with 10 000 mg/kg in the diet for four weeks.
[e] Oxidative damage was not found in rat kidney DNA; 8-OH-dG, 8-hydroxydeoxyguanosine

4.5 Mechanistic considerations

Some general considerations about the role of peroxisome proliferation as a mechanism of carcinogenicity are presented in the General Remarks section of this volume. Studies of this mechanism are reviewed fully in Section 4.5 of the monograph on di(2-ethylhexyl) phthalate in this volume.

The weight of evidence for di(2-ethylhexyl) adipate, and for other rodent peroxisome proliferators in general, demonstrates that they do not act as direct DNA-damaging agents.

Chronic administration of peroxisome proliferators to rodents results in sustained oxidative stress due to overproduction of peroxisomal hydrogen peroxide. This can theoretically generate reactive oxygen species which can damage DNA and other intracellular targets. The induction of peroxisomal fatty acid β-oxidation by di(2-ethylhexyl) adipate *in vivo* under carcinogenicity testing conditions in rats and mice (Lake *et al*., 1997) supports this hypothesis. Limited supporting data on induction of oxidative stress (formation of 8-OH-dG in DNA) in rat liver by di(2-ethylhexyl) adipate are available (Takagi *et al*., 1990); however, there are no data for mouse liver.

Similarly, the modulation of hepatocellular proliferation by peroxisome proliferators has been implicated in the mechanism of carcinogenesis. This can theoretically result in increased levels of mutation by increasing the frequency of replicative DNA synthesis as well as increasing the number of hepatocytes at risk. Furthermore, hepatocellular proliferation is likely to be involved in the promotion of growth of preneoplastic hepatocytes. There is clear evidence that di(2-ethylhexyl) adipate causes acute and sustained hepatocellular proliferation under bioassay conditions which resulted in liver tumours in mice. Interestingly, the duration of hepatocellular proliferation was limited in rats, which did not respond with liver tumours in the bioassay as did the mice (Lake *et al*., 1997).

Marked species differences in hepatic peroxisome proliferation have been reported (Ashby *et al*., 1994; IARC, 1995; Lake, 1995a,b; Cattley *et al*., 1998). In biopsies from humans receiving hypolipidaemic drugs, there was no effect or changes were much smaller than those that would be produced in rodent hepatocytes at equivalent dose levels (Lake, 1995a,b; Cattley *et al*., 1998). While peroxisome proliferation may be readily demonstrated in cultured rat and mouse hepatocytes, such effects are not observed in hepatocytes from non-responsive species including guinea-pigs, primates and humans. No study has yet compared the responsiveness of human versus rodent livers *in vivo* or hepatocytes *in vitro* to di(2-ethylhexyl) adipate; however, a growing body of evidence concerning the molecular basis of peroxisome proliferation, summarized below, indicates that human livers and hepatocytes would be refractory to induction of peroxisome proliferation by di(2-ethylhexyl) adipate.

Studies of PPARα activation *in vitro* or in PPARα knock-out mice *in vivo* have not yet been conducted with di(2-ethylhexyl) adipate; however, given that the receptor mediates the same response for a variety of other peroxisome proliferators, it is likely

to mediate the hepatic effects of di(2-ethylhexyl) adipate. Stated another way, induction of peroxisome proliferation by a PPARα-independent mechanism would be unprecedented.

Cultured hepatocytes from non-human primates (marmosets and macaques) and humans have been similarly unresponsive to a variety of peroxisome proliferators (reviewed in Doull *et al*., 1999). No evaluation of peroxisome proliferation in human hepatocytes treated with di(2-ethylhexyl) adipate metabolites *in vitro* has been published. The lack of peroxisome proliferation in hepatocytes from marmosets suggests that human hepatocytes also would be unresponsive (Cornu *et al*., 1992). These negative results were significant in that the same metabolites induced typical induction of peroxisomal (cyanide-insensitive) palmitoyl-CoA oxidation activity in rat and mouse hepatocytes.

The insensitivity of human hepatocytes towards peroxisome proliferators is reflected in the guinea-pig. The guinea-pig is also refractory to the hepatic effects of rodent peroxisome proliferators (reviewed in Doull *et al*., 1999), including di(2-ethylhexyl) adipate metabolites (Cornu *et al*., 1992), and like humans expresses similar low levels of PPARα (Bell *et al*., 1998; Tugwood *et al*., 1998). Significant responses (peroxisome proliferation, induction of fatty acid oxidizing enzymes and the stimulation of replicative DNA synthesis) believed to be associated with hepatocarcinogenesis in rodents are not observed in humans and guinea-pigs. However, these species do exhibit hypolipidaemic responses when exposed to some rodent peroxisome proliferators (Lake, 1995a ; Bell *et al*., 1998; Cattley *et al*., 1998). This hypolipidaemic response, which is not associated with any hypertrophic or hyperplastic response, has been attributed to PPARα-mediated regulation of genes encoding lipoprotein lipase and various lipoproteins. The existence of such a response, in spite of low levels of PPARα, may be explained by differences in the mechanism of action of PPARα in relation to these hypolipidaemic genes compared to those that regulate hypertrophic and hyperplastic responses: the former may have a lower threshold of activation and may require lower concentrations of receptor due to different binding affinities for different PPREs. Differences in the activation properties for different PPRE-containing promoters have been demonstrated (Hsu *et al.*, 1995).

In summary:

1. Di(2-ethylhexyl) adipate does not show evidence of genotoxicity.
2. Di(2-ethylhexyl) adipate produces liver tumours in mice.
3. Under conditions of the bioassays, di(2-ethylhexyl) adipate induces peroxisome proliferation and cell replication in liver that are characteristic of a peroxisome proliferator in mice and, to a limited extent, in rats.
4. Rodent peroxisome proliferators exercise their pleiotropic effects due to activation of PPARα. This process is essential for liver hypertrophy and hyperplasia and eventual hepatocarcinogenesis in response to peroxisome proliferators.

5. The absence of significant response of human liver to induction of peroxisome proliferation and hepatocellular proliferation is explained by several aspects of PPARα-mediated regulation of gene expression.
6. Hepatic peroxisome proliferation has not been evaluated in studies of human subjects or systems treated with di(2-ethylhexyl) adipate. However, interspecies comparisons with other peroxisome proliferators, along with the role of PPARα in this response, indicate that humans can reasonably be predicted to be refractory to induction of peroxisome proliferation and hepatocellular proliferation by di(2-ethylhexyl) adipate.
7. Overall, these findings suggest that the increased incidence of liver tumours in mice treated with di(2-ethylhexyl) adipate results from a mechanism that does not operate in humans. However, studies of di(2-ethylhexyl) adipate or its metabolites regarding peroxisome proliferation in human cells are not available.

5. Summary of Data Reported and Evaluation

5.1 Exposure data

Di(2-ethylhexyl) adipate is a liquid of low volatility, widely used as a plasticizer in flexible poly(vinyl chloride) products, notably food films, as well as in other plastics and in a number of other minor applications, such as lubricants and cosmetics. Occupational exposure may occur by inhalation of di(2-ethylhexyl) adipate as an aerosol during its manufacture and its use. Meat-wrapping workers may be exposed while cutting poly(vinyl chloride) film across a heated cutter. Food is the major source of exposure of the general population to di(2-ethylhexyl) adipate because of migration from poly(vinyl chloride) packaging, particularly into fatty foods such as cheese and meat.

5.2 Human carcinogenicity data

No data were available to the Working Group.

5.3 Animal carcinogenicity data

Di(2-ethylhexyl) adipate was tested for carcinogenicity by oral administration in one experiment in mice and one experiment in rats. In mice, liver adenomas and carcinomas were produced in both males and females. No treatment-related tumours were observed in rats.

5.4 Other relevant data

Di(2-ethylhexyl) adipate is rapidly and completely absorbed after oral administration, rapidly and extensively metabolized and rapidly excreted in humans and experimental animals. It is hydrolysed in the gastrointestinal tract before absorption.

No data on the toxic effects of di(2-ethylhexyl) adipate in humans were available to the Working Group.

In mice and rats, di(2-ethylhexyl) adipate induced hepatic markers of peroxisome proliferation (ultrastructural and biochemical) as well as hepatomegaly and increased replicative DNA synthesis. The species differences in carcinogenicity assays of di(2-ethylhexyl) adipate (increased hepatocellular tumours in mice, not rats) are consistent with a higher intake of di(2-ethylhexyl) adipate and a greater extent of peroxisome proliferation and associated responses in livers of mice compared with rats fed the same dietary doses.

In hepatocytes isolated from rats and mice, treatment of primary cultures with metabolites of di(2-ethylhexyl) adipate increased peroxisomal palmitoyl-coenzyme A oxidation activity. The same treatment of primary cultures of hepatocytes from guinea-pigs and marmosets failed to cause any similar increase in activity.

No data on reproductive and developmental effects in humans were available to the Working Group.

Exposure of rats to di(2-ethylhexyl) adipate during organogenesis caused an increased frequency of variations and retardations in the fetuses at doses below the maternally toxic range.

No effects on male or female fertility were found in rats given di(2-ethylhexyl) adipate in the feed. The body weight gain of the pups at the highest dose was reduced throughout the postnatal period. In mice, a single high intraperitoneal dose given to males before mating was associated with a reduced percentage of pregnancies and increased number of fetal deaths.

No data on the genetic and related effects of di(2-ethylhexyl) adipate in humans or human cells were available to the Working Group.

Di(2-ethylhexyl) adipate did not bind covalently to mouse liver DNA *in vivo*. One report showed evidence of oxidative damage in rat liver DNA *in vivo* but not in rat kidney DNA. A weak dominant lethal effect has been reported in male mice. Analyses of mouse bone marrow after treatment with di(2-ethylhexyl) adipate *in vivo* found no induction of micronuclei in one study and no induction of chromosomal aberrations in one study. Urine from rats treated with di(2-ethylhexyl) adipate by gavage was not mutagenic to *Salmonella typhimurium*.

Di(2-ethylhexyl) adipate did not induce gene mutations, sister chromatid exchanges, chromosomal aberrations or micronuclei in rodent cells *in vitro*. It did not induce sex-linked recessive lethal mutations in *Drosophila* when administered either by diet or injection. Di(2-ethylhexyl) adipate was not mutagenic to either *Photo-*

bacterium phosphoreum or *Salmonella typhimurium* in the presence or absence of exogenous metabolic activation.

These data indicate that di(2-ethylhexyl) adipate is not genotoxic.

5.5 Evaluation

No epidemiological data relevant to the carcinogenicity of di(2-ethylhexyl) adipate were available.

There is *limited evidence* in experimental animals for the carcinogenicity of di(2-ethylhexyl) adipate.

Overall evaluation

Di(2-ethylhexyl) adipate is *not classifiable as to its carcinogenicity to humans (Group 3)*.

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