DI(2-ETHYLHEXYL) PHTHALATE

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 into the monograph and taken into consideration in the present evaluation.

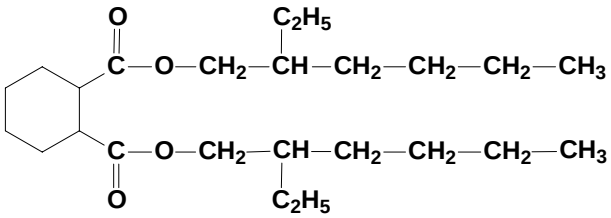
1. Exposure Data

1.1 Chemical and physical data

1.1.1 *Nomenclature*

Chem. Abstr. Serv. Reg. No.: 117-81-7
Deleted CAS Reg. Nos: 8033-53-2; 40120-69-2; 50885-87-5; 126639-29-0; 137718-37-7; 205180-59-2
Chem. Abstr. Name: 1,2-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester
IUPAC Systematic Names: Bis(2-ethylhexyl) phthalate; phthalic acid, bis(2-ethylhexyl) ester
Synonyms: Bis(2-ethylhexyl) 1,2-benzenedicarboxylate; bis(2-ethylhexyl) *ortho*-phthalate; DEHP; dioctyl phthalate; di-sec octyl phthalate; ethylhexyl phthalate; 2-ethylhexyl phthalate; octyl phthalate; phthalic acid di(2-ethylhexyl) ester; phthalic acid dioctyl ester

1.1.2 *Structural and molecular formulae and relative molecular mass*



$C_{24}H_{38}O_4$ Relative molecular mass: 390.56

1.1.3 *Chemical and physical properties of the pure substance*

(*a*) *Description*: Light-coloured liquid with a slight odour (Agency for Toxic Substances and Disease Registry, 1993; Verschueren, 1996)
(*b*) *Boiling-point*: 384 °C (Lide, 1999)
(*c*) *Melting-point*: –55 °C (Lide, 1999)
(*d*) *Density*: 0.981 g/cm^3 at 25 °C (Lide, 1999)
(*e*) *Spectroscopy data*: Infrared (prism [28]; grating [18401]), ultraviolet [22080], nuclear magnetic resonance (proton [9392]; C-13 [5201]) and mass [NIST, 43511] spectral data have been reported (Sadtler Research Laboratories, 1980; Lide & Milne, 1996)
(*f*) *Solubility*: Slightly soluble in water (0.285 mg/L at 24 °C)[1]; slightly soluble in carbon tetrachloride (Environmental Protection Agency, 1998; Lide, 1999)
(*g*) *Volatility*: Vapour pressure, 8.6×10^{-4} Pa at 25 °C, 160 Pa at 200 °C; relative vapour density (air = 1), 13.4 (Howard *et al.*, 1985; Verschueren, 1996)
(*h*) *Octanol/water partition coefficient (P)*: log P, 7.45 (Hansch *et al.*, 1995)
(*i*) *Conversion factor*[2]: mg/m^3 = 16.0 × ppm

1.1.4 *Technical products and impurities*

Di(2-ethylhexyl) phthalate is available in a variety of technical grades (including a special grade for capacitor applications and a low residuals grade). Typical specifications are: minimal ester content, 99.0–99.6%; maximal moisture content, 0.1%; and acidity (as acetic acid or phthalic acid), 0.007–0.01% (Aristech Chemical Corp., 1992; WHO, 1992).

Trade names for di(2-ethylhexyl) phthalate include: Bisoflex; Compound 889; Diacizer DOP; DOP; Eastman DOP Plasticizer; Ergoplast; Etalon; Eviplast; Fleximel; Flexol DOP; Good-rite GP 264; Hatco-DOP; Kodaflex DOP; Monocizer DOP; Octoil; Palatinol AH; Pittsburgh PX 138; Plasthall DOP; Platinol AH; Reomol D 79P; Sicol 150; Staflex DOP; Truflex DOP; Vestinol AH; Vinycizer; Witcizer 312 (National Toxicology Program, 1991; WHO, 1992; American Conference of Governmental Industrial Hygienists, 1999).

1.1.5 *Analysis*

Detection and quantification of very low levels of di(2-ethylhexyl) phthalate are seriously limited by the presence of this compound as a contaminant in almost all laboratory equipment and reagents. Plastics, glassware, aluminium foil, cork, rubber, glass wool, solvents and Teflon® sheets have all been found to be contaminated (Agency for Toxic Substances and Disease Registry, 1993).

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[1] Lower values have been proposed, based on models (Staples *et al.*, 1997).
[2] Calculated from: mg/m^3 = (relative molecular mass/24.45) × ppm, assuming a temperature of 25 °C and a pressure of 101 kPa

Determination of di(2-ethylhexyl) phthalate in air, water, soil/sediments and food is usually accomplished by gas chromatographic analysis; a high-performance liquid chromatography method for food has also been developed. Di(2-ethylhexyl) phthalate is usually extracted from environmental samples with an organic solvent such as hexane, chloroform, dichloromethane or acetonitrile. Air samples are drawn through a solid sorbent material and desorbed with carbon disulfide. A purge-and-trap method has been developed for separation of di(2-ethylhexyl) phthalate from the fat in foods (Agency for Toxic Substances and Disease Registry, 1993).

Selected methods for the analysis of di(2-ethylhexyl) phthalate in various matrices are presented in Table 1.

1.2 Production

The worldwide production of di(2-ethylhexyl) phthalate has been increasing during recent decades and in the late 1980s amounted to approximately 1 million tonnes per year (WHO, 1992). Production of di(2-ethylhexyl) phthalate in the United States increased during the 1980s, from approximately 114 000 tonnes in 1982 to over 130 000 tonnes in 1986 (Environmental Protection Agency, 1998). In 1994, production of di(2-ethylhexyl) phthalate in the United States was 117 500 tonnes; production in Japan in 1995 was 298 000 tonnes; production in Taiwan in 1995 was 207 000 tonnes, down from 241 000 tonnes in 1994 (Anon., 1996).

Di(2-ethylhexyl) phthalate is produced commercially by the reaction of excess 2-ethylhexanol with phthalic anhydride in the presence of an acid catalyst such as sulfuric acid or *para*-toluenesulfonic acid. It was first produced in commercial quantities in Japan around 1933 and in the United States in 1939 (IARC, 1982).

Information available in 1999 indicated that di(2-ethylhexyl) phthalate was produced by 30 companies in China, 15 companies in India, 12 companies in Japan, eight companies in Mexico, seven companies in Taiwan, five companies each in Germany and the Russian Federation, four companies each in Argentina, Brazil, the Philippines and the United States, three companies each in Canada, Chile, Spain, Thailand, Turkey and Venezuela, two companies each in Belgium, Colombia, Ecuador, France, Indonesia, Iran, Italy, Korea (Republic of), Malaysia and Poland, and one company each in Albania, the Czech Republic, Finland, Kazakhstan, Pakistan, Peru, Romania, South Africa, Sweden, Ukraine, the United Kingdom and Viet Nam (Chemical Information Services, 1999).

1.3 Use

Di(2-ethylhexyl) phthalate is widely used as a plasticizer in flexible vinyl products. Plastics may contain from 1 to 40% di(2-ethylhexyl) phthalate by weight and are used in consumer products such as imitation leather, rainwear, footwear, upholstery, flooring, wire and cable, tablecloths, shower curtains, food packaging materials and children's

Table 1. Selected methods for the analysis of di(2-ethylhexyl) phthalate

Sample matrix	Sample preparation	Assay procedure[a]	Limit of detection	Reference
Air	Collect on cellulose ester membrane filter; desorb with carbon disulfide	GC/FID	10 µg/sample	Eller (1994) [Method 5020]
Drinking-water, and source water	Extract in liquid–solid extractor; elute with dichloromethane; concentrate by evaporation	GC/MS	0.5 µg/L	Environmental Protection Agency (1995a) [Method 525.2]
Drinking-water	Extract in liquid–liquid extractor; isolate; dry; concentrate	GC/PID	2.25 µg/L	Environmental Protection Agency (1995b) [Method 506]
Wastewater, municipal and industrial	Extract with dichloromethane; dry; exchange to hexane and concentrate	GC/ECD	2.0 µg/L	Environmental Protection Agency (1999a) [Method 606]
	Extract with dichloromethane; dry; concentrate	GC/MS	2.5 µg/L	Environmental Protection Agency (1999b) [Method 625]
	Add isotope-labelled analogue; extract with dichloromethane; dry over sodium sulfate; concentrate	GC/MS	10 µg/L	Environmental Protection Agency (1999c) [Method 1625]
Groundwater, leachate, soil, sludge, sediment	Aqueous sample: extract with dichloromethane; elute with acetonitrile; exchange to hexane; Solid sample: extract with dichloromethane/acetone (1:1) or hexane/acetone (1:1); clean-up	GC/ECD	0.27 µg/L (aqueous)	Environmental Protection Agency (1996) [Method 8061A]

[a] Abbreviations: GC, gas chromatography; ECD, electron capture detection; FID, flame ionization detection; MS, mass spectrometry; PID, photoionization detection

toys. Poly(vinyl chloride) (PVC) containing di(2-ethylhexyl) phthalate is also used for tubing and containers for blood products and transfusions. Di(2-ethylhexyl) phthalate is also used as a hydraulic fluid and as a dielectric fluid (a non-conductor of electric current) in electrical capacitors (Agency for Toxic Substances and Disease Registry, 1989). Other uses are in rubbing alcohol, liquid detergents, decorative inks, munitions, industrial and lubricating oils and defoaming agents during paper and paperboard manufacture (Environmental Protection Agency, 1998). It is also used as an acaricide in

orchards, an inert ingredient in pesticides, a detector for leaks in respirators, in the testing of air filtration systems and as a component of cosmetic products (National Toxicology Program, 1991).

World consumption of phthalates in the early 1990s was estimated to be 3.25 million tonnes, of which di(2-ethylhexyl) phthalate accounted for approximately 2.1 million tonnes. The estimated total consumption of di(2-ethylhexyl) phthalate by geographical region was (thousand tonnes): western Europe, 465; North America, 155; eastern Asia, 490; Japan, 245; and others, 765 (Towae *et al.*, 1992).

1.4 Occurrence

Concern regarding exposure to di(2-ethylhexyl) phthalate rose to prominence when Jaeger and Rubin (1970) reported its presence in human blood that had been stored in PVC bags. The same authors later reported the presence of di(2-ethylhexyl) phthalate in tissue samples of the lung, liver and spleen from patients who had received blood transfusions before death (Jaeger & Rubin, 1972). While occupational inhalation is a significant potential route of exposure, medical procedures such as haemodialysis, extracorporeal membrane oxygenation, blood transfusion, umbilical catheterization and short-term cardiopulmonary by-pass can also result in high exposures (Huber *et al*., 1996; Karle *et al*., 1997). Patients undergoing haemodialysis are considered to have the highest exposure, due to the chronic nature of the treatment. Further, because of the widespread use of di(2-ethylhexyl) phthalate in plastic containers and its ability to leach out of PVC, humans are exposed to this substance on a regular basis. The extensive manufacture of di(2-ethylhexyl) phthalate-containing plastics has resulted in its becoming a ubiquitous environmental contaminant (Huber *et al*., 1996).

1.4.1 *Natural occurrence*

Di(2-ethylhexyl) phthalate is not known to occur naturally.

1.4.2 *Occupational exposure*

According to the 1981–83 US National Occupational Exposure Survey, approximately 341 000 workers in the United States were potentially exposed to di(2-ethylhexyl) phthalate. Occupational exposure to di(2-ethylhexyl) phthalate may occur during its manufacture and its use mostly as a plasticizer of PVC (compounding, calendering and coating operations). Printing and painting occupations account also for a large number of workers potentially exposed (NOES, 1999). Occupational exposure to di(2-ethylhexyl) phthalate occurs by inhalation, essentially in the form of an aerosol (mist) because of the very low vapour pressure of the substance (Fishbein, 1992). Indeed, di(2-ethylhexyl) phthalate aerosols are used to test the efficiency of high-efficiency particulate air filters during their manufacture (Roberts, 1983).

Few data are available on levels of occupational exposure to di(2-ethylhexyl) phthalate (Table 2). Huber *et al.* (1996) observed that concentrations in air reported in older studies were well above (up to 60 mg/m^3) those determined later; these older studies, however, reported concentrations of total phthalates.

Urinary levels of di(2-ethylhexyl) phthalate, its metabolites and total phthalates have been shown in a few studies to be higher in di(2-ethylhexyl) phthalate-exposed workers than in non-exposed workers and in post-shift samples than in pre-shift samples. No standard method has been proposed for biological monitoring of exposure to di(2-ethylhexyl) phthalate (Liss *et al.*, 1985; Nielsen *et al.*, 1985; Dirven *et al.*, 1993a).

Other exposures may occur concurrently with exposure to di(2-ethylhexyl) phthalate, e.g., phthalic anhydride, other phthalates and hydrogen chloride, depending on the type of industry (Liss *et al.*, 1985; Nielsen *et al.*, 1985; Vainiotalo & Pfäffli, 1990).

1.4.3 *Environmental occurrence*

The environmental fate of phthalate esters has been reviewed (Staples *et al.*, 1997).

Di(2-ethylhexyl) phthalate is considered a priority and/or hazardous pollutant in the United States (Environmental Protection Agency, 1984; Kelly *et al*., 1994), Canada (Meek & Chan, 1994; Meek *et al*., 1994; Environment Canada, 1997) and the Netherlands (Wams, 1987) because of the very large quantities that have been emitted during its production, use and disposal and its ubiquitous occurrence and stability in the environment. It is known to be widely distributed, generally at very low levels, in air, precipitation, water, sediment soil and biota (with the highest levels found in industrial areas), in food samples and in human and animal tissues (Peterson & Freeman, 1982; Giam *et al*., 1984; Wams, 1987; WHO, 1992; Agency for Toxic Substances and Disease Registry, 1993; Kelly *et al*., 1994; Sharman *et al*., 1994; Huber *et al*., 1996). The principal route by which it enters the environment is via transport in air or via leaching from plastics and plasticizer plants or other sources such as sewage treatment plants, paper and textile mills and refuse incinerators. Patients receiving blood products or undergoing treatments requiring extracorporeal blood circulation may be exposed by leaching of di(2-ethylhexyl) phthalate from PVC bags and tubing (Wams, 1987; WHO, 1992; Agency for Toxic Substances and Disease Registry, 1993). Human daily intakes of di(2-ethylhexyl) phthalate from various exposure pathways have been estimated (Table 3) (Meek & Chan, 1994).

(*a*) *Air*

According to the Toxics Release Inventory (Environmental Protection Agency, 1999d), air emissions of di(2-ethylhexyl) phthalate from 298 industrial facilities in the United States amounted to 107 tonnes in 1997.

In Canada, 27 tonnes of di(2-ethylhexyl) phthalate were released to air in 1995, according to the Canadian National Pollutant Release Inventory (Environment Canada, 1997).

Table 2. Workplace air levels of di(2-ethylhexyl) phthalate

Production	Country	Air concentration (mg/m^3)		Sampling	No. of samples	Reference
		Mean	Range			
Di(2-ethylhexyl) phthalate manufacturing plant	USA			Personal—whole-shift		Liss & Hartle (1983)
Chemical operators, technicians and maintenance workers			ND–4.11[a]		50[b]	
Poly(vinyl chloride) processing industry	Sweden			Personal—2-h		Nielsen *et al.* (1985)
Thick film department: calender operators/machine attendants		0.4[c]	0.1–0.8		16	
Poly(vinyl chloride) processing plants	Finland			Area—1.5–3 h		Vainiotalo & Pfäffli (1990)
Extrusion		0.05	± 0.03		4	
Extrusion		0.3	± 0.2		5	
Calendering		0.5	± 0.5		7	
Hot embossing		0.05	± 0.02		5	
Welding		0.3	± 0.05		4	
Injection moulding		0.02	± 0.01		2	
Compounding		0.02	± 0.01		5	
Thermoforming		0.02	± 0.02		2	
High-frequency welding		< 0.02				
Poly(vinyl chloride) processing plants	Netherlands			Personal—2-h		Dirven *et al.* (1993a)
Boot factory						
Mixing process		0.26	0.1–1.2		16	
Extruder process		0.12	0.05–0.28		11	
Cable factory						
Mixing process		0.18	0.01–0.81		8	
Extruder process		0.24	0.01–1.27		13	

Table 2 (contd)

Production	Country	Air concentration (mg/m^3)		Sampling	No. of samples	Reference
		Mean	Range			
Various plants	USA			Personal—4–5 h		Roberts (1983)
Di(2-ethylhexyl) phthalate manufacture		ND			8	
Two aerosol filter testing facilities			0.01–0.14		4	
Poly(vinyl chloride) sheet processing plant			0.06–0.29		3	

[a] ND, not detected
[b] Only six measurements were above the detection limit
[c] Presented as total phthalates, but where di(2-ethylhexyl) phthalate was the main plasticizer

Table 3. Estimated daily intake of di(2-ethylhexyl) phthalate by the population of Canada

Substrate/medium	Estimated intake for various age ranges (ng/kg bw per day)				
	0–0.5 years[a]	0.5–4 years[b]	5–11 years[c]	12–19 years[d]	20–70 years[e]
Ambient air: Great Lakes region	0.03–0.3	0.03–0. 3	0.04–0.4	0.03–0.3	0.03–0.3
Indoor air	860	990	1200	950	850
Drinking-water	130–380	60–180	30–100	20–70	20–60
Food	7900	18 000	13 000	7200	4900
Soil	0.064	0.042	0.014	0.04	0.03
Total estimated intake	8900–9100	19 000	14 000	8200	5800

From Meek & Chan (1994)
[a] Assumed to weigh 6 kg, breathe 2 m^3 air, drink 0.75 L water and ingest 35 mg soil
[b] Assumed to weigh 13 kg, breathe 5 m^3 air, drink 0.8 L water and ingest 50 mg soil
[c] Assumed to weigh 27 kg, breathe 12 m^3 air, drink 0.9 L water and ingest 35 mg soil
[d] Assumed to weigh 57 kg, breathe 21 m^3 air, drink 1.3 L water and ingest 20 mg soil
[e] Assumed to weigh 70 kg, breathe 23 m^3 air, drink 1.5 L water and ingest 20 mg soil

Di(2-ethylhexyl) phthalate concentrations of up to 790 ng/m^3 have been found in urban and polluted air, but usually the levels in ambient air are well below 100 ng/m^3 (WHO, 1992: Agency for Toxic Substances and Disease Registry, 1993).

Di(2-ethylhexyl) phthalate released into air can be carried for long distances in the troposphere and it has been detected over the Atlantic and Pacific Oceans; wash-out by rain appears to be a significant removal process (Atlas & Giam, 1981; Giam *et al*., 1984; WHO, 1992).

Di(2-ethylhexyl) phthalate in air has been monitored in the North Atlantic, the Gulf of Mexico and on Enewetak Atoll in the North Pacific and levels ranged from not detectable to 4.1 ng/m^3 (Giam *et al.*, 1978, 1980; Atlas & Giam, 1981). Concentrations of di(2-ethylhexyl) phthalate in the atmosphere of the northwestern Gulf of Mexico averaged 1.16 ng/m^3 for ten samples, with 57% of the compound measured in the vapour phase only. The concentration was one to two orders of magnitude lower in maritime air than in continental atmospheres (Giam *et al.*, 1978, 1980).

Somewhat similar levels in air, between 0.5 and 5 ng/m^3 (mean, 2 ng/m^3) of di(2-ethylhexyl) phthalate have been found in the Great Lakes ecosystem (Canada and United States). The concentration of di(2-ethylhexyl) phthalate in precipitation ranged from 4 to 10 ng/L (mean, 6 ng/L). Atmospheric fluxes to the Great Lakes are a combination of dry and wet removal processes. The total deposition of di(2-ethylhexyl) phthalate into Lakes Superior, Michigan, Huron, Erie and Ontario was estimated to amount to 16, 11, 12, 5.0 and 3.7 tonnes per year, respectively (Eisenreich *et al*., 1981).

In Sweden, air concentrations of di(2-ethylhexyl) phthalate were measured at 14 monitoring stations (53 samples). The median air concentration was 2.0 ng/m^3 (range, 0.3–77 ng/m^3) and the average fallout was 23.8 μg/m^2 per month (range, 6.0–195.5 μg/m^2 per month). The total annual fallout of di(2-ethylhexyl) phthalate in Sweden was estimated to be 130 tonnes (Thurén & Larsson, 1990).

During 1995, four sets of samples from two monitoring stations of the breathable fraction of atmospheric particulates including phthalates were measured in the air of Rieti urban area in Italy. The concentrations of di(2-ethylhexyl) phthalate ranged from 20.5 to 31 ng/m^3 (normalized) and from 34.8 to 503.5 ng/m^3 (normalized), respectively, at the two monitoring stations (Guidotti *et al*., 1998).

The concentration of di(2-ethylhexyl) phthalate in air at Lyngby, Denmark was calculated to be 22 ng/m^3 based on the analysis of snow samples (Løkke & Rasmussen, 1983). Levels of 26–132 ng/m^3 were measured in four samples from a residential area in Antwerp, Belgium (Cautreels *et al*., 1977). The yearly average concentrations at three air sampling stations in New York City in 1978 ranged from 10 to 17 ng/m^3, respectively (Bove *et al*., 1978).

There is a paucity of data concerning concentrations of di(2-ethylhexyl) phthalate concentrations in indoor air, although its volatilization from plastic products has been noted (Wams, 1987; Agency for Toxic Substances and Disease Registry, 1993).

Di(2-ethylhexyl) phthalate has been shown to account for 69 and 52% of the total amount of phthalates adsorbed to sedimented dust and particulate matter, respectively, in a number of Oslo dwellings. It was found at levels of 11–210 μg/100 mg [110–2100 mg/kg] sedimented dust in 38 dwellings and at levels of 24–94 μg/100 mg [240–940 mg/kg] suspended particulate matter (mean ± SD, 60 ± 30) in six dwellings. It was suggested that suspended particulate exposure to di(2-ethylhexyl) phthalate is one- to three-fold higher than the estimated vapour phase exposure (Øie *et al*., 1997).

(*b*) *Water and sediments*

In general, concentrations of di(2-ethylhexyl) phthalate in fresh waters are in the range of < 0.1–10 μg/L, although occasionally much higher values have been observed (~ 100 μg/L) when water basins are surrounded by heavy concentrations of industry (WHO, 1992; Agency for Toxic Substances and Disease Registry, 1993).

Surface water discharges of di(2-ethylhexyl) phthalate from 298 industrial facilities in 1994 in the United States amounted to 264 kg, as reported in the Environmental Protection Agency Toxics Release Inventory (Environmental Protection Agency, 1999d).

Di(2-ethylhexyl) phthalate has been detected in 24% of 901 surface water supplies at a median concentration of 10 μg/L and in 40% of 367 sediment samples at a median concentration of 1000 μg/kg in samples recorded in the STORET database in the United States (Staples *et al*., 1985). Di(2-ethylhexyl) phthalate concentrations in water from Galveston Bay, Texas, ranged from < 2 to 12 000 ng/L (average, 600 ng/L) (Murray *et al*., 1981), somewhat higher than those found earlier for the Mississippi Delta

(23–225 ng/L; average, 70 ng/L) and the Gulf of Mexico coast (6–316 ng/L; average, 130 ng/L) (Giam *et al*., 1978). Levels of di(2-ethylhexyl) phthalate up to 720 ng/L were found in two sampling stations of the Mississippi River in the summer of 1984 (DeLeon *et al*., 1986).

Levels of dissolved di(2-ethylhexyl) phthalate in samples from the River Mersey estuary, Liverpool, United Kingdom, ranged from 0.125 to 0.693 µg/L (Preston & Al-Omran, 1989).

Levels of up to 1.9 µg/L di(2-ethylhexyl) phthalate were found in rivers of the greater Manchester area, United Kingdom (Fatoki & Vernon, 1990) and at unspecified levels as contaminants of the Elbe River and its tributaries in Germany during the period 1992–94 (Franke *et al*., 1995).

Levels of di(2-ethylhexyl) phthalate in two rivers in southern Sweden were 0.32–3.10 µg/L and 0.39–1.98 µg/L. The highest value was in samples taken near an industrial effluent discharge (Thurén, 1986).

In a 12-day survey, di(2-ethylhexyl) phthalate at levels ranging from 0.2 to 0.6 µg/L was found in the River Rhine near Lobith and levels ranging from < 0.1 to 0.3 ng/L were found in the IJsselmeer, The Netherlands (Ritsema *et al*., 1989).

Levels of di(2-ethylhexyl) phthalate in water samples from 12 stations in the Klang River Basin in central Malaysia ranged from 3.1 to 64.3 µg/L between January 1992 and February 1993. The highest levels of phthalates in the water and sediment samples were collected near industrial areas where direct discharge points were found (Tan, 1995).

Di(2-ethylhexyl) phthalate has been reported in the leachate from municipal and industrial landfills at levels ranging from < 0.01 to 150 µg/mL (Ghassemi *et al*., 1984). It has also been detected in 13% of 86 samples of urban storm water runoff evaluated for the National Urban Runoff Program at concentrations ranging from 7 to 39 µg/L (Cole *et al*., 1984).

Since di(2-ethylhexyl) phthalate is lipophilic, it tends to be absorbed onto sediment, which serves as a sink (WHO, 1992). Di(2-ethylhexyl) phthalate has been measured in rivers and lake sediments in Europe (Schwartz *et al*., 1979; Giam & Atlas, 1980; Thurén, 1986; Preston & Al-Omeron, 1989; Ritsema *et al*., 1989) and in river and bay sediments in the United States (Peterson & Freeman, 1982; Ray *et al*., 1983; Hollyfield & Sharma, 1995). Concentrations ranged from 0.029 to 70 mg/kg. Near direct discharge points from industry in Sweden and Malaysia, concentrations of di(2-ethylhexyl) phthalate in sediments were above 1000 mg/kg (Thurén, 1986; Tan, 1995), and ranged from 190 to 700 µg/kg near industrial discharges in marine sediments around coastal Taiwan (Jeng, 1986).

In experimental studies of a marine environment of Narragansett Bay, Rhode Island, United States, it was shown that biodegradation by the surface microlayer biota accounted for at least 30% of the removal of di(2-ethylhexyl) phthalate (Davey *et al*., 1990).

Water solubility is a major factor limiting the degradation of phthalate esters under methanogenic conditions. In a study of the degradation of di(2-ethylhexyl) phthalate

and its intermediate hydrolysis products, 2-ethylhexanol and mono(2-ethylhexyl) phthalate in a methanogenic phthalic acid ester-degrading enrichment culture at 37 °C, the culture readily degraded 2-ethylhexanol via 2-ethylhexanoic acid to methane, mono(2-ethylhexyl) phthalate was degraded to stoichiometric amounts of methane with phthalic acid as a transient intermediate, while di(2-ethylhexyl) phthalate remained unaffected throughout the 330-day experimental period (Ejlertsson & Svensson, 1996; Ejlertsson *et al*., 1997).

(*c*) *Soil*

The principal source of di(2-ethylhexyl) phthalate release to land is disposal of industrial and municipal waste to landfills (Agency for Toxic Substances and Disease Registry, 1993; Bauer & Herrmann, 1997). Additionally, di(2-ethylhexyl) phthalate from various sources such as food wraps is released to municipal waste. Waste disposal of PVC products containing varying amounts of di(2-ethylhexyl) phthalate to landfills is another source (Swedish Environmental Protection Agency, 1996). Releases of di(2-ethylhexyl) phthalate to land from 298 industrial facilities in the United States in 1997 amounted to 32 137 kg (Environmental Protection Agency, 1999d). According to the Canadian National Pollutant Release Inventory, 33 tonnes of di(2-ethylhexyl) phthalate were released from Canadian facilities to land (Environment Canada, 1997).

Five soils and leachate-sprayed soils from the Susquehanna River basin in Pennsylvania and New York had levels of di(2-ethylhexyl) phthalate ranging from 0.001 to 1.2 mg/kg (Russell & McDuffie, 1983). Contaminated soil in the Netherlands was found to contain up to 1.5 mg/kg dry matter (Wams, 1987). Residues of di(2-ethylhexyl) phthalate in soil collected in the vicinity of a di(2-ethylhexyl) phthalate manufacturing plant amounted to up to 0.5 mg/kg (Persson *et al*., 1978).

(*d*) *Foods*

The most common route of human exposure to di(2-ethylhexyl) phthalate is through food contamination. The average daily exposure from food in the United States has been estimated to be about 0.3 mg/day per individual, with a maximum exposure of 2 mg/day (WHO, 1992; Agency for Toxic Substances and Disease Registry, 1993).

Di(2-ethylhexyl) phthalate has been found at generally low levels in a broad variety of foods, including milk, cheese, margarine, butter, meat, cereals, fish and other seafood (Cocchieri, 1986; Giam & Wong, 1987; Castle *et al*., 1990; Petersen, 1991; WHO, 1992; Agency for Toxic Substances and Disease Registry, 1993; Gilbert, 1994). It can originate from PVC wrapping materials, manufacturing processes or from the animals which had produced the milk or meat (Giam & Wong, 1987; Gilbert, 1994; Sharman *et al*., 1994). The highest levels of di(2-ethylhexyl) phthalate have been measured in milk products, meat and fish as well as in other products which have a high fat content. The use of di(2-ethylhexyl) phthalate in food contact applications is reported to be decreasing (Page & Lacroix, 1995).

Di(2-ethylhexyl) phthalate was determined in milk, cream, butter and cheese samples from a variety of sources from Norway, Spain and the United Kingdom. Samples of Norwegian milk obtained at various stages during collection, transportation and packaging operations showed no apparent trends in phthalate contamination, with total phthalate levels (expressed as di(2-ethylhexyl) phthalate equivalents) in the raw milk of between 0.12 and 0.28 μg/kg. On processing the di(2-ethylhexyl) phthalate was concentrated in the cream at levels up to 1.93 μg/g, whereas low-fat milk contained from < 0.01 to 0.07 μg/g [mg/L]. In the United Kingdom, pooled milk samples from doorstep delivery in different regions of the country contained < 0.01–0.09 μg/g [mg/L] di(2-ethylhexyl) phthalate. Concentrations of di(2-ethylhexyl) phthalate in 10 samples of retail cream and 10 samples of butter obtained in the United Kingdom ranged from 0.2 to 2.7 μg/g and 2.5 to 7.4 μg/g, respectively. Thirteen retail milk and cream products from Spain had levels of di(2-ethylhexyl) phthalate ranging from < 0.01 to 0.55 μg/g (Sharman *et al*., 1994).

Milk samples were collected from a dairy in Norway at various stages of the milking process to determine the extent of migration of di(2-ethylhexyl) phthalate from plasticized tubing used in commercial milking equipment. Concentrations for each individual cow averaged 0.03 μg/g [mg/L] and rose to 0.05 μg/g [mg/L] in the central collecting tank. In control milk samples obtained by hand-milking, the concentration of di(2-ethylhexyl) phthalate was below 0.005 μg/g [mg/L]. In Norway and the United Kingdom, di(2-ethylhexyl) phthalate in milk tubing has been replaced by other types of plasticizers, such as di(2-ethylhexyl) adipate (see monograph in this volume) and diisodecyl phthalate (Castle *et al*., 1990).

An investigation of residues of di(2-ethylhexyl) phthalate in retail whole milk samples from 14 Danish dairies about six months after the use of di(2-ethylhexyl) phthalate-plasticized milk tubing was banned in Denmark in August 1989 revealed a mean concentration lower than 50 μg/L (Petersen, 1991).

Retail samples of Canadian butter and margarine wrapped in aluminium foil–paper laminate were found to contain di(2-ethylhexyl) phthalate at levels up to 11.9 mg/kg. Ten samples of butter (454 g each) had levels of di(2-ethylhexyl) phthalate ranging from 2.9 to 11.9 mg/kg and six samples of margarine (454 g each) had levels ranging from 0.8 to 11.3 mg/kg. Analysis of the wrappers showed little correlation between the levels of di(2-ethylhexyl) phthalate in the total wrapper and the corresponding food. When di(2-ethylhexyl) phthalate was not present in the wrapper, a background level of di(2-ethylhexyl) phthalate from about 3 to 7 mg/kg was found in butter while, with di(2-ethylhexyl) phthalate present in the wrapper, an average level in the butter of 9.4 mg/kg of the phthalate was found (Page & Lacroix, 1992).

Di(2-ethylhexyl) phthalate was found in both the packaging and in a number of contacted foods sampled in a 1985–89 survey as part of the Canadian Health Protection Branch Total Diet Program. Low levels (65 μg/kg [L] average in beverages and 29 μg/kg average in foods) associated with the use of di(2-ethylhexyl) phthalate-plasticized cap or lid seals were found in a variety of glass-packaged foods. It was

found in 14 types of cheese at levels up to 5.5 mg/kg [average, 2.2 mg/kg] and, on a butter–fat basis, these levels averaged about 8 mg/kg di(2-ethylhexyl) phthalate. Levels in factory-packaged fish were 0.2 mg/kg in halibut and 2.1 mg/kg in pollack and in two smoked salmon samples were 0.3 and 3.9 mg/kg. Di(2-ethylhexyl) phthalate was found in nine varieties of factory-packaged non-frozen meats at levels that ranged from 0.1 to 3.7 mg/kg (Page & Lacroix, 1995).

Analysis of dairy food composite samples showed the presence of di(2-ethylhexyl) phthalate in all samples at 0.1–3.4 mg/kg. The levels in total diet samples of meat, poultry and fish ranged from 0.1 to 2.6 mg/kg and, in total diet cereal products, ranged from 0.02 to 1.5 mg/kg. Low incidence and low levels of di(2-ethylhexyl) phthalate were found in total diet samples of fruits and vegetables (mostly not detected to 0.07 mg/kg) (Page & Lacroix, 1995).

Di(2-ethylhexyl) phthalate was detected in 80, 71, 84 and 52% of Italian plastic packaged salted meat, jam, baby food and milk samples, respectively, and in all the cheese and vegetable soups samples. The mean di(2-ethylhexyl) phthalate levels ranged between 0.21 and 2.38 mg/kg (Cocchieri, 1986).

A German study in which 22 samples of baby milk, baby food, mothers' milk and cows' milk were analysed for their content of phthalates found a relatively narrow range of 50–210 mg/kg di(2-ethylhexyl) phthalate, with hardly any differences between the food items (Gruber *et al*., 1998).

Di(2-ethylhexyl) phthalate was found (mg/kg wet weight) in the following commercial fish (pooled samples of 10 individuals each): herring (fillets), 4.71; mackerel (fillets), 6.50; cod (liver), 5.19; plaice (fillets), < 0.010; and redfish (fillets), < 0.010 (Musial *et al*., 1981).

In an investigation of 2-ethyl-1-hexanol as a contaminant in some samples of bottled Italian drinking-water, di(2-ethylhexyl) phthalate was also found in 12 glass bottled drinking-water samples (sealed with caps with plastic internal gasket) at levels ranging from 2.4 to 17.7 μg/L (mean, 6.0 μg/L). It was also found in 13 poly(ethylene terephthalate) bottled drinking-water samples (sealed with caps with plastic internal gasket) at levels ranging from 2.7 to 31.8 μg/L (mean, 10.5 μg/L) (Vitali *et al*., 1993).

(*e*) *Exposure from medical devices*

Di(2-ethylhexyl) phthalate at concentrations up to 40% by weight is generally used as a plasticizer in PVC materials which have been widely used for a variety of medical purposes (e.g., infusion–transfusion, dialysis systems or feeding tubes and catheters in disposable medical devices). It is known to leach from PVC blood packs into whole blood, platelet concentrates and plasma during storage; the concentration of di(2-ethylhexyl) phthalate increases with storage time and it is converted by a plasma enzyme to a more toxic metabolite, mono(2-ethylhexyl) phthalate (Rock *et al.*, 1978). Di(2-ethylhexyl) phthalate has been detected in the blood and tissues of patients receiving blood transfusions and haemodialysis treatments (Jaeger & Rubin, 1972; Rock *et al*., 1978; Sasakawa & Mitomi, 1978; Cole *et al*., 1981; Rock *et al*.,

1986; Christensson *et al*., 1991; Dine *et al*., 1991; Huber *et al*., 1996; Mettang *et al*., 1996a).

Di(2-ethylhexyl) phthalate was detected in whole blood at levels ranging from 16.8 to 46.1 µg/mL [mg/L] and in packed cells at levels ranging from 32.6 to 55.5 µg/mL [mg/L] in PVC blood bags stored at 5 °C. These levels increased with storage. The average content was 6.7 ± 4.6 µg/mL in cryoprecipitate and 7.4 ± 2.8 µg/mL in fresh frozen plasma. Both values were independent of the storage period (Sasakawa & Mitomi, 1978).

The accumulation of di(2-ethylhexyl) phthalate in platelet-poor plasma stored for seven and 14 days in PVC bags sterilized by steam, ethylene oxide or irradiation revealed seven-day storage levels of di(2-ethylhexyl) phthalate of 378 ± 19, 362 ± 10 and 275 ± 15 mg/L, respectively, and 14-day storage levels of 432 ± 24, 428 ± 22 and 356 ± 23 mg/L, respectively (Dine *et al*., 1991).

In one study of newborn infants who received exchange transfusion, the plasma levels of di(2-ethylhexyl) phthalate in six patients varied between 3.4 and 11.1 mg/L, while mono(2-ethylhexyl) phthalate levels in the corresponding samples ranged from 2.4 to 15.1 mg/L. In newborn infants subjected to a single exchange transfusion, concentrations of di(2-ethylhexyl) phthalate in plasma from the blood taken from the transfusion set varied between 36.8 and 84.9 mg/L, while mono(2-ethylhexyl) phthalate in these samples ranged between 3.0 and 15.6 mg/mL (Sjöberg *et al*., 1985a). The concentrations in blood of both di- and mono(2-ethylhexyl) phthalates were similar in PVC bags stored for four days or less (Rock *et al*., 1986).

In an additional study to investigate further the disposition of di(2-ethylhexyl) phthalate and mono(2-ethylhexyl) phthalate during a single exchange transfusion in four newborn infants, the amounts of di(2-ethylhexyl) phthalate and mono(2-ethylhexyl) phthalate infused ranged from 0.8 to 3.3 and 0.05 to 0.20 mg/kg bw, respectively. There were indications that about 30% of the infused di(2-ethylhexyl) phthalate originated from parts of the transfusion set other than the blood bag. Approximately 30% of the infused amount of di(2-ethylhexyl) phthalate was withdrawn during the course of each transfusion. Immediately after the transfusions, the plasma levels of di(2-ethylhexyl) phthalate levels ranged between 5.8 and 19.6 mg/L and subsequently declined rapidly (reflecting its distribution within the body), followed by a slower elimination phase. The half-life of this phase was approximately 10 h (Sjöberg *et al*., 1985b).

Serum levels of di(2-ethylhexyl) phthalate in 16 newborn infants undergoing exchange transfusion indicated an undetectable level (< 1 mg/L) before exchange but levels ranging from 6.1 to 21.6 mg/L serum (average, 12.5 ± 6.2 mg/L) after a single exchange transfusion. In 13 newborn infants receiving a second blood unit, the serum levels of di(2-ethylhexyl) phthalate ranged from 12.3 to 87.8 mg/L and, in six patients receiving a third blood unit, the serum levels ranged from 24.9 to 93.1 mg/L (Plonait *et al*., 1993).

The degree of exposure to di(2-ethylhexyl) phthalate was assessed in 11 patients undergoing haemodialysis for treatment of renal failure and showed that on average,

an estimated 105 mg di(2-ethylhexyl) phthalate was extracted from the dialyser during a single 4-h dialysis session, with a range of 23.8–360 mg. Time-averaged circulating concentrations of mono(2-ethylhexyl) phthalate during the session (1.33 ± 0.58 mg/L) were similar to those of di(2-ethylhexyl) phthalate (1.91 ± 2.11 mg/L). Assuming a schedule of treatment three times per week, the average patient in the study would have received approximately 16 g di(2-ethylhexyl) phthalate over the course of a year, with a range of 3.7–56 g (Pollack *et al*., 1985).

Di(2-ethylhexyl) phthalate was found at concentrations ranging from 0.8 to 4.2 mg/L serum in 17 haemodialysis patients after dialysis and 0.1–0.9 mg/L in four of seven continuous ambulatory peritoneal dialysis (CAPD) patients. In three of the CAPD patients and in all of the predialysis patients, di(2-ethylhexyl) phthalate was not detected (< 0.1 mg/L); in no case could the hydrolysis product mono(2-ethylhexyl) phthalate be detected (< 0.4 mg/L) (Nässberger *et al*., 1987).

A comparative evaluation of haemodialysis tubing plasticized with di(2-ethylhexyl) phthalate and with tri-2-ethylhexyl trimellitate was made in 11 patients (10 men, one woman) with chronic renal failure on haemodialysis for a period of six months. During treatment with tubing containing di(2-ethylhexyl) phthalate, the plasma level of di(2-ethylhexyl) phthalate rose from 0.10 mg/L (range, < 0.05–0.17) to 0.70 mg/L (range, 0.30–1.6) (detection limit, 0.05 mg/L) (Christensson *et al*., 1991).

The degree of exposure to and the fate of di(2-ethylhexyl) phthalate and derived mono(2-ethylhexyl) phthalate, 2-ethylhexanol and phthalic acid in seven elderly patients undergoing regular CAPD were compared with those in six aged-matched healthy controls during a 4-h dwell period. Serum concentrations of di(2-ethylhexyl) phthalate and phthalic acid were significantly higher ($p = 0.027$ and $p = 0.026$, respectively) in patients (median, 0.079 mg/L; range, 0.032–0.210 mg/L; and 0.167 mg/L; range, 0.097–0.231 mg/L, respectively) than in controls (median, 0.0195 mg/L; range, 0.016–0.025 mg/L; and 0.012 mg/L; range, 0.006–0.034 mg/L, respectively). The concentration of mono(2-ethylhexyl) phthalate in the fluid of CAPD bags before use was four times higher than that of di(2-ethylhexyl) phthalate. During the first 4 h of dwell time, the concentrations of mono(2-ethylhexyl) phthalate and 2-ethylhexanol in dialysate consistently decreased from 0.177 mg/L (range, 0.137–0.239 mg/L) to 0.022 mg/L (range, 0.005–0.058 mg/L) ($p = 0.017$), and from 0.087 mg/L (range, 0.075–0.097 mg/L) to 0.05 mg/mL (range, 0.023–0.064 mg/L) ($p = 0.017$), respectively, while the concentration of di(2-ethylhexyl) phthalate remained stable. Remarkably high concentrations of phthalic acid (0.129 mg/L; range, 0.038–0.466 mg/L) were found in the CAPD bags before use, and these concentrations tended to increase during dwell time but without statistical significance (0.135 mg/L; range, 0.073–0.659 mg/L; $p = 0.062$) (Mettang *et al.,* 1996a).

Levels of di(2-ethylhexyl) phthalate ranging from < 1 to 4100 μg/mL [mg/L] in the condensate from water traps of six respirators have been reported. Estimation of the inhalatory di(2-ethylhexyl) phthalate exposure to five artificially ventilated preterm infants over a 24-h period yielded values ranging between 1 μg/h and 4200 μg/h. Di(2-

ethylhexyl) phthalate (0.23 mg/kg wet weight) was found in the lung tissue of one infant who died of pneumothorax soon after birth following artificial ventilation (Roth *et al*., 1988)

Serum samples and autopsy specimens were examined from two infants with congenital diaphragmatic hernia who had received life support with extracorporeal membrane oxygenation (ECMO). The serum levels of di(2-ethylhexyl) phthalate after 14 and 24 days of ECMO support were 26.8 and 33.5 mg/L respectively, and levels of 3.5, 1.0 and 0.4 mg/kg di(2-ethylhexyl) phthalate were found in liver, heart and testicular tissues, respectively, and trace quantities were found in the brain. The rate of di(2-ethylhexyl) phthalate extraction from the model PVC circuits was linear with time (rate, 3.5 and 4.1 mg/L per hour). The exposure to di(2-ethylhexyl) phthalate for a 4-kg infant on ECMO support for 3–10 days was estimated to be 42–140 mg/kg (Shneider *et al*., 1989).

A more recent study of 18 infants on ECMO life support also reported leaching of di(2-ethylhexyl) phthalate from the PVC circuits at linear rates that were dependent on the surface area of the circuit. For standard 3–10-day treatment courses, the mean peak plasma concentration of di(2-ethylhexyl) phthalate was 8.3 ± 5.7 mg/L, and the estimated exposure over 3–10 days was 10–35 mg/kg bw. No leaching of di(2-ethylhexyl) phthalate from heparin-coated PVC circuits was detected (Karle *et al.*, 1997).

Exposure of children to di(2-ethylhexyl) phthalate by migration from PVC toys and other articles into saliva has been reported. Until the early 1980s, di(2-ethylhexyl) phthalate was the predominant plasticizer used in soft PVC children's products. Since then, it has been replaced in most countries by other plasticizers, in particular di(iso-nonyl) phthalate (Steiner *et al*., 1998; Wilkinson & Lamb, 1999).

1.5 Regulations and guidelines

Occupational exposure limits for di(2-ethylhexyl) phthalate are given in Table 4.

The World Health Organization has established an international drinking-water guideline for di(2-ethylhexyl) phthalate of 8 μg/L (WHO, 1993). The Environmental Protection Agency (1998) has set the maximum contaminant level (MCL) for di(2-ethylhexyl) phthalate in drinking-water at 6 μg/L in the United States.

The Czech Republic has set a maximum limit for plastic materials for di(2-ethyl-hexyl) phthalate of 50 mg/g as a component of plastic products permitted for contact with food (UNEP, 1999).

The Food and Drug Administration (1999) permits the use of di(2-ethylhexyl) phthalate in the United States as a component of adhesives used in food packaging, as a plasticizer in resinous and polymeric coatings used in food packaging, as a component of defoaming agents used in the manufacture of paper and paperboard used in food packaging, as a flow promoter in food contact surfaces not to exceed 3 wt% based on monomers, as a component of cellophane where total phthalates do

not exceed 5%, as a component of surface lubricants used in the manufacture of metallic articles that contact food and as a food-packaging plasticizer for foods of high water content.

The European Pharmacopoeia identifies di(2-ethylhexyl) phthalate as a substance that may be used in the manufacture of PVC plasticized containers and tubing for human blood and blood components, at a level of not more than 40% in the plastic (Council of Europe, 1997).

Table 4. Occupational exposure limits for di(2-ethylhexyl) phthalate[a]

Country[b]	Year	Concentration (mg/m^3)[b]	Interpretation[b]
Argentina	1991	5	TWA
		10	STEL
Australia	1993	5	TWA
		10	STEL
Belgium	1993	5	TWA
		10	STEL
Canada	1994	5	TWA
		10	STEL
Czech Republic	1993	5	TWA
		10	STEL
Denmark	1993	5 (Ca)	TWA
Finland	1998	5 (sk)	TWA
		10	STEL
France	1993	5	TWA
Germany	1999	10	TWA
Hungary	1993	5 (sk)	TWA
		10	STEL
Ireland	1997	5	TWA
		10	STEL
Japan	1998	5	TWA
Netherlands	1993	5	TWA
Philippines	1993	5	TWA
Poland	1998	1	TWA
		5	STEL
Russian Federation	1993	1	STEL
Slovakia	1993	5	TWA
		10	STEL
Sweden	1993	3	TWA
		5	STEL
Switzerland	1993	5	TWA
United Kingdom	1993	5	TWA
		10	STEL

Table 4 (contd)

Country[b]	Year	Concentration (mg/m^3)[b]	Interpretation[b]
United States			
OSHA (PEL)	1999	5	TWA
NIOSH (REL)	1997	5 (Ca)	TWA
		10	STEL
ACGIH[c] (TLV)	1999	5 (A3)	TWA

[a] From Finnish Ministry of Social Affairs and Health (1998); National Library of Medicine (1998); Deutsche Forschungsgemeinschaft (1999); Occupational Safety and Health Standards (1999); UNEP (1999)
[b] Abbreviations: TWA, time-weighted average; STEL, short-term exposure limit; A3, animal carcinogen; C, suspected of being a carcinogen; Ca, potential occupational carcinogen; PEL, permissible exposure limit; REL, recommended exposure limit; sk, skin designation; TLV, threshold limit value
[c] The following countries follow the exposure limits suggested by the ACGIH: Bulgaria, Colombia, Jordan, New Zealand, Republic of Korea, Singapore and Viet Nam

In the United States, there is a voluntary industry standard that states that pacifiers, rattles and teethers shall not intentionally contain di(2-ethylhexyl) phthalate [ASTM F 963-96a] (American Society of Testing and Materials, 1997).

The European Union has temporarily banned the use of six phthalates, including di(2-ethylhexyl) phthalate, in toys and other articles intended for children aged under three years of age and designed to be put in the mouth. Several countries in Europe also have proposed, or are considering, restrictions on use of phthalates as plasticizers (softeners) in PVC toys and baby care items (Anon., 1999).

2. Studies of Cancer in Humans

Cohort study

Occupational exposure

The mortality of 221 workers in a di(2-ethylhexyl) phthalate production plant in Germany was followed between 1940 and 1976. Most subjects (135/221) were hired after 1965 and the process was completely enclosed in 1966. No information on level of exposure was provided. Information on vital status for foreigners [number not stated] was obtained for only 55% of them, but appeared to be complete for the remaining cohort. Reference rates were obtained from local populations (the city of Ludwigshafen, the Rheinhessen-Pfalz *land*) and national rates. Altogether, eight deaths

occurred during the follow-up period versus 15.9 expected using local rates [standardized mortality ratio, 0.50; 95% confidence interval, 0.22–0.99] and 17.0 expected using national rates. One death from pancreatic cancer (0.13 expected) and one from bladder papilloma (0.01 expected) occurred among workers with a long exposure time (≥ 20 years). No further report on a longer follow-up for this cohort was available to the Working Group (Thiess *et al*., 1978). [The Working Group noted that the majority of the cohort members were employed after exposure levels had been considerably reduced, and that the methods for this study were poorly described.]

Dialysis patients

Long-term dialysis patients are likely to experience elevated exposures to di(2-ethylhexyl) phthalate, through frequent and protracted exposure to substances leached from surgical tubing during dialysis (see Section 1.4.3(*e*)).

Cancer risk among dialysis patients has been specifically studied because of concern about medical conditions (for example, immunodeficiency) or incidental exposures from treatment (viruses, drugs) (Inamoto *et al.*, 1991). However, exposure to di(2-ethylhexyl) phthalate resulting from treatment has not been studied as such in relation to cancer risk. Due to the medical condition of this population, follow-up is usually very short and incompatible with induction of chemical carcinogenesis. In conclusion, the Working Group was not aware of any study of dialysis patients for which study methods were suitable for the evaluation of carcinogenic risk associated with di(2-ethylhexyl) phthalate.

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 3000 or 6000 mg/kg diet (ppm) di(2-ethylhexyl) phthalate (> 99% pure) for 103 weeks. All surviving mice were killed at 104–105 weeks. There was a clear dose-related decrease in body weight gain in females. Survival at the end of the study was more than 60% in males and more than 50% in females. High-dose males had a slightly decreased body weight gain. In male mice, significant increases in the incidence of hepatocellular carcinomas were observed (control, 9/50; low-dose, 14/48; high-dose, 19/50; $p = 0.022$, Fisher's exact test). The Cochran–Armitage test also indicated a significant trend ($p = 0.018$). The incidence of hepatocellular adenomas and carcinomas combined was also increased in males (control, 14/50; low-dose, 25/48, $p = 0.013$; high-dose, 29/50, $p = 0.002$, Fisher's exact test). In females, significant increases in the incidence of hepatocellular carcinomas were seen (control, 0/50; low-dose, 7/50, $p = 0.006$;

high-dose, 17/50, $p < 0.001$, Fisher's exact test) and of hepatocellular adenoma and carcinoma combined (control, 1/50; low-dose, 12/50; high-dose, 18/50, $p < 0.001$, trend and Fisher's exact tests) (Kluwe *et al.*,1982; National Toxicology Program, 1982; Kluwe *et al.*, 1983, 1985).

Groups of 65 male and 65 female B6C3F$_1$ mice, six weeks of age, were fed diets containing 0, 100, 500, 1500 or 6000 ppm di(2-ethylhexyl) phthalate (purity, 99.7%) for 104 weeks. Another group of 65 male and 65 female mice received the highest concentration for 78 weeks and then control diet for a further 26 weeks (recovery group). Ten of these animals per group and sex were killed at week 105 for biochemical analyses of peroxisome proliferation. Two subgroups of 15 additional mice in the 0 and 6000 ppm groups were designated for measurement of cell proliferation, biochemical analysis for peroxisome proliferation and histopathological evaluation and were killed at week 79. These additional animals were included in the final analysis. All surviving mice were killed at 105 weeks for histopathological examination. There was a clear dose-related decrease in body weight gain in females. The survival of male mice treated with 6000 ppm was significantly lower than that of the controls; about 80% of the untreated controls and treated animals in the other groups survived. In females, more than 60% of the animals survived until the end of the study. Hepatocellular tumour incidences in males were: 8/70 (control), 14/60 (100 ppm), 21/65 (500 ppm), 27/65 (1500 ppm), 37/70 (6000 ppm) and 15/55 (recovery group). All but the 100 ppm group differed significantly ($p < 0.05$, Fisher's exact test) from the control group. The respective incidences in females were 3/70, 4/60, 7/65, 19/65, 44/70 and 30/55, with the 1500-ppm, 6000-ppm and recovery groups differing significantly from the concurrent control group ($p < 0.05$, Fisher's exact test) (David *et al.*, 1999)

3.1.2 *Rat*

Groups of 50 male and 50 female Fischer 344 rats, five to six weeks of age, were fed diets containing 6000 or 12 000 mg/kg diet (ppm) di(2-ethylhexyl) phthalate (> 99% pure) for 103 weeks. All surviving rats were killed at 104–105 weeks. Other groups of 50 males and 50 females served as controls. There was a dose-related decrease in body weight gain in both sexes but no effect on survival. More than 60% of the animals survived to the end of the study. High-dose male rats had significant increases ($p = 0.01$, Fisher's exact test) in the combined incidence of hepatocellular carcinomas and neoplastic nodules (control, 3/50; low-dose, 6/49; high-dose, 12/49). The Cochran–Armitage test also indicated a significant trend ($p = 0.007$). [The Working Group noted that the term neoplastic nodule is now generally assumed to represent hepatocellular adenomas.] The incidence of hepatocellular carcinomas alone or neoplastic nodules alone was not significantly increased. In female rats, the incidence of hepatocellular carcinomas was increased in high-dose rats (8/50; $p = 0.003$, Fisher's exact test) compared with controls (0/50) and that of neoplastic nodules was also

increased in high-dose females (5/50; $p < 0.028$) compared with controls (0/50). The incidence of hepatocellular carcinomas and neoplastic nodules combined was also increased in low-dose (6/49; $p = 0.012$) and high-dose (13/50; $p < 0.001$) females compared with controls (0/50) (Kluwe *et al*., 1982; National Toxicology Program, 1982; Kluwe *et al*., 1983, 1985).

Groups of 65 male and 65 female Fischer 344 rats, six weeks of age, were fed diets containing 0, 100, 500, 2500 or 12 500 ppm di(2-ethylhexyl) phthalate (purity, 99.7%) for up to 104 weeks. Another group of 65 male and 65 female rats received the highest concentration for 78 weeks and then control diet for a further 26 weeks (recovery group). Ten of these animals per group and sex were killed at week 105 for biochemical analyses of peroxisome proliferation. Fifteen additional rats in the 0-, 2500- and 12 500-ppm groups were designated for measurement of cell proliferation, biochemical analyses for peroxisome proliferation and histopathological evaluation and were killed at 79 weeks, and these animals were included in the final tumour analyses. About 65% of males and females survived until the end of the study. All surviving rats were killed at 105 weeks for histopathological examination. For group comparison, the Fisher's exact test was used. Hepatocellular tumour incidences in males were: 5/80 (control), 5/50 (100 ppm), 4/55 (500 ppm), 11/65 (2500 ppm, $p < 0.05$), 34/80 (12 500 ppm, $p < 0.05$) and 18/55 (recovery group, $p < 0.05$). The respective incidences in females were 0/80, 4/50 ($p < 0.05$), 1/55, 3/65, 22/80 ($p < 0.05$) and 10/55 ($p < 0.05$) (David *et al*., 1999).

Several studies using smaller numbers of animals have also been reported, some of which were not designed for carcinogenicity testing. These studies are reviewed below.

Groups of 20 female Fischer 344 rats, eight weeks of age, were fed a diet containing 0 (control), 0.03, 0.10 or 1.2% di(2-ethylhexyl) phthalate [purity not specified] for two years. Neoplastic nodules or hepatocellular carcinomas were seen in 0/18 control, 1/18 low-dose, 1/19 mid-dose and 6/20 high-dose rats ($p < 0.01$). Di(2-ethylhexyl) phthalate did not induce foci of altered hepatocytes as judged by basophilia, ATPase-deficiency or glucose-6-phosphatase-deficiency (Cattley *et al*., 1987).

Groups of 10 male Fischer 344 rats, six weeks of age, were fed a diet containing 2% di(2-ethylhexyl) phthalate (purity, 98%) for 95 weeks. Neoplastic nodules and/or hepatocellular carcinomas were found in 0/18 controls and 6/10 rats fed di(2-ethylhexyl) phthalate ($p < 0.005$). Both the neoplastic nodules and hepatocellular carcinomas were negative for γ-glutamyltranspeptidase (γ-GT) (Rao *et al*., 1987).

Groups of 10–14 male Fischer 344 rats were fed a diet containing 0 (control) or 2% di(2-ethylhexyl) phthalate (98% pure) for 108 weeks. All liver lobes were sliced at 1–2-mm intervals and the number and size of grossly visible lesions recorded. Neoplastic nodules and/or hepatocellular carcinomas were observed in 1/10 controls and 11/14 treated rats [no statistical analysis given] (Rao *et al*., 1990). [The authors suggest that their finding of a high incidence of liver tumours was due to their gross slicing technique, although they do not report a comparison of their findings with conventional liver trimming and sectioning techniques].

A total of 520 male Sprague-Dawley rats (180 g) were fed diets containing 0, 0.02, 0.2 or 2% (200, 2000 or 20 000 ppm) di(2-ethylhexyl) phthalate (purity, > 99%) for 102 weeks [group sizes not specified]. There was a significant, dose-dependent decrease in body weights in the mid- and high-dose groups, but not in the low-dose animals. Data on survival were not reported. For biochemical assays and histopathological examinations, 7–18 rats were killed at weeks 24, 48, 72 and 96. The number of animals at terminal sacrifice was not given. No hyperplastic nodules or hepatocellular carcinomas were reported in either the control or treated animals (Ganning *et al*., 1991) [The Working Group noted the inadequate reporting.]

As part of a larger experiment for studying the characteristics of hepatocarcinogenesis, 17 male Fischer 344 rats were fed a diet containing 2% (20 000 ppm) di(2-ethylhexyl) phthalate [purity unspecified] for up to 78 weeks. A group of 18 untreated animals served as controls. At 52 weeks, no liver tumours had developed in 10 di(2-ethylhexyl) phthalate-treated rats or in 10 controls, while at 78 weeks, hepatocellular carcinomas or neoplastic nodules were found in 3/7 di(2-ethylhexyl) phthalate-treated rats and 0/8 controls (Hayashi *et al*., 1994).

3.2 Inhalation exposure

Hamster: Groups of 65–80 male or female Syrian golden hamsters, 12 weeks of age, were exposed by whole-body inhalation to 15 μg/m^3 di(2-ethylhexyl) phthalate vapour (> 99% pure) for 24 h per day on five days per week until natural death. Total exposure over the lifetime was 7–10 mg/kg bw. No difference in survival was seen between controls and the di(2-ethylhexyl) phthalate-treated groups. Median survival in controls was 709 days in males and 507 days in females and that in treated animals was 703 days in males and 522 days in females. Tumour incidence was not increased in di(2-ethylhexyl) phthalate-treated hamsters (Schmezer *et al*., 1988). [The Working Group noted the low concentration and that it was selected to simulate occupational exposure.]

3.3 Intraperitoneal administration

Hamster: Groups of 50 male and 50 female Syrian golden hamsters, six weeks of age, were administered 0 (control) or 3 g/kg bw di(2-ethylhexyl) phthalate (> 99% pure) by intraperitoneal injection once per week, once per two weeks or once per month (total doses, 24–54 g/kg bw). Hamsters were maintained for their natural lifespan. There were no differences in survival between groups (range of median survival being 629–686 days for males and 465–495 days for females). The incidence of tumours was not increased by treatment (Schmezer *et al*., 1988). [The Working Group noted limitations in the dosing schedule.]

3.4 Administration with known carcinogens and modifying agents

There are numerous reports of studies of the initiating or promoting activities of di(2-ethylhexyl) phthalate given in combination with known carcinogens or promoting agents. Selected studies (complex protocols involving multiple promoting agents and special procedures were excluded) are summarized below and in Tables 5 and 6.

3.4.1 *Mouse*

Liver: Male B6C3F_1 mice, four weeks of age, received a single intraperitoneal injection of 80 mg/kg bw *N*-nitrosodiethylamine (NDEA) in tricaprylin. Two weeks later, the mice were fed diets containing 0, 3000, 6000 or 12 000 ppm di(2-ethylhexyl) phthalate for up to six months. Groups of 10 mice were killed at two, four and six months after NDEA treatment. Few hepatocellular foci were seen at two, four or six months in mice treated with NDEA alone or di(2-ethylhexyl) phthalate alone, while numerous foci and neoplasms were seen in mice given di(2-ethylhexyl) phthalate after NDEA. No tumours were found at six months in mice receiving NDEA alone. By the end of the study, the number of foci per unit volume of liver was similar in mice at all doses of di(2-ethylhexyl) phthalate, but there was an increase in the volume of the foci (0, 1.4, 0.6, 9.4 mm^3 for the control, 3000-, 6000- and 12 000-ppm groups, respectively) (Ward *et al*., 1983).

The differential effects of short- or long-term exposure to di(2-ethylhexyl) phthalate were studied in male B6C3F_1 mice. Mice were given an intraperitoneal injection of 80 mg/kg bw NDEA at four weeks of age. At five weeks of age, the mice were fed diets containing 3000 ppm di(2-ethylhexyl) phthalate for periods of one, seven, 28, 84 or 168 days and were killed at 168 days. When di(2-ethylhexyl) phthalate was fed after NDEA treatment for 28 or more days, there was an increase in incidences of hepatocellular foci (45, 50, 67%) and adenomas (20.6, 17.8 and 46.6%) compared with those in mice receiving NDEA alone (foci, 20%; adenomas, 6.6%). There was also an increase in lesion number and size (Ward *et al*., 1984).

Male B6C3F_1 mice, four weeks of age, received a single intraperitoneal injection of 80 mg/kg bw NDEA in tricaprylin and, two weeks later, were fed diets containing 0, 3000, 6000 or 12 000 ppm di(2-ethylhexyl) phthalate (purity, 99%) for up to 18 months. Groups of 10–20 mice were killed at two, four, six or 18 months. The numbers of mice with hepatocellular foci, adenomas and carcinomas were determined. All doses of di(2-ethylhexyl) phthalate increased the numbers of all lesions at the time periods studied compared with mice receiving NDEA alone. At 18 months, carcinomas were found in 3/10 mice treated with NDEA alone and in 10/10 and 18/20 mice treated with NDEA + di(2-ethylhexyl) phthalate at the low and mid doses, respectively. All mice given 12 000 ppm di(2-ethylhexyl) phthalate with NDEA initiation died by nine months and 11/20 had liver carcinomas. In mice treated with di(2-ethylhexyl) phthalate alone, 2/30 had liver carcinomas (Ward *et al.*, 1986).

Table 5. Selected promotion studies on di(2-ethylhexyl) phthalate (DEHP) with known carcinogens and modifying factors

Tumour type Species/strain (sex)	Known carcinogen (initiator)	Route of administration	Interval between initiator and promoter	Dose and duration of DEHP	Route of administration	Promoting activity for DEHP	Reference
Liver							
$B6C3F_1$ mice (M)	80 mg/kg bw NDEA	i.p.	2 weeks	3000 mg/kg diet/6 months	Oral	+	Ward *et al.* (1983)
$B6C3F_1$ mice (M)	80 mg/kg bw NDEA	i.p.	2 weeks	6000 mg/kg diet/6 months	Oral	+	Ward *et al.* (1983)
$B6C3F_1$ mice (M)	80 mg/kg bw NDEA	i.p.	2 weeks	12 000 mg/kg diet/6 months	Oral	+	Ward *et al.* (1983)
$B6C3F_1$ mice (M)	80 mg/kg bw NDEA	i.p.	1 week	3000 mg/kg diet/28 days	Oral	+	Ward *et al.* (1984)
$B6C3F_1$ mice (M)	80 mg/kg bw NDEA	i.p.	1 week	3000 mg/kg diet/84 days	Oral	+	Ward *et al.* (1984)
$B6C3F_1$ mice (M)	80 mg/kg bw NDEA	i.p.	1 week	3000 mg/kg diet/168 days	Oral	+	Ward *et al.* (1984)
$B6C3F_1$ mice (M)	80 mg/kg bw NDEA	i.p.	2 weeks	3000 mg/kg diet/18 months	Oral	+	Ward *et al.* (1986)
$B6C3F_1$ mice (M)	80 mg/kg bw NDEA	i.p.	2 weeks	6000 mg/kg diet/18 months	Oral	+	Ward *et al.* (1986)
$B6C3F_1$ mice (M)	80 mg/kg bw NDEA	i.p.	2 weeks	12 000 mg/kg diet/18 months	Oral	+	Ward *et al.* (1986)
C3H/HeNCr mice (M,F)	5 mg/kg bw NDEA	i.p.	2 weeks	12 000 mg/kg diet/24 weeks	Oral	+	Weghorst *et al.* (1993/94)
Fischer 344 rats (M)	150 mg/kg bw NDEA	i.p.	3 weeks	12 000 mg/kg diet/6 months	Oral	–	Popp *et al.* (1985)
Fischer 344 rats (M)	282 mg/kg bw NDEA	i.p.	2 weeks	12 000 mg/kg diet/14 weeks	Oral	–	Ward *et al.* (1986)
Fischer 344 rats (M)	200 mg/kg diet AAF 7 weeks	Oral	4 weeks	12 000 mg/kg diet/31 weeks	Oral	–	Williams *et al.* (1987)
Fischer 344 rats (M)	PH/200 mg/kg NDEA	i.p.	2 weeks	3000 mg/kg diet/6 weeks	Oral	–	Ito *et al.* (1988)
Sprague-Dawley rats (F)	8 mg/kg NDEA	i.g.	1 week	10 mg/kg 3 × weekly/ 11 weeks	i.g.	–	Oesterle & Deml. (1988)
Sprague-Dawley rats (F)	8 mg/kg NDEA	i.g.	1 week	100 mg/kg 3 × weekly/ 11 weeks	i.g.	–	Oesterle & Deml (1988)
Sprague-Dawley rats (F)	8 mg/kg NDEA	i.g.	1 week	200 mg/kg 3 × weekly/ 11 weeks	i.g.	+	Oesterle & Deml (1988)
Sprague-Dawley rats (F)	8 mg/kg NDEA	i.g.	1 week	500 mg/kg 3 × weekly/ 11 weeks	i.g.	+	Oesterle & Deml (1988)
Fischer 344 rats (M)	200 ppm AAF 7 weeks	Oral	4 weeks	12 000 ppm /24 weeks	Oral	–	Maruyama *et al.* (1990)

Table 5 (contd)

Tumour type Species/strain (sex)	Known carcinogen (initiator)	Route of adminis-tration	Interval between initiator and promoter	Dose and duration of DEHP	Route of adminis-tration	Promoting activity for DEHP	Reference
Kidney							
Fischer 344 rats (M)	500 mg/kg diet EHEN/2 weeks	Oral	0 weeks	12 000 ppm/24 weeks	Oral	+	Kurokawa *et al*. (1988)
Urinary bladder							
Fischer 344 rats (M)	5000 ppm BBN weeks 1–4; 30 000 ppm uracil weeks 8–11	Oral	0 weeks	3000 ppm/16 weeks	Oral	–	Hagiwara *et al*. (1990)

AAF, 2-acetylaminofluorene; BBN, *N*-butyl-*N*-(4-hydroxybutyl)nitrosamine; EHEN, *N*-ethyl-*N*-hydroxyethylnitrosamine; NDEA, *N*-nitrosodiethylamine; F, female; M, male; i.p., intraperitoneal injection; i.g., intragastric; PH, partial hepatectomy

Table 6. Initiation studies on di(2-ethylhexyl) phthalate (DEHP) with promoting agents

Species/strain	DEHP initiation	Route of administration	Interval between initiation and promotion	Dose and duration of promoter	Route of administration	Initiating activity of DEHP	Reference
Mouse B6C3F$_1$ (M)	25 or 50 g/kg bw	Gavage	2 weeks	500 mg/L phenobarbital 6 or 18 months	Drinking-water	– –	Ward *et al.* (1986)
Fischer 344 rats (F)	10 g/kg bw DEHP 6, 12, 24 h after PH	Oral	2 weeks	200 ppm AAF 2 weeks 1.5 mL/kg CCl_4 once	Diet Gavage		Garvey *et al.* (1987)
Fischer 344 rats (F)	12 000 mg/kg diet DEHP 12 weeks	Oral	2 weeks	500 mg/kg diet phenobarbital, 39 weeks	Diet	–	Garvey *et al.* (1987)

PH, partial hepatectomy; AAF, 2-acetylaminofluorene; CCl_4, carbon tetrachloride

In a study to test di(2-ethylhexyl) phthalate for initiating activity, groups of 7–20 male B6C3F_1 mice, four weeks of age, were given a single intragastric dose of 25 or 50 g/kg bw di(2-ethylhexyl) phthalate (99% pure). Groups of 10–17 controls were used. Two weeks later, phenobarbital was given as a promoting agent at a concentration of 500 mg/L in the drinking-water for six or 18 months. At 18 months, hepatocellular carcinomas were found in 0/7 mice given 50 g/kg di(2-ethylhexyl) phthalate, 2/15 mice given 50 g/kg bw di(2-ethylhexyl) phthalate + phenobarbital, 1/10 mice given 25 g/kg di(2-ethylhexyl) phthalate, 2/20 mice given 25 g/kg di(2-ethylhexyl) phthalate + phenobarbital, 3/17 mice given phenobarbital alone and 0/10 untreated mice [statistical analysis not given]. Thus the study showed no evidence of initiating activity of di(2-ethylhexyl) phthalate (Ward *et al.*, 1986).

Groups of 10 male and five female C3H/HeNCr mice, 15 days of age, received either a single intraperitoneal injection of 5 mg/kg bw NDEA or saline. At weaning (four weeks of age), mice were divided into two groups and fed diets containing either 0 or 12 000 ppm di(2-ethylhexyl) phthalate [purity unspecified] for 24 weeks. All mice were killed at 28 weeks of age and the number and size of hepatic foci were measured. Di(2-ethylhexyl) phthalate in combination with NDEA increased the average numbers of foci per liver (NDEA-treated males, 176; NDEA + di(2-ethylhexyl) phthalate-treated males, 366; NDEA-treated females, 47; NDEA + di(2-ethylhexyl) phthalate-treated females, 169). The numbers of adenomas per liver were also increased (NDEA-treated males, 7; NDEA + di(2-ethylhexyl) phthalate-treated males, 15.8; NDEA-treated females, 0; NDEA + di(2-ethylhexyl) phthalate-treated females, 2). In male mice, treatment with NDEA + di(2-ethylhexyl) phthalate yielded larger adenomas than those seen in mice treated with NDEA alone (2.4 mm^3 versus 1.3 mm^3) (Weghorst *et al.*, 1993/94).

Skin: Groups of 25 female SENCAR mice, seven weeks of age, received a single application of 20 μg 7,12-dimethylbenz[*a*]anthracene (DMBA) in 0.2 mL acetone on the skin of the back. One week later, mice received applications of 100 mg per animal di(2-ethylhexyl) phthalate (99% pure) twice weekly for 28 weeks. 12-*O*-Tetradecanoylphorbol 13-acetate (TPA) control groups received 2 μg TPA. To test di(2-ethylhexyl) phthalate as a second-stage promoter, mice received TPA for two weeks followed by di(2-ethylhexyl) phthalate for 26 weeks. Appropriate acetone, TPA and di(2-ethylhexyl) phthalate controls were included. Di(2-ethylhexyl) phthalate, when tested as a complete promoter (28 weeks of exposure), enhanced only slightly the numbers of papillomas (0.88 per mouse versus DMBA alone 0 per mouse) but significantly ($p < 0.01$) enhanced papillomas when given for 26 weeks after two weeks of TPA first-stage promotion (6.44 versus 2.2). The authors concluded that di(2-ethylhexyl) phthalate was a second-stage promoter (Diwan *et al.*, 1985; Ward *et al.*, 1986).

3.4.2 *Rat*

Liver: Groups of 10 female Fischer 344 rats, six to eight weeks of age, received a single intraperitoneal injection of 150 mg/kg bw NDEA followed three weeks later by

a diet containing 1.2% di(2-ethylhexyl) phthalate (99.5% pure) for three or six months. No neoplasms or nodules were identified. Di(2-ethylhexyl) phthalate did not increase the number of foci or the mean volume of the foci, as identified by five different histological markers (Popp *et al*.,1985).

The initiating activity of di(2-ethylhexyl) phthalate was examined after single and sub-chronic dosing. Di(2-ethylhexyl) phthalate (99.5% pure) was administered as a single oral dose (10 g/kg bw) or by 12 weeks of feeding at a concentration of 1.2% in the diet followed by various known promotion regimens, such as phenobarbital treatment or partial hepatectomy. There was no increase in number or mean volume of foci in liver sections examined using multiple histological markers and no tumours were identified, indicating that di(2-ethylhexyl) phthalate had no initiating activity (Garvey *et al*., 1987).

Groups of 18–20 male Fischer rats (weighing 160 g) were given a single intraperitoneal injection of 200 mg/kg bw NDEA. Two weeks later, they were fed a diet containing 3000 ppm di(2-ethylhexyl) phthalate [purity unspecified] for six weeks. At week 3, they were subjected to a partial hepatectomy. All rats were killed at week 8. Di(2-ethylhexyl) phthalate-treated rats had no increase in foci staining positively for glutathione *S*-transferase placental form (8.5 per cm^2 versus 11.6 for NDEA alone) (Ito *et al*., 1988).

Male Fischer 344 rats were fed 200 ppm 2-acetylaminofluorene (AAF) for seven weeks to induce hepatocellular altered foci, and were subsequently fed 0 or 12 000 ppm di(2-ethylhexyl) phthalate (98% pure) in the diet. No evidence of induction of hepatocellular altered foci or hepatic neoplasms was found when di(2-ethylhexyl) phthalate was given alone for 24 weeks. Di(2-ethylhexyl) phthalate fed for 24 weeks increased basophilic foci, but showed no promoting effect on iron-excluding altered hepatic foci induced by AAF, and produced no significant enhancement of the occurrence of AAF-induced liver neoplasms (3/6 compared with 3/12) (Williams *et al*., 1987).

Di(2-ethylhexyl) phthalate exerted weak promoting activity in weanling female Sprague-Dawley rats after doses of 200 or 500 mg/kg bw, given three times per week by gavage for 11 consecutive weeks after initiation with a single oral dose of 8 mg/kg bw NDEA. Lower doses were ineffective. The incidence of ATPase-deficient foci was enhanced about two-fold compared with rats treated with NDEA alone. The incidence of foci with expression of γ-GT was not affected by di(2-ethylhexyl) phthalate treatment (Oesterle & Deml, 1988).

Male Fischer 344 rats were fed diets containing 200 ppm AAF for seven weeks to induce hepatocellular altered foci, and were then fed diets containing either 0 or 12 000 ppm di(2-ethylhexyl) phthalate (purity, 98%) for 24 weeks. In foci that were induced by AAF, di(2-ethylhexyl) phthalate reduced the activity of γ-GT, as detected histochemically, but did not increase the number, mean volume or volume percentage of foci detected by deficiencies in iron storage, glucose-6-phosphatase, adenosine triphosphatase or fibronectin. Although the numbers of haematoxylin/eosin-stained foci were increased in di(2-ethylhexyl) phthalate-treated rats, the volume percentage was not

increased and no difference in the numbers of iron storage foci was seen (Maruyama *et al.*, 1990).

Urinary system: Groups of 20 male Fischer 344 rats were given 0.05% *N*-ethyl-*N*-hydroxyethylnitrosamine (EHEN) for two weeks in the diet followed by di(2-ethylhexyl) phthalate [purity unspecified] at a concentration of 0 or 1.2% in the diet for 24 weeks. Rats were killed at 27 weeks. Di(2-ethylhexyl) phthalate increased the numbers of rats with renal (tubular) cell tumours (EHEN + di(2-ethylhexyl) phthalate 65% versus 20% for EHEN alone; $p < 0.01$) and the mean number of tumours per kidney (EHEN + di(2-ethylhexyl) phthalate 1.1 versus EHEN alone 0.2, $p < 0.01$) (Kurokawa *et al.*, 1988).

The modifying potential of di(2-ethylhexyl) phthalate on second-stage *N*-butyl-*N*-(4-hydroxybutyl)nitrosamine (BBN)-initiated urinary bladder carcinogenesis was investigated in male Fischer 344 rats, using a uracil-accelerated transitional-cell proliferation model. Six-week-old animals received 0.05% BBN in their drinking-water for four weeks followed by di(2-ethylhexyl) phthalate [purity unspecified] (0, 0.3, 0.6 or 1.2% in the diet) for experimental weeks 5–8 and weeks 12–20. Uracil was administered during weeks 9–11 at a dietary level of 3.0%. Surviving animals were killed at the end of week 20 of the experiment. Di(2-ethylhexyl) phthalate did not promote hyperplastic lesions (papillary or nodular) of the urinary bladder or papillomas induced by BBN (Hagiwara *et al.*, 1990).

3.4.3 *Hamster*

Groups of 50 male and 50 female Syrian golden hamsters, six weeks old, were given intraperitoneal injections of 3 g/kg bw di(2-ethylhexyl) phthalate (> 99% pure) either once, or once per week for two or four weeks. *N*-Nitrosodimethylamine (NDMA) was given orally at 1.67 mg/kg bw once [exact week of dosing for both chemicals not given]. Hamsters were maintained for their natural lifespan. Survival was reduced among hamsters receiving NDMA. Di(2-ethylhexyl) phthalate did not affect tumour yield (liver tumours: 16/50 and 9/50 in di(2-ethylhexyl) phthalate + NDMA and NDMA males; and 6/50 and 6/50 in di(2-ethylhexyl) phthalate + NDMA and NDMA females) [statistical analysis not given] (Schmezer *et al.*, 1988).

3.5 Carcinogenicity of the metabolite 2-ethylhexanol

3.5.1 *Mouse*

Groups of 50 male and 50 female B6C3F_1 mice, seven weeks of age, were given 2-ethylhexanol by gavage five times weekly at doses of 0, 50, 200 and 250 mg/kg bw for 18 months. Body weight gain was reduced by 24–26% in the high-dose group and mortality was dose-related. In females, liver carcinomas occurred in 0/50 control, 1/50 low-dose, 3/50 mid-dose and 5/50 ($p < 0.05$ Fisher's exact test) high-dose mice (Astill *et al.*, 1996).

3.5.2 *Rat*

Groups of 50 male and 50 female Fischer 344 rats, six weeks of age, were given 2-ethylhexanol by gavage, five times weekly, at doses of 0, 50, 150 or 500 mg/kg bw for 104 weeks. A dose-related depression of body weight gain in male and female rats and increased mortality in high-dose female rats were observed. There was no increase in the incidence of tumours in any treated group (Astill *et al.*, 1996).

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

4.1 Absorption, distribution, metabolism and excretion

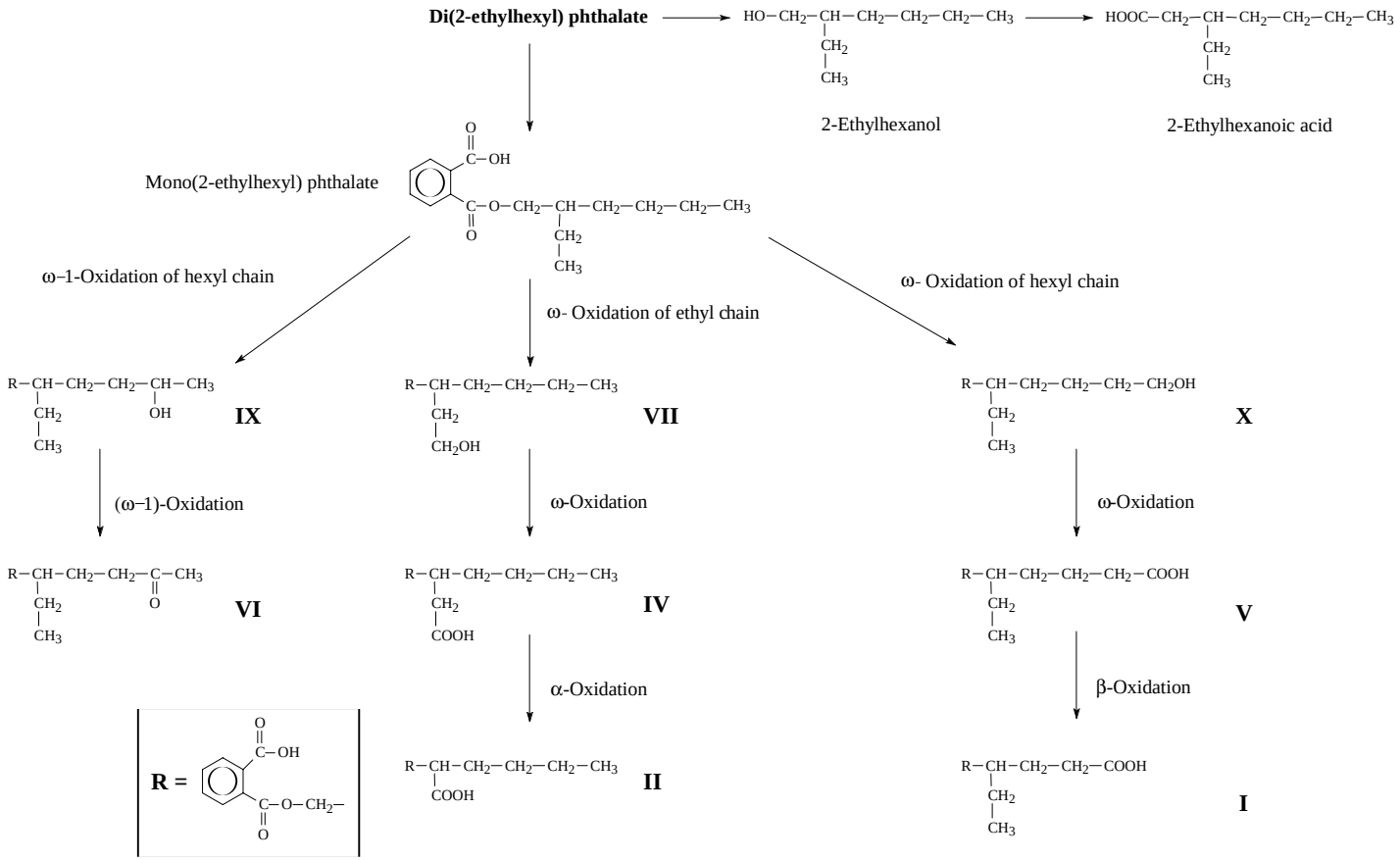
4.1.1 *Humans*

Human exposure to di(2-ethylhexyl) phthalate can occur via the dermal, inhalation, oral and intravenous routes. The high level of exposure has prompted many studies on the absorption, distribution, metabolism and excretion of di(2-ethylhexyl) phthalate in humans (Lawrence & Tuell, 1979; Thomas & Thomas, 1984; Burg, 1988; Albro & Lavenhar, 1989; Kamrin & Mayor, 1991; Huber *et al*., 1996; Doull *et al*., 1999).

In a very early study, Shaffer *et al*. (1945) administered single oral doses of 5 and 10 g di(2-ethylhexyl) phthalate to two human subjects and reported that approximately 4.5% of the dose was excreted in the urine within 24 h. Schmid and Schlatter (1985) also administered di(2-ethylhexyl) phthalate orally to two human subjects, but at the much lower dose of 30 mg per person. These authors reported that 11–15% of the dose was excreted in the urine and a urinary elimination half-life of about 12 h can be estimated from the data. In the same study, the two volunteers also received 10 mg di(2-ethylhexyl) phthalate daily for four days, there being no evidence of accumulation, with 11 and 33 % of the dose recovered each day in the urine. In contrast, Rubin and Schiffer (1976) reported data from two patients receiving platelet transfusions from bags containing di(2-ethylhexyl) phthalate, who excreted between 60 and 90% of the infused dose in the urine collected for 24 h after transfusion.

Figure 1 summarizes the metabolic pathways responsible for the metabolism of di-(2-ethylhexyl) phthalate in humans and in animals, using the widely accepted metabolite nomenclature of Albro and Lavenhar (1989). The quantitative urinary metabolic profile of di(2-ethylhexyl) phthalate reported by Schmid and Schlatter (1985) is quite similar to that found by Albro *et al*. (1982), who determined the urinary metabolites of di(2-ethylhexyl) phthalate in leukaemia patients who received platelet transfusions from bags containing di(2-ethylhexyl) phthalate. These data are also in good agreement with the results of Dirven *et al*. (1993a), who studied the excretion of di(2-ethylhexyl) phthalate metabolites in five workers occupationally exposed to di(2-ethylhexyl) phthalate. The major phase I metabolites of di(2-ethylhexyl) phthalate in human urine are mono(2-

Figure 1. Major pathways involved in the metabolism of di(2-ethylhexyl) phthalate



The numbering of the metabolites is based on the nomenclature of Albro and Lavenhar (1989). This chart is based on the work of Huber *et al.* (1996) and Astill (1989). I, phthalic acid, mono(4-carboxy-2-ethylhexyl) ester; II, phthalic acid, mono(2-carboxyhexyl) ester; IV, phthalic acid, mono(2-(carboxymethyl)hexyl) ester; V, phthalic acid, mono(5-carboxy-2-ethylpentyl) ester; VI, phthalic acid, mono(2-ethyl-5-oxohexyl) ester; VII, phthalic acid, mono(2-(2-hydroxyethyl)hexyl) ester; IX, phthalic acid, mono(2-ethyl-5-hydroxyhexyl) ester; X, phthalic acid, mono(2-ethyl-6-hydroxyhexyl) ester

ethylhexyl) phthalate, and compounds V, VI and IX (Figure 1; Albro *et al*., 1982; Schmid & Schlatter, 1985, Dirven *et al*., 1993b). Dirven *et al*. (1993b) found that the urinary concentration of metabolites VI and IX was 1.7 times higher than the concentration of metabolite V, and concluded that metabolism via (ω–1)-hydroxylation is favoured over ω-hydroxylation in humans. Metabolites VI and IX were reported by Dirven *et al*. (1993b) to be almost completely conjugated, while only 32–45% of metabolite V was present in the conjugated form. The same authors found a marked interindividual variation in the conjugation of mono(2-ethylhexyl) phthalate. In two of the five subjects studied, 77–100% of mono(2-ethylhexyl) phthalate was in the free form, whereas in urine samples from the other three subjects, only 20–38% of mono(2-ethylhexyl) phthalate was not conjugated. Both Albro *et al*. (1982) and Schmidt and Schlatter (1985) reported that up to 80% of the urinary metabolites of di(2-ethylhexyl) phthalate in humans were excreted as glucuronide conjugates. Conjugates of di(2-ethylhexyl) phthalate metabolites with sulfate, taurine and glycine have not been found in any species studied to date.

Rubin and Schiffer (1976) reported peak blood plasma levels of di(2-ethylhexyl) phthalate in adult patients transfused with platelets ranging from 3 to 8 mg/L and described the kinetics of plasma disappearance as mono-exponential, with a half-life of 28 min. The plasma levels ranged from 0.3 to 1.2 mg/kg bw di(2-ethylhexyl) phthalate. This is a low level compared with di(2-ethylhexyl) phthalate levels in whole blood stored in blood bags for up to 21 days (Jaeger & Rubin, 1972; Rock *et al*., 1978; Peck *et al*., 1979), from which a transfusion of 2.5 L of blood to a 70-kg person would give an exposure of 1.3–2.6 mg/kg bw di(2-ethylhexyl) phthalate. Similar levels of exposure (1.7 to 4.2 mg/kg bw) have been reported in newborn infants during single exchange transfusions (Sjöberg *et al*., 1985a).

Lewis *et al*. (1978) determined the disappearance of di(2-ethylhexyl) phthalate in seven adult patients following dialysis. The mean serum levels were 606 μg/L immediately after dialysis, 323 μg/L at 30 min, 167 μg/L at 1 h and 145 μg/L at 3 h after completion of dialysis. They concluded that most of the di(2-ethylhexyl) phthalate present in serum at the completion of dialysis is likely to disappear within 5–7 h. Sjöberg *et al*. (1985b) and Plonait *et al*. (1993) also described a rapid decline in serum di(2-ethylhexyl) phthalate levels in term newborns similar to that seen in adults. Sjöberg *et al*. (1985b) reported that plasma levels of di(2-ethylhexyl) phthalate declined biexponentially after transfusions in newborn infants, with the half-life of the terminal phase being approximately 10 h. The terminal half-life of the active metabolite of di(2-ethylhexyl) phthalate, mono(2-ethylhexyl) phthalate, in newborn infants was similar (Sjöberg *et al*., 1985b). However, in preterm infants, di(2-ethylhexyl) phthalate can persist in serum for a much longer time and it is postulated that either there are different kinetics resulting from immature metabolism or there is a biphasic disappearance pattern, as Stern *et al.* (1977) and Sjöberg *et al.* (1985c,d) observed in rats (Plonait *et al.*, 1993). In adult haemodialysis patients, circulating levels of mono(2-ethylhexyl) phthalate are similar to

those of di(2-ethylhexyl) phthalate, showing that this metabolite is formed after systemic intake in humans (Pollack *et al*., 1985). Further, di(2-ethylhexyl) phthalate can be hydrolysed by plasma proteins in blood products to mono(2-ethylhexyl) phthalate. Hence, patients undergoing transfusion may be exposed to mono(2-ethylhexyl) phthalate at doses of up to one tenth those of di(2-ethylhexyl) phthalate (Rock *et al*., 1978).

Absorption of di(2-ethylhexyl) phthalate via the lungs is considered to be the major route of occupational exposure, as dermal absorption through preparations of human skin *in vitro* is low (Scott *et al*., 1987; Dirven *et al*., 1993a; Huber *et al*., 1996). Occupational exposure to di(2-ethylhexyl) phthalate based on personal air sampling data in the range 9–1266 μg/m^3 has been reported (Dirven *et al*., 1993a). Furthermore, Dirven *et al*. (1993a) evaluated the inhalation exposure to di(2-ethylhexyl) phthalate in a group of nine volunteers in a boot factory in an attempt to evaluate the absorption and disposition of the plasticizer. They reported that the median values of three major di(2-ethylhexyl) phthalate metabolites studied (VI, IX and V; see Figure 1) were significantly increased (1.2–2.3-fold) in urine samples collected at the end of the workday compared with urine samples collected at the start of the workday. However, a similar comparison in six cable factory workers detected no statistically significant increase in post-shift urinary di(2-ethylhexyl) phthalate metabolite concentrations compared with pre-shift values, although the airborne exposure levels were within a similar range. As pointed out by the authors, a meaningful interpretation of these data is impeded by the lack of understanding of the toxicokinetics of di(2-ethylhexyl) phthalate metabolites in humans.

4.1.2 *Experimental systems*

The absorption and disposition of di(2-ethylhexyl) phthalate has been extensively investigated in laboratory animals (Thomas & Thomas, 1984; Albro & Lavenhar, 1989; Astill, 1989; Huber *et al*., 1996; Doull *et al*., 1999). The first step in the metabolism of di(2-ethylhexyl) phthalate in all species is the hydrolysis of one of the two ethylhexyl side-chains to yield mono(2-ethylhexyl) phthalate and 2-ethylhexanol. After oral administration of di(2-ethylhexyl) phthalate, this pathway is primarily catalysed by pancreatic lipase. The level of pancreatic lipase in the intestine of laboratory animals has been shown to exhibit marked species and strain differences (Albro & Thomas, 1973). Lower levels of di(2-ethylhexyl) phthalate-hydrolysing enzyme activity are expressed in the liver, blood and other tissues (Albro, 1986; Huber *et al*., 1996). It is generally accepted that in all mammalian species the majority of di(2-ethylhexyl) phthalate reaching the intestine is absorbed as hydrolysis products rather than as the intact diester (Albro & Lavenhar, 1989). For example, Teirlynck and Belpaire (1985) studied the disposition of both di(2-ethylhexyl) phthalate and mono(2-ethylhexyl) phthalate in immature male Wistar rats and reported that, after a single oral dose of di(2-ethylhexyl) phthalate (2.8 g/kg), plasma concentrations of 8.8 ± 1.7 μg/mL di(2-ethylhexyl) phthalate and 63.2 ± 8.7 μg/mL mono(2-ethylhexyl) phthalate were reached

after 3 h. The plasma concentration of mono(2-ethylhexyl) phthalate declined with a half-life of 5.2 h, whereas the corresponding value for di(2-ethylhexyl) phthalate could not be estimated due to very low levels of the parent diester.

The efficiency of di(2-ethylhexyl) phthalate elimination from the gastrointestinal tract differs between species. Ikeda *et al*. (1980) fed 50 mg/kg bw di(2-ethylhexyl) phthalate per day to male Sprague-Dawley rats, male pure-bred beagles and male immature swine of the Hormel strain for 21–28 days and then administered a single dose of ^{14}C-labelled di(2-ethylhexyl) phthalate at 50 mg/kg bw on the last day. In rats, 37.5 and 53.2% of the administered radioactivity were excreted in the urine and faeces, respectively, within four days. In dogs, the figures showed that the faecal route of excretion dominated (75%), with the urinary route accounting for 20.7% of the radioactivity. The opposite was the case in pigs, with the faecal and urinary routes accounting for 25.7% and 79.4%, respectively. In male Wistar rats given a single 2.9 mg/kg bw oral dose of [^{14}C]di(2-ethylhexyl) phthalate, Daniel and Bratt (1974) found 42% and 57% of the dose in the urine and faeces within seven days. In the same study, in rats fed 2.6 mg/kg bw [^{14}C]di(2-ethylhexyl) phthalate, 14% of the dose was excreted in the bile.

Rhodes *et al*. (1986) found that the absorption of di(2-ethylhexyl) phthalate was considerably lower in marmosets than in rats. These authors reported that 45% and 7% of di(2-ethylhexyl) phthalate were absorbed by marmosets at doses of 100 mg/kg bw and 2000 mg/kg bw, respectively. However, Astill (1989) observed that, after a single 100 mg/kg bw gavage dose of di(2-ethylhexyl) [*carbonyl*-^{14}C]phthalate, faecal elimination accounted for approximately 50% of the dose in cynomolgus monkeys, male Fischer 344 rats and male B6C3F$_1$ mice. Urinary excretion levels were also similar across the three species, ranging from 28% in monkeys, 33% in rats to 37% in mice over approximately the first 96 h. According to this study, the excretion of di(2-ethylhexyl) phthalate in rats varied depending on whether it was administered by gavage or in the diet. In African green monkeys, di(2-ethylhexyl) phthalate was rapidly and extensively metabolized, with urinary excretion being 80% in the form of glucuronide conjugates (Albro *et al*., 1981, 1982). Peck and Albro (1982) also showed that di(2-ethylhexyl) phthalate was rapidly and extensively metabolized in both African green monkeys and humans. Di(2-ethylhexyl) phthalate is excreted largely in the urine by both African green monkeys and humans (> 60% in 24 h) as glucuronide conjugates (~80%) of the oxidation products of mono(2-ethylhexyl) phthalate. The predominant hydrolysable urinary metabolites were metabolite IX (26.1%) and mono-(2-ethylhexyl) phthalate (19.6%) and faecal excretion in monkeys accounted for approximately 8% of the administered di(2-ethylhexyl) phthalate 48 h after infusion. In contrast to the extent of conjugation of urinary metabolites in African green monkeys, only 16% of the urinary metabolites of di(2-ethylhexyl) phthalate in cynomolgus monkeys were in the form of hydrolysable products (Astill, 1989). Di(2-ethylhexyl) phthalate excretion in urine and faeces and the subsequent metabolic patterns of di(2-ethylhexyl) phthalate were stated to be quantitatively similar in cynomolgus monkeys, rats and mice.

In making species comparisons, careful interpretation of data is required, as the physical state of the di(2-ethylhexyl) phthalate used can affect the outcome (Thomas & Thomas, 1984; Albro & Lavenhar, 1989; Astill, 1989). For example, the disposition half-life in the rat of [^{14}C]di(2-ethylhexyl) phthalate solubilized in aqueous polysorbate emulsion, in plasma containing polysorbate emulsion, in plasma containing ethanol or in the plasticizer leached from the plastic into the plasma was 263, 83, 181 and 31 min, respectively (Miripol *et al*., 1975, cited in Thomas & Thomas, 1984). Albro and Lavenhar (1989) reported that intact di(2-ethylhexyl) phthalate was not absorbed until the dose reached 500 mg/kg bw in CD-1 mice and 450 mg/kg bw in Fischer 344 rats. In contrast, no threshold was seen in B6C3F$_1$ mice: the amount of di(2-ethylhexyl) phthalate absorbed was directly proportional to the dose down to a dose of 20 mg/kg bw. Further, Astill (1989) reported that prolonged feeding or the use of high doses of up to 1000 mg/kg bw did not influence the absorption of di(2-ethylhexyl) phthalate from the gastrointestinal tract of male Fischer 344 rats. While most of a dose of di(2-ethylhexyl) phthalate is absorbed as mono(2-ethylhexyl) phthalate, more of the parent diester reaches the circulation at high doses (Albro *et al*., 1982).

Because distribution studies have monitored total radioactivity, our understanding of the distribution of intact di(2-ethylhexyl) phthalate is limited. Chu *et al*. (1978) studied the metabolism and distribution of mono(2-ethylhexyl) phthalate in rats after oral dosing and found that the intestine contained the highest tissues levels after 24 h. The liver, heart, lung and muscle each contained approximately half the level in the intestine. They also reported that 80% of the ^{14}C-dose of mono(2-ethylhexyl) phthalate was eliminated 24 h after oral administration, 72% in the urine and 8% in the faeces. Twenty minutes after the administration of an intravenous dose of [^{14}C]mono(2-ethylhexyl) phthalate, Chu *et al*. (1978) found comparable levels in the liver, kidney and bladder, with other organs containing approximately 10–25% of the level of the liver.

Species differences in the metabolism of di(2-ethylhexyl) phthalate have been reported and attempts have been made to explain the susceptibility of animals to di(2-ethylhexyl) phthalate-induced hepatic peroxisome proliferation based on their metabolic profiles (Doull *et al*., 1999). As mentioned above, the bulk of a di(2-ethylhexyl) phthalate dose is absorbed as the mono-ester, mono(2-ethylhexyl) phthalate, and following absorption this metabolite is subjected to extensive oxidative metabolism mediated by cytochrome P450 enzymes (Albro & Lavenhar, 1989; Astill, 1989; Huber *et al*., 1996; Doull *et al*., 1999). The metabolism of mono(2-ethylhexyl) phthalate has been summarized by Doull *et al*. (1999) as follows (see Figure 1):

1. hydroxylation of the terminal carbon atoms (ω-oxidation) of both the hexyl and ethyl side-chains and the penultimate carbon (ω–1-oxidation) of the hexyl chain;
2. conversion of these hydroxyl groups to either a carboxylic acid (ω-oxidation) or a ketone (ω–1-oxidation); and
3. further metabolism (shortening of the carbon chains) of the dicarboxylic acid products of ω-oxidation by α- or β-oxidation.

Mono(2-ethylhexyl) phthalate and metabolites VI and IX are considered to be primarily responsible for the peroxisome-proliferating activity of di(2-ethylhexyl) phthalate (Mitchell *et al*., 1985; Elcombe & Mitchell, 1986; Cornu *et al*., 1992; Keith *et al*., 1992; Elcombe *et al*., 1996; Doull *et al*., 1999). These are the keto and hydroxyl metabolites of mono(2-ethylhexyl) phthalate that are produced via ω–1-oxidation. Mono(2-ethylhexyl) phthalate undergoes extensive metabolism in rats, with approximately 75% of all hydrolysable urinary metabolites consisting of dicarboxylic acids, mainly metabolited V, resulting from two oxidative steps, which accounts for 51.3% of the urinary metabolites of di(2-ethylhexyl) phthalate in rats (Albro *et al*., 1981). African green monkeys, guinea-pigs and humans excrete approximately 5% of a dose of di(2-ethylhexyl) phthalate in the urine as metabolite V, while mice and hamsters excrete 1.1% and 14%, respectively (Albro *et al*., 1981, 1982). Interestingly, two other studies have reported that this metabolite comprises approximately 20–30% of urinary metabolites in humans (Schmid & Schlatter, 1985; Dirven *et al*., 1993b). In another comparative study, Astill (1989) reported that 5.7%, 8.4% and 0.3% of a 100-mg/kg bw single gavage dose of di(2-ethylhexyl) phthalate was excreted in the urine in the first 24 h as metabolite V by cynomolgus monkeys, rats and mice, respectively. In mice, 18.6% of hydrolysable metabolites of di(2-ethylhexyl) phthalate was excreted as mono(2-ethylhexyl) phthalate and this metabolite ranged from 71.2% in guinea-pigs, 28.9% in African green monkeys, 18.3% in humans to 4.5% in hamsters (Albro *et al*., 1981, 1982). In contrast, mono(2-ethylhexyl) phthalate was present only in trace amounts in rat urine. In rats and mice, metabolite IX accounted for 13.3% and 12.3% of hydrolysable urinary metabolites, whereas in African green monkeys, hamsters and humans, this metabolite could account for as much as 30% of urinary metabolites (Albro *et al*., 1982). In contrast, Astill (1989) reported that similar amounts of metabolite IX were excreted in the urine of cynomolgus monkeys, rats and mice. It should be emphasized that the above data for African green monkeys and humans in the studies by Albro and coworkers (Albro *et al*., 1981, 1982) were collected following intravenous administration of di(2-ethylhexyl) phthalate. The study by Dirven *et al*. (1993b) involved occupational exposure of humans via inhalation, whereas all the remaining studies reported findings following a single oral dose. Therefore, the possibility that the route of administration affected the metabolic profile cannot be ruled out.

Dermal absorption of di(2-ethylhexyl) phthalate is slow in rats (Melnick *et al*., 1987; Scott *et al*., 1987; Albro & Lavenhar, 1989; Chu *et al*., 1996; Deisinger *et al*., 1998). Fetal exposure to [^{14}C]di(2-ethylhexyl) phthalate or its metabolites occurs following intraperitoneal administration to pregnant rats (Singh *et al*., 1975). Liver microsomes from fetuses (21 days of gestation) and neonates (one- and five-day-old) *in vitro* are capable of metabolizing mono(2-ethylhexyl) phthalate. Further, liver microsomes from mature animals metabolize mono(2-ethylhexyl) phthalate at a rate similar to those from five-day-old rats, demonstrating rapid postnatal development of mono(2-ethylhexyl) phthalate (ω–1)-hydroxylase activity in rats (Sjöberg *et al*., 1988). In rats given high oral doses of di(2-ethylhexyl) phthalate (2000 mg/kg bw)

during lactation, large amounts of di(2-ethylhexyl) phthalate (e.g., 216 μg/mL 6 h after dosing) and smaller, yet significant amounts of mono(2-ethylhexyl) phthalate (e.g., 25 μg/mL 6 h after dosing) were transported into the milk (Dostal *et al*., 1987a). While occupational exposure via inhalation is considered to be a major route of human exposure to di(2-ethylhexyl) phthalate, there is no information available on the toxicokinetics or metabolic disposition of this compound in animals exposed via this route.

Placental transfer of di(2-ethylhexyl) phthalate has been observed following intraperitoneal administration of di(2-ethylhexyl) [*carbonyl*-^{14}C]phthalate on gestational day 5 or 10 in rats (Singh *et al*., 1975). The dams were killed at 24-h intervals starting on days 8 and 11 until day 20 of gestation. Radioactivity was detected in fetal tissues, amniotic fluid and placenta at all time points. The radioactivity peaked at 48 h and declined rapidly thereafter. The concentration was less than that in maternal blood and less than 1% of the administered dose.

Radiolabelled di(2-ethylhexyl) phthalate (10 mL/kg) was administered by oral gavage to ddY-SLC mice on gestation day 8. Three and 12 h after exposure, levels of di(2-ethylhexyl) phthalate in the fetuses were 522 μg/g and 426 μg/g, respectively. The level of mono(2-ethylhexyl) phthalate in the fetus was approximately 1% that of di(2-ethylhexyl) phthalate (Tomita *et al.*, 1986).

Sjöberg *et al*. (1985d) also studied the kinetics of orally administered di(2-ethylhexyl) phthalate and its excretion in groups of 10 rats 25, 40 or 60 days old. The AUC (area under the plasma curve) for 0–30 h was significantly higher (about two-fold) in the 25-day-old rats than in the older rats; the half-life was, however, not significantly higher in the 25-day-old rats (3.9 h compared with 3.1 h and 2.8 h at 40 and 60 days, respectively). The amount of di(2-ethylhexyl) phthalate-derived products excreted in the urine was twice as high in 25- as in 60-day-old rats. It was concluded that the toxicokinetic differences may in part explain the age-dependent effect of di(2-ethylhexyl) phthalate on the testes (see Section 4.3.2).

4.2 Toxic effects

4.2.1 *Humans*

Dermally applied di(2-ethylhexyl) phthalate was considered to be moderately irritating, but only slightly or non-sensitizing to human skin (Shaffer *et al.*, 1945; Mallette & von Haam, 1952). Two adults given single oral doses of either 5 or 10 g di(2-ethylhexyl) phthalate exhibited no untoward effects apart from mild gastric disturbances and moderate diarrhoea at the higher dose (Shaffer *et al*., 1945). There are few data on effects of occupational exposure specifically to di(2-ethylhexyl) phthalate (WHO, 1992). In a study involving workers at a Swedish PVC-processing factory, peripheral nervous system symptoms and signs were investigated in 54 workers exposed to di(2-ethylhexyl) phthalate and other phthalate diesters. Some workers showed various peripheral nervous system symptoms and signs, but these

were not related to the level of exposure to phthalate diesters. None of the workers reported symptoms indicating work-related obstructive lung disease and conventional lung function tests showed no association with exposure to phthalate diesters (Nielsen *et al.*, 1985). One case of occupational exposure to di(2-ethylhexyl) phthalate was associated with asthma in a worker at a PVC-processing plant (WHO, 1992).

Di(2-ethylhexyl) phthalate is leached in significant amounts from the PVC tubing used in transfusion and dialysis. Dialysis patients were studied for evidence of liver peroxisome proliferation in biopsy samples (Ganning *et al*., 1984, 1987). Based on subjective ultrastructural evaluation of one subject, no effect was seen after one month of dialysis. However, in a liver biopsy from another subject after 12 months of dialysis, an increased number of peroxisomes was reported to be present. Others have suggested that more cautious evaluation, including objective measurements, increased numbers of biopsy intervals, and appropriate controls, would be needed to determine conclusively whether peroxisome proliferation due to di(2-ethylhexyl) phthalate occurs in dialysis patients (Huber *et al*., 1996). [The Working Group noted that biopsy study of Ganning *et al.* (1984, 1987) is not adequate for evaluation.]

The toxicity of di(2-ethylhexyl) phthalate was evaluated in 28 term infants with respiratory failure, 18 of whom received extracorporeal membrane oxygenation (ECMO) and were compared with 10 untreated infants. Various clinical parameters of liver, pulmonary and cardiac dysfunction were found to be unaffected in treated infants, even though the rate of administration ranged up to 2 mg/kg bw di(2-ethylhexyl) phthalate over 3–10 days (mean peak plasma concentration, 8 μg/mL). ECMO is considered to be the clinical intervention that results in the highest intravenous dose of di(2-ethylhexyl) phthalate (Karle *et al*., 1997).

Mettang *et al*. (1996b) investigated the relationship between di(2-ethylhexyl) phthalate exposure and uraemic pruritus in dialysis patients. There was no relationship between severity of pruritus and post-dialysis serum concentrations of di(2-ethylhexyl) phthalate, mono(2-ethylhexyl) phthalate, phthalic acid or 2-ethylhexanol. Furthermore, serum concentrations of di(2-ethylhexyl) phthalate and these related compounds were not significantly different between patients with or without uraemic pruritus.

4.2.2 *Experimental systems*

In-vivo studies of general toxic effects

Acute oral LD_{50} values for di(2-ethylhexyl) phthalate ranging from 26.3 to 33.9 g/kg bw in rats, mice, guinea-pigs and rabbits have been reported (Shaffer *et al*., 1945; Lawrence *et al*., 1975; IARC, 1982). LD_{50} values after intraperitoneal administration were 30.7 g/kg bw in rats and 14.2 and 37.8 g/kg bw in mice (Shaffer *et al*., 1945; Calley *et al*., 1966; Lawrence *et al*., 1975).

Nair *et al*. (1998) evaluated the systemic toxicity of di(2-ethylhexyl) phthalate (0–7.5 mg/kg bw given up to six times by intraperitoneal injection on alternate days) in male Wistar rats (approximately 150 g bw). Animals were evaluated by organ

weight (testis and liver), light microscopy (liver, heart, brain and testis) and plasma clinical chemistry (γ-GT, lactic dehydrogenase, alanine aminotransferase, alkaline phosphatase). No evidence of toxicity was observed.

In rats, age may be an important factor in sensitivity to the lethal effects of oral di(2-ethylhexyl) phthalate. Dostal *et al*. (1987b) evaluated the effects of di(2-ethylhexyl) phthalate (five daily oral doses of 0, 10, 100, 1000 or 2000 mg/kg bw) in male Sprague-Dawley rats, beginning at 6, 14, 16, 21, 42 or 86 days of age. Suckling rats (starting at 6–21 days of age) suffered severe growth retardation at doses of 1000 mg/kg bw and death at 2000 mg/kg bw, while older rats showed only decreased weight gain at 2000 mg/kg bw. Lethality was observed at doses of 1000 mg/kg bw starting at 14 days of age but not at six days or ≥ 16 days.

A number of other studies have examined the toxic effects of di(2-ethylhexyl) phthalate in rodents and other species following oral administration. Young, post-weanling male and female Sprague-Dawley (CD) rats were fed diets containing 0.2, 1.0 and 2.0% di(2-ethylhexyl) phthalate for 17 weeks, resulting in mean di(2-ethylhexyl) phthalate intakes of 143, 737 or 1440 mg/kg bw per day in males and 154, 797 or 1414 mg/kg bw per day in females, respectively (Gray *et al*., 1977). Significant dose-dependent increases in relative liver weight to 116–204% of control values were observed in both males and females at all levels of di(2-ethylhexyl) phthalate treatment. Di(2-ethylhexyl) phthalate levels of 1.0 and 2.0% resulted in reduced testis weights in male rats, associated with marked seminiferous tubule atrophy. Apart from the liver, no relative organ weights were affected in male and female rats given 0.2% di(2-ethylhexyl) phthalate in the diet.

In another study, young male and female Sprague-Dawley rats (10 per sex per group) were fed diets containing 5, 50, 500 or 5000 ppm di(2-ethylhexyl) phthalate for 13 weeks (Poon *et al*., 1997). Mean di(2-ethylhexyl) phthalate intakes were 0.4, 3.7, 38 and 375 mg/kg bw per day in males and 0.4, 4.2, 42 and 419 mg/kg bw per day in females, respectively. No clinical signs of toxicity were observed, and body weight gain and food consumption were not affected. Significant increases in relative liver weight, to 146 and 124% of control values in male and female rats, respectively, were observed only in animals given 5000 ppm di(2-ethylhexyl) phthalate. Relative testis weight was reduced in male rats fed 5000 ppm di(2-ethylhexyl) phthalate. Morphological examination revealed minimal to mild centrilobular hypertrophy in the liver and mild to moderate seminiferous tubule atrophy in the testis in male rats fed 5000 ppm di(2-ethylhexyl) phthalate and Sertoli cell vacuolation of minimal nature in male rats fed 500 ppm di(2-ethylhexyl) phthalate.

In-vivo studies of hepatic peroxisome proliferation in rats and mice

Many studies have examined the hepatic biochemical and morphological effects of di(2-ethylhexyl) phthalate (reviewed in Huber *et al*., 1996). Such effects in rodents include peroxisome proliferation, which is characterized by increases in the number of

hepatocellular peroxisomes, the induction of peroxisomal and microsomal fatty acid-oxidizing enzymes and hepatocellular hyperplasia.

Young male Wistar rats were given 2000 mg/kg bw di(2-ethylhexyl) phthalate per day by gavage for periods of 3–21 days (Lake *et al*., 1975). Treatment caused increases in relative liver weight and in microsomal cytochrome P450 content. Ultrastructural examination revealed marked peroxisome proliferation and a dilation of the smooth and rough endoplasmic reticulum. Rats were also treated with mono(2-ethylhexyl) phthalate, 2-ethylhexanol and phthalic acid at doses equimolar to 2000 mg/kg bw per day di(2-ethylhexyl) phthalate for seven days. While phthalic acid had no effect, both mono(2-ethylhexyl) phthalate and 2-ethylhexanol increased relative liver weight and produced hepatic peroxisome proliferation.

Male Fischer 344 rats (body weight, 100–150 g) were fed 0.5–4% di(2-ethylhexyl) phthalate in the diet for one or four weeks and male Swiss Webster mice (20–30 g) were fed 2 or 4% di(2-ethylhexyl) phthalate in the diet for one or four weeks (Reddy *et al*., 1976). Di(2-ethylhexyl) phthalate increased relative liver weights and markedly induced hepatic carnitine acetyltransferase activity in both species (up to 25-fold in rats and 10-fold in mice). Some increase in hepatic catalase activity (approximately two-fold) was observed and subjective (non-morphometric) ultrastructural examination revealed marked peroxisome proliferation. This study also demonstrated that di(2-ethylhexyl) phthalate was a hypolipidaemic agent, as serum triglyceride levels were reduced to one seventh of control values in rats and one third of control values in mice.

Male and female Fischer 344 rats and B6C3F_1 mice were fed di(2-ethylhexyl) phthalate for up to 13 weeks (David *et al*., 1999). In rats fed 12 500 ppm di(2-ethylhexyl) phthalate, there was an increase in hepatocyte replicative DNA synthesis (measured after continuous bromodeoxyuridine administration (osmotic pump) for three days before sampling) after one week (but not after two or 13 weeks) and an increase in hepatic peroxisomal β-oxidation activity after one, two and 13 weeks' administration. In mice fed 10 000 and 17 500 ppm di(2-ethylhexyl) phthalate, there was no increase in hepatocyte replicative DNA synthesis (measured after continuous bromodeoxyuridine three days before sampling) after one, two or 13 weeks of administration, but there was an increase in hepatic peroxisomal β-oxidation activity after one, two and 13 weeks' administration. In mice fed 1000 ppm di(2-ethylhexyl) phthalate, there was no statistically significant increase in hepatic peroxisomal β-oxidation activity after one, two or 13 weeks' administration (bromodeoxyuridine labelling was not evaluated at this lower dietary concentration of di(2-ethylhexyl) phthalate).

Treatment of five-week-old male Sprague-Dawley rats with 1000 mg/kg bw di(2-ethylhexyl) phthalate per day by gavage for 14 days caused increased relative liver weight and hepatic peroxisome proliferation (Lake *et al*., 1984). In addition, di(2-ethylhexyl) phthalate treatment induced cyanide-insensitive palmitoyl-coenzyme A (CoA) oxidation activity and microsomal lauric acid 12-hydroxylase activity in the

liver. While the former is considered to be a specific marker of the peroxisomal fatty acid β-oxidation cycle, the latter reflects induction of cytochrome P450 isoforms in the CYP4A subfamily. CYP4A induction is generally associated with peroxisome proliferation (Huber *et al.*, 1996). Induction of these two enzymatic markers has also been observed in other studies. Di(2-ethylhexyl) phthalate at dietary levels of 0.01–2.5% to young male and female Fischer 344 rats for 21 days produced dose-related increases in relative liver weight and in cyanide-insensitive palmitoyl-CoA oxidation and lauric acid 12-hydroxylase activities (Barber *et al.*, 1987). Reddy *et al*. (1986) fed diets containing 0.25–2.0% di(2-ethylhexyl) phthalate to young male Fischer 344 rats for 30 days. Dose-related increases in relative liver weight, cyanide-insensitive palmitoyl-CoA oxidation activity and peroxisome volume density were observed. In this study, an excellent correlation between the enzymatic marker of the peroxisomal fatty acid β-oxidation cycle and changes in peroxisome morphometry was observed, demonstrating that peroxisomal cyanide-insensitive palmitoyl-CoA oxidation is a good marker for peroxisome proliferation in rodent liver.

Di(2-ethylhexyl) phthalate-induced hepatic peroxisome proliferation in rodents is not due to the parent diester *per se*, but rather to its metabolites. With mono(2-ethylhexyl) phthalate, the two 'proximate' peroxisome proliferators were found to be the (ω–1)-hydroxy (IX) and keto (VI) metabolites, while for 2-ethylhexanol the 'proximate' peroxisome proliferator was 2-ethylhexanoic acid (Mitchell *et al*., 1985; Elcombe & Mitchell, 1986; Cornu *et al.*, 1992; Lewis & Lake, 1993).

Studies of peroxisome proliferator-activated receptor α (PPARα)

It has been established that the peroxisome proliferation and hepatocellular proliferation effects of di(2-ethylhexyl) phthalate and other peroxisome proliferators in rodent liver are mediated through activation of the peroxisome proliferator-activated receptor α (PPARα). This nuclear receptor is a member of the steroid hormone receptor superfamily. Its role in mediating the hepatic effects of peroxisome proliferators was demonstrated with knock-out mice that do not express the PPARα receptor (Lee *et al.*, 1995; Peters *et al*., 1997). Knock-out mice fed di(2-ethylhexyl) phthalate at 1.2% in the diet for 24 weeks showed none of the hepatic effects (liver weight increase, induction of mRNA expression for peroxisomal acyl CoA oxidase and microsomal CYP4A) that were observed in SV129 strain control mice (Ward *et al*., 1998). This relatively high intake of di(2-ethylhexyl) phthalate induced histological evidence of testicular and renal toxicity in both strains of mice, although earlier onset and greater severity were seen in the SV129 strain control mice than in the knock-out mice. These results establish an absolute requirement for PPARα in the manifestation of hepatic effects of di(2-ethylhexyl) phthalate, and indicate a contributory role of the receptor in mediating extrahepatic toxicity.

In non-hepatocytic systems *in vitro*, addition of mono(2-ethylhexyl) phthalate results in activation of PPARα (Issemann & Green, 1990; Isseman *et al.*, 1993). Such

systems were also used to study the ability of di(2-ethylhexyl) phthalate and its metabolites to activate nuclear receptors PPARα from mouse and human (Maloney & Waxman, 1999). In COS-1 cells transfected to express high levels of mouse or human PPARα, mono(2-ethylhexyl) phthalate and 2-ethylhexanoic acid, but not di(2-ethylhexyl) phthalate, caused transactivation of a responsive reporter gene construct.

Other hepatic studies in vivo

Other hepatic effects of di(2-ethylhexyl) phthalate in rodent liver include effects on replicative DNA synthesis and oxidative stress. Male Fischer 344 rats (seven to nine weeks old) were fed di(2-ethylhexyl) phthalate in the diet for various periods up to 365 days (Marsman *et al*., 1988; Conway *et al*., 1989). Replicative DNA synthesis was studied employing subcutaneously implanted osmotic pumps to continuously infuse [^{3}H]thymidine over seven-day periods (Marsman *et al*., 1988). Liver sections were processed for autoradiography and subsequent determination of the hepatocyte labelling index. Di(2-ethylhexyl) phthalate produced a burst of replicative DNA synthesis during treatment days 1–8, whereas at later time points, rates of replicative DNA synthesis were similar to those in control animals. Over the 365-day treatment period, di(2-ethylhexyl) phthalate produced a sustained stimulation of peroxisome proliferation, as demonstrated by cyanide-insensitive palmitoyl-CoA oxidation activity and peroxisome morphometry. Di(2-ethylhexyl) phthalate was found to increase levels of lipofuscin, as a marker of oxidative stress, three-fold after 39 days of treatment, this level being maintained throughout the rest of the treatment period (Conway *et al*., 1989). In another study, male Sprague-Dawley rats were fed a diet containing 2% di(2-ethylhexyl) phthalate for two years (Lake *et al*., 1987). Levels of conjugated dienes were increased in liver homogenates and morphological examination of liver sections revealed increased lipofuscin deposition in non-nodular/tumorous areas of the liver. Takagi *et al*. (1990a,b) investigated the relationship between hepatic peroxisome proliferation and levels of 8-hydroxydeoxyguanosine in hepatic DNA. Male Fischer 344 rats (six weeks old) were fed 1.2% di(2-ethylhexyl) phthalate in the diet for periods of 1–12 months. Treatment with di(2-ethylhexyl) phthalate resulted in sustained stimulation of cyanide-insensitive palmitoyl-CoA activity and produced up to a twofold increase in levels of 8-hydroxydeoxyguanosine in hepatic DNA.

Two studies have demonstrated that oral administration of di(2-ethylhexyl) phthalate to mice results in increased replicative DNA synthesis in hepatocytes. In the first study, administration of oral di(2-ethylhexyl) phthalate (1150 mg/kg bw per day) to male B6C3F$_1$ mice for two days resulted in a 2.4-fold increase in replicative DNA synthesis (James *et al*., 1998). In the second study, feeding a diet containing 6000 ppm di(2-ethylhexyl) phthalate to male B6C3F$_1$ mice for seven days resulted in a seven-fold increase in replicative DNA synthesis in hepatocytes, while no increase was observed when the animals were fed for 14 or 28 days (Smith-Oliver & Butterworth, 1987).

Other hepatic effects have also been attributed to di(2-ethylhexyl) phthalate. Increases of about 30% in hepatic phosphatidylcholine and phosphatidylethanolamine were observed in male Wistar rats fed 2% di(2-ethylhexyl) phthalate in the diet for seven days (Mizuguchi *et al*., 1999).

Interspecies comparisons in vivo

Many studies have demonstrated marked species differences in hepatic peroxisome proliferation. Male Wistar rats (250–350 g), male and female Sprague-Dawley rats (140–170 g), male mice (20–30 g) and male guinea-pigs (320–340 g) were fed 2% di(2-ethylhexyl) phthalate in the diet for two weeks (Osumi & Hashimoto, 1978). Hepatic cyanide-insensitive palmitoyl-CoA oxidation activity was significantly increased in both rat strains, with a more marked effect in male than in female Sprague-Dawley rats. Marked induction of enzyme activity was also observed in the mice, but none in guinea-pigs. In another study, male Fischer 344 rats and male Dunkin-Hartley guinea-pigs were given 950 mg/kg bw per day by gavage for four days (Hasmall *et al*., 2000). Significant increases in liver weight, hepatic β-oxidation activity and hepatocyte DNA replication, and decreases in hepatocyte apoptosis were observed in the rats but not the guinea-pigs. In another study, male Sprague-Dawley rats (five weeks old) and male Syrian hamsters (five weeks old) were given di(2-ethylhexyl) phthalate at doses of 25–1000 mg/kg bw per day by gavage for 14 days (Lake *et al*., 1984). In rats, treatment with di(2-ethylhexyl) phthalate produced dose-related increases in relative liver weight and hepatic cyanide-insensitive palmitoyl-CoA oxidation and carnitine acetyltransferase activities. While some effect was also observed in Syrian hamsters, this species was clearly less responsive to di(2-ethylhexyl) phthalate-induced hepatic peroxisome proliferation than rats (liver weight: up to 176% increase in rats versus 122% increase in hamsters; cyanide-insensitive palmitoyl-CoA oxidation: up to 1400% increase in rats versus 200% increase in hamsters; carnitine acetyltransferase: up to 1000% increase in rats versus 180% increase in hamsters). In the same study, rats and Syrian hamsters were also treated with 500 mg/kg bw mono(2-ethylhexyl) phthalate per day. As with di(2-ethylhexyl) phthalate, mono(2-ethylhexyl) phthalate produced a greater increase in relative liver weight and a greater stimulation of enzyme activities in rats than in Syrian hamsters (liver weight: 65% increase in rats versus 10% increase in hamsters; cyanide-insensitive palmitoyl-CoA oxidation: 1380% increase in rats versus 171% increase in hamsters; carnitine acetyltransferase: 2100% increase in rats versus 162% increase in hamsters).

A number of studies have compared the ability of di(2-ethylhexyl) phthalate to induce hepatic peroxisome proliferation in rats and primates. Male and female Wistar-derived rats (six to eight weeks old) and male and female marmosets (12–18 months old) were given 2000 mg/kg bw per day di(2-ethylhexyl) phthalate by gavage for 14 days (Rhodes *et al*., 1986). Male and female marmosets (24 months old) were also given 1000 mg/kg bw per day di(2-ethylhexyl) phthalate by daily intraperitoneal

injection for 14 days. In rats, treatment with di(2-ethylhexyl) phthalate increased relative liver weight and produced hepatic peroxisome proliferation, as demonstrated by ultrastructural examination and increased cyanide-insensitive palmitoyl-CoA oxidation and lauric acid hydroxylation. No such effects were observed in marmosets given di(2-ethylhexyl) phthalate by either oral or intraperitoneal administration. Groups of four male and four female marmosets (final body weight, around 360 g) were given 100, 500 or 2500 mg/kg bw di(2-ethylhexyl) phthalate per day by gavage for 13 weeks (Kurata *et al*., 1998). A preliminary dose-setting study indicated that 2500 mg/kg bw di(2-ethylhexyl) phthalate per day was close to the maximum tolerated dose in this species. Significant suppression of body weight gain was observed in male marmosets given 2500 mg/kg di(2-ethylhexyl) phthalate per day. Treatment with di(2-ethylhexyl) phthalate did not affect relative liver weight or hepatic cyanide-insensitive palmitoyl-CoA oxidation and carnitine acetyltransferase activities. Ultrastructural examination did not reveal significant changes in peroxisome numbers per hepatocyte or in peroxisome volume density, although small increases in mean peroxisomal volume were noted in male marmosets given 500 or 2500 mg/kg bw di(2-ethylhexyl) phthalate per day. As peroxisomal volume density is considered the most accurate morphometric measurement of peroxisome proliferation by ultrastructural evaluation (IARC, 1995), the results are considered not to have demonstrated peroxisome proliferation. Furthermore, di(2-ethylhexyl) phthalate did not produce morphological changes in the livers of male and female marmosets or in the testes of the males. Thus, in addition to demonstrating a lack of hepatic peroxisome proliferation, these results indicate that di(2-ethylhexyl) phthalate treatment did not produce testicular damage in adult marmosets.

Male Fischer 344 rats were fed diets containing 100–25 000 ppm di(2-ethylhexyl) phthalate for 21 days, while male cynomolgus monkeys were given 100 or 500 mg/kg bw di(2-ethylhexyl) phthalate per day by gavage for 25 days (Short *et al*., 1987). In rats, di(2-ethylhexyl) phthalate treatment produced a dose-related increase in relative liver weight and in enzymatic markers and ultrastructural evidence (subjective evaluation) of hepatic peroxisome proliferation. Treatment with di(2-ethylhexyl) phthalate did not affect relative liver weight or activities of cyanide-insensitive palmitoyl-CoA oxidation, carnitine acetyltransferase and lauric acid 12-hydroxylase in male cynomolgus monkeys. In addition, no treatment-related changes were observed by light or electron microscopic examination of liver sections.

Interspecies comparisons with hepatocytes in vitro

Peroxisome proliferation may also be demonstrated *in vitro* in cultured rat and mouse hepatocytes. Many of the known characteristics of peroxisome proliferation *in vivo*, including increased number and size of peroxisomes, differential induction of peroxisomal enzyme activities and stimulation of replicative DNA synthesis, have been demonstrated in cultured rat and mouse hepatocytes (IARC, 1995).

Hepatocytes isolated from male Wistar rats (180–250 g) were treated with 0.2 mM mono(2-ethylhexyl) phthalate or 1 mM 2-ethylhexanol for 48 h (Gray *et al*., 1982). Both di(2-ethylhexyl) phthalate metabolites increased carnitine acetyltransferase activity about nine-fold. In studies with hepatocytes from male Sprague-Dawley rats (180–220 g), treatment with 0.2 mM mono(2-ethylhexyl) phthalate and 1.0 mM 2-ethylhexanol for 48 h resulted in induction of carnitine acetyltransferase activity about 15-fold and six-fold, respectively (Gray *et al*., 1983). Mono(2-ethylhexyl) phthalate was also shown to induce cyanide-insensitive palmitoyl-CoA oxidation and, by ultrastructural examination, to increase numbers of peroxisomes. Hepatocytes were isolated from Wistar-derived rats (180–220 g) and treated for 72 h with 0–0.5 mM mono(2-ethylhexyl) phthalate and some mono(2-ethylhexyl) phthalate metabolites (Mitchell *et al*., 1985). Treatment with mono(2-ethylhexyl) phthalate and metabolites VI and IX (see Figure 1) resulted in a concentration-dependent induction of cyanide-insensitive palmitoyl-CoA oxidation. In addition, 0–0.5 mM mono(2-ethylhexyl) phthalate and 0–1.0 mM metabolite VI produced concentration-dependent increases in lauric acid hydroxylation. Treatment with metabolites I and V resulted in only small effects on the enzymatic markers of peroxisome proliferation. In another study with hepatocytes from Wistar-derived rats (180–220 g), metabolite VI was shown by subjective ultrastructural examination to cause proliferation of peroxisomes (Elcombe & Mitchell, 1986).

Primary hepatocyte cultures may also be employed to study species differences in hepatic peroxisome proliferation (IARC, 1995; Doull *et al*., 1999). Hepatocytes were isolated from male Sprague–Dawley rats (180–220 g), male Syrian hamsters (70–80 g) and male Dunkin-Hartley guinea-pigs (400–450 g). Treatment with 20–200 μM mono(2-ethylhexyl) phthalate for 70 h caused strong induction of cyanide-insensitive palmitoyl-CoA oxidation activity in rat hepatocytes (up to 600% of control levels), while no marked effect was observed in Syrian hamster (up to 120% of control) or guinea-pig (down to 80% of control) hepatocytes (Lake *et al*., 1986).

Hepatocytes were isolated from male Wistar-derived rats (180–220 g) and male Alderley Park guinea-pigs (400–500 g) and treated with 0–0.5 mM mono(2-ethylhexyl) phthalate or metabolite IX for 72 h (Mitchell *et al*., 1985).While both caused concentration-dependent induction of cyanide-insensitive palmitoyl-CoA oxidation in rat hepatocytes, no such effect was observed in guinea-pig hepatocytes.

Species comparisons of hepatic peroxisomal proliferation have also included studies of human and non-human primate primary hepatocyte cultures. Hepatocytes isolated from Wistar-derived rats (180–220 g), male Alderley Park guinea-pigs (400–500 g), male marmosets (350–500 g) and three human liver samples (renal transplant donors) were treated with 0–0.5 mM mono(2-ethylhexyl) phthalate for 72 h (Elcombe & Mitchell, 1986). While there was a concentration-dependent induction of cyanide-insensitive palmitoyl-CoA oxidation in rat hepatocytes, no induction was observed in guinea-pig or human hepatocytes and only small non-concentration-dependent effects were observed in marmoset hepatocytes. Metabolite VI induced cyanide-insensitive palmitoyl-CoA oxidation and lauric acid hydroxylation in cultured

rat hepatocytes. In contrast, treatment of marmoset hepatocytes with 0–1.0 mM metabolite VI and guinea-pig and human hepatocytes with 0–2.0 mM metabolite VI resulted in no induction of cyanide-insensitive palmitoyl-CoA oxidation activity. Similarly, lauric acid hydroxylation activity was not induced in marmoset or human hepatocytes treated with 0–2.0 mM metabolite VI.

Hepatocytes were isolated from male Wistar rats (200 g), male Dunkin-Hartley guinea-pigs (350 g), male New Zealand rabbits (2500 g) and cynomolgus monkeys (two to three years old) and treated with mono(2-ethylhexyl) phthalate or with metabolites VI or V for 72 h (Dirven *et al*., 1993c). The treatment of rat hepatocytes with 0–300 μM of mono(2-ethylhexyl) phthalate or metabolite VI resulted in a concentration-dependent induction of cyanide-insensitive palmitoyl-CoA oxidase and lauric acid 12-hydroxylase activities; metabolite V had very little effect. In guinea-pig, rabbit and cynomolgus monkey hepatocytes, neither mono(2-ethylhexyl) phthalate nor metabolite VI had any effect on cyanide-insensitive palmitoyl-CoA oxidase activity at concentrations of up to 300 μM. Small increases in cyanide-insensitive palmitoyl-CoA oxidase activity were observed at mono(2-ethylhexyl) phthalate and metabolite VI concentrations of 600 μM, whereas treament with 0–600 μM of either compound had no effect on lauric acid 12-hydroxylase activity in guinea-pig, rabbit or cynomolgus monkey hepatocyte cultures.

Hepatocytes were isolated from male Wistar-derived rats, male Alderley Park guinea-pigs and three human liver samples (liver transplant donors). Treatment with up to 1100 μM metabolite VI for 72 h caused a concentration-dependent induction of cyanide-insensitive palmitoyl-CoA oxidation in rat hepatocytes, but had no significant effect in either guinea-pig or human hepatocytes. Ultrastructural examination revealed an increase in the numbers of peroxisomes in rat hepatocytes, but no such effect was observed in cultured human hepatocytes (Elcombe *et al*., 1996).

Hepatocytes were isolated from male Fischer 344 rats and from two human liver samples (liver surgery patients). Treatment with 200 μM mono(2-ethylhexyl) phthalate for either 48 or 72 h induced carnitine acetyltransferase activity in cultured rat but not human hepatocytes (Butterworth *et al.*, 1989).

Hepatocytes were isolated from male Fischer 344 rats and from three human liver (liver transplantation donors). Treatment with mono(2-ethylhexyl) phthalate induced β-oxidation activity, replicative DNA synthesis and inhibited apoptosis induced by transforming growth factor β (TGFβ) in cultured rat but not human hepatocytes (Hasmall *et al*., 1999).

Hepatocytes were isolated from male Wistar rats, two dogs (age, breed and sex not stated) and two human subjects (69–71 years of age, sex not stated) (Hildebrand *et al*., 1999). In collagen sandwich cultures, the rat hepatocytes responded to di(2-ethylhexyl) phthalate in the culture medium with slightly increased carnitine acetyltransferase activity, while dog and human hepatocytes did not respond.

Hepatocytes were isolated from male Alderley Park (Wistar-derived) rats, male Alderley Park (Swiss) mice, male Alderley Park (Dunkin-Hartley) guinea-pigs and

male captive bred (ICI, Alderley Park) marmosets. Treatment with either 2-ethylhexanol or 2-ethylhexanoic acid for 72 h produced a concentration-dependent induction of cyanide-insensitive palmitoyl-CoA oxidation in rat and mouse but not guinea-pig or marmoset hepatocytes (Cornu *et al.*, 1992).

Peroxisome proliferators have also been shown to induce replicative DNA synthesis in cultured rodent hepatocytes (IARC, 1995). In contrast, several peroxisome proliferators have failed to induce replicative DNA synthesis in human hepatocyte cultures (Doull *et al*., 1999). Hepatocytes were isolated from male Wistar-derived rats and from three human liver samples (liver transplantation donors) and treated with 2-ethylhexanoic acid and some other peroxisome proliferators for 72 h (Elcombe *et al*., 1996). While 2-ethylhexanoic acid induced replicative DNA synthesis in cultured rat hepatocytes, no effect was observed in human hepatocytes. Hepatocytes were isolated from male Fischer 344 rats and three humans and treated in culture with 250–2000 μM mono(2-ethylhexyl) phthalate (Hasmall *et al.*, 1999). Increased peroxisomal ω-oxidation (at 250–750 μM), replicative DNA synthesis (at 500–1000 μM), and inhibition of apoptosis (at 250–1000 μM) were observed in rat hepatocytes. None of these parameters was affected by mono(2-ethylhexyl) phthalate in human hepatocytes.

Other effects

The effect of di(2-ethylhexyl) phthalate in diet (2% for 21 days) on lipoprotein metabolism in male Wistar rats was evaluated (Mocchiutti & Bernal, 1997). The observed reduction in plasma triglyceride levels was associated with (and attributed to) increased activity of extrahepatic lipoprotein lipase.

An effect of di(2-ethylhexyl) phthalate on estrogen metabolism has been reported (Eagon *et al.*, 1994). Male Fischer 344 rats fed diets containing 1.2% di(2-ethylhexyl) phthalate for four, eight or 16 weeks had significantly increased serum estradiol levels. This was explained by the observation that these rats showed significant loss of hepatic activity of a major male estrogen-metabolizing enzyme, estrogen 2-hydroxylase, and a male-specific estrogen-sequestering protein.

The testicular toxicity of di(2-ethylhexyl) phthalate is described in Section 4.3.

Female Fischer 344 rats treated with a single oral dose (up to 5000 mg/kg bw) or with repeated doses (up to 1500 mg/kg bw per day for 14 days) of di(2-ethylhexyl) phthalate showed no neurobehavioural effects, as evaluated by functional observational battery and motor activity testing (Moser *et al.*, 1995).

4.3 Reproductive and developmental effects

4.3.1 *Humans*

No data were available to the Working Group.

4.3.2 *Experimental systems*

The teratogenicity and reproductive toxicity of di(2-ethylhexyl) phthalate have been reviewed (Huber *et al*., 1996). It was noted that di(2-ethylhexyl) phthalate impairs the fertility in both sexes of adult rats at doses above 100 mg/kg bw per day, and that several studies indicate that di(2-ethylhexyl) phthalate is embryotoxic and teratogenic in rodents. Di(2-ethylhexyl) phthalate was associated with a reduction in relative testis weight, decreases in sperm production and a depletion of testicular zinc. The testicular response appeared to be species-specific, but was not consistent with the species sensitivity to peroxisome proliferation (e.g., guinea-pigs were more sensitive to the testicular effects than were Syrian hamsters). The metabolite mono(2-ethylhexyl) phthalate was judged to be more potent in causing both teratogenicity and reproductive toxicity. Both the embryotoxic and testicular effects in adults were considered to be observed at doses above those at which peroxisome proliferation was recorded, but no mechanism for either response was identified. Effects on testicular development in rats following exposure to di(2-ethylhexyl) phthalate prenatally and during suckling or during adolescence at dose levels below those associated with peroxisome proliferation have been reported (Poon *et al*., 1997; Arcadi *et al*., 1998).

(*a*) *Developmental toxicity studies*

(i) *Rats*

Sprague-Dawley rats were administered intraperitoneal injections of 5 or 10 mL/kg bw [4930 or 9860 mg/kg] di(2-ethylhexyl) phthalate on gestation days 5, 10 and 15. Fetal body weight was reduced in both treated groups, resorptions increased from about 8% in controls and the low-dose group to 27% in the high-dose group and an increase in the incidence of abnormalities (e.g., haemangiomas of the legs) but not skeletal abnormalities was also evident in the high-dose group (Singh *et al*., 1972).

Groups of 25 Wistar rats were exposed by head–nose inhalation to di(2-ethylhexyl) phthalate (> 99% pure) at aerosol concentrations (mass median aerodynamic diameter (MADD) 50% of < 1.2 μm) of 0, 0.1, 0.05 and 0.3 mg/L [a range-finding study noted a dose-related trend for hepatic peroxisome proliferation at levels of 0.2, 0.5 and 1.0 mg/L (the maximum concentration that could be achieved)] for 6 h per day on gestation days 6–15. Twenty females were killed on gestation day 20 and their fetuses examined for viability, growth and malformations. The only dose-related effect noted was an increase in the extent of retarded development (renal pelvic dilatations) in the highest-exposure group. The remaining five females per group were allowed to litter and their offspring were examined for postnatal growth and viability. No dose-related effects were noted. The only maternal effect seen was a reduction in body weight on post-partum day 21 in the high-dose group (Merkle *et al*., 1988).

Groups of 22–25 Fischer 344 rats were fed diets containing 0, 0.5, 1.0, 1.5 or 2.0% di(2-ethylhexyl) phthalate (> 99% pure) on days 0–20 of gestation (Tyl *et al*., 1988). Fetuses were examined on gestation day 20 for growth, viability, external, visceral and

skeletal malformations and variations. Maternal food intake was decreased and water consumption and liver weights were increased at all dose levels. Maternal body weight gain effects and reduced fetal weights were evident at levels of 1.0% and above. Fetal viability was reduced at the highest exposure level. No significant increase in the incidence of variations or malformations was observed.

The effects on testicular development in the offspring of rats exposed to di(2-ethylhexyl) phthalate *in utero* were studied by Tandon *et al*. (1991). Groups of six Wistar rats were exposed to 0 or 1000 mg/kg di(2-ethylhexyl) phthalate by gavage for the entire period of gestation and spermatogenesis was assessed in the offspring at 31, 61 and 91 days of age. Testicular weight was significantly reduced in the offspring only at 31 days of age. Alterations in the activity of testicular γ-GT, sorbitol dehydrogenase, lactate dehydrogenase and β-glucuronidase were noted, especially at the youngest age, suggesting alterations in Sertoli cell function and germ cell maturation. Epididymal sperm counts were reduced by about 22% at 91 days of age.

In another study, Fischer 344 rats were exposed to 0, 0.25, 0.5 or 1% di(2-ethylhexyl) phthalate in the diet during gestation and offspring were evaluated for reproductive function and fertility. In the summary of the study, it was reported that the high-dose level reduced the body weight of the parental generation by 25%, while reductions in food consumption were seen at the mid- and high-dose level. The only sign of reproductive toxicity was an 8% decrease in pup birth weight (National Toxicology Program, 1997a).

In a screening assay for developmental toxicity, groups of 16–21 Fischer 344 rats were exposed to di(2-ethylhexyl) phthalate (98% pure) by oral gavage at doses of 0, 1125 or 1500 mg/kg bw per day on gestation days 6–19 (Narotsky & Kavlock, 1995). The litters were followed postnatally for growth, viability and malformations. Exposure to either level resulted in decreased maternal weight gain on gestation days 6–8 and a significant increase in females with fully resorbed litters. No treated pups survived to postnatal day 6 and malformations (e.g., microphthalmia, missing innominate artery and cleft palate) were present in a few pups that were found dead. In a follow-up study, groups of 10–12 Fischer 344 rats were exposed to 0, 333, 500, 750 or 1125 mg/kg bw di(2-ethylhexyl) phthalate per day by gavage on gestation days 6–15. There were no effects upon maternal body weight or clinical findings and the only developmental effects were a delay in parturition and micro/anophthalmia at 750 and 1125 mg/kg bw. These effects were in contrast to the earlier study, so a comparison study was made using dose levels of 1125 and 1500 mg/kg bw administered on gestational days 6–15 or 6–19. Detailed results were not presented, but the authors stated that in the 6–15-day groups, there were significant maternal weight loss, increased prenatal mortality, delay in parturition and eye malformations (Narotsky *et al*., 1995).

Di(2-ethylhexyl) phthalate was one of several phthalate esters evaluated for prenatal toxicity in Wistar rats (Hellwig *et al*., 1997). Groups of 10 females received di(2-ethylhexyl) phthalate at doses of 0, 40, 200 and 1000 mg/kg bw per day by oral

gavage on gestation days 6–15. The highest dose level decreased food consumption and body weight gains, and increased relative liver and kidney weights at term; two dams in this group showed vaginal haemorrhage during treatment. Also in the high-dose group, there were pronounced postimplantation loss, reduced numbers of live fetuses and an increase in malformations, predominantly of the tail, brain, urinary tract, gonads, vertebral column and sternum.

There are reports indicating major organ toxicity in the offspring including irreversible testicular effects following exposure to di(2-ethylhexyl) phthalate prenatally and during suckling (Arcadi *et al*., 1998). Groups of 12 female Long-Evans rats were exposed daily to drinking water containing 32.5 or 325 µL/L di(2-ethylhexyl) phthalate from day 1 of pregnancy to day 21 after the delivery. Based on estimated water intake, the exposure was roughly calculated to correspond to 3.0–3.5 and 30–35 mg/kg di(2-ethylhexyl) phthalate per day during pregnancy; during suckling this value increased by at least 30%, which was assumed to be due to increased water intake. At different times after delivery (21, 28, 35, 42, 56 days), eight pups per group were killed. Pup body weight gain and kidney, liver and testis weights were measured. Histopathology of the kidneys, liver and testes was also studied. Female pups were used for behavioural assessment 30 days after birth in the 'beam walking' test, designed to assess the locomotor activity by employing a learned avoidance test. Pregnancy rate, body weight gain and gross appearance in the dams were not affected by the treatment. Perinatal exposure produced no significant changes in body weight gain in the pups. A reduction in kidney weight (absolute and relative) was observed at both dose levels, accompanied by histopathological findings (shrinkage of renal glomeruli with signs of glomerulonephritis, dilation of renal tubuli and light fibrosis) between weeks 0 and 4 of age. The alterations were less pronounced at week 8. The increase in liver weight was not dose-related. A dose-dependent reduction in testicular weight (absolute and relative) was observed and did not appear to return to normal with growth. The perinatal exposure caused severe histological damage to the testes. At 21 and 28 days of age, there was a gross disorganization of the seminiferous tubular structure, detachment of the spermatogonial cells from basal membrane and absence of spermatocytes in both exposure groups. At the end of the observation period, at 56 days, there were still severe histopathological changes in the testes of pups. Low-dose rats exhibited only a few elongated spermatids in tubules showing a pervious lumen. In high-dose animals, the histological findings included a generalized disorganization of the tubular epithelium, with spermatogonia detached from the basal membrane, absence of elongated spermatids and spermatozoa and tubular lumen filled with cellular deposits. Four adult male rats exposed to the same levels via the drinking-water for 42 days did not show any significant change in either kidney, liver or testis relative weights, and only minor histological damage of the testes. Female pups exposed perinatally to 325 µL/L di(2-ethylhexyl) phthalate showed a significantly increased time necessary to perform the beam walking test, indicating a behavioural effect expressed as reduced locomotor activity.

(ii) *Postnatal studies in rats*

Parmar *et al*. (1985) exposed groups of five dams (albino rats) to 0 or 2000 mg/kg bw di(2-ethylhexyl) phthalate. Litters of seven pups were dosed with di(2-ethylhexyl) phthalate through mothers' milk throughout the lactation period (from parturition to day 21). Pup body weights were recorded at five-day intervals and on day 21 when the pups were killed. Pooled liver homogenates were prepared for an assay of the activities of arylhydrocarbon hydroxylase, aniline hydroxylase and ethylmorphine *N*-demethylase, and concentration of cytochrome P450. The body weight of the di(2-ethylhexyl) phthalate-treated pups was lower than that of the control group throughout the whole period. Absolute liver weight was significantly decreased in the di(2-ethylhexyl) phthalate-treated pups; relative liver weight was similar in the two groups. All four biochemical parameters showed significant decreases in the di(2-ethylhexyl) phthalate-treated pups relative to control. In the livers of the pups (5), a concentration of 25.7 ± 0.3 μg/g di(2-ethylhexyl) phthalate was found.

Female Sprague-Dawley rats were given five oral doses of 2000 mg/kg bw per day di(2-ethylhexyl) phthalate (> 99% pure) in corn oil by gavage on days 2–6, 6–10 or 14–18 of lactation (Dostal *et al*., 1987a). The rats were killed 24 h after the last dose. The body weights of lactating rats and of their suckling pups were significantly reduced at all treatment intervals. Food consumption was reduced in the dams dosed on days 14–18. Relative liver weights were increased in the lactating dams at all three stages of lactation but not in the suckling pups. The hepatic peroxisomal enzyme activities (cyanide-insensitive palmitoyl-CoA oxidase and carnitine acetyltransferase) were increased five- to eight-fold in treated dams at all three stages of lactation. Twofold increases in these enzyme activities were also observed in pups suckling the treated dams. Hypolipidaemia was observed in treated lactating rats at all three stages of lactation. Plasma cholesterol and triglyceride concentrations were decreased by 30–50%.

The transfer of di(2-ethylhexyl) phthalate into the milk of lactating rats was shown in groups of female Sprague-Dawley rats given three oral doses of 2000 mg/kg bw per day di(2-ethylhexyl) phthalate in corn oil by gavage on days 15–17 of lactation. Two hours after dosing on day 17, 10 pups per litter were removed from the dams to allow milk to accumulate. Six hours after the last dose, the dams were killed and milk and mammary glands were collected. Two pups from each litter were killed 3–4 h after the third dose. Increased activities of cyanide-insensitive palmitoyl-CoA oxidase and carnitine acetyltransferase in dams and pups were observed. In treated rats, mammary gland weights, both absolute and relative, were significantly reduced, and total milk solids, lipid, and protein were increased relative to control rats, whereas milk lactose was significantly decreased. Milk collected 6 h after the third dose contained 216 μg/mL di(2-ethylhexyl) phthalate and 25 μg/mL mono(2-ethylhexyl) phthalate. In contrast, plasma contained virtually no di(2-ethylhexyl) phthalate (< 0.5 μg/mL) but substantial amounts of mono(2-ethylhexyl) phthalate (76 μg/mL), resulting in a high milk:plasma ratio for di(2-ethylhexyl) phthalate and a low milk:plasma ratio for

mono(2-ethylhexyl) phthalate. Di(2-ethylhexyl) phthalate and mono(2-ethylhexyl) phthalate were not detected in the plasma of the pups (Dostal *et al.*, 1987a).

Groups of 10 male Sprague-Dawley rats were given 0, 10, 100, 1000 or 2000 mg/kg bw per day di(2-ethylhexyl) phthalate (> 99% pure) in corn oil by gavage for five days, beginning at the age of six (one week old), 14–16 (two weeks old), 21 (three weeks old), 42 (six weeks old) or 86 (12 weeks old) days (Dostal *et al*., 1987b). The control group was given the vehicle. After two doses of 2000 mg/kg bw per day, virtually all pups in the three youngest age groups died, whereas six- and 12-week-old rats showed significantly decreased body weight but no fatalities. Five daily doses of 1000 mg/kg bw per day caused significant decreases in body weight gain in one-, two- and three-week-old rats. Absolute and relative liver weights were significantly increased at 100 mg/kg bw per day in all age groups (except for one-week-old rats) and in all age groups at higher dose levels. Absolute kidney weight was reduced in some cases, whereas relative kidney weight was increased at doses of 1000 mg/kg bw per day or more in rats three or more weeks old. Morphological examination revealed increased peroxisome proliferation in neonatal as well as in adult rats. The activities of cyanide-insensitive palmitoyl-CoA oxidase and carnitine acetyltransferase were increased in a dose-dependent manner in all age groups. The activities of these enzymes were similar in control rats of all ages. Plasma cholesterol concentrations were higher in suckling control rats (one- and two-week-old) than in weanling (three-week-old) and adult controls. In di(2-ethylhexyl) phthalate-treated rats, plasma cholesterol concentrations were significantly reduced in weanling and adult rats given doses of 1000 mg/kg bw per day or more. In suckling rats, plasma cholesterol levels were increased at 1000 mg/kg bw per day. Plasma triglyceride levels in the control group were similar at all ages, whereas significant decreases in plasma triglycerides were observed in weanling and adult rats; in suckling rats only small decreases (not significant) occurred.

Tandon *et al*. (1990) performed a study on testis development in male rats after exposure during the suckling period. Groups of four female rats were given vehicle (groundnut oil) or 2000 mg/kg bw per day di(2-ethylhexyl) phthalate orally for 21 days from parturition. The pups were killed at the age of 31, 61 or 91 days when testes, epididymis, prostate and seminal vesicles were examined. The offspring of di(2-ethylhexyl) phthalate-treated mothers showed a significant increase in the activity of testicular γ-GT, lactate dehydrogenase and β-glucuronidase, and a significant decrease in the activity of acid phosphatase and sorbitol dehydrogenase at 31 and 61 days of age compared with controls. No effect on these testicular enzymes was seen in 91-day-old rats. The authors concluded that exposure to di(2-ethylhexyl) phthalate during early life through mother's milk causes biochemical alterations which may affect the functional development of the testis.

In a neonatal rat model used to assess di(2-ethylhexyl) phthalate toxicity following intravenous administration, groups of 12 neonates, two to four days of age, were injected intravenously with 30.8, 91.7 or 164.8 mg/kg bw di(2-ethylhexyl)

phthalate in 4% bovine serum albumin (BSA) solution for 18 consecutive days (Greener *et al*., 1987). Control neonates were injected with a solution of 4% BSA or saline, or were untreated. Neonates were examined for signs of toxicity immediately after treatment and again 1–3 h later. Animals were killed 24 h after the last treatment (postnatal days 20–23) and a complete necropsy was performed and selected tissues (brain, heart, lungs, liver, spleen, kidneys, injection site, eyes, stomach, duodenum and caecum) were prepared for histopathological evaluation. Body weight gains and average weight gain per day were significantly and dose-dependently decreased from days 4 to 21 of the treatment period. Absolute and relative liver weights were significantly increased in a dose-related manner. No conclusive histopathological alterations were detected in the tissues.

(iii) *Mice*

In a study designed to identify the gestational days when mice are particularly sensitive to di(2-ethylhexyl) phthalate, groups of three to eight female ddY-Slc(SPF) mice, seven to eight weeks of age, were given 0, 1.0, 2.5, 5, 7.5, 10 or 30 mL/kg bw di(2-ethylhexyl) phthalate (> 99% pure; corresponding to 1/30, 1/12, 1/6, 1/4, 1/3 or 1/1 of the acute oral LD_{50}) by gavage on day 6, 7, 8, 9 or 10 of gestation (Yagi *et al*., 1980). The average body weights of the fetuses were decreased at all dose levels, regardless of the day of maternal exposure. The number of resorptions was increased largely depending on the dose and particularly on the day of dosing. A high and dose-related increase was observed in animals dosed on days 7 and 8 of gestation at all dose levels tested. Doses of 5 or 10 mL/kg bw given on day 7 led to 100% fatality of the fetuses. The incidences of fetal deaths were 8% and 5%, respectively, when 10 mL/kg bw di(2-ethylhexyl) phthalate was administered on day 9 or 10 of gestation. Dose levels of 2.5 or 7.5 mL/kg di(2-ethylhexyl) phthalate given to mice on day 7 or 8 of gestation induced a high incidence of gross and skeletal abnormalities including encephaly, open eyelid and club foot. There was no information on maternal toxicity.

Groups of 6–19 ddY-Slc × CBA mice were administered 0.05–30 mL/kg di(2-ethylhexyl) phthalate on gestational day 6, 7, 8, 9 or 10 (Tomita *et al*., 1982a). Dose- and stage-dependent effects on fetal development were observed. The numbers of live fetuses were greatly reduced by treatment on gestational days 7–9, but particularly by 2.5 mL/kg bw on day 7. High rates of external (e.g., exencephaly, open eyelid, club foot and bent tail) and skeletal anomalies (e.g., abnormal vertebrae and ribs) were noted in groups receiving 2.5 or 7.5 mL/kg bw on day 7 or 8 of gestation.

Di(2-ethylhexyl) phthalate was administered in the diet to groups of 7–24 ICR mice at levels of 0, 0.05, 0.1, 0.2, 0.4 or 1.0% by weight throughout gestation and effects on the developing fetus were assessed (Shiota & Nishimura, 1982). Maternal body weight at the end of pregnancy was reduced at dose levels of 0.2% and higher. Dose levels of 0.1% and above significantly reduced embryonic viability. In the 0.2% di(2-ethylhexyl) phthalate group, malformations such as exencephaly, myeloschisis and tail anomalies were observed.

Groups of 9–11 Slc-ICR mice were exposed to di(2-ethylhexyl) phthalate by oral gavage at concentrations of 0, 250, 500, 1000 or 2000 mg/kg bw or by intraperitoneal injection (3–9 per group) at doses of 0, 500, 1000, 2000, 4000 or 8000 mg/kg bw on days 7–9 of gestation (Shiota & Mima, 1985). Controls received the olive oil vehicle. When given by the oral route, di(2-ethylhexyl) phthalate exposure caused resorptions, reduced fetal weight and an increased incidence of malformations (exencephaly, anencephaly, open eyelids, tail anomalies) at doses of 1000 mg/kg and above. One of 11 females died in the highest-dose group. No effects on the fetuses were observed following intraperitoneal exposure, although two of three females exposed to the high dose died. Using a similar experimental design, these authors reported that mono(2-ethylhexyl) phthalate was not toxic to development in the mouse.

Groups of 24–30 CD-1 mice were fed diets containing 0, 0.025, 0.05, 0.10 or 0.15% di(2-ethylhexyl) phthalate (> 99% pure) on days 0–17 of gestation (Tyl *et al*., 1988). Fetuses were examined on gestational day 17 for growth, viability, external, visceral and skeletal malformations and variations. Maternal body weight gain was reduced and liver weights were increased at 0.10% and above. Fetal body weight was reduced in females at 0.10%, whereas this effect was not observed until the 0.15% level in male offspring. Concentrations of 0.10% and above reduced fetal viability and, beginning at the 0.05% level, increased the incidence of malformations (affecting the eye, central nervous system, heart, axial skeleton and tail).

In another study, CD-1 mice were exposed via the diet to 0. 0.01, 0.025 or 0.05% di(2-ethylhexyl) phthalate in the diet on days 0–17 of gestation, and the offspring were evaluated for acquisition of developmental landmarks, spontaneous locomotor activity and fertility. The summary report of the study indicates that no effects were observed on reproductive toxicity in either generation (National Toxicology Program, 1997b).

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

Groups of 7–10 Wistar rats were dosed by gavage with undiluted di(2-ethylhexyl) phthalate (12.5 or 25 mmol/kg), 2-ethylhexanol (6.25 or 12.5 mmol/kg) or 2-ethylhexanoic acid (6.25 or 12.5 mmol/kg) on day 12 of gestation and fetuses were examined for viability, growth and morphology on day 20 of gestation (Ritter *et al*., 1987). Control females were untreated [no maternal effects were reported]. All three chemicals induced malformations (hydronephrosis, laevocardia, septal defects, short and kinky tails, ectrodactyly, misplaced digits and bowed radius), with the order of potency suggesting that 2-ethylhexanoic acid may be the proximate teratogen [malformation data not shown].

Fetal body weights were reduced by about 23% and relative liver weights increased by about 22% in rats exposed by oral gavage to 1000 mg/kg bw di(2-ethylhexyl) phthalate on gestational days 6–15. This dose also reduced maternal weight gain during pregnancy and decreased the activity of several enzymes (dehydrogenase, malate dehydrogenase, adenosine triphosphatase and cytochrome c oxidase) in fetal livers, although no malformations were observed (Srivastava *et al*., 1989).

Di(2-ethylhexyl) phthalate (0, 0.5, 1, 2.5 or 5 g/kg per day) was administered to Fischer 344 rats by oral gavage from birth through lactation day 21 and the activity of several peroxisomal enzymes was determined in the livers, kidneys and brains of the females and their offspring (Cimini *et al*., 1994). No pups survived exposure to doses of 2.5 g/kg per day. Pup growth was impaired at the two lowest doses. In the liver, cyanide-insensitive palmitoyl-CoA oxidase activity showed similar increases in pups and adult females.

Groups of 10–13 pregnant female mice, either homozygous wild type (+/+) or PPARα null (–/–), were administered di(2-ethylhexyl) phthalate by gavage at 0 or 1000 mg/kg on days 8–9 of gestation. Offspring were evaluated on gestational days 10 and 18. Similar developmental toxicicity (resorptions, growth retardation, incidence of malformations) was seen in mice of both genotypes, suggesting that the developmental effects are not PPARα-mediated. Additional analysis showed that di(2-ethylhexyl) phthalate induced maternal hepatic CYP4A1 mRNA in the wild-type females only, and both genotypes showed induced MT-1 and zinc levels in the livers and reduced serum and fetal concentrations after exposure to di(2-ethylhexyl) phthalate (Peters *et al*., 1997a,b).

The reproductive effects of 10 known or suspected anti-androgens on sexual differentiation were investigated in male rats. Groups of 10 Long-Evans hooded rats were administered 0 or 750 mg/kg di(2-ethylhexyl) phthalate by gavage from day 14 of pregnancy to day 3 of lactation. In the offspring, di(2-ethylhexyl) phthalate induced lower body weight, decreased anogenital distance, retained nipples and high levels of testicular and epididymal abnormalities, including atrophy and agenesis. In eight-day-old pups, several males from different litters displayed haemorrhagic testes that were visible by gross examination of the inguinal region. Testicular lesions of this nature have not been observed with known anti-androgens, which indicates that di(2-ethylhexyl) phthalate affects the developing male reproductive system by a mechanism that is distinct from that of previously described reproductive toxicants. The profile of the effects induced by di(2-ethylhexyl) phthalate was different from that induced by some known androgen-receptor antagonists (i.e., vinclozolin, procymidone, *p,p′*-DDE (1,1-dichloro-2,2-bis(*para*-chlorophenyl)ethylene)) (Gray *et al*., 1999).

(*c*) *Reproductive toxicity studies*

(i) *Rats*

Sprague-Dawley rats received daily intraperitoneal injections of 50 or 100 mg/kg bw di(2-ethylhexyl) phthalate on alternate days for 20 days. Prostatic and testicular zinc levels were reduced by this treatment (Curto & Thomas, 1982).

Groups of five or 15 male and female Sprague-Dawley rats were exposed to di(2-ethylhexyl) phthalate in the diet at concentrations of 0, 0.2, 1.0, or 2.0% (0, 143, 737 or 1440 mg/kg bw per day in males) for two, six or 17 weeks. The absolute and relative testicular weights of rats in the mid- and high-dose groups were lower than

those of control rats. Histological examination revealed severe seminiferous tubular atrophy and cessation of spermatogenesis related to the dietary level of di(2-ethylhexyl) phthalate. These changes were observed after exposure for two weeks (Gray *et al*., 1977).

Testicular effects were also investigated after oral administration of 2000 mg/kg bw di(2-ethylhexyl) phthalate for seven consecutive days to 13-week-old male Wistar rats (Saxena *et al*., 1985). Degeneration was observed in about 40% of the seminiferous tubules. Loss of succinic dehydrogenase, NADH-diaphorase and acid phosphatase activity and increases in adenosine triphosphatase, glucose-6-phosphate dehydrogenase and alkaline phosphatase activity were observed in treated rats.

The reversibility of testicular effects was studied after oral administration of 2000 mg/kg bw per day di(2-ethylhexyl) phthalate for 14 days (Oishi, 1985). One day after the last administration, 10 treated animals were killed and compared with 10 rats fed control diet for an additional 45 days without further administration of di(2-ethylhexyl) phthalate. Testicular morphology was characterized by a marked shrinkage of the seminiferous tubules, the germinal epithelium consisting of Sertoli cells, very few spermatogonia and several multinucleated cells. The interstitial tissue and Leydig cells appeared normal. After a recovery period of 45 days after termination of di(2-ethylhexyl) phthalate administration, the majority of tubules showed a lack of spermatogenesis, but some tubules had intact epithelium. The percentage of spermatogenic tubules in a representative cross-section was 0 (total atrophy) or 12.8%.

Oral administration of di(2-ethylhexyl) phthalate at levels of 0, 250, 500 or 1000 mg/kg bw to adult albino rats for 15 days resulted in a significant decrease in sperm count in the epididymus and increased activity of β-glucuronidase, γ-GT and lactate dehydrogenase and a decrease in the activity of acid phosphatase in the testes. The authors interpreted these findings as indicating germ-cell depletion and deterioration of the germinal epithelium in the testes (Parmar *et al*., 1986).

Dietary exposure of groups of 24 adult male Fischer 344 rats to 0, 320, 1250, 5000 or 20 000 ppm di(2-ethylhexyl) phthalate (> 99% pure) for 60 days resulted in dose-dependent reductions in body weight and testis, epididymis and prostate weights beginning at 5000 ppm. Histopathologically, the testes showed severe atrophy of the seminiferous epithelium and loss of spermatogonia at the highest dose. At this dose level, there were reductions in the sperm density in the epididymus and percentage of motile sperm, while abnormal sperm shapes, testicular zinc and serum follicle-stimulating hormone (FSH) and luteinizing hormone (LH) were elevated. The size of the litters at birth was significantly reduced at the highest dose (Agarwal *et al*., 1986).

In a 102-week study, adult male Sprague-Dawley rats were exposed to di(2-ethylhexyl) phthalate in the diet at dose levels of 0, 0.02, 0.2 or 2% (0, 7, 70, or 700 mg/kg bw per day) (Ganning *et al*., 1991). In all dose groups, di(2-ethylhexyl) phthalate inhibited spermatogenesis and general seminiferous tubular atrophy at the end of the study was reported. [The Working Group noted that the study was designed to study the effects of phthalates on the liver and there was little information on testicular effects].

Male Sprague-Dawley rats, aged one, two, three, six or 12 weeks of age, received five daily doses ranging from 10 to 2000 mg/kg bw per day di(2-ethylhexyl) phthalate by oral gavage and testicular effects were examined 24 h after the last dose (Dostal *et al*., 1988). Relative testicular weight was reduced at doses of 1000 mg/kg bw per day in one-, two-, three- and six-week-old rats, while 2000 mg/kg was required for this effect when the animals were 12 weeks of age. Sertoli cell number was transiently reduced in rats exposed to 1000 mg/kg bw per day at one week of age. Offspring from this treatment showed normal fertility at six weeks of age, although there was histological evidence of decreased maturation of spermatids at doses as low as 500 mg/kg bw per day. Losses of spermatocytes were evident in rats exposed to 1000 or 2000 mg/kg bw per day at ages of two weeks or older. Decreased testicular zinc levels did not appear to be related to the effect on spermatogenesis.

Groups of 10 male Wistar rats, nine weeks of age, were exposed by inhalation to 0, 0.01, 0.05 or 1.0 mg/L di(2-ethylhexyl) phthalate for 6 h per day on five days per week for 28 days and had normal fertility when mated with untreated females two and six weeks later (Klimisch *et al*., 1992). At the end of the exposure, relative liver weights were increased in the high-dose males, but there was no evidence of testicular histopathological effects.

Adult male albino rats received 0, 500 or 1000 mg/kg di(2-ethylhexyl) phthalate by gavage for 15 days and were killed 24 h later (Siddiqui & Srivastava, 1992). Relative testis weight and epididymal sperm counts were reduced at the highest dose, as was the activity of several enzymes relevant to spermatogenesis (aldose reductase and sorbitol dehydrogenase). The activity of testicular lactate dehydrogenase was increased at this dose.

Effects on female reproductive function were evaluated in groups of six to nine Sprague-Dawley rats exposed by gavage to 2 g/kg di(2-ethylhexyl) phthalate (purity, > 99%) by gavage daily for 1–12 days (Davis *et al*., 1994). Di(2-ethylhexyl) phthalate-exposed females had prolonged estrous cycles, suppressed or delayed ovulations and smaller preovulatory follicles. Endocrinologically, the treated females had reduced preovulatory granulosa cell estrogen production, with secondary increases in FSH and insufficient LH surge for ovulation.

In a 90-day study, groups of 10 young male Sprague-Dawley rats (105–130 g at initiation of dosing) were given 0, 5, 50, 500 or 5000 ppm (0, 0.4, 3.7, 37.6 or 375.2 mg/kg bw) di(2-ethylhexyl) phthalate per day in the diet for 13 weeks. No clinical signs of toxicity were observed. Feed consumption and body weight gain were not affected. At 5000 ppm, rats had significantly increased absolute and relative liver weights and relative kidney weight. In the 500-ppm dose group, a high incidence of minimal to mild Sertoli cell vacuolation was observed in 7/10 rats. No other effects were noted at this dose level. At 5000 ppm, the absolute and relative testis weights were significantly reduced. Microscopic examination revealed mild to moderate, bilateral, multifocal or complete atrophy of the seminiferous tubules with complete loss of spermatogenesis and cytoplasmic vacuolation of the Sertoli cells lining the tubules in 9/10 rats (Poon *et al*., 1997).

(ii) *Mice*

Adult Swiss Webster mice received daily intraperitoneal injections of 50 or 100 mg/kg bw di(2-ethylhexyl) phthalate for five days or alternate daily intraperitoneal injections of 50 or 100 mg/kg bw di(2-ethylhexyl) phthalate for 20 days. Prostatic and testicular zinc levels were not affected by treatment with di(2-ethylhexyl) phthalate (Curto & Thomas, 1982).

In a fertility assessment by continuous breeding, groups of 20 male and 20 female CD-1 mice were fed di(2-ethylhexyl) phthalate (> 99% pure) at dietary levels of 0.01, 0.1 or 0.3% (equivalent to 20, 200 or 600 mg/kg bw per day, respectively) and a control group of 40 male and 40 female mice received basal diet (Lamb *et al*., 1987). Both male and female mice were exposed during a seven-day premating period and were then randomly grouped as mating pairs. Treatment continued for the 98-day cohabitation period and for 21 days thereafter. Reproductive function was evaluated by measuring the number of litters per breeding pair, number of pups per litter, proportion of pups born alive and mean pup weight. Dietary levels of 0.1 and 0.3% di(2-ethylhexyl) phthalate produced dose-dependent and significant decreases in fertility and in the number and proportion of pups born alive. In males, 0.3% di(2-ethylhexyl) phthalate caused significantly reduced weights of the testes, epididymis, prostate and seminal vesicles. All high-dose males but one showed some degree of bilateral atrophy of the seminiferous tubules. Sperm analysis showed a significant decrease in the percentage of motile sperm and a significantly decreased sperm concentration in cauda epididymis. Exposure to 0.3% di(2-ethylhexyl) phthalate also caused an increased incidence of abnormal sperm forms. Di(2-ethylhexyl) phthalate did not significantly decrease body-weight gain in the high-dose group. A cross-over mating trial conducted with F_0 mice showed a decrease in fertility for both treated males and treated females. Only four litters out of 20 were born to treated males mated with control females and the proportion of pups born alive was decreased. No pups were born when treated females were mated with control males.

A two-generation study in CD-1 mice was performed by feeding 0.01, 0.025 or 0.05% di(2-ethylhexyl) phthalate (> 99% pure) in the diet (NTIS, 1988). The doses were equivalent to about 19, 48 or 95 mg/kg bw per day. The study was carried out to examine the effect of prenatally administered di(2-ethylhexyl) phthalate on the growth, development and reproductive performance of the F_1 generation. The F_1 generation was mated within dose groups at sexual maturity and F_2 offspring were evaluated for viability and growth at postnatal day 4. For F_1 litters, the percentage of prenatal mortality was increased at the high dose from 9% to 26.4%. During the neonatal period, the percentage of viable pups was significantly decreased at the dose of 0.05% di(2-ethylhexyl) phthalate. No other effects of di(2-ethylhexyl) phthalate upon growth, viability, age of acquisition for developmental landmarks (incisor eruption, wire grasping, eye opening, testis descent, or vaginal opening or spontaneous locomotor activity) were observed on postnatal days 14, 21 or 50.

Groups of male and female ICR mice received doses of di(2-ethylhexyl) phthalate ranging from 1 to 100 mL/kg bw by subcutaneous injections on experimental days 1, 5 and 10 and were evaluated on day 21 (Agarwal *et al*., 1989). Doses of 10 mL/kg or greater reduced the fertility of both males and females. At 15 mL/kg, relative testis weights were reduced, while changes in ovarian and testicular ATPase and lysosomal enzymes occurred at 10 mL/kg. Histopathological changes were observed in the testes from 10 mL/kg and in the ovaries from 1 mL/kg.

Thirty mice of an inbred colony were used to study the effect of di(2-ethylhexyl) phthalate on reproductive function (Jain & Joshi, 1991). Fifteen mice were dosed orally with 1000 mg/kg bw di(2-ethylhexyl) phthalate [purity not specified] in 0.1 mL olive oil for one week. The fertility (evaluated by the ability of the motile spermatozoa to fertilize normal cycling females) was reduced from 90 to 75%. Sperm density and sperm motility were also significantly reduced.

(iii) *Marmosets*

In a 13-week study, groups of four mature male marmosets were given daily doses of 0, 100, 500 or 2500 mg/kg bw di(2-ethylhexyl) phthalate. Body-weight gain was significantly depressed at 2500 mg/kg bw. No significant changes were observed in testis weights or histopathology of the testis, epididymis, seminal vesicles or prostate (Kurata *et al*., 1998).

(*d*) *Mechanistic-based reproductive toxicity studies*

The Sertoli cell appears to be the primary site of phthalate toxicity in the testes, and theories have been proposed related to (1) reduced testicular zinc levels, (2) altered hormonal status, (3) altered metabolic function and (4) altered FSH reactivity. None of these alone appears to account for the observed testicular effects (reviewed in Boekelheide, 1993). Younger rats tend to be more sensitive to the testicular effects of phthalates, although this difference appears to be related to changes in absorption, metabolism and distribution rather than to changes in tissue sensitivity (Heindel & Powell, 1992). Administration of 2 g/kg mono(2-ethylhexyl) phthalate by oral gavage to 20-day-old Fischer 344 rats resulted in a collapse of Sertoli cell vimentin filaments within 3 h of exposure. This response was accompanied at first by a decrease, and then several hours later by an increase, in germ-cell apoptosis. The authors suggested that these events may be linked via signal transduction events (Richburg & Boekelheide, 1996). Subsequently, it has been demonstrated that expression of Fas, a trans-membrane receptor protein, and of the Fas ligand (FasL), which occur in the germ and Sertoli cells, respectively, is up-regulated in the Fischer 344 rat testes following exposure to 2 g/kg mono(2-ethylhexyl) phthalate. Expression of Fas in the germ cell is related to apoptosis, and terminal deoxynucleotide transferase (TdT)-mediated dUTP-biotin nick-end labelling (TUNEL)-positive cells (i.e., those showing the staining reaction indicative of apoptosis) were present in same regions as the heightened Fas expression. This suggests that damage to Sertoli cells increases the

output of FasL, leading to increased germ-cell loss in an effort to maintain testicular homeostasis (Lee *et al*., 1997).

The estrogenic activities of phthalates were investigated in competitive ligand-binding assays, yeast and mammalian gene expression assays and in a uterotrophic assay. Di(2-ethylhexyl) phthalate did not compete for estrogen receptors, induce luciferase activity in transfected MCF-7 cells or stably transfected HeLa cells, support estrogen-inducible growth in yeast cells or demonstrate any estrogenic activity in mammalian assays *in vivo* (Zacharewski *et al*., 1998).

The involvement of testosterone in the testicular atrophy caused by di(2-ethylhexyl) phthalate was examined by co-administration of testosterone (1 mg/kg bw) subcutaneously with 2000 mg/kg bw di(2-ethylhexyl) phthalate [purity not specified] in groundnut oil to adult male Wistar rats for 15 days (Parmar *et al*., 1987). Administration of di(2-ethylhexyl) phthalate reduced the sperm count and also significantly increased the activity of γ-GT, lactate dehydrogenase and β-glucuronidase and decreased the activity of sorbitol dehydrogenase and acid phosphatase. Co-administration of testosterone seemed to normalize the sperm count and the activity of testicular enzymes. The role of testosterone in the testicular toxicity of di(2-ethylhexyl) phthalate has not been fully elucidated. Several reports refer to increased or decreased testosterone levels in plasma and testicular tissue.

A study of the influence of a low-protein diet on the testicular toxicity of di(2-ethylhexyl) phthalate was performed in adult male Wistar rats (12 weeks old) (Tandon *et al*., 1992). One group received a synthetic diet containing 20% casein and the other a diet containing 8% starch. After 15 days of consumption, half of the rats in each group received 1000 mg/kg bw di(2-ethylhexyl) phthalate [purity not stated] in 0.2 mL groundnut oil orally for 15 days. The other half served as a control group. The group on the low-protein diet had a more severe response to di(2-ethylhexyl) phthalate in terms of sperm count, and of increased activity of β-glucuronidase and γ-GT.

Gray and Butterworth (1980) found an age-dependent induction of testicular atrophy in rats, with younger rats being more sensitive than older ones. Four-, 10- and 15-week-old Wistar rats were administered 2800 mg/kg bw di(2-ethylhexyl) phthalate [purity not specified] dissolved in corn oil by gavage for 10 days. In some experiments, testosterone propionate (200 μg/kg per day in corn oil) or FSH was given subcutaneously. In four-week-old rats, di(2-ethylhexyl) phthalate produced uniform seminiferous tubular atrophy, comprising loss of spermatids and spermatocytes. In 10-week old rats, 5–50% of the tubules were atrophic; the testicular weight was not affected. A marked decrease in testicular weight (% of control) was seen in four-week-old rats. The weights of the seminal vesicles and ventral prostate were reduced in the four- and 10-week-old males. In four-week-old rats fed a diet containing 2.0% di(2-ethylhexyl) phthalate, the testicular effects were reversible within 12 weeks when treatment was stopped before puberty, but recovery was slower when treatment continued throughout puberty. Simultaneous administration of testosterone or FSH with di(2-ethylhexyl) phthalate did not affect the development of testicular atrophy,

but prevented the reduction of accessory gland weights. The interstitial tissue appeared to be unaffected in all dosed animals. No effects were observed in 15-week-old rats.

Groups of eight male Sprague-Dawley rats (25, 40 or 60 days old) were dosed with 0 or 1000 mg/kg bw di(2-ethylhexyl) phthalate in corn oil by gavage for 14 days (Sjöberg *et al*., 1985d). The liver weight was increased in all three age groups. In the 25-day-old rats, the absolute testicular weight was decreased and histopathological examination showed severe testicular damage, whereas the older animals were unaffected. In the youngest age group, there were a marked reduction in the number of germ cells, a high occurrence of degenerating cells and a reduction in the tubular diameter. There was also a marked reduction in the number of spermatogonia. The authors suggested that this indicated that the damage was not completely reversible. In some animals that were allowed to survive for 100 days, the majority of tubules were totally devoid of germ cells.

To determine which compound or compounds were responsible for the testicular damage after oral administration of di(2-ethylhexyl) phthalate, Sjöberg *et al*. (1986a) administered di(2-ethylhexyl) phthalate and five of its major metabolites (mono(2-ethylhexyl) phthalate, 2-ethylhexanol and three identified metabolites (V, VI, or IX) of mono(2-ethylhexyl) phthalate) for five days. Groups of six male Sprague-Dawley rats (35 days old at the start of the experiment) were given 2.7 mmol/kg bw di(2-ethylhexyl) phthalate (1055 mg/kg bw) or one of the metabolites. Counting of degenerated cells per tubular cross-section was carried out. No testicular damage was observed following oral doses of di(2-ethylhexyl) phthalate or 2-ethylhexanol. The number of degenerated spermatocytes and spermatids was increased in animals receiving mono-(2-ethylhexyl) phthalate; no such effects were seen in animals given the mono(2-ethylhexyl) phthalate-derived metabolites.

In another study, Sjöberg *et al*. (1986b) examined the age-dependent testicular toxicity of di(2-ethylhexyl) phthalate (1000 and 1700 mg/kg bw in the diet for 14 days) in rats at 25, 40 and 60 days of age. Body weight gain was retarded in all treated groups. Testicular weight was markedly reduced and severe testicular damage occurred in the 25- and 40-day-old rats given doses of 1700 mg/kg bw. No changes were found in the 60-day-old rats. Only a few seminiferous tubules (1–10%) were affected in the 1000 mg/kg bw group at any age of exposure. The authors concluded that the difference in response to di(2-ethylhexyl) phthalate of male rats of different ages may be due to higher absorption of the metabolite mono(2-ethylhexyl) phthalate in younger animals.

Groups of eight male Sprague-Dawley rats (four, 10 or 15 weeks of age) were used to study the age-dependence of effects on male reproductive organs (Gray & Gangolli, 1986). The rats were given 2800 mg/kg bw di(2-ethylhexyl) phthalate orally for 10 days. Administration to four-week-old rats produced a marked reduction in absolute weights of the testes, seminal vesicles and prostate. There was only a slight reduction in testis weight in 10-week-old rats, but the seminal vesicle and prostate weights were significantly reduced. Di(2-ethylhexyl) phthalate had no effect in 15-week-old rats. Histologically, the testes of the four-week-old rats showed severe atrophy affecting

virtually all the tubules, which were populated only by Sertoli cells, spermatogonia and occasional primary spermatocytes. In the 10-week-old rats, these histological changes were evident in 5–50% of the tubules, the remainder appearing to be essentially normal. No histological abnormalities were seen in testes from the 15-week-old rats. In the same study, the effects of mono(2-ethylhexyl) phthalate on Sertoli cell function in immature rats were studied by measuring the secretion of seminiferous tubule fluid and androgen-binding protein. A single dose of 1000 mg/kg bw mono(2-ethylhexyl) phthalate reduced fluid and protein production to around 50% of the concurrent control group and to 25% after three repeated doses.

To elucidate further the mechanisms responsible for the enhanced sensitivity of the testes of developing animals to di(2-ethylhexyl) phthalate, the activities of the testicular enzymes associated with spermatogenesis including lactate dehydrogenase, γ-GT, sorbitol dehydrogenase, β-glucuronidase and acid phosphatase were studied in 25-day-old male Wistar rats (Parmar *et al*., 1995). Doses of 0, 50, 100, 250 or 500 mg/kg bw di(2-ethylhexyl) phthalate in groundnut oil were given for 30 consecutive days to groups of six male rats. There was an exposure-related and significant decrease of absolute and relative testicular weight at all dose levels. From 50 mg/kg bw, a dose-dependent and significant increase in the activities of lactate dehydrogenase and γ-GT was noted, while activity of sorbitol dehydrogenase decreased. β-Glucuronidase activity was elevated at 250 or 500 mg/kg bw, while acid phosphatase decreased at the same dose levels. The administration also resulted in marked destructive changes in the advanced germ cell layers and marked degrees of vacuolar degeneration in the testes at 250 and 500 mg/kg bw. The significant alterations in the activities of sorbitol dehydrogenase, lactate dehydrogenase and γ-GT occurred thus at much lower levels of di(2-ethylhexyl) phthalate and before the histopathological changes. The Leydig cells and the fibroblasts appeared to be normal.

4.4 Genetic and related effects

Di(2-ethylhexyl) phthalate was one of the compounds used in the IPCS evaluation of short-term tests for carcinogenicity (IPCS, 1985). The data from this and other evaluations of the genetic and related effects of di(2-ethylhexyl) phthalate have been reviewed (Huber *et al*., 1996).

4.4.1 *Humans*

No data were available to the Working Group.

4.4.2 *Experimental systems* (see Table 7 for references)

Di(2-ethylhexyl) phthalate was not mutagenic to *Salmonella typhimurium* strains TA100, TA102, TA1535, TA1537, TA1538, TA98, TA97 or the TA7000 series in the

Table 7. Genetic and related effects of di(2-ethylhexyl) phthalate

Test system	Result [a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Bacillus subtilis rec, differential toxicity	–	NT	500 µg/disc	Tomita *et al*. (1982b)
Salmonella typhimurium, forward mutation	–	–	500	Liber (1985)
Salmonella typhimurium TA100, TA1535, TA1537, TA1538, TA98, reverse mutation	–	–	9860	Kirby *et al*. (1983)
Salmonella typhimurium TA100, TA98, reverse mutation	–	–	4000	Robertson *et al*. (1983)
Salmonella typhimurium TA100, TA98, reverse mutation	–	–	2000	Yoshikawa *et al*. (1983)
Salmonella typhimurium TA100, TA102, TA98, TA97, reverse mutation	–	–	10 000	Baker & Bonin (1985)
Salmonella typhimurium TA100, TA102, TA98, TA97, reverse mutation	–	–	5000	Matsushima *et al*. (1985)
Salmonella typhimurium TA100, TA102, TA98, TA97, reverse mutation	–	–	10 000	Nohmi *et al*. (1985)
Salmonella typhimurium TA100, TA1535, TA1537, TA1538, TA98, reverse mutation	–	–	5000	Rexroat & Probst (1985)
Salmonella typhimurium TA100, TA1535, TA98, TA97, reverse mutation	–	–	10 000	Zeiger & Haworth (1985)
Salmonella typhimurium TA100, TA1535, TA1537, TA98, reverse mutation	–	–	10 000	Zeiger *et al*. (1985)
Salmonella typhimurium TA1537, TA98, TA7001, TA7002, TA7003, TA7004, TA7005, TA7006, reverse mutation	–	–	1000	Gee *et al.* (1998)
Escherichia coli WP2 uvrA, reverse mutation	–	–	2000	Yoshikawa *et al*. (1983)
Saccharomyces cerevisiae, gene conversion	(+)	(+)	5000	Arni (1985)
Saccharomyces cerevisiae, gene conversion	–	–	2000	Brooks *et al*. (1985)
Saccharomyces cerevisiae, gene conversion	–	–	1000	Inge-Vechtomov *et al*. (1985)

Table 7 (contd)

Test system	Result [a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Saccharomyces cerevisiae, gene conversion	+	+	1500	Mehta & von Borstel (1985)
Saccharomyces cerevisiae D6, aneuploidy	+	+	5000	Parry & Eckardt (1985)
Saccharomyces cerevisiae, gene conversion	–	–	5000	Parry & Eckardt (1985)
Saccharomyces cerevisiae, homozygosis	–	–	5000	Arni (1985)
Saccharomyces cerevisiae, homozygosis	–	–	1000	Inge-Vechtomov *et al.* (1985)
Aspergillus nidulans haploid, mutation	–	NT	9900	Carere *et al.* (1985)
Aspergillus nidulans non-disjunction	–	NT	9900	Carere *et al.* (1985)
Aspergillus nidulans, mitotic crossing-over	–	NT	9900	Carere *et al*. (1985)
Saccharomyces cerevisiae, forward mutation	–	–	1000	Inge-Vechtomov *et al.* (1985)
Saccharomyces cerevisiae, reverse mutation	–	–	5000	Arni (1985)
Saccharomyces cerevisiae, reverse mutation	–	–	1000	Inge-Vechtomov *et al.* (1985)
Saccharomyces cerevisiae, reverse mutation	+	+	1500	Mehta & von Borstel (1985)
Saccharomyces cerevisiae, reverse mutation	–	–	5000	Parry & Eckardt (1985)
Saccharomyces pombe, forward mutation	?	–	5900	Loprieno *et al*. (1985)
Saccharomyces cerevisiae DEL assay, and ICR recombination	–	–	200 000	Carls & Schiestl (1994)
Drosophila melanogaster, crossing-over/recombination	–		39 000 µg/g food	Würgler *et al*. (1985)
Drosophila melanogaster, somatic mutation	(+)		6930 µg/cm^2 food surface	Fujikawa *et al*. (1985)
Drosophila melanogaster, somatic mutation	(+)		780 µg/g food	Vogel (1985)
Drosophila melanogaster, somatic mutation	–		39 000 µg/g food	Würgler *et al*. (1985)

Table 7 (contd)

Test system	Result [a]		Dose [b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Drosophila melanogaster, sex-linked recessive lethal mutation	–		20 inj.	Yoon *et al.* (1985)
Drosophila melanogaster, sex-linked recessive lethal mutation	–		18 600 µg/g food	Zimmering *et al.* (1989)
Drosophila melanogaster, DNA double strand breakage *in vivo*	–		7540 µg/g food	Kawai (1998)
Drosophila melanogaster, DNA repair test *in vivo*	–		7540 µg/g food	Kawai (1998)
Drosophila melanogaster, wing spot test, mutation *in vivo*	–		7540 µg/g food	Kawai (1998)
DNA single-strand breaks, rat hepatocytes *in vitro*	–	NT	3900	Bradley (1985)
DNA strand breaks, Chinese hamster ovary cells *in vitro*	–	–	39 000	Douglas *et al*. (1985)
DNA single-strand breaks, rat or Syrian hamster hepatocytes *in vitro*	–	NT	9750	Schmezer *et al.* (1988)
Unscheduled DNA synthesis, rat primary hepatocytes	–	NT	3900	Butterworth *et al*. (1984)
Unscheduled DNA synthesis, rat primary hepatocytes	–	NT	3900	Kornbrust *et al*. (1984)
Unscheduled DNA synthesis, rat primary hepatocytes	–	NT	3900	Probst & Hill (1985)
URP, Unscheduled DNA synthesis, rat primary hepatocytes	–	NT	10 000	Williams *et al*. (1985)
URP, Unscheduled DNA synthesis, rat primary hepatocytes	–	NT	1000	Astill *et al*. (1986)
UIA, Unscheduled DNA synthesis, B6C3F$_1$ mouse primary hepatocytes *in vitro*	–	NT	390	Smith-Oliver & Butterworth (1987)
Gene mutation, mouse lymphoma L5178Y cells, *Tk* locus *in vitro*	(+)	–	980	Kirby *et al.* (1983)
Gene mutation, mouse lymphoma L5178Y cells, *Tk* locus *in vitro*	?	–	2500	Amacher & Turner (1985)
Gene mutation, mouse lymphoma L5178Y cells, *Tk* locus *in vitro*	–	–	4900	Myhr *et al*. (1985)
Gene mutation, mouse lymphoma L5178Y cells, *Tk* locus *in vitro*	(+)	(+)	7.5	Oberly *et al*. (1985)

Table 7 (contd)

Test system	Result [a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Gene mutation, mouse lymphoma L5178Y cells, *Tk* locus *in vitro*	–	–	9800	Styles *et al*. (1985)
Gene mutation, mouse lymphoma L5178Y cells, *Tk* locus *in vitro*	–	–	250	Astill *et al*. (1986)
Gene mutation, mouse lymphoma L5178Y cells, ouabain resistance *in vitro*	–	–	200	Garner & Campbell (1985)
Gene mutation, mouse lymphoma L5178Y cells, ouabain resistance *in vitro*	–	–	9800	Styles *et al*. (1985)
Gene mutation, BALB/c-3T3 mouse cells, ouabain resistance *in vitro*	NT	–	1960	Matthews *et al*. (1985)
Sister chromatid exchange, Chinese hamster Don cells *in vitro*	–	NT	3900	Abe & Sasaki (1977)
Sister chromatid exchange, Chinese hamster ovary cells *in vitro*	–	–	3900	Douglas *et al*. (1985)
Sister chromatid exchange, Chinese hamster ovary cells *in vitro*	(+)	–	5000	Gulati *et al*. (1985)
Sister chromatid exchange, rat liver RL4 cells *in vitro*	–	NT	1000	Priston & Dean (1985)
Micronucleus formation, Chinese hamster ovary cells *in vitro*	–	–	3900	Douglas *et al*. (1985)
Micronucleus formation, rat hepatocytes *in vitro*	–	NT	3900	Müller-Tegethoff *et al*. (1995)
Micronucleus formation, Syrian hamster embryo cells, *in vitro*	+	NT	NG	Fritzenschaf *et al.* (1993)
Chromosomal aberrations, Chinese hamster Don cells *in vitro*	–	NT	3900	Abe & Sasaki (1977)
Chromosomal aberrations, Chinese hamster lung cells *in vitro*	–	NT	160	Ishidate & Odashima (1977)
Chromosomal aberrations, Chinese hamster ovary cells *in vitro*	–	NT	781	Phillips *et al*. (1982)
Chromosomal aberrations, Chinese hamster liver cells *in vitro*	–	NT	50	Danford (1985)
Chromosomal aberrations, Chinese hamster ovary cells *in vitro*	–	–	5000	Gulati *et al*. (1985)
Chromosomal aberrations, Chinese hamster lung cells *in vitro*	–	–	4130	Ishidate & Sofuni (1985)

Table 7 (contd)

Test system	Result [a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Chromosomal aberrations, rat liver RL4 cells *in vitro*	–	NT	1000	Priston & Dean (1985)
Chromosomal aberrations, Syrian hamster embryo cells *in vitro*	–	+	39	Tsutsui *et al*. (1993)
Aneuploidy, Chinese hamster liver cells *in vitro*	(+)	NT	50	Danford (1985)
Mitotic aberrations, Chinese hamster primary liver cells *in vitro*	(+)	NT	50	Parry (1985)
Aneuploidy, rat liver RL4 cells *in vitro*	–	NT	1000	Priston & Dean (1985)
Cell transformation, BALB/3T3 mouse cells	–	–	25 000	Matthews *et al*. (1985)
Cell transformation, BALB/3T3 mouse cells	–	–	20	Astill *et al*. (1986)
Cell transformation, C3H10T½ mouse cells	(+)	(+)	40	Lawrence & McGregor (1985)
Cell transformation, C3H10T½ mouse cells	–	NT	3.9	Sanchez *et al*. (1987)
Cell transformation, Syrian hamster embryo cells, clonal assay	+	NT	1	Barrett & Lamb (1985)
Cell transformation, Syrian hamster embryo cells, clonal assay	+	NT	4	Sanner & Rivedal (1985)
Cell transformation, Syrian hamster embryo cells, clonal assay	+	NT	10	Mikalsen *et al*. (1990)
Cell transformation, Syrian hamster embryo cells, clonal assay	+	NT	30	Mikalsen & Sanner (1993)
Cell transformation, Syrian hamster embryo cells, clonal assay	(+)	+	1.2	Tsutsui *et al*. (1993)
Ornithine decarboxylase superinduction, Syrian hamster embryo cells[d]	–	NT	39	Dhalluin *et al*. (1998)
Cell transformation, RLV/Fischer rat	+	NT	1000	Suk & Humphreys (1985)
Cell transformation, SA7/Syrian hamster embryo cells	+	NT	500	Hatch & Anderson (1985)
Cell transformation, Syrian hamster embryo cells	+	NT	39	Dhalluin *et al*. (1998)
Unscheduled DNA synthesis, human hepatocytes *in vitro*	–	NT	3900	Butterworth *et al*. (1984)
Gene mutation, human lymphocytes, *TK* and *HPRT* loci *in vitro*	–	–	1000	Crespi *et al*. (1985)
Sister chromatid exchange, human lymphocytes *in vitro*	–	–	1000	Obe *et al*. (1985)
Sister chromatid exchange, human lymphocytes (metabolic activation by co-cultured with rat liver cells) *in vitro*	–	(+)	39	Lindahl-Kiessling *et al*. (1989)
Chromosomal aberrations, human lymphocytes *in vitro*	–	NT	75	Turner *et al*. (1974)

Table 7 (contd)

Test system	Result [a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Chromosomal aberrations, human lymphocytes *in vitro*	–	NT	60	Stenchever *et al*. (1976)
Chromosomal aberrations, human lymphocytes *in vitro*	–	NT	160	Tsuchiya & Hattori (1976)
Aneuploidy, human fetal lung cells *in vitro*	–	NT	6	Stenchever *et al*. (1976)
Comet assay on human blood *in vitro*	+	–	156	Anderson *et al.* (1999)
Body fluids, Sprague Dawley rat urine, microbial mutagenicity	–	–	2000 × 15 po	DiVincenzo *et al*. (1985)
DNA strand breaks, Wistar rat liver *in vivo*	–		2000 × 28 po	Elliott & Elcombe (1987)
DNA oxidative damage, Fischer 344 rat liver *in vivo*	+ [c]		12 000 mg/kg diet 1 y	Takagi *et al*. (1990a)
DNA oxidative damage, Fischer 344 rat liver *in vivo*	+ [c]		12 000 mg/kg diet, 1–2 w	Takagi *et al*. (1990b)
DNA single-strand breaks, Fischer 344 rat liver *in vivo*	–		20 000 mg/kg diet 78w	Tamura *et al*. (1991)
DNA oxidative damage, Fischer 344 rat liver *in vivo*	–		12 000 mg/kg diet 22w	Cattley & Glover (1993)
Unscheduled DNA synthesis, Fischer 344 rat hepatocytes *in vivo*	–		500 × 1 po, 150 × 14 po or 12 000 ppm diet, 30 days + 500 × 1 po	Butterworth *et al*. (1984)
Unscheduled DNA synthesis, Sprague-Dawley rat hepatocytes *in vivo*	–		5000 × 1 po	Kornbrust *et al*. (1984)
Unscheduled DNA synthesis, Fischer 344 rat hepatocytes *in vivo*	–		12 000 mg/kg diet 28 d	Cattley *et al*. (1988)
Unscheduled DNA synthesis, B6C3F_1 mouse hepatocytes *in vivo*	–		6000 mg/kg diet 28 d	Smith-Oliver & Butterworth (1987)

Table 7 (contd)

Test system	Result [a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Gene mutation, *lac*I transgenic C57BL/6 mouse liver *in vivo*	–		6000 mg/kg diet 120 d	Gunz *et al*. (1993)
Micronucleus formation, mice *in vivo*	–		5000 × l po	Astill *et al*. (1986)
Micronucleus formation, B6C3F$_1$ mouse erythrocytes *in vivo*	–		6000 × 5 ip	Douglas *et al*. (1986)
Chromosomal aberrations, Fischer 344 rat bone marrow *in vivo*	–		4900 × 5 po	Putman *et al*. (1983)
Chromosomal aberrations, Syrian hamster embryos *in vivo*	+		7500 × 1 po	Tomita *et al*. (1982b)
Dominant lethal test, ICR Swiss mice *in vivo*	+		12 780 × 1 ip	Singh *et al*. (1974)
Dominant lethal test, mice *in vivo* [strain not specified]	+		980 × 3 sc	Autian (1982)
Dominant lethal test, ICR Swiss mice *in vivo*	+		980 × 3 sc	Agarwal *et al*. (1985)
Aneuploidy, Fischer 344 rat hepatocytes *in vivo*	–		12 000 mg/kg diet 7 d	Hasmall & Roberts (1997)
Cell transformation, Syrian hamster embryos *in vivo/in vitro*	+		7500 po	Tomita *et al*. (1982b)
Binding (covalent) to Fischer 344 rat hepatocyte DNA *in vitro*	–	NT	390	Gupta *et al*. (1985)
Binding (covalent) to Fischer 344 rat liver DNA *in vivo*	–		10 000 mg/kg diet 11 d	Albro *et al*. (1982)
Binding (covalent) to Fischer 344 rat liver DNA *in vivo*	–		10 000 mg/kg diet 4 w	Däniken *et al*. (1984)
Binding (covalent) to Fischer 344 rat liver DNA *in vivo*	–		2000 × 3 po	Gupta *et al*. (1985)
Binding (covalent) to Fischer 344 rat liver DNA *in vivo*	–		500 × 1 po	Lutz (1986)
Inhibition of gap-junctional intercellular communication, cynomolgus monkey liver cells *in vivo*	–		500 × 14 po	Pugh *et al.* (1999)
Inhibition of gap-junctional intercellular communication, Chinese hamster V79 cells *in vitro*	+	NT	3	Malcolm *et al*. (1983)
Inhibition of gap-junctional intercellular communication, Chinese hamster V79 cells *in vitro*	–	NT	0.1	Kornbrust *et al*. (1984)

Table 7 (contd)

Test system	Result [a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Inhibition of gap-junctional intercellular communication, Chinese hamster V79 cells *in vitro*	+	NT	5	Elmore *et al*. (1985)
Inhibition of gap-junctional intercellular communication, Chinese hamster V79 cells *in vitro*	+	NT	10	Malcolm & Mills (1989)
Inhibition of gap-junctional intercellular communication, Syrian hamster embryo cells *in vitro*	+	NT	30	Mikalsen & Sanner (1993)
Inhibition of gap-junctional intercellular communication, Chinese hamster V79 cells *in vitro*	+	NT	78	Vang *et al*. (1993)
Inhibition of gap-junctional intercellular communication, Chinese hamster V79 cells and Syrian hamster embryo cells *in vitro*	+	NT	10	Cruciani *et al*. (1997)
Sperm morphology, B6C3F_1 mice *in vivo*	–		6000 × 5 ip	Douglas *et al*. (1986)
Sperm morphology, Sprague Dawley rats *in vivo*	–		5200 × 5 ip	Douglas *et al*. (1986)
Metabolites				
Mono(2-ethylhexyl) phthalate (MEHP)				
Bacillus subtilis rec, differential toxicity	+	NT	400 µg/disc	Tomita *et al.* (1982b)
Salmonella typhimurium TA100, reverse mutation	–	–	1250 µg/plate	Tomita *et al.* (1982b)
Salmonella typhimurium TA100, TA1535, TA1537, TA1538, TA98, reverse mutation	–	–	0.2 µL/plate	Kirby *et al.* (1983)
Salmonella typhimurium TA100, TA102, TA 98, TA 97, reverse mutation	–	–	1000 µg/plate	Dirven *et al.* (1991)
Unscheduled DNA synthesis, B6C3F_1 mouse primary hepatocytes *in vitro*	–	NT	139	Smith-Oliver & Butterworth (1987)

Table 7 (contd)

Test system	Result [a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Gene mutation, mouse lymphoma L5178Y cells, *Tk* locus *in vitro*	–	–	0.3 µL/mL	Kirby *et al.* (1983)
Sister chromatid exchange, Chinese hamster V79 cells *in vitro*	+	NT	25	Tomita *et al.* (1982b)
Chromosomal aberrations in Syrian hamster embryo cells *in vitro*	–	+	2.8	Tsutsui *et al.* (1993)
Cellular transformation in Syrian hamster embryo cells *in vitro*	–	(+)	56	Tsutsui *et al.* (1993)
Cell transformation, Syrian hamster embryo cells, clonal assay	+	NT	23	Mikalsen *et al.* (1990)
Cell transformation, C3H10T½ mouse cells	–	NT	417	Sanchez *et al.* (1987)
Inhibition of gap-junctional intercellular communication, Chinese hamster V79 cells and Syrian hamster embryo cells *in vitro*	+	NT	28	Cruciani *et al.* (1997)
Unscheduled DNA synthesis, human primary hepatocytes *in vitro*	–	NT	139	Butterworth *et al.* (1984)
DNA strand breaks, Wistar rat liver *in vivo*	–	NT	500 po × 14	Elliot & Elcombe (1987)
Mono(2-ethyl 5-hydroxyhexyl) phthalate (IX)				
Salmonella typhimurium TA100, TA102, TA98, TA97, reverse mutation	–	–	1000 µg/plate	Dirven *et al.* (1991)
Mono(2-ethyl 5-oxohexyl) phthalate (VI)				
Salmonella typhimurium TA100, TA102, TA98, TA97, reverse mutation	–	–	1000 µg/plate	Dirven *et al.* (1991)
Mono(5-carboxyl 2-ethylpentyl) phthalate (V)				
Salmonella typhimurium TA100, TA102, TA98, TA97, reverse mutation	–	–	1000 µg/plate	Dirven *et al.* (1991)

Table 7 (contd)

Test system	Result [a]		Dose [b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
2-Ethylhexanol				
Bacillus subtilis rec, differential toxicity	–	NT	500	Tomita *et al.* (1982b)
Salmonella typhimurium TA100, TA1535, TA1537, TA1538, TA98, reverse mutation	–	–	1 µL/plate	Kirby *et al.* (1983)
Gene mutation, mouse lymphoma L5178 cells, *Tk* locus *in vitro*	–	–	0.3 µL/mL	Kirby *et al.* (1983)
Phthalic acid				
Bacillus subtilis rec, differential toxicity	–	NT	500	Tomita *et al.* (1982b)

[a] +, positive; (+), weakly positive; –, negative; NT, not tested; ?, inconclusive

[b] LED, lowest effective dose; HID, highest ineffective dose; in-vitro tests, µg/mL; in-vivo tests, mg/kg bw/day; po, oral; ip, intraperitoneal; d, day; w, week; y, year; sc, subcutaneous

[c] No oxidative damage in kidney DNA

[d] Positive if followed by 5 h 0.16 µM 12-*O*-tetradecanoylphorbol 13-acetate

presence or absence of exogenous metabolic activation. It was also not mutagenic to *Escherichia coli* WP2 *uvr*A in the presence or absence of exogenous metabolic activation. It did not induce gene mutations in *Saccharomyces cerevisiae* strain D7 in four of five studies. It induced aneuploidy in a single study. Mitotic gene conversion was induced in two out of five studies with *S. cerevisiae*. In a single study, mitotic recombination was not induced in *S. cerevisiae* in the presence or absence of exogenous metabolic activation. Neither mutation nor genetic crossing-over was induced in *Aspergillus nidulans* cultured with di(2-ethylhexyl) phthalate in the absence of exogenous metabolic activation in one study.

A small increase in somatic mutation frequency was reported in the eye-colour spot test with *Drosophila melanogaster* exposed to di(2-ethylhexyl) phthalate in the feed but no effect was observed in two independent wing-spot tests. In a single study, mitotic recombination was not induced by di(2-ethylhexyl) phthalate; neither was sex-linked recessive lethal mutation induced in two studies in *D. melanogaster* treated with di(2-ethylhexyl) phthalate in the feed or by injection. When administered to *D. melanogaster*, di(2-ethylhexyl) phthalate and *N*-nitrosodiethylamine induced DNA double-strand breakage and DNA repair, although neither compound was active when administered alone.

DNA single-strand breaks were not induced by di(2-ethylhexyl) phthalate in primary cultures of rat or Syrian hamster hepatocytes or in Chinese hamster ovary (CHO) cells. Unscheduled DNA synthesis was not induced in rat or mouse primary hepatocytes exposed to di(2-ethylhexyl) phthalate. Only one of six studies reported induction of gene mutations at the *Tk* locus in mouse lymphoma L5178Y cells exposed to di(2-ethylhexyl) phthalate in the absence of an exogenous metabolic activation system. Ouabain-resistant mutants were not induced in mouse lymphoma or BALB/c-3T3 cells.

In one of two studies, di(2-ethylhexyl) phthalate induced a small increase in sister chromatid exchange frequencies in Chinese hamster ovary cells cultured without but not with exogenous metabolic activation. In other studies conducted only without metabolic activation, it caused no increase in sister chromatid exchanges in either Chinese hamster Don cells or rat liver RL4 cells. Di(2-ethylhexyl) phthalate did not induce micronuclei in Chinese hamster ovary cells or in cultured rat hepatocytes, whereas the induction of micronuclei by di(2-ethylhexyl) phthalate in Syrian hamster embryo cells has been reported.

Chromosomal aberrations were not induced by di(2-ethylhexyl) phthalate in any of eight studies in various types of cultured cells in the absence of metabolic activation. Only three of these studies for chromosomal aberrations included an exogenous metabolic activation system. Of these, one, using Syrian hamster embryo cells, found an increase in aberration frequency. Weak effects were detected for the induction of aneuploidy and mitotic division aberrations in Chinese hamster lung cells.

Di(2-ethylhexyl) phthalate induced cell transformation in the Syrian hamster embryo clonal assay, the virally enhanced SA7/Syrian hamster embryo assay and

RLV/Fischer rat assay without the addition of an exogenous metabolic activation system. In a single study, mouse JB6 epidermal cells were transformed by di(2-ethylhexyl) phthalate without activation and in one of two studies a weak response was reported in the C3H10T½ cell transformation assay with di(2-ethylhexyl) phthalate in either the absence or presence of exogenous metabolic activation. BALB/c-3T3 cells were not transformed by di(2-ethylhexyl) phthalate with or without metabolic activation. Di(2-ethylhexyl) phthalate inhibited gap-junctional intercellular communication in Chinese hamster V79 cells in six of seven studies, but not in one study of liver cells of cynomolgus monkeys *in vivo*. Di(2-ethylhexyl) phthalate treatment of Syrian hamster embryo cells in a two-stage exposure with 12-*O*-tetradecanoylphorbol 13-acetate resulted in superinduction of ornithine decarboxylase, an early event in morphological transformation; no effect was seen after a one-stage treatment with di(2-ethylhexyl) phthalate alone.

Di(2-ethylhexyl) phthalate did not increase the frequency of chromosomal aberrations in human lymphocytes in the absence of exogenous metabolic activation *in vitro*. In single studies with human lymphocytes *in vitro*, di(2-ethylhexyl) phthalate did not induce unscheduled DNA synthesis or gene mutations at the *TK* or *HPRT* loci without exogenous metabolic activation, or aneuploidy in human fetal lung cells cultured without metabolic activation. One single study using human lymphocytes found that di(2-ethylhexyl) phthalate did not induce sister chromatid exchange, while another study found weak induction when the lymphocytes were co-cultured with rat liver cells. A single study investigating the effect of di(2-ethylhexyl) phthalate on human blood cells by the Comet assay reported induction of DNA damage but none in the presence of exogenous metabolic activation.

Urine samples from rats treated by gavage with 15 daily doses of di(2-ethylhexyl) phthalate were not mutagenic to *S. typhimurium* strains TA100, TA1535, TA1537, TA1538 or TA98. In one study, the formation of 8-hydroxydeoxyguanosine (8-OH-dG) was measured as an indicator of DNA oxidative damage in liver and kidney of rats exposed to di(2-ethylhexyl) phthalate in the diet for one to two weeks: 44–64% increased levels of 8-OH-dG were observed in liver but not kidney DNA. Similar results were reported in a follow-up study in which rats received di(2-ethylhexyl) phthalate in the diet for one year. A significant increase in 8-OH-dG was measured in liver DNA at the one- and 12-month sampling times but not at three, six or nine months. Di(2-ethylhexyl) phthalate did not induce DNA strand breaks in rat liver or unscheduled DNA synthesis in rat or mouse liver following single or multiple oral treatments, nor did it give rise to covalent DNA binding in rat hepatocytes *in vitro* or rat liver *in vivo*.

Di(2-ethylhexyl)phthalate did not increase the mutation frequency in the *lac*I gene of liver DNA isolated from female *lac*I transgenic mice given di(2-ethylhexyl) phthalate in the feed for 120 days. Micronuclei were not induced in mice fed with di(2-ethylhexyl) phthalate. Di(2-ethylhexyl)phthalate did not induce chromosomal aberrations in rat bone marrow sampled 6 h after the last of five daily treatments

administered by gavage and it did not induce aneuploidy in hepatocytes of rats fed a diet containing di(2-ethylhexyl) phthalate for seven days. One study reported that di(2-ethylhexyl) phthalate was antimutagenic and co-recombinogenic in combination with *N*-ethyl-*N*-nitrosourea in the mouse spot test (Fahrig & Steinkamp-Zucht, 1996). Chromosomal aberrations and cell transformations were induced in Syrian golden hamster embryos exposed transplacentally to di(2-ethylhexyl) phthalate for 24 h after pregnant females were treated on day 11 of gestation. Di(2-ethylhexyl) phthalate was reported to induce dominant lethal mutations in mice in three studies. Two re-evaluations (Adler & Ashby, 1989; Ashby & Clapp, 1995) of these studies considered that cytotoxicity can interfere with the recognition of a dominant lethal effect, so that this reported mutagenic activity of di(2-ethylhexyl) phthalate is questionable. Di(2-ethylhexyl) phthalate did not induce changes in sperm morphology in mice or rats.

Metabolites

Five metabolites of di(2-ethylhexyl) phthalate, mono(2-ethylhexyl) phthalate, mono(2-ethyl-5-hydroxyhexyl) phthalate (IX), mono(2-ethyl-5-oxohexyl) phthalate (VI), mono(5-carboxy-2-ethylpentyl) phthalate (V) and 2-ethylhexanol, were tested in genotoxicity assays. None induced mutations in *S. typhimurium*.

Negative results were also obtained in the *Bacillus subtilis* rec assay with mono(2-ethylhexyl) phthalate, 2-ethylhexanol and phthalic acid.

Unscheduled DNA synthesis was not induced in either mouse or human primary hepatocyte cultures with mono(2-ethylhexyl) phthalate, and neither this metabolite nor 2-ethylhexanol induced mutations in mouse lymphoma cells *in vitro*. Mono(2-ethylhexyl) phthalate induced sister-chromatid exchange in Chinese hamster V79 cells and chromosomal aberrations in Syrian hamster embryo cells. It also induced transformation in Syrian hamster embryo cells, but not in mouse C3H10T½ cells. Gap-junctional intercellular communication was inhibited by mono(2-ethylhexyl) phthalate in Syrian hamster embryo cells and in Chinese hamster V79 cells. As reported in an abstract (Baker *et al.*, 1996), this function was also inhibited in rat and mouse hepatocytes, but not in Syrian hamster or human hepatocytes.

In the single study with mono(2-ethylhexyl) phthalate, DNA strand breaks were not induced in rat liver *in vivo*.

4.5 Mechanistic considerations

Significant species differences have been observed in the absorption and disposition of di(2-ethylhexyl) phthalate. The peroxisome-proliferating effect of di(2-ethylhexyl) phthalate has been related most specifically in susceptible species to metabolites VI, IX and mono(2-ethylhexyl) phthalate; however, analysis of the disposition data does not provide an explanation for the observed species differences in di(2-ethylhexyl) phthalate-induced hepatic peroxisome proliferation.

The weight of evidence for di(2-ethylhexyl) phthalate 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 and increases in peroxisomal volume density by di(2-ethylhexyl) phthalate *in vivo* under bioassay conditions (Marsman *et al*., 1988; Lake *et al*., 1987) supports this hypothesis. Supporting data on induction of oxidative stress (lipofuscin accumulation, 8-OH-dG in DNA) in rat liver by di(2-ethylhexyl) phthalate are available (Conway *et al*., 1989; Takagi *et al*., 1990a); however, there are no data for mouse liver.

Similarly, 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 probably involved in the promotion of growth of preneoplastic hepatocytes. There is clear evidence that di(2-ethylhexyl) phthalate causes acute and sustained hepatocellular proliferation under bioassay conditions which resulted in liver tumours in rats (Marsman *et al*., 1988).

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 to di(2-ethylhexyl) phthalate of human versus rodent livers *in vivo*. Evidence from humans, rats and mice indicates that differences in disposition, including formation of active metabolites, are of a quantitative, not qualitative nature. Taken together, these disposition data do not provide an explanation for the species differences in response to di(2-ethylhexyl) phthalate. However, several studies using human hepatocytes, unlike those with hepatocytes from mice and rats, have demonstrated that administration of di(2-ethylhexyl) phthalate metabolites does not lead to peroxisome proliferation *in vitro*. A growing body of evidence concerning the molecular basis of peroxisome proliferation, summarized below, indicates why human livers and hepatocytes would be expected to be refractory to induction of peroxisome proliferation and carcinogenesis by di(2-ethylhexyl) phthalate.

Several lines of investigation have enhanced our understanding of the molecular basis of peroxisome proliferation, with important implications for cancer risk and species differences. In rodent liver, increased organ weight, peroxisome proliferation, increased replicative DNA synthesis and induction of peroxisomal and microsomal fatty

acid-oxidizing enzymes require the expression of functional PPARα. This nuclear receptor is a member of the steroid hormone receptor superfamily and binds to DNA as a heterodimer with the retinoid X receptor (RXR). Peroxisome proliferator response elements (PPREs) have been found in genes for both peroxisomal and microsomal fatty acid-oxidizing enzymes (Lake, 1995b; Cattley *et al*., 1998). Studies with PPARα knock-out mice have demonstrated that all the above effects are lost in these mice (Lee *et al*., 1995; Peters *et al*., 1997a; Aoyama *et al*., 1998; Ward *et al*., 1998). Moreover, unlike wild-type mice, the potent peroxisome proliferator Wy-14,643 did not produce liver tumours in PPARα-deficient mice (Peters *et al*., 1997a). In this study, WY-14,643 at 0.1% in the diet or control diet was fed for 11 months to PPARα-deficient and wild-type mice and the livers were removed and examined. Of nine PPARα-deficient mice fed WY-14,643, three died and were lost to evaluation and one was euthanized and examined when moribund before 11 months. WY-14,643 produced multiple hepatocellular tumours in 6/6 wild-type mice; in three of these mice, some of these tumours were malignant. In contrast, the same treatment produced no tumours in PPARα-deficient mice (0/9), and no preneoplastic lesions were found by microscopy. No gross tumours or microscopic evidence of preneoplasia were seen in either wild-type mice or PPARα-deficient mice on the control diet (incidence, 0/9 and 0/9, respectively). Taken together, these results demonstrate that peroxisome proliferation, related hepatic effects and carcinogenesis induced by peroxisome proliferators are all absolutely dependent on functional PPARα.

Studies of PPARα activation *in vitro* have shown that di(2-ethylhexyl) phthalate metabolites are capable of activating PPARα. A study of PPARα knock-out mice treated with di(2-ethylhexyl) phthalate *in vivo* demonstrated that the increased liver weights and induction of peroxisomal and microsomal enzymes are absolutely dependent on PPARα (Ward *et al*., 1998).

The species differences, particularly with respect to humans compared to rats and mice, can be potentially attributed to several aspects of PPARα-mediated regulation of gene expression. These include the level of expression and functional capability of PPARα, the presence or absence of active PPREs in the promoter region of specific genes, and other aspects of interaction with transcriptional regulatory proteins.

Recent evidence confirms that species differences can involve more than one aspect of PPARα-mediated regulation of gene expression. The insensitivity of human liver to rodent peroxisome proliferators is associated with low levels of expression of PPARα in human liver. Marked species differences in the expression of PPARα mRNA have been demonstrated between rodent and human liver, with the latter expressing 1–10% of the levels found in mouse or rat liver (Palmer *et al*., 1994; Tugwood *et al*., 1996; Palmer *et al*., 1998). Using a sensitive and specific immuno/DNA binding assay, Palmer *et al*. (1998) have shown that active PPARα protein is expressed at variable concentrations in human livers. The study compared 20 different human livers and found that those with the highest levels of PPARα protein expression contained less than 10% of the level in mice. Most of the samples (13/20) contained no detectable PPARα activity, but did

exhibit PPRE-binding activity that did not correspond to PPARα. All six subjects aged 10 years or less lacked detectable PPARα activity. Such data support a 'threshold through competition' concept. In most human samples studied, it was found that PPREs are mainly bound by other competing proteins that may block peroxisome proliferator responsiveness. In addition, the low levels of PPARα protein detected in human liver were lower than those estimated from RNA analysis and this was explained by the finding that a significant fraction of PPARα mRNA is mis-spliced in human liver. It is possible that the low level of PPARα in human liver may explain, at least in part, the apparent lack of responsiveness of human hepatocytes to peroxisome proliferators. In support of this hypothesis, Palmer *et al.* (1998) have shown that transcriptional responses to peroxisome proliferators are not evident in the Huh cell line, which contains similar levels of PPARα and RXR to those found in intact human liver. However, increasing PPARα levels by heterologous expression in the Huh cell line conferred responsiveness to peroxisome proliferators, suggesting that the low level of endogenous PPARα is the major factor in the non-responsiveness of these cells.

The truncated human PPARα resulting from mRNA mis-splicing, identified by Palmer *et al.* (1998), has been further characterized by Gervois *et al.* (1999). They found that the truncated PPARα mRNA accounted for 25–50% of total PPARα mRNA in 10 human liver samples, while no truncated PPARα mRNA was found in livers of rats and mice. The truncated human PPARα mRNA was expressed *in vitro*, where it was shown to (*a*) fail to bind to PPRE, a necessary step for gene activation and (*b*) interfere with gene activation by expressed full-length human PPARα, in part due to titration of coactivator CREB-binding protein, an additional element of transcriptional regulation. Taken together, these results demonstrate that a certain fraction of human PPARα is probably truncated and inactive and may interfere with the function of any full-length human PPARα present in human hepatocytes.

Peroxisome proliferator responsiveness is unlikely to be a linear function of PPARα expression levels. This may be due to competition for binding to PPREs by other transcription factors such as HNF4, ARP-1 and TR (Palmer *et al*., 1994; Miyamoto *et al*., 1997), binding of PPARα to other transcription factors (e.g., LXRα) leading to formation of non-functional heterodimers (Miyata *et al*., 1996), competition of PPARα with other hormone receptors (e.g., TR) for dimerization with RXR (Juge-Aubry *et al*., 1995) or lack of co-activating proteins such as steroid receptor co-activator-1 (SRC-1) and PPAR-binding protein (PBP) (Zhu *et al*., 1997). Other human PPARs may compete with PPARα; for example PPAR* (hNUC1) can repress the activation of hPPARα (Jow & Mukherjee, 1995).

In addition to the role of receptor expression and other transcriptional regulators, differential species sensitivity to peroxisome proliferators could depend on gene-specific factors. In the case of peroxisomal acyl-coenzyme A oxidase, the promoter regions containing PPRE responsible for transcriptional activation of the rodent gene are not present in the promoter region of the human gene (Lambe *et al.*, 1999; Woodyatt *et al*., 1999). It is possible that similar differences in promoter regions of

other genes could also help to explain differences between rodent and human responses to peroxisome proliferators.

The insensitivity towards peroxisome proliferators exhibited by human hepatocytes is reflected in guinea-pigs. Guinea-pigs are also refractory to the hepatic effects of rodent peroxisome proliferators (reviewed in Doull *et al*., 1999), including di(2-ethylhexyl) phthalate metabolites (Elcombe & Mitchell, 1986; Dirven *et al*., 1993c; Elcombe *et al*., 1996) and, like humans, express similar low levels of PPARα (Bell *et al*., 1998; Tugwood *et al*., 1998). Significant responses (peroxisome proliferation, induction of fatty acid-oxidizing enzymes and stimulation of replicative DNA synthesis) believed to be associated with hepatocarcinogenesis in rodents are not observed in humans or 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). The 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 with those that regulate hypertrophic or hyperplastic responses: the former may have a lower threshold of activation and may require lower concentrations of receptors 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 addition to results from guinea-pigs, recent studies with rabbits suggest a further potential model for the refractory nature of human liver to peroxisome proliferation (Staels & Auwerx, 1998). In humans, fibrates increase plasma levels of high-density lipoprotein (HDL) via induction of human *apo A-I* gene expression. The fibrate effect on human *apo A-I* is mediated by PPARα that interacts with a PPRE in its promoter. In normal rabbits, plasma lipoprotein levels are not changed by fibrate treatment. However, fibrate treatment of transgenic apo A-I rabbits results in increased plasma HDL and human apo A-I concentrations due to induction of the human apoA-I transgene expression in the rabbit liver. Significantly, this induction of lipoprotein gene expression occurs without affecting liver weight or peroxisomal acyl-coenzyme A oxidase activity. This demonstrates that PPARα-mediated effects of fibrates on human lipoprotein gene expression can occur in the absence of peroxisome proliferation.

In summary:

1. The weight of evidence for di(2-ethylhexyl) phthalate and its metabolic products demonstrates that they do not act as direct DNA-damaging agents.
2. Di(2-ethylhexyl) phthalate produces liver tumours in rats and mice.
3. Under conditions of the bioassays, di(2-ethylhexyl) phthalate induces peroxisome proliferation and cell replication in liver that are characteristic of a peroxisome proliferator in mice and rats.
4. Rodent peroxisome proliferators exercise their pleiotropic effects in liver due to activation of PPARα. This process is essential for liver hypertrophy and

hyperplasia and eventual hepatocarcinogenesis in response to peroxisome proliferators.

5. Hepatic peroxisome proliferation has not been adequately evaluated in studies of human livers following exposure to di(2-ethylhexyl) phthalate *in vivo*; however, the effect of treatment of human and mouse hepatocytes with di(2-ethylhexyl) phthalate metabolites which are active in rat hepatocytes, as well as other peroxisome proliferators, indicate that humans can reasonably be predicted to be refractory to induction of peroxisome proliferation and hepatocellular proliferation by di(2-ethylhexyl) phthalate. The evidence indicates that the mechanism of peroxisome proliferation induced by di(2-ethylhexyl) phthalate in rat hepatocytes does not operate in humans.
6. The absence of a 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.
7. Overall, these findings indicate that the increased incidence of liver tumours in mice and rats treated with di(2-ethylhexyl) phthalate results from a mechanism that does not operate in humans.

5. Summary of Data Reported and Evaluation

5.1 Exposure data

Di(2-ethylhexyl) phthalate is a liquid of low volatility, widely used as a plasticizer in flexible poly(vinyl chloride) products at concentrations of up to 40%, as well as in a number of other minor applications. Occupational exposure occurs mainly by inhalation as an aerosol during its manufacture and its use as a plasticizer in poly(vinyl chloride) product manufacturing plants, at concentrations usually below 1 mg/m^3.

Di(2-ethylhexyl) phthalate is ubiquitous in the general environment as a result of its widespread use in poly(vinyl chloride) products. It is found in ambient air at levels usually below 100 ng/m^3. The highest levels of di(2-ethylhexyl) phthalate in foods are found in milk products, meat and fish and in other products with a high fat content, where concentrations up to 10 mg/kg have been reported. The leaching of di(2-ethylhexyl) phthalate from flexible plastics used in medical devices, such as during dialysis and transfusion, can result in large direct exposures.

5.2 Human carcinogenicity data

One small study of workers in a di(2-ethylhexyl) phthalate production plant did not show any excess of cancer mortality. However, this study did not have adequate power to detect a potential excess risk.

5.3 Animal carcinogenicity data

Di(2-ethylhexyl) phthalate was tested for carcinogenicity by oral administration in the diet in two experiments in mice and six experiments in rats. Hepatocellular tumours were produced consistently in both species.

In a number of initiation/promotion studies in strains of mice susceptible to liver carcinogenesis, administration of di(2-ethylhexyl) phthalate following administration with known carcinogens enhanced the incidences of hepatocellular preneoplastic foci, adenomas and carcinomas. In a number of similar studies in rats and in one study in hamsters, in general, no promoting activity of di(2-ethylhexyl) phthalate was demonstrated. No initiating activity of di(2-ethylhexyl) phthalate was found in the liver of mice or rats. In two *N*-nitrosamine-initiation target organ models in rats, one showed enhancement of renal tubule tumours by di(2-ethylhexyl) phthalate, whereas the other showed no promotion of urinary bladder tumours.

5.4 Other relevant data

The absorption and disposition of di(2-ethylhexyl) phthalate has been investigated extensively in humans and laboratory animals. In all species studied, the compound underwent rapid metabolism, with the urine and faeces being the major routes of excretion. Following oral administration, the bulk of a di(2-ethylhexyl) phthalate dose was absorbed as the monoester, mono(2-ethylhexyl) phthalate. This ester is also formed by esterases in the body following intravenous administration and is subject to extensive oxidative metabolism by the cytochrome P450 system.

The peroxisome-proliferating effects of di(2-ethylhexyl) phthalate in susceptible species (e.g., rats and mice) have primarily been related to mono(2-ethylhexyl) phthalate and two other specific metabolites. However, while species differences have been observed in the absorption and disposition of di(2-ethylhexyl) phthalate, they do not provide an explanation for the species differences in hepatic peroxisome-proliferating activity.

The literature on potential toxic effects of di(2-ethylhexyl) phthalate following human exposure is limited. Taken together, the data indicate that di(2-ethylhexyl) phthalate does not cause observable toxicity following oral and intravenous exposure, but do not contribute information relevant to the evaluation of human carcinogenicity.

A considerable amount of information on the hepatic effects of orally administered di(2-ethylhexyl) phthalate indicates that it causes hepatic peroxisome proliferation (ultrastructural effects and enzyme induction), hepatomegaly and increased replicative DNA synthesis in rats and mice. At a lower magnitude in Syrian hamsters, enzyme induction and hepatomegaly have been observed (ultrastructural effects and replicative DNA synthesis have not been evaluated). Guinea-pigs, marmosets and cynomolgus monkeys evaluated under the same or similar experimental conditions did not exhibit peroxisome proliferation responses. Studies of di(2-ethylhexyl) phthalate

metabolites in primary rat, mouse and, to a lesser extent, Syrian hamster hepatocyte cultures *in vitro* elicited markers of peroxisome proliferation, while the same or similar experimental conditions did not elicit markers of peroxisome proliferation in primary cultures of either guinea-pig, rabbit, dog, cynomolgus monkey, marmoset or, most notably, human hepatocytes.

Hepatic peroxisome proliferation depends on a nuclear receptor, PPARα, to mediate these responses in mice, based on lack of response to peroxisome proliferators in PPARα-deficient mice. In one study with another peroxisome proliferator, WY-14,643, carcinogenesis was shown to be dependent on the same receptor. Oral administration of di(2-ethylhexyl) phthalate failed to elicit markers of peroxisome proliferation in PPARα-deficient mice, while the same treatment elicited this response in normal mice. Metabolites of di(2-ethylhexyl) phthalate caused activation of PPARα-mediated gene expression in mammalian cell co-transfection assays. Differences between responsive rodents and humans in various aspects of PPARα-mediated regulation of gene expression are consistent with the lack of activity of di(2-ethylhexyl) phthalate metabolites in hepatocyte cultures from 12 people studied to date.

No data on reproductive and developmental effects in humans were available.

Oral exposure of rats and mice to di(2-ethylhexyl) phthalate during organogenesis caused malformations and fetal death. A study in knock-out mice suggested that the developmental effects are not PPARα-mediated.

Irreversible testicular damage has been observed in male rat pups exposed prenatally and during suckling via maternal exposure to drinking water containing the compound.

Oral exposure of adult rats and mice caused effects on fertility in males and females and serious effects on the testicles. Young animals were much more sensitive to gonadal effects than adults and in some cases, the onset of occurrence of the testicular effects was earlier in young animals. Dose-dependent testicular effects were seen in young rats exposed to di(2-ethylhexyl) phthalate in the diet.

In one study using small groups of adult marmosets, oral exposure did not cause testicular toxicity at doses higher than those producing testicular effects in adult rats.

The Sertoli cells in the testes appear to be the main target of the testicular toxicity. Proposed mechanistic hypotheses relate to reduced testicular zinc levels, altered hormonal status, altered metabolic function and altered follicle-stimulating hormone reactivity.

Di(2-ethylhexyl) phthalate has been studied extensively for its genotoxic effects in a wide range of test systems, both *in vitro* and *in vivo*. The majority of these studies did not reveal any activity. No mutagenic activity was observed in bacteria. In fungi, all but two studies failed to show any evidence of recombinational events or mutation. A single study in yeast for aneuploidy was positive. Low levels of mutation were induced in *Drosophila melanogaster* in somatic cells in some studies, but no germ-cell mutations or DNA damage were induced in these insects. In cultured mammalian cells, no primary DNA damage, mutation, sister chromatid exchange or chromosomal

aberrations were induced (except in a single study for DNA strand breakage), whereas transformation of cells was induced in a number of different systems.

In vivo, neither covalent binding to DNA nor DNA strand breakage was induced in several studies on rat liver, and unscheduled DNA synthesis was not induced in the liver of either rats or mice. Gene mutations were not induced in the liver of dosed mice in a single study and there was no evidence for induction of chromosomal aberrations in mice or rats. Aberrations were induced, however, in the embryos of dosed pregnant Syrian hamsters. Dominant lethal effects were reported to be induced in male mice, but re-evaluation of these data did not confirm this conclusion.

5.5 Evaluation

There is *inadequate evidence* in humans for the carcinogenicity of di(2-ethylhexyl) phthalate.

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

Overall evaluation

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

In making its overall evaluation of the carcinogenicity to humans of di(2-ethylhexyl) phthalate, the Working Group took into consideration that (*a*) di(2-ethylhexyl) phthalate produces liver tumours in rats and mice by a non-DNA-reactive mechanism involving peroxisome proliferation; (*b*) peroxisome proliferation and hepatocellular proliferation have been demonstrated under the conditions of the carcinogenicity studies of di(2-ethylhexyl) phthalate in rats and mice; and (*c*) peroxisome proliferation has not been documented in human hepatocyte cultures exposed to di(2-ethylhexyl) phthalate nor in the liver of exposed non-human primates. Therefore, the mechanism by which di(2-ethylhexyl) phthalate increases the incidence of hepatocellular tumours in rats and mice is not relevant to humans.

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