M

World Health Organization

SOME CHEMICALS PRESENT IN INDUSTRIAL AND CONSUMER PRODUCTS, FOOD AND DRINKING-WATER

VOLUME 101

This publication represents the views and expert opinions of an IARC Working Group on the Evaluation of Carcinogenic Risks to Humans, which met in Lyon, 15-22 February 2011

LYON, FRANCE - 2013

IARC MONOGRAPHS ON THE EVALUATION OF CARCINOGENIC RISKS TO HUMANS

International Agency for Research on Cancer

World Health Organization

DI(2-ETHYLHEXYL) PHTHALATE

This substance was considered by previous Working Groups, in October 1981 (IARC, 1982), March 1987 (IARC, 1987) and February 2000 (IARC, 2000). 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

From ATSDR (2002), HSDB (2010) and Lide (2010).

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; 109630-52-6;
126639-29-0; 137718-37-7; 205180-59-2;
275818-89-8; 607374-50-5
Chem. Abstr. Name: Di(2-ethylhexyl) phthalate
IUPAC Systematic Name: Bis(2-ethylhexyl) phthalate
Synonyms: 1,2-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester; 1,2-benzenedicarboxylic acid, 1,2-bis(2-ethylhexyl) ester; bis(2-ethylhexyl) benzene-1,2-dicarboxylate; bis(2-ethylhexyl) 1,2-benzenedicarboxylate; bis(2-ethylhexyl) *o*-phthalate; DEHP; diethylhexyl phthalate; di-2-ethylhexyl phthalate; dioctyl phthalate; di-sec-octyl phthalate; ethylhexyl phthalate; 2-ethylhexyl phthalate; octyl phthalate; phthalic acid, bis(2-ethylhexyl) ester; phthalic acid di(2-ethylhexyl) ester; phthalic acid dioctyl ester

1.1.2 Structural and molecular formulae and relative molecular mass
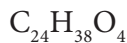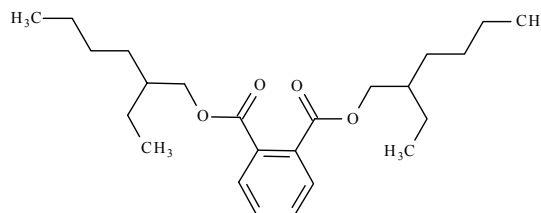
$C_{24}H_{38}O_4$
Relative molecular mass: 390.56

1.1.3 Chemical and physical properties of the pure substance

Description: Colourless liquid with almost no odour
Boiling-point: 384 °C
Melting-point: –55 °C
Density: 0.981 g/cm³ at 25 °C
Solubility: Sparingly soluble in water (0.27 mg/L at 25 °C); slightly soluble in carbon tetrachloride; soluble in blood and

fluids containing lipoproteins; miscible with mineral oil and hexane

Volatility: Vapour pressure, 1.42×10^{-7} mm Hg at 25 °C

Octanol/water partition coefficient: log K_{ow}, 7.6

Conversion factor in air: 1 ppm = 15.94 mg/m^3

1.1.4 Technical products and impurities

Technical products are generally reported to be of high purity (> 99.7%). The impurities found are mainly other phthalates. Some technical formulations may contain bisphenol A (CAS No. 80-05-7) as an additive in the range 0.025–0.5% (European Commission, 2008).

Trade names for di(2-ethylhexyl) phthalate (DEHP) include: Bisoflex 81; Bisoflex DOP; Compound 889; Corflex 400; DEHP; Diacizer DOP; Dioctyl phthalate; DOF; DOF (Russian plasticizer); DOP; ESBO-D 82; Ergoplast FDO; Ergoplast FDO-S; Etalon; Etalon (plasticizer); Eviplast 80; Eviplast 81; Fleximel; Flexol DOD; Flexol DOP; Garbeflex DOP-D 40; Good-rite GP 264; Hatco DOP; Jayflex DOP; Kodaflex DEHP; Kodaflex DOP; Monocizer DOP; NSC 17069; Octoil; Octyl phthalate; Palatinol AH; Palatinol AH-L; Palatinol DOP; Pittsburgh PX 138; Plasthall DOP; Reomol D 79P; Sansocizer DOP; Sansocizer R 8000; Sconamoll DOP; Sicol 150; Staflex DOP; Truflex DOP; Vestinol AH; Vinycizer 80; Vinycizer 80K; Witcizer 312 (SciFinder, 2010).

1.1.5 Analysis

Detection and quantification of very low levels of DEHP 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 (ATSDR, 2002).

Selected methods for the analysis of DEHP in various matrices are presented in Table 1.1.

1.2 Production and use

1.2.1 Production

DEHP 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 (ATSDR, 2002). It was first produced in commercial quantities in Japan around 1933 and in the United States of America in 1939 (IARC, 2000).

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

The global production of DEHP in 1994 was estimated to be between 1 and 4 million tonnes. The production volume of DEHP in western Europe was 595 000 tonnes/year in 1997 but had decreased to 221 000 in 2004. Some 800 plants in the European Union (EU) use DEHP or preparations that contain DEHP (European Commission, 2008). The European Commission reported that 1 million tonnes of DEHP were used in Europe in 2000 (IUCLID DataBase, 2000).

DEHP was first used commercially in the USA in 1949. During the period 1950–54, its production in the USA was 106 thousand tonnes, and, by 1965–69, had risen to 655 thousand tonnes (Peakall, 1975). From 1982 to 1986, production of DEHP in the USA increased from 114 to 134 thousand tonnes, but, in 1994, was 117 500 tonnes (Anon., 1996). Production in Japan in 1995 was 298 000 tonnes and that in Taiwan, China, in 1995 was 207 000 tonnes, down from 241 000 tonnes in 1994 (Anon., 1996). It was estimated that 109 thousand tonnes of dioctyl

Table 1.1 Selected methods for the analysis of di(2-ethylhexyl) phthalate

Sample matrix	Sample preparation	Assay procedure	Limit of detection	Reference
Air	Collect on cellulose ester membrane filter; desorb with carbon disulfide	GC/FID	10 µg/sample	NIOSH (2003) [Method 5020]
Drinking-water and source water	Extract in liquid–solid extractor; elute with dichloromethane; concentrate by evaporation	GC/MS	0.5 µg/L	EPA (1995a) [Method 525.2]
Drinking-water	Extract in liquid–liquid extractor; isolate; dry; concentrate	GC/PID	2.25 µg/L	EPA (1995b) [Method 506]
Wastewater, municipal and industrial	Extract with dichloromethane; dry; exchange to hexane and concentrate	GC/ECD	2.0 µg/L	EPA (1999a) [Method 606]
	Extract with dichloromethane; dry; concentrate	GC/MS	2.5 µg/L	EPA (1999b) [Method 625]
	Add isotope-labelled analogue; extract with dichloromethane; dry over sodium sulfate; concentrate	GC/MS	10 µg/L	EPA (1999c) [Method 1625]
Groundwater, leachate, soil, sludge and 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)	EPA (1996) [Method 8061A]
Serum	Precipitate proteins; add internal standard; extract with *n*-heptane	GC/MS	10 µg/L	Buchta *et al.* (2003)

GC, gas chromatography; ECD, electron capture detection; FID, flame ionization detection; MS, mass spectrometry; PID, photoionization detection

phthalates were produced in the USA in 1999 (ATSDR, 2002).

Information available in 2010 indicated that DEHP was produced by 23 companies in the USA, 19 companies in Mexico, nine companies in China, four companies in the United Kingdom, three companies in Germany, two companies each in China (Hong Kong Special Administrative Region), India and Japan, and one company each in Belgium, Bulgaria, Canada, the Czech Republic, France, the former state union of Serbia and Montenegro, South Africa and Switzerland (Chemical Sources International, 2010). A European source indicated that DEHP was produced by five companies each in Germany and Italy, four companies each in the Netherlands and the United Kingdom, three companies each in Austria and France, two companies in Belgium and one company each in Finland, Spain and Sweden (IUCLID DataBase, 2000).

1.2.2 Use

As a plasticizer, the primary function of DEHP is to soften otherwise rigid plastics and polymers. Plastics may contain from 1 to 40% DEHP by weight. An estimated 90% of DEHP is used as a plasticizer for polyvinyl chloride (PVC) polymers (Toxics Use Reduction Institute, 2005). In the EU, 95% of DEHP is used as a plasticizer in polymer products (European Commission, 2008).

The uses of DEHP fall into two major categories: polymer uses (e.g. consumer products such as footwear, shower curtains and toys, medical devices and commercial/industrial uses) and non-polymer uses (e.g. dielectric fluids, paints, adhesives and inks). Non-polymer uses represent less than 5% of the total DEHP used in the USA. Approximately 45% of total consumption of DEHP in the USA is for plasticizing various industrial and commercial products. Industrial and commercial uses of DEHP include resilient flooring, wall covering, roofing, aluminium foil coating/laminating, paper coating, extrudable moulds and profiles, electronic component parts, and wire and cable coating and jacketing. Medical devices comprise approximately 25% of total manufacturing use of DEHP in the USA. Medical devices that contain DEHP include PVC sheet materials such as intravenous bags, and tubing used in a variety of medical applications (Toxics Use Reduction Institute, 2005).

1.3 Occurrence

Concern regarding exposure to DEHP rose to prominence when Jaeger & Rubin (1970) reported its presence in human blood that had been stored in PVC bags. The same authors later reported the presence of DEHP in tissue samples of the lung, liver and spleen from patients who had received blood transfusions (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 DEHP 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 DEHP-containing plastics has resulted in its becoming a ubiquitous environmental contaminant (Huber et al., 1996). Many data sources show that nearly all populations absorb DEHP and excrete its metabolites in their urine in measurable amounts. Within these general population studies, concentrations in body fluids (and presumably exposures) vary substantially, and the 95th percentile exposure is 10-fold or higher than the median (CDC, 2009).

1.3.1 Natural occurrence

DEHP is not known to occur naturally.

1.3.2 Occupational exposure

According to the 1981–83 National Occupational Exposure Survey, 341 800 workers in the USA were potentially exposed to DEHP. Occupational exposure to DEHP may occur during its manufacture and its use, mostly as a plasticizer of PVC (compounding, calendering and coating operations). Printing and painting occupations also account for a large number of workers being potentially exposed (NOES, 1999). Occupational exposure to DEHP occurs by inhalation, essentially in the form of an aerosol (mist), because of its very low vapour pressure (Fishbein, 1992). Indeed, DEHP aerosols are used to test the efficacy of high-efficiency particulate air filters during their manufacture (Roberts, 1983).

Few data are available on levels of occupational exposure to DEHP (Table 1.2). Huber *et al.* (1996) observed that concentrations in air reported in older studies were well above (up to 60 mg/m³) those determined later; these older studies, however, reported concentrations of total phthalates.

Urinary levels of DEHP, its metabolites and total phthalates have been shown in a few studies to be higher in DEHP-exposed workers than in unexposed workers and in post-shift samples than in pre-shift samples (Liss *et al.*, 1985; Nielsen *et al.*, 1985; Dirven *et al.*, 1993).

Exposure to DEHP may occur concurrently with that to other compounds, 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).

A biological monitoring survey of workers exposed to DEHP was conducted in a factory using PVC plastisols. Three urinary metabolites of DEHP — mono(2-ethylhexyl) phthalate (MEHP), mono(2-ethyl-5-carboxypentyl) phthalate (MECPP) and 2-ethylhexanoic acid (2-EHA) — were quantified in five workers using a plastisol (containing 33% DEHP) and in five unexposed workers (controls) during 5 days with pre- and post-shift sampling. In the first stage, plastisols are prepared in a closed workshop where PVC resins are blended with DEHP and other ingredients in containers, then routed to the neighbouring workshop where glass bottles are automatically dipped into vats filled with plastisols. The bottles are then dried after passage in an oven at 160 °C. Around 100 tonnes of DEHP were consumed by the factory per year. Every day, two of the investigated workers, who wore gloves and protective clothes, were involved in the preparation of plastisols. The other participating workers oversaw the automatic chain and fed plastisols into where flasks were dipped. Median concentrations of pre- and post-shift urinary samples in the exposed workers ($n = 62$) were 12.6 and 28.7 µg/L for MEHP, 38.6 and 84.4 µg/L for MECPP and 20.4 and 70.6 µg/L for 2-EHA, respectively. In the controls ($n = 29$), the corresponding values were 4.8 and 4.7 µg/L for MEHP, 15.1 and 12.4 µg/L for MECPP and 21.8 and 20.5 µg/L for 2-EHA, respectively. There was a significant increase (Mann–Whitney U-test, $P < 0.05$) in post-shift excretion by exposed workers versus unexposed controls and in post-shift versus pre-shift concentrations only in the exposed workers. No air samples were reported (Gaudin *et al.*, 2010).

In 2003–05, 156 workers were recruited from eight sectors in which materials containing diethyl phthalate (DEP), dibutyl phthalate (DBP) and/or DEHP were used during part of their regular job duties. Companies included one company from each of seven manufacturing sectors: phthalate manufacture, PVC film, PVC compounding, vehicle filters, rubber hoses, rubber gaskets and rubber boots; and 13 companies from nail-only salons. For MEHP, geometric mean concentrations of mid-shift to end-shift

Table 1.2 Workplace air levels of di(2-ethylhexyl) phthalate (DEHP)

Production	Country	Air concentration (mg/m³)		Sampling	No. of samples	Reference
		Mean	**Range**			
DEHP-manufacturing plant	USA			Personal, whole-shift		Liss & Hartle (1983)
Chemical operators, technicians and maintenance workers			ND–4.11		50[a]	
Polyvinyl chloride (PVC)-processing industry	Sweden			Personal, 2 h		Nielsen *et al.* (1985)
Thick film department: calender operators/ machine attendants		0.4[b]	0.1–0.8		16	
PVC-processing plants	Finland			Area, 1.5–3 h		Vainiotalo & Pfäffli (1990)
Extrusion		0.05	0.02–0.08		4	
Extrusion		0.3	0.1–0.5		5	
Calendering		0.5	0–1		7	
Hot embossing		0.05	0.03–0.07		5	
Welding		0.3	0.25–0.35		4	
Injection moulding		0.02	0.01–0.03		2	
Compounding		0.02	0.01–0.03		5	
Thermoforming		0.02	0–0.04		2	
High-frequency welding		< 0.02				
PVC-processing plants	Netherlands			Personal, 2 h		Dirven *et al.* (1993)
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	
Various plants	USA			Personal, 4–5 h		Roberts (1983)
Two aerosol filter testing facilities			0.01–0.14			
PVC sheet-processing plant			0.06–0.29		3	

[a] Only six measurements were above the detection limit.
[b] Presented as total phthalates, but DEHP was the main plasticizer.
ND, not detected

Table 1.3 Estimated daily intake of di(2-ethylhexyl) phthalate by the population of Canada

Substrate/medium	Estimated intake for various age ranges (ng/kg body weight 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

[a] Assumed to weigh 7 kg, breathe 2 m³ air, drink 0.75 L water and ingest 35 mg soil
[b] Assumed to weigh 13 kg, breathe 5 m³ air, drink 0.8 L water and ingest 50 mg soil
[c] Assumed to weigh 27 kg, breathe 12 m³ air, drink 0.9 L water and ingest 35 mg soil
[d] Assumed to weigh 57 kg, breathe 21 m³ air, drink 1.3 L water and ingest 20 mg soil
[e] Assumed to weigh 70 kg, breathe 23 m³ air, drink 1.5 L water and ingest 20 mg soil
Calculated by the Working Group based on the assumptions by Meek & Chan (1994)

samples (in µg/L) were significantly increased in PVC compounding (from 13.0 to 24.0), rubber hose manufacture (from 6.08 to 8.70) and rubber boot manufacture (from 4.98 to 9.21). Increases were also observed in PVC film manufacture, rubber gasket manufacture and nail salons, but these did not achieve statistical significance (Hines *et al.*, 2009).

Using the same data set, phthalate metabolite concentrations measured in the workers' end-shift urine samples were used in a simple pharmacokinetic model to estimate daily phthalate intake. DEHP intake estimates based on three of its metabolites combined were 0.6–850 µg/kg per day; the two highest geometric mean intakes occurred in PVC film manufacture (17 µg/kg per day) and PVC compounding (12 µg/kg per day) (Hines *et al.*, 2011).

1.3.3 Environmental occurrence

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

DEHP is considered a priority and/or hazardous pollutant in Canada (Meek & Chan, 1994; Meek *et al.*, 1994; Environment Canada, 1995), the Netherlands (Wams, 1987) and the USA (Kelly *et al.*, 1994), 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, precipitations, 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; ATSDR, 2002; 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.

Human daily intakes of DEHP from various exposure pathways have been estimated (see Table 1.3).

(a) Biota

DEHP was measured in water, sediment and in several species of fish at six sites from the Ogun river catchments, Ketu, Lagos (Nigeria). The concentration of DEHP in water ranged from 255 to 390 µg/L at the six sites, and that in sediment ranged from 20 to 820 µg/kg. The concentration of DEHP in fish species ranged from 40 to 150 µg/kg in *Tilapia* sp., from 40 to 110 µg/kg

in *Chrysichthys* sp. and from 30 to 300 µg/kg in *Synodontis* sp. The concentration of phthalates in fish did not differ between the species and was not correlated with water or sediment concentration (Adeniyi *et al.*, 2011).

Phthalate compounds in sediments and fishes were investigated in 17 rivers in Taiwan, China, to determine the relationships between levels of phthalates in sediment and aquatic factors, and biota–sediment accumulation factor for phthalates. The highest concentrations of DEHP in fish samples were found in *Liza subviridis* (253.9 mg/kg dry weight) and *Oreochromis miloticus niloticus* (129.5 mg/kg dry weight). The biota–sediment accumulation factors of DEHP in *L. subviridis* (13.8–40.9) and *O. miloticus niloticus* (2.4–28.5) were higher than those in other fish species, indicating that the living habits of fish and physical–chemical properties of phthalates, such as their octanol/water partition coefficient, may influence the bioavailability of phthalates in fish. Mean concentrations (range) of DEHP during the low-flow season and high-flow season were 4.1 (< 0.05–46.5) and 1.2 (< 0.05–13.1) mg/kg body weight (bw), respectively. Concentrations of DEHP in sediments were significantly affected by temperature, suspended solids, ammonia–nitrogen and chemical oxygen demand (Huang *et al.*, 2008).

Levels of DEHP were measured in blood samples obtained from 33 breeding monkeys and 10 wild monkeys in Japan. In breeding monkeys, the total average amount of DEHP was 0.1939 ± 0.1050 µg/mL, and was slightly higher in male monkeys (0.2348 ± 0.0955 µg/mL) than in female monkeys (0.1642 ± 0.1057 µg/mL). In wild monkeys, the total average amount of DEHP was 0.4674 ± 0.1346 µg/mL, and was slightly higher in males (0.4703 ± 0.1436 µg/mL) than in females (0.4443 µg/mL) (Asaoka *et al.*, 2000).

(b) Air

The Environmental Protection Agency (EPA) Toxic Release Inventory reported that 86 tonnes of DEHP were released into the environment in the USA in 2008, of which 72 tonnes were released into the air from 215 facilities that manufactured and used this chemical, combined with 1588.6 tonnes that were transferred offsite (see Table 1.4). In 1997, air emissions of DEHP from 312 industrial facilities in the USA amounted to 110.5 tonnes (National Library of Medicine, 2011). In Canada, 27 tonnes of DEHP were released into the air in 1995, according to the Canadian National Pollutant Release Inventory (Environment Canada, 1995). DEHP concentrations of up to 300 ng/m^3 have been found in urban and polluted air, and levels between 0.5 and 5 ng/m^3 have been reported in the air of oceanic areas (WHO, 1992).

DEHP released into the air can be carried for long distances in the troposphere and 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).

DEHP in air has been monitored in the North Atlantic, the Gulf of Mexico and on Enewetak Atoll in the North Pacific at levels that ranged from not detectable to 4.1 ng/m^3 (Giam *et al.*, 1978, 1980; Atlas & Giam, 1981). Concentrations of DEHP in the atmosphere of the northwestern Gulf of Mexico averaged 1.16 ng/m^3 for 10 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).

Similar levels of DEHP in air (between 0.5 and 5 ng/m^3; mean, 2 ng/m^3) have been found in the Great Lakes ecosystem (Canada and the USA). The concentration of DEHP in precipitation ranged from 4 to 10 ng/L (mean, 6 ng/L). Atmospheric fluxes to the Great Lakes are a

Table 1.4 Environmental Protection Agency Toxic Release Inventory, 2008, results for 215 facilities in the USA

On-site environmental release (form R)	Pounds	Tonnes
Total air release	159 506	72
Total water release	4 163	1.9
Total underground injection release	0	0
Total land release	25 830	11.7
Total disposal (environmental release)	**189 499**	**86**
Off-site waste transfer (form R)	**Pounds**	**Tonnes**
Total publicly owned treatment works transfer	4 222	1.9
Total other off-site locations transfer	3 502 285	1588.6
Total off-site waste transfer	**3 506 507**	**1590.5**
Total environmental release and off-site waste transfer	**3 696 006**	**1676.5**

From National Library of Medicine (2011)

combination of dry and wet removal processes. The total deposition of DEHP 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, DEHP was measured at 14 monitoring stations (53 samples), and the mean air concentration was 2.0 ng/m³ (range, 0.3–77 ng/m³), with an average fallout of 23.8 µg/m² per month (range, 6.0–195.5 µg/m² per month). The total annual fallout of DEHP in Sweden was estimated to be 130 tonnes (Thurén & Larsson, 1990).

During 1995, four sets of samples of the breathable fraction of atmospheric particulates, including phthalates, from two monitoring stations were measured in the air of the Rieti urban area in Italy. The concentrations of DEHP ranged from 20.5 to 31 ng/m³ (normalized) and from 34.8 to 503.5 ng/m³ (normalized) at the two monitoring stations, respectively (Guidotti *et al.*, 1998).

The concentration of DEHP in the air at Lyngby, Denmark, was calculated to be 22 ng/m³ based on the analysis of snow samples (Løkke & Rasmussen, 1983), and levels of 26–132 ng/m³ 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, USA, in 1978 ranged from 10 to 17 ng/m³ (Bove *et al.,* 1978).

There is a paucity of data concerning concentrations of DEHP in indoor air, although its volatilization from plastic products has been noted (Wams, 1987). DEHP has been shown to account for 69 and 52% of the total amount of phthalates adsorbed to sedimented dust and particulate matter, respectively, in several Oslo (Norway) dwellings. It was found at levels of 11–210 µg/100 mg sedimented dust in 38 dwellings and at levels of 24–94 µg/100 mg suspended particulate matter (mean ± standard deviation [SD], 60 ± 30) in six dwellings. It was suggested that suspended particulate exposure to DEHP is one- to threefold higher than the estimated vapour-phase exposure (Øie *et al.*, 1997).

Levels of phthalates were measured in 48-hour personal air samples collected from parallel cohorts of pregnant women in New York City, USA ($n = 30$), and in Krakow, Poland ($n = 30$). Spot urine samples were collected during the same 48-hour period from the New York women ($n = 25$). DEHP was present in 100% of the air and urine samples. The air concentrations of DEHP (shown in Table 1.5) were higher in Krakow

157

Table 1.5 Airborne exposure of pregnant women to di(2-ethylhexyl) phthalate (µg/m³)

Area	No.	Median	Mean ± SD	Range
New York City, USA	30	0.22	0.22 ± 0.10	0.05–0.41
Krakow, Poland	30	0.37	0.43 ± 0.24	0.08–1.1

SD, standard deviation
From Adibi et al. (2003)

(median, 0.37 µg/m³) than in New York (median, 0.22 µg/m³). The urinary concentrations of the metabolite MEHP among the New York women were (µg/g creatinine): median, 4.60; mean, 40.5 ± 98.4; range, 1.80–449. MEHP levels were not correlated with measured personal levels of DEHP (Adibi et al., 2003).

The same investigators measured airborne DEHP and DEHP metabolite concentrations in spot urine samples collected from 246 pregnant Dominican and African-American women in New York City, USA. Other phthalates were also measured, and 48-hour personal air samples (n = 96 women) and repeated indoor air samples (n = 32 homes) were also collected. DEHP was detected in 100% of personal air samples (mean, 0.18 µg/m³; range, 0.16–0.21 µg/m³). Indoor air sample results were: mean, 0.09 µg/m³; range, 0.08–0.10 µg/m³. Urine samples for DEHP metabolites showed an approximately 50-fold increased range from the lowest 5th percentile to the upper 95th percentile. For MEHP (the least concentrated of the metabolites), the results were: mean, 4.8 ng/mL; range, 4.0–5.8 ng/mL. The urinary levels of MEHP were not correlated with measured airborne concentrations (Adibi et al., 2008).

(c)   Water and sediments

In general, concentrations of DEHP in freshwater 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 large numbers of industrial plants (WHO, 1992).

Surface water discharges of DEHP from 195 industrial facilities in 2010 in the USA amounted to 555 kg, as reported in the EPA Toxic Release Inventory (National Library of Medicine, 2011). DEHP 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 USA (Staples et al., 1985). DEHP 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 DEHP 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 DEHP 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 DEHP were found in rivers of the greater Manchester area, United Kingdom (Fatoki & Vernon, 1990), and at unspecified levels as contaminants in all the samplings of the Elbe River and its tributaries in Germany during the period 1992–94 (Franke et al., 1995). Levels of DEHP 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 in the Netherlands, DEHP levels ranging from 0.2 to 0.6 µg/L were found in the River Rhine near Lobith and levels ranging from < 0.1

to 0.3 ng/L were found in Lake Yssel (Ritsema et al., 1989).

Levels of DEHP 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).

DEHP has been reported in the leachate from municipal and industrial landfills at levels ranging from < 0.01 to 200 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).

Because DEHP is lipophilic, it tends to be adsorbed onto sediment, which serves as a sink (WHO, 1992). It has been measured in rivers and lake sediments in Europe (Schwartz et al., 1979; Giam & Atlas, 1980; Preston & Al-Omran, 1989; Ritsema et al., 1989) and in river and bay sediments in the USA (Peterson & Freeman, 1982; Ray et al., 1983; Hollyfield & Sharma, 1995), at concentrations that ranged from 0.021 to 70 mg/kg. Near direct discharge points from industry in Sweden and Malaysia, concentrations of DEHP 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, China (Jeng, 1986).

In experimental studies of a marine environment of Narragansett Bay, RI, USA, biodegradation by the surface microlayer biota was shown to account for at least 30% of the removal of DEHP (Davey et al., 1990).

Water solubility is a major factor that limits the degradation of phthalate esters under methanogenic conditions. In a study of the degradation of DEHP and its intermediate hydrolysis products, 2-ethylhexanol (2-EH) and MEHP in a methanogenic phthalic acid ester-degrading enrichment culture at 37 °C, the culture readily degraded 2-EH via 2-EHA to methane; MEHP was degraded to stoichiometric amounts of methane with phthalic acid as a transient intermediate; while DEHP remained unaffected throughout the 330-day experimental period (Ejlertsson & Svensson, 1996).

In a study of treatment efficiency, the fate of six phthalates was investigated throughout all the processes in the wastewater-treatment plant of Marne Aval (France). The plant treats wastewater from a highly populated area and was used as a pilot station for the development of nitrification processes. At each step of treatment, DEHP was always the major compound (9–44 µg/L) present in wastewater. In sludge, the prevailing compound was also DEHP (72 µg/g). For the studied period, the removal efficiency of DEHP from wastewater was about 78%. Downstream of the treatment plant discharge, DEHP concentrations remained below the European norm for surface water (1.3 µg/L) (Dargnat et al., 2009).

In a study of a large number of organic pollutants, water samples were collected at 15 sites from five main stream sections of the Yangtze River in Jiangsu Province (China). In three of the main sections, DEHP concentrations were [mean (range) µg/L]: 0.836 (0.469–1.33); 0.771 (0.352–1.07); and 1.123 (0.582–2.05). In the remaining two sections, DEHP was below the limit of quantitation (He et al., 2011).

Phthalate ester plasticizers were determined in rivers and dams of the Venda region, South Africa. Generally, the highest concentrations of phthalates were found as DBP and DEHP. DEHP levels at nine sites ranged from 0.3 to 2.18 mg/L (Fatoki et al., 2010).

A selection of 30 primarily estrogenic organic wastewater contaminants was measured in several influent/effluent wastewater samples from four municipal wastewater treatment plants and effluents from one bleached kraft pulp mill in Canada. DEHP was detected at 6–7 µg/L in municipal effluents (Fernandez et al., 2007).

A series of xenoestrogens, including DEHP, was measured in various matrices collected in Germany: 116 surface water samples, 35 sediments from rivers, lakes and channels, 39 sewage effluents, and 38 sewage sludges. DEHP was the dominant phthalate, concentrations of which ranged from 0.33 to 97.8 µg/L (surface water), 1.74 to 182 µg/L (sewage effluents), 27.9 to 154 mg/kg dry weight (sewage sludge) and 0.21 to 8.44 mg/kg (sediment) (Fromme *et al.*, 2002).

Water and soil samples were taken from different agricultural areas in Almeria (Spain). The level of DEHP was below the limit of quantification (0.05 mg/kg) in soil samples whereas it was detected in water samples at concentrations ranging from 0.19 to 0.88 µg/L (Garrido Frenich *et al.*, 2009). A study was designed to provide information on both the occurrence and concentration build-up of phthalate plasticizers along a heavily urbanized transect of the Seine River in Paris, France. For surface waters, eight or nine sampling sessions were performed at six Seine River locations and at one Marne River site. Surface water samples were manually sampled from July 2006 to November 2007, with a sampling frequency of about 2 months. For settleable particles, sediment traps were set during a 4-week period at four sampling sites. Of the four frequently observed pollutants, DEHP was predominant and exhibited the highest concentrations, the median of which was 1.00 µg/L and the maximum was 14.63 µg/L (Gasperi *et al.*, 2009).

Surface sediment samples from five tidal marshes along the coast of California, USA, were analysed for organic pollutants. In sediments from Stege Marsh, all phthalate compounds measured in the study were found at levels higher than other contaminants. Creek bank sediment at one station exhibited a very high concentration of DEHP (32 000 ng/g) compared with other stations. Excluding this station, DEHP was most abundant with concentration levels of 235–3000 ng/g (median, 1630 ng/g) in Stege Marsh sediments. DEHP concentrations found in sediments from most stations fell within the range found in other contaminated areas but were much higher than ambient San Francisco Bay sediment levels (200 ng/g) (Hwang *et al.*, 2006).

(d) Soil

The principal source of DEHP release to land is disposal of industrial and municipal waste to landfills (Swedish Environmental Protection Agency, 1996; Bauer & Herrmann, 1997; ATSDR, 2002). Releases of DEHP to land from 298 industrial facilities in the USA in 1997 amounted to 32 tonnes (National Library of Medicine, 2011). According to the Canadian National Pollutant Release Inventory, 33 tonnes of DEHP were released from Canadian facilities onto the land (Environment Canada, 1995).

Five soils and leachate-sprayed soils from the Susquehanna River basin in the states of Pennsylvania and New York (USA) had levels of DEHP 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 DEHP in soil collected in the vicinity of a DEHP manufacturing plant amounted to up to 0.5 mg/kg (Persson *et al.*, 1978).

DEHP has been identified in at least 737 of the 1613 hazardous waste sites that have been proposed for inclusion on the EPA National Priorities List. However, the number of sites evaluated for DEHP is not known (ATSDR, 2002).

One hundred and thirty-nine samples from 20 wastewater-treatment plants, seven sludge-treatment (thermal drying) and three composting sludge plants in Catalonia (Spain) were taken between 2001 and 2003. Of these, 92.8% had DEHP concentrations that ranged from 1.9 to 100 mg/kg dry matter. Ten samples had concentrations from above 100 to 3513.8 mg/kg dry matter (Abad *et al.*, 2005).

Soil samples collected randomly within the Muledane open dump, Thohoyandou, Limpopo province, South Africa, had mean values for DEHP of 0.03 ± 0.01 mg/kg (Adeniyi *et al.*, 2008).

Contamination by phthalates in 23 arable soils throughout China was investigated to evaluate the present pollution situation. Among the phthalates, DEHP was dominant and was detected in all 23 soils at concentrations that ranged from 0.2 to 5.98 mg/kg. A distinct feature of phthalate pollution in China was that the average concentration in northern China was higher than that in southern China. A close relationship was observed between the concentration of phthalates in soils and the consumption of agricultural film which suggests that the application of agriculture film might be a significant source of phthalate pollution in arable soils in China (Hu *et al.*, 2003).

(e)   Food

The most common route of human exposure to DEHP is through food contamination. The average daily exposure from food in the USA was estimated in 1992 to be about 0.3 mg/day per individual, with a maximum exposure of 2 mg/day (WHO, 1992).

DEHP 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; Gilbert, 1994). It can originate from PVC wrapping materials, manufacturing processes or from the animals that produced the milk or meat (Giam & Wong, 1987; Gilbert, 1994; Sharman *et al.*, 1994). The highest levels of DEHP have been measured in milk products, meat and fish as well as in other products that have a high fat content. The use of DEHP in food contact applications is reported to be decreasing (Page & Lacroix, 1995).

DEHP 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 levels of phthalates (expressed as DEHP equivalents) in the raw milk of between 0.12 and 0.28 mg/kg. After processing, DEHP was concentrated in cream at levels of up to 1.93 mg/kg, whereas low-fat milk contained < 0.01–0.07 mg/kg. In the United Kingdom, pooled milk samples from doorstep delivery in different regions of the country contained < 0.01–0.09 mg/kg DEHP. Concentrations of DEHP in 10 samples of retail cream and 10 samples of butter obtained in the United Kingdom ranged from 0.2 to 2.7 mg/kg and 2.5 to 7.4 mg/kg, respectively. Thirteen retail milk and cream products from Spain had levels of DEHP ranging from < 0.01 to 0.55 mg/kg (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 DEHP from plasticized tubing used in commercial milking equipment. Concentrations for each individual cow averaged 0.03 mg/kg and rose to 0.05 mg/kg in the central collecting tank. In control milk samples obtained by hand-milking, the concentration of DEHP was below 0.005 mg/kg. In Norway and the United Kingdom, DEHP in milk tubing has been replaced by other types of plasticizer, such as di(2-ethylhexyl) adipate and diisodecyl phthalate (Castle *et al.*, 1990). An investigation of residues of DEHP in retail whole milk samples from 14 Danish dairies in August 1989 (about 6 months after the use of DEHP-plasticized milk tubing was banned in Denmark) revealed a mean concentration below 50 µg/L (Petersen, 1991).

Retail samples of Canadian butter and margarine wrapped in aluminium foil–paper laminate were found to contain DEHP at levels of up to 11.9 mg/kg. Ten samples of butter (454 g each) had levels of DEHP 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 DEHP in the total wrapper and the corresponding food. When DEHP was not present in the wrapper, a background level of DEHP of about 3–7 mg/kg was found in butter while, when it was present, an average level of 9.4 mg/kg was found (Page & Lacroix, 1992).

DEHP was found in both the packaging and in several contacted foods sampled in a 1985–89 survey as part of the Canadian Health Protection Branch Total Diet Program. Average levels of 65 µg/kg DEHP in beverages and 290 µg/kg in foods were associated with the use of DEHP-plasticized cap or lid seals in a variety of glass-packaged foods. DEHP was found in 14 types of cheese at levels of up to 5.5 mg/kg [average, 2.4 mg/kg] and, on a butter–fat basis, these levels averaged about 8 mg/kg DEHP. Levels in factory-packaged fish were 0.2 mg/kg in halibut and 2.1 mg/kg in pollack and those in two smoked salmon samples were 0.3 and 3.9 mg/kg. DEHP 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 DEHP 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 those in total diet cereal products ranged from 0.02 to 1.5 mg/kg. A low incidence and low levels of DEHP were found in total diet samples of fruit and vegetables (mostly not detected to 0.07 mg/kg) (Page & Lacroix, 1995). DEHP was detected in 80, 71, 94 and 52% of Italian plastic-packaged salted meat, jam, baby food and milk samples, respectively, and in all the cheese and vegetable soup samples. The mean DEHP 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 DEHP, with hardly any differences between the food items (Gruber et al., 1998). DEHP was found in commercial fish (pooled samples of 10 individuals each; mg/kg wet weight): 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).

DEHP was found in 12 glass-bottled drinking-water samples (sealed with caps with a plastic internal gasket) at levels ranging from 2.4 to 17.7 µg/L (mean, 6.0 µg/L). It was also found in 12 polyethylene terephthalate-bottled drinking-water samples (sealed with caps with a plastic internal gasket) at levels ranging from 2.7 to 31.8 µg/L (mean, 10.5 µg/L) (Vitali et al., 1993).

A study designed to quantify dietary intake of phthalates and of diethylhexyl adipate, using duplicate diet samples, and to compare these data with those calculated based on urinary levels of primary and secondary phthalate metabolites, was conducted in Germany. Twenty-seven female and 23 male healthy subjects aged 14–60 years collected daily duplicate diet samples over 7 consecutive days. The median (95th percentile) daily intake of DEHP via food was 2.4 (4.0) µg/kg bw. Its metabolite, MEHP was detectable only at minor concentrations in the food samples, thus conversion of DEHP to MEHP and dietary intake of MEHP were negligible. When comparing dietary DEHP intake from the previous day with intake of DEHP calculated from concentrations of urinary DEHP metabolites, significant correlations were observed for most of the metabolites. The investigators concluded that food was the major source of intake of DEHP (Fromme et al., 2007a).

DEHP was analysed in 29 total diet samples, in 11 samples of baby food and in 11 samples of infant formulae from Denmark. Twenty-nine 'copies' of a 24-hour daily diet were prepared and collected by randomly selected adults. A selection of 11 different types of ready-to-use baby food in glass jars were sampled in retail shops and analysed before their last day of use. Different types of fruit, cereal, rice mixed with fruit or

meat mixed with vegetables were represented. The 11 types of infant formulae were bought in retail shops. In the total diet samples, DEHP was present at levels above the limit of quantitation in 11/29 samples; in a further six samples the level was above the limit of detection but below the limit of quantification. In baby food and infant formulae, two of 11 samples were above the limit of quantification for each category. Maximum and minimum mean concentrations in the total diet samples were 0.11 and 0.18 mg/kg DEHP, respectively (Petersen & Breindahl 2000).

In a study to estimate the influence of air concentrations of DEHP on its levels in food, concentrations in the atmosphere and in four vegetable crops cultivated on land surrounding a plastics production factory in China were determined. The DEHP concentrations (means) in air at sites 0.2, 0.4, 0.8 and 1.6 km from the production building were 9.4–12.8, 5.8–9.6, 1.6–5.0 and 0.04–0.27 µg/m^3 dry weight, respectively, and were highest downwind and lowest upwind from the factory. Similarly, vegetables accumulated the highest DEHP contents downwind and the lowest quantities upwind from the plant. The highest accumulated DEHP contents of field mustard, bok choy, eggplant and cowpea were 52.0 ± 3.1, 43.1 ± 2.2, 36.2 ± 2.8 and 19.4 ± 0.47 mg/kg dry weight, respectively (Du et al., 2010).

In a study designed to evaluate the migration of six phthalic acid esters in orange juice packaged in PVC bottles, the concentration of DEHP in the orange juice was lower than the limit of detection (1.11 ng/mL) during the first 2 months of storage after production, but was detectable after 3 months. The concentration increased with duration of storage and reached 662 ng/mL after 12 months, at which time the expiration date came into force (Guo et al., 2010).

Packaging materials intended for direct food contact were acquired from the Brazilian retail market and analysed for their plasticizer content. DEHP was identified in films and closure seals at concentrations ranging from 15 to 44% (w/w) (Freire et al., 2006).

A total of 98 samples of cap-sealing resins for bottled foods from in and around Yokohama City (Japan) were purchased in 1993, 1995 and 1997–99. DEHP was detected in seven of 16 samples purchased in 1993 or 1995, at concentrations ranging from 8.4 to 48.2%; the seven positive samples were all from imported bottled foods. For 1997–99, among the 61 samples of imported bottled foods, 11 contained DEHP. DEHP was also detected in seven of the 21 samples of domestic (Japanese) bottled foods at concentrations of 19.6–31.2% (Hirayama et al., 2001).

(f) Exposure from medical devices

DEHP at concentrations of up to 40% by weight is generally used as a plasticizer in PVC materials that 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) (Toxics Use Reduction Institute, 2005). It is known to leach from PVC blood packs into whole blood, platelet concentrates and plasma during storage; DEHP increases in concentration with storage time and is converted by a plasma enzyme to the metabolite, MEHP (Rock et al., 1978). DEHP 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). Patients receiving blood products or undergoing treatments requiring extracorporeal blood circulation may be exposed by leaching of DEHP from PVC bags and tubing (Wams, 1987; WHO, 1992).

DEHP was detected in whole blood at levels ranging from 16.8 to 52.6 mg/L and in packed cells at levels ranging from 32.6 to 55.5 mg/L in PVC blood bags stored at 5 °C. These levels increased with duration of storage. The average

content was 6.7 ± 4.6 mg/L in cryoprecipitate and 7.4 ± 2.8 mg/L in fresh frozen plasma. Both values were independent of the storage period (Sasakawa & Mitomi, 1978).

The accumulation of DEHP in platelet-poor plasma stored for seven and 14 days in PVC bags sterilized by steam, ethylene oxide or irradiation revealed 7-day storage levels of DEHP 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 DEHP in six patients varied between 3.4 and 11.1 mg/L, while those of MEHP ranged from 2.4 to 15.1 mg/L. In newborn infants subjected to a single exchange transfusion, concentrations of DEHP in plasma from the blood taken from the transfusion set varied between 36.8 and 84.9 mg/L, while those of MEHP ranged between 3.0 and 15.6 mg/mL (Sjöberg et al., 1985a). The concentrations in blood of both DEHP and MEHP were similar in PVC bags stored for 4 days or less (Rock et al., 1986).

The degree of exposure to DEHP was assessed in 11 patients undergoing haemodialysis treatment for renal failure and showed that, on average, an estimated 105 mg DEHP was extracted from the dialyser during a single 4-hour dialysis session, with a range of 23.8–360 mg. Time-averaged circulating concentrations of MEHP during the session (1.33 ± 0.58 mg/L) were similar to those of DEHP (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 DEHP over the course of a year, with a range of 3.7–56 g (Pollack et al., 1985). DEHP 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 pre-dialysis patients, DEHP was not detected (< 0.1 mg/L); in no case was the hydrolysis product MEHP detected (< 0.4 mg/L) (Nässberger et al., 1987). A comparative evaluation of haemodialysis tubing plasticized with DEHP and that plasticized with tri-2-ethylhexyl trimellitate was made in 11 patients (10 men, one woman) with chronic renal failure undergoing haemodialysis for a period of 6 months. During treatment with tubing containing DEHP, the plasma level of DEHP 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 DEHP and its derivatives MEHP, 2-EH and phthalic acid in seven elderly patients undergoing regular CAPD were compared with those in six aged-matched healthy controls during a 4-hour dwell period. Serum concentrations of DEHP 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 MEHP in the fluid of CAPD bags before use was four times higher than that of the parent compound DEHP. During the first 4 hours of dwell time, the concentrations of MEHP and 2-EH 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 DEHP 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 not statistically significantly (0.135 mg/L; range, 0.073–0.659 mg/L; P = 0.062) (Mettang et al., 1996a).

Levels of DEHP ranging from < 1 to 4100 mg/L have been reported in the condensate from water traps of six respirators. Estimation of the inhalation exposure to DEHP of five artificially ventilated pre-term infants over a 24-hour period yielded values ranging between 1 μg/h and 4200 μg/h. DEHP (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)

Plateletpheresis concentrates were transfused to thrombocytopenic adult haematooncological patients (seven men, five women) in Austria. Serum DEHP levels were assessed before and after transfusion and after storage for 5 days. The median DEHP concentration in plateletpheresis concentrates was 10.9 mg/L (range, 5.7–23.7), representing a median total dose of 3.5 mg DEHP (range, 1.6–8.8 mg) to the patient. Median serum DEHP levels increased from 192 ng/mL (range, 10–532 ng/mL) at baseline to 478 ng/mL (range, 142–1236 ng/mL) after transfusion. DEHP was also measured on days 1, 3, 4 and 5 in plateletpheresis concentrates that were either stored in donor plasma ($n = 5$) or with addition of T-Sol ($n = 5$). In samples taken shortly after the plateletpheresis procedure, a mean level of 1.88 μg/mL (range, 0.41–3.2 μg/mL) DEHP was found in concentrates suspended in donor plasma compared with 6.59 μg/mL (range, 2.09–10.67 μg/mL) on day 5 ($P = 0.0002$). Similarly, mean DEHP levels increased from 0.75 μg/mL (range, 0.29–1.0 μg/mL) in concentrates suspended in 35% of donor plasma to 1.9 μg/mL (range, 0.5–3.25 μg/mL; $P = 0.0023$) in those suspended in 65% of the T-Sol additive solution (Buchta et al., 2005).

DEHP and MEHP levels, migration patterns and metabolism in blood products were examined for the detailed assessment of exposure to DEHP. From the Japanese Red Cross Society, 78 blood products (red blood cell concentrate, $n = 18$; irradiated red blood cell concentrate, $n = 18$; whole blood, $n = 18$; blood platelets, $n = 18$; and frozen plasma, $n = 6$) were sampled in January 2003 for use in this study. The levels of DEHP and MEHP detected and the ratio of MEHP to DEHP ([MEHP concentration per DEHP concentration in samples] × 100%) were: 6.8–36.5 μg/mL, 0.3–4.3 μg/mL and 6.0 ± 2.6%, respectively, in red blood cell products released from medical blood bags; 7.4–36.1 μg/mL, 0.3–3.3 μg/mL and 5.7 ± 2.0%, respectively, in irradiated red blood cell concentrate products; 15.0–83.2 μg/mL, 0.5–9.7 μg/mL and 7.8 ± 4.1%, respectively, in whole blood products; 1.8–15.0 μg/mL, 0.1–4.6 μg/mL and 15.2 ± 8.4%, respectively, in platelet products; and 11.6–18.5 μg/mL, 1.1–2.5 μg/mL and 10.5 ± 2.3%, respectively, in fresh frozen plasma products. The levels of DEHP and MEHP in all blood products ranged from 1.8 to 83.2 μg/mL and from 0.1 to 9.7 μg/mL, respectively, and increased with increasing storage time. In addition, whole blood products stored in PVC bags had the highest DEHP levels compared with other blood products. These results indicate that the maximum level of human exposure to DEHP released from blood bags would be 0.7 mg/kg bw/time (Inoue et al., 2005).

Sixteen patients undergoing coronary artery bypass grafting in Japan were randomly divided into two groups of eight. Group A had tubing containing DEHP in the circuit, and group B had no DEHP in the tubing. The plasma level of DEHP at the end of the operation was significantly increased compared with that before anaesthesia in both groups (group A: from 103 ± 60 to 2094 ± 1046 ng/mL; group B: from 135 ± 60 to 472 ± 141 ng/mL), and was significantly higher in group A than in group B (Takahashi et al., 2008).

DEHP and MEHP were measured in the supernatant of centrifuged samples of 10 whole blood units collected in citrate-phosphate-dextrose buffer from healthy adult blood donors. The level of DEHP significantly increased 12.6-fold from 34.3 ± 20.0 (SD) μM [13.4 ± 7.8 (SD) mg/L] on day 1 to 433.2 ± 131.2 (SD) μM

[169.2 ± 51.2 (SD) mg/L] on day 42, and that of MEHP significantly increased 20.2-fold from 3.7 ± 2.8 (SD) μM [1 ± 0.8 (SD) mg/L] on day 1 to 74.0 ± 19.1 (SD) μM [20.6 ± 5.3 (SD) mg/L] on day 42 (Rael et al., 2009).

The exposure of six plasma donors, six discontinuous-flow platelet donors and six continuous flow platelet donors to DEHP was measured in Germany, where each donor can perform up to 26 plateletpheresis or up to 40 plasmapheresis procedures per year. Exposure was calculated by determining three specific metabolites in urine: mono(2-ethyl-5-hydroxyhexyl) phthalate [MEHHP]; mono(2-ethyl-5-oxohexyl)phthalate [MEOHP] and MEHP. Maximum concentrations in urine samples were found after the continuous-flow plateletpheresis procedure: 826 μg/L (range, 241–1346 μg/L) MEHHP, 774 μg/L (range, 302.3–1153 μg/L) MEOHP and 266 μg/L (range, 69.2–536.5 μg/L) MEHP (means for the six volunteers). The corresponding mean values for discontinuous-flow donors, plasma donors and controls were: 416.3, 416.3 and 148.1 μg/L; 86.3, 91.6 and 41.9 μg/L; and 52.3, 38.2 and 15.5 μg/L, respectively. The excretion of metabolites was significantly ($P < 0.0001$) higher for both plateletpheresis techniques compared with plasmapheresis and controls, and continuous-flow plateletpheresis led to significantly higher ($P < 0.0001$) levels of excretion than discontinuous-flow plateletpheresis. Mean absolute exposures to DEHP were 1.2 mg for discontinuous- and 2.1 mg for continuous-flow plateletpheresis, and those for plasmapheresis (0.37 mg) were in the range of the controls (0.41 mg). Mean doses of DEHP for both plateletpheresis techniques (18.1 and 32.3 μg/kg per day) were close to or exceeded the reference dose of the EPA and the tolerable daily intake value of the EU on the day of the apheresis (Koch et al., 2005a).

(g) Building materials

Afshari et al. (2004) measured phthalate emissions from PVC flooring, polyolefine flooring, a refrigerator list, two electric cables, PVC skirting and floor wax. Samples were taken in exhaust air from the chambers after 6, 35, 62, 105 and 150 days from the start of the experiment. PVC flooring was tested for an additional 100 days. Polyolefine covered with wax did not emit DEHP. The other materials resulted in a maximum concentration of approximately 1 μg/m³ DEHP. The concentration of DEHP in each chamber increased slowly until a rather stable level was reached after 150 days.

In a field study of floor dust from 15 Danish schools, the mean concentration of DEHP was 3.214 mg/g dust, the 90th percentile value was 6.404 mg/g and the 95th percentile was 7.063 mg/g (Clausen et al., 2003).

Room air samples from 59 apartments and 74 kindergartens in Berlin (Germany) were tested in 2000 and 2001 for the presence of phthalates. These substances were also measured in household dust from 30 apartments. For air samples in apartments, the levels of DEHP at 59 sites were: 191 (mean), 156 (median), 390 (95%) and 615 ng/m³ (maximum). In kindergartens, the levels in 73 samples were: 599 (mean), 458 (median), 1510 (95%) and 2253 ng/m³ (maximum). With a contribution of approximately 80% of all values, the main phthalate in house dust was DEHP, with median values of 703 mg/kg (range, 231–1763 mg/kg). No statistically significant correlation could be found between air and dust concentration. The estimated daily intake of DEHP for an adult (in μg/kg per day) was 0.05 from indoor air and 0.11 from dust. For a child, the estimated daily intake (in μg/kg per day) was 0.06 for air and 5.97 for dust (Fromme et al., 2004).

The presence of a target set of phthalates was investigated in the interior of 23 used private cars during the summer and winter in Italy. DEHP

was detected in six of the vehicles at detectable concentrations that ranged from 200 to 1400 ng/m³ (Geiss *et al.*, 2009).

In 2003, in a village in a rural part of southern Germany, internal exposure to DEHP of 36 nursery schoolchildren (aged 2 ± 6 years), 15 parents and four teachers was determined and compared. The DEHP metabolites MEHHP, MEOHP and MEHP were determined in the first morning urine. The sum of the three DEHP metabolites in the urine of children and adults was 90.0 and 59.1 µg/L, respectively (median values; $P = 0.074$). Concentrations of the secondary metabolites MEHHP (median, 49.6 versus 32.1 µg/L; $P = 0.038$) and MEOHP (median, 33.8 versus 19.6 µg/L; $P = 0.015$) were significantly higher in children than in adults, whereas those of MEHP were low in both adults and children (median, 6.6 µg/L versus 9.0 µg/L). Creatinine-adjusted values more accurately reflected the dose taken up (with respect to body weight) in children and adults. Total creatinine-adjusted levels of DEHP metabolites in urine were significantly higher in children than in adults (median values, 98.8 versus 50.9 µg/g creatinine; $P < 0.0001$), as were those of both secondary metabolites, MEHHP (55.8 versus 28.1 µg/g creatinine; $P < 0.0001$) and MEOHP (38.3 versus 17.2 µg/g creatinine; $P < 0.0001$). Creatinine-corrected concentrations for the monoester MEHP in children and adults were very similar (8.7 versus 8.6 µg/g creatinine (Koch *et al.*, 2004a).

Urine samples of the general German population were examined for human-specific metabolites of phthalates. The study subjects were inhabitants of Erlangen, a city in southern Germany, and its vicinity. Eighty-five subjects were sampled (median age, 33 years; range, 7–64 years; 53 women: median age, 29 years; 32 men: median age, 36 years). First morning urine was collected in mid-April 2002, and metabolites of DEHP — MEHP, MEHHP and MEOHP — were measured in urine, from which the daily intake of DEHP was determined. A median intake of 13.8 µg/kg bw per day and an intake at the 95th percentile of 52.1 µg/kg bw per day were estimated. Twelve per cent of the subjects (10/85 samples) within this group had intake greater than 37 µg/kg bw per day, and 31% (26/85 samples) had values higher than the reference dose (20 µg/kg bw per day) of the US Environmental Protection Agency (Koch *et al.*, 2003a).

(h) Sludge

Organic residues from four wastewater-treatment plants in Seville, Spain, were analysed between January and October 2005. All the treatment plants carried out anaerobic biological stabilization of the sludge. DEHP was found in all of the sludge samples analysed at concentrations of 12–345 mg/kg dry mass. Mean concentrations of DEHP in primary, secondary and anaerobically-digested dehydrated sludges and in compost samples were 53, 65, 159 and 75 mg/kg dry mass, respectively (Aparicio *et al.*, 2009).

Concentrations of DEHP and its metabolites were measured in the sludges from several sewage-treatment plants in the Province of Québec, Canada. DEHP was found at concentrations ranging from 15 to 346 mg/kg in primary, secondary, digested, dewatered or dried sludges. Metabolites were detected in almost all sludges, except those that had undergone a drying process at high temperature (Beauchesne *et al.*, 2008).

In Switzerland, sewage sludges from different catchment areas were sampled: four samples containing predominantly domestic sewage were obtained from separate sewer systems (type A); six samples containing a mixture of domestic sewage, stormwater runoff and small amounts of industrial wastewater were obtained from combined sewer systems (type B); and two samples containing a mixture of domestic sewage, stormwater runoff and higher amounts of industrial wastewater were obtained from combined sewer systems (type C). DEHP was

found in all 12 samples at concentrations ranging from 21 to 114 mg/kg dry mass. There was no apparent difference in concentration between the three types of source (Berset & Etter-Holzer, 2001).

The presence of nonylphenols, nonylphenol ethoxylates and eight phthalates was analysed in urban stormwater and sediment from three catchment areas in Sweden. Emission loads for these substances were then calculated for a specific urban catchment area. A level of ≤ 48 µg/g DEHP was found in the sediment, and aqueous concentrations of up to 5.0 µg/L DEHP were detected (Björklund et al., 2009).

A variety of contaminants, including phthalates, was measured in source-separated compost and digestate samples from Switzerland that were selected to cover a wide variety of variables that may influence organic pollutant concentrations, including treatment processes (composting/digestion), origin (urban/rural) and composition of input material (green waste with/without crude organic kitchen waste), and season of collection. The median concentration of DEHP was 280 µg/kg dry weight (n = 6). The highest value (1990 µg/kg dry weight) was detected in an urban digestate sample containing organic kitchen waste, as well as fruit and vegetable residues from grocery stores (Brändli et al., 2007).

In a study that measured the occurrence of 43 semi-volatile organic compounds in sewage sludges collected from 11 wastewater-treatment plants in mainland and Hong Kong Special Administrative Region, China, DEHP was detected in the sludge of nine of the 11 sites. The mean DEHP concentration was 21 mg/kg dry weight, with detectable values ranging from 4.4 to 108 mg/kg dry weight (Cai et al., 2007).

Seventeen sewage sludges were studied for organic pollutants that the EU has proposed be controlled for land application. Samples were collected in 2002–03 from different wastewater-treatment plants of the Valencian Community (Spain) by third parties (inspectors of the regional

government) at the points they considered to be representative during 1 day of sampling. Results by type of treatment were: anaerobic (10 facilities), range 40–325 mg/kg dry weight; aerobic (three facilities), 2–80 mg/kg; and without digestion (four facilities), 50–350 mg/kg (Gomez-Rico et al., 2007).

(i) Total exposure of the general population

The Centers for Disease Control and Prevention (CDC, 2009) in the USA analysed urine samples from a representative population sample of 2605 persons for DEHP metabolites, as part of the National Health and Nutrition Examination Survey for 2003–4. The metabolites measured were MEHP, MEHHP, MEOHP and MCEPP. Results were presented as geometric means and selected percentile (in µg/g creatinine) by age group, sex and ethnic group for each metabolite. Results for MEHP are presented in Table 1.6. For the total population, the geometric mean was 2.20 µg/g (95% confidence interval [CI], 2.01–2.41 µg/g), with a 95th percentile value of 25.4 µg/g (95%CI: 16.7–34.7 µg/g). For each subgroup, the ratio of the 95th percentile to the median value was 10-fold or greater. Urinary concentrations in the youngest age group (6–11 years) were somewhat higher than those of adults: 3.00 µg/g (95%CI: 2.30–3.93 µg/g) versus 2.14 µg/g (95%CI: 1.98–2.31 µg/g). Comparisons between sex and ethnicity are also presented in Table 1.6. Geometric mean and percentile values for the four metabolites are compared in Table 1.7. Compared with the geometric mean value of 2.20 µg/g (95%CI: 2.01–2.41 µg/g) for MEHP, the concentrations of the other metabolites were substantially higher: MEHHP, 20.4 µg/g (95%CI: 18.7–22.3 µg/g); MEOHP, 13.6 µg/g (95%CI: 12.4–14.8 µg/g); and MCEPP, 32.6 µg/g (95%CI: 29.6–36.0 µg/g). Similar 10-fold or greater ratios of the 95th percentile to the median values, and similar relative values for children compared with adults were observed for each of the metabolites.

Table 1.6 Urinary mono(2-ethylhexyl) phthalate (in µg/g of creatinine) for the population in the USA, 2003–4

| | Geometric mean (95% CI) | Selected percentiles (95% CI) | | | | |
		50th	75th	90th	95th	No.
Total	2.20 (2.01–2.41)	1.89 (1.68–2.19)	4.31 (3.84–4.74)	10.8 (8.72–13.8)	25.4 (16.7–34.7)	2605
Age group						
6–11 yr	3.00 (2.30–3.93)	2.80 (1.93–4.09)	5.86 (4.69–7.70)	14.3 (8.54–24.4)	28.7 (14.1–45.3)	342
12–19 yr	2.07 (1.74–2.48)	1.88 (1.60–2.23)	4.25 (3.19–5.62)	11.6 (6.83–23.2)	24.8 (11.6–37.9)	729
> 20	2.14 (1.98–2.31)	1.84 (1.63–2.08)	4.14 (3.78–4.40)	10.5 (8.38–12.9)	25.6 (15.9–36.3)	1534
Sex						
Male	2.01 (1.82–2.21)	1.71 (1.46–1.89)	4.14 (3.49–4.81)	10.4 (7.68–16.2)	23.3 (15.1–41.1)	1250
Female	2.40 (2.15–2.69)	2.16 (1.84–2.40)	4.40 (3.97–4.89)	10.9 (8.27–16.0)	27.0 (17.5–34.6)	1355
Ethnic group						
Mexican Americans	2.12 (1.74–2.59)	1.94 (1.50–2.42)	4.06 (3.29–4.93)	9.38 (5.72–15.4)	16.8 (9.86–38.6)	652
Non-Hispanic blacks	2.56 (2.24–2.92)	2.28 (2.02–2.78)	5.17 (4.48–6.83)	13.2 (10.5–16.2)	27.5 (18.4–36.0)	699
Non-Hispanic whites	2.12 (1.91–2.35)	1.82 (1.60–2.13)	4.11 (3.49–4.42)	10.7 (7.42–15.1)	27.0 (15.1–37.4)	1088

CI, confidence interval; yr, years
Adapted from CDC (2009)

Table 1.7 Urinary concentrations of selected di(2-ethylhexyl) phthalate metabolites (in μg/g of creatinine) for the total population in the USA, 2003–4

	Geometric mean (95% CI)	Selected percentiles (95% CI)				
		50th	**75th**	**90th**	**95th**	**No.**
Mono(2-ethylhexyl) phthalate	2.20 (2.01–2.41)	1.89 (1.68–2.19)	4.31 (3.84–4.74)	10.8 (8.72–13.8)	25.4 (16.7–34.7)	2605
Mono(2-ethyl-5-hydroxyhexyl) phthalate	20.4 (18.7–22.3)	17.7 (16.3–19.6)	35.8 (30.5–43.3)	93.5 (74.0–128)	182 (134–262)	2605
Mono(2-ethyl-5-oxohexyl) phthalate	13.6 (12.4–14.8)	12.1 (11.0–12.9)	24.3 (20.9–27.8)	63.0 (47.8–75.8)	118 (94.1–153)	2605
Mono(2-ethyl-5-carboxypentyl) phthalate	32.6 (29.6–36.0)	27.0 (24.3–30.6)	54.6 (48.0–63.5)	139 (109–186)	251 (192–356)	2605

CI, confidence interval

Adapted from CDC (2009)

In a study to assess the contribution of different food types to phthalate exposure, associations between dietary intake (assessed by a 24-hour dietary recall) for a range of food types (meat, poultry, fish, fruit, vegetables and dairy) and phthalate metabolites measured in the urine were analysed using multiple linear regression modelling. MEHP levels in the urinary samples (in ng/g creatinine) were: median, 1.9; mean, 6.5; geometric mean, 2.2; and range, 0.09–294. Other metabolites showed a similar skewed distribution. The levels of metabolites of DEHP were associated with the consumption of poultry (Colacino *et al.*, 2010).

Median daily intakes of DEHP among German university students were calculated to be between 2.4 and 4.2 µg/kg bw per day based on DEHP metabolites measured in the urine, and were associated with the industrial production of DEHP in Germany with a correlation coefficient of > 0.9 (Helm, 2007).

Daily intake of phthalates was estimated from urinary metabolite levels in Japan between late May and early June 2004. Thirty-six volunteers (35 adults and one child; aged 4–70 years), 26 of whom were aged 20–29 years and 25 of whom were men, participated. Most of the participants lived in the Tokyo-Yokohama area. The median concentration of MEHP was 5.0 µg/L (geometric SD, 2.52 µg/L). From this, the investigators estimated a mean DEHP intake of 2.7 (range, 2.0–3.3) µg/kg bw per day (Itoh *et al.*, 2007).

Wittassek & Angerer (2008) reported that, based on urinary phthalate metabolite concentrations estimated in 102 German subjects aged 6–80 years, the median daily intake of DEHP was 2.7 µg/kg bw per day. They noted that, in general, children had higher exposures than adults (Wittassek & Angerer 2008).

In a retrospective human biomonitoring study, 24-hour urine samples taken from the German Environmental Specimen Bank for Human Tissues were analysed for a series of phthalate ester metabolites. The samples were collected from 634 subjects (predominantly students; age range, 20–29 years; 326 women, 308 men) between 1988 and 2003 ($n \geq 60$ per year). Based on urinary metabolite excretion, the daily intakes of the parent phthalates were estimated and the chronological course of phthalate exposure was investigated. Metabolites of all five phthalates were detectable in over 98% of the urine samples. For the DEHP metabolite, MEHP, the median value declined from 9.8 µg/g creatinine in 1988 to 5.5 µg/g creatinine in 2003. The median over the whole period was 7.3 µg/g creatinine for the total cohort, with similar values for men and women. Other metabolites showed similar trends. For the entire study period, the daily intake estimated from the metabolite levels (µg/kg bw per day) was: median, 3.5; upper 95th percentile, 10.1; and range, 0.19–39.8. These levels decreased from 1988 (median, 3.9; upper 95th percentile, 9.9; range, 0.78–39.8) to 2003 (median, 2.4; upper 95th percentile, 5.7; range, 0.82–7.1) (Wittassek *et al.*, 2007a).

The excretion of DEHP and its metabolites were studied by analysing first morning urine samples from 53 women and 32 men, aged 7–64 years (median, 34.2 years), living in northern Bavaria (Germany) who were not occupationally exposed to phthalates. Concentrations were found to vary considerably between subjects with differences spanning more than three orders of magnitude. Median concentrations of excreted DEHP metabolites were 46.8 µg/L MEHHP (range, 0.5–818 µg/L), 36.5 µg/L MEOHP (range, 0.5–544 µg/L), and 10.3 µg/L MEHP (range, < 0.5 [limit of quantification]–177 µg/L). A strong correlation was found between the excretion of MEHHP and MEOHP (correlation coefficient r = 0.991) indicating close metabolic proximity of these two parameters but also the absence of any contaminating interference (Koch *et al.*, 2003b).

(j) Exposure of infants and children

Zhang et al. (2009) assessed maternal–fetal exposure to phthalates to investigate whether in-utero exposure to phthalates is associated with low birth weight (LBW). They examined phthalate concentrations in specimens of maternal blood, cord blood and meconium from a total of 201 newborn–mother pairs (88 LBW cases and 113 controls) residing in Shanghai (China) during 2005–06 in a nested case–control study on pregnancy outcomes. Median DEHP concentrations in maternal blood and cord blood varied from 0.5 to 0.7 mg/L and did not differ between controls and mothers with LBW infants. In contrast, highly significant differences were found between control and LBW mothers for the levels of the metabolite MEHP. Median MEHP levels in maternal blood were: controls, 1.4 mg/L (range, 1.2–2.1 mg/L); and LBW, 2.9 mg/L (range,1.8–3.5 mg/L). Levels in cord blood were: controls, 1.1 mg/L (range, 0.9–1.7 mg/L); and LBW, 2.5 mg/L (range, 1.6–3.4 mg/L). Levels in neonatal meconium were: controls, 2.9 mg/g (range, 1.8–4.4 mg/g); and LBW, 5.5 mg/g (range, 3.4–9.3 mg/g).

Exposure of children to DEHP by migration from PVC toys and other articles into saliva has been reported. Until the early 1980s, DEHP was the predominant plasticizer used in soft PVC products for children. Since that time, it has been replaced in most countries by other plasticizers, in particular di(isononyl) phthalate (Steiner et al., 1998; Wilkinson & Lamb, 1999).

The levels of phthalate esters were analysed in a total of 86 human milk samples collected among 21 breast-feeding mothers over a 6-month postpartum time, in the city of Kingston, Ontario, Canada, in 2003–04. DEHP was the predominant ester with an arithmetic mean value of 222 ng/g (range, 156–398 ng/g). Weak correlations between lipid content and levels of phthalate esters were observed. The levels of phthalate esters in human milk fluctuated over the 6-month period. The mean daily intake of DEHP over the first 6-month period for a 7-kg infant consuming 750 g milk (breast-feeding) was estimated at 167 µg/day (Zhu et al., 2006).

The presence of several phthalate metabolites was analysed in breast milk from healthy mothers living in southern Italy. Milk samples from 62 healthy mothers (mean age ± SD, 29.42 ± 5.28 years; range, 18–41 years) from the Brindisi and Tricase areas were randomly collected within approximately 7 days postpartum during March 2006 and September 2006. Women enrolled in the study did not undergo surgical interventions and/or ongoing medical treatments, including intravenous infusions or blood transfusions, during the period immediately preceding the sample collection. MEHP was found in all samples: median, 8.4 µg/L (95%CI: 7.6–10.0 µg/L); 95th percentile, 28.5 µg/L; maximum, 109 µg/L. No oxidized metabolites of DEHP were found (Latini et al., 2009).

In 2001, 42 primiparae from southern Sweden (median age, 29 years; range, 23–39 years) provided breast milk, blood and urine samples 2–3 weeks after delivery. In breast milk, DEHP was detected in 39/42 samples (median, 9.0 ng/mL; range, 0.45–305 ng/mL) and MEHP was detected in 16/42 samples (median, 0.49 ng/mL; maximum, 6.5 ng/mL). In blood, DEHP was detected in 17/36 samples (median, 0.50 ng/mL; maximum, 129 ng/mL) and MEHP was detected in six of 36 samples (median, 0.49 ng/mL). MEHP was detected in all 38 samples of urine (median, 9 ng/mL; range, 2.9–57 ng/mL). No correlations existed between urinary concentrations and those found in milk or blood/serum for individual phthalate metabolites (Högberg et al., 2008).

The disposition of DEHP and MEHP during a single exchange transfusion was investigated in four newborn infants. The amounts of DEHP and MEHP 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 DEHP

originated from parts of the transfusion set other than the blood bag. Immediately after the transfusions, the plasma levels of DEHP 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 hours (Sjöberg et al., 1985b).

Measurement of serum levels of DEHP in 16 newborn infants undergoing exchange transfusion indicated an undetectable level (< 1 mg/L) before the exchange but levels ranging from 6.1 to 21.6 mg/L (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 DEHP ranged from 12.3 to 87.8 mg/L and, in six newborn infants receiving a third blood unit, the serum levels ranged from 24.9 to 93.1 mg/L (Plonait et al., 1993).

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 DEHP after 14 and 24 days of ECMO support were 26.8 and 33.5 mg/L, respectively. DEHP levels of 3.5, 1.0 and 0.4 mg/kg were found in the liver, heart and testicular tissues, respectively, and trace quantities were found in the brain. The rate of DEHP extraction from the model PVC circuits was linear with time (rate, 3.5 and 4.1 mg/L per hour). The exposure to DEHP for a 4-kg infant on ECMO support for 3–10 days was estimated to be 42–140 mg/kg bw (Shneider et al., 1989).

A more recent study of 18 infants on ECMO life support also reported leaching of DEHP 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 DEHP was 8.3 ± 5.7 mg/L. For a 4-kg infant, the estimated exposure over 3–10 days was 10–35 mg/kg bw. No leaching of DEHP from heparin-coated PVC circuits was detected (Karle et al., 1997).

Exposure to DEHP in six critically ill premature newborns was assessed by measuring the levels of three DEHP metabolites in 41 urine samples. MEHHP and MEOHP were detected in all 41 samples, and MEHP was detected in 33. For the 33 samples, the geometric mean, 5% percentile and 95% percentile urinary concentrations were: MEOHP, 1617, 243 and 10 413 ng/mL; MEHHP, 2003, 290 and 13 161 ng/mL; and MEHP, 100, 6.22 and 704 ng/mL. Concentrations per gram of creatinine were approximately eightfold higher than results in nanograms per millilitre (Calafat et al., 2004).

Green et al. (2005) studied 54 neonates admitted to neonatal intensive care units in Boston, MA, USA, for at least 3 days between 1 March and 30 April 2003. The exposures of infants to DEHP were classified based on medical products used: the low-exposure group included infants receiving primarily bottle and/or gavage feedings; the medium-exposure group included infants receiving enteral feedings, intravenous hyperalimentation and/or nasal continuous positive airway pressure; and the high-exposure group included infants receiving umbilical vessel catheterization, endotracheal intubation, intravenous hyperalimentation and in-dwelling gavage tube. Urinary MEHP levels increased monotonically with DEHP exposure. For the low-, medium- and high-exposure groups, median (interquartile range) MEHP levels were 4 (18), 28 (58) and 86 (150) ng/mL, respectively ($P = 0.004$). After adjustment for institution and sex, urinary MEHP levels among infants in the high-exposure group were 5.1 times those among infants in the low-exposure group ($P = 0.03$).

In a follow-up report, the levels of three metabolites of DEHP were measured in the urine of the 54 infants. Urinary concentrations stratified by intensiveness (in ng/mL) were: MEHP – low, 4; medium, 28; high, 86; MEHHP – low, 27; medium, 307; high, 555; and MEOHP – low, 29; medium, 286; high, 598. Urinary concentrations of MEHHP and MEOHP among infants in

the high-intensiveness group were 13–14 times those among infants in the low-intensiveness group ($P \leq 0.007$). A structural equation model confirmed the specific monotonic association between intensiveness of product use and biological measures of DEHP. Inclusion of the oxidative metabolites, MEHHP and MEOHP, strengthened the association between intensiveness of product use and biological indices of DEHP exposure over that observed with MEHP alone (Weuve et al., 2006).

In Germany, urine samples were taken from 254 children aged 3–14 years during 2001 and 2002, and were analysed for concentrations of the DEHP metabolites, MEHP, MEHHP and MEOHP. In addition, DEHP was analysed in house dust samples collected with vacuum cleaners in the homes of the children. The geometric means of the urinary levels of MEHP, MEHHP and MEOHP (in μg/L) were 7.9, 52.1 and 39.9, respectively. MEHHP and MEOHP concentrations were highly correlated (r = 0.98). The correlations of MEHHP and MEOHP with MEHP were also high (r = 0.72 and 0.70, respectively). The concentrations of MEHHP and MEOHP were 8.0-fold and 6.2-fold those of MEHP. The ratios of MEHHP to MEOHP and MEOHP to MEHP decreased with increasing age. Boys showed higher urinary concentrations than girls for all three metabolites of DEHP. Children aged 13–14 years had the lowest mean urinary concentrations of the secondary metabolites. The house dust analyses revealed DEHP contamination of all samples with a geometric mean of 508 mg/kg dust. No correlation could be observed between the levels of any of the urinary DEHP metabolites and those of DEHP in house dust (Becker et al., 2004).

Another study estimated the daily DEHP intake of children who lived in two different urban areas in the city of Berlin and in two rural villages in the northern part of Germany. First morning urine samples for the analysis of the DEHP metabolites MEHHP, MEOHP and

MEHP and individual data (age, body weight, body height) from 239 children (aged 2–14 years; median, 8.5 years) were collected between March 2001 and March 2002. Two calculation models based upon the volume and the creatinine-related urinary metabolite concentrations were applied. Applying the volume-based model, a median daily DEHP intake of 7.8 μg/kg bw per day, a 95th percentile of 25.2 μg/kg bw per day and a maximum of 140 μg/kg bw per day were estimated. Using the creatinine-based model, the median daily intake was estimated at 4.3 μg/kg bw per day with a 95th percentile of 15.2 μg/kg bw per day and a maximum of 409 μg/kg bw per day. In general, exposure to DEHP decreased with increasing age. The median for children aged 2–4 years was 5.7 (creatinine-based) or 10.7 (volume-based) μg/kg bw per day. For children aged 12–14 years, the median was 2.7 (creatinine-based) or 4.8 (volume-based) μg/kg bw per day. Comparing boys and girls in the whole age range, values were slightly higher in boys than in girls ($P = 0.023$ in the creatinine-based model and $P = 0.097$ in the volume-based model). While median values were comparable in both estimation models (4.9 versus 3.9 μg/kg bw per day in the creatinine-based model and 8.4 versus 7.4 μg/kg bw per day in the volume-based model), the 95th percentile was about twice as high for boys as for girls. Differences in gender were particularly pronounced in the youngest children, between 2 and 4 years of age ($P < 0.004$ in both calculation models) (Wittassek et al., 2007b).

1.4 Regulations and guidelines

Occupational exposure limits and guidelines for DEHP in several countries are presented in Table 1.8.

In 1989, the EPA required that DEHP be subject to registration as a pesticide in the USA under the Federal Insecticide, Fungicide, and Rodenticide Act. This requirement was cancelled in 1998 (RTECS, 2009).

Table 1.8 Occupational exposure limits and guidelines for di(2-ethylhexyl) phthalate

Country	Year	Concentration (mg/m³)	Interpretation
Argentina[a]	2007	5	TWA
Australia	2008	5	TWA
		10	STEL
Belgium	2002	5	TWA
		10	STEL
Bulgaria[a]	2007	5	TWA
Colombia[a]	2007	5	TWA
Denmark	2002	3	TWA
Finland	1999	5	TWA
		10 (sk)	STEL
France	2006	5	VME
Germany	2005	10	MAK
Hungary	2000	10	TWA
		40 (sk)	STEL
Japan	2007	5	OEL
Jordan[a]	2007	5	TWA
Korea, Republic of	2006	5	TWA
		10	STEL
Mexico	2004	5	TWA
		10	STEL
New Zealand	2002	5	TWA
		10	STEL
Norway	1999	3	TWA
Philippines	1993	5	TWA
Poland	1999	1	MAK (TWA)
		5	MAK (STEL)
Russian Federation	2003	1	STEL
Singapore[a]	2007	5	TWA
Sweden	2005	3	TWA
		5	STEL
Switzerland	2006	5	MAK-wk
United Kingdom	2005	5	TWA
		10	STEL
USA			
ACGIH (TLV)	2007	5	TWA
OSHA (PEL)	1994	5 (general industry, shipyards, construction, federal contractors)	TWA
NIOSH (REL air)	1992	5	TWA
		10	STEL
MSHA (air)	1971	5	TWA
Viet Nam[a]	2007	5	TWA

a These countries follow the recommendations of the ACGIH threshold limit values.
ACGIH, American Conference of Governmental Industrial Hygienists; MAK, maximale Arbeitsplatz-Konzentration; MSHA, Mine Safety and Health Administration; NIOSH, National Institute of Occupational Safety and Health; OEL, occupational exposure limit; OSHA, Occupational Safety and Health Administration; PEL, permissible exposure limit; REL, recommended exposure limit; sk, absorption through the skin may be a significant source of exposure; STEL, short-term exposure limit; TLV, threshold limit value; TWA, time-weighted average; VME, valeur moyenne d'exposition [average exposure value]; wk, week
From IUCLID DataBase (2000); RTECS (2009); ACGIH (2010)

The World Health Organization established an international drinking-water guideline for DEHP of 8 µg/L in 1993 (WHO, 2008). The EPA (1998) has set the maximum contaminant level for DEHP in drinking-water at 6 µg/L in the USA.

The Food and Drug Administration (1999) permits the use of DEHP in the USA 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% weight 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 come into contact with food and as a food-packaging plasticizer for foods with a high water content.

The European Union has ruled that DEHP shall not be used in toys and childcare articles at concentrations greater than 0.1% by mass of the plasticised material (Council of Europe, 2005).

A reference dose of 20 µg/kg bw per day was set by the EPA (2007).

2. Cancer in Humans

The epidemiological studies that evaluated cancer risk and exposure specifically to DEHP are very limited, and include an occupational cohort study of DEHP production workers that was reviewed by a previous IARC Working Group (IARC, 2000), and a case–control study of breast cancer. However, several studies evaluated cancer risk and exposure to plastics, particularly in occupational settings and the PVC industry. Workers who make PVC plastics or products are potentially exposed to phthalate plasticizers, including DEHP, which is the most common, and these products can contain 1–40% DEHP

by weight. Some studies of workers making PVC plastics are briefly reviewed in this section if they included analyses for exposure to phthalate plasticizers (Hagmar et al., 1990) or phthalates (Heineman et al., 1992), or if they specifically mentioned that exposure to DEHP occurred or that DEHP was a suspected etiological agent (Hagmar et al., 1990; Selenskas et al., 1995; Hardell et al., 1997, 2004). These comprise one cohort study, three case–control studies of testicular cancer, one case–control study of pancreatic cancer and one case–control study of multiple myeloma.

Long-term dialysis patients are liable to experience elevated exposures to DEHP, through frequent and protracted exposure to substances leached from surgical tubing during dialysis; however, the Working Group was not aware of any study of dialysis patients for which study methods were adequate for the evaluation of carcinogenic risks associated with DEHP.

Exposure to DEHP has been associated with some hormone-related outcomes (e.g. endometriosis, thyroid hormone disruption and testicular dysgenesis syndrome), thus studies of breast and testicular cancer are of special interest.

2.1 Studies specific for exposure to DEHP

2.1.1 Occupational exposure

Thiess et al. (1978) evaluated the mortality of 221 workers in a DEHP production plant in Germany, who were followed between 1940 and 1976. Most subjects (135/221) were hired after 1965 and the process was completely enclosed in 1966. No information on the level of exposure was provided. Only eight deaths from all causes occurred during the follow-up period; one from pancreatic cancer (0.13 expected) and one from urinary bladder papilloma (0.01 expected) occurred among workers with a long duration of exposure (≥ 20 years). No further report on a

longer follow-up for this cohort was available to the Working Group. [The Working Group noted that the majority of the cohort members were employed after exposure levels had been considerably reduced. The methods for this study were poorly described and power was inadequate to detect a potential excess risk.]

2.1.2 Case–control study

(a) Cancer of the breast

López-Carrillo *et al.* (2010) conducted a case–control study in northern Mexico to evaluate the association between urinary levels of nine phthalate metabolites and breast cancer (see Table 2.1). They interviewed 233 women with breast cancer and 221 age-matched controls from 2007 to 2008, and collected sociodemographic and reproductive characteristics and first morning void urine samples before any treatment. Exposure assessment was based on the measurement of biomarkers: no data on personal habits involving exposure to phthalates were available. Phthalate metabolites, detected in at least 82% of all women, were measured in urine samples by isotope dilution/high-performance liquid chromatography coupled to tandem mass spectrometry. After adjusting for risk factors and other phthalates, increased odds ratios for breast cancer were associated with urinary concentrations of four DEHP metabolites: MEHP, MEHHP, MEOHP, and MECPP; however, this increased risk was only statistically significant for MECPP, with a dose–response trend ($P = 0.047$). A non-significant negative association was observed for MEOHP. With regard to other phthalate metabolites, urinary concentrations of DEP and monoethyl phthalate (MEP) metabolites were positively associated with breast cancer (odds ratio of highest versus lowest tertile, 2.20; 95%CI: 1.33–3.63; *P* for trend < 0.01). In contrast, significant negative associations were found for monoisobutyl phthalate (MiBP), monobenzyl phthalate (MBzP) and mono(3-carboxylpropyl)

phthalate (MCPP) metabolites. The odds ratios for the sum of all nine metabolites (including five non-DEHP phthalate metabolites), for the 2nd and 3rd versus the 1st tertile were 0.94 (95%CI: 0.57–1.56) and 1.41 (95%CI: 0.86–2.31; *P* for trend = 0.114).

[The Working Group considered that this study had an appropriate design, although the timing of exposure assessment was a concern. Biological samples to measure DEHP metabolites were obtained after diagnosis among cases, before any treatment; metabolites were measured in the urine, and it is not known whether disease status could have affected metabolite levels. A limitation of this study was the lack of consistency in effect between the four DEHP metabolites measured and the lack of a dose–response for all metabolites. Further, it is unclear which metabolite is the best biomarker for exposure to DEHP.]

2.2 Occupational exposure to phthalate plasticizers

2.2.1 Cohort study

See Table 2.2

Hagmar *et al.* (1990) reported on the mortality of 2031 Swedish workers at a PVC-processing factory that produced flooring, film and pipes from PVC. DEHP was the major plasticizer used in all of these products. A significant excess of total cancer morbidity (standard incidence ratio, 1.28; 95%CI: 1.01–1.61; 75 cases) and respiratory cancer morbidity (SIR, 2.13; 95%CI: 1.27–3.46; 17 cases) was observed among the PVC-processing workers, but no statistically significant association was found with cumulative exposure to plasticizers. [The Working Group noted that only 6% of the cohort was exposed only to plasticizers.] Respiratory cancer risk was increased in individuals who were exposed to both asbestos and plasticizers but not to vinyl chloride (SIR, 10.70; 95%CI: 2.20–31.20; three cases) and in

Table 2.1 Case–control study of cancer and di(2-ethylhexyl) phthalate (DEHP)

Reference, study location, period	Total cases	Total controls	Control source (hospital, population)	Exposure assessment	Organ site	Exposure categories	Exposed cases	Relative risk (95% CI)	Covariates	Comments
López-Carrillo *et al.* (2010), Northern Mexico, 2007–08	233 cases identified in 25 tertiary hospitals, aged ≥ 18 yr; response rate, 94.8%; histopathologically confirmed [Note: incident status of cases unspecified, but because urine samples were obtained before any treatment, incident cases are assumed]	221	Population-based selected from lists of the Health Department national surveys; matched 1:1 by age (± 5 yr) and residency; response rate, 99.5%	Measurement of four DEHP metabolites in first morning void urine samples: MEHP, MEHHP, MEOHP, MECPP	Breast (unspecified)	MEHP (µg/g creatinine) 0.22–3.42 3.43–7.51 7.52–257.08 MEHHP (µg/g creatinine) 2.69–35.61 35.62–63.38 63.39–1014.60 MEOHP (µg/g creatinine) 2.10–23.90 24.91–43.10 43.11–1230.94 MECPP (µg/g creatinine) 11.59–57.88 57.89–97.67 97.68–1742.92	 81 67 83 90 53 88 108 51 72 69 73 89	 1.00 1.03 (0.62–1.69) 1.23 (0.75–2.01) *P* trend = 0.383 1.00 0.77 (0.46–1.28) 1.37 (0.84–2.24) *P* trend = 0.106 1.00 0.60 (0.36–1.00) 0.84 (0.52–1.36) *P* trend = 0.651 1.00 1.27 (0.77–2.10) 1.68 (1.01–2.78) *P* trend = 0.047	Current age, age at menarche, parity, menopausal status, non-DEHP phthalate metabolites	Results are also described for metabolites of MEP, MnBP, MiBP, MBzP, MCPP

CI, confidence interval; MnBP, mono(*n*-butyl) phthalate; MBzP, monobenzyl phthalate; MCPP, mono(3-carboxypropyl) phthalate; MECPP, mono(2-ethyl-5-carboxypentyl) phthalate; MEHHP, mono(2-ethyl-5-hydroxyhexyl) phthalate; MEHP, mono(2-ethylhexyl) phthalate; MEOHP, mono (2-ethyl-5-oxohexyl) phthalate; MEP, monoethyl phthalate; MiBP, monoisobutyl phthalate; yr, year or years

Table 2.2 Cohort study of workers potentially exposed to di(2-ethylhexyl) phthalate (DEHP), phthalates or polyvinyl chloride (PVC) plastics

Reference, period	Study population	Follow-up period	Exposure assessment	Organ site	Exposure categories	No. of cases/ deaths	Relative risk (95% CI)	Comments
Hagmar *et al.* (1990), 1945–80	2031 PVC-processing workers	1945–85	Workers were exposed to DEHP in addition to other phthalates, PVC, and vinyl chloride	All cancer Respiratory cancer	Exposed to DEHP in addition to other phthalates, PVC and vinyl chloride	75 17	**Morbidity** 1.28 (1.01–1.61) 2.13 (1.27–3.46)	Expected number of deaths and cancer morbidity based on national rates; SMRs and 95% CIs calculated using Poisson distribution; exposure–response relationship were based on person–yr; potential confounding by tobacco smoking and exposure to other agents; limited power to detect exposure relationships and risk of cancer at individual sites; no overall risk estimates given for plasticizers.
				All cancer Respiratory cancer		38 10	**Mortality** 1.22 (0.88–1.71) 1.53 (0.73–2.80)	
			Workers categorized into four levels (not exposed, low, moderate and high) of exposure to plasticizers, vinyl chloride monomer and asbestos by experts	Respiratory cancer	**Cumulative exposure to plasticizers** (mg–yr) Low (≤ 0.05) Moderate (> 0.05–0.5) High (> 0.5) Test for trend	 1 5 5	**Morbidity** 0.52 [0.01–2.90] 3.73 [1.22–8.71] 2.13 [0.69- 4.97] $P > 0.05$	

CI, confidence interval; SMR, standardized mortality ratio; yr, year or years

workers not exposed to any of the three agents. [The limitations of this study were the small number of workers and exposed deaths/cases of site-specific cancers, potential confounding by tobacco smoking or other risk factors and crude exposure estimates.]

2.2.2 Case–control studies

See Table 2.3

Three population-based case–control studies — two in Sweden and one in Denmark — evaluated the association between occupational exposure to PVC plastics or products and testicular cancer. A small Swedish study found an increased risk for testicular cancer among men exposed to PVC (OR, 6.6; 95%CI: 1.4–32; seven exposed cases and two exposed controls) (Hardell et al., 1997). [The Working Group noted that the results were only from living subjects, which could introduce bias if the risk factor were associated with poor prognosis.] In a larger Danish study, no increased risk for testicular cancer was observed for men ever exposed to mainly PVC (OR, 0.7; 95%CI: 0.5–1.2) or plastics in general (OR, 1.0; 95%CI: 0.8–1.2) (Hansen, 1999). [However, this study had limited statistical power because of small numbers of exposed cases; the prevalence of exposure to PVC for 1 year or more among controls was only 0.5%.] In the second Swedish study (Hardell et al., 2004; Westberg et al., 2005) of 791 men with germ-cell testicular cancer and 791 matched controls, ever exposure to PVC plastics was associated with an increased risk for testicular cancer (OR, 1.35; 95%CI: 1.06–1.71); a non-significant increased risk was reported for exposure to soft (containing plasticizer) plastics (OR, 1.48; 95%CI: 0.94–2.34; 54 cases and 37 controls) but not to rigid plastics (OR, 1.06; 95%CI: 0.55–2.01; 23 cases and 26 controls). The risk was elevated among workers with a 10-year latency (OR, 1.45; 95%CI: 1.06–1.98). However, odds ratios for exposure decreased with increasing exposure for all four measures of exposure (duration, maximum intensity, median intensity over the subject work history and cumulative median intensity). [The questions on exposure were focused on PVC in general and not on exposure to specific substances, which could decrease the possibility of detecting an effect due to phthalates.]

Selenskas et al. (1995) conducted a nested case–control study of pancreatic cancer among a cohort of workers employed at a plastics manufacturing and research and development plant in New Jersey, USA (Dell & Teta, 1995). Individuals with potential exposure to phthalates worked in either the vinyl- and polyethylene-processing department, or the fibres and fabrics department. Vinyl processing involved the compounding and calendering (a fabrication step) of PVC polymers and copolymers that are produced at other locations. Potential exposure to DEHP, specifically mentioned as being used in this plant, occurred in the production of flexible plastics. A significantly increased risk for pancreatic cancer was observed only in the vinyl- and polyethylene-processing workers (relative risk, 7.15; 95%CI: 1.28–40.1; five exposed cases who had worked for more than 16 years). No trend of increasing risk with increasing duration or latency was observed. [However, there were only nine cases and 40 controls in the combined production areas, so that the number of cases and control in each exposure stratum (duration or latency) was small.] Most of the cases (eight of nine) and controls (34/40) in the vinyl- and polyethylene-processing areas worked in the vinyl- and polyethylene-processing subdepartment in that area. Among these workers, an elevated risk for pancreatic cancer was observed among those exposed for more than 18 years (relative risk, 8.98; 95%CI: 0.90–89.8). All of the cases of pancreatic cancer that occurred in the vinyl- and polyethylene-processing department worked in the building where both vinyl and polyethylene were processed and none of the cases occurred among workers in the building where only polyethylene was processed. [The

Table 2.3 Case-control studies of workers potentially exposed to di2-ethylhexyl) phthalate (DEHP), phthalates or polyvinyl chloride (PVC) plastics

Reference, location	Study population and methods	Exposure	Effects	Comments
Hardell *et al.* (1997); Ohlson & Hardell (2000), Sweden	*Population-based, testicular cancer* *Cases:* 148 men aged 30–75 yr with testicular cancer reported to the Swedish Cancer Registry between 1989 and 1992 *Controls:* 315 men selected from the Swedish Population Registry who were born the same yr as the cases; ORs calculated using conditional logistic regression and analyses made with latency times of 1 and 5 yr	Occupational exposure to PVC plastics assessed via a detailed questionnaire; cumulative exposure calculated by multiplying the exposure level by portion of days worked and number of yr of exposure	Odds ratios (95% CI); cases/controls *Exposure to PVC plastics* All: 6.6 (1.4–32); 7/2 Low: 2.6 (0.3–32); 2/2 High: NR; 5/0	Small number of exposed cases and controls; retroactive exposure assessment; self-administered questionnaire
Hansen (1999), Denmark	*Population-based, testicular cancer* *Cases:* 3745 men aged 16–75 yr with testicular cancer, identified in the Danish Cancer registry between 1970 and 1989, and members of the national pension fund *Controls:* 7490 men without cancer randomly selected from the national pension fund and matched to cases on age; odds ratios calculated using conditional regression models adjusting for socioeconomic status and analyses made using lag times of 0, 1, 5, 10 and 15 yr	Employment history obtained from the national pension fund records, and socioeconomic status inferred from occupational titles; workers in the cable manufacturing industry assumed to be exposed to PVC (industry is the largest consumer of PVC and phthalate plasticizers in Denmark)	Odds ratios (95% CI); cases/controls *Ever exposed (yr) to mainly PVC* All: 0.7 (0.5–1.2); 26/71 < 1: 0.9 (0.5–1.6); 28/39 1–2: 1.3 (0.4–4.7); 4/6 > 2: 0.4 (0.1–1.0); 4/26	Limited documentation; study reported as a peer-reviewed letter to the editor; small number of subjects exposed to mainly PVC for more than 1 yr; potential misclassification of exposure; potential confounding

Table 2.3 (continued)

Reference, location	Study population and methods	Exposure	Effects	Comments
Hardell *et al.* (2004); Westberg *et al.* (2005) Sweden	*Population-based, testicular cancer Cases:* 791 men with testicular cancer reported to the Swedish Cancer Registry from 1993 to 1997 *Controls:* 791 matched men selected from the Swedish population registry; odds ratios calculated by conditional logistic regression model for matched studies and analyses made using lag times, of > 1 yr and > 10 yr; cases and controls with an exposure time shorter than 1 yr before diagnosis classified as unexposed; exposure time dichotomized by the median exposure time of controls (8 yr)	Exposure to PVC plastics obtained from detailed questionnaire on entire working history and exposure specifically to PVC; an expert assessment performed using questionnaire data and five semi-quantatitive exposure categories developed: unexposed, insignificant, low, medium and high; five different exposure measures then derived based on the qualitative and quantitative categorization: ever/never exposed, duration, maximum intensity, median intensity, cumulative median intensity	Odds ratios (95% CI); cases/controls *Hardell et al. (2004)* **Ever exposed (latency) to PVC** > 1 yr: 1.35 (1.06–1.71); 200/59 > 10 yr: 1.45 (1.06–1.98); 123/97 **Type of PVC plastic** Rigid plastic: 1.06 (0.55–2.01); 23/26 Soft plastic: 1.48 (0.94–2.34); 54/37 **Median intensity of exposures with a 10-yr latency** Insignificant: 1.75 (0.51–5.98); 8/6 Low: 1.50 (0.89–2.51); 51/39 Medium: 1.52 (0.98–2.35); 57/46 High: 0.67 (0.19–2.36); 7/6 *Westberg et al. (2005)* **Insignificant, low, medium, high** Duration: 1.4, 1.6, 1.1, 1.2 Maximum intensity: 2.3, 1.2, 1.4*, 0.9 Median intensity: 2.6*, 1.3, 1.4, 0.9 Cumulative median intensity: 1.6*, 1.5*, 1.1, 1.0	The study population did not overlap with Hardell *et al.* (1997); ever exposure to PVC occurred in 160 subjects as reported by the subjects and 360 subjects as assessed by the expert assessment; 360 subjects were used for the analysis; small number of exposed subjects in highest exposure category; lack of exposure measures for work tasks * 95% CI does not include 1.0

Table 2.3 (continued)

Reference, location	Study population and methods	Exposure	Effects	Comments
Selenskas et al. (1995), New Jersey, USA	*Nested, pancreatic cancer* *Cohort*: 5594 male workers employed at least 7 mo between 1946 and 1967 at a plastics manufacturing plant (Dell & Teta, 1995) *Cases:* 28 men who died from pancreatic cancer *Controls:* 140 men randomly selected from the cohort and matched to the case for yr of birth and survival; risk estimates calculated by conditional logistic regression models for matched studies; exposures within 10 yr of case death excluded	Occupational exposure assessed by employment history and department of work; individuals classified into major production and non-production areas	Relative risk (95% CI) for pancreatic cancer *Vinyl and polyethylene processing (9 cases/40 controls)* **Duration of employment (yr)** ≤ 1: 0.54 (0.06–4.57) 1–5: 0.84 (0.16–4.30) 5–16: 0.47 (0.06–3.84) > 16: 7.15 (1.28–40.1) No trend observed with time since first employment *Other departments* At least five cases of pancreatic cancer also observed in resin pulverizing, resins and varnish, and plant service and maintenance, but no associations observed in the duration of employment or latency strata	Individuals with potential exposure to DEHP or phthalates worked in either the vinyl- and polyethylene-processing department or the fibres and fabrics department; small numbers of subjects; limited power to detect effects of duration of employment and latency because only nine cases and 40 vinyl- and polyethylene-processing workers stratified into five duration categories or five latency categories

Table 2.3 (continued)

Reference, location	Study population and methods	Exposure	Effects	Comments
Heineman et al. (1992), Denmark	*Population-based, multiple myeloma* *Cases*: 1098 men over 18 yr, and diagnosed with multiple myeloma from 1970 to 1984 *Controls*: 4169 men selected at random from the Danish Central Population Registry and matched to the case on age and yr in which the case was diagnosed; maximum likelihood estimates of the odds ratios and 95% CI calculated adjusting for age; risks for specific substances calculated using a referent group not exposed to that substance; effects of multiple exposure calculated using logistic regression that adjusted for exposure to several agents and age	Employment history obtained from the pension fund and job titles on tax records; information used by experts to assess and assign individuals to categories of exposures to specific substances	Odds ratios (95% CI); cases/control *Exposure to phthalates (overall)* Possible: 1.3 (0.9–2.0); 34/99 Probable: 2.0 (0.9–4.4); 11/21 *Duration of exposure, no lagging* **Possible** 1 mo– < 5 yr: 1.1 (0.6–2.3); 12/41 ≥ 5 yr: 1.2 (0.7–2.2); 17/54 Test for trend $P = 0.21$ **Probable** 1 mo– < 5 yr: 1.9 (0.4–8.9); 3/6 ≥ 5 yr: 2.5 (0.9–7.0); 7/11 Test for trend $P = 0.02$ When exposure lagged for 10 yr, risk increased with duration of possible exposure but not probable exposure; however, test for trend not significant. *Logistic regression adjusting for exposure to vinyl chloride, engine exhaust and gasoline* No increase in risk observed with increasing duration of possible or probable exposure under both lagging conditions	Specific estimates given for exposure to phthalates; small numbers of exposed cases and controls in each stratum, especially for probable exposure; some attempt to adjust for multiple exposures, especially vinyl chloride

CI, confidence interval; mo, month or months; NR, not reported; yr, year or years

Working Group noted that the limitations of this study include the small numbers of exposed cases, the categorization of exposure that was not specific to DEHP, the lack of quantitative exposure measures and potential confounding from exposure to occupational agents, tobacco smoking or other risk factors.]

The relationship between multiple myeloma and exposure to phthalates (and other occupational agents) was evaluated in a population-based case–control study among Danish men (Heineman *et al.*, 1992). Exposure to phthalates was associated with elevated but non-significant odds ratios for multiple myeloma, with a higher risk estimate for probable exposure (OR, 2.0; 95%CI: 0.9–4.4; 11 cases and 21 controls) than possible exposure (OR, 1.3; 95%CI: 0.9–2.0; 34 cases and 94 controls). Risk estimates for probable exposure increased with increasing duration of exposure when latency was not considered (OR for probable exposure greater than 5 years, 2.5; 95%CI: 0.9–7.0; *P* for trend = 0.02). When time since first exposure was lagged for 10 years, risks increased with duration of exposure for possible but not probable exposure. Exposure to vinyl chloride was also associated with an increased risk for multiple myeloma, and the risk estimate increased with increasing duration of exposure. Stratified analysis was conducted to separate the effects of exposure to phthalates from exposure to vinyl chloride. Increased risk estimates were observed for: exposure to phthalates but never to vinyl chloride (OR for 5 or more years, 2.0; 95%CI: 0.1–27; one case); exposure to vinyl chloride but never to phthalates (OR for 5 or more years, 2.6; 95%CI: 0.3–19.2; two cases); and exposure to both (OR, 5.2; 95%CI: 1.0–29.5; four cases). However, the numbers of exposed cases in each stratum were small. In logistic regression analyses that controlled for exposure to vinyl chloride, engine exhaust and gasoline, risk estimates for exposure to phthalates no longer increased with duration of exposure. [If duration of exposure were a poor surrogate for cumulative

exposure, this would lead to a misclassification of exposure and thus make it hard to detect an exposure–response relationship.]

3. Cancer in Experimental Animals

3.1 Inhalation

3.1.1 Hamster

Groups of 65 male and 65 female Syrian golden hamsters, 12 weeks of age, were exposed continuously by whole-body inhalation to DEHP vapour at a concentration of 15 ± 5 µg/m^3 until natural death (almost continuously for 23 months up to a total exposure of 7–10 mg/kg bw per hamster). Controls (80 males and 80 females) were untreated. No significant difference in tumour incidence was observed between the controls and the DEHP-exposed group (Schmezer *et al.*, 1988).

3.2 Oral administration

See Table 3.1.

3.2.1 Mouse

Groups of 50 male and 50 female B6C3F$_1$ mice, 5 weeks of age, were maintained on a diet containing 0 (controls), 3000 or 6000 ppm DEHP for 103 weeks. A dose-related decrease in mean body weight gain was observed in female mice from week 25 to the end of the study. The incidence of hepatocellular carcinoma was significantly increased in high-dose males with a significant trend. In females, both the low-dose and the high-dose groups showed an increase in the incidence of hepatocellular carcinoma with a significant trend. The incidence of hepatocellular carcinoma or adenoma (combined) was increased in low- and high-dose males and females compared to their respective controls.

Table 3.1 Carcinogenicity studies of exposure to di(2-ethylhexyl) phthalate (DEHP) in the diet in experimental animals

Species, strain (sex) Duration Reference	Dosing regimen Animals/group at start	Incidence of tumours	Significance	Comments
Mouse, B6C3F$_1$ (M) 103 wk Kluwe *et al.* (1982); NTP (1982); Kluwe *et al.* (1985)	0, 3000 or 6000 ppm 50	Liver (hepatocellular carcinoma): 9/50, 14/48, 19/50* Liver (hepatocellular adenoma or carcinoma, combined): 14/50, 25/48*, 29/50**	*P = 0.022 P = 0.018 (trend) *P = 0.013 ** P = 0.002	> 99.5% pure
Mouse, B6C3F$_1$ (F) 103 wk Kluwe *et al.* (1982); NTP (1982); Kluwe *et al.* (1985)	0, 3000 or 6000 ppm 50	Liver (hepatocellular carcinoma): 0/50, 7/50*, 17/50** Liver (hepatocellular adenoma or carcinoma, combined): 1/50, 12/50*, 18/50**	* P = 0.006 ** P < 0.001 P < 0.001 (trend) * P = 0.001 ** P < 0.001	> 99.5% pure
Mouse, B6C3F$_1$ (M) 104 wk David *et al.* (1999, 2000a)	0, 100, 500, 1500 or 6000 ppm 70, 60, 65, 65 and 70	Liver (hepatocellular adenoma or carcinoma, combined): 8/70, 14/60, 21/65*, 27/65*, 37/70* Liver (hepatocellular adenoma): 4/70, 10/60, 13/65, 14/65, 19/70 Liver (hepatocellular carcinoma): 4/70, 5/60, 9/65, 14/65, 22/70	*P < 0.05 [P < 0.0005, trend] [P < 0.0005, trend]	> 99.7% pure; 10–15 animals per group were killed during wk 79 and the remaining animals were autopsied at wk 105; survival was reduced in mice receiving 6000 ppm DEHP.
Mouse, B6C3F$_1$ (F) 104 wk David *et al.* (1999, 2000a)	0, 100, 500, 1500 or 6000 ppm 70, 60, 65, 65 and 70	Liver (hepatocellular adenoma or carcinoma, combined): 3/70, 4/60, 7/65, 19/65*, 44/70* Liver (hepatocellular adenoma): 0/70, 2/60, 4/65, 9/65, 34/70 Liver (hepatocellular carcinoma): 3/70, 2/60/ 3/65, 16/65, 16/70	*P < 0.05 [P < 0.0005, trend] [P < 0.0005, trend]	> 99.7% pure; 10–15 animals per group were killed during wk 79 and the remaining animals were autopsied at wk 105; survival was reduced in mice receiving 6000 ppm DEHP
Mouse, Sv/129 wild-type or Sv/129 *Ppara*-null (M) 22 mo Ito *et al.* (2007a)	0, 0.01 or 0.05% NR	Liver (tumours): *Ppara*-null–1/25, 1/25, 8/31 Liver (tumours): wild-type–0/24, 2/23, 2/20	[NS], see comments	Purity NR; according to the authors, the incidence of liver tumours was higher (P < 0.05) in *Ppara*-null mice exposed to 0.05% DEHP (8/31; six hepatocellular adenomas, one hepatocellular carcinoma and one cholangiocellular carcinoma) than in those exposed to 0% DEHP (1/25; one hepatocellular carcinoma). The Working Group noted the unusual grouping of cholangiocellular and hepatocellular tumours. When comparing hepatocellular tumours only (7/31 versus 1/25), there was no statistical difference [P = 0.052, Fisher's one-tailed test.]

Table 3.1 (continued)

Species, strain (sex) Duration Reference	Dosing regimen Animals/group at start	Incidence of tumours	Significance	Comments
Rat, F344 (M) 103 wk Kluwe et al. (1982); NTP (1982); Kluwe et al. (1985)	0, 6000 or 12000 ppm 50	Liver (hepatocellular adenoma or carcinoma, combined): 3/50, 6/49, 12/49*	* P < 0.02	99.5% pure
Rat, F344 (F) 103 wk Kluwe et al. (1982); NTP (1982); Kluwe et al. (1985)	0, 6000 or 12000 ppm 50	Liver (hepatocellular adenoma): 0/50, 4/49, 5/50*	* P < 0.05	99.5% pure
		Liver (hepatocellular carcinoma): 0/50, 2/49, 8/50**	**P < 0.01	
		Liver (hepatocellular adenoma or carcinoma, combined): 0/50, 6/49***, 13/50****	***P < 0.02 ****P < 0.001	
Rat, F344 (M) 104 wk David et al. (1999, 2000b)	0, 100, 500, 2500 and 12500 ppm 80, 50, 55, 65 and 80	Liver (hepatocellular adenoma or carcinoma, combined): 5/80, 5/50, 4/55, 11/65*, 34/80*	*P < 0.05	> 99.7% pure
		Liver (hepatocellular adenoma): 4/80, 5/50, 3/55, 8/65, 21/80	[P < 0.0005, trend]	
		Liver (hepatocellular carcinoma): 1/80, 0/50, 1/55, 3/65, 24/80	[P < 0.0005, trend]	
		Pancreas (acinar-cell adenoma): 0/60 (control), 5/59* (high dose)	*P < 0.05	
Rat, F344 (F) 104 wk David et al. (1999, 2000b)	0, 100, 500, 2500 and 12500 ppm 80, 50, 55, 65 and 80	Liver (hepatocellular adenoma or carcinoma, combined): 0/80, 4/50*, 1/55, 3/65, 22/80*	*P < 0.05	> 99.7% pure
		Liver (hepatocellular adenoma): 0/80, 3/50, 1/55, 2/65, 8/80	[P < 0.0005, trend]	
		Liver (hepatocellular carcinoma): 0/80, 1/50, 0/55, 1/65, 14/80	[P < 0.0005, trend]	

Di(2-ethylhexyl) phthalate

Table 3.1 (continued)

Species, strain (sex) Duration Reference	Dosing regimen Animals/group at start	Incidence of tumours	Significance	Comments
Rat, Sprague-Dawley (SD-CD) (M) 159 wk Voss *et al.* (2005)	0, 600, 1897 and 6000 ppm 390, 180, 100 and 60	Testicle (benign Leydig-cell tumour): 64/390, 34/180, 21/100, 17/60*	* $P < 0.05$ $P < 0.019$ (trend)	99.7% pure
		Liver (hepatocellular adenoma): 13/167, 3/84, 4/53, 6/31	[NS]	
		Liver (hepatocellular carcinoma): 2/167, 3/84, 0/53, 3/31*	*[$P < 0.05$]	
		Liver (hepatocellular adenoma or carcinoma, combined): 15/167, 6/84, 4/53, 9/31*	*$P < 0.005$ $P = 0.001$ (trend)	

F, female; M, male; mo, months; NR, not reported; NS, not significant; wk, week or weeks

Multiple liver tumours occurred more frequently in exposed mice than in controls. (Kluwe et al., 1982; NTP, 1982; Kluwe et al., 1985).

Five groups of 70, 60, 65, 65 and 70 male and 70, 60, 65, 65 and 70 female B6C3F$_1$ mice, 6 weeks of age, were fed 0 (controls), 100, 500, 1500 or 6000 ppm DEHP in the diet for up to 104 weeks. Ten to 15 animals per group were killed during week 79. The remaining animals were autopsied at week 105. Survival was reduced in male and female mice receiving 6000 ppm DEHP. Overall weight gain was significantly lower in 6000-ppm males. Significantly higher liver weight was observed in 500-, 1500- and 6000-ppm males and 6000-ppm females. A significantly higher incidence of hepatocellular adenoma or carcinoma (combined) was observed in 500- (males only), 1500- and 6000-ppm groups (incidences at week 79 and 105 were combined). The incidence of hepatocellular adenoma and of hepatocellular carcinoma was increased in a dose-related manner [P for trend < 0.0005] in males and females (David et al., 1999, 2000a).

3.2.2 Rat

Groups of 50 male and 50 female Fischer 344 rats, 4–5 weeks of age, were maintained on a diet containing 0 (controls), 6000 or 12 000 ppm DEHP for 103 weeks. A dose-related decrease in mean body weight gain was observed throughout the study in males. In females, body weight gain was reduced in the high-dose group only. In males, the incidence of hepatocellular adenoma and of hepatocellular carcinoma did not differ significantly among the groups. However, in males of the 12 000-ppm group, the incidence of hepatocellular adenoma or carcinoma (combined) differed significantly from that in controls. In females, the incidence of hepatocellular adenoma and of hepatocellular carcinoma was significantly higher in the high-dose groups compared with controls. In addition, the incidence of hepatocellular adenoma or carcinoma

(combined) was significantly higher in low-dose and high-dose females (Kluwe et al., 1982; NTP, 1982; Kluwe et al., 1985).

Two groups of 10 male Fischer 344 rats, 6 weeks of age, were fed a diet containing 0% (controls) or 2% DEHP (purity, 98%) for 95 weeks. Hepatocellular adenoma or carcinoma (combined) developed in six of 10 rats fed DEHP and none were found in eight controls (P < 0.005, χ^2 test) (Rao et al., 1987). [The Working Group noted the small number of animals per group and the limited reporting of the study.]

Groups of 80, 50, 55, 65 and 80 male and 80, 50, 55, 65 and 80 female Fischer 344 rats, 6 weeks of age, were fed a diet containing 0 (controls), 100, 500, 2500 and 12 500 ppm DEHP for 104 weeks. No significant differences in survival were observed among the groups. Body weight and food consumption were significantly lower (P < 0.05) in males and females of the 12 500-ppm group. The incidence of hepatocellular carcinoma or hepatocellular adenoma (combined) was significantly higher in 100-ppm females, 2500-ppm males and 12 500-ppm males and females compared with controls. The incidence of hepatocellular adenoma and of hepatocellular carcinoma was increased in a dose-related manner [P for trend < 0.0005] in males and females. In males of the high-dose group (12 500 ppm), but not in females or in other groups of males, the incidence of acinar-cell adenoma of the pancreas was significantly increased compared with controls (David et al., 1999, 2000b).

Seven hundred and thirty male Sprague-Dawley (SD-CD) rats were divided into four groups. DEHP was mixed with the diet at 0 (controls), 600, 1897 and 6000 ppm. DEHP-treated animals were fed 5 g DEHP-diet/100 g bw daily on 6 days per week for 159 weeks and received DEHP-free diet on the 7th day only after they had consumed the rest of their DEHP-containing diet. On this basis, the DEHP-treated rats were exposed to 30, 95 and 300 mg/kg bw, respectively. Controls were fed an equicaloric

DEHP-free diet. No difference in survival was observed among the groups. In the high-dose group, the incidence of hepatocellular adenoma or carcinoma (combined) and of benign Leydig-cell tumours was significantly increased and showed a dose-related trend. Time to tumour analysis revealed that DEHP-induced Leydig-cell tumours developed earlier in life (Voss *et al.*, 2005). [The Working Group noted the large variability in the number of animals per group.]

3.3 Intraperitoneal injection

3.3.1 Hamster

Three groups of 25 male and 25 female Syrian golden hamsters, 6 weeks of age, received intraperitoneal injections of 3 g/kg bw DEHP once a week, once every 2 weeks or once every 4 weeks. A group of 25 males and 25 females served as untreated controls. The animals were observed for life or were killed when moribund. No significant difference in tumour incidence was observed between the DEHP-treated groups and the controls (Schmezer *et al.*, 1988).

3.4 Genetically modified mouse

3.4.1 Oral administration

Groups of 15 male and 15 female CB6F1-rasH2 mice, 6 weeks of age, were fed DEHP (purity, > 99%) in the diet at concentrations of 0 (controls), 1500, 3000 or 6000 ppm, and groups of 15 male and 15 female wild-type mice were fed concentrations of 0 (controls) or 6000 ppm DEHP in the diet, for 26 weeks. DEHP treatment induced hepatocellular adenomas in 1/15, 2/15 and 4/15 ($P < 0.05$, Fisher's exact probability test) male CB6F1-rasH2 mice in the 1500-, 3000- and 6000-ppm groups, respectively, compared with 0/15 controls. No hepatocellular tumours were observed in females or wild-type mice (Toyosawa *et al.*, 2001; Usui *et al.*, 2001).

Groups of 15 male and 15 female Tg.AC mice were fed a diet containing 0 (controls), 1500, 3000 or 6000 ppm DEHP for 26 weeks. No increase in the incidence of tumours was observed (Eastin *et al.*, 2001).

Groups of 15 male and 15 female $Xpa^{-/-}$ mice were fed a diet containing 0 (controls), 1500, 3000 or 6000 ppm DEHP, and groups of 15 male and 15 female wild-type (C57BL/6) or $Xpa^{-/-}/P53^{+/-}$ were fed a diet containing 0 (controls) or 6000 ppm DEHP. The observation period for all groups was 39 weeks. No significant difference in tumour incidence was observed between the DEHP-treated groups and their respective controls (Mortensen *et al.*, 2002).

DEHP-induced tumorigenesis was compared in wild-type and $Ppar\alpha$-null Sv/129 mice. Mice of each genotype, 3 weeks of age, were divided into three groups and fed diets containing 0 (controls), 0.01 or 0.05% DEHP [purity unspecified] for 22 months. The liver tumour incidence in the $Ppar\alpha$-null mice was 1/25, 1/25 and 8/31 for the 0, 0.01 and 0.05% doses, respectively. In the wild-type mice, the corresponding incidence was 0/24, 2/23 and 2/20 for the 0, 0.01 or 0.05% doses, respectively. According to the authors, the incidence of liver tumours was significantly higher ($P < 0.05$) in $Ppar\alpha$-null mice exposed to 0.05% DEHP (8/31; six hepatocellular adenomas, one hepatocellular carcinoma, one cholangiocellular carcinoma) than in control $Ppar\alpha$-null mice (1/25; one hepatocellular carcinoma). [The Working Group noted the unusual grouping of cholangiocellular and hepatocellular tumours. When comparing hepatocellular tumours only (7/31 versus 1/25), there was no statistical difference ($P = 0.052$, Fisher's one-tailed test)] (Ito *et al.*, 2007a).

3.4.2 Skin application

Groups of 15 male and 15 female Tg.AC mice, 8–9 weeks of age, received daily topical applications of 0 (controls), 100, 200 or 400 mg/kg bw

DEHP (in acetone) on 5 days per week for 28 weeks. DEHP did not increase the incidence of tumours at the site of application (Eastin et al., 2001). [The Working Group noted the limited reporting of the study.]

3.5 Co-exposure with modifying agents

3.5.1 Mouse

(a) Oral administration

Male B6C3F$_1$ mice, 4 weeks of age, received a single intraperitoneal injection of 80 mg/kg bw N-nitrosodiethylamine (NDEA). At 5 weeks of age, the mice were fed a diet containing 0 (controls) or 3000 ppm DEHP (Aldrich Chemical Co. [purity unspecified]) for 1, 7, 28, 84 or 168 days (and killed after 168 days), or for 168 days (and killed after 252 days). When DEHP was fed for 28, 84 or 168 days (killed after 168 days), or 168 days (killed after 252 days), the incidence of hepatocellular adenoma was increased compared with mice receiving NDEA only (6/29, 5/28, 14/30 [P < 0.01] and 5/11 [P < 0.01], respectively, versus 6/30 controls) (Ward et al., 1984).

Groups of 10–20 male B6C3F$_1$ mice, 4 weeks of age, received a single intraperitoneal injection of 80 mg/kg bw NDEA followed 2 weeks later by exposure to 0 (controls), 3000, 6000 or 12 000 ppm DEHP (Aldrich Chemical, Co. [purity unspecified]) in the diet for 18 months. DEHP increased the incidence of hepatocellular carcinoma (3/10, 10/10 [P < 0.01], 18/20 [P < 0.01] and 11/20, respectively) (Ward et al., 1986). [The Working Group noted that the mean survival of the animals treated with 12 000 ppm DEHP was much lower (8.7 months) than that in the 3000-ppm (> 18 months) and 6000-ppm (> 17 months) groups.]

(b) Skin application

Two groups of 25 female SENCAR mice, 7 weeks of age, received a single topical application of 20 μg/animal 7,12-dimethylbenz[a] anthracene. Starting 1 week later, one group received twice-weekly applications of 12-O-tetradecanoylphorbol 13-acetate (TPA) (2 μg/application) for 2 weeks and then multiple twice-weekly applications of 100 mg DEHP (purity, 99%) for 26 weeks; the other group received TPA only and served as the control group. The animals were killed at experimental week 29. DEHP acted as a second-stage promoter in enhancing skin tumorigenesis (total number of papillomas: 161 versus 55 in controls [P < 0.01]; 6.44 papilloma/mouse versus 2.2/control mouse) (Diwan et al., 1985).

3.5.2 Rat

Groups of 10 female CF-344/CrlBR rats, 6–8 weeks of age, received a single intraperitoneal injection of 150 mg/kg bw NDEA. Two weeks later, they were maintained on a basal diet containing 0 or 1.2% DEHP. Five animals from each group were killed at 3 and 6 months. No liver tumours were observed (Popp et al., 1985).

Groups of 10 female F344/NCr rats, 5 weeks of age, received a single intraperitoneal injection of 282 mg/kg bw NDEA followed 2 weeks later by exposure to 0 (controls), 3000, 6000 or 12 000 ppm DEHP in the diet for 14 weeks. DEHP did not increase the number or size [details not given] of NDEA-induced hepatocellular proliferative lesions, including hyperplastic foci and hepatocellular tumours (Ward et al., 1986). [The Working Group noted the limited reporting of the results.]

Two groups of 6–12 male F344 rats were fed 200 ppm 2-acetylaminofluorene in the diet for 7 weeks and were subsequently given 0 (controls) or 12 000 ppm DEHP in the diet for 24 weeks. DEHP did not enhance the occurrence of

2-acetylaminofluorene-induced liver neoplasms (Williams *et al.*, 1987).

Groups of 20 male F344 rats, 5 weeks of age, were fed 0.05% *N*-ethyl-*N*-hydroxyethylnitrosamine in the diet for 2 weeks and were subsequently maintained on a diet containing 0% (controls) or 1.2% DEHP (Wako Pure Chemical Ind. Ltd [purity unspecified]) for 24 weeks. The incidence (13/20 versus 4/20 in controls) and multiplicity (1.10 ± 1.12 versus 0.20 ± 0.41 in controls) of renal tubule adenoma or carcinoma (combined) were significantly ($P < 0.01$) increased compared with controls (Kurokawa *et al.*, 1988).

Groups of 15 male F344/DuCrj rats, 6 weeks of age, were given drinking-water containing 0.05%   *N*-butyl-*N*-(4-hydroxybutyl)nitrosamine for 4 weeks and were then maintained on diets containing 0 (controls), 0.3, 0.6 or 1.2% DEHP. The diets contained also 3.0% uracil for 2 weeks. Surviving animals were killed at the end of experimental week 20. DEHP did not increase the incidence of urinary bladder papilloma (Hagiwara *et al.*, 1990).

3.5.3 Hamster

Two groups of 25 male and 20–25 female Syrian golden hamsters, 6 weeks of age, were exposed to DEHP vapour in the breathing air at a concentration of 0 (controls) or 15 ± 5 µg/m^3 continuously from 12 weeks of age until natural death. The animals were also given an oral dose of 0.3 mg *N*-nitrosodimethylamine (NDMA) in saline once a week for 30 weeks. DEHP did not increase the incidence of NDMA-induced liver tumours (Schmezer *et al.*, 1988).

A group of 25 male and 25 female Syrian golden hamsters, 6 weeks of age, received intra-peritoneal injections of 3 g/kg bw DEHP once every 4 weeks and were also treated once a week with an oral dose of 1.67 mg/kg bw NDMA. A control group of 25 males and 25 females was treated with NDMA, but no DEHP. The animals

were observed for life or were killed when moribund. DEHP did not increase the incidence of NDMA-induced liver tumours (Schmezer *et al.*, 1988).
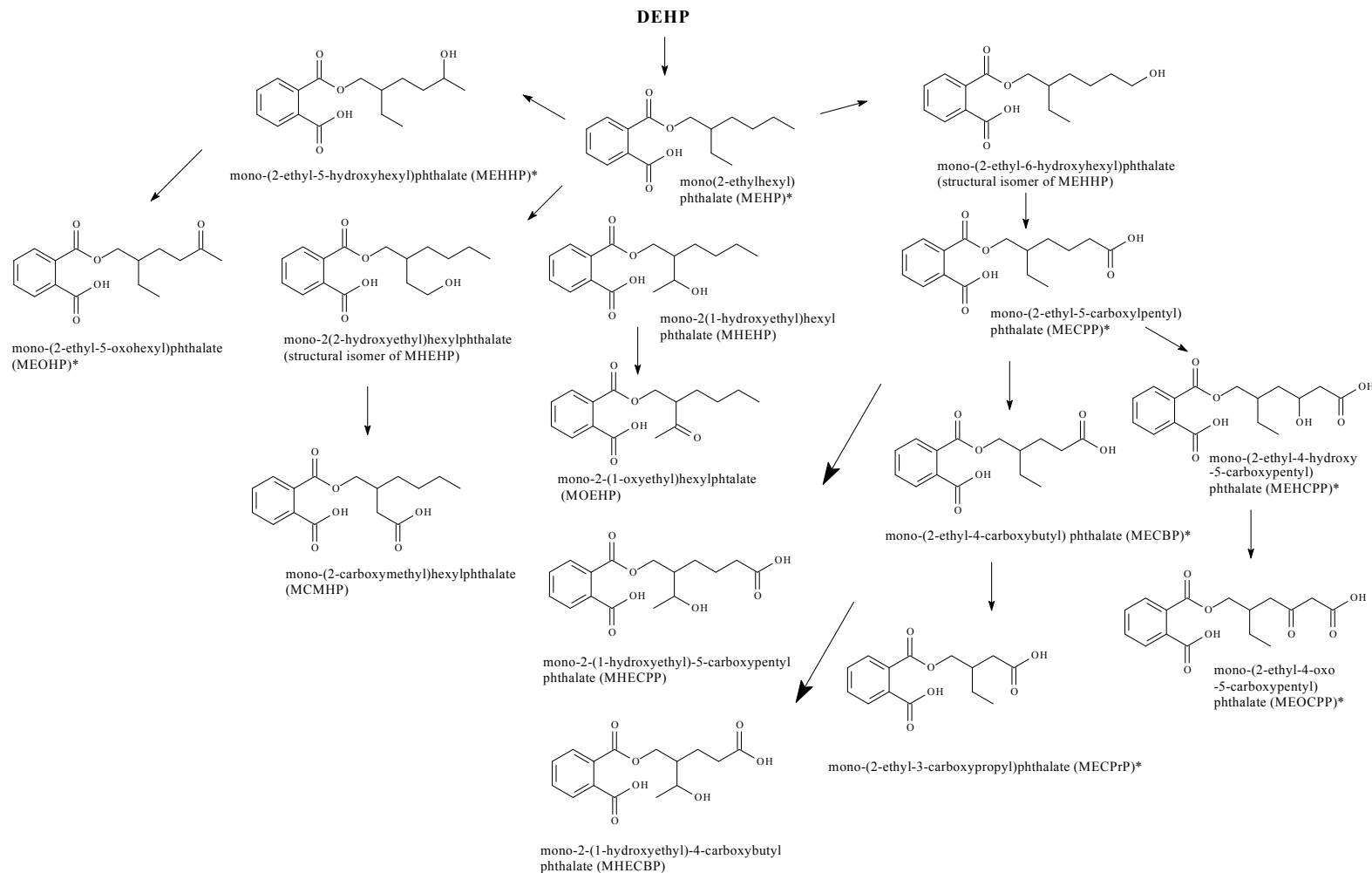
4. Other Relevant Data

4.1 Absorption, distribution, metabolism and excretion

Human exposure to DEHP can occur via the dermal, inhalation, oral and intravenous routes, and may be high when the compound is released from medical equipment to patients in neonatal intensive care units (Silva *et al.*, 2006). Since the last review (IARC, 2000), several studies have been published on absorption, distribution, metabolism and excretion of DEHP in rodents and, more importantly, in humans. the reproductive and developmental toxicity of DEHP has been evaluated twice by an expert panel (Kavlock *et al.*, 2002, 2006) that provides comments on such studies.

Much attention has been paid to the DEHP metabolite, MEHP. Upon ingestion, DEHP is rapidly metabolized to MEHP by pancreatic lipases in the lumen of the gut in both rodents and humans, before being further converted into oxidative metabolites and glucuronidated for excretion in the urine and faeces. The review by Silva *et al.* (2006) shows the metabolic schema for humans and rodents (Fig. 4.1).

The approaches for studying the absorption, distribution, metabolism and excretion of DEHP are hampered by its ubiquitous presence in the environment and laboratory equipment, and by its hydrolysis under abiotic conditions to monoesters (Fromme *et al.*, 2007b). Studies that used radiolabelled DEHP provided the most accurate estimation of its absorption, distribution, metabolism and excretion and metabolites. Two sets of data are most frequently cited and

Fig. 4.1 Suggested mechanisms for di(2-ethylhexyl) phthalate (DEHP) metabolism in humans

DEHP

mono-(2-ethyl-5-hydroxyhexyl)phthalate (MEHHP)*

mono(2-ethylhexyl) phthalate (MEHP)*

mono-(2-ethyl-6-hydroxyhexyl)phthalate (structural isomer of MEHHP)

mono-(2-ethyl-5-oxohexyl)phthalate (MEOHP)*

mono-2(2-hydroxyethyl)hexylphthalate (structural isomer of MHEHP)

mono-2(1-hydroxyethyl)hexyl phthalate (MHEHP)

mono-(2-ethyl-5-carboxylpentyl) phthalate (MECPP)*

mono-(2-carboxymethyl)hexylphthalate (MCMHP)

mono-2-(1-oxyethyl)hexylphtalate (MOEHP)

mono-(2-ethyl-4-carboxybutyl) phthalate (MECBP)*

mono-(2-ethyl-4-hydroxy-5-carboxypentyl) phthalate (MEHCPP)*

mono-2-(1-hydroxyethyl)-5-carboxypentyl phthalate (MHECPP)

mono-(2-ethyl-4-oxo-5-carboxypentyl) phthalate (MEOCPP)*

mono-(2-ethyl-3-carboxypropyl)phthalate (MECPrP)*

mono-2-(1-hydroxyethyl)-4-carboxybutyl phthalate (MHECBP)

Di(2-ethylhexyl) phthalate

are most applicable to the characterization of the absorption, distribution, metabolism and excretion of DEHP in humans (Koch *et al.*, 2004b, 2005b, 2006) and in rodents (Albro & Thomas, 1973; Albro *et al.*, 1983; Albro, 1986; Albro & Lavenhar, 1989). As discussed below, studies in marmosets have attempted to reflect the human situation and the possible risk of effects of DEHP, but the ability to metabolize and absorb DEHP apparently differs between the two species. Emerging evidence indicates that the effects of DEHP on the metabolism of endogenous and xenobiotic compounds are relevant to its toxicity. The activities of several metabolic enzymes have been shown to be both enhanced and inhibited by exposure to DEHP in experimental animals. As discussed in Section 4.3, DEHP may specifically affect general metabolism through the constitutive androstane receptor (CAR)-2, a nuclear receptor transcription factor. Only one physiologically based pharmacokinetic model is available for predictions on DEHP and MEHP in rats (Keys *et al.*, 1999). The adequacy of this model is discussed below.

4.1.1 Humans

(a) Absorption, distribution and excretion

Urinary and serum levels of DEHP metabolites were evaluated in a human male volunteer after a single oral dose (48.10 mg; 641 µg/kg bw) of deuterium-labelled DEHP (i.e. the D4-ring-labelled DEHP) to avoid background interference (Koch *et al.*, 2004b, 2005b). The excretion of two oxidative metabolites, MEHHP and MEOHP, and MEHP was monitored for 44 hours in urine (i.e. 25 samples every 1–4 hours after dosing) and for 8 hours in serum (i.e. five samples before and every 2 hours after dosing). Peak concentrations of the three metabolites were found in serum after 2 hours and of oxidative metabolites in the urine after 4 hours. In serum, all metabolites were unconjugated and the major metabolite was MEHP at a much higher concentration (4.95 mg/L)

than the oxidative metabolites (0.20 mg/L MEHHP and 0.06 mg/L MEOHP). The half-lives for all metabolites in serum were estimated to be shorter than 2 hours. Excretion in the urine followed a multiphase elimination model in which the absorption and distribution phase lasted approximately 4–8 hours. MEHP oxidation products are polar metabolites and consequently showed higher urinary elimination: after 44 hours, 47% of the DEHP dose was excreted in the urine in the form of MEHHP (peak concentration, 10.04 mg/L), MEOHP (6.34 mg/L) and MEHP (3.63 mg/L).

The clearance of DEHP through excretion of its metabolites was studied after three doses of D4-ring-labelled DEHP (low, 4.7 µg/kg bw; medium, 28.7 µg/kg bw; and high, 650 µg/kg bw) were administered to the same volunteer at 1-week intervals (Koch *et al.*, 2005b). The study identified two new ω-oxidation products, MECPP and mono(2-carboxymethyl)hexyl phthalate (MCMHP). Monitoring was carried out for 44 hours in urine and 8 hours in blood plus in 24-hour urine samples for the medium and low doses. On a molar basis, after 24 hours, 70.5% of the high DEHP dose was excreted in the urine (24.1% as MEHHP, 20.7% as MECPP, 14.6% as MEOHP, 7.3% as MEHP and 3.8% as MCMHP). An additional 3.8% of DEHP was excreted as metabolites on day 2 to give a total of 74.3% DEHP excreted in the urine after 2 days. The elimination half-lives were 5 hours for MEHP, 10 hours for MEHHP and MEOHP, 12–15 hours for MECPP and 24 hours for MCMHP. The proportional metabolite excretion rate relative to DEHP did not vary by dose, and therefore metabolism and excretion were not dose-dependent. Thus, most of the orally administered DEHP was absorbed and then excreted in the urine. Unlabelled metabolites found in the urine of the volunteer in this study indicated that DEHP metabolism was comparable with that found in the general population. [However, the Working Group recognized that higher exposure

levels than the range tested may result in differences in metabolism and excretion as well as other factors related to potentially susceptible subpopulations.]

The above results suggested that the use of secondary DEHP metabolites in urine may give a more accurate estimate of DEHP exposure and dose than MEHP in the blood or urine because of the short half-life of MEHP. However, Koch et al. (2005b) noted that serum MEHP levels in humans were of the same order of magnitude as those in animal studies, despite lower doses in human studies and a dose-normalized area under the curve (AUC) in blood which was 15–100 times higher in the human volunteer than in animals. Koch et al. (2005b) stated that, if it is assumed that MEHP in blood is a surrogate for toxic potential, DEHP would be 15–100 times more toxic in humans than in marmosets or rats.

Previous estimates of human absorption have been reported in two volunteers exposed to non-labelled DEHP (Schmid & Schlatter, 1985) and extrapolated from studies of absorption in rats (Rhodes et al., 1986). The data of Koch et al. (2004b, 2005b) showed a much higher fraction of absorption using labelled than non-labelled DEHP. The authors also stressed that, in previous human studies, standard substances were not used to quantify the metabolites and to exclude confounding due to contamination. Koch et al. (2004b) noted that Peck & Albro (1982) found that, 24 hours after intravenous infusion of a platelet concentrate containing DEHP to a cancer patient, almost 60% of the infused dose appeared in the urine as DEHP metabolites.

Several DEHP exposure studies have also noted a greater proportion of oxidative metabolites than MEHP in monitored urine (Barr et al., 2003; Silva et al., 2006; Weuve et al., 2006; Fromme et al., 2007b; Wittassek et al., 2007a, b). Silva et al. (2006) identified urinary oxidative metabolites of DEHP from individuals with MEHP concentrations about 100 times higher than the median concentrations in the general population of the

USA. Three additional oxidative metabolites were identified: mono(2-ethyl-3-carboxypropyl) phthalate, mono(2-ethyl-4-carboxybutyl) phthalate and mono-2-(1-oxoethyl)hexyl phthalate; the presence of urinary DEHP metabolites containing less than eight carbons in the alkyl chain, that had previously been identified in rodents, were also reported in humans (Albro et al., 1983), indicating a similar oxidative metabolism of DEHP in humans and rodents. All of these metabolites were identified in human urine based on their chromatographic and mass spectrometric behaviour. Moreover, Silva et al. (2006) noted that all of the above metabolites were identified in three adult volunteers who were not exposed to DEHP via the intravenous route, with MEHHP and MEOHP levels above 500 ng/mL. They concluded that the presence of similar metabolites regardless of dose and route of exposure may indicate a uniform metabolism of DEHP in humans.

Glucuronidation increases urinary clearance of DEHP metabolites, but Koch et al. (2004b, 2005b) noted the absence of glucuronidated MEHP in the blood of the volunteer (results in urine were not reported). Albro et al. (1982) cite urinary data from the two humans given non-radioactive DEHP by Peck et al. (1978) as the basis for species comparisons of the glucuronidation of urinary DEHP metabolites. The levels of free versus conjugated metabolites were reported as 20% versus 80% with no specific information with regard to the form. Peck & Albro (1982) reported the chemical structure of the urinary metabolites in only one of the two patients, that MEHP represented only 11% of the given dose (174.3 mg DEHP), and that approximately 80% of the urinary metabolites were glucuronidated. Therefore, from the available human data, oxidative metabolites and not MEHP appear to be specifically glucuronidated.

In a variability study, MEHP was present in 95% of the samples with secondary metabolites detectable in 100% of the samples (Fromme et al.,

2007b). The authors reported substantial day-to-day and within-subject variability also after adjustment for creatinine (a reasonable surrogate for bw-adjusted dose). Hauser et al. (2004) found substantial variation in individual day-to-day excretion levels. Such variation in urinary metabolism reflects differences in exposure as well as absorption, distribution, metabolism and excretion between subjects.

Silva et al. (2006) reported that exposure to DEHP in the general population occurs via inhalation rather than dermal absorption and that MEHP may be found in human amniotic fluid (Silva et al., 2004). Moreover, Frederiksen et al. (2007) cited a body of literature indicating that DEHP is found in human breast milk (unmetabolized or as its primary monoester). [The presence of oxidative metabolites in free (unconjugated) form in breast milk and amniotic fluid may pose additional risks (Kavlock et al., 2006).]

Exposure studies in humans that measured primary and secondary urinary metabolites suggested age-related differences in their production and/or clearance, with younger children producing higher proportions of MEHHP and MEOHP compared with MEHP (Kavlock et al., 2006; Wittassek et al., 2007a). Neonates show a further deviation in oxidative DEHP metabolism, with MECPP being the most predominant (Wittassek & Angerer, 2008). A reduced potential for glucuronidation may lead to slower excretion and higher concentrations of DEHP metabolites in neonates than in older children and adults (Weuve et al., 2006).

(b) Metabolism

After ingestion, DEHP is rapidly metabolized to MEHP by pancreatic lipases in the lumen of the gut in multiple species (Albro & Thomas, 1973; Albro & Lavenhar, 1989), and is further converted to oxidative metabolites in both rats and humans (Silva et al., 2006). The oxidation of MEHP can occur via cytochrome P450 (CYP) 4A, alcohol dehydrogenase (ADH) and aldehyde dehydrogenase (ALDH), and yields multiple oxidation products of 2-ethylhexanol (2-EH) and of the aliphatic side chain of MEHP (Fay et al., 1999). Both ω-1 and ω-2 oxidation occur in humans; however, the forms of CYP or other enzymes that are responsible for this oxidative metabolic activity are not known.

Koch et al. (2005b) reported rapid degradation of all DEHP metabolites in blood at room temperature. Kato et al. (2004) reported that human serum lipase activity could almost totally convert DEHP to MEHP. Lipases are also present in breast milk (Kavlock et al., 2006). Weuve et al. (2006) noted that neonates have elevated gastric lipase activity (Hamosh, 1990), which may enhance their ability to convert DEHP to MEHP as well as to increase their digestion of milk fats. Because the concentrations of excreted substances are dependent on water intake, toxicokinetics are easier to interpret from measurements in blood than from those in urine. However, measurements of phthalate monoesters are susceptible to contamination in blood from the parent phthalates, which are also hydrolysed to their respective monoesters by serum enzymes (Kato et al., 2004).

(c) Toxicokinetic models

No pharmacologically based pharmacokinetic model is available in humans to predict the absorption, distribution, metabolism and excretion of DEHP or its metabolites from a given exposure concentration. Wittassek et al. (2007a, b) have extrapolated exposure concentrations from data on urinary excretion in children and adults (Koch et al., 2005b). The authors did not take into account differences in lipase activity and absorption, distribution, metabolism and excretion between age groups. [The Working Group did not present the results of this extrapolation because the products are estimates of exposure concentration and not outputs of absorption, distribution, metabolism and excretion.]

4.1.2 Experimental systems

(a) Absorption, distribution and excretion

Relatively little information is available regarding the extent to which DEHP is metabolized before or during absorption from the gastrointestinal tract and the available data are limited to rodents. In general, a high level of DEHP hydrolase activity has been found in pancreatic juice, intestinal contents and/or intestinal tissue of many mammalian species (Albro & Lavenhar, 1989). Most if not all DEHP that reaches the intestines is probably absorbed as hydrolysis products rather than intact diester.

Studies of labelled DEHP in several species have reported that the majority of an oral dose is absorbed and excreted, and that the levels of absorption are different among the species (Albro *et al.*, 1982; Albro, 1986; Rhodes *et al.*, 1986; Albro & Lavenhar, 1989; Kessler *et al.*, 2004). Ikeda *et al.* (1980) fed 50 mg/kg bw DEHP to rats, dogs and miniature pigs for 21–28 days and then gave ^{14}C-labelled DEHP on the last day by gavage. By 24 hours, rats were reported to have excreted 84% of the label (urine and faeces), whereas dogs excreted 67% and pigs excreted 37%. [The Working Group noted that the small sample size of these studies limited interpretations regarding interindividual variability and reduced the confidence in comparisons between species.]

Calafat *et al.* (2006) gave unlabelled DEHP to pregnant Sprague-Dawley rats at different doses (11, 33, 100 or 300 mg/kg bw by gavage) and reported urinary concentrations of 1.62, 3.19, 8.11 and 15.9 μg/mL MEHP, respectively. They also provided creatinine-adjusted values.

Koo & Lee (2007) administered a single dose of [^{14}C]DEHP to assess serum and urinary excretion of DEHP and MEHP in 4-week-old Sprague-Dawley rats. Total radioactivity peaked at 24 hours in the urine and after 24 hours in the serum. Urinary DEHP and MEHP levels peaked at 24 hours only for the highest dose tested. [Results

in the text of the report for urinary concentrations of MEHP at 24 hours differed from those displayed graphically.] Serum concentrations at 24 hours appeared to be proportional to the dose for MEHP (6.22, 36.3 and 169.19 μg/mL following doses of 40, 200 and 1000 mg/kg bw, respectively) but not for DEHP (1.78, 2.38 and 5.3 μg/mL following the same doses, respectively). The peak concentration, the time to reach peak concentration and the AUC were greater for MEHP than for DEHP in both urine and serum but elimination half-lives of DEHP were greater than those of MEHP. Kessler *et al.* (2004) conducted a similar study in Sprague-Dawley rats treated orally with 30–500 mg per day unlabelled DEHP. Normalized AUCs of DEHP were at least one order of magnitude smaller than those of MEHP. Results from the two studies differed in terms of the time at which peak concentrations were achieved in blood or serum samples. Quantitatively, the magnitude of peak concentration achieved was comparable at the highest dose (1000 mg/kg). [Inconsistencies exist between the concentrations of DEHP reported in the blood by Koo & Lee (2007) and Kessler *et al.* (2004).]

In contrast to earlier reports (Albro *et al.*, 1982), Calafat *et al.* (2006) administered DEHP (0, 11, 33, 100 or 300 mg/kg bw per day) to pregnant Sprague-Dawley rats and reported that MEHP was mostly glucuronidated (87%) in the urine whereas free MEHP predominated in the amniotic fluid (88.2%). They suggested that maternal urinary MEHP levels may be a useful surrogate marker for fetal exposure to DEHP because these values were inter-correlated. It was noted that although glucuronidation increases the hydrophilic properties of the phthalate metabolites, thus enhancing their urinary excretion, it is not known whether their conjugated species are biologically inactive.

Studies of the distribution of labelled DEHP in the tissues of experimental animals have been reported, but in most cases only total radioactivity was monitored with no distinction between

DEHP and its metabolites (Lindgren *et al.*, 1982; Gaunt & Butterworth, 1982; Rhodes *et al.*, 1986; Isenberg *et al.*, 2000; Ono *et al.*, 2004). Therefore, the tissue distribution of parent DEHP following exposure cannot be ascertained by any route. The liver appears to acquire an initially higher load of DEHP and/or its metabolites (Albro & Lavenhar 1989; Pugh *et al.*, 2000; Ito *et al.*, 2007a) with second highest specific activity in adipose tissue (Williams & Blanchfield, 1974; Ikeda *et al.*, 1980). Ikeda *et al.* (1980) reported radioactivity in the bile of DEHP-exposed dogs and pigs for several days suggesting the possibility of entero-hepatic recirculation. Studies of unlabelled DEHP showed no consistent rise in different tissues in animals (Ljungvall *et al.*, 2004; Rhind *et al.*, 2009).

(b) Metabolism

Albro (1986) reported that pancreatic tissue has the greatest ability to hydrolyse DEHP in CD rats through competition of non-specific lipases that require a phthalate monoester as a substrate. Pancreatic lipase activity per gram of tissue was 400-fold that in intestinal mucosa and 240-fold that in the liver. The only esterase reported to metabolize MEHP was located in liver microsomes and was assumed to be responsible for the formation of urinary phthalic acid found in rats and mice. Liver and kidney oxidation of MEHP appeared to occur via microsomal monooxygenases analogous (or identical) to the CYP-associated fatty acid ω- and (ω-1) hydroxylases (Albro & Lavenhar, 1989).

Lake *et al.* (1977) evaluated the rates of intestinal hydrolysis of phthalates, including DEHP, among species and reported them to be: ferrets > rats > humans; baboons have a rate threefold higher than that of ferrets.

Ito *et al.* (2005) measured the activity of four DEHP-metabolizing enzymes (lipase, uridine 5′-diphospho-glucuronosyl transferase (UGT), ADH and ALDH) in several organs (liver, lungs, kidneys and small intestine) of CD-1 mice, rats

and marmosets. The authors reported that lipase activity was highest in the small intestines of mice, UGT activity was highest in mice, and ADH and ALDH activities were 1.6–3.9 times greater in the livers of marmosets than in those of rats or mice. [Interpretation of the study is limited by the lack of measurement of pancreatic lipase.]

Studies of the effects of age on metabolism are limited and results are contradictory (Gollamudi *et al.*, 1983; Sjöberg *et al.*, 1985c).

(c) Toxicokinetic models

Currently, only one physiologically based pharmacokinetic model is available for DEHP and MEHP in rats (Keys *et al.*, 1999). [The adequacy of this model is limited by the data available to the authors at the time it was published and by the difficulties in obtaining important information with which to construct and test the model. However, it also has methodological issues regarding its construction and validation.] Tissue:blood partition coefficients for DEHP were estimated from the *n*-octanol:water partition coefficient, while partition coefficients for MEHP were determined experimentally using a vial equilibration technique. All other parameters were either found in the literature or were estimated. The flow-limited model failed to simulate the available data adequately. Alternative plausible models were explored, including diffusion-limited membrane transport, enterohepatic circulation and MEHP ionization (pH-trapping model), which significantly improved predictions of DEHP and MEHP blood concentrations with respect to the flow-limited model predictions. In the pH-trapping model, non-ionized MEHP is assumed to pass into intracellular compartments where it is mostly ionized and trapped intracellularly until it is de-ionized and released. [No references were given by the authors and there are no known references in the literature stating that MEHP is ionized. The authors appear to base their assumption on structure–activity relationships. It is not readily apparent why MEHP is

assumed to be ionized but DEHP is not (because they do not differ greatly by molecular weight).]

4.2 Genetic and related effects

An examination of the current literature since the publication of the previous *Monograph* (IARC, 2000) showed that, although in most bacterial systems DEHP gives negative results, a robust response is shown in cell transformation and DNA damage assays.

The information from the original Table 7 of the previous *Monograph* (IARC, 2000) is provided for reference in Table 4.1 (i.e. genetic and related effects of DEHP, MEHP and other DEHP metabolites), which contains some additional information, as well as some corrections made to the originally reported results and new studies. The genotoxicity of DEHP oxidative metabolites other than MEHP has not been adequately characterized experimentally and no new studies are available.

4.2.1 Humans

(a) DNA adducts

No specific studies were identified regarding DNA adduct formation following exposure to DEHP or its metabolites.

(b) DNA strand breaks

Exposure to DEHP, as detected by its metabolite MEHP, has been associated with increased DNA damage in humans. DEHP is suspected to contribute to the increasing incidence of testicular dysgenesis syndrome. Hauser *et al.* (2007) assessed human sperm DNA damage following environmental exposure to DEHP using the neutral Comet assay. The urinary levels of phthalate metabolites were similar to those reported among the general population of the USA: the 50th and 95th percentiles were 7.7 ng/mL and 112 ng/mL for MEHP, and 48.5 ng/mL and

601 ng/mL for MEHHP. MEHP concentration was positively associated with a 12% increase in tail DNA relative to the study population median, and with sperm DNA damage after adjusting for MEHP oxidative metabolites. The authors suggested that the negative association of Comet results with the concentrations of oxidative metabolites may serve as a phenotypic marker for DEHP metabolism, indicating lower toxicity if a person is able to metabolize DEHP/MEHP oxidatively to a greater extent. An earlier report (Duty *et al.*, 2003) with fewer samples and no adjustment for oxidative metabolites did not find a relationship between MEHP and sperm DNA strand breaks.

DEHP has been studied *in vitro* in various human tissues using Comet assays. Anderson *et al.* (1999a) reported dose-related increased median tail moments (alkaline Comet assay) in both leukocytes at non-toxic DEHP concentrations and in blood lymphocytes. The addition of metabolic activation abolished the effect in the positive leukocyte experiment. The length of exposure was not given. Biscardi *et al.* (2003) found increased Comet length in human leukocytes exposed for 1 hour to organic extracts of water lyophilisates stored in polyethylene terephthalate bottles. The study was successful in detecting the presence of DEHP, although concentrations were not reported. Choi *et al.* (2010) reported increased Olive tail moment 24 and 48 hours after exposure to DEHP (0.97 µg/mL) in human hepatocyte HepG2 cells without cytotoxicity. Whether the assay was alkaline or neutral was not clear. Erkekoğlu *et al.* (2010a) reported increased tail intensity and moment in LNCaP prostate adenocarcinoma cells (24-hour exposure, alkaline Comet assay). The experiments were conducted at half maximal inhibitory concentrations (IC_{50}) that induced cell cytotoxicity (1170 µg/mL). Park & Choi (2007) reported increased tail moment in HeLa cervical epithelial carcinoma cells (24-hour exposure). There was a dose-related increase in tail moments from

Table 4.1 Genetic and related effects of di(2-ethylhexyl) phthalate (DEHP), mono(2-ethylhexyl) phthalate (MEHP) and other DEHP metabolites

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.* (1982a)
Salmonella typhimurium, reverse mutation	NT	+	5 mg/plate	Tomita *et al.* (1982a)
Salmonella typhimurium, forward mutation	–	–	500	Liber (1985)
Salmonella typhimurium TA100, TA1535, TA1537, TA1538, TA98, reverse mutation	–	–	9860 μg/plate	Kirby *et al.* (1983)
Salmonella typhimurium TA100, TA98, reverse mutation	–	–	4000 μg/plate	Robertson *et al.* (1983)
Salmonella typhimurium TA100, TA98, reverse mutation	–	–	2000 μg/plate	Yoshikawa *et al.* (1983)
Salmonella typhimurium TA100, TA102, TA98, TA97, reverse mutation	–	–	10 000 μg/plate	Baker & Bonin (1985)
Salmonella typhimurium TA100, TA102, TA98, TA97, reverse mutation	–	–	5000 μg/plate	Matsushima *et al.* (1985)
Salmonella typhimurium TA100, TA102, TA98, TA97, reverse mutation	–	–	10 000 μg/plate	Nohmi *et al.* (1985)
Salmonella typhimurium TA100, TA1535, TA1537, TA1538, TA98, reverse mutation	–	–	5000 μg/plate	Rexroat & Probst (1985)
Salmonella typhimurium TA100, TA1535, TA98, TA97, reverse mutation	–	–	10 000 μg/plate	Zeiger & Haworth (1985)
Salmonella typhimurium TA100, TA1535, TA1537, TA98, reverse mutation	–	–	10 000 μg/plate	Zeiger *et al.* (1985)
Salmonella typhimurium TA1537, TA98, TA7001, TA7002, TA7003, TA7004, TA7005, TA7006, reverse mutation	–	–	1000 μg/plate	Gee *et al.* (1998)
Salmonella typhimurium TA1535/pSK 1002, *umu* C gene expression system with commercial rat liver metabolic system (*n* = 3)	–	+	780	Okai & Higashi-Okai (2000)
Salmonella typhimurium TA1535/pSK 1002, *umu* C gene expression system with fresh Sprague-Dawley rat liver metabolic system (*n* = 3)	NT	+	780	Okai & Higashi-Okai (2000)
Salmonella typhimurium TA1535/pSK 1002, *umu* C gene expression system with fresh Sprague-Dawley rat pancreas metabolic system (*n* = 3)	NT	+	780	Okai & Higashi-Okai (2000)
Salmonella typhimurium TA1535/pSK 1002, *umu* C gene expression system with fresh Sprague-Dawley rat intestine metabolic system (*n* = 3)	NT	+	780	Okai & Higashi-Okai (2000)
Salmonella typhimurium TA1535/pSK 1002, *umu* C gene expression system with fresh Sprague-Dawley rat kidney metabolic system (*n* = 3)	NT	–	780	Okai & Higashi-Okai (2000)
Salmonella typhimurium TA1535/pSK 1002, *umu* C gene expression system with fresh Sprague-Dawley rat lung metabolic system (*n* = 3)	NT	–	780	Okai & Higashi-Okai (2000)
Salmonella typhimurium TA1535/pSK 1002, *umu* C gene expression system with hog pancreatic lipase (*n* = 3) + 1 mM cholic acid	NT	+	780	Okai & Higashi-Okai (2000)

Table 4.1 (continued)

Test system	Result[a]		Dose[b]	Reference
	Without exogenous metabolic system	With exogenous metabolic system	(LED or HID)	
Salmonella typhimurium TA1535/pSK 1002, *umu* C gene expression system with hog pancreatic lipase (*n* = 3) + 5 mM cholic acid	NT	+	780	Okai & Higashi-Okai (2000)
Salmonella typhimurium TA1535/pSK 1002, *umu* C gene expression system with hog pancreatic lipase (*n* = 3) + 1 mM deoxycholic acid	NT	+	780	Okai & Higashi-Okai (2000)
Salmonella typhimurium TA1535/pSK 1002, *umu* C gene expression system with hog pancreatic lipase (*n* = 3) + 1 mM deoxycholic acid	NT	+	780	Okai & Higashi-Okai (2000)
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)
Saccharomyces cerevisiae D7–144, gene conversion[c]	+	NT	3100	Mehta & von Borstel (1985)
Saccharomyces cerevisiae D7–144, gene conversion[c]	NT	+	1500	Mehta & von Borstel (1985)
Saccharomyces cerevisiae XV185-^{14}C, point mutation[c]	+	NT	12 300	Mehta & von Borstel (1985)
Saccharomyces cerevisiae XV185-^{14}C, point mutation[c]	NT	+	3100	Mehta & von Borstel (1985)
Saccharomyces cerevisiae RM52, point mutation[c]	+	–	12 300	Mehta & von Borstel (1985)
Saccharomyces cerevisiae, gene conversion	–	–	5000	Parry & Eckardt (1985)
Saccharomyces cerevisiae D6, aneuploidy	+	+	5000	Parry & Eckardt (1985)
Saccharomyces cerevisiae D7, mitotic segregation	–	+	5000	Parry & Eckardt (1985)
Saccharomyces cerevisiae, homozygosis	–	–	5000	Arni (1985)
Saccharomyces cerevisiae, homozygosis	–	–	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	–	–	5000	Parry & Eckardt (1985)
Saccharomyces cerevisiae, forward mutation	–	–	1000	Inge-Vechtomov *et al.* (1985)
Saccharomyces pombe, forward mutation	?	–	5900	Loprieno *et al.* (1985)
Saccharomyces cerevisiae DEL assay and ICR recombination	–	–	200 000	Carls & Schiestl (1994)
Aspergillus nidulans, haploid mutation, non-disjunction and mitotic crossing-over	–	NT	9900	Carere *et al.* (1985)
Allium cepa (rooting onion), total chromosomal aberration, 96 h immersion (*n* = 5500 cells) *in vivo*	–		50	Rank *et al.* (2002)
Allium cepa (rooting onion), total chromosomal aberration, 96 h immersion (*n* = 5500 cells) *in vivo*	+		5	Rank *et al.* (2002)

Table 4.1 (continued)

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Tradsecantia pollen cells, micronucleus formation, plant mutagenicity test (Trad/MCN test)	+		?[d]	Biscardi *et al.* (2003)
Drosophila melanogaster, crossing-over/recombination	–		39 000 μg/g food	Würgler *et al.* (1985)
Drosophila melanogaster, somatic mutation	(+)		6930 μg/cm² [53300 μg/mL]	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)
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)
Chironomus riparius larvae, mouthpart deformities, *in vivo* (10 d) (*n* = 13/ dish × 3)	+		1	Park & Kwak (2008)
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)
DNA single- and double-strand breaks[e], Comet assay (alkaline) tail intensity, MA-10 mouse Leydig tumour cell line (24 h) *in vitro*	+	NT	1170	Erkekoğlu *et al.* (2010a)
DNA single- and double-strand breaks[e], Comet assay (alkaline) tail moment, MA-10 mouse Leydig tumour cell line (24 h) *in vitro*	+	NT	1170	Erkekoğlu *et al.* (2010a)
Cell viability (24 h), MA-10 mouse Leydig tumour cell line *in vitro*	+	NT	3.9	Erkekoğlu *et al.* (2010a)
Reactive oxygen species production (24 h), MA-10 mouse Leydig tumour cell line *in vitro*	+	NT	1170	Erkekoğlu *et al.* (2010a)
Unscheduled DNA synthesis, rat primary hepatocytes *in vitro*	–	NT	3900	Butterworth *et al.* (1984); Kornbrust *et al.* (1984); Probst & Hill (1985)
Unscheduled DNA synthesis, rat primary hepatocytes *in vitro*	–	NT	10 000	Williams *et al.* (1985)
Unscheduled DNA synthesis, rat primary hepatocytes *in vitro*	–	NT	1000	Astill *et al.* (1986)
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)

Table 4.1 (continued)

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*	−	−	4900	Myhr *et al.* (1985)
Gene mutation, mouse lymphoma L5178Y cells, *Tk*^{+/−} locus *in vitro*	(+)	(+)	7.5	Oberly *et al.* (1985)
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 V79 cells *in vitro*	+	NT	25	Tomita *et al.* (1982a)
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	NR	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)
Chromosomal aberrations, rat liver RL4 cells *in vitro*	−	NT	1000	Priston & Dean (1985)
Chromosomal aberrations, Syrian hamster embryo cells *in vitro*[f]	−	NT	30 μM [12] 24-h	Tsutsui *et al.* (1993)
Chromosomal aberrations, Syrian hamster embryo cells *in vitro*[f]	NT	+	1 μM [0.4] 2-h	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, C3H 10T½ mouse cells	−	NT	3.9	Sanchez *et al.* (1987)

Table 4.1 (continued)

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Cell transformation, Syrian hamster embryo cells, clonal assay	+	NT	1[g]	Barrett & Lamb (1985)
Cell transformation, Syrian hamster embryo cells, clonal assay[h]	+	NT	100	Sanner & Rivedal (1985)
Cell transformation, Syrian hamster embryo cells, clonal assay[h]	+	NT	4	Sanner & Rivedal (1985)
Cell transformation, Syrian hamster embryo cells, clonal assay[h]	+	NT	0.8	Sanner & Rivedal (1985)
Cell transformation, SA7/Syrian hamster embryo cells[i]	+	NT	78	Hatch & Anderson (1985)
Cell transformation, SA7/Syrian hamster embryo cells[i]	+	NT	507	Hatch & Anderson (1985)
Cell transformation, Syrian hamster embryo cells, clonal assay	+	NT	4 μM [1.5 μg/mL]	Mikalsen et al. (1990)
Cell transformation, Syrian hamster embryo cells, clonal assay	+	NT	30 μM [12 μg/mL]	Mikalsen & Sanner (1993)
Cell transformation, Syrian hamster embryo cells, clonal assay[f]	+	NT	3 μM [1.2] 48-h	Tsutsui et al. (1993)
Cell transformation, Syrian hamster embryo cells, clonal assay[f]	NT	+	30 μM [12] 2-h	Tsutsui et al. (1993)
Cell transformation, Syrian hamster embryo cells, clonal assay[f]	NT	+	3 μM [1.2] 3-h	Tsutsui et al. (1993)
Cell transformation, Syrian hamster embryo cells, clonal assay (traditional X-radiated feeder layer [j])	+	NT	5	Pant et al. (2010)
Cell transformation, Syrian hamster embryo cells, clonal assay (conditioned media)	+	NT	2.5	Pant et al. (2010)
Cell transformation, RLV/Fischer rat embryo cells, anchorage independent cell growth[k]	?	NT	NR	Suk & Humphreys (1985)
Cell transformation, RLV/Fischer rat embryo cells, anchorage independent cell growth[k]	+	NT	2000	Suk & Humphreys (1985)
Ornithine decarboxylase superinduction, Syrian hamster embryo cells[l]	–	NT	39	Dhalluin et al. (1998)
DNA strand breaks, Comet assay (alkaline), human leukocytes in vitro (median tail moment)	–, +	NT	390, 3900[m]	Anderson et al. (1999a)
DNA strand breaks, Comet assay (alkaline), human leukocytes in vitro (median tail moment)	+	–	31 and 156[m]	Anderson et al. (1999a)
DNA strand breaks, Comet assay (alkaline), human lymphocytes in vitro (median tail moment)	+	–	3.9 and 195[n]	Anderson et al. (1999a)
DNA single and double-strand breaks[d], Comet assay, human leukocytes in vitro (total comet length; 1 h)	+	NT	NR	Biscardi et al. (2003)
DNA single and double-strand breaks[d], Comet assay, human leukocytes in vitro (number of cells > 96th distribution; 1 h)	+	NT	NR	Biscardi et al. (2003)
DNA single and double-strand breaks, Comet assay, human hepatocyte HepG2 cell line in vitro (Olive tail moment)	+	NT	0.97 24-h	Choi et al. (2010)

Table 4.1 (continued)

Test system	Result[a] Without exogenous metabolic system	Result[a] With exogenous metabolic system	Dose[b] (LED or HID)	Reference
DNA single and double-strand breaks, Comet assay, human hepatocyte HepG2 cell line *in vitro* (Olive tail moment)	+	NT	0.97 48-h	Choi *et al.* (2010)
DNA single and double-strand breaks, Comet assay, LNCaP human prostate adenocarcinoma cell line *in vitro* (tail intensity)[e]	+	NT	1170 24-h	Erkekoğlu *et al.* (2010b)
DNA single and double-strand breaks, Comet assay, LNCaP human prostate adenocarcinoma cell line *in vitro* (tail moment)[e]	+	NT	1170 24-h	Erkekoğlu *et al.* (2010b)
DNA single and double-strand breaks, Comet assay (alkaline), human HeLa cells *in vitro* (tail moment; *n* = 3)[o]	+	NT	38 24-h	Park & Choi (2007)
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 (co-culture with rat liver cells) *in vitro*	–	(+)	39	Lindahl-Kiessling *et al.* (1989)
Chromosomal aberrations, human lymphocytes *in vitro*	–	NT	75	Turner *et al.* (1974)
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)
DNA strand breaks, Wistar rat liver *in vivo*	–		2000 po × 28	Elliott & Elcombe (1987)
DNA oxidative damage, F344 rat liver *in vivo*	+[p]		12 000 mg/kg diet, 1 yr	Takagi *et al.* (1990a)
DNA oxidative damage, F344 rat liver *in vivo*	+[p]		12 000 mg/kg diet, 1–2 wk	Takagi *et al.* (1990b)
DNA single-strand breaks, F344 rat liver *in vivo*	–		20 000 mg/kg diet, 78 wk	Tamura *et al.* (1991)
DNA oxidative damage, F344 rat liver *in vivo*	–		12 000 mg/kg diet, 22 wk	Cattley & Glover (1993)
DNA oxidative damage, 8-OHdG in the liver, male Sprague-Dawley rats *in vivo*	+		1000 po × 14 d	Seo *et al.* (2004)
DNA single-strand breaks (quantification of hydroxy DNA ends) in liver, male F344 rats *in vivo*	–		1.2% diet [12 000 mg/kg] × 5 mo	Pogribny *et al.* (2008)
Unscheduled DNA synthesis, F344 rat hepatocytes *in vivo*	–		500 po, 150 po × 14, or 12 000 mg/kg diet, 30 d + 500 po	Butterworth *et al.* (1984)

Table 4.1 (continued)

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Unscheduled DNA synthesis, Sprague-Dawley rat hepatocytes *in vivo*	–		5000 po	Kornbrust *et al.* (1984)
Unscheduled DNA synthesis, F344 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)
Gene mutation, *lac*I transgenic C57BL/6 mouse liver *in vivo*	–		6000 mg/kg diet, 120 d	Gunz *et al.* (1993)
Gene mutation, *lacZ* gene, *lacZ* transgenic mouse liver, *in vivo* (2 wk)	+, +		2333 po × 6	Boerrigter (2004)
Gene mutation, *lacZ* gene, *lacZ* transgenic mouse kidney, *in vivo* (2 wk)	–, –		2333 po × 6	Boerrigter (2004)
Gene mutation, *lacZ* gene, *lacZ* transgenic mouse spleen, *in vivo* (2 wk)	–, –		2333 po × 6	Boerrigter (2004)
Gene mutation, G*pt* mutant frequency in liver DNA, G*pt* delta transgenic Sprague-Dawley female rats *in vivo*	–		12 000 ppm [1440 mg/kg/d] × 13 wk (diet)	Kanki *et al.* (2005)
Micronucleus formation, mice *in vivo*	–		5000 po	Astill *et al.* (1986)
Micronucleus formation, B6C3F$_1$ mouse erythrocytes *in vivo*	–		6000 ip × 5	Douglas *et al.* (1986)
Micronucleus formation, male rat (Sprague-Dawley or F344) hepatocytes, *in vivo*[q]	?		1000 po	Suzuki *et al.* (2005)
Micronucleus formation, male rat (Sprague-Dawley or F344) reticulocytes, *in vivo*[q]			1000 po	Suzuki *et al.* (2005)
Chromosomal aberrations (aberrant metaphase), Syrian hamster exposed *in vivo*, embryos cultured *in vitro*[r]	+		7500 po	Tomita *et al.* (1982a)
Chromosomal aberrations, F344 rat bone marrow *in vivo*	–		4900 po × 5	Putman *et al.* (1983)
Aneuploidy, F344 rat hepatocytes *in vivo*	–		12 000 diet, 7 d	Hasmall & Roberts (1997)
Proportion in any ploidy class, F344 rat hepatocytes (*n* = 4) analysed 30 h after first dose	?		950 po × 2	Hasmall & Roberts (2000)
Proliferation in all ploidy classes (greatest in higher classes of octoploid cells), F344 rat hepatocytes (*n* = 4) analysed 30 h after first dose	+		950 po × 2	Hasmall & Roberts (2000)
Cell transformation, Syrian hamster exposed *in vivo*, embryos cultured *in vitro*[r]	+		7500 po	Tomita *et al.* (1982a)
Sperm morphology, B6C3F$_1$ mice *in vivo*	–		6000 ip × 5	Douglas *et al.* (1986)
Sperm morphology, Sprague-Dawley rats *in vivo*	–		5200 ip × 5	Douglas *et al.* (1986)
Dominant lethal test, ICR Swiss male mice *in vivo*	+		12.78 mL/kg [12 780 mg/kg] ip	Singh *et al.* (1974)

Table 4.1 (continued)

Test system	Result[a]		Dose[b]	Reference
	Without exogenous metabolic system	With exogenous metabolic system	(LED or HID)	
Dominant lethal test, male mice *in vivo* (d 1,5,10)	+	NT	1 mL/kg [1000 mg/kg] sc × 3	Autian (1982)
Dominant lethal test, ICR Swiss male mice *in vivo* (d 1,5,10)	+	NT	1 mL/kg [1000 mg/kg] sc × 3	Agarwal *et al.* (1985)
Body fluids, Sprague-Dawley rat urine, microbial mutagenicity	–	–	2000 po × 15	DiVincenzo *et al.* (1985)
Binding (covalent) to F344 rat hepatocyte DNA *in vitro*	–	NT	390	Gupta *et al.* (1985)
Binding (covalent) to DNA liver, F344 rat *in vivo*	–		10 000 mg/kg diet, 11 d	Albro *et al.* (1982)
Binding (covalent) to DNA liver, F344 rat *in vivo*	–		10 000 mg/kg diet, 4 wk	von Däniken *et al.* (1984)
Binding (covalent) to DNA liver, F344 rat *in vivo*	–		2000 po × 3	Gupta *et al.* (1985)
Binding (covalent) to DNA liver, F344 rat *in vivo*	–		500 po	Lutz (1986)
Mono(2-ethylhexyl) phthalate (MEHP)				
Bacillus subtilis rec, differential toxicity	+	NT	400 μg/disc [400 μg/mL]	Tomita *et al.* (1982a)
Salmonella typhimurium TA100, reverse mutation	+	–	1250 μg/plate [350 μg/mL]	Tomita *et al.* (1982a)
Salmonella typhimurium TA100, reverse mutation	+ (with cytotoxic-city)	NT	2500 μg/plate [700 μg/mL	Tomita *et al.* (1982a)
Salmonella typhimurium TA100, TA1535, TA1537, TA1538, TA98, reverse mutation	–	–	0.2 μl/plate [100 μg/mL]	Kirby *et al.* (1983)
Salmonella typhimurium TA100, TA102, TA98, TA97, reverse mutation	–	–	1000 μg/plate [500 μg/mL]	Dirven *et al.* (1991)
Escherichia coli WP2 B/r	+	NT	700 (without cytotoxicity)	Tomita *et al.* (1982a)
Saccharomyces cerevisae strain Bj3505, yeast-based estrogen receptor gene transcription assay (methylated sequences in the ER promoter region)	–	NT	280	Kang & Lee (2005)
Unscheduled DNA synthesis, B6C3F$_1$ mouse primary hepatocytes *in vitro*	–	NT	139	Smith-Oliver & Butterworth (1987)
Gene mutation, mouse lymphoma L5178Y cells, *Tk*$^{+/-}$ locus *in vitro*	–	–	0.3 μL/mL [300 μg/mL]	Kirby *et al.* (1983)
Sister chromatid exchange, Chinese hamster V79 cells *in vitro*	+	NT	25	Tomita *et al.* (1982a)

Table 4.1 (continued)

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	**With exogenous metabolic system**		
Chromosomal aberrations, Syrian hamster embryo cells *in vitro*	–	+	10 µM [2.78 µg/mL]	Tsutsui *et al.* (1993)
Chromosomal aberrations, Syrian hamster embryo cells *in vitro*[f]	–	NT	83.4 24-h	Tsutsui *et al.* (1993)
Chromosomal aberrations, Syrian hamster embryo cells *in vitro*[f]	NT	+	0.84 2-h	Tsutsui *et al.* (1993)
Cell transformation, Syrian hamster embryo cells[f]	(+)	NT	28 48-h	Tsutsui *et al.* (1993)
Cell transformation, Syrian hamster embryo cells[f]	NT	+	56 2-h	Tsutsui *et al.* (1993)
Cel transformation in Syrian hamster embryo cells	–	(+)	100 µM [27.8 µg/mL]	
Cell transformation, Syrian hamster embryo cells, clonal assay	+	NT	23 µM [6.4 µg/mL]	Mikalsen *et al.* (1990)
Cell transformation, C3H10T½ mouse cells	–	NT	417	Sanchez *et al.* (1987)
DNA single- and double-strand breaks, Comet assay (alkaline), MA-10 mouse Leydig tumour cell line (24 h)[e] (tail intensity)	+	NT	0.84	Erkekoğlu *et al.* (2010a)
DNA single- and double-strand breaks, Comet assay (alkaline), MA-10 mouse Leydig tumour cell line (24 h)[e] (tail moment)	+	NT	0.84	Erkekoğlu *et al.* (2010a)
DNA single- and double-strand breaks, Comet assay (alkaline), MA-10 mouse Leydig tumour cell line (24 h)[e] (cell viability)	–	NT	0.84	Erkekoğlu *et al.* (2010a)
DNA single- and double-strand breaks, Comet assay (alkaline), MA-10 mouse Leydig tumour cell line (24 h)[e] (reactive oxygen species production)	–	NT	0.84	Erkekoğlu *et al.* (2010a)
DNA strand breaks, Comet assay, human leukocytes *in vitro* (median tail moment)	+	NT	28	Anderson *et al.* (1999a)
DNA single- and double-strand breaks, Comet assay, LNCaP human prostate adenocarcinoma cell line *in vitro* (24 h)[e] (tail intensity and moment)	+	NT	0.84	Erkekoğlu *et al.* (2010b)
DNA single- and double-strand breaks, Comet assay, human inferior nasal turbinate epithelial cells *in vitro*, slow migration of Olive tail moments (% DNA in tail × median migration) > 2	+		1400	Kleinsasser *et al.* (2004a)
DNA single- and double-strand breaks, Comet assay, human inferior nasal turbinate mucosal cells *in vitro*, slow migration of Olive tail moments (% DNA in tail × median migration) > 2 and % of DNA in tail	+		28	Kleinsasser *et al.* (2004b)
DNA single- and double-strand breaks, Comet assay, human peripheral lymphocytes *in vitro*, slow migration of Olive tail moments (% DNA in tail × median migration) > 2 and % of DNA in tail	+		28	Kleinsasser *et al.* (2004b)
Unscheduled DNA synthesis, human primary hepatocytes *in vitro*	–	NT	139	Butterworth *et al.* (1984)
DNA strand breaks, Wistar rat liver *in vivo*	–	NT	500	Elliott & Elcombe (1987)
Chromosomal aberrations, Syrian hamster exposed *in vivo*, embryos cultured *in vitro*	+		375 × 1 po	Tomita *et al.* (1982a)

Table 4.1 (continued)

Test system	Result[a]		Dose[b]	Reference
	Without exogenous metabolic system	With exogenous metabolic system	(LED or HID)	
Cell transformation, Syrian hamster exposed *in vivo* embryos cultured *in vitro*	+		375 × 1 po	Tomita *et al.* (1982a)
DNA single- and double-strand breaks, Comet assay (neutral), human sperm *in vivo*[s] (comet extent, % DNA in tail, total distributed moment)	–		IQR increase in urinary concentration (max., 0.4 μg/mL urine)	Duty *et al.* (2003)
DNA single and double-strand breaks, Comet assay, human sperm *in vivo*[s] (comet extent, % DNA in tail, total distributed moment)	+		IQR increase in urinary concentration (max., 0.9 μg/mL urine)	Hauser *et al.* (2007)
Mono(2-ethyl 5-hydroxyhexyl) phthalate				
Salmonella typhimurium TA100, TA102, TA98, TA97, reverse mutation	–	–	1000 μg/plate [500 μg/mL]	Dirven *et al.* (1991)
Mono(2-ethyl 5-oxohexyl) phthalate				
Salmonella typhimurium TA100, TA102, TA98, TA97, reverse mutation	–	–	1000 μg/plate [500 μg/mL]	Dirven *et al.* (1991)
Mono(5-carboxyl 2-ethylpentyl) phthalate				
Salmonella typhimurium TA100, TA102, TA98, TA97, reverse mutation	–	–	1000 μg/plate [500 μg/mL]	Dirven *et al.* (1991)
2-Ethylhexanol				
Bacillus subtilis rec, differential toxicity	–	NT	500	Tomita *et al.* (1982a)
Salmonella typhimurium TA100, TA1535, TA1537, TA1538, TA98, reverse mutation	–	–	1 μL/plate [500 μg/mL]	Kirby *et al.* (1983)
Gene mutation, mouse lymphoma L5178 cells, *Tk* locus *in vitro*	–	–	0.3 μL/mL [300 μg/mL]	Kirby *et al.* (1983)
Phthalic acid				
Bacillus subtilis rec, differential toxicity	–	NT	500 μg/disk	Tomita *et al.* (1982a)

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

[b] LED, lowest effective dose; HID, highest ineffective dose; in-vitro tests, μg/mL; in-vivo tests, mg/kg bw per day

[c] The authors only recognized a response greater than twofold as positive.

[d] The authors examined extracts of lyophilisates of mineral water contaminated with DEHP after storage in plastic (polyethylene terephthalate) bottles but the concentrations of DEHP in the lyophilisates were not given by the authors and a dose–response cannot therefore be assessed.

[e] The authors conducted the Comet assay at the half maximal inhibitory concentration only at which cytotoxicity was observed.

[f] There was an increase at every dose of DEHP tested (1–300 μM) for aberrant metaphase including chromatid gaps and isochromatid gaps after 2 h of exposure. The authors conducted

Table 4.1 (continued)

a trend test for chromosomal aberrations which was positive for DEHP. In the same experiment, MEHP induced an increase in the percentage of transformed colonies between 2.8 and 28 μg/mL after exposure for 48 hours but the number of colonies scored was half that induced by DEHP and the increases were not statistically significant for MEHP.

[g] Dose-related increases starting at 1 μg/mL; no statistical analysis made by authors

[h] The authors did not conduct statistical analyses of their data but considered a result to be positive if it was greater than 1% transformation. For one experiment, the lowest concentration of DEHP (0.8 μg/mL) gave a positive result of 0.79% which was threefold greater than the concurrent control for that experiment.

[i] The authors only recognized a response greater than twofold as positive; in one experiment, there was cytotoxicity as higher levels and the increase at 78 μg/mL gave the same magnitude of response as exposure to 507 μg/mL in another experiment with no cytotoxicity.

[j] The authors reported a similar result for 2.5 μg/mL DEHP as that for 5.0 μg/mL using the standard method, but it failed to reach significance.

[k] The authors did not conduct statistical analyses of their data and considered a result with a twofold enhancement of survival to be positive and, at two or more concentrations, to be a positive dose–response. The positive control was lost due to contamination; the authors therefore considered one experiment to be null. In a second experiment, DEHP-induced anchorage-independent survival showed a dose–response at 1000 and 2000 μg/mL (61% and 161%). No trend test was made.

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

[m] Similar responses, one of which was while the other was not statistically significant; no trend test made in the second experiment. In one experiment, a positive response was found at 3900 μg/mL but with significant toxicity and the response was negative at 390 μg/mL.

[n] Dose-related increases started at 3.9 μg/mL and were statistically significant at 195 μg/mL, but no trend test was made.

[o] The authors did not carry out a trend test but the response was increased in a dose-related manner with statistical significance reached at the half maximal inhibitory concentration (toxicity).

[p] No oxidative damage in kidney DNA

[q] The authors used 50% and 25% of the half maximal lethal dose. In one laboratory, there was 70% cytotoxicity at 1000 mg/kg and ~60% cytotoxicity in another in the liver (these were the lowest doses tested). In one laboratory, the total number of reticulocytes was reduced. Cytotoxicity rendered the results of this study suspect.

[r] Only two doses were tested, with the lowest dose giving a 2.7-fold increase in aberrant metaphase above control levels (3750 mg/kg) but the higher dose (7500 mg/kg) giving a result that reached statistical significance. A similar result was reported for transformation.

[s] Sperm DNA damage was associated with monoethylphthalate and MEHP after adjusting for DEHP oxidative metabolites, which may serve as phenotypic markers for DEHP metabolism to 'less toxic' metabolites. Results are given as adjusted regression coefficients (95% confidence interval) for common parameters associated with an interquartile range increase in MEHP and mono(2-ethyl-5-hydroxyhexyl) phthalate when both compounds are included in the same model; 89% of MEHP in the urine was above the level of detection (1.2 ng/mL). The authors hypothesized that % DNA in tail may indicate single-strand breaks compared with comet extent and tail distributed moment (double-strand breaks).

d, day or days; Gpt, guanine phosphoribosyltransferase; h, hour or hours; ip, intraperitoneal; IQR, interquartile ranges; max, maximum; mo, month or months; NR, not reported; 8OH-dG, 8-hydroxydeoxyguanosine; po, oral; sc, subcutaneous; Tk, thymidine kinase; wk, week or weeks; yr, year or years

9.4 µg/mL DEHP (a concentration that induced little cytotoxicity) to 38 µg/mL. The authors did not carry out a trend test and only reported statistical significance at the IC_{50} concentration (38 µg/mL).

MEHP has also been studied in human tissues *in vitro* using the Comet assay. Kleinsasser *et al.* (2004a) used three-dimensional mini-organ cultures of inferior nasal turbinate epithelia from 25 donors. Olive tail moment > 2 was increased in the mini-organ cultures and in single-cell epithelial cultures, which were more sensitive to MEHP than the mini-organ cultures at relatively high concentrations (1400 µg/mL).

In a separate study, Kleinsasser *et al.* (2004b) reported increased Olive tail moments and percentage DNA in tail, in inferior nasal turbinate cultures and in peripheral lymphocytes exposed to MEHP (28 µg/mL). Anderson *et al.* (1999a) reported increases in median tail moment after exposure to MEHP (28 µg/mL) in human leukocytes.

Hauser *et al.* (2007) did not report reactive oxygen species or apoptosis in sperm.

Positive results from Comet assays in several human tissues or cell lines were reported at concentrations that did not induce cytotoxicity or apoptosis, resulting in loss of cell viability for DEHP and MEHP. For most of the in-vitro studies, positive Comet results were obtained at ~1–10 µg/mL for DEHP and ~30 µg/mL for MEHP. Thus, the parent compound appeared to be more efficient for this end-point.

(c) Mutations

No specific studies were identified regarding the induction of mutations in human tissues after exposure to DEHP or its metabolites.

(d) Chromosomal effects

The results of sister chromatid exchange and chromosomal aberration assays of human lymphocytes treated *in vitro* with DEHP have

been largely negative with the exception of Lindahl-Kiessling *et al.* (1989) (see Table 4.1).

(e) Changes in DNA methylation pattern

No DEHP-specific data on DNA methylation in humans were available to the Working Group.

4.2.2 Experimental systems

A large number of in-vitro systems have tested the ability of DEHP to induce mutation, transformation and epigenetic changes. Overlaps in context with assays already discussed in Section 4.2.1 are not repeated here. There is an especially robust database for Syrian hamster embryo (SHE) transformation (see Table 4.1). Overall, tests in *Salmonella* assays have given negative results for DEHP. Below, epigenetic and mutational changes in DEHP-induced tumours are discussed to elucidate the mechanisms of DEHP carcinogenicity.

(a) DNA oxidative damage

No covalent binding following exposure to DEHP *in vivo* has been reported in rat hepatocytes; DNA oxidative damage was previously reported at high concentrations of DEHP (IARC, 2000).

More recently, Seo *et al.* (2004) reported that administration of DEHP (14-day exposure) to Sprague-Dawley rats increased levels of 8-hydroxydeoxyguanosine (8-OHdG) (at 1000 mg/kg bw) and malondialdehyde (at 50 mg/kg bw) in liver DNA. Neither end-point was correlated with patterns of enzyme induction associated with peroxisomal proliferation or with the observed decreases in CYP1A1, -1A2, -3A4, UGT or glutathione *S*-transferase (GST). DEHP (12000 ppm [12000 mg/kg diet] for 22 days) induced expression of DNA repair enzymes (8-oxoguanine glycosylase/lyase, apurinic/apyrimidinic endonuclease, mammalian *N*-methylpurine-DNA glycosylase and polymerase β) in F334 rats (Rusyn *et al.*, 2000).

(b) DNA strand breaks

Pogribny et al. (2008) reported no increased DNA strand breaks as measured by the number of 3′-hydroxy DNA ends in F334 rats (1.2% DEHP in the diet for 5 months). Exposure to DEHP (1170 µg/mL) *in vitro* increased tail moment and intensity in the Comet assay in MA-10 mouse Leydig cell tumour lines (Erkekoğlu et al., 2010b). MA-10 cells showed ~80–60% survival at 3.9–195 µg/mL DEHP, increased tail moment and intensity and decreased cell viability at 0.84 µg/mL MEHP.

(c) Cell transformation

One of the most robust databases for the effects of DEHP, as measured in the SHE cell assay, was the study of neoplastic transformation by chemical carcinogens. Tsutsui et al. (1993) reported that a low concentration of DEHP (1.2 µg/mL) was effective after a longer period of exposure (48 hours) in the absence of exogenous metabolic activity and after shorter time periods in the presence of exogenous activity, with no effect on survival. Barrett & Lamb (1985) and Sanner & Rivedal (1985) also reported a positive response without metabolic activation at low levels of exposure (~1 µg/mL). Pant et al. (2010) demonstrated the feasibility of conducting the SHE cell transformation assay without using an X-ray-irradiated feeder layer and including conditioned media to find a greatly enhanced response at lower exposures to DEHP. Tomita et al. (1982a) exposed Syrian hamsters *in utero* to a single dose of DEHP (7500 mg/kg) and reported a positive transformation assay and chromosomal aberrations in the embryonic cells (see Section 4.2.2(*e*)).

Tsutsui et al. (1993) reported that MEHP gave negative results in the SHE assay after 48 hours and did not affect survival (up to 28 µg/mL), while it gave positive results for transformation in the presence of exogenous metabolic activation and at a higher concentration (56 µg/mL).

Mikalsen et al. (1990) reported that MEHP was positive at 5.6 µg/mL.

Park & Kwak (2008) exposed *Chironomus riparius* larvae to DEHP *in vivo* and reported increased mouthpart deformities and upregulation of heat shock protein (which interacts with certain cellular proteins including steroid hormone receptors) at a concentration of 1 µg/mL.

(d) Mutations

Most assays using *Salmonella typhimurium* have given negative results for DEHP (see Table 4.1). Tomita et al. (1982a) were able to induce a positive response with a large dose (5 mg/plate). Using the *umu* C gene expression system in *S. typhimurium*, Okai & Higashi-Okai (2000) reported that exposures to DEHP (780 µg/mL) gave negative results in the absence of activation and weakly positive results in the presence of a commercial metabolic activation mixture. Results were positive in the presence of metabolic activation from rat pancreas; weak but significant activities were reported with metabolic activation from liver and intestine; and no significant activities were observed with metabolic activation from lung and kidney. Significant *umu* C gene expression was obtained with highly purified lipase from porcine pancreas and was enhanced in the presence of bile acids. Metabolic lipase activity in various organs correlated with DEHP genotoxic activity.

Tomita et al. (1982a) reported positive results in *S. typhimurium* exposed to MEHP (700 µg/mL) and concurrent cytotoxicity, in the *Escherichia coli WP2 B/r* test system with no cytotoxicity at the same concentration and in the *Bacillus subtilis* differential toxicity test at 400 µg/disc.

Parry & Eckardt (1985) presented positive results for gene conversion and aneuploidy at cytotoxic concentrations of DEHP (5000 µg/mL). Gene conversions, and point and reverse mutations were induced in three strains of *Saccharomyces cerevisiae* D7-144 in the presence of metabolic activation at 1500 µg/mL

DEHP (Mehta & von Borstel, 1985). Largely negative results have been reported for mutations in *Drosophila melanogaster* exposed to DEHP.

In-vitro results on gene mutation in mouse lymphoma L5178Y cells at the thymidine kinase (*Tk*^{+/−}) locus have been mixed. Oberly *et al.* (1985) reported weakly positive results at 7.5 μg/mL DEHP, but several other studies reported negative results at much higher concentrations.

The dominant lethal test for mutation was positive in several studies in mice *in vivo* (Singh *et al.*, 1974; Autian, 1982; Agarwal *et al.*, 1985), with either repeated exposures (three subcutaneous injections of 1 mL/kg bw [1000 mg/kg bw]) or a single dose (injection of 12.78 mL/kg bw [12 780 mg/kg bw]). Dominant lethal mutations in parent germ cells may induce failure of implantation or early death due to unscheduled DNA synthesis, chromosomal breaks or elimination of aberrant chromosomes.

Boerrigter (2004) investigated the response of male and female *lacZ*-plasmid transgenic mice to six doses of 2333 mg/kg bw DEHP, 200 mg/kg bw 4-chloro-6-(2,3-xylidino)-2-pyrimidinylthioacetic acid (WY-14 643) or 90 mg/kg bw clofibrate. Mutant frequencies were significantly elevated (~40%) in the liver but not in kidney or spleen after 21 days of exposure to DEHP and WY-14 643 but not to clofibrate. The pattern of mutation induction matched that of tumour induction in mice for all the three compounds (Boerrigter, 2004).

Kanki *et al.* (2005) studied mutagenicity and mutation spectra *in vivo* in an animal model developed for genotoxicity analysis (i.e. guanine phosphoribosyltransferase delta transgenic rats). In the DEHP-treated (12000 ppm [12000 mg/kg diet], ~187 mg/rat per day) rats, about 10 random copies of the transgene lambda EG10 per haploid gene and marked hepatomegaly were reported, although only a few GST-placental form-positive liver cell foci were observed (GST-placental form staining was negative), and no mutagenic activity of DEHP was detected in the liver.

(e) Chromosomal effects

Sister chromatid exchange and micronucleus formation after exposure to DEHP *in vitro* have been investigated in Chinese hamster cells with mostly negative results. Suzuki *et al.* (2005) reported negative results for micronuclei and micronucleated reticulocytes at cytotoxic concentrations in 4-week-old rats. Fritzenschaf *et al.* (1993) reported positive micronucleus formation in SHE cells but the exposure level was not given. MEHP (25 μg/mL) increased sister chromatid exchange in Chinese hamster V79 cells (Tomita *et al.*, 1982a).

Exposure to DEHP *in vitro* did not induce chromosomal aberrations in several assays in Chinese hamster cells. However, chromosomal aberrations were increased in SHE cells in the presence of exogenous metabolic activity after exposure to 0.4 μg/mL DEHP or 0.84 μg/mL MEHP (Tsutsui *et al.*, 1993). MEHP (375 mg/kg bw) also increased chromosomal aberrations in embryo cells of pregnant Syrian hamsters (Tomita *et al.*, 1982a). The same cells gave positive results in the SHE transformation assay (see Section 4.2.2(*c*)).

Results for chromosomal aberration in the *Allium cepa* assay after in-vivo exposure to 5–50 μg/mL DEHP were inconclusive (Rank *et al.*, 2002).

No alteration of hepatocyte ploidy was reported in hepatocyates of adult F344 rats exposed *in vivo* to DEHP for 7 days (Hasmall & Roberts, 1997). However, two doses of 950 mg/kg bw DEHP have been reported to increase DNA synthesis after 30 hours of exposure in all ploidy classes and in particular in the octoploid classes (normally representing a small proportion of polyploidy cell in the liver) (Hasmall & Roberts, 2000).

(f) Changes in DNA methylation pattern

Pogribny *et al.* (2008) reported no changes in global methylation of total F334 rat liver DNA after exposure to 1.2% DEHP in the diet for 5 months. No statistically significant change in the methylation status of the promoter region of the *GST-pi* gene was reported. Western blot analysis showed an increase in DNA methyltransferase I and c-myc expression but not H3K9 or H4K20 histone trimethylation nor changes in the metabolism of methyl donors (*S*-adenosylmethionine and *S*-adenosylhomocysteine content in liver).

Wu *et al.* (2010) reported a DEHP-induced increase in global DNA methylation status and significantly upregulated RNA expressions of DNA methyltransferases (*Dnmt1*, *Dnmt3a* and *Dnmt 3b*) in the testes of male Kunming mouse fetuses after 1 week of maternal exposure to 500 mg/kg bw per day.

4.3 Mechanistic data

4.3.1 Hepatocyte-specific events

(a) Effects of DEHP on peroxisome proliferator-activated receptor (PPAR) activation

Phthalates, including DEHP, are ligands for nuclear receptors PPARα, -β and -γ (Issemann & Green, 1990; Dreyer *et al.*, 1992; Göttlicher *et al.*, 1992; Sher *et al.*, 1993; Corton & Lapinskas, 2005).

(i) Humans

No studies could be found which showed evidence that DEHP activates PPARα in human liver *in vivo*.

In vitro, trans-activation assays were used to assess the activation potential of DEHP, MEHP and 2-EHA for either full-length PPAR subtype (Maloney & Waxman, 1999; Hurst & Waxman, 2003; Lampen *et al.*, 2003; Lapinskas *et al.*, 2005) or hybrid transcription factors consisting of the PPAR ligand-binding domain fused with DNA-binding domains of the glucocorticoid receptor (Lampen *et al.*, 2003) or the transcriptor factor GAL4 (Bility *et al.*, 2004). DEHP did not activate human PPARα or human PPARγ (Maloney & Waxman, 1999). However, several studies showed that all three human PPAR subtypes were activated by MEHP (Maloney & Waxman, 1999; Hurst & Waxman, 2003; Lampen *et al.*, 2003; Bility *et al.*, 2004). In addition, both human PPARγ isoforms -γ1 and -γ2 were activated by MEHP (Feige *et al.*, 2007). The DEHP metabolite 2-EHA weakly activated human PPARα but not human PPARγ (Maloney & Waxman, 1999). No studies have examined the activation of human PPARβ by DEHP or 2-EHA.

Rotroff *et al.* (2010) examined the ability of DEHP to activate several response elements in a multiplex human response element transactivation assay in the hepatocellular carcinoma cell line, HepG2. In addition, a modification of the approach was used to generate the human ligand-binding domain of nuclear receptors expressed as a chimera with the yeast GAL4 DNA-binding domain that activated in trans a 5X-UAS$_G$-TATA promoter linked to a reporter sequence. DEHP was shown to activate PPARα and PPARγ significantly (effective concentration for half-maximal response in µM): PPARg_TRANS (46), PPRE_CIS (48), PPARa_TRANS (50). The PPARβ TRANS assay was negative for DEHP (Martin *et al.*, 2010; http://actor.epa.gov/actor/faces/ToxCastDB/assay.jsp).

Lapinskas *et al.* (2005) determined whether phthalate esters interact directly with human PPARα or PPARγ using the scintillation proximity assay. The binding affinity for MEHP to bind to the α and γ subtypes were 15 µM and 12 µM, respectively. DEHP was negative in this assay (i.e. binding affinity > 150 µM). The PPARβ subtype was not examined.

(ii) Experimental systems

The DEHP metabolite MEHP activates mouse PPARα, PPARγ and, to a lesser extent, PPARβ in trans-activation assays (Maloney & Waxman, 1999; Hurst & Waxman, 2003; Lampen *et al.*, 2003; Lapinskas *et al.*, 2005; Feige *et al.*, 2007). In addition, both mouse PPARγ isoforms — -γ1 and -γ2 — were activated by MEHP (Feige *et al.*, 2007). 2-EHA weakly activated mouse PPARα, -β and -γ (Lampen *et al.*, 2003; Lapinskas *et al.*, 2005). In one study, 2-EHA failed to activate mouse PPARγ (Maloney & Waxman, 1999). DEHP activated PPARα and PPARγ, albeit weakly, but not mouse PPARβ (Lampen *et al.*, 2003; Lapinskas *et al.*, 2005).

(iii) Species differences

DEHP treatment increased the expression of PPARα in mice and rats but not significantly, while the level of PPARα appeared to be reduced in marmosets (Ito *et al.*, 2007b).

[Important species differences in expression and molecular signalling for PPARα have been reported, and were considered to be of critical importance for the evaluation of human cancer risk from DEHP in the previous IARC evaluation (IARC, 2000). More recent studies are detailed below to illustrate the state of science in this area.]

Mice and rats express PPARα at high levels in the liver, whereas human PPARα is expressed at a lower level in human liver (Palmer *et al.*, 1998). Palmer *et al.* (1998) used electrophoretic mobility shift assays to determine the level of PPARα that binds to a peroxisome proliferator response element (PPRE) from the *CYP4A6* gene. In seven lysates in which PPARα could be detected by the assay, the amounts were about 10 times lower than those detected in the livers of CD-1 or BALB/cByJ mice; for the remainder of the 13 samples, the amount was below the level of detection (more than 20 times lower than in mouse liver). Using the RNase protection assay, a threefold variation in the expression

of full-length *PPARα* messenger RNA (mRNA) between human samples was observed. [The Working Group noted that expression of human PPARα has not been determined in a sufficient number of samples to conclude unequivocally that all populations express less PPARα than responsive rodents.]

In one study, the expression of human PPARα protein in one of six humans appeared to approach levels found in mouse livers (Walgren *et al.*, 2000). [The Working Group noted that, in this study, human PPARα protein expression was not normalized to housekeeping or loading controls that would help to evaluate whether the proteins in the sample were intact.]

Ito *et al.* (2007b) reported no differences in constitutive *PPARα* mRNA expression in CD-1 mice and Sprague-Dawley rats but a lower level of expression in marmosets (*Calithrix jacchus*) (i.e. ~25% of the level in mice).

There is evidence that human and rodent PPARα differ in their ability to be activated by PPARα agonists, as would be expected given that the amino acid sequences within the ligand-binding domains differ between species. The mouse and rat PPARα ligand-binding domains are 94% similar to that of human PPARα (Sher *et al.*, 1993; Mukherjee *et al.*, 1994; Tugwood *et al.*, 1996). The activation of the human PPARs by MEHP required higher concentrations for to achieve the same levels as the corresponding mouse receptor (Maloney & Waxman, 1999; Hurst & Waxman, 2003; Lampen *et al.*, 2003; Bility *et al.*, 2004).

A truncated human PPARα variant exists that may determine responses between species. This truncated form, identified in several laboratories and called hPPARα-8/14 (Tugwood *et al.*, 1996), hPPAR$_{SV}$ (Palmer *et al.*, 1998), PPARα$_{tr}$ (Gervois *et al.*, 1999) and PPARα2 (Hanselman *et al.*, 2001), lacks exon 6 due to alternative splicing, resulting in a human PPARα that lacks the hinge region and ligand-binding domain. In transactivation assays *in vitro*, this form acts as a dominant

negative, inhibiting the ability of the wild-type receptor to activate transcription, possibly by titrating out limiting amounts of co-activators such as C/EBP binding protein/p300 (Gervois *et al.*, 1999). The level of the truncated human PPARα mRNA ranges from 10 to 50% of full-length human PPARα mRNA (Palmer *et al.*, 1998; Gervois *et al.*, 1999; Roberts *et al.*, 2000; Hanselman *et al.*, 2001). In the Palmer *et al.* (1998) study, the authors used RNase protection assays to determine that the truncated form accounts for 28–42% of the protected fragments in each of 10 samples. In mice, rats and rabbits, this level is below 10%, whereas, in cynomolgus monkeys, the ratios of the truncated to full-length form approach those in humans (Hanselman *et al.*, 2001). One study concluded that the level of the truncated form does not correlate with responsiveness to PPARα agonists (Hanselman *et al.*, 2001); however, this study only measured primary human hepatocyte mRNA levels of the acyl-coenzyme A oxidase gene, an inappropriate biomarker of human PPARα activity given that this gene does not appear to be regulated by PPARα in the same way as the rodent gene.

(b) Effects of DEHP on markers of PPAR activation

PPARα activation in the liver has been also characterized indirectly by assessing: (1) increases in the size and/or numbers of peroxisomes in cells; (2) increases in acyl-coenzyme A oxidase expression, protein or activity; (3) increased expression, protein levels or activity of CYP4A protein, a ω-lauric acid hydroxylase; and/or (4) increases in the levels of carnitine acyl-coenzyme A transferase (CAT). [The Working Group noted that these indirect markers have been shown to be activated even in the absence of PPARα, i.e. in PPARα-null mice exposed to peroxisome proliferators (Rosen *et al.*, 2008); thus, the results from these indirect assays should be interpreted with caution if no evidence of PPARα activity is available.]

(i) Humans

No study assessed markers of PPARα activation by DEHP. Studies are available from people who may have been exposed to DEHP and other agents that leached from plastics used in medical devices. 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 1 month of dialysis. However, in a liver biopsy from one 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 DEHP occurs in dialysis patients (Huber *et al.*, 1996). [The Working Group noted that these data should be interpreted with extreme caution.]

(ii) Experimental systems

In-vivo studies

Pugh *et al.* (2000) evaluated the effects of DEHP in young adult male cynomolgus monkeys. Groups of four monkeys received DEHP (500 mg/kg bw per day) or vehicle (0.5% methyl cellulose, 10 mL/kg bw per day) by intragastric intubation for 14 consecutive days. Clofibrate (250 mg/kg bw per day), a hypolipidaemic drug used for cholesterol reduction in human patients, was used as a reference substance. None of the test substances had any effect on body weight or liver weights. Histopathological examination of tissues from these animals revealed no distinctive treatment-related effects in the liver, kidney or testes. There were also no changes in any of the hepatic markers for peroxisomal proliferation, including peroxisomal β-oxidation.

Tomonari *et al.* (2006) treated male and female marmosets daily with 0, 100, 500 or 2500 mg/kg bw DEHP by gavage for 65 weeks from weaning

(3 months of age) to sexual maturity (18 months) and examined several enzyme activities. The ages at commencement of treatment were 90–115 days and body weights on the day of initiation of the dosing varied significantly (range, 95–180 g for males and 116–188 g for females). Five to six animals per group were examined for hepatic enzyme activity. No change in cyanide-insensitive palmitoyl coenzyme A β oxidation was observed in males but a large variability was seen in females especially at the 500-mg/kg dose, although the 95% increase reported was not statistically significant. For CAT, there was a large variability in control levels and across dose groups, especially in females. Carnitine palmitoyltransferase activity showed a similar pattern of variability in activity for the 2500-mg/kg group with a SD similar in magnitude to the mean. For lauric acid ω-1-hydrolase activity, females had dose-related increases compared with controls that were statistically significant at 500 mg/kg. Males showed an increase in every dose group that was not statistically significant (~40% increase).

Reddy *et al.* (1986) fed diets containing 0.25–2.0% DEHP to young male F344 rats for 30 days. Dose-related increases in relative liver weight, cyanide-insensitive palmitoyl-coenzyme A oxidation activity and peroxisome volume density were observed. In this study, a 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-coenzyme A oxidation is a good marker for peroxisome proliferation in rodent liver.

Wong & Gill (2002) treated male C57BL/6 mice with 1.0% DEHP in the diet for 13 weeks. Microarray analysis identified 51 DEHP-regulated genes involved in peroxisome proliferation, xenobiotic detoxification, oxidative stress response, immune function, steroid hormone metabolism, testis development and pheromone transport.

Currie *et al.* (2005) dosed male B6C3F$_1$ mice (*n* = 6) with DEHP (10 mL/kg bw) by gavage every 24 hours for 3 days (1150 mg/kg bw per day) or with an equivalent volume of corn oil. Gene expression levels were measured 2, 8, 24 and 72 hours after the first exposure. DEHP treatment induced a statistically significant increase in liver weight 48 hours and 72 hours after the first exposure. Histological analysis revealed an increase in eosinophilic staining of the smooth endoplasmic reticulum in centrilobular hepatocytes at both these times (indicative of peroxisome proliferation) and hypertrophy of these cells. There was an increased rate of hepatocyte DNA synthesis 48 hours and 72 hours after the first dosing, consistent with an increase in S-phase progression in the periportal area of livers of mice exposed to DEHP. There was a considerable increase in *Cyp4a10* expression from the earliest time-point sampled. As well as a coordinate induction of genes involved in fatty acid metabolism confirmed by the over-representation of Gene Ontology terms, Protein Analysis Through Evolutionary Relationships (PANTHER) and Gen Map Annotator and Pathway Profiler (GenMAPP) pathways involved in the metabolism of lipids (e.g. acyl-coenzyme A metabolism, fatty acid β-oxidation, pantothenate and coenzyme A biosynthesis, or coenzyme metabolism).

Yamazaki *et al.* (2009) examined the effects of 0.5% DEHP in the diet for 7 days on peroxisomal β-oxidation and several acyl transferases including 1-acyl-2-lysophospholipid acyl transferases (1-acylglycerophosphoethanolamine acyl transferase, 1-acylglycerophosphoinositol acyl transferase, 1-acylglycerophosphoserine acyl transferase and 1-acylglycerophophocholine acyl transferase) in the kidneys and livers of male Wistar rats (5 weeks of age). Exposure to DEHP considerably increased the activities of the acyl

transferases and peroxisomal β-oxidation in the microsomes of rat kidneys and livers.

In-vitro studies

Hepatocytes isolated from male Wistar rats and Sprague-Dawley rats (180–250 g) were treated with 0.2 mM [55.7 µg/mL] MEHP or 1 mM 2-EH [130.2 µg/mL] for 48 hours (Gray *et al.,* 1982, 1983). Both DEHP metabolites increased CAT activity about 6–15 fold.

The effects of MEOHP, a DEHP metabolite, on mitochondrial β-oxidation were investigated (Grolier & Elcombe, 1993). In isolated rat hepatocytes, MEOHP inhibited long-chain fatty acid oxidation and had no effect on the ketogenesis of short-chain fatty acids, suggesting that the inhibition occurred at the site of carnitine-dependent transport across the mitochondrial inner membrane. In rat liver mitochondria, MEOHP inhibited CAT I competitively with the substrates palmitoyl-coenzyme A and octanoyl-coenzyme A. An analogous treatment of mouse mitochondria produced a similar competitive inhibition of palmitoyl-coenzyme A transport whereas exposure of guinea-pig and human liver mitochondria with MEOHP revealed little or no effect. The addition of clofibric acid, nafenopin or methylclofenopate revealed no direct effects upon CAT I activity. Inhibition of transferase activity by MEOHP was reversed in mitochondria that had been solubilized with octyl glucoside to expose the latent form of CAT II, suggesting that the inhibition was specific for CAT I. The authors concluded that, *in vitro*, MEOHP inhibits fatty acid oxidation in rat liver at the site of transport across the mitochondrial inner membrane with a marked species difference and support the idea that induction of peroxisome proliferation could be due to an initial biochemical lesion of the fatty acid metabolism.

(iii) Species differences

Elcombe & Mitchell (1986) made species comparisons of hepatic peroxisomal proliferation in primary hepatocyte cultures. Hepatocytes isolated from Wistar-derived rats, male Alderley Park guinea-pigs, male marmosets and three human liver samples (renal transplant donors) were treated with 0–0.5 mM MEHP for 72 hours. While there was a concentration-dependent induction of cyanide-insensitive palmitoyl-coenzyme A 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 [MEOHP] induced cyanide-insensitive palmitoyl-coenzyme A 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-coenzyme A oxidation activity. Similarly, lauric acid hydroxylase activity was not induced in marmoset or human hepatocytes treated with 0–2.0 mM metabolite VI.

Goll *et al.* (2000) examined the effects of various peroxisome proliferators including DEHP on peroxisomal enzyme activities in rat FaO and human HepG2 hepatoma cell lines. Both growing and confluent cultures were treated with peroxisome proliferators (250 µM) for 48 or 72 hours. In accordance with previous observations in peroxisome proliferator-treated primary hepatocyte cultures of rat and human origin, the various peroxisome proliferators increased peroxisomal enzyme activities in rat FaO cells but not in human HepG2 cells.

Rat hepatocytes treated *in vitro* with MEHP (250, 500 and 750 µM) exhibited increased peroxisomal β-oxidation. In contrast, there was no response of human hepatocytes to 250, 500 or 750 µM MEHP (Hasmall *et al.,* 2000a).

(c)   Effects of DEHP on PPAR-independent receptor-mediated events

(i)   Humans

The estrogenic activities of phthalates were investigated in competitive ligand-binding assays, yeast and mammalian gene expression assays and a uterotrophic assay. DEHP did not compete for estrogen receptors or induce luciferase activity in MCF-7 cells transiently transfected with the Gal-4 human estrogen receptor (ER) construct or the Gal4-regulated luciferase reporter gene or HeLa cells stably transfected with the Gal4-human ER construct (Zacharewski *et al.*, 1998).

Phthalate esters have been tested for their ability to interact with sex hormone receptors. DEHP and its active metabolite, MEHP, do not bind to the human androgen receptor (AR) in the monkey kidney cell line, COS, transiently transfected with a human AR vector (Parks *et al.*, 2000).

Ghisari & Bonefeld-Jorgensen (2009) investigated the in-vitro estrogenic activities of a range of widely used plasticizers and phenols, including DEHP, in human MVLN cells, derived from the breast cancer MCF-7 cell line stably transfected with an ER element luciferase reporter vector. Furthermore, the combined effect of a multicomponent mixture of six plasticizers was evaluated for its estrogenic activities. DEHP antagonized the 17β-estradiol (E2)-induced ER function at concentrations of $\geq$ 10 µM. The chemicals were tested in the ER trans-activation assay alone and after co-treatment with 25 pM E2 (corresponding to the half maximum effect concentration[EC_{50}]). No significant agonistic ER activity was observed for DEHP.

Transfection assays were performed with a human pregnane X receptor (PXR; also called steroid-xenobiotic receptor) expression plasmid and a reporter plasmid containing the xenobiotic response elements (XREs) in the *CYP3A4* gene promoter in HepG2 cells. DEHP activated human PXR-mediated transcription on the XREs. The study indicated that DEHP may be an inducer of the *CYP3A4* gene through PXR, and may influence the metabolism of endogenous steroids, drugs and other xenobiotics (Takeshita *et al.*, 2001).

Takeuchi *et al.* (2005) characterized the activities of human ERα, ERβ and AR in the presence of 22 phthalates, including three of their metabolites, using reporter gene assays in host Chinese hamster ovary cells. Of the 22 compounds tested, several phthalate diesters with alkyl chains ranging in length from C3 to C6 exhibited not only human ERα-mediated estrogenic activity, but also human ERβ-mediated anti-estrogenic activity in a dose-dependent manner. DEHP but not MEHP activated ERα. Neither DEHP nor MEHP activated ERβ. DEHP but not MEHP had antagonistic effects on ERβ. Neither DEHP nor MEHP was antagonistic towards human AR.

Takeshita *et al.* (2006) tested the hypothesis that leaching of DEHP during parenteral chemotherapy for cancer patients may facilitate PXR-mediated multidrug resistance 1 (MDR1) expression in various tissues, including cancer cells, which may promote drug resistance. DEHP was studied in the human colon adenocarcinoma-derived cell line, LS174T, which endogenously expresses PXR, and increased PXR-mediated transcription of the *MDR1* gene in luciferase-reporter assays. The induction by DEHP was abrogated when a reporter plasmid containing a mutated DR+4 motif in the XRE was used. In a mammalian two-hybrid assay, DEHP recruited steroid receptor co-activator-1 to the ligand-binding domain of PXR. Using real-time reverse transcriptase-polymerase chain reaction (RT-PCR), DEHP increased *MDR1* gene expression in a dose-dependent manner. The data support the activation of the *MDR1* gene by DEHP through PXR.

DEHP activated human PXR in stably transfected HGPXR cells, that are derived from HeLa cells and express luciferase under the control of a

chimeric human PXR at an EC_{50} value of 2.5 µM. DEHP also induced CYP3A4 and -2B6 expression in two preparations of primary cultured human hepatocytes at 10 µM (eight- and 38-fold for CYP3A4 and two- and fourfold for CYP2B6). The activation of CYP2B6 could also indicate the activation of the CAR (Mnif *et al.*, 2007).

Krüger *et al.* (2008) determined the effect of several chemicals, including DEHP, alone or in mixtures of selected compounds, on the human aryl hydrocarbon receptor (AhR) and human AR function using chemically activated luciferase gene expression (CALUX) bioassays in recombinant mouse hepatoma Hepa1.12cR cells (AhR-CALUX) or in transiently transfected Chinese hamster ovary cells (AR-CALUX). Weakly induced AhR activities were observed for DEHP, reaching 1.75-fold above the solvent control at the highest concentration tested (100 µM). DEHP did not affect AR activation in this assay.

DEHP and MEHP induced PXR-mediated transcription of the CYP3A4 promoter in a dose-dependent fashion in HepG2 cells. Co-exposure to either MEHP or DEHP and dexamethasone resulted in enhanced CYP3A4 promoter activity. This induction was abrogated by both the glucocorticoid receptor antagonist, RU486, and glucocorticoid receptor small interfering RNA. Dexamethasone induced PXR protein expression in human hepatocytes and a liver-derived rat cell line, H4IIE-C3. CYP3A4 protein was strongly induced by co-administration of dexamethasone and DEHP in human primary hepatocyte cultures. Enhanced 6β-hydroxytestosterone formation in human primary hepatocytes co-treated with dexamethasone and DEHP or MEHP confirmed CYP3A4 enzyme induction. Concomitant exposure to glucocorticoids and phthalates resulted in enhanced metabolic activity of CYP3A4, which may play a role in the altered efficacy of pharmaceutical agents (Cooper *et al.*, 2008).

DeKeyser *et al.* (2009) examined a novel CAR — CAR2 — that, unlike the constitutively active reference form of the receptor, is a ligand-activated receptor that comprises approximately 30% of the reference transcript levels in human hepatocytes. CAR2 transcripts are not generated in mice, rats or marmosets. CAR2 was activated in cells transfected with the receptor at an EC_{50} of 211 nM [0.085 µg/mL] DEHP. The authors reported that MEHP was a weak CAR2 activator at 10 µM [2.8 µg/mL] in their in-vitro reporter assays and concluded that the parent compound DEHP is the most active modulator of CAR2. Primary human hepatocyte cultures from three donors, cultured in such a way as to preserve differentiation, had very different responses to CAR-responsive enzyme (CYP2B6 and CYP3A4) mRNA induction. The concentrations of DEHP that induced these enzymes were 0.039 µg/mL, 0.39 µg/mL and 19.5 µg/mL for the three donors. [Cytotoxicity was not noted.]

Induction of *CYP2B6* gene expression, indicative of CAR activation in primary human hepatocyte cultures [culture conditions not specified], was increased in cultures from two patients exposed to 19.5 µg/mL DEHP (Eveillard *et al.*, 2009). [Cytotoxicity was not noted.]

Rotroff *et al.* (2010) examined the ability of DEHP to activate several response elements in a multiplex transcription factor assay in HepG2 cells. DEHP significantly activated at the following elements indicative of CAR and/or PXR activation (EC_{50} values in µM): PXRE_CIS (37), PXR_TRANS (38), CAR_TRANS (50) (Martin *et al.*, 2010; http://actor.epa.gov/actor/faces/ToxCastDB/Assay.jsp).

Rotroff *et al.* (2010) also examined the ability of DEHP to activate several genes associated with the activation of PPARα, CAR, PXR, farnesoid X receptor and AhR. Usually one preparation of primary hepatocytes from one human donor was used. Cells were treated with a range of doses and harvested after 6, 24 and 48 hours. DEHP significantly increased the expression of the

CAR-responsive gene *CYP2B6* only at all three time-points.

DeKeyser *et al.* (2011) examined the interaction of alternatively spliced human CARs and PXR with a range of suspected endocrine disruptors, including phthalates. Transactivation studies in COS-1 cells revealed that DEHP had EC_{50} values for the activation of CAR2 and PXR of 0.1 μM and 3.8 μM, respectively. DEHP does not activate the CAR3 isoform. Studies with primary human hepatocytes showed DEHP induced CYP2B6 and CYP3A4 expression. Mutation analysis of CAR2, in-silico modelling and ligand docking studies suggested that the SPTV amino acid insertion of CAR2 creates a unique ligand-binding pocket, and that this alternative gene splicing results in variant CARs that selectively recognize phthalates.

(ii) Experimental systems

Transgenic mice with hepatocyte-specific constitutively active *Pparα* in the absence of ligand were generated and observed for up to 11 months of age (Yang *et al.*, 2007). In absence of treatment, these transgenic mice exhibited various responses that mimic wild-type mice treated with peroxisome proliferators, including a significant decrease in serum fatty acids, and numerous liver effects: hepatomegaly, hepatocyte hypertrophy, increased rate of cell proliferation, marked induction of *Pparα* target genes encoding fatty acid oxidation enzymes and increased accumulation of triglycerides. Although these phenotypic changes were similar (and of comparable magnitude) to those induced in wild-type mice by the potent peroxisome proliferator WY-14 643 (0.1% w/w diet), no liver tumours were detected in untreated transgenic mice by 11 months of age whereas all treated wild-type mice developed liver tumours.

Two additional mouse models have been created to evaluate the role of PPARα in responses to peroxisome proliferators. One study used hepatocyte transplantation to generate chimeric livers composed of *Pparα*-null and -positive hepatocytes in *Pparα*-null or -positive mice (Weglarz & Sandgren, 2004). The relationship between *Pparα* status and the ability of hepatocytes to proliferate in response to WY-14 643 was examined *in vivo*. When treated with WY-14 643 for 7 days, both *Pparα*-null and -positive hepatocytes in chimeric livers displayed elevated DNA synthesis regardless of host receptor status, as long as at least some hepatocytes contained the receptor. These findings suggest that the mitogenic response to peroxisome proliferators does not require the presence of active PPARα in all hepatocytes.

A transgenic mouse line that overexpresses human *Pparα* in a *Pparα*-null mouse was used in subchronic studies with WY-14 643 and fenofibrate (Cheung *et al.*, 2004) and a chronic feeding study with WY-14 643 (Morimura *et al.*, 2006). In these studies, PPARα-humanized mice did not exhibit hepatocellular proliferation, hepatomegaly or liver tumours when treated with peroxisome proliferators; however, typical markers of fatty acid β-oxidation were induced. [These mouse models have not been evaluated with DEHP and no toxicity markers were evaluated in the reports.]

Groups of 18–22-week-old male *Pparα*-null mice and corresponding wild-type mice (C57BL/6J strain) were treated with DEHP (0, 20 or 200 mg/kg bw per day) by gavage for 21 days (Eveillard *et al.*, 2009). While this study did not report on liver weight or liver histopathology, gene expression profiling was performed on liver tissues, and the authors reported that several prototypic Car target genes were induced by DEHP in *Pparα*-null mice. There is only weak evidence that DEHP activates mouse AhR, although *Cyp1a1*, a marker gene for AhR, was increased in *Pparα*-null mice but not wild-type mice in this study.

Ren *et al.* (2010) identified PPARα and CAR as targets of DEHP indirectly through meta-analysis of transcript profiles of livers from rats

treated with nuclear receptor activators, and directly through transcriptional analysis in wild-type mice and mice nullizygous for these nuclear receptors. Microarray analysis showed an overlap in the profiles of DEHP-, valproic acid- and clofibrate-treated rats with a classical activator of CAR (phenobarbital) and, to a lesser extent, an activator of PXR (pregnenolone-16-α-carbonitrile). The overlapping genes included *CYP* gene families that are often considered to be signature genes for nuclear receptor activation. In addition, groups of wild-type, *Pparα*-null and *Car*-null mice were treated with 0, 200 or 1150 mg/kg bw DEHP in corn oil by gavage daily for 4 days and liver gene expression was compared. A microarray gene expression comparison of DEHP-treated wild-type and *Pparα*-null mice revealed that PPARα was required for ~94% of all transcriptional changes in wild-type mice. The remaining 6% of the genes were dominated by those involved in xenobiotic metabolism and are known target genes of CAR or PXR (Stanley *et al.*, 2006; Timsit & Negishi, 2007), and those involved in cholesterol biosynthesis and are regulated by several transcription factors including the retinoid X receptor (Anderson *et al.*, 2004a). Xenobiotic metabolism enzymes, including *Cyp2b10, Cyp3a11* and *Cyp3a41a,* as well as metallothionine-1 (*Mt1*) were induced by DEHP partially or completely, depending on CAR, but not PPARα as determined in wild-type mice and mice nullizygous for *Pparα* or *Car*. The expression of the *Car* gene itself was increased by DEHP in *Pparα*-null but not in wild-type mice. Several putative CAR and PXR targets exhibited PPARα- and CAR-independent induction, including *Cyp8b1*, GST M4 (*Gstm4*) and *Gstm7*. It was concluded that DEHP requires CAR for the induction of a small subset of genes (compared with PPARα) and that some liver transcriptional effects may be PPARα-independent.

Kim *et al.* (2010) examined the effects of DEHP on nuclear receptor expression and phospholipase D (PLD), an enzyme that catalyses the hydrolysis of phosphatidyl choline to generate phosphatidic acid and choline. PLD is believed to play an important role in cell proliferation, survival signalling, cell transformation and tumour progression. DEHP (500 mg/kg bw per day) was administered orally to prepubertal rats (4 weeks of age) for 1, 7 or 28 days. Protein expression levels of PLD1/2, PPAR and CYP were determined by Western blot analysis using specific antibodies. Liver weight was significantly increased in the DEHP-treated groups. A significant rise in PLD1/2 expression was observed in the liver of DEHP-exposed rats after 7 days. The authors stated that PPARα, CAR, PXR and CYP2B1 protein expression levels were markedly elevated in DEHP-treated groups. [The Working Group noted that no quantitation of these data was performed.]

Hurst & Waxman (2004) investigated the effects of phthalates on the PXR, which mediates the induction of enzymes involved in steroid metabolism and xenobiotic detoxification. The ability of phthalate monoesters to activate PXR-mediated transcription was assayed in a HepG2 cell reporter assay following transfection with mouse PXR (mPXR), human PXR (hPXR) or the *hPXR* allelic variants V140M, D163G and A370T. MEHP increased the transcriptional activity of both mPXR and hPXR (five- and 15-fold, respectively) with EC_{50} values of 7–8 μM. hPXR-V140M and hPXR-A370T exhibited patterns of phthalate responses similar to the wild-type receptor. In contrast, hPXR-D163G was unresponsive to all phthalate monoesters tested.

Baldwin & Roling (2009) performed CAR transactivation assays using mouse CAR in HepG2 cells using a variety of environmental chemicals, steroid hormones and bile acids at 10 μM. 1,4-Bis-2-(3,5-dichloropyridyloxyl)benzene activated dihydroandrosterone-repressed CAR activity nearly fourfold. MEHP also increased CAR activity more than 3.9-fold at 100 μM.

Ghisari & Bonefeld-Jorgensen (2009) investigated the thyroid hormone-like activities *in vitro* of a range of widely used plasticizers and phenols, including DEHP. Thyroid hormone-disrupting potential was determined by the effect on the proliferation of thyroid hormone-dependent rat pituitary GH3 cells using the T-screen assay. All of the compounds tested, including DEHP, significantly affected GH3 cell proliferation at concentrations below levels that were cytotoxic.

(iii) Species differences

DeKeyser *et al.* (2009) evaluated whether mice, rats and marmosets could generate the *Car2* transcript that encodes the DEHP-responsive isoform of CAR. Protein sequences were retrieved from the National Center for Biotechnology Information database and aligned. The results showed that the rhesus monkey sequence included a four-amino acid insertion very similar to that of human CAR2, suggesting that the splice variant is conserved across multiple species. To determine whether other species could generate similar transcripts, a genomic alignment was performed for three species of rodents and six primates using the University of California Santa Cruz genome browser with mouse CAR as the reference sequence. Although the splice acceptor site for CAR1 was conserved across each species, the CAR2 site was not conserved in marmosets, mice or rats, indicating that these species are incapable of generating a CAR2-like, four-amino acid insertion protein.

(d) Effects of DEHP on PPAR-independent metabolism

(i) Humans

Primary human hepatocytes, cultured in such a way as to preserve differentiation, had increased CYP3A4 protein levels after exposure for 3 days to 2.0 µg/mL (5 µM) DEHP. This effect was greatly enhanced by co-incubation with 0.01 µM dexamethasone. Testosterone 6β-hydroxylase activity, a measure of CYP3A4

activity, was only slightly increased by exposure to 195 µg/mL (500 µM) DEHP; in the presence of 0.1 µM dexamethasone, however, this activity was considerably increased at 19.5 µg/mL (50 µM). Similarly, testosterone hydroxylase activity was not increased by 14 µg/mL (50 µM) MEHP in primary cultures of human hepatocytes but was increased by 1.4 µg/mL MEHP (5 µM) in the presence of dexamethasone. Cytotoxicity was not noted at these concentrations (Cooper *et al.*, 2008; see Section 4.2.2(*c*)).

The ability of DEHP to activate CAR-dependent target genes was examined in primary hepatocyte cultures derived from two patients. DEHP dose-dependently increased the expression of CYP2B6, a human homologue and a CAR target, at 50 and 100 µM (Eveillard *et al.*, 2009).

Kang & Lee (2005) reported increased expression of ERβ in MCF7 human breast-cancer cells exposed to DEHP (3.9 µg/mL; 10^{-5} M).

Expression of *MDR1* in the human colon adenocarcinoma-derived LS174T cell line was increased after a 24-hour exposure to DEHP at 0.39 µg/mL (10^{-6} M) (Takeshita *et al.*, 2006).

Increased expression of matrix metalloproteinases -2 and -9, and reduced tissue inhibitor of matrix metalloproteinase-2 mRNA and protein expression were observed in human neuroblastoma SK-N-SH cells treated with 50 µM DEHP. DEHP-induced phosphorylation at Ser473 of the serine/threonine kinase protein was also noted. Cytotoxicity was not reported (Zhu *et al.*, 2010).

Turan *et al.* (2008) reported the results of a genome-wide gene-expression analysis based on steady-state mRNA levels in human medulloblastoma TE671 cells exposed for 24 hours to DEHP (0.2 µg/mL; 0.5 µM) *in vitro*. In a microarray of 47 000 transcripts and variants, DEHP caused alterations in 6.2% of transcripts with an overall reduction in mRNAs from genes associated with cell proliferation/survival, signal transduction, development/growth and various other categories, including those associated with

oxidation resistance, histone deacetylase 3 and human α-catenin. RT-PCR confirmed the reduction of gene expression for several selected genes. Increased gene expression was only observed for ER-60 protease, which was confirmed by RT-PCR. Cytotoxicity was not reported.

(ii) Experimental systems

Tomonari *et al.* (2006) treated male and female marmosets daily by gavage with 0, 100, 500 or 2500 mg/kg bw DEHP for 65 weeks from weaning (3 months of age) to sexual maturity (18 months) and examined serum hormone levels and several enzyme activities. No obvious treatment-related changes in testosterone levels were found in any treatment group, but there were large variations in individual values (serum testosterone in males varied over 100-fold). Significantly elevated levels of E2 were recorded in all female marmosets by week 65 in the 500-mg/kg group. Increased ovarian and uterine weights were observed in the two highest-dose groups of females (500 and 2500 mg/kg bw). No treatment-related differences in glutathione (GSH) content, or in the activities of sorbitol dehydrogenase, γ-glutamyl transpeptidase and GSH peroxidase were observed in the testis. GST activity and zinc content in the 100- and 500-mg/kg bw treatment groups were significantly reduced. Testosterone 6β-hydroxylase activity (CYP3A) in the liver was significantly higher in females of the 2500-mg/kg bw group, but was not statistically significantly increased in males.

Administration of 2000 mg/kg bw DEHP for a period of 7 or 15 days to rats, mice, guinea-pigs and rabbits produced differential effects, as judged by alterations in body weight gain, liver weight and activities of mixed-function oxidases. Exposure to DEHP for 7 days caused an increase in the activities of aniline hydroxylase, arylhydrocarbon hydroxylase (guinea-pigs only) and ethylmorphine *N*-demethylase in rats, mice and guinea-pigs, but caused a decrease in these activities in rabbits. However, exposure for 15 days caused a (smaller) increase in the activity of ethylmorphine *N*-demethylase and aniline hydroxylase in rats and mice and produced a decrease in the activity of these two enzymes in guinea-pigs. The activity of arylhydrocarbon hydroxylase was decreased in all three species [data in rabbits not shown] (Parmar *et al.*, 1988).

In mice, Poole *et al.* (2001) examined the expression of the non-specific carboxylesterases (EC.3.1.1.1), a large group of enzymes that play an important role in the metabolism of xenobiotics and endogenous lipids, including activators of PPARα. After dietary exposure of male SV129 wild-type and *Pparα*-null mice to DEHP (0.6% in the diet) for 3 weeks, the expression of carboxylesterases ES-4 and ES-10 was downregulated in the kidney, but not in the liver of wild-type mice. The decrease in carboxylesterase expression in the kidney was not observed in *Pparα*-null mice, which demonstrates the involvement of PPARα in these changes. These studies show that carboxylesterase ES-protein expression is under the complex control of peroxisome proliferators.

Decreased liver lysosomal enzyme activity was eliminated in C3H mice fed a diet containing 2% DEHP for 3 weeks. After 2 weeks, vacuolar H^+-adenine triphosphatase (V-ATPase) was decreased and at 3 weeks the liver lysosomal compartment was completely negative for this enzyme, as shown by immunoblot analysis. Enzyme cytochemical staining showed that acid phosphatase was present in lysosomes; the number of late autophagosomes containing this enzyme was increased after DEHP treatment. These data suggest that the DEHP-induced reduction in V-ATPase in the lysosomal compartment of the liver may result in an inability to degrade excess cell organelles (Wang *et al.*, 2001).

Nicotinamide adenine dinucleotide phosphate (NADPH)-CYP oxidoreductase (P450R) is an often rate-limiting component in CYP-dependent reactions. In male wild-type SV129 mice, P450R mRNA levels increased in the liver after exposure to DEHP. This induction

of mRNA was not observed in the liver of mice that had no functional PPARα. In wild-type male mice, P450R protein was decreased ninefold in the liver after treatment with DEHP, but increased twofold in *Pparα*-deficient mice. This study demonstrates the complex regulation of P450R expression by DEHP at two different levels, both of which are dependent upon PPARα: upregulation of mRNA transcript levels and downregulation of protein levels (Fan *et al.*, 2003).

Fan *et al.* (2004) reported that, in male SV129 wild-type and *Pparα*-deficient mice fed 0.6% DEHP in the diet for 3 weeks, the expression of the 6α-testosterone hydroxylase *Cyp3a11* gene was increased in the liver. The increase caused by DEHP was PPARα-independent.

ICR mice (6 weeks of age) were fed a niacin-free, 20%-casein diet supplemented with DEHP (0, 0.1, 0.5, 1.0 or 2%) for 21 days. The mice showed increased urinary excretion of quinolinic acid and of lower metabolites of the tryptophan-niacin pathway. This urinary excretion increased with the dose of DEHP (statistically significant at 0.5, 1.0 and 2.0%) (Ohta *et al.*, 2004).

Takashima *et al.* (2008) conducted a follow-up to the study of the carcinogenic effects of DEHP in wild-type and *Pparα*-null male mice exposed to 0.01 or 0.05% DEHP in the feed for 22 months (Ito *et al.*, 2007a); transcript profiling and RT-PCR were used to examine gene expression in the tumours from the two types of mice. Microarray analysis by RT-PCR showed the expression of five genes involved in carcinogenesis. Significant increases were observed in the expression of the growth arrest and DNA damage 45α and apoptotic protease-activating factor 1 genes in tumour tissue versus control tissue in wild-type mice. In *Pparα*-null mice, cyclin B2 and myeloid-cell leukaemia sequence 1 genes were significantly increased in tumour tissue versus control tissue. [The Working Group noted that the differences are difficult to interpret because they could be due to treatment with DEHP or to differences between the tumours and surrounding tissue, or both. The increase in liver tumours could be related to the higher incidence of spontaneous liver tumours in control *Pparα*-null mice compared with control wild-type mice (Howroyd *et al.*, 2004).]

Eveillard *et al.* (2009) examined whole-liver gene expression in wild-type and *Pparα*-null C57BL/6 mice exposed by gavage to 0, 20 or 200 mg/kg bw DEHP daily for 21 days (*n* = 10/group). Fatty acid homeostasis and xenobiotic metabolism were the most represented pathways in terms of altered gene expression. A total of 56 transcripts were differentially expressed between wild-type and *Pparα*-deficient control mice. DEHP (high dose) altered the expression of 49 transcripts in wild-type mice, and of 16 genes in *Pparα*-null livers. The four genes that were similarly altered in both strains encoded acyl-coenzyme A oxidase 1, ALDH family 1 subfamily 1a1, aminolevulinic acid synthase 1 and Cyp2c29. Thus, many of the genes were altered by DEHP in wild-type, but not in *Pparα*-deficient mice.

Male F344 rats fed diets containing 1.2% DEHP for 4, 8 or 16 weeks had significantly increased E2 concentrations in serum, but ER activity in the liver was strongly reduced. These rats also showed a significant loss of hepatic activity of the male estrogen-metabolizing enzyme, estrogen 2-hydroxylase, and of the male-specific estrogen-sequestering protein. In contrast, the expression of mRNAs for ER and for the oncogene *fos* (but not *myc* or *ras*) increased significantly after exposure to DEHP (8 and 16 weeks only), as did the expression of the proliferating cell nuclear antigen (Eagon *et al.*, 1994).

Seo *et al.* (2004) examined the differences in oxidative damage caused by DEHP and three other phthalates and correlations between changes in Phase-I and Phase-II enzymes in groups of 8–10 male Sprague-Dawley rats given daily doses of 50, 200 or 1000 mg/kg bw in corn oil for 14 days. The peroxisomal markers, cyanide-insensitive palmytoyl-coenzyme A oxidation and CAT, were significantly increased at all doses.

Malondialdehyde concentrations in the liver were substantially increased at all doses, while oxidative damage in DNA (8-hydroxydeoxyguanosine; 8-OH-dG) was moderately increased at the highest dose only. CYP1A2 and -3A4, UGT and GST activities were decreased at the two highest doses, with no correlation between inhibitory effects on metabolizing enzymes and peroxisome proliferation. DEHP-induced effects on xenobiotic metabolizing enzymes may thus be independent of peroxisomal proliferation and oxidative stress.

Prepubertal Wistar male rats (4 weeks of age) received an oral dose of DEHP (100 or 1000 mg/kg bw) daily for 5 days. This treatment induced a significant reduction in the activity of cytosolic phospholipase A_2, the state-limiting enzyme in the synthesis of arachidonic acid and eicosanoids, in the testis. There was increased expression of 12-lipoxygenase in the testis, but no change in the activity of cyclo-oxygenase-2. DEHP increased CYP4A1 expression in the testis in a dose-dependent manner. A dose-related decrease in serum testosterone was statistically significant at 1000 mg/kg bw. Concentrations of arachidonic acid in serum were significantly decreased at both doses. Testicular atrophy (62% reduction in testis weight) was observed at the 1000-mg/kg bw dose with no significant change in body weight (Kim *et al.*, 2004a).

Changes in expression of several proteins that regulate the homeostasis of essential fatty acids were studied in female Sprague-Dawley rats treated with oral doses of DEHP (750 or 1500 mg/kg bw per day) on gestational days 0–19. Expression of PPARα, PPARγ, fatty acid translocase, fatty acid transport protein 1, heart cytoplasmic fatty acid-binding protein and CYP4A1 were upregulated in the placenta while cyclo-oxygenase-2 was downregulated (Xu *et al.*, 2008).

van Ravenzwaay *et al.* (2010) examined the metabolite profile in plasma of fasted male and female Wistar rats exposed to DEHP (3000 ppm in the diet) in 28-day studies. Induction of cyanide-insensitive palmitoyl-coenzyme A oxidation per gram of liver was greater in males than in females.

(iii) Species differences

The ability of MEHP to induce CYP1A1, a known AhR target gene, was demonstrated in human CaCo-2 (derived from colon adenocarcinoma), HepG2 (hepatoma) and A549 (lung adenocarcinoma) cell lines, as well as primary human keratinocytes (Sérée *et al.*, 2004). The induction specifically involved PPARα and required two PPRE sites that were located within the CYP1A1 promoter. Whether this also occurs in normal human hepatocytes has yet to be established. CYP1A1 is a major CYP isoform that is responsible for the bioactivation of many environmental pro-carcinogens. Compared with hepatocytes from control rats, those isolated from rats treated with DEHP and other peroxisome proliferators showed enhanced DNA-adduct formation when incubated with benzo[*a*]pyrene-7,8-dihydrodiol, a proximate carcinogenic metabolite of benzo[*a*]pyrene. This enhanced adduct formation was the result of the higher activity of CYP1A1 in DEHP-treated animals (Voskoboinik *et al.*, 1997). Thus, the fact that the regulation of CYP1A1 by MEHP is mediated by PPARα in human cells may have significant implications; however, other studies have shown that another peroxisome proliferator, clofibrate, inhibits expression of CYP1A1 and CYP1A2 in the liver of treated rats (Shaban *et al.*, 2004).

(e) Cell proliferation

(i) Humans

No data on the effects of DEHP on cell proliferation in the human liver were available to the Working Group.

Studies with cultured human hepatocytes failed to produce evidence of increased peroxisome proliferation after exposure to DEHP. Some of these studies directly compared the results in human hepatocytes exposed to comparable

doses of DEHP (Goll *et al.*, 1999; Hasmall *et al.*, 1999; Roberts, 1999).

[The Working Group noted that only a limited number of human donors were sampled in these studies, which may have affected the significance levels of the results due to presumably higher genetic variability in the human population. In addition, the unknown condition of the livers available for the isolation of hepatocytes may be considered as a confounding variable.]

(ii) Experimental systems

Groups of four cynomolgus monkeys received di-isononyl phthalate (DINP; 500 mg/kg bw), DEHP (500 mg/kg bw) or vehicle (0.5% methyl cellulose, 10 mL/kg bw) by intragastric intubation daily for 14 consecutive days. Clofibrate (250 mg/kg bw per day) was used as a reference substance, because of its peroxisome proliferation-related effects in the liver of rodents (Doull *et al.*, 1999). There were no changes in any of the hepatic markers for peroxisomal proliferation, including peroxisomal β-oxidation and replicative DNA synthesis. None of the test chemicals – including the reference substance – produced any toxicologically important changes in urine analysis, haematology or clinical chemistry (Pugh *et al.*, 2000).

DEHP (1.2% in the diet) and WY-14 643 (0.1% in the diet) were fed to male F344 rats for up to 365 days. At the end of this period, all rats fed WY-14 643 had numerous grossly visible nodules in the liver, while no nodules were seen in DEHP-fed animals or in controls. Despite this difference, both DEHP and WY-14 643 increased the peroxisomal volume density (percentage of cytoplasm occupied by peroxisomes) four- to sixfold during the treatment. Activities of enzymes involved in peroxisome proliferation and β-oxidation were increased eightfold by both DEHP and WY-14 643 during the first 18 days of treatment. These enzyme activities remained about 25% higher in the livers of WY-14 643-fed rats than in rats that received DEHP. DEHP or

WY-14 643 increased absolute liver weights by 50–75% above those of controls. Labelling of the hepatocyte nuclei with a [3H]thymidine pulse given 2 hours before the animals were killed revealed a rapid increase in DNA replication in both groups of rats, with a labelling index that reached a maximum on day 2 for the DEHP-treated group and on day 1 for the WY-14 643-treated group. The labelling index returned to control levels by day 4 in both groups. Implantation of 7-day osmotic pumps containing [3H] thymidine showed a five- to 10-fold increase in replicative DNA synthesis in rats receiving WY-14 643 for 39–365 days. DEHP induced a four- to fivefold increase in hepatocyte proliferation on day 8. The ability of these two agents to induce a persistent increase in replicative DNA synthesis correlated with their relative strengths as hepatocarcinogens in rodents (Marsman *et al.*, 1988).

A diet containing 6000 ppm DEHP was given to male B6C3F$_1$ mice for 7 days. Pulse-labelling with [3H]thymidine resulted in a sevenfold increase in replicative DNA synthesis in hepatocytes, while no increase was seen when the animals had been fed for 14 or 28 days (Smith-Oliver & Butterworth, 1987).

Male F344 rats (7–9 weeks of age) were fed a diet containing 1.2% DEHP for various periods up to 365 days. Over the entire treatment period, DEHP produced a sustained stimulation of peroxisome proliferation, as demonstrated by cyanide-insensitive palmitoyl-coenzyme A oxidation activity and peroxisome morphometry. DEHP caused a threefold increase in the level of lipofuscin — a marker of oxidative stress — after 39 days of treatment; this level decreased during the rest of the treatment period but remained higher than that in the controls (Conway *et al.*, 1989).

Flow cytometry was used to investigate the effects of DEHP, chlorendic acid and 1,4-dichlorobenzene on hepatocyte ploidy, nuclearity and labelling-index distribution. Male F344 rats received 12 000 ppm DEHP in the diet for 7 days. The dose and route of administration

corresponded with those used in the NTP cancer bioassays. DEHP increased the mean hepatic labelling index (23 ± 3% compared with 1.4 ± 0.4% in controls). This index was increased in all hepatocyte ploidy/nuclearity classes except the binucleated tetraploid cells and was highest in the mononucleated octoploid population (49 ± 14% versus 1.3 ± 0.4% of octoploid hepatocytes for DEHP and controls, respectively). DEHP tended to induce DNA synthesis in a greater proportion of diploid and binucleated tetraploid cells (Hasmall & Roberts, 1997).

Oral administration of DEHP (1150 mg/kg bw per day for 2 days) in corn oil to male B6C3F$_1$ mice resulted in a 2.4-fold increase in replicative DNA synthesis, measured by immunochemical detection of bromodeoxyuridine incorporated into newly synthesized DNA. In a similar experiment in male F344 rats that were given 950 mg/kg bw DEHP for 2 days, the increase in replicative DNA synthesis was more than 13-fold (James et al., 1998a).

Male and female F344 rats and B6C3F$_1$ mice were fed a diet containing DEHP for up to 13 weeks (David et al., 1999). In rats that received 12 500 ppm DEHP, there was an increase in hepatocyte replicative DNA synthesis (measured after continuous bromodeoxyuridine administration for 3 days before sampling) after 1 week (but not after 2 or 13 weeks) and an increase in hepatic peroxisomal β-oxidation (palmitoyl-coenzyme A oxidation) activity after 1, 2 and 13 weeks of treatment. In mice fed 10 000 and 17 500 ppm DEHP, there was no increase in hepatocyte replicative DNA synthesis at any time-point. However, at these two dose levels, there was an increase in hepatic peroxisomal β-oxidation activity after 1, 2 and 13 weeks, which was not observed at the 1000-ppm dose.

The response of mice transgenic for hepatocyte-specific expression of a constitutively activated form of PPARα (VP16PPARα) was compared with that in wild-type mice that were treated with WY-14 643, a PPARα ligand.

Expression of VP16PPARα in the transgenic animals led to increases in hepatocyte proliferation in the absence of non-parenchymal cell proliferation. In contrast, treatment with WY-14 643 led to increased replication of both hepatocytes and non-parenchymal cells. Importantly, chronic activation of VP16PPARα did not increase the incidence of liver tumours in transgenic mice (Yang et al., 2007). [The Working Group noted that these results indicate that non-parenchymal cell activation is important for hepatocarcinogenesis and that PPARα-mediated hepatocyte proliferation by itself is not sufficient to induce liver cancer.]

Daily oral doses of 500 mg/kg bw DEHP were given to prepubertal rats (4 weeks of age, weighing approximately 70–90 g) for 1, 7 or 28 days. Liver weight was significantly increased in the DEHP-treated groups compared with controls. Immunohistochemical analysis demonstrated that DEHP caused strong staining of proliferating cell nuclear antigen after 28 days of exposure, suggestive of hepatocyte proliferation (Kim et al., 2010). [The Working Group noted the lack of quantification of the antigen staining.]

Rat hepatocyte cultures were treated with various peroxisome proliferators at 100–500 µM for 72 hours. Dependent on the agent used, there was an increase in acyl-coenzyme A oxidase and CAT activities, markers of peroxisome proliferation, with the following potencies: ciprofibrate = nafenopin > bezafibrate > clofibric acid > DEHP (negative for acyl-coenzyme A oxidase). DEHP induced a concentration-dependent increase in DNA synthesis (measured as incorporation of bromodeoxyuridine) and a decrease in spontaneous apoptosis after 48 hours of treatment, with no dose–response. The reduced apoptosis was also observed morphologically. Furthermore, DEHP inhibited apoptosis induced by transforming growth factor β (TGFβ) but not that induced by tumour necrosis factor α/α amanitine (TNFα/αAma) (Goll et al., 1999).

Hasmall *et al.* (1999) examined differences in the response of rat and human hepatocytes to MEHP – the principal metabolite of DEHP – and DINP *in vitro*. In rat hepatocytes, both DINP and MEHP caused a concentration-dependent induction of DNA synthesis and suppression of both spontaneous apoptosis and apoptosis induced by TGFβ1. Similarly, both compounds caused a concentration-dependent induction of peroxisomal β-oxidation, although the response to DINP was weaker. None of these effects were seen in human hepatocytes.

[The Working Group noted that peroxisome proliferators have not been shown to exhibit a marked effect on replicative DNA synthesis *in vitro* in purified hepatocyte cultures. The magnitude of such a response is much lower than that seen in rat and mouse liver *in vivo* and it has been hypothesized that other cells in the liver (e.g. Kupffer cells) may play an important role in potentiating the proliferative response of the hepatocytes by producing mitogenic cytokines. It was also noted that the human liver may be more refractory than the rodent liver to mitogenic stimuli.]

(iii) Species differences

Male F344 rats and male Dunkin-Hartley guinea-pigs were given 950 mg/kg bw per day DEHP by gavage for 4 days (Hasmall *et al.*, 2000b). Significant increases in liver weight, hepatic β-oxidation activity and hepatocyte DNA replication, and reductions in hepatocyte apoptosis were observed in rats but not guinea-pigs.

(f) Apoptosis

(i) Humans

Cultured human hepatocytes are non-responsive to the anti-apoptotic activities of DEHP, its principal metabolite MEHP and DINP (Hasmall *et al.*, 1999). DINP and MEHP did not cause induction of β-oxidation, stimulation of DNA synthesis or suppression of apoptosis in cultured human hepatocytes obtained from three separate donors. These effects had been observed in rat hepatocytes. The lack of an effect in the human cells was consistent for both spontaneous and cytokine-induced (TGFβ1 or TNFα/α-Ama) apoptosis. [The Working Group noted that the human hepatocyte cultures used in these experiments may have been contaminated with up to 5% of non-parenchymal cells (Goll *et al.*, 1999).]

Viability of human monocytic leukaemia U937 cells was decreased after 20 hours of exposure to MEHP (range, 150–1000 µM), with concurrent increases in DNA fragmentation (Yokoyama *et al.*, 2003). Treatment with MEHP increased caspase-3 activity, which was diminished by 50% by pretreatment with selective PPARγ inhibitors and antagonists (BADGE, GW9662) but was increased twofold by pretreatment with a PPARγ ligand (rosiglitazone). A PPARα antagonist (MK886) had no effect on MEHP-induced caspase-3 activity. In these MEHP-treated U937 cells, the mRNA levels were decreased for B-cell lymphoma-2 (Bcl-2) protein and increased for Bcl-2-associated X (BaX) protein, resulting in a decrease in the Bcl-2/BaX protein ratio.

(ii) Experimental systems

Mice were given 1150 mg/kg bw DEHP per day by gavage in corn oil for 2 days. The treatment resulted in a significant reduction in both spontaneous and TGFβ1-induced apoptosis and a strong induction of DNA synthesis (James *et al.*, 1998a). In the same study, rats were given 950 mg/kg bw DEHP per day by gavage in corn oil for 2 days. The treatment induced DNA synthesis and suppressed both spontaneous and TGFβ1-induced apoptosis in the hepatocytes. Apoptosis was reduced to undetectable levels in three of five animals. The same authors reported that MEHP was able to suppress apoptosis and induce DNA synthesis in mouse hepatocytes. Those effects were comparable with those observed in rat hepatocytes (James *et al.*, 1998a).

MEHP (500 µM) or exogenous TNFα (5000 U/mL) induced hepatocyte proliferation and

suppressed apoptosis in mouse primary hepato-cytes (Hasmall *et al.*, 2002).

Kim *et al.* (2004b) reported that exposure to DEHP increased the proliferation of MCF-7 (ER-positive) cells but not MDA-MB-231 (ER-negative) cells at the same concentration. DEHP mimicked estrogen in the inhibition of tamoxifen-induced apoptosis in MCF-7 cells, measured by the TUNEL assay. Bcl-2/BaX ratios were decreased by treatment with tamoxifen. Pre-incubation of the MCF-7 cells with DEHP before exposure to tamoxifen reduced this decrease in Bcl-2/BaX ratio.

The survival of human cultured NCTC 2554 keratinocytes was decreased by DEHP-induced necrosis (not apoptosis) at 97 μg/mL after 4 hours of exposure and 20 μg/mL after 24 or 48 hours of exposure (Martinasso *et al.*, 2006). Necrosis was inhibited by the addition of an antisense oligonu-cleotide against PPARβ showing the involvement of PPARβ in this effect. Western blot analysis showed decreased expression of the proteins pErk1, Erk2 and c-myc and increased the expres-sion of PPARβ with increasing doses of DEHP. After an initial increase, PPARα expression was reduced at higher doses of DEHP.

(g) Oxidative stress

(i) Humans

No data were available to the Working Group.

(ii) Experimental systems

Several studies have been carried out in rats given DEHP in which end-points indicative of oxidative damage in the liver were measured. These include increases in lipofuscin (Rao *et al.*, 1982, 1987; Hinton *et al.*, 1986; Cattley *et al.*, 1987; Lake *et al.*, 1987; Conway *et al.*, 1989) and malondialdehyde (Seo *et al.*, 2004).

Male Sprague-Dawley rats were fed a diet containing 2% DEHP for 2 years (Lake *et al.*, 1987). Levels of conjugated dienes indicative of lipid peroxidation were increased in liver homogenates, and morphological examination of liver sections revealed increased lipofuscin deposition in non-nodular but not in nodular areas of the liver.

Takagi *et al.* (1990a, b) investigated the rela-tionship between hepatic peroxisome prolifera-tion and levels of 8-OHdG in hepatic DNA. Male F344 rats (6 weeks of age) were fed 1.2% DEHP in the diet for periods of 1–12 months. Treatment with DEHP resulted in sustained stimulation of cyanide-insensitive palmitoyl-coenzyme A activity and produced up to a twofold increase in levels of 8-OHdG in hepatic DNA.

Rusyn *et al.* (2001) used the spin-trapping technique and electron spin resonance spectro-scopy to provide evidence of oxidative stress in liver of rats administered DEHP acutely. The spin trapping agent α-(4-pyridyl-1-oxide)-*N-tert*-butylnitrone (POBN) was also administered and bile samples were collected for 4 hours. Under these conditions, the intensity of the six-line radical adduct signal increased to a maximum value of 2.5-fold 2 hours after administration of DEHP, before peroxisomal oxidases were induced. Furthermore, DEHP given with $[^{13}C_2]$ dimethyl sulfoxide produced a 12-line electron spin resonance spectrum, providing evidence that DEHP stimulates hydroxyl radical formation *in vivo*. Furthermore, when rats were pretreated with dietary glycine, which inactivates Kupffer cells, DEHP did not increase radical signals. Moreover, similar treatments were performed in knockout mice deficient in NADPH oxidase (p47[phox] subunit). Importantly, DEHP increased oxidant production in wild-type but not in NADPH oxidase-deficient mice. These data provide evidence for the hypothesis that the molecular source of free radicals induced by peroxisome proliferators is NADPH oxidase in Kupffer cells. Radical adduct formation was not affected in *Pparα* knockout mice. These obser-vations represent in-vivo evidence that DEHP increases free radicals in liver before peroxisomal oxidases are induced.

In a follow-up study, Woods *et al.* (2007) hypothesized that continuous treatment with peroxisome proliferators will cause a sustained formation of POBN radical adducts in the liver. Mice were fed diets containing either WY-14 643 (0.05% w/w) or DEHP (0.6% w/w) for up to 3 weeks. Liver-derived radical production was assessed in bile samples by measuring POBN radical adducts using electron spin resonance. WY-14 643 caused a sustained increase in POBN radical adducts in mouse liver and this effect was greater than that of DEHP. Free radical production, induced by administration of DEHP, occurred after 2 hours and 3 weeks but not after 3 days or 1 week of exposure. To understand the molecular source of these radical species, NADPH oxidase-deficient ($p47^{phox}$-null) and *Pparα*-null mice were examined after treatment with WY-14 643. No increase in radicals was observed in *Pparα*-null mice that were treated with WY-14 643 for 3 weeks, while the response in $p47^{phox}$-null mice was similar to that in wild-type mice. These results show that PPARα, but not NADPH oxidase, is critical for a sustained increase in POBN radical production caused by peroxisome proliferators in rodent liver. [Therefore, the production of POBN radicals induced by peroxisome proliferators in Kupffer cells may be limited to an acute response to these compounds in mouse liver.]

Male rats were administered peroxisome proliferators (DEHP, DBP and *n*-butylbenzyl phthalate: 50, 200 and 1000 mg/kg bw per day; clofibrate: 100 mg/kg bw per day) orally for 14 days, and the activities of metabolizing enzymes and peroxisomal enzymes were investigated. Oxidative damage was measured as 8-OHdG in the DNA and as 4,4′-methylenedianiline level in the liver. The four peroxisome proliferators significantly increased the relative liver weights, cyanide-insensitive palmitoyl-coenzyme A oxidation and activity of CAT. DEHP was found to be the most potent peroxisome proliferator among the three phthalates. A dramatic and dose-dependent increase in hepatic levels of 4,4′-methylenedianiline was observed in clofibrate-, DEHP- ($\geq$ 50 mg/kg), DBP- and *n*-butylbenzyl phthalate- ($\geq$ 200 mg/kg) treated groups. However, the level of 8-OHdG in hepatic DNA was increased only in DEHP- (1000 mg/kg) and clofibrate-treated groups (Seo *et al.*, 2004). The authors indicate that 8-OHdG positively correlates with the carcinogenic potential of peroxisome proliferators, but other factors, as well as peroxisomal peroxidase could be involved in the generation of 8-OHdG and the carcinogenesis of peroxisome proliferators.

A chronic (22-month) feeding study with DEHP was conducted in wild-type (Sv/129 strain) and *Pparα*-null mice (Ito *et al.*, 2007a). Groups of mice were fed diets containing 0, 0.01 or 0.05% DEHP. No significant effect on the body or liver weight, or serum alanine aminotransferase activity was observed in either dose or genotype group at the commencement of the study. Treatment-related induction of oxidative DNA damage indicated by an increase in 8-OHdG and expression of the inflammatory marker, nuclear factor κ B, were observed in both groups, although the changes were more pronounced in *Pparα*-null mice exposed to DEHP.

Primary monolayer cultures of hepatocytes isolated from male F344 rats were incubated in medium containing one of three different peroxisome proliferators and examined for the induction of peroxisomal coenzyme A oxidase activity and lipid peroxidation. The latter parameter was determined by measuring levels of conjugated dienes in lipid fractions extracted from harvested cells. The peroxisome proliferators used in these studies were nafenopin and clofibric acid (two hypolipidaemic drugs) and MEHP. The relative specific activity of peroxisomal acyl coenzyme A oxidase was increased by about 300% after incubation for 44 hours with 200 μM (62 μg/mL) nafenopin; lower levels of induction were observed with clofibric acid or MEHP. Relative to controls, the level of conjugated dienes was increased approximately

twofold after incubation with 200 μM (62 μg/mL) nafenopin; there was no apparent increase in conjugated dienes after incubation with up to 200 μM (55 μg/mL) MEHP or 400 μM (86 μg/mL) clofibric acid (Tomaszewski et al., 1990).

(h) Gap-junctional intercellular communication

(i) Humans

No data were available to the Working Group.

(ii) Experimental systems

Pugh et al. (2000) investigated the effects of the peroxisome proliferators DINP (500 mg/kg bw per day) and DEHP (500 mg/kg bw per day) or vehicle (0.5% methyl cellulose, 10 mL/kg) administered to male cynomolgus monkeys by intragastric intubation for 14 consecutive days. In situ dye transfer studies using fresh liver slices revealed that DINP and DEHP had no effect on gap-junctional intercellular communication.

Isenberg et al. (2000) reported inhibition of dye transfer in situ for liver strips from male B6C3F$_1$ mice treated with 500 ppm DEHP at 2 weeks and 6000 ppm at 4 weeks of exposure, and from male F334 rats treated in vivo with DEHP (6000 ppm in the diet) from 1 to 6 weeks. [Food consumption was not recorded and the rats and mice were fed ad libitum; therefore the dose in milligrams per kilogram of body weight per day could not be calculated.]

Dietary administration of DEHP at a dose of 20 000 mg/kg diet to male F344 rats for 2 weeks decreased gap-junctional intercellular communication (67% of control) and enhanced replicative DNA synthesis (4.8-fold the control). Elevation of the relative liver weight and the induction of peroxisomal β oxidation were also observed following treatment with DEHP. Following administration of DEHP to F344 rats and B6C3F$_1$ mice at a dose of 6000 mg/kg diet for 18 months, inhibition of gap-junctional intercellular communication persisted, and the relative liver weight and induction of peroxisomal β oxidation remained elevated in both

species. In recovery studies in which DEHP was administered to male F344 rats for 2 weeks and then withdrawn, the relative liver weight, rate of peroxisomal β oxidation, increase in replicative DNA synthesis and inhibition of gap-junctional intercellular communication returned to control values within 2–4 weeks after cessation of treatment. Recovery studies with phenobarbital produced similar results. The primary active metabolite of DEHP, MEHP, was detected in the livers of rats and mice treated with DEHP for more than 2 weeks. However, it could not be detected after withdrawal of DEHP from the diet after 2 weeks. This study demonstrated that inhibition of gap-junctional intercellular communication, together with indicators of peroxisomal proliferation, including increased relative liver weight and enhanced peroxisomal β oxidation, persist while DEHP treatment continues but are reversed when treatment is stopped. Studies with phenobarbital produced a similar pattern of response (Isenberg et al., 2001).

Inhibition of gap-junctional intercellular communication (inhibition of metabolic cooperation) in Chinese hamster V79 lung fibroblast cells was reported after exposure to DEHP in vitro. Elmore et al. (1985) reported a positive response following exposure to DEHP (5 μg/mL) in one of three experiments that did not also lead to cytotoxicity. In a limited report, Malcolm et al. (1983) stated that DEHP (4 μg/mL) gave positive results for this end-point. In a subsequent study, Malcolm & Mills (1989) reported that DEHP (1, 10, 15 μg/mL) gave a positive response in two experiments. Other studies reported positive results at higher concentrations in Chinese hamster V79 cells.

Inhibition of gap-junctional intercellular communication (inhibition of dye transfer) was also studied in SHE cells exposed to DEHP. Mikalsen & Sanner (1993) reported that normal SHE cells exposed to DEHP (77 μM [30 μg/mL]) for 24 hours gave positive results in the assay and that 5 days of exposure of transformed SHE

cells to DEHP (30 μg/mL) also gave a positive response. Cruciani et al. (1997) reported transient inhibition of dye transfer in SHE cells after exposure to DEHP (9.7 μg/mL). Therefore, metabolic cooperation in Chinese hamster V79 cells appeared to be more sensitive than dye transfer in SHE cells as a metric of gap-junctional intercellular communication after exposure to DEHP.

The only available study of gap-junctional intercellular communication following exposure to MEHP is that of Cruciani et al. (1997) who reported inhibition of metabolic cooperation and inhibition of dye transfer in exposed Chinese hamster V79 cells. The concentrations tested that inhibited metabolic cooperation (28, 56 and 112 μg/mL [100, 200 and 400 μM]) also decreased cell survival which was already at 60%. Cell survival was not noted in the study of inhibition of dye transfer of MEHP but 112 μg/mL (400 μM) MEHP was reported to cause this effect in V79 cells. Cruciani et al. (1997) also studied inhibition of dye transfer in SHE cells and reported a transient inhibition at 7 μg/mL (25 μM) MEHP.

(i) Liver toxicity

(i) Humans

The subchronic toxicity of DEHP was evaluated in 28 term infants with respiratory failure, 18 of whom received 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, although the rate of administration ranged up to 2 mg/kg bw DEHP 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 DEHP (Karle et al., 1997).

One study compared cholestasis in premature and newborn infants who received parenteral nutrition through PVC-containing or PVC-free infusion systems (von Rettberg et al., 2009). A retrospective analysis, before and after changing from PVC-containing to PVC-free infusion systems, was conducted on two groups of 30 and 46 patients, respectively. It was found that the use of PVC-containing lines correlated strongly with the development of cholestasis ($P = 0.0004$) and that the incidence of cholestasis decreased from 50 to 13% after PVC-containing infusion systems were discontinued.

(ii) Experimental systems

Subchronic liver toxicity has been reported in non-human primates that were subjected to chronic transfusions through PVC tubing containing DEHP. Abnormal liver function (e.g. bromosulfophthalein clearance) and cholestasis have been reported in rhesus monkeys in chronic experiments that mimicked conditions of patients undergoing repeated blood or platelet transfusions through PVC-containing tubing, an effect that was absent when polyethylene containers were used (Jacobson et al., 1977). The average cumulative amount of DEHP infused in 1 year was 69.3 mg (or 21.3 mg/kg bw) which the authors found to be comparable or even lower than that in humans on chronic transfusion therapy.

A subsequent study in rhesus monkeys by the same group evaluated hepatic function and liver histology up to 26 months after cessation of transfusions (Kevy & Jacobson, 1982). It was reported that abnormal liver function tests and histological abnormalities (e.g. disturbances of hepatic architecture, the presence of round-cell infiltration and multinucleated giant cells) in liver biopsies persisted throughout transfusion and the follow-up period. [While these studies implicate DEHP as a potential toxic ingredient of plastic medical devices, these associations need to be verified further. Similar hepatotoxic effects of DEHP do not appear to be observed in rodents and may be a phenomenon of route of exposure (intravenous versus dietary) or may comprise another set of important species differences.]

The effects of the peroxisome proliferators DINP and DEHP were evaluated in young adult male cynomolgus monkeys, with emphasis on the detection of hepatic and other effects seen in rats and mice after treatment with high doses of phthalates. Groups of four monkeys received DINP (500 mg/kg bw per day), DEHP (500 mg/kg bw per day) or vehicle (0.5% methyl cellulose, 10 mL/kg bw) by intragastric intubation for 14 consecutive days. Clofibrate (250 mg/kg bw per day), a hypolipidaemic drug used for cholesterol reduction in human patients, was used as a reference substance. None of the test substances had any effect on body weight or liver weights. Histopathological examination of tissues from these animals revealed no distinctive treatment-related effects in the liver, kidney or testes (Pugh et al., 2000).

Diets containing 2% DEHP were fed to male Crlj:CD1(ICR) mice for 10 days (daily dose of DEHP was 0.90 ± 0.52 mg/mouse). The testes, livers, kidneys and pancreata were examined for the presence of MEHP and nitrogen oxides (NO_x) produced by the peroxidation of nitric oxide with free radicals, and lipid peroxidation induced by the chain reaction of free radicals. Histological observations and serum analyses showed the presence of liver dysfunction and dehydration. Unexpectedly, the concentration of MEHP in the testes was extremely low compared with that in the liver. However, the concentration of the NO_x in the testes was as high as the hepatic concentration. Furthermore, free radical-induced lipid peroxidation was detected histochemically in the testes but not in the liver (Miura et al., 2007). [The Working Group noted that this study did not report data on controls for NO_x. The authors state that NO_x concentrations in all four organs in control mice were less than 10 pmol/10 μL, and that those in the liver of treated mice were ~45 pmol/10 μL but that it was not possible to determine the extent of the increase.]

González et al. (2009) examined the effects of exposure to peroxisome proliferators on rat α2-macroglobulin, an important acute-phase protein, of which normal adult rats present low serum levels but pregnant rats display high amounts. To determine whether the effects were mediated by Pparα, wild-type mice and Pparα-null mice were used and treated with WY-14 643 or DEHP. WY-14 643, but not DEHP, reduced α2-macroglobulin and γ-fibrinogen (another acute-phase protein) expression in the livers of wild-type mice, but had no effect in Pparα-null mice. WY-14 643 or DEHP did not affect expression of complement C3 protein, another acute-phase response protein.

Anderson et al. (1999b) examined the expression of acute-phase proteins in mouse liver after exposure to peroxisome proliferators. Mice treated with either WY-14 643 or DEHP for 3 weeks had decreased hepatic α-1 antitrypsin expression but increased expression of ceruloplasmin and haptoglobin. Pparα-null mice showed no hepatic acute-phase protein gene alteration after treatment with peroxisome proliferators but had higher basal expression than did wild-type controls. It was concluded that Pparα activation by different peroxisome proliferators leads to dysregulation of hepatic acute-phase protein gene expression in mice.

A chronic (22-month) study was conducted in groups of wild-type (Sv/129 strain) and Pparα-null mice (Ito et al., 2007a) fed diets containing 0, 0.01 or 0.05% DEHP. No significant effect on the body or liver weights, or serum alanine aminotransferase activity was observed in either dose or genotype group.

A study by Ward et al. (1998) showed that the mean liver weight of DEHP-treated (12 000 ppm for up to 24 weeks) wild-type mice was significantly greater than that of untreated wild-type mice at all time-points. The mean liver weight of treated Pparα-null mice did not differ from that of untreated controls at any time-point. Livers from wild-type mice fed DEHP had marked diffuse hepatocytomegaly and cytoplasmic granular hepatocyte eosinophilia, the severity of

which was time-related. These lesions were not present in DEHP-treated *Pparα*-null mice at any time-point. Glycogen deposits were present in hepatocytes in untreated wild-type mice, while treated and untreated *Pparα*-null mice had much less glycogen present. *Pparα*-null mice on control diet developed moderate centrilobular fatty changes beginning at week 8, while no fatty changes were observed in DEHP-treated *Pparα*-null mice.

[The Working Group noted that although no studies have evaluated cholestasis due to DEHP in rats or mice, other peroxisome proliferators are known to have a protective effect against cholestasis. For example, cholestasis was observed in bezafibrate-fed *Pparα*-null, but not wild-type mice (Hays *et al.*, 2005).]

In a 104-week feeding study of DEHP (100, 500, 1500 or 6000 ppm) in B6C3F$_1$ mice, an increase in the following signs of liver damage were reported: hepatocyte pigmentation, increased cytoplasmic eosinophilia and chronic inflammation were observed in all high-dose males and females. No signs of these histopathological changes were found in control and lower-dose groups (David *et al.*, 2000a).

Nair *et al.* (1998) evaluated the systemic toxicity of DEHP (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-glutamyl transpeptidase, lactate dehydrogenase, alanine aminotransferase and alkaline phosphatase). No evidence of toxicity was observed.

Young male and female Sprague-Dawley rats (10 per sex per group) were fed diets containing 5, 50, 500 or 5000 ppm DEHP for 13 weeks (Poon *et al.*, 1997). Mean DEHP 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 141 and 120% of control values in male and female rats, respectively, were observed only in animals given 5000 ppm DEHP.

In a 104-week feeding study in F344 rats, significant increases in the incidence of Kupffer cell/hepatocyte pigmentation was reported in male and female rats fed 12 500 ppm DEHP and of spongiosis hepatis in male rats fed 2500 and 12 500 ppm compared with controls (David *et al.*, 2000b).

The hepatic effects of low and high concentrations of DEHP (1000 and 6000 ppm) were examined in male Syrian golden hamsters (refractory to peroxisome proliferator-induced tumorigenicity). A slight increase in the relative liver weight, and peroxisomal β-oxidation activity and replicative DNA synthesis in the liver was observed. However, these effects were not of the same magnitude or consistency as those observed in rats or mice. Furthermore, DEHP had no effect on gap-junctional intercellular communication in hamster liver at any of the time-points examined (2 and 4 weeks). No changes in markers of liver damage were reported (Isenberg *et al.*, 2000).

The ability of DEHP to induce hepatic microsomal carboxylesterase isozymes in hamsters was studied by measuring hydrolase activities and by immunoblot analysis using specific antibodies. Animals were given 2% (w/w) DEHP in the diet for 7 days. No changes in markers of liver damage were reported (Hosokawa *et al.*, 1994).

After in-vitro exposure of male Wistar rat primary hepatocytes to DEHP (Ghosh *et al.*, 2010), decreased cell viability and increased intracellular reactive oxygen species were observed at 39 µg/mL DEHP. The decreased cell viability was reversed by the addition of catalase. Apoptosis but not necrosis was associated with decreased cell survival at exposure to 9.8 µg/mL DEHP and was consistent with increased caspase-3 activity and changes in mitochondrial membrane potential.

4.3.2 Non-parenchymal cell-specific events

Rose *et al.* (1999) tested the hypothesis that Kupffer cells are activated directly by peroxisome proliferators, including DEHP and MEHP. Kupffer cell superoxide production after 30 minutes of exposure was measured following treatment *in vitro*. WY-14 643 increased superoxide production in a dose-dependent manner (0.1 and 50 μM) with half-maximal stimulation at 2.5 μM. DEHP and its metabolite, 2-EH, did not increase superoxide production even at doses 50 times higher than those of WY-14 643; however, its key metabolite, MEHP, activated superoxide production as effectively as WY-14 643 with half-maximal stimulation at 5 μM. In-vivo treatment of rats with WY-14 643 in the diet (0.1%) for 21 days caused a twofold increase in Kupffer cell superoxide production while treatment with DEHP (1.2%) did not. Pretreatment of Kupffer cells with staurosporine (0.01–10 pM), an inhibitor of protein kinase C, completely blocked generation of superoxide demonstrating that protein kinase C is a prerequisite. Moreover, WY-14 643 increased Kupffer cell calcium-dependent protein kinase C activity threefold. Pretreatment of Kupffer cells with the amino acid glycine (0.01–3 mM), which blunts calcium signalling, inhibited both WY-14 643-stimulated protein kinase C activity and superoxide production completely. The authors stated that these data are consistent with the hypothesis that potent peroxisome proliferators (WY-14 643 and MEHP) activate Kupffer cell production of oxidants directly via mechanisms that involve protein kinase C.

Evidence for Kupffer cell-mediated increased oxidant free radical production *in vivo* after treatment with DEHP was obtained using a spin-trapping technique and electron spin resonance spectroscopy (Rusyn *et al.*, 2001). Specifically, when rats were given DEHP acutely for 2 hours, a radical adduct signal was detected. No increase in the radical signal due to DEHP was observed when Kupffer cells were inactivated *in vivo* with glycine pretreatment, or in NADPH oxidase-deficient mice ($p47^{phox}$-null mice). The authors suggested that the molecular source of free radicals induced by peroxisome proliferators is NADPH oxidase in Kupffer cells. The rapid DEHP-induced production of free radicals *in vivo* occurred long before peroxidase-generating enzymes in peroxisomes were induced, and was not dependent on PPARα status.

A gene expression profiling study examined transcriptional changes induced by DEHP in mouse liver (Currie *et al.*, 2005). In addition to many genes that have traditionally been associated with hepatocyte-specific responses to peroxisome proliferators, several known components of the TNF/interleukin-1 (IL-1) signalling pathways, including the IL-1 receptor-associated kinase-like 2, myeloid differentiation primary response gene 88 (inhibitor of nuclear factor κB kinase gamma) and other genes were induced very early (2 hours) and declined at later times (24 hours) after acute treatment with DEHP, consistent with other studies showing a time-course of Kupffer cell activation.

4.3.3 Dose–response for the mechanistic end-points

Exposure to DEHP caused increases in the incidence of liver tumours in male and female B6C3F$_1$ mice and F344 rats (NTP, 1982; Cattley *et al.*, 1987; David *et al.*, 1999, 2000a, b). Dose–response characteristics for liver tumour induction are summarized in Fig. 4.2 and 4.3 for mice and rats, respectively. In male and female mice, consistent induction of liver tumours was observed at doses of 1500 ppm DEHP in the diet and higher (NTP, 1982; David *et al.*, 1999), and a small increase in liver tumour incidence occurred in male but not female mice at 500 ppm in one study (David *et al.*, 1999). In rats, consistent induction of liver tumours was observed at 6000 ppm and higher (NTP, 1982; Cattley *et al.*, 1987; David

et al., 1999). A minimal increase in the incidence of liver tumours occurred in female but not male rats at 100 ppm, no increase occurred in either sex at 500 ppm and an increase was observed in males but not females at 2500 ppm (David *et al.*, 1999). In a separate study, liver tumours were observed at 12 000 ppm but not at 350 ppm or 1000 ppm (Cattley *et al.*, 1987). DEHP also increased the incidence of pancreatic acinar-cell tumours in male rats but only at the highest dose (12 500 ppm) (David *et al.*, 2000b).

Markers of PPARα activation have been quantitated in mice and rats after exposure to DEHP and compared with increases in the incidence of liver tumours (Fig. 4.2 and 4.3). There was a good correlation between liver tumour induction and several well characterized indicators of PPARα activation. These indicators include: 1) increases in the size and/or numbers of peroxisomes; 2) increases in acyl-coenzyme A oxidase encoding a peroxisomal palmitoyl-coenzyme A oxidase (a marker of peroxisome proliferation and the rate-limiting enzyme of fatty acid β-oxidation); 3) increases in the levels of CYP4A protein, a ω-lauric acid hydroxylase; and/or 4) increases in the levels of CAT (also known as carnitine palmitoyl transferase) involved in fatty acid transport into the mitochondria. In mice and rats, the induction of the markers of PPARα occurred at doses coincident with or usually lower than those that induce liver cancer, as predicted if a PPARα-dependent mechanism is operational. The data indicated that a relatively good correlation exists between liver tumour induction and induction of markers of PPARα activation in mice and rats, supporting a role for PPARα in liver tumour induction by DEHP.

Fig. 4.2 Dose-dependent relationships between liver cancer and end-points associated with peroxisome proliferator-activated receptor α activation after exposure to di(2-ethylhexyl) phthalate (DEHP) in mice
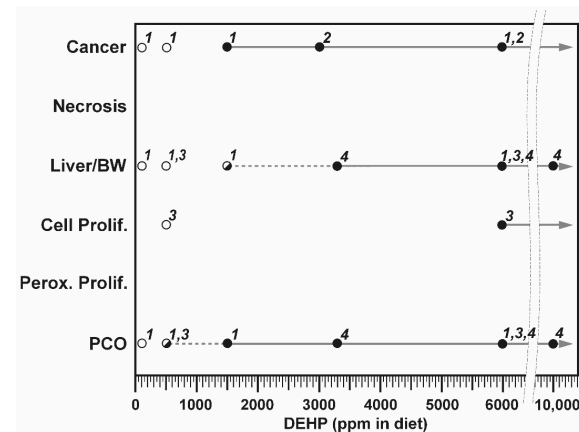
End-points evaluated in this figure include those associated with typical peroxisome proliferation including peroxisome proliferation (measured as increases in volume or number) and increases in palmitoyl-coenzyme A oxidase (PCO) activity. Also shown are the dose–response data for end-points relevant to the mode of action of several liver carcinogens: increases in liver to body weights, hepatocyte proliferation and hepatocyte necrosis. The times in parentheses after the literature citation refer to the time of exposure relevant for measurement of changes in cell proliferation. Figures show the doses at which the measured end-point has (filled circles) or has not (open circles) exhibited statistically significant increases. Half-filled circles indicate conditions where there are conflicting studies in which the end-point was or was not observed at the same dose. Solid lines indicate doses that consistently induce the indicated end-point whereas dotted lines indicate conditions that result in inconsistent effects. Data were taken from feeding studies at the indicated concentrations.
BW, body weight
From Corton (2008). © Informa Healthcare. Adapted with permission of Informa Healthcare.
[1] From David *et al.* (1999)
[2] From NTP (1982)
[3] From Isenberg *et al.* (2000) (2, 4 weeks)
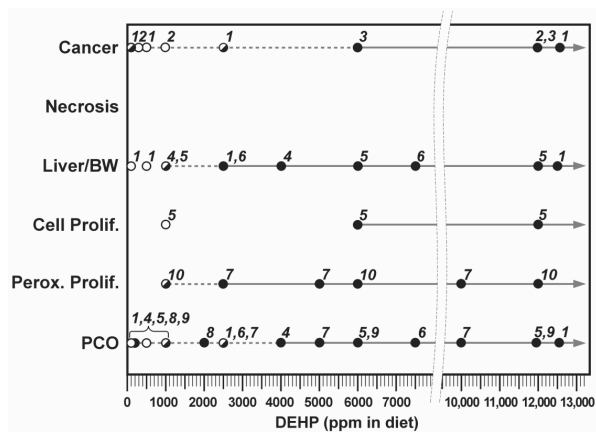[4] From Ochs *et al.* (1992)

4.3.4 Other relevant data

(a) Acute toxicity

(i) Humans

Dermal application of DEHP was moderately irritating, but only slightly sensitizing to human skin (Shaffer *et al.*, 1945; Mallette & Von Haam, 1952). Two adults given single oral doses of either 5 or 10 g DEHP did not show adverse effects other

Fig. 4.3 Dose-dependent relationships between liver cancer and end-points associated with peroxisome proliferator-activated receptor α activation after exposure to di(2-ethylhexyl) phthalate (DEHP) in rats

End-points evaluated in this figure include those associated with typical peroxisome proliferation including peroxisome proliferation (measured as increases in volume or number) and increases in palmitoyl-coenzyme A oxidase (PCO) activity. Also shown are the dose–response data for end-points relevant to the mode of action of several liver carcinogens: increases in liver to body weights, hepatocyte proliferation and hepatocyte necrosis. The times in parentheses after the literature citation refer to the time of exposure relevant to measurement of changes in cell proliferation. Figures show the doses at which the measured end-point has (filled circles) or has not (open circles) exhibited statistically significant increases. Half-filled circles indicate conditions where there are conflicting studies in which the end-point was or was not observed at the same dose. Solid lines indicate doses that consistently induce the indicated end-point whereas dotted lines indicate conditions that give inconsistent data. Data were taken from feeding studies at the indicated concentrations. BW, body weight
From Corton (2008). © Informa Healthcare. Adapted with permission of Informa Healthcare.
[1] From David et al. (1999)
[2] From Cattley et al. (1987)
[3] From NTP (1982)
[4] From Mitchell et al. (1985)
[5] From Isenberg et al. (2000) (1, 2, 4 weeks)
[6] From Dirven et al. (1990)
[7] From Reddy et al. (1986)
[8] From Ganning et al. (1990)
[9] From Short et al. (1987)
[10] From Barber et al. (1987)

than mild gastric disturbances and moderate diarrhoea at the higher dose (Shaffer et al., 1945).

Few data on the effects of occupational exposure specifically to DEHP are available (WHO, 1992). In a study involving workers at a Swedish PVC-processing factory, 54 workers exposed to DEHP and other phthalate diesters

(0.02–2 mg/m³) were studied clinically. 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 the results of conventional lung function tests showed no relation with exposure (Nielsen et al., 1985). Occupational exposure to DEHP was associated with asthma in one worker at a PVC-processing plant (WHO, 1992).

Several surgical procedures involve the use of PVC-containing tubing that comes into contact with patients' blood. This was shown to result in peri-operative exposure to various plasticizers, including a seven- to 10-fold increase in blood concentrations of DEHP in infants undergoing corrective operations for congenital defects, and in adults after cardiopulmonary bypass surgery (Barry et al., 1989).

PVC-containing tubing is also used in artificial ventilation devices. DEHP was measured in the inspired air of five mechanically ventilated pre-term infants; exposures ranged from 1 to 4200 mg DEHP/h (estimated from water traps in the ventilators). In three of the five cases studied, signs of advanced hyaline membrane lung disease were detected. One patient died of pneumothorax soon after being ventilated (DEHP was detected in the lung tissue of this patient), and the other two recovered after the PVC tubing was replaced with ethylene vinyl acetate tubing (Roth et al., 1988).

(ii) Experimental systems

Acute oral median lethal dose values for DEHP ranged from 26 to 34 g/kg bw in rats, mice, guinea-pigs and rabbits. Median lethal dose values after intraperitoneal administration were 30.7 g/kg bw in rats and 14–38 g/kg bw in mice (Shaffer et al., 1945; Calley et al., 1966; Lawrence et al., 1975; IARC, 1982).

Acute inhalation exposure to MEHP was studied in BALB/c mice. The breathing pattern

of these animals was monitored during exposure to 0.3–43.6 mg/m³ MEHP for 60 minutes, and inflammatory effects were studied by analysis of bronchoalveolar lavage fluid. Lower airway irritation was reported with a no-observed-effect level (NOEL) of 0.3 mg/m³. The NOEL for inflammatory markers in bronchoalveolar lavage fluid was 1.7 mg/m³, and the number of macrophages in this fluid reached a maximum about 16 hours after exposure (Larsen *et al.*, 2004).

The effects of intravenous injection of up to 300 mg/kg bw DEHP were studied in male Wistar rats. The DEHP was solubilized in aqueous solutions of several Tween surfactants. Dose-dependent lethality was observed with death generally occurring within 90 minutes after injection. The DEHP:Tween-treated animals had enlarged and generally darkened lungs, and in some cases showed haemorrhagic congestion. Histological examination of the lungs revealed an oedematous swelling of the alveolar wall and a marked infiltration of polymorphonuclear leukocytes. The effects were observed at doses as low as 50 mg/kg bw. Intravenous administration of aqueous Tween solutions alone did not cause any adverse effects (Schulz *et al.*, 1975).

The systemic toxicity of DEHP was investigated in male Wistar rats that received 0–7.5 mg/kg bw in six intraperitoneal injections on alternate days. Animals were evaluated by organ weight (liver and testis), light microscopy (liver, heart, brain and testis) and plasma clinical chemistry (γ-glutamyl transpeptidase, lactate dehydrogenase, alanine aminotransferase and alkaline phosphatase). No evidence of toxicity was observed (Nair *et al.*, 1998).

The acute testicular toxicity of MEHP was investigated in 28-day-old male Wistar rats 3, 6 and 12 hours after a single oral dose (by gavage) of 400 mg/kg bw. Detachment and sloughing of germ cells was reported (Dalgaard *et al.*, 2001).

The acute cardiac effects of increasing doses of MEHP were studied in anaesthetized rats injected via the femoral artery. There was a steady and significant decrease in heart rate, beginning after a total dose > 20 mg, and a decline in blood pressure after a total dose > 40 mg (Rock *et al.*, 1987).

Female F344 rats treated with a single oral dose (up to 5000 mg/kg bw) of DEHP showed no neurobehavioural effects, as evaluated by a functional observational test battery and by motor-activity testing (Moser *et al.*, 1995).

(b) Subchronic and chronic toxicity

(i) Humans

Kidney

The relationship between exposure to DEHP and uraemic pruritus was studied in patients undergoing regular continuous (4 hours) ambulatory peritoneal dialysis, during which exposure to DEHP and related compounds may occur. The post-dialysis serum concentrations of DEHP, MEHP and 2-EH were significantly higher than the corresponding values before dialysis. There was no relationship between severity of pruritus and post-dialysis serum concentrations of DEHP, MEHP, phthalic acid or 2-EH. Furthermore, the serum concentrations of these compounds did not differ significantly between patients with or without uraemic pruritus (Mettang *et al.*, 1996b). [The Working Group noted potential difficulties in exposure assessment of phthalates in biological fluids. Additional details are provided in Section 4.1].

Lung

A cross-sectional human study assessed the association between phthalate exposure — in terms of phthalate metabolite concentrations in urine — and four pulmonary function parameters (forced vital capacity, forced expiratory volume in one second, peak expiratory flow and maximum mid-expiratory flow) among 240 adult participants in the Third National Health and Nutrition Examination Survey (Hoppin *et al.*, 2004). Concentrations of MEHP in urine were not associated with any of the pulmonary

function parameters evaluated. [The Working Group noted potential difficulties in exposure assessment of phthalates in biological fluids. Additional details are provided in Section 4.1].

Immune function (in-vitro studies)

The human epithelial A549 cell line was exposed to 15.6–2000 µg/mL MEHP and concentrations of the pro-inflammatory cytokines IL-6 and IL-8 were measured in the cell culture supernatant. A concentration-dependent increase in cytokine production was observed at the lower (100–200 µg/mL) concentrations, whereas cytokine production was suppressed at higher (~1000 µg/mL) concentrations (Jepsen *et al.*, 2004). Similar observations have been reported for other phthalate esters (Larsen *et al.*, 2002).

A study in human peripheral blood mononuclear cells (containing 0.1–1% basophils) was aimed to assess a possible effect of plasticizers on immunoglobulin (Ig) E and IgG release in the basophil histamine-release assay, which models the inflammatory part of allergic disease (Glue *et al.*, 2005). Concentrations of 5, 50 and 500 µM MEHP and DEHP did not induce histamine release *per se*. However, when cross-binding of the high-affinity IgE receptor (FcεRI) was performed by stimulation with anti-IgE antibody, an increased dose-dependent histamine release was obtained.

(ii) Experimental systems

General toxicity

Young male and female Sprague-Dawley rats (10 per sex per group) were fed diets containing 5, 50, 500 or 5000 ppm DEHP for 13 weeks (Poon *et al.*, 1997). Mean DEHP 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 1.4- and 1.2-fold the control values in male and female rats, respectively, were observed only in animals given 5000 ppm DEHP. Relative testis weight was significantly reduced in male rats fed 5000 ppm. 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, and mild Sertoli-cell vacuolation in male rats fed 500 ppm DEHP.

Subchronic (13-week) feeding studies were conducted in F344 rats and B6C3F$_1$ mice (NTP, 1982). Diets containing 0, 1600, 3100, 6300, 12 500 or 25 000 ppm DEHP were fed to male and female rats, and male and female mice received diets containing 0, 800, 1600, 3100, 6300 or 12 500 ppm. A reduction in mean body-weight gain of male and female rats (29% and 53%, respectively) was observed in the 25 000-ppm group. Testicular atrophy was observed in all male rats fed 25 000 ppm and was present, but less pronounced, in rats fed 12 500 ppm. No other compound-related histopathological effects were observed. In mice, a reduction in mean body-weight gain of 10% or more was observed in males fed 3100, 6300 or 12 500 ppm, and in all treated females except those fed 1600 ppm. No other compound-related effects were observed.

Two-year cancer bioassays (feeding studies) were conducted in F344 rats and B6C3F$_1$ mice (NTP, 1982). Diets containing 0, 6000 or 12 000 ppm DEHP were fed to male and female rats, and male and female mice received diets containing 0, 3000 or 6000 ppm. In male rats, pituitary hypertrophy and testicular atrophy were observed in the 12 000-ppm group. In male mice, chronic kidney inflammatory changes and testicular degeneration were reported in the 6000-ppm group. No general toxicity was reported in female mice.

The effects of DEHP were studied in male Sv/129 mice (6 weeks of age at the beginning of the feeding study) that were homozygous wild-type or *Pparα*-null and fed 12000 ppm for up to 24 weeks (Ward *et al.*, 1998). General toxicity, evident from high lethality and considerably retarded body weight gain, was most evident in

treated wild-type mice. No animal in this group survived past 16 weeks. In treated *Pparα*-null mice, the weight gain became retarded only from week 16 and the mice began to lose weight by week 24. Throughout this study, the mean kidney weight of DEHP-treated wild-type mice was significantly greater than that of untreated control mice. In treated *Pparα*-null mice, kidney weight did not differ from that of untreated controls at any time-point. On necropsy, DEHP-treated wild-type mice had developed nephropathy, the severity of which was time-related. After 4 weeks of feeding, focal tubular degeneration, atrophy and regenerative tubular hyperplasia were observed, which became more diffuse with age. In DEHP-treated mice that died between 8 and 16 weeks, severe cystic renal tubules were seen, which were especially prominent in the straight portion of the proximal tubules. In the stomach, lung, heart or bone, no lesion indicative of renal failure was noted in this group. DEHP-treated *Pparα*-null mice also showed focal renal tubular lesions after 4–8 weeks, that were less pronounced than those in wild-type mice. Severe diffuse lesions that were morphologically similar to those found earlier in DEHP-treated wild-type mice were observed by week 24 in the *Pparα*-null animals.

A study in which rats were exposed to DEHP was designed to mimic the dose to which humans on haemodialysis would be subjected during each dialysis session. Three groups of rats were administered (by gavage three times a week for 3, 6, 9 and 12 months) sesame seed oil (control), 2.14 mg/kg bw (150 mg/70 kg) DEHP or 2.14 mg/kg bw (150 mg/70 kg) of a leachate obtained from a phthalate-containing plastic artificial kidney. Body weight, serum creatinine and kidney histopathology were evaluated. No significant weight loss was found in any of the groups at any time point. Significantly reduced kidney function, denoted by reduced clearance of creatinine, was found in the DEHP-treated group at 12 months. In both treated groups, a statistically significant increase in focal cysts in the kidneys was observed at 12 months (Crocker et al., 1988).

In a 104-week feeding study, B6C3F$_1$ mice received DEHP at 0, 100, 500, 1500 or 6000 ppm in the diet. At 104 weeks, mean relative kidney weights were significantly lower in males in the three highest-dose groups compared with controls. Chronic progressive nephropathy was seen in males in all dose groups, including the controls. In female mice, chronic progressive nephropathy was significantly increased at 78 and 104 weeks in the 1500- and 6000-ppm groups. Similar nephrotoxic effects were observed in a 104-week feeding study in F344 rats. In males, there was an increase in mean relative kidney weight, which was significant at 2500 and 12 500 ppm, while in females this increase was found to be significant only at the highest dose. In males, a significantly higher incidence of mineralization of the renal papilla was found in the 500-, 2500- and 12 500-ppm groups at 104 weeks (David et al., 2000a, b).

Immune function

Several studies have been performed to assess whether various phthalates, including DEHP and MEHP, act as sensitizers. These have been reviewed (Jaakkola & Knight, 2008) and some are discussed below.

Subcutaneous injection, not a probable route of exposure in humans yet an important mode of administration in studies on immune function, was performed in several studies. Ovalbumin was frequently used as the model antigen and was injected subcutaneously into the neck region of BALB/cJ mice, with or without the test substance. MEHP was shown (Larsen et al., 2001) to have an immunosuppressive effect, measured as a statistically significant reduction in IgE and IgG1 antibody production at a dose of 1000 μg/mL, and an adjuvant effect, measured as a statistically significant increase in IgE antibody concentration at a dose of 10 μg/mL. In a follow-up study,

the adjuvant effect of DEHP, defined as a statistically significant increase in IgG$_1$ concentration, was observed at a dose of 2000 µg/mL (Thor Larsen et al., 2001).

In a study that tested whether PPARα may be involved in the adjuvant effect of DEHP, *Pparα*-deficient 129/Sv mice were exposed intraperitoneally to a mixture of ovalbumin and DEHP (2000 µg/mL), and the ovalbumin-specific IgE, IgG1 and IgG2a responses were compared with the corresponding responses in wild-type mice. Compared with mice only given ovalbumin, DEHP induced a strong increase in ovalbumin-specific IgG1 and IgG2a, both in the wild-type and in the *Pparα*-null mice, indicating that DEHP is a mixed T-helper cell adjuvant and that its activity as an adjuvant is mediated through a *Pparα*-independent mechanism (Larsen & Nielsen, 2007).

[The Working Group noted that the findings that DEHP can act as an adjuvant have been challenged in a study (Dearman et al., 2008) in which topical administration of DEHP had no impact on antibody responses, regardless of whether the compound was applied locally or distant to the site of immunization with ovalbumin. Several methodological differences from the original studies have been identified in the latter work by the group who originally reported the findings (Hansen et al., 2008).]

The immuno-modulatory effects of MEHP on the immune response of BALB/c mice to ovalbumin were studied after inhalation exposure (60 minutes; 0.3–43.6 mg/m³). A concentration-dependent decrease in tidal volume and an increased number of alveolar macrophages were reported, but no changes in the numbers of neutrophils, lymphocytes, eosinophils or epithelial cells in bronchoalveolar lavage fluid were observed (Larsen et al., 2004).

A study in female B6C3F$_1$ mice aimed to assess the potential of DEHP to cause general and respiratory sensitization following topical administration. The positive (25% trimellitic anhydride) and reference (1% dinitrochlorobenzene) control animals were initiated by dermal application of DEHP (50 µL/flank; 25%, 50% and 100% solutions). The challenge dose which was applied 7 days later involved application to both ears at a dose of 25 µL/ear. Seven days later, the animals were killed and IgE was determined. This study showed no significant increases in IgE or cytokines after exposure to DEHP (Butala et al., 2004).

The effects of DEHP (50–500 µM) on antigen-induced degranulation of rat basophilic leukaemia (RBL-2H3) cells was evaluated. Without antigen stimulation, DEHP did not cause any significant increase in degranulation; however, it significantly potentiated the release of β-hexosaminidase induced by dinitrophenylated bovine serum albumin (Nakamura et al., 2002).

Lung

A 28-day toxicity study of DEHP was carried out in 9-week-old male and female Wistar rats that were exposed via head-nose inhalation to aerosols for 6 hours per day on 5 days per week for 4 weeks; target concentrations in air were 0, 0.01, 0.05 or 1.0 mg/L and estimated doses were 2.30, 11 or 230 and 3.6, 18 or 360 mg/kg bw per day in males and females, respectively (Klimisch et al., 1992). No general toxicity, based on clinical investigation and blood-chemistry parameters, was observed. A statistically significant (16%) increase in relative lung weights, accompanied by increased foam-cell proliferation and thickening of the alveolar septi, was reported in the highest-dose males.

In a 104-week feeding study, B6C3F$_1$ mice received 0, 100, 500, 1500 and 6000 ppm DEHP in the diet. At 104 weeks, mean relative lung weights were significantly increased in highest-dose males (6000 ppm). A similar effect was observed at the end of a 104-week feeding study in male F344 rats fed 2500 and 12 500 ppm DEHP (David et al., 2000a, b).

Brain and neurobehavioural effects

Female F344 rats treated with repeated doses (up to 1500 mg/kg bw per day for 14 days) of DEHP showed no neurobehavioural effects, as evaluated by a functional observational test battery and by motor-activity testing (Moser *et al.*, 1995).

In the 104-week feeding studies mentioned above, DEHP caused an increase in mean relative brain weights in male B6C3F$_1$ mice at the highest dose (6000 ppm). A similar effect was observed in male and female F344 rats at the highest dose of 12 500 ppm (David *et al.*, 2000a, b).

(c) Reproductive and developmental effects

(i) Humans

While no human studies were available at the time of the previous evaluation (IARC, 2000), several studies of reproductive and developmental effects of DEHP have been published since then. [The Working Group noted that the human studies detailed below, which showed either positive or negative associations, relied exclusively on concentrations of MEHP in biological fluids as a biomarker of exposure to DEHP and should be interpreted with caution. Assessment of oxidative metabolites may be necessary to evaluate in-vivo exposures to DEHP fully. Additional details are provided in Section 4.1.]

Developmental toxicity

A study of 84 newborns in Perrino Hospital, Brindisi, Italy, evaluated in-utero exposures to DEHP and gestational age. DEHP and MEHP were detectable in 88% of the samples of serum (cord blood). This finding was interpreted as evidence of in-utero exposure to DEHP in humans. Furthermore, the authors compared gestational age between MEHP-positive [the difference in levels was not taken into account] and -negative newborns and found shorter pregnancies in the MEHP-positive group (Latini *et al.*, 2003).

A possible association between pre-term birth and exposure to phthalates was investigated in a Mexican birth cohort study in which third-trimester urinary concentrations of phthalate metabolites in 30 women who delivered pre-term (< 37 weeks of gestation) were compared with those of 30 controls (≥ 37 weeks of gestation). Pre-term birth cases had significantly higher odds ratios for the presence of urinary DEHP metabolites — MEHP (OR, 3.5; 95%CI: 1.0–12.9), MEHHP (OR, 4.6; 95%CI: 1.3–16.7) and MEOHP (OR, 7.1; 95%CI: 1.9–26.5) — and for the sum of four DEHP metabolites (OR, 5.0; 95%CI: 1.4–18.0). After correction of metabolite concentrations by creatinine, only the association with the four DEHP metabolites remained significant (OR, 4.1; 95%CI: 1.0–17.5) (Meeker *et al.*, 2009a).

A study of 201 newborn–mother pairs from Shanghai, China, were investigated in a nested case–control study of in-utero exposure to phthalates, including DEHP, and birth outcomes. Of the newborns, 88 had low body weight and 113 had normal body weight at birth. Higher levels of phthalates in maternal and cord blood were found in low-body weight cases compared with controls. While some associations were found between prenatal exposures to phthalates and low body weight and body length at birth, these were not significant for DEHP after adjustment for potential confounders (Zhang *et al.*, 2009).

A cross-sectional study investigated the relationship between the levels of prenatal exposure to phthalate esters and birth outcomes among 149 pregnant Japanese women. Urinary concentrations of nine phthalate ester metabolites — monomethyl phthalate (MMP), monoethyl phthalate (MEP), mono-*n*-butyl phthalate (MnBP), MBzP, MEHP, MEHHP, MEOHP, mono-iso-nonyl phthalate and mono-*n*-octyl phthalate — were analysed in spot urine samples collected from the pregnant women. The health outcomes evaluated were birth weight, birth length, head circumference and gestational age,

and the relationships between prenatal exposure to phthalate esters and these outcomes were not significant (Suzuki *et al.*, 2010).

Data from a prospective Danish-Finnish cohort study on cryptorchidism collected from 1997 to 2001 were used to analyse individual breast milk samples collected 1–3 months postnatally as additive aliquots (*n* = 130; 62 cryptorchid/68 healthy boys) for phthalate monoesters, including MEHP. Serum samples (obtained from 74% of all boys) were analysed for gonadotropins, sex-hormone binding globulin, testosterone and inhibin B. No association was found between phthalate monoester levels and cryptorchidism. MEHP concentrations in breast milk did not show significant associations with any of the hormones evaluated (Main *et al.*, 2006).

A study examined anogenital distance (AGD), an end-point associated with impaired testicular function, and other genital measurements in 134 boys 2–36 months of age (Swan *et al.*, 2005). Shortening of the AGD has been shown in male rodents to be related to prenatal exposure to phthalates (Parks *et al.*, 2000). Swan *et al.* (2005) reported that AGD was significantly correlated with penile volume (*R* = 0.27; *P* = 0.001) and the proportion of boys with incomplete testicular descent (*R* = 0.20; *P* = 0.02). Urinary concentrations of four phthalate metabolites (MEP, MnBP, MBzP and mono-iso-butyl phthalate [MiBP]) were measured and correlated with the anogenital index (AGI) [AGI = AGD/weight (mm/kg)]. Comparing boys with prenatal MnBP concentrations in the highest quartile with those in the lowest quartile, the odds ratio for a smaller than expected AGI was 10.2 (95%CI: 2.5–42.2). The corresponding odds ratios for MEP, MBzP and MiBP were 4.7, 3.8, and 9.1, respectively (all *P*-values < 0.05). The age-adjusted AGI decreased significantly (*P*-value for slope = 0.009) with increasing summary phthalate score (joint exposure to the four phthalate metabolites). The authors concluded that the associations between

male genital development and exposure to phthalates seen here are consistent with the phthalate-related syndrome of incomplete virilization that has been reported in prenatally exposed rodents, which supports the hypothesis that prenatal exposure to phthalates at environmental levels can adversely affect male reproductive development in humans.

However, another similar study found no association between in-utero exposure to phthalates and either the AGD or AGI in male newborns (Huang *et al.*, 2009). The authors evaluated the association between maternal urinary excretion of phthalates, exposure of the fetus to phthalates in amniotic fluid and the health of newborns. Amniotic fluid and urine samples from pregnant women were collected and five phthalate monoesters, including MEHP, were measured. The birth weight, gestational age and AGD of newborns were also recorded. No effects of MEHP were found. However, a significantly negative correlation between MnBP in amniotic fluid, AGD (*R* = −0.31; *P* < 0.06) and AGI adjusted by birth weight (*R* = −0.32; *P* < 0.05) was found only in female newborns. The authors concluded that in-utero exposure to phthalates may have anti-androgenic effects on the fetus.

Male reproductive toxicity

A randomized controlled study of 21 infertile men (low sperm counts and abnormal sperm morphology) and 32 control men (normal semen quality and evidence of conception) investigated the relationship between seminal plasma concentrations of phthalate esters and semen quality (Rozati *et al.*, 2002). Concentrations of phthalate esters in infertile men were higher than those in controls. Sperm quality parameters (total motile sperm count) were inversely proportional to concentrations of phthalates.

A study conducted in Shanghai, China, investigated semen concentration of phthalates, including DEHP, and semen quality in men. A positive significant association was reported

between semen concentrations of all three phthalates tested and liquefaction time of semen. No correlations were found for other sperm quality parameters, such as sperm density or viability. No adjustment for confounders was performed in this study (Zhang *et al.*, 2006).

A study of 220 male partners of subfertile couples, aged between 20 and 54 years, investigated the relationship between sperm movement characteristics (straight-line velocity, curvilinear velocity and linearity) and urinary levels of phthalates (Duty *et al.*, 2004). Urinary concentrations of the phthalate monoesters — MEP, MBzP, MnBP, MEHP and MMP — were measured. While no consistent and statistically significant associations were found between urinary levels of phthalates and sperm motility end-points, negative trends were reported for MEHP.

A study of 234 young Swedish men (normal population) investigated the relationship between urinary concentrations of five phthalates, including MEHP, serum levels of reproductive hormones and male reproductive end-points (semen volume, sperm concentration and motility) (Jönsson *et al.*, 2005). No significant associations were found between any of the reproductive biomarkers and urinary levels of phthalates.

A study of 463 men who were partners in subfertile couples included detailed semen analysis (sperm concentration, motility and morphology) and single spot urinary concentrations of three DEHP metabolites (Hauser *et al.*, 2006). No relationships were found between DEHP metabolites and semen parameters.

Sperm DNA damage and urinary phthalate metabolites were assessed in male subjects (*n* = 379) who were patients at an infertility clinic (Hauser *et al.*, 2007). DNA damage was assessed with the neutral comet assay and it was reported that sperm DNA damage was associated with urinary MEHP after adjusting for DEHP oxidative metabolites; however, this study did not include control subjects (with no infertility).

A study of 349 men who were partners in subfertile couples included detailed semen analysis (sperm concentration, motility and morphology) and single spot urinary concentrations of four DEHP metabolites: MEOHP, MEHHP, MECPP and MEHP (Herr *et al.*, 2009). The evaluation of human reproductive parameters in this study (from semen analyses) did not show significant associations with concentrations of DEHP metabolites determined in spot urine sampled at the day of andrological examination.

A study of 74 male workers exposed to phthalates at the workplace (production of unfoamed PVC-containing flooring) and a matched group of 63 construction workers (no known occupational exposure to phthalates) investigated urinary concentrations of MnBP and MEHP and serum concentrations of gonadotropin, luteinizing hormone, follicle-stimulating hormone, free testosterone and E2 (Pan *et al.*, 2006). In exposed workers, urinary levels of both phthalates were significantly higher and serum free testosterone was significantly lower than those in controls. Phthalate concentrations were significantly negatively correlated with free testosterone.

A study of 408 infertile men assessed urinary levels of MEHP and DEHP and serum concentrations of free thyroxine and total tri-iodo thyronine. Urinary MEHP concentration was found to be inversely correlated with serum free thyroxine and total tri-iodo thyronine (Meeker *et al.*, 2007).

In a study of 425 men recruited through an infertility clinic in the USA, urinary concentrations of MEHP and several other phthalate monoester metabolites were measured, together with serum levels of testosterone, E2, sex hormone-binding globulin, follicle-stimulating hormone, luteinizing hormone, inhibin B and prolactin. The authors reported that, after adjusting for potential confounders, urinary levels of MEHP were inversely associated with levels of testosterone and E2 and the free

androgen index (molar ratio of total testosterone to sex hormone-binding globulin). The ratio of testosterone to E2 was positively associated with MEHP concentration (P = 0.07) and MEHP percentage (proportion of DEHP metabolite) (P = 0.007), which was interpreted as suggestive of a potential relationship with aromatase suppression (Meeker et al., 2009b).

A study of 13 healthy male adolescents (14–16 years of age) who received ECMO as neonates, a procedure performed with medical devices that contain high levels of phthalates, investigated sexual development (testicular volume, phallic length and serum levels of sex hormones), as well as thyroid, liver and renal function (Rais-Bahrami et al., 2004). All subjects were found to have normal growth percentile for age and sex, had normal internal organ function and exhibited sexual development (including serum hormone levels) appropriate for the stage of pubertal maturity.

Female reproductive toxicity

A study of 41 subjects with premature breast development, defined as growth of mammary tissue in girls under 8 years of age with no other manifestations of puberty, and 35 age-matched control female subjects assessed serum concentrations of phthalates (Colón et al., 2000). In 28 of 41 subjects, compared with one of 35 controls, DEHP and MEHP were detectable. This effect was challenged, however, due to the possible contamination of samples (McKee, 2004).

A study of six female adolescents (14–16 years of age) who received ECMO as neonates, a procedure performed with medical devices that contain high levels of phthalates, investigated sexual development (serum levels of sex hormones), as well as thyroid, liver and renal function (Rais-Bahrami et al., 2004). All female subjects were found to have a normal growth percentile for age and sex, had normal internal organ function and exhibited sexual development (including serum

hormone levels) appropriate for their stage of pubertal maturity.

A study of 55 women with endometriosis and 24 age-matched control women investigated concentrations of DEHP and MEHP in plasma and peritoneal fluid (Cobellis et al., 2003). Significantly higher plasma levels of DEHP were observed in women with endometriosis, and both phthalates were detected in the peritoneal fluid in > 90% of cases with endometriosis. However, there was no association between phthalate levels and stage of the disease or time of diagnosis.

A prospective case–control study of 49 infertile women with endometriosis, 38 age-matched women without endometriosis but with infertility related to tubal defects, fibroids, polycystic ovaries, idiopathic infertility and pelvic inflammatory diseases diagnosed by laparoscopy and 21 age-matched women with proven fertility and no evidence of endometriosis or other gynaecological disorders was conducted in Hyderabad, Andhra Pradesh state, India (Reddy et al., 2006). Serum concentrations of di(n-butyl) phthalate, butyl benzyl phthalate, di(n-octyl) phthalate and DEHP were measured. Significant differences in the serum concentrations of phthalates were observed between women with and without endometriosis. A significant correlation between serum concentrations of phthalates, including DEHP, and different levels of severity of endometriosis was observed.

A cross-sectional study of urinary concentrations of phthalate metabolites obtained from the US National Health and Nutrition Examination Survey (1999–2004) examined their relation to self-reported history of endometriosis and uterine leiomyomata among 1227 women 20–54 years of age (Weuve et al., 2010). Four phthalate metabolites, including MEHP, were examined. Eighty-seven (7%) and 151 (12%) women reported diagnoses of endometriosis and leiomyomata, respectively. After comparing the highest versus lowest three quartiles of urinary MEHP, there were no significant associations

with endometriosis or leiomyomata. A significant inverse association (OR, 0.59; 95%CI: 0.37–0.95) was found for both conditions combined.

A prospective case–control study of 97 women with advanced-stage endometriosis and 169 control women was performed in the Republic of Korea (Kim et al., 2011). Plasma concentrations of MEHP and DEHP were determined using liquid chromatography–tandem mass spectrometry. The study reported that the concentrations of MEHP and DEHP were significantly higher in women with advanced-stage endometriosis.

(ii) Experimental systems

The teratogenicity and reproductive toxicity of DEHP have been reviewed (Huber et al., 1996; Lyche et al., 2009). It was noted that DEHP impairs fertility in both sexes of adult rats at doses above 100 mg/kg bw per day, and that several studies indicate that DEHP is embryotoxic and teratogenic in rodents. DEHP was associated with a reduction in relative testis weight, decreases in sperm production and a depletion of testicular zinc. The testicular response appears to be somewhat species-specific (among the experimental animal systems tested), but is not consistent with the reported species sensitivity to peroxisome proliferation (e.g. guinea-pigs were more sensitive to the testicular effects than Syrian hamsters). The metabolite MEHP was judged to be more potent in causing both teratogenicity and reproductive toxicity. Both embryotoxic and testicular effects in adults were 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 prenatal exposure to DEHP and exposure 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). Detailed summaries of most of the studies published before 2000 were included in the previous evaluation (IARC, 2000) and are not repeated here (unless a particular study was omitted previously).

Developmental toxicity

Gestational exposure to DEHP was reported to lead to decreased maternal and fetal body weight, fetal resorptions and decreased viability of the pups (Singh et al., 1972; Tyl et al., 1988; Narotsky & Kavlock, 1995; Hellwig et al., 1997; NTP, 1997). Some but not all studies reported an increase in the incidence of abnormalities (e.g. haemangiomas of the legs) (Singh et al., 1972; Narotsky & Kavlock, 1995) and retarded development (renal pelvic dilatations) (Merkle et al., 1988). A comparative study of the teratogenicity of DEHP, 2-EH and 2-EHA in rats showed that all three chemicals induced malformations (hydronephrosis, laevocardia, septal defects, short and kinky tails, ectrodactyly, misplaced digits and bowed radius), and 2-EHA was the most potent (Ritter et al., 1987).

DEHP and several other phthalates were administered orally to pregnant rats at 750 mg/kg bw from gestational day 14 to postnatal day 3. None of the phthalates induced overt maternal toxicity or reduced litter sizes. DEHP treatment reduced maternal weight gain by about 15 g during the entire dosing period, reduced pregnancy weight gain to gestational day 21 by 24 g and reduced pup weight at birth (15%). Male pups from the DEHP-treated group displayed shortened AGDs (about 30%) and reduced testis weights (about 35%). As infants, males in the DEHP-treated group displayed female-like areolas/nipples (87%; $P < 0.01$) and a significant incidence of reproductive malformations (82%; $P < 0.0001$). The authors concluded that DEHP altered sexual differentiation in rats (Gray et al., 2000).

Female Sprague-Dawley rats were given oral doses of 0, 500 or 1000 mg/kg bw per day DEHP on gestation days 7–18. Fetal deaths were recorded at gestation days 12, 14, 16, 18 and 20. At all of these time-points, fetal deaths (20–36%)

were detected in the highest-dose group. Multiple abnormalities in male germ cells in fetal testis were also reported in DEHP-treated groups. In a follow-up experiment, lower doses (125, 250 and 500 mg/kg bw per day) were tested using the same protocol. In the 250- and 500-mg/kg groups, adverse effects on male testis development were observed; however, in the 125-mg/kg group, no effect was detected (Shirota *et al.*, 2005).

DEHP was administered orally to female Sprague-Dawley rats from gestation day 3 to postnatal day 21 at doses of 0, 375, 750 or 1500 mg/kg bw per day (Moore *et al.*, 2001). Male offspring were investigated for end-points of reproductive health and sexual behaviour at postnatal days 21, 63 or 105–112. Dose-related adverse effects in male sex organ development and sperm quality were found, as well as low sexual activity. These effects were most pronounced in groups exposed prenatally to 750 and 1500 mg/kg bw per day.

Lactational exposures to DEHP were shown to result in a decrease in body weight, as well as alterations in liver weight and function in rat pups (Parmar *et al.*, 1985; Dostal *et al.*, 1987). Parmar *et al.* (1985) showed that lactational exposure is indicated by a significant quantity of DEHP in the liver of pups, which shows that DEHP can be transferred through the mother's milk and is not hydrolysed as it is in adults (Parmar *et al.*, 1985). Exposure to DEHP during early life through mother's milk causes biochemical alterations which may affect the functional development of the testis (Tandon *et al.*, 1990).

Exposure of newborn rats to DEHP induced both general toxicity (e.g. reduced body weight and body weight gain) and hepatic effects (e.g. hepatomegaly and induction of peroxisomal enzymes) regardless of the route of exposure or age (Greener *et al.*, 1987; Cimini *et al.*, 1994). Male Sprague-Dawley rats (3–5 days of age) were administered DEHP by intravenous injections (0, 60, 300 or 600 mg/kg bw per day) or gavage (0, 300 or 600 mg/kg bw per day) for 21 days.

Organ weights and reproductive organ toxicity were evaluated at the end of treatment or after rats reached sexual maturity (90 days of age). No effects were observed in the low-dose/intravenous group. Abnormal histology was reported in testis in all other treated groups after the 21-day dosing period regardless of the route of administration. A dose-dependent increase in the severity of testicular lesions was observed and the effect was modestly stronger in rats exposed orally. No effects on sperm count, sperm morphology or sperm motility were observed in treated rats at 90 days of age (Cammack *et al.*, 2003).

Adverse effects on testicular development in male rat offspring after exposure of female rats to DEHP during gestation and suckling have been reported (Tandon *et al.*, 1991; Arcadi *et al.*, 1998). The pathological effects in the testis (e.g. decreased AGD, retained nipples and high levels of testicular and epididymal abnormalities, including atrophy and agenesis) induced by DEHP differed from those induced by other known androgen-receptor antagonists (i.e. vinclozolin, procymidone and 1,1-dichloro-2,2-bis(*para*-chlorophenyl)ethylene) (Wolf *et al.*, 1999).

Groups of Long-Evans rats were administered DEHP (0 or 10 mg/kg bw per day) orally from postnatal day 21 (weaning) to postnatal day 120 and showed no signs of overt toxicity, although Leydig-cell hyperplasia, reduced Leydig-cell testosterone production ex vivo, and increased levels of serum luteinizing hormone, testosterone and E2 were observed (Akingbemi *et al.*, 2004). The rise in testosterone levels found in this study contrasts with the decrease in testosterone observed in studies of prenatal exposure to DEHP. In a follow-up study (Ge *et al.*, 2007), a wider dose-range of DEHP (0, 10, 500 or 750 mg/kg bw per day) was administered to male rats starting at postnatal day 21 for 28 days. Pubertal onset was significantly decreased in the 10-mg/kg group, while it was significantly delayed in the 750-mg/kg group compared with controls. Furthermore,

similar bi-modal effects of the low- (10 mg/kg bw per day) versus high- (750 mg/kg bw per day) dose effects were seen for serum testosterone, as well as testis and body weight. The authors tested the effects of various concentrations of the DEHP metabolite MEHP *in vitro* in Leydig cell cultures. MEHP at 100 μM (27.8 μg/mL) increased luteinizing hormone-stimulated testosterone production, while 10 mM (2780 μg/mL) was inhibitory.

Gestational effects of DEHP similar to those observed in rats have been also reported in mice (Yagi *et al.*, 1980; Shiota & Nishimura, 1982; Tomita *et al.*, 1982b; Shiota & Mima, 1985; Tyl *et al.*, 1988).

Groups of 10–13 pregnant female homozygous wild-type or *Pparα*-null mice were administered DEHP by gavage at 0 or 1000 mg/kg bw on days 8–9 of gestation. Offspring were evaluated on gestational days 10 and 18. Similar developmental toxicity (resorptions, growth retardation and incidence of malformations) was seen in mice of both genotypes, suggesting that the developmental effects are not PPARα-mediated. Additional analysis showed that DEHP induced maternal hepatic *CYP4A1* mRNA in the wild-type females only; both genotypes showed DEHP-induced metallothionein-1 and zinc levels in the maternal livers and reduced zinc concentration in maternal serum and in the fetus after exposure (Peters *et al.*, 1997).

Reproductive toxicity

Exposure of adolescent male rats to DEHP has been shown to lead to the reduction in absolute and relative testicular weights, seminiferous tubular atrophy and cessation of spermatogenesis, leading to smaller litters (Gray *et al.*, 1977; Agarwal *et al.*, 1986; Parmar *et al.*, 1986; Dostal *et al.*, 1988; Ganning *et al.*, 1990; Siddiqui & Srivastava, 1992; Poon *et al.*, 1997). These effects appear to persist even after cessation of exposure (Oishi, 1985; Sjöberg *et al.*, 1985c).

A study of female reproductive function following exposure to DEHP reported prolonged estrous cycles, suppressed or delayed ovulation and smaller pre-ovulatory follicles, reduced pre-ovulatory granulosa-cell estrogen production, with secondary increases in follicle-stimulating hormone and insufficient luteinizing hormone surge for ovulation (Davis *et al.*, 1994).

In a 104-week feeding study of DEHP in male and female F344 rats, a statistically significant decrease in mean relative testes weight was observed with the highest dose tested (12 500 ppm). Bilateral aspermatogenesis was observed at 78 weeks in the 12 500-ppm group and at 104 weeks in the 500-, 2500- and 12 500-ppm groups. No effect on mean relative uterine weight was observed in this study (David *et al.*, 2000b).

A dose–response study following in-utero and lactational exposure to DEHP was conducted to investigate effects on androgenic status, developmental landmarks and testicular histology in male offspring rats. Female Wistar rats were treated daily by gavage with DEHP from gestation day 6 to lactation day 21 at doses of 0.015, 0.045, 0.135, 0.405 or 1.215 mg/kg bw per day (low doses) and 5, 15, 45, 135 or 405 mg/kg bw per day (high doses). Nipple retention and reduced AGD were observed in male offspring exposed *in utero* and during lactation to the highest dose (405 mg/kg bw per day). Delayed preputial separation was observed in animals exposed to 15 mg/kg bw per day or higher doses. The presence of bi- and multinucleated gonocytes and reduced germ-cell differentiation in seminiferous tubules were observed during histopathological examination of the testis on postnatal days 1 and 22 at doses of 135 and 405 mg/kg bw per day (Andrade *et al.*, 2006a).

A companion dose–response study following in-utero and lactational exposure to DEHP was conducted in rats to investigate reproductive toxicity in female offspring. The study design and dose range were identical to those detailed above (Andrade *et al.*, 2006a). No effects on organ (liver, kidney, spleen, thymus, thyroid, ovary and uterus) or body weights were detected in female

offspring, which presented a normal pattern of estrous cyclicity with no hormonal alterations (serum E2 and progesterone). An increase in the number of ovarian atretic tertiary follicles (observed only at the highest dose of 405 mg/kg bw per day) was the only effect observed in adult female offspring exposed to DEHP *in utero* and during lactation (Grande *et al.*, 2007).

Evidence for the toxicity of DEHP in both male (e.g. testicular effects) and female (e.g. effects on the uterus and hormone levels) mice, as well as reductions in litter size have been reported (NTIS, 1988; Lamb *et al.*, 1987; Agarwal *et al.*, 1989; Jain & Joshi, 1991).

In a 104-week feeding study of DEHP in B6C3F$_1$ mice (David *et al.*, 2000a), a decrease in mean relative testes weight was reported in male mice fed doses of 500, 1500 or 6000 ppm. Significant increases in immature/abnormal epididymal sperm and bilateral hypospermia of the testes were observed in male mice that received 6000 ppm at 78 weeks. Similar effects were observed at 104 weeks in male mice treated with 1500 or 6000 ppm. A significant reduction in mean relative uterus weight was observed in female mice at the highest dose tested (6000 ppm).

In a 13-week study, groups of four mature male marmosets were given daily doses of 0, 100, 500 or 2500 mg/kg bw DEHP. 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).

In a 65-week study, groups of male and female marmosets were given daily oral doses of 0, 100, 500 or 2500 mg/kg bw DEHP by gavage throughout the pre- and peri-adolescent period, from weaning (3 months of age) through to sexual maturity (18 months). No significant effect of DEHP on male organ weights was observed, and no microscopic changes were found in male gonads or secondary sex organs. The

authors evaluated sperm head counts, zinc levels, GSH levels and testicular enzyme activities. Furthermore, electron microscopic examination revealed no treatment-related abnormalities in Leydig, Sertoli or spermatogenic cells. Histochemical examination of the testis after 3β-hydroxysteroid dehydrogenase staining did not reveal any alterations in steroid synthesis in the Leydig cells. In females, increased ovarian and uterine weights and elevated blood E2 levels were observed in groups treated with 500 and 2500 mg/kg bw. In addition, the activity of several liver enzymes involved in the biosynthesis of sex hormones (CYP contents, testosterone 6β-hydroxylase and lauric acid ω-1-hydroxylase) was increased in males and/or females of either the mid- or high-dose groups, but no consistent dose-related trend was evident (Tomonari *et al.*, 2006).

Mechanistic-based reproductive toxicity

Ward *et al.* (1998) observed that the mean testis weight in DEHP-treated (12 000 ppm for up to 24 weeks) wild-type and *Pparα*-null Sv/129 mice was significantly lower than that of controls after 4 and 8 weeks of feeding. Focal tubular degenerative lesions were found in all wild-type mice fed DEHP. Spermatogenesis was also diminished and giant cells were found within the epididymis in this group by 8–16 weeks. DEHP-fed *Pparα*-null mice had primarily normal testes after 4–8 weeks except for a few tubules on the outer portion of the testis that lacked normal indicators of spermatogenesis. However, after 24 weeks, most DEHP-treated *Pparα*-null mice had severe tubular lesions. Tubules of untreated wild-type and *Pparα*-null mice were normal.

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 follicle-stimulating hormone reactivity. None of these

factors alone appears to account for the observed testicular effects (reviewed in Boekelheide, 1993).

The mode of cell death in the testicular toxicity of MEHP has been suggested to be germ-cell apoptosis (Richburg & Boekelheide, 1996; Lee *et al.*, 1997).

Gray & Butterworth (1980) and Sjöberg *et al.* (1986a) found age-dependent induction of testicular atrophy in rats; younger rats were more sensitive to DEHP than older rats. However, this difference may be related to changes in absorption, metabolism and distribution rather than to changes in tissue sensitivity (Heindel & Powell, 1992).

To determine which compound was responsible for the testicular damage after oral administration of DEHP, Sjöberg *et al.* (1986b) administered DEHP and five of its major metabolites (MEHP, 2-EH and three identified metabolites of MEHP — MEHHP, MEOHP and MECPP) for 5 days. No testicular damage was observed following oral doses of DEHP or 2-EH. The number of degenerated spermatocytes and spermatids was increased in rats receiving MEHP; no such effects were seen in animals given the MEHP-derived metabolites.

To investigate whether the anti-androgenic action of DEHP occurs through the inhibition of testosterone production or inhibition of androgenic action by binding to the AR, maternal treatment with DEHP (750 mg/kg bw per day from gestational day 14 to postnatal day 3) was investigated (Parks *et al.*, 2000). Exposure to DEHP caused a reduction in testosterone production, and reduced testicular and whole-body testosterone levels in fetal and neonatal male rats from gestational day 17 to postnatal day 2. AGD on postnatal day 2 was reduced by 36% in exposed male but not female offspring. By gestational day 20, DEHP treatment also reduced testis weight. Testes in the DEHP-treated group displayed enhanced 3β-hydroxysteroid dehydrogenase staining and increased numbers of multifocal areas of Leydig-cell hyperplasia as well as multinucleated gonocytes compared with controls at gestational day 20 and postnatal day 3. Neither DEHP nor MEHP displayed affinity for the human AR at concentrations up to 10 μM (3.9 μg/mL) *in vitro*. The authors concluded that DEHP disrupts male rat sexual differentiation by reducing testosterone in the fetus to female levels during a critical stage of reproductive tract differentiation.

Suppression of aromatase activity, an enzyme that catalyses the conversion of testosterone to E2 and plays a critical role in brain sexual differentiation, has been suggested as a mechanism for the interference of DEHP with estrogen metabolism (Andrade *et al.*, 2006b). Wistar rat dams were treated daily with DEHP (0.015, 0.045, 0.135, 0.405 or 1.215 mg/kg bw per day, low doses; and 5, 15, 45, 135 or 405 mg/kg bw per day, high doses) by gavage from gestation day 6 to lactation day 21. Aromatase activity was determined in hypothalamic/preoptic area brain sections from male and female pups on postnatal days 1 and 22. In males on postnatal day 1, aromatase activity was inhibited at low doses and increased at high doses resulting in a non-monotonic dose–response profile that resembled a J-shaped curve. Inhibition was statistically significant at 0.135 and 0.405 mg/kg bw per day, while statistically significant increased activity was observed at 15, 45 and 405 mg/kg bw per day. In contrast to findings on postnatal day 1, aromatase activity at weaning (postnatal day 22) was more strongly affected in females than in males. An increase in aromatase activity was observed at only one dose in males (0.405 mg/kg bw per day) while an increase in activity was observed at all doses in the females except for 0.045 and 5 mg/kg bw per day.

4.4 Susceptibility

4.4.1 Genetic polymorphisms

The human PPARα is indistinguishable from the rodent *Pparα* in overall structure (Sher *et al.*, 1993; Mukherjee *et al.*, 1994; Tugwood *et al.*, 1996), but several allelic variants of human PPARα have been isolated which possess properties that differ from those of the originally cloned human PPARα. The L162V variant that contains an amino acid change in the DNA-binding domain is found at an allelic frequency of ~0.025–0.073 in ethnically diverse populations (Flavell *et al.*, 2000; Lacquemant *et al.*, 2000; Tai *et al.*, 2002). In North Indians, this allele is found at high frequencies (0.745) (Sapone *et al.*, 2000). The human PPARα L162V variant exhibits no response to low doses of WY-14 643 but greater ligand-induced activity (up to ~four-fold) at higher doses compared with the wild-type receptor (Flavell *et al.*, 2000; Sapone *et al.*, 2000). Humans carrying this variant exhibit greater decreases in total serum cholesterol after treatment with the hypolipidaemic, bezafibrate (Flavell *et al.*, 2000). Three different Asian populations carry a human PPARα variant (V227A) within the hinge region between the DNA-binding and ligand-binding domains at frequencies of 0.003–0.051 (Yamakawa-Kobayashi *et al.*, 2002; Chan *et al.*, 2006). This allele has been associated with decreases in serum cholesterol and triglycerides in a Japanese population (Yamakawa-Kobayashi *et al.*, 2002) and in Chinese women (Chan *et al.*, 2006). Because of increased interactions with the nuclear receptor corepressor, this variant exhibits decreased responsiveness to PPARα activators (Liu *et al.*, 2008). The human PPARα-6/29 variant containing four amino acid substitutions is a dominant negative that binds to a PPRE but cannot be activated by PPARα activators (James *et al.*, 1998b). The human PPARα-6/29 variant is probably very rare, because it was not detected in any of the 173 human subjects investigated in two studies (Roberts, 1999; Sapone *et al.*, 2000). Overall, some *PPARα* allelic heterogeneity exists in human populations, but no variants have been identified that are more sensitive to low, environmentally-relevant doses of PPARα activators than the 'wild-type' human receptor. This topic would benefit from a contiguous comparison of wild-type and human PPARα variants in trans-activation assays to determine dose–response relationships of PPARα activators.

4.4.2 Identification of groups or subpopulations with an enhanced susceptibility to DEHP with a focus on fetal and neonatal responses

Cimini *et al.* (1994) treated F344 rat dams with 1 g/kg bw per day DEHP by gavage for up to 21 days from the day of delivery through to lactation. Pups were killed on days 14, 21 or 35 following a 14-day recovery period (of treatment withdrawal). Relative liver weights increased 1.65-fold in the dams at weaning and 1.47-fold in 14- and 21-day pups. At day 21, palmitoyl coenzyme A oxidase activity in the liver increased 9.3-fold in dams, while it increased sixfold in the nursing pups at 14 days and 4.85-fold at 21 days. However, this activity was substantially lower in the pups than in the dams treated with DEHP (pups, 1.2 mU/mg protein at 14 days; dams, 34.4 mU/mg protein at 21 days). Dihydroxyacetone phosphate acyltransferase in the liver was increased about twofold in 14- and 21-day neonates, but levels were unaffected in DEHP-treated dams. Catalase activity was increased about twofold in 14-day and 21-day neonates and adults. Following 14 days of recovery, most enzyme levels returned to normal in the dams and pups, although catalase activity remained slightly higher.

In a separate study, pregnant lactating F344 rat dams were given 1 g/kg bw per day DEHP by gavage for 21 days beginning on the day of delivery until weaning, and the pups nursed by treated dams were killed after 2–3 weeks or

following a 14-day recovery period (Stefanini *et al.*, 1995). The numerical density or volume density of peroxisomes was increased marginally (less than twofold) relative to controls in both pup groups. Dams treated for 21 days with DEHP showed a more pronounced increase in the volume density of peroxisomes (about twofold), but the numerical density of peroxisomes was increased in the dams to the same degree as that in the 2–3-week-old pups. The increases in volume density or numerical density of peroxisomes did not decline to control levels in the 3-week-old pups after a 14-day recovery period. Volume density of peroxisomes apparently declined to near control levels after a recovery period of 8 days in dams treated for 3 weeks, but there was no apparent decline in the numerical density of peroxisomes. Relative liver weights were increased about equally in 2–3-week-old pups and dams (1.5–1.6-fold).

A study designed to investigate the effects of a PPARα agonist on neonatal rats of different ages was conducted by Dostal *et al.* (1987). Male Sprague-Dawley rats that were 6, 14, 16, 21, 42 or 86 days of age were given daily doses of 0, 10, 100, 1000, or 2000 mg/kg bw DEHP by gavage for 5 days; 24 hours after they were killed, the activities of hepatic peroxisomal enzymes, palmitoyl coenzyme A oxidase and CAT were determined. Administration of 1000 mg/kg bw per day caused significant decreases in body weight and caused mortality (66–70%) in pups 14–18 days of age, and administration of 2000 mg/kg bw per day caused mortality in virtually all pups of these ages. At a non-lethal dose level of 100 mg/kg bw per day, increases in absolute liver weight relative to the controls were 0, 17, 3, 10 and 14% for 6–10-, 14–18-, 21–25-, 42–46- and 86–89-day old pups and adults, respectively. At this dose level, measurements of palmitoyl coenzyme A activity showed that there was a greater increase only in the 14–18-day-old pups compared with 86–90-day-old adults (6.9-fold versus 3.98-fold). A greater increase in CAT also was shown at this dose level only for 14–18-day-old pups compared with 86–90-day-old adults (7.8-fold versus 4.4-fold). The data on increased liver weights and peroxisomal enzyme activities from this study indicated that neonatal or young adult rats do not differ greatly from adult rats in their response to treatment with DEHP, although the palmitoyl coenzyme A and CAT activities were higher in the 14–18-day-old pups than in adults.

4.5 Mechanistic considerations

4.5.1 Effects on the liver

The effects of DEHP on the liver — a primary target organ for the pleiotropic effects of DEHP and other peroxisome proliferators in rodents — have been the focus of scientific debate for the last three decades. In the liver of rodents, parenchymal cells (also called hepatocytes) are the major cell type that is responsive to DEHP; however, other cells, such as resident hepatic macrophages (called Kupffer cells) may also play an important role. The increase in the number and size of peroxisomes in hepatocytes — peroxisome proliferation that results in elevation of fatty acid metabolism — is a hallmark response to DEHP and other peroxisome proliferators in the liver of susceptible species. A link between peroxisome proliferation and liver tumour response has been a predominant theory to explain the cause of a hepatocarcinogenic effect, although the experimental data are not unequivocal. Other molecular events, such as the induction of cell proliferation, decreased apoptosis, oxidative DNA damage and selective clonal expansion of the initiated cells, have also been proposed to be critically involved.

Overall, it is believed that the events that occur relative to DEHP-induced liver carcinogenesis in rodents involve the following, whereby the combination of the molecular signals and multiple pathways rather than a single hallmark event (such as activation of PPARα, peroxisome

proliferation or cell proliferation) contribute to the formation of tumours: (1) rapid metabolism of the parent compound to primary and secondary bioactive metabolites that are readily absorbed and distributed throughout the body; (2) receptor-independent activation of hepatic macrophages and production of oxidants; (3) activation of PPARα in hepatocytes and sustained increases in the expression of peroxisomal and non-peroxisomal metabolism-related genes; (4) enlargement of many hepatocellular organelles (e.g. peroxisomes, mitochondria); (5) a rapid but transient increase in cell proliferation and a decrease in apoptosis; (6) sustained hepatomegaly; (7) chronic low-level oxidative stress and accumulation of DNA damage; (8) selective clonal expansion of initiated cells; (9) appearance of preneoplastic nodules; and (10) development of adenomas and carcinomas.

Despite the wide use of phthalates, including DEHP, which leads to appreciable exposure of the general population, only limited data are available for consideration of the possible adverse health effects of DEHP in human populations. The majority of experimental human data comes from in-vitro studies in cultured human liver cells; however, results of these studies suggest that human cells do not respond to DEHP or its metabolites in manner that parallels responses observed in cultured rodent liver cells. At the same time, even rodent liver cells do not replicate many of the events observed in rodent livers *in vivo*.

Major differences in the metabolism and molecular signalling events elicited by DEHP in the liver, such as the activation of PPARα, have been observed between species. A previous evaluation (IARC, 2000) considered DEHP and concluded that, in rodents, peroxisome proliferators exercise their pleiotropic effects in the liver due to the activation of PPARα and that this process is essential for liver hypertrophy and hyperplasia and eventual hepatocarcinogenesis. This conclusion was based on data from a variety of studies that considered the molecular biology of PPARα signalling in different species, transactivation potency of PPARα from different species, in-vivo studies in non-human primates and studies in genetically modified mice.

One of the key pieces of evidence reviewed previously (IARC, 2000) was the chronic feeding study with the peroxisome proliferator WY-14 643 in wild-type and *Ppara*-null mice that showed that the null mice were completely refractory to liver carcinogenesis. Although this study made one of the most significant contributions to mechanistic research on peroxisome proliferators, the large dose of the agent used, a duration of exposure of less than 2 years and the relatively small numbers of animals evaluated somewhat limit the utility of these data. Several additional studies that used the same and other genetically engineered mice have been completed during the past 4 years, and these provide important additional data key for consideration of the relevance of the PPARα mode of action to rodent and human liver carcinogenesis. These include, but are not limited to, studies in *Ppara*-null mice, *PPARα* humanized transgenic mice and hepatocyte-specific constitutively activated *Ppara* transgenic mice (Yang *et al.*, 2007). The data from these animal models suggest that, although the activation of PPARα and the subsequent downstream events mediated by this transcription factor represent one key mechanism of action, it is evident that several additional molecular signals and multiple pathways in several cell types in the liver, rather than a single molecular event, contribute to the formation of liver tumours in rats and mice.

Furthermore, it should be noted that although important species differences in the activation of PPARα or its signalling network by peroxisome proliferators exist, human cells express PPARα and are not devoid of transactivation responses to many peroxisome proliferators, including MEHP. Important interindividual differences in PPARα expression have been reported, suggesting

that the differences in expression between species may need to be verified using larger samples of both humans and animal strains. Thus, although quantitative differences between species may well exist, qualitative similarities cannot be ignored, especially because DEHP and other PPARα activators are known to induce molecular responses independent of PPARα activation. It remains a possibility that these pathways contribute to human risk in ways that differ somewhat from those postulated for liver cancer in rats and mice.

Overall, the mechanisms for the induction of cancer by DEHP have not been established entirely and are certainly complex. In the following section, some of the diverse effects that could contribute are summarized. Although it is becoming more evident that the complex molecular events that lead to cancer may not always lend themselves to unequivocal assignment to one of the stages of carcinogenesis (i.e. initiation, promotion or progression), this established paradigm provides a useful framework for a discussion of most of the mechanistic data on DEHP that are relevant to the liver.

(a) Tumour initiation

(i) Genotoxic and related events elicited by DEHP or its metabolites

DEHP and its metabolites generally give negative results in bacterial mutagenicity assays in the presence or absence of metabolic activation. However, several recent studies in human and other mammalian primary cells or established cell lines showed that in-vitro exposures to DEHP or its primary metabolite, MEHP, produced DNA strand breaks detectable in the Comet assay or induced cell transformation. One mutation study in transgenic mice has shown evidence for the in-vivo genotoxicity of DEHP, while another gave negative results. In one study, DEHP did not exhibit initiation activity. It is not clear whether these effects that indicate genotoxicity are a result of a direct reaction of DEHP or its metabolites with DNA or could be due to secondary oxidative stress or other events.

(ii) Oxidative stress

It is widely accepted that in-vivo exposure of rats or mice to DEHP leads to increased oxidative stress in the liver. The induction of peroxisomal and microsomal enzymes, a pathway largely dependent on the activation of PPARα, contributes to an increase in reactive oxygen species formed in the hepatocytes. Oxidative DNA damage, generation of lipid peroxidation products that can form DNA adducts and the induction of base-excision DNA repair genes (presumably in response to increased oxidative DNA damage) in the liver has been observed in both rats and mice. An alternative PPARα-independent mechanism for increased oxidative stress has been shown to involve the activation of resident liver macrophages (Kupffer cells) which have been shown to be activated by MEHP *in vitro* to generate oxidants. Also, several studies *in vivo* in both rats and mice have shown that Kupffer cells produce oxidants that, in turn, may damage DNA. There may be important differences in the cellular source of oxidants in the liver that are dependent on the duration of exposure to DEHP. Although the peroxisomal- and microsomal-derived oxidants may be generated continuously throughout treatment, the role of Kupffer cells is best established after only short-term or acute exposures to DEHP. Although the peroxisome proliferator-induced activation of Kupffer cells has been shown to generate oxidants in rodent cells, no data are available to determine whether this PPARα-independent mechanisms is also operative in human cells.

(iii) Effects on toxicokinetics of other carcinogens

DEHP has a major effect on liver metabolism. Although most of the effects pertain to lipid biotransformation, several studies have shown that drug metabolism enzymes other than the CYP4A family are also affected by exposure to

DEHP. These effects occur in both rodents and humans, and PPARα-independent effects on metabolism genes have been are observed.

(b) Tumour promotion

(i) Cell proliferation

The rate of hepatocellular proliferation is markedly increased even after administration of a single dose of peroxisome proliferators, including DEHP, to rats or mice. There is clear evidence that DEHP causes an acute increase in hepatocellular proliferation in both rats and mice. The elevation in cell replication rate in the liver has been implicated in the mechanism of carcinogenesis, because it may result in higher levels of mutation by increasing the frequency of replicative DNA synthesis as well as the number of hepatocytes at risk.

A cell-proliferation response has not been observed in non-human primates or in some rodent species exposed to DEHP, but no studies in humans have addressed this potential mechanism. The acute cell-proliferation response in the liver of rats and mice *in vivo* is a whole-liver phenomenon, and has not been observed in rodent hepatocytes *in vitro* even when the cells were properly purified, suggesting that factors produced by other cell types in the whole liver are also a prerequisite. Although there appears to be a relationship between the activation of PPARα in rats and mice and an increase in cell proliferation, the molecular mechanisms of this association are not well understood.

Cell-cycle genes do not appear to be under the transcriptional control of PPARα. Rather, several indirect mechanisms have been proposed, including the involvement of microRNAs, the activation of p38 mitogen-activated kinase or the activation of *Ras*-like proto-oncogenes via post-translational modification. None of these mechanisms has been investigated with regard to DEHP in susceptible species, and their relevance to human hazard has yet to be elucidated.

In addition, one study showed that, although constitutive activation of PPARα in mouse liver (through genetic means and without chemical treatment) leads to increased cell proliferation, it does not lead to hepatocarcinogenesis.

Numerous studies that used an initiation–promotion protocol — with NDEA or another agent as an initiator and DEHP or phenobarbital as a promoter — showed that DEHP can promote genotoxic carcinogenesis; however, it is not clear whether this effect is due to an effect of DEHP on carcinogen metabolism, the induction of cell proliferation or other events. In addition, because the increase in cell proliferation in rat and mouse liver caused by DEHP is an acute phenomenon, its relevance to DEHP-induced liver carcinogenesis needs further study.

(ii) Suppression of apoptosis

Suppression of apoptosis in the liver has been suggested as an additional mechanism through which the normal balance of cell turnover may be impaired to create conditions that promote neoplastic growth. *In vivo*, little evidence exists to support this hypothesis, even in rats and mice. In cultured hepatocytes, several peroxisome proliferators, including DEHP, have been shown to inhibit pro-apoptotic signalling events. This has not been observed in cultured human hepatocytes, but the database on human studies is limited to a single report. The potential role for this mechanism is further complicated by the observation of an increase in apoptotic signalling after continuous exposure to WY-14 643.

(iii) Activation of nuclear receptors other than PPARα

Recent studies showed that DEHP and other peroxisome proliferators can induce several drug-metabolizing genes that are not known PPARα targets. In human primary hepatocytes and cancer cell lines, some studies have shown that DEHP can increase activity and/or expression of the CYP3A, CYP2B and CYP1A family of

enzymes. In mice, comparative analysis of gene expression profiles in the liver of DEHP-treated wild type animals with those in DEHP-treated *Pparα*-null animals showed that, although PPARα-dependent events are a dominant transcriptional response, the induction of other genes, many of which are homologous to those affected in human cells, was also observed. There is evidence in both mice and humans that other nuclear receptors are targets for DEHP including CAR and PXR.

4.5.2 Effects on testes

Many rodent studies have observed that chronic administration of DEHP leads to testicular toxicity. Various pre- and postnatal study designs showed that DEHP is a reproductive and developmental toxicant in rats and mice. It has also been demonstrated that the testicular toxicity of DEHP is a PPARα-independent phenomenon, because identical, although slightly delayed, effects were observed in *Pparα*-null mice. Two bioassays (103–104 weeks in duration) in F344 rats and B6C3F$_1$ mice did not show evidence of testicular tumours although consistent observations of testicular toxicity were reported. Lifelong (up to 159 weeks) exposure to DEHP in a different strain of rats (Sprague-Dawley) showed a significant increase in testicular (as well as liver) tumours, and it was noted that latency for the testicular tumours was even shorter than that for liver tumours. In humans, the database of the reproductive and developmental effects of phthalates has been growing rapidly over the past decade, and numerous studies reported data suggestive of an association between exposure to DEHP and/or other phthalates and adverse effects on both male and female reproduction.

4.5.3 Pancreatic tumours

One chronic bioassay found that DEHP can induce acinar-cell adenoma of the exocrine pancreas of F344 rats. No mechanistic or other follow-up studies are available to determine whether evidence in animals may be relevant to humans.

5. Summary of Data Reported

5.1 Exposure data

Di(2-ethylhexyl) phthalate has been produced since the 1930s by the reaction of 2-ethylhexanol with phthalic anhydride. It is widely used as a plasticizer to soften polyvinyl chloride plastics (used in medical devices, tubing, footwear, food packaging, wire and cable coverings and toys) and, to a lesser extent, non-polymers (used in dielectric fluids, paints, adhesives and inks). Occupational exposure to di(2-ethylhexyl) phthalate generally occurs by inhalation during its manufacture and use as plasticizer of polyvinyl chloride. Exposure of the general population to di(2-ethylhexyl) phthalate occurs from the use of medical devices, such as blood bags and medical tubing, its presence as a contaminant of food and, to a lesser extent, its presence in the environment (air, water and soil).

Human intake of di(2-ethylhexyl) phthalate can be estimated by measurement of di(2-ethylhexyl) phthalate and its total metabolites in blood and urine; particularly high concentrations of urinary metabolites were reported in neonates in intensive care.

5.2 Human carcinogenicity data

The only analytical epidemiological study that measured exposure to di(2-ethylhexyl) phthalate specifically was a case–control study of female breast cancer and exposure to phthalates, including di(2-ethylhexyl) phthalate. Phthalate

metabolites were measured in the urine and an increased risk for breast cancer was identified for one of the four di(2-ethylhexyl) phthalate metabolites evaluated (mono(2-ethyl-5-carboxypentyl) phthalate). No association was found for the other three di(2-ethylhexyl) phthalate metabolites: mono(2-ethylhexyl) phthalate, mono(2-ethyl-5-hydroxyhexyl) phthalate and mono(2-ethyl-5-oxohexyl) phthalate.

One cohort study and one nested case–control study assessed pancreatic cancer in workers potentially exposed to di(2-ethylhexyl) phthalate. In the nested–case–control study, a large excess of pancreatic cancer was observed among men who had worked for more than 16 years in vinyl and polyethylene production areas, where di(2-ethylhexyl) phthalate was used. However, this excess was based on five exposed cases only. One small study of workers in a di(2-ethylhexyl) phthalate production plant did not show any excess mortality from cancer. However, this cohort study did not have adequate power to detect a potential excess risk. Only eight deaths from any cause occurred and the one observed cancer death was from pancreatic cancer.

Two of three case–control studies of testicular cancer reported a statistically significant association with occupational exposure to polyvinyl chloride; although there was no positive exposure–response relationship in one study. The third study was limited by the small number of men exposed to polyvinyl chloride for more than 1 year. These workers were potentially exposed to phthalate plasticizers, including the most common (i.e. di(2-ethylhexyl) phthalate), but none of these studies evaluated exposure to di(2-ethylhexyl) phthalate specifically.

In a cohort study of polyvinyl chloride processing workers, moderate and high cumulative exposure to plasticizers was associated with an increased risk for respiratory cancer but no statistically significant positive exposure–response was observed. Di(2-ethylhexyl) phthalate was the main plasticizer used in this study.

In a population-based case–control study, the risk for multiple myeloma increased with increasing duration of probable occupational exposure to phthalates.

5.3 Animal carcinogenicity data

Di(2-ethylhexyl) phthalate was tested for carcinogenicity by oral administration in the diet in male and female mice in two studies, in male and female rats in two studies, and in one study in male rats. The incidence of hepatocellular adenoma and hepatocellular carcinoma was consistently increased in both species. In one study in rats, a significant increase in the incidence of pancreatic acinar-cell adenoma was observed in males. In another study in rats, the incidence of benign Leydig-cell tumours was increased, and was dose-related with early onset.

In two initiation–promotion studies in mice, exposure to di(2-ethylhexyl) phthalate following administration of N-nitrosodiethylamine enhanced the incidence of hepatocellular adenoma or hepatocellular carcinoma. In several initiation–promotion studies in rats and in two such studies in hamsters, in general, no promoting activity of di(2-ethylhexyl) phthalate was demonstrated but, in one initiation–promotion study in rats with N-ethyl-N-hydroxyethylnitrosamine, the incidence and multiplicity of renal tubule adenoma or carcinoma (combined) were increased by di(2-ethylhexyl) phthalate.

Tumours of the exocrine pancreas are rare spontaneous neoplasms in experimental animals.

5.4 Other relevant data

In humans and rodents, di(2-ethylhexyl) phthalate is metabolized by lipases, ubiquitous enzymes in various tissues, to mono(2-ethylhexyl) phthalate, which is then oxidatively metabolized to several compounds that are subsequently excreted in the urine. Pancreatic lipase plays a major role, especially after oral exposure. Species

differences in lipase activity between tissues have been identified and may play a role in species differences in the effects of di(2-ethylhexyl) phthalate. In humans, urinary metabolites include mono(2-ethyl-5-hydroxyhexyl) phthalate, mono(2-ethyl-5-oxohexyl) phthalate, mono(2-ethylhexyl) phthalate, mono(2-ethyl-5-carboxypentyl) phthalate and mono(2-carboxymethyl)hexyl phthalate. The major metabolites excreted in the urine are mono(2-ethyl-5-oxohexyl) phthalate, mono(2-ethyl-5-carboxypentyl) phthalate and mono(2-ethyl-5-oxohexyl) phthalate. Due to the complexity of secondary metabolites formed, no single metabolite has been identified as a good biomarker for exposure to di(2-ethylhexyl) phthalate. The forms of cytochrome P450 or other enzymes responsible for oxidative metabolism are not well understood.

Studies of the absorption, distribution, excretion and metabolism of di(2-ethylhexyl) phthalate are hampered by its ubiquitous presence in the environment and laboratory equipment, and that it can be hydrolysed under abiotic conditions. Studies of the radiolabelled compound provide the most accurate indications of the absorption and distribution of di(2-ethylhexyl) phthalate and its metabolites. Variation in urinary metabolites of di(2-ethylhexyl) phthalate in humans is large and may reflect differences in exposure, as well as its absorption, distribution, excretion and metabolism between subjects.

Di(2-ethylhexyl) phthalate and its metabolites have been extensively tested in bacterial mutagenicity assays in the presence or absence of metabolic activation. The results of these studies have been generally negative. Studies in human and other mammalian primary cells or established cell lines provide evidence that in-vitro exposure to di(2-ethylhexyl) phthalate or its primary metabolite, mono(2-ethylhexyl) phthalate, may result in DNA strand breaks or induce cell transformation. It is not clear whether these effects are a result of the direct reaction of di(2-ethylhexyl) phthalate or its metabolites with DNA or could be due to secondary oxidative stress or other events. Studies of in-vivo mutagenicity in two different transgenic mouse models have been conducted, but the results are conflicting, which confounds the interpretation of these findings.

The molecular events associated with the reproductive and developmental effects of di(2-ethylhexyl) phthalate and other phthalates are not well characterized, but many studies suggest that the effects of phthalates on metabolism and other cellular functions lead to disruption of steroidogenesis, increased oxidative stress, increased apoptosis and other events. Data also suggest that both Sertoli and Leydig cells are targets for the toxicity of di(2-ethylhexyl) phthalate.

Since the previous evaluation, important additional mechanistic information has become available, including, but not limited to, subacute, subchronic and chronic studies with di(2-ethylhexyl) phthalate in peroxisome proliferator-activated receptor α-null mice, as well as findings from several transgenic (peroxisome proliferator-activated receptor α-humanized and hepatocyte-specific constitutively activated peroxisome proliferator-activated receptor α mouse lines. Activation of peroxisome proliferator-activated receptor α and the subsequent downstream events mediated by this transcription factor represent an important mechanism of action for di(2-ethylhexyl) phthalate in rats and mice. However, additional data from animal models and studies in humans exposed to di(2-ethylhexyl) phthalate from the environment suggest that multiple molecular signals and pathways in several cell types in the liver, rather than a single molecular event, contribute to the induction of cancer in rats and mice. Thus, the relevance to human cancer of the molecular events that lead to cancer elicited by di(2-ethylhexyl) phthalate in several target tissues (e.g. the liver and testis) in rats and mice cannot be ruled out.

6. Evaluation

6.1 Cancer in humans

No data were available to the Working Group.

6.2 Cancer in experimental animals

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

6.3 Overall evaluation

Di(2-ethylhexyl) phthalate is *possibly carcinogenic to humans (Group 2B).*

References

Abad E, Martínez K, Planas C *et al.* (2005). Priority organic pollutant assessment of sludges for agricultural purposes. *Chemosphere*, 61: 1358–1369. doi:10.1016/j.chemosphere.2005.03.018 PMID:16291406

Abe S & Sasaki M (1977). Chromosome aberrations and sister chromatid exchanges in Chinese hamster cells exposed to various chemicals. *J Natl Cancer Inst*, 58: 1635–1641. PMID:864744

ACGIH (2010). *2010 TLVs and BEIs* [CD-ROM]. Cincinnati, OH: American Conference of Governmental Industrial Hygienists.

Adeniyi A, Dayomi M, Siebe P, Okedeyi O (2008). An assessment of the levels of phthalate esters and metals in the Muledane open dump, Thohoyandou, Limpopo Province, South Africa. *Chem Cent J*, 2: 9 doi:10.1186/1752-153X-2-9 PMID:18474112

Adeniyi AA, Okedeyi OO, Yusuf KA (2011). Flame ionization gas chromatographic determination of phthalate esters in water, surface sediments and fish species in the Ogun river catchments, Ketu, Lagos, Nigeria. *Environ Monit Assess*, 172: 561–569. doi:10.1007/s10661-010-1354-2 PMID:20221801

Adibi JJ, Perera FP, Jedrychowski W *et al.* (2003). Prenatal exposures to phthalates among women in New York City and Krakow, Poland. *Environ Health Perspect*, 111: 1719–1722. doi:10.1289/ehp.6235 PMID:14594621

Adibi JJ, Whyatt RM, Williams PL *et al.* (2008). Characterization of phthalate exposure among pregnant women assessed by repeat air and urine samples. *Environ Health Perspect*, 116: 467–473. PMID:18414628

Afshari A, Gunnarsen L, Clausen PA, Hansen V (2004). Emission of phthalates from PVC and other materials. *Indoor Air*, 14: 120–128. doi:10.1046/j.1600-0668.2003.00220.x PMID:15009418

Agarwal DK, Eustis S, Lamb JC 4th *et al.* (1986). Effects of di(2-ethylhexyl) phthalate on the gonadal pathophysiology, sperm morphology, and reproductive performance of male rats. *Environ Health Perspect*, 65: 343–350. doi:10.2307/3430202 PMID:3709461

Agarwal DK, Lawrence WH, Autian J (1985). Antifertility and mutagenic effects in mice from parenteral administration of di-2-ethylhexyl phthalate (DEHP). *J Toxicol Environ Health*, 16: 71–84. doi:10.1080/15287398509530720 PMID:4068057

Agarwal DK, Lawrence WH, Turner JE, Autian J (1989). Effects of parenteral di-(2-ethylhexyl)phthalate (DEHP) on gonadal biochemistry, pathology, and reproductive performance of mice. *J Toxicol Environ Health*, 26: 39–59. doi:10.1080/15287398909531232 PMID:2913333

Akingbemi BT, Ge R, Klinefelter GR *et al.* (2004). Phthalate-induced Leydig cell hyperplasia is associated with multiple endocrine disturbances. *Proc Natl Acad Sci USA*, 101: 775–780. doi:10.1073/pnas.0305977101 PMID:14715905

Albro PW (1986). Absorption, metabolism, and excretion of di(2-ethylhexyl) phthalate by rats and mice. *Environ Health Perspect*, 65: 293–298. doi:10.2307/3430196 PMID:3086077

Albro PW, Corbett JT, Schroeder JL *et al.* (1982). Pharmacokinetics, interactions with macromolecules and species differences in metabolism of DEHP. *Environ Health Perspect*, 45: 19–25. doi:10.2307/3429379 PMID:7140694

Albro PW & Lavenhar SR (1989). Metabolism of di(2-ethylhexyl)phthalate. *Drug Metab Rev*, 21: 13–34. doi:10.3109/03602538909029953 PMID:2696633

Albro PW & Thomas RO (1973). Enzymatic hydrolysis of di-(2-ethylhexyl) phthalate by lipases. *Biochim Biophys Acta*, 306: 380–390. PMID:4726865

Albro PW, Tondeur I, Marbury D *et al.* (1983). Polar metabolites of di-(2-ethylhexyl)phthalate in the rat. *Biochim Biophys Acta*, 760: 283–292. PMID:6626575

Amacher DE, Turner GN (1985). *Tests for gene mutational activity in the L5178Y/TK assay system.* In: *Evaluation of Short-term Tests for Carcinogens: Report of the International Programme on Chemical Safety's Collaborative Study on In Vitro Assays.* Ashby J, de Serres FJ, Draper M *et al.*, editors. Amsterdam: Elsevier, pp. 487–496.

Anderson D, Yu TW, Hinçal F (1999a). Effect of some phthalate esters in human cells in the comet assay. *Teratog Carcinog Mutagen*, 19: 275–280. doi:10.1002/(SICI)1520-6866(1999)19:4<275::AID-TCM4>3.0.CO;2-1 PMID:10406891

Anderson SP, Cattley RC, Corton JC (1999b). Hepatic expression of acute-phase protein genes during carcinogenesis induced by peroxisome proliferators. *Mol Carcinog*, 26: 226–238. doi:10.1002/(SICI)1098-2744(199912)26:4<226::AID-MC2>3.0.CO;2-Q PMID:10569800

Anderson SP, Dunn C, Laughter A *et al.* (2004a). Overlapping transcriptional programs regulated by the nuclear receptors peroxisome proliferator-activated receptor alpha, retinoid X receptor, and liver X receptor in mouse liver. *Mol Pharmacol*, 66: 1440–1452. doi:10.1124/mol.104.005496 PMID:15371561

Andrade AJ, Grande SW, Talsness CE *et al.* (2006a). A dose-response study following in utero and lactational exposure to di-(2-ethylhexyl) phthalate (DEHP): effects on androgenic status, developmental landmarks and testicular histology in male offspring rats. *Toxicology*, 225: 64–74. doi:10.1016/j.tox.2006.05.007 PMID:16806631

Andrade AJ, Grande SW, Talsness CE *et al.* (2006b). A dose-response study following in utero and lactational exposure to di-(2-ethylhexyl)-phthalate (DEHP): non-monotonic dose-response and low dose effects on rat brain aromatase activity. *Toxicology*, 227: 185–192. doi:10.1016/j.tox.2006.07.022 PMID:16949715

Anon. (1996). Facts and figures for the chemical industry. *Chem Eng News*, 74: 38–79.

Aparicio I, Santos JL, Alonso E (2009). Limitation of the concentration of organic pollutants in sewage sludge for agricultural purposes: A case study in South Spain. *Waste Manag*, 29: 1747–1753. doi:10.1016/j.wasman.2008.11.003 PMID:19135349

Arcadi FA, Costa C, Imperatore C *et al.* (1998). Oral toxicity of bis(2-ethylhexyl) phthalate during pregnancy and suckling in the Long-Evans rat. *Food Chem Toxicol*, 36: 963–970. doi:10.1016/S0278-6915(98)00065-9 PMID:9771559

Arni P (1985). *Induction of various genetic effects in the yeast Saccharomyces cerevisiae strain D7*. In: *Evaluation of Short-term Tests for Carcinogens: Report of the International Programme on Chemical Safety's Collaborative Study on In Vitro Assays*. Ashby J, de Serres FJ, Draper M *et al.*, editors. Amsterdam: Elsevier, pp. 217–224.

Asaoka K, Hagihara K, Kabaya H *et al.* (2000). Uptake of phthalate esters, di(n-butyl)phthalate and di(2-ethylhexyl)phthalate, as environmental chemicals in monkeys in Japan. *Bull Environ Contam Toxicol*, 64: 679–685. doi:10.1007/s001280000057 PMID:10857451

Astill B, Barber E, Lington A *et al.* (1986). Chemical industry voluntary test program for phthalate esters: health effects studies. *Environ Health Perspect*, 65: 329–336. doi:10.2307/3430200 PMID:3709458

Atlas E & Giam CS (1981). Global transport of organic pollutants: ambient concentrations in the remote marine atmosphere. *Science*, 211: 163–165. doi:10.1126/science.211.4478.163 PMID:17757266

ATSDR (2002). *Toxicological Profile: Di(2-ethylhexyl) Phthalate*. Atlanta, GA: Agency for Toxic Substances and Disease Registry.

Autian J (1982). Antifertility effects and dominant lethal assays for mutagenic effects of DEHP. *Environ Health Perspect*, 45: 115–118. doi:10.2307/3429393 PMID:7140683

Baker RS, Bonin AM (1985). *Tests with the Salmonella plate-incorporation assay*. In: *Evaluation of Short-term Tests for Carcinogens: Report of the International Programme on Chemical Safety's Collaborative Study on In Vitro Assays*. Ashby J, de Serres FJ, Draper M *et al.*, editors. Amsterdam: Elsevier, pp. 177–180.

Baldwin WS & Roling JA (2009). A concentration addition model for the activation of the constitutive androstane receptor by xenobiotic mixtures. *Toxicol Sci*, 107: 93–105. doi:10.1093/toxsci/kfn206 PMID:18832183

Barber ED, Astill BD, Moran EJ *et al.* (1987). Peroxisome induction studies on seven phthalate esters. *Toxicol Ind Health*, 3: 7–24. PMID:3617071

Barr DB, Silva MJ, Kato K *et al.* (2003). Assessing human exposure to phthalates using monoesters and their oxidized metabolites as biomarkers. *Environ Health Perspect*, 111: 1148–1151. doi:10.1289/ehp.6074 PMID:12842765

Barrett JC & Lamb PW (1985). Tests with the Syrian hamster embryo cell transformation assay. *Prog Mutat Res*, 5: 623–628.

Barry YA, Labow RS, Keon WJ *et al.* (1989). Perioperative exposure to plasticizers in patients undergoing cardiopulmonary bypass. *J Thorac Cardiovasc Surg*, 97: 900–905. PMID:2657224

Bauer MJ & Herrmann R (1997). Estimation of the environmental contamination by phthalic acid esters leaching from household wastes. *Sci Total Environ*, 208: 49–57. doi:10.1016/S0048-9697(97)00272-6 PMID:9496648

Beauchesne I, Barnabé S, Cooper DG, Nicell JA (2008). Plasticizers and related toxic degradation products in wastewater sludges. *Water Sci Technol*, 57: 367–374. doi:10.2166/wst.2008.001 PMID:18309214

Becker K, Seiwert M, Angerer J *et al.* (2004). DEHP metabolites in urine of children and DEHP in house dust. *Int J Hyg Environ Health*, 207: 409–417. doi:10.1078/1438-4639-00309 PMID:15575555

Berset JD & Etter-Holzer R (2001). Determination of phthalates in crude extracts of sewage sludges by high-resolution capillary gas chromatography with mass spectrometric detection. *J AOAC Int*, 84: 383–391. PMID:11324602

Bility MT, Thompson JT, McKee RH *et al.* (2004). Activation of mouse and human peroxisome proliferator-activated receptors (PPARs) by phthalate monoesters. *Toxicol Sci*, 82: 170–182. doi:10.1093/toxsci/kfh253 PMID:15310864

Biscardi D, Monarca S, De Fusco R *et al.* (2003). Evaluation of the migration of mutagens/carcinogens from PET bottles into mineral water by Tradescantia/micronuclei test, Comet assay on leukocytes and GC/MS. *Sci Total Environ*, 302: 101–108. doi:10.1016/S0048-9697(02)00349-2 PMID:12526902

Björklund K, Cousins AP, Strömvall A-M, Malmqvist P-A (2009). Phthalates and nonylphenols in urban runoff: occurrence, distribution and area emission factors. *Sci Total Environ*, 407: 4665–4672. doi:10.1016/j.scitotenv.2009.04.040 PMID:19457546

Boekelheide K (1993). *Sertoli cell toxicants. In: The Sertoli Cell.* Clearwater, FL: Cache River Press

Boerrigter ME (2004). Mutagenicity of the peroxisome proliferators clofibrate, Wyeth 14,643 and di-2-ethylhexyl phthalate in the lacZ plasmid-based transgenic mouse mutation assay. *J Carcinog*, 3: 7 doi:10.1186/1477-3163-3-7 PMID:15128457

Bove JL, Dalven P, Kukreja VP (1978). Airborne di-butyl and di(2-ethylhexyl)phthalate at three New York City air sampling stations *Int J environ Chem*, 5: 189–194. doi:10.1080/03067317808071144

Bradley MO (1985). *Measurement of DNA single-strand breaks by alkaline elution in rat hepatocytes.* In: *Evaluation of Short-term Tests for Carcinogens: Report of the International Programme on Chemical Safety's Collaborative Study on In Vitro Assays.* Ashby J, de Serres FJ, Draper M *et al.*, editors. Amsterdam: Elsevier, pp. 353–357.

Brändli RC, Kupper T, Bucheli TD *et al.* (2007). Organic pollutants in compost and digestate. Part 2. Polychlorinated dibenzo-p-dioxins, and -furans, dioxin-like polychlorinated biphenyls, brominated flame retardants, perfluorinated alkyl substances, pesticides, and other compounds. *J Environ Monit*, 9: 465–472. doi:10.1039/b617103f PMID:17492092

Brooks TM, Gonzalez LP, Calvert R *et al.* (1985). *The induction of mitotic gene conversion in the yeast Saccharomyces cerevisiae strain JD1.* In: *Evaluation of Short-term Tests for Carcinogens: Report of the International Programme on Chemical Safety's Collaborative Study on In Vitro Assays.* Ashby J, de Serres FJ, Draper M *et al.*, editors. Amsterdam: Elsevier, pp. 225–228.

Buchta C, Bittner C, Heinzl H *et al.* (2005). Transfusion-related exposure to the plasticizer di(2-ethylhexyl)phthalate in patients receiving plateletpheresis concentrates. *Transfusion*, 45: 798–802. doi:10.1111/j.1537-2995.2005.04380.x PMID:15847671

Buchta C, Bittner C, Höcker P *et al.* (2003). Donor exposure to the plasticizer di(2-ethylhexyl)phthalate during plateletpheresis. *Transfusion*, 43: 1115–1120. doi:10.1046/j.1537-2995.2003.00479.x PMID:12869118

Butala JH, David RM, Gans G *et al.* (2004). Phthalate treatment does not influence levels of IgE or Th2 cytokines in B6C3F$_1$ mice. *Toxicology*, 201: 77–85. doi:10.1016/j.tox.2004.04.004 PMID:15297022

Butterworth BE, Bermudez E, Smith-Oliver T *et al.* (1984). Lack of genotoxic activity of di(2-ethylhexyl)phthalate (DEHP) in rat and human hepatocytes. *Carcinogenesis*, 5: 1329–1335. doi:10.1093/carcin/5.10.1329 PMID:6488454

Cai QY, Mo CH, Wu QT *et al.* (2007). Occurrence of organic contaminants in sewage sludges from eleven wastewater treatment plants, China. *Chemosphere*, 68: 1751–1762. doi:10.1016/j.chemosphere.2007.03.041 PMID:17509650

Calafat AM, Brock JW, Silva MJ *et al.* (2006). Urinary and amniotic fluid levels of phthalate monoesters in rats after the oral administration of di(2-ethylhexyl) phthalate and di-n-butyl phthalate. *Toxicology*, 217: 22–30. doi:10.1016/j.tox.2005.08.013 PMID:16171919

Calafat AM, Needham LL, Silva MJ, Lambert G (2004). Exposure to di-(2-ethylhexyl) phthalate among premature neonates in a neonatal intensive care unit. *Pediatrics*, 113: e429–e434. doi:10.1542/peds.113.5.e429 PMID:15121985

Calley D, Autian J, Guess WL (1966). Toxicology of a series of phthalate esters. *J Pharm Sci*, 55: 158–162. doi:10.1002/jps.2600550206 PMID:5923262

Cammack JN, White RD, Gordon D *et al.* (2003). Evaluation of reproductive development following intravenous and oral exposure to DEHP in male neonatal rats. *Int J Toxicol*, 22: 159–174. doi:10.1080/10915810305098 PMID:12851149

Carere A, Conti G, Conti L *et al.* (1985). *Assays in Aspergillus nidulans for the induction of forward-mutation in haploid strain 35 and for mitotic nondisjunction, haploidization and crossing-over in diploid strain P1.* In: *Evaluation of Short-term Tests for Carcinogens: Report of the International Programme on Chemical Safety's Collaborative Study on In Vitro Assays.* Ashby J, de Serres FJ, Draper M *et al.*, editors. Amsterdam: Elsevier, pp. 307–312.

Carls N & Schiestl RH (1994). Evaluation of the yeast DEL assay with 10 compounds selected by the International Program on Chemical Safety for the evaluation of short-term tests for carcinogens. *Mutat Res*, 320: 293–303. doi:10.1016/0165-1218(94)90082-5 PMID:7508555

Castle L, Gilbert J, Eklund T (1990). Migration of plasticizer from poly(vinyl chloride) milk tubing. *Food Addit Contam*, 7: 591–596. doi:10.1080/02652039009373924 PMID:2253803

Cattley RC, Conway JG, Popp JA (1987). Association of persistent peroxisome proliferation and oxidative injury with hepatocarcinogenicity in female F-344 rats fed di(2-ethylhexyl)phthalate for 2 years. *Cancer Lett*, 38: 15–22. doi:10.1016/0304-3835(87)90195-9 PMID:3690505

Cattley RC & Glover SE (1993). Elevated 8-hydroxydeoxyguanosine in hepatic DNA of rats following exposure to peroxisome proliferators: relationship to carcinogenesis

and nuclear localization. *Carcinogenesis*, 14: 2495–2499. doi:10.1093/carcin/14.12.2495 PMID:8269617

Cattley RC, Smith-Oliver T, Butterworth BE, Popp JA (1988). Failure of the peroxisome proliferator WY-14,643 to induce unscheduled DNA synthesis in rat hepatocytes following in vivo treatment. *Carcinogenesis*, 9: 1179–1184. doi:10.1093/carcin/9.7.1179 PMID:3383337

Cautreels W, Van Cauwenberghe KV, Guzman LA (1977). Comparison between the organic fraction of suspended matter at a background and urban air station. *Sci Total Environ*, 8: 79–88. doi:10.1016/0048-9697(77)90063-8

CDC (2009). *Fourth National Report on Human Exposure to Environmental Chemicals*. Department of Health and Human Services, Centers for Disease Control and Prevention.

Chan E, Tan CS, Deurenberg-Yap M *et al.* (2006). The V227A polymorphism at the *PPARA* locus is associated with serum lipid concentrations and modulates the association between dietary polyunsaturated fatty acid intake and serum high density lipoprotein concentrations in Chinese women. *Atherosclerosis*, 187: 309–315. doi:10.1016/j.atherosclerosis.2005.10.002 PMID:16288935

Chemical Sources International (2010). *Chem Sources-Online,* Clemson, SC. Available at: http://www.chemsources.com/index.html

Cheung C, Akiyama TE, Ward JM *et al.* (2004). Diminished hepatocellular proliferation in mice humanized for the nuclear receptor peroxisome proliferator-activated receptor alpha. *Cancer Res*, 64: 3849–3854. doi:10.1158/0008-5472.CAN-04-0322 PMID:15172993

Choi S, Park SY, Jeong J *et al.* (2010). Identification of toxicological biomarkers of di(2-ethylhexyl) phthalate in proteins secreted by HepG2 cells using proteomic analysis. *Proteomics*, 10: 1831–1846. doi:10.1002/pmic.200900674 PMID:20198640

Christensson A, Ljunggren L, Nilsson-Thorell C *et al.* (1991). In vivo comparative evaluation of hemodialysis tubing plasticized with DEHP and TEHTM. *Int J Artif Organs*, 14: 407–410. PMID:1889893

Cimini AM, Sulli A, Stefanini S *et al.* (1994). Effects of di-(2-ethylhexyl)phthalate on peroxisomes of liver, kidney and brain of lactating rats and their pups. *Cell Mol Biol (Noisy-le-grand)*, 40: 1063–1076. PMID:7873979

Clausen PA, Lindeberg Bille RL, Nilsson T *et al.* (2003). Simultaneous extraction of di(2-ethylhexyl) phthalate and nonionic surfactants from house dust. Concentrations in floor dust from 15 Danish schools. *J Chromatogr A*, 986: 179–190. PMID:12597625

Cobellis L, Latini G, De Felice C *et al.* (2003). High plasma concentrations of di-(2-ethylhexyl)-phthalate in women with endometriosis. *Hum Reprod*, 18: 1512–1515. doi:10.1093/humrep/deg254 PMID:12832380

Cocchieri RA (1986). Occurrence of phthalate esters in Italian packaged foods. *J Food Prot*, 49: 265–266.

Colacino JA, Harris TR, Schecter A (2010). Dietary intake is associated with phthalate body burden in a nationally representative sample. *Environ Health Perspect*, 118: 998–1003. doi:10.1289/ehp.0901712 PMID:20392686

Cole RH, Frederick RE, Healy RP, Rolan RG (1984). Preliminary findings of the priority pollutant monitoring project of the nationwide urban runoff program. *J Water Pollut Control Fed*, 56: 898–908.

Cole RS, Tocchi M, Wye E *et al.* (1981). Contamination of commercial blood products by di-2-ethylhexyl phthalate and mono-2-ethylhexyl phthalate. *Vox Sang*, 40: 317–322. doi:10.1111/j.1423-0410.1981.tb00715.x PMID:7245717

Colón I, Caro D, Bourdony CJ, Rosario O (2000). Identification of phthalate esters in the serum of young Puerto Rican girls with premature breast development. *Environ Health Perspect*, 108: 895–900. doi:10.1289/ehp.00108895 PMID:11017896

Conway JG, Tomaszewski KE, Olson MJ *et al.* (1989). Relationship of oxidative damage to the hepatocarcinogenicity of the peroxisome proliferators di(2-ethylhexyl)phthalate and Wy-14,643. *Carcinogenesis*, 10: 513–519. doi:10.1093/carcin/10.3.513 PMID:2924396

Cooper BW, Cho TM, Thompson PM, Wallace AD (2008). Phthalate induction of CYP3A4 is dependent on glucocorticoid regulation of PXR expression. *Toxicol Sci*, 103: 268–277. doi:10.1093/toxsci/kfn047 PMID:18332045

Corton JC (2008). Evaluation of the role of peroxisome proliferator-activated receptor alpha (PPARalpha) in mouse liver tumor induction by trichloroethylene and metabolites. *Crit Rev Toxicol*, 38: 857–875. doi:10.1080/10408440802209796 PMID:18821149

Corton JC & Lapinskas PJ (2005). Peroxisome proliferator-activated receptors: mediators of phthalate ester-induced effects in the male reproductive tract? *Toxicol Sci*, 83: 4–17. doi:10.1093/toxsci/kfi011 PMID:15496498

Council of Europe (2005). *Directive on the Restriction of Phthalates 2005/84/EC*, Official Journal of the European Union, Strasbourg, p. 4.

Crespi CL, Ryan CG, Seixas GM *et al.* (1985). *Tests for mutagenic activity using mutation assays at two loci in the human lymphoblast cell lines TK6 and AHH-1.* In: *Evaluation of Short-term Tests for Carcinogens: Report of the International Programme on Chemical Safety's Collaborative Study on In Vitro Assays.* Ashby J, de Serres FJ, Draper M *et al.*, editors. Amsterdam: Elsevier, pp. 497–516.

Crocker JF, Safe SH, Acott P (1988). Effects of chronic phthalate exposure on the kidney. *J Toxicol Environ Health*, 23: 433–444. doi:10.1080/15287398809531126 PMID:3361614

Cruciani V, Mikalsen SO, Vasseur P, Sanner T (1997). Effects of peroxisome proliferators and 12-O-tetradecanoyl phorbol-13-acetate on intercellular communication and connexin43 in two hamster fibroblast systems. *Int J Cancer*, 73: 240–248. doi:10.1002/

(SICI)1097-0215(19971009)73:2<240::AID-IJC14>3.0.CO;2-J PMID:9335450

Currie RA, Bombail V, Oliver JD *et al.* (2005). Gene ontology mapping as an unbiased method for identifying molecular pathways and processes affected by toxicant exposure: application to acute effects caused by the rodent non-genotoxic carcinogen diethylhexylphthalate. *Toxicol Sci*, 86: 453–469. doi:10.1093/toxsci/kfi207 PMID:15901911

Dalgaard M, Nellemann C, Lam HR *et al.* (2001). The acute effects of mono(2-ethylhexyl)phthalate (MEHP) on testes of prepubertal Wistar rats. *Toxicol Lett*, 122: 69–79. doi:10.1016/S0378-4274(01)00348-4 PMID:11397558

Danford N (1985). *Tests for chromosome aberrations and aneuploidy in the Chinese hamster fibroblast cell line CH1-L*. In: *Evaluation of Short-term Tests for Carcinogens: Report of the International Programme on Chemical Safety's Collaborative Study on In Vitro Assays*. Ashby J, de Serres FJ, Draper M *et al.*, editors. Amsterdam: Elsevier, pp. 397–411.

Dargnat C, Teil MJ, Chevreuil M, Blanchard M (2009). Phthalate removal throughout wastewater treatment plant: case study of Marne Aval station (France). *Sci Total Environ*, 407: 1235–1244. doi:10.1016/j.scitotenv.2008.10.027 PMID:19036415

Davey EW, Perez KT, Soper AE *et al.* (1990). Significance of the surface microlayer to the environmental fate of di(2-ethylhexyl)phthalate predicted from marine microcosms. *Mar Chem*, 31: 231–269. doi:10.1016/0304-4203(90)90041-A

David RM, Moore MR, Cifone MA *et al.* (1999). Chronic peroxisome proliferation and hepatomegaly associated with the hepatocellular tumorigenesis of di(2-ethylhexyl)phthalate and the effects of recovery. *Toxicol Sci*, 50: 195–205. doi:10.1093/toxsci/50.2.195 PMID:10478855

David RM, Moore MR, Finney DC, Guest D (2000a). Chronic toxicity of di(2-ethylhexyl)phthalate in mice. *Toxicol Sci*, 58: 377–385. doi:10.1093/toxsci/58.2.377 PMID:11099649

David RM, Moore MR, Finney DC, Guest D (2000b). Chronic toxicity of di(2-ethylhexyl)phthalate in rats. *Toxicol Sci*, 55: 433–443. doi:10.1093/toxsci/55.2.433 PMID:10828276

Davis BJ, Maronpot RR, Heindel JJ (1994). Di-(2-ethylhexyl) phthalate suppresses estradiol and ovulation in cycling rats. *Toxicol Appl Pharmacol*, 128: 216–223. doi:10.1006/taap.1994.1200 PMID:7940536

Dearman RJ, Beresford L, Bailey L *et al.* (2008). Di-(2-ethylhexyl) phthalate is without adjuvant effect in mice on ovalbumin. *Toxicology*, 244: 231–241. doi:10.1016/j.tox.2007.11.017 PMID:18179854

DeKeyser JG, Laurenzana EM, Peterson EC *et al.* (2011). Selective phthalate activation of naturally occurring human constitutive androstane receptor splice variants and the pregnane X receptor. *Toxicol Sci*, 120: 381–391. doi:10.1093/toxsci/kfq394 PMID:21227907

DeKeyser JG, Stagliano MC, Auerbach SS *et al.* (2009). Di(2-ethylhexyl) phthalate is a highly potent agonist for the human constitutive androstane receptor splice variant CAR2. *Mol Pharmacol*, 75: 1005–1013. doi:10.1124/mol.108.053702 PMID:19211671

DeLeon IR, Byrne CJ, Peuler EA *et al.* (1986). Trace organic and heavy metal pollution in the Mississippi River. *Chemosphere*, 15: 795–805. doi:10.1016/0045-6535(86)90047-0

Dell L & Teta MJ (1995). Mortality among workers at a plastics manufacturing and research and development facility: 1946–1988. *Am J Ind Med*, 28: 373–384. doi:10.1002/ajim.4700280307 PMID:7485191

Dhalluin S, Elias Z, Cruciani V *et al.* (1998). Two-stage exposure of Syrian-hamster-embryo cells to environmental carcinogens: superinduction of ornithine decarboxylase correlates with increase of morphological-transformation frequency. *Int J Cancer*, 75: 744–749. doi:10.1002/(SICI)1097-0215(19980302)75:5<744::AID-IJC13>3.0.CO;2-6 PMID:9495243

Dine T, Luyckx M, Cazin M *et al.* (1991). Rapid determination by high performance liquid chromatography of di-2-ethylhexyl phthalate in plasma stored in plastic bags. *Biomed Chromatogr*, 5: 94–97. doi:10.1002/bmc.1130050211 PMID:1868266

Dirven HA, Theuws JL, Jongeneelen FJ, Bos RP (1991). Non-mutagenicity of 4 metabolites of di(2-ethylhexyl) phthalate (DEHP) and 3 structurally related derivatives of di(2-ethylhexyl)adipate (DEHA) in the Salmonella mutagenicity assay. *Mutat Res*, 260: 121–130. doi:10.1016/0165-1218(91)90088-4 PMID:2027336

Dirven HA, van den Broek PH, Jongeneelen FJ (1990). Effect of di(2-ethylhexyl)phthalate on enzyme activity levels in liver and serum of rats. *Toxicology*, 65: 199–207. doi:10.1016/0300-483X(90)90089-Y PMID:2274965

Dirven HAAM, van den Broek PHH, Arends AMM *et al.* (1993). Metabolites of the plasticizer di(2-ethylhexyl) phthalate in urine samples of workers in polyvinylchloride processing industries. *Int Arch Occup Environ Health*, 64: 549–554. doi:10.1007/BF00517699 PMID:8314612

DiVincenzo GD, Hamilton ML, Mueller KR *et al.* (1985). Bacterial mutagenicity testing of urine from rats dosed with 2-ethylhexanol derived plasticizers. *Toxicology*, 34: 247–259. doi:10.1016/0300-483X(85)90175-1 PMID:3883574

Diwan BA, Ward JM, Rice JM *et al.* (1985). Tumor-promoting effects of di(2-ethylhexyl)phthalate in JB6 mouse epidermal cells and mouse skin. *Carcinogenesis*, 6: 343–347. doi:10.1093/carcin/6.3.343 PMID:3919955

Dostal LA, Chapin RE, Stefanski SA *et al.* (1988). Testicular toxicity and reduced Sertoli cell numbers in neonatal rats by di(2-ethylhexyl)phthalate and the recovery of

fertility as adults. *Toxicol Appl Pharmacol*, 95: 104–121. doi:10.1016/S0041-008X(88)80012-7 PMID:3413790

Dostal LA, Jenkins WL, Schwetz BA (1987). Hepatic peroxisome proliferation and hypolipidemic effects of di(2-ethylhexyl)phthalate in neonatal and adult rats. *Toxicol Appl Pharmacol*, 87: 81–90. doi:10.1016/0041-008X(87)90086-X PMID:3798454

Douglas GR, Blakey DH, Liu-lee VW *et al.* (1985). *Alkaline sucrose sedimentation, sister-chromatid exchange and micronucleus assays in CHO cells.* In: *Evaluation of Short-term Tests for Carcinogens: Report of the International Programme on Chemical Safety's Collaborative Study on In Vitro Assays.* Ashby J, de Serres FJ, Draper M *et al.*, editors. Amsterdam: Elsevier, pp. 359–366.

Douglas GR, Hugenholtz AP, Blakey DH (1986). Genetic toxicology of phthalate esters: mutagenic and other genotoxic effects. *Environ Health Perspect*, 65: 255–262. doi:10.2307/3430191 PMID:3709450

Doull J, Cattley R, Elcombe C *et al.* (1999). A cancer risk assessment of di(2-ethylhexyl)phthalate: application of the new U.S. EPA Risk Assessment Guidelines. *Regul Toxicol Pharmacol*, 29: 327–357. doi:10.1006/rtph.1999.1296 PMID:10388618

Dreyer C, Krey G, Keller H *et al.* (1992). Control of the peroxisomal beta-oxidation pathway by a novel family of nuclear hormone receptors. *Cell*, 68: 879–887. doi:10.1016/0092-8674(92)90031-7 PMID:1312391

Du QZ, Wang JW, Fu XW, Xia HL (2010). Diffusion and accumulation in cultivated vegetable plants of di-(2-ethylhexyl) phthalate (DEHP) from a plastic production factory. *Food Addit Contam Part A Chem Anal Control Expo Risk Assess*, 27: 1186–1192. PMID:20496185

Duty SM, Calafat AM, Silva MJ *et al.* (2004). The relationship between environmental exposure to phthalates and computer-aided sperm analysis motion parameters. *J Androl*, 25: 293–302. PMID:14760016

Duty SM, Singh NP, Silva MJ *et al.* (2003). The relationship between environmental exposures to phthalates and DNA damage in human sperm using the neutral comet assay. *Environ Health Perspect*, 111: 1164–1169. doi:10.1289/ehp.5756 PMID:12842768

Eagon PK, Chandar N, Epley MJ *et al.* (1994). Di(2-ethylhexyl)phthalate-induced changes in liver estrogen metabolism and hyperplasia. *Int J Cancer*, 58: 736–743. doi:10.1002/ijc.2910580519 PMID:7915705

Eastin WC, Mennear JH, Tennant RW *et al.* (2001). Tg.AC genetically altered mouse: assay working group overview of available data. *Toxicol Pathol*, 29: Suppl 60–80. doi:10.1080/019262301753178483 PMID:11695563

Eisenreich SJ, Looney BB, Thornton JD (1981). Airborne organic contaminants in the Great Lakes ecosystem. *Environ Sci Technol*, 15: 30–38. doi:10.1021/es00083a002

Ejlertsson J & Svensson BH (1996). Degradation of bis(2-ethylhexyl) phthalate constituents under methanogenic conditions. *Biodegradation*, 7: 501–506. doi:10.1007/BF00115296 PMID:9188196

Elcombe CR & Mitchell AM (1986). Peroxisome proliferation due to di(2-ethylhexyl) phthalate (DEHP): species differences and possible mechanisms. *Environ Health Perspect*, 70: 211–219. doi:10.1289/ehp.8670211 PMID:3104023

Elliott BM & Elcombe CR (1987). Lack of DNA damage or lipid peroxidation measured in vivo in the rat liver following treatment with peroxisomal proliferators. *Carcinogenesis*, 8: 1213–1218. doi:10.1093/carcin/8.9.1213 PMID:3621460

Elmore E, Korytynski EA, Smith MP (1985). *Tests with the Chinese hamster V79 inhibition of metabolic cooperation assay.* In: *Evaluation of Short-term Tests for Carcinogens : Report of the International Programme on Chemical Safety's Collaborative Study on In Vitro Assays.* Ashby J, de Serres FJ, Draper M *et al.*, editors. Amsterdam: Elsevier, pp. 597-612

Environment Canada (1995). *National Pollutant Release Inventory, Summary Report 1995.* Ottawa, Canada: Minister of Public Works and Government Services.

EPA; Environmental Protection Agency (1995a). Method 525.2. Determination of organic compounds in drinking water by liquid-solid extraction and capillary column gas chromatography/mass spectrometry [Rev. 2.0]. In: *Methods for the Determination of Organic Compounds in Drinking Water, Supplement III* (EPA Report No. EPA-600/R-95/131; NTIS PB-216616). Cincinnati, OH: Environmental Monitoring Systems Laboratory.

EPA; Environmental Protection Agency (1995b). Method 506. Determination of phthalate and adipate esters in drinking water by liquid-liquid extraction or liquid-solid extraction and gas chromatography with photoionization detection [Rev. 1.1]. In: *Methods for the Determination of Organic Compounds in Drinking Water, Supplement III* (EPA Report No. EPA-600/R-95/131; NTIS PB95–261616). Cincinnati, OH: Environmental Monitoring Systems Laboratory.

EPA; Environmental Protection Agency (1996). Method 8061A. Phthalate esters by gas chromatography with electron capture detection (GC/ECD) [Rev 1]. In: *Test Methods for Evaluating Solid Waste - Physical/Chemical Methods* (EPA No. SW-846), Washington DC: Office of Solid Waste.

EPA; Environmental Protection Agency (1998). *Technical Factsheet on: Di(2-ethylhexyl) Phthalate (DEHP).* Washington DC: Office of Ground Water and Drinking Water.

EPA; Environmental Protection Agency (1999a). Methods for organic chemical analysis of municipal and industrial wastewater. Method 606 - Phthalate ester. *US Code Fed. Regul.*, Title 40, Part 136, App. A, pp. 91–101.

EPA; Environmental Protection Agency (1999b). Methods for organic chemical analysis of municipal and industrial wastewater. Method 625 - Base/neutrals and acids. *US Code Fed. Regul.*, Title 40, Part 136, App. A, pp. 202–228.

EPA; Environmental Protection Agency (1999c). Method 1625 Revision B - Semivolatile organic compounds by isotope dilution GC/MS. *US Code Fed. Regul.*, Title 40, Part 136, App. A, pp. 286–306.

EPA; Environmental Protection Agency (2007). *Toxicity and Exposure Assessment for Children's Health.* Phthalates – TEACH Chemical Summary.

Erkekoğlu P, Rachidi W, De Rosa V *et al.* (2010a). Protective effect of selenium supplementation on the genotoxicity of di(2-ethylhexyl)phthalate and mono(2-ethylhexyl)phthalate treatment in LNCaP cells. *Free Radic Biol Med*, 49: 559–566. doi:10.1016/j.freeradbiomed.2010.04.038 PMID:20466057

Erkekoğlu P, Rachidi W, Yuzugullu OG *et al.* (2010b). Evaluation cytoxicity and oxidative DNA damaging effects of di(2-ethylhexyl)-phthalate (DEHP) and mono (2-ethylhexyl)-phthalate (MEHP) on MA-10 Leydig cells and protection by selenium. *Toxicol and Pharmacol*, 248: 52–62.

European Commission (2008). *Bis(2-ethylhexyl)phthalate (DEHP)* – Summary Risk assessment Report EUR 23384EN/2-European Chemicals Bureau-Institute for Health and Consumer Protection – Toxicology and Chemical Substance (TCS). I-21027 Ispra (VA), Italy.

Eveillard A, Mselli-Lakhal L, Mogha A *et al.* (2009). Di-(2-ethylhexyl)-phthalate (DEHP) activates the constitutive androstane receptor (CAR): a novel signalling pathway sensitive to phthalates. *Biochem Pharmacol*, 77: 1735–1746. doi:10.1016/j.bcp.2009.02.023 PMID:19428328

Fan LQ, Coley J, Miller RT *et al.* (2003). Opposing mechanisms of NADPH-cytochrome P450 oxidoreductase regulation by peroxisome proliferators. *Biochem Pharmacol*, 65: 949–959. doi:10.1016/S0006-2952(03)00004-2 PMID:12623126

Fan LQ, You L, Brown-Borg H *et al.* (2004). Regulation of phase I and phase II steroid metabolism enzymes by PPAR alpha activators. *Toxicology*, 204: 109–121. doi:10.1016/j.tox.2004.06.018 PMID:15388238

Fatoki OS, Bornman M, Ravandhalala L *et al.* (2010). Phthalate ester plasticizers in freshwater systems of Venda, South Africa and potential health effects. *Water SA*, 36: 117–125. doi:10.4314/wsa.v36i1.50916

Fatoki OS & Vernon F (1990). Phthalate esters in rivers of the greater Manchester area, U.K. *Sci Total Environ*, 95: 227–232. doi:10.1016/0048-9697(90)90067-5

Fay M, Donohue JM, De Rosa CAgency for Toxic Substances and Disease Registry (1999). ATSDR evaluation of health effects of chemicals. VI. Di(2-ethylhexyl)phthalate. *Toxicol Ind Health*, 15: 651–746. PMID:10786378

Feige JN, Gelman L, Rossi D *et al.* (2007). The endocrine disruptor monoethyl-hexyl-phthalate is a selective peroxisome proliferator-activated receptor gamma modulator that promotes adipogenesis. *J Biol Chem*, 282: 19152–19166. doi:10.1074/jbc.M702724200 PMID:17468099

Fernandez MP, Ikonomou MG, Buchanan I (2007). An assessment of estrogenic organic contaminants in Canadian wastewaters. *Sci Total Environ*, 373: 250–269. doi:10.1016/j.scitotenv.2006.11.018 PMID:17197011

Fishbein L (1992). Exposure from occupational versus other sources. *Scand J Work Environ Health*, 18: Suppl 15–16. PMID:1411379

Flavell DM, Pineda Torra I, Jamshidi Y *et al.* (2000). Variation in the PPARalpha gene is associated with altered function in vitro and plasma lipid concentrations in Type II diabetic subjects. *Diabetologia*, 43: 673–680. doi:10.1007/s001250051357 PMID:10855543

Food and Drug Administration (1999). *Food and drugs*. US Code Fed. Regul., Title 21, Parts 175.105, 175.300, 176.210, 177.1010, 177.1200, 178.3910, 181.27, pp. 138–165, 211–213, 216–222, 227–230, 405–410, 424.

Franke S, Hildebrandt S, Schwarzbauer J *et al.* (1995). Organic compounds as contaminants of the Elbe River and its tributaries. Part II: GC/MS screening for contaminants of the Elbe water. *Fresenius J Anal Chem*, 353: 39–49. doi:10.1007/BF00322888

Frederiksen H, Skakkebaek NE, Andersson AM (2007). Metabolism of phthalates in humans. *Mol Nutr Food Res*, 51: 899–911. doi:10.1002/mnfr.200600243 PMID:17604388

Freire MTDA, Santana IA, Reyes FGR (2006). Plasticizers in Brazilian food-packaging materials acquired on the retail market. *Food Addit Contam*, 23: 93–99. doi:10.1080/02652030500241686 PMID:16393819

Fritzenschaf H, Kohlpoth M, Rusche B, Schiffmann D (1993). Testing of known carcinogens and noncarcinogens in the Syrian hamster embryo (SHE) micronucleus test in vitro; correlations with in vivo micronucleus formation and cell transformation. *Mutat Res*, 319: 47–53. doi:10.1016/0165-1218(93)90029-D PMID:7690458

Fromme H, Bolte G, Koch HM *et al.* (2007b). Occurrence and daily variation of phthalate metabolites in the urine of an adult population. *Int J Hyg Environ Health*, 210: 21–33. doi:10.1016/j.ijheh.2006.09.005 PMID:17182278

Fromme H, Gruber L, Schlummer M *et al.* (2007a). Intake of phthalates and di(2-ethylhexyl)adipate: results of the Integrated Exposure Assessment Survey based on duplicate diet samples and biomonitoring data. *Environ Int*, 33: 1012–1020. doi:10.1016/j.envint.2007.05.006 PMID:17610953

Fromme H, Küchler T, Otto T *et al.* (2002). Occurrence of phthalates and bisphenol A and F in the environment. *Water Res*, 36: 1429–1438. doi:10.1016/S0043-1354(01)00367-0 PMID:11996333

Fromme H, Lahrz T, Piloty M *et al.* (2004). Occurrence of phthalates and musk fragrances in indoor air and dust from apartments and kindergartens in Berlin (Germany). *Indoor Air*, 14: 188–195. doi:10.1111/j.1600-0668.2004.00223.x PMID:15104786

Fujikawa K, Ryo H, Kondo S (1985). *The Drosophila reversion assay using the unstable zeste-white somatic eye color system*. In: *Evaluation of Short-term Tests for Carcinogens: Report of the International Programme on Chemical Safety's Collaborative Study on In Vitro Assays*. Ashby J, de Serres FJ, Draper M *et al.*, editors. Amsterdam: Elsevier, pp. 319–324.

Ganning AE, Brunk U, Dallner G (1984). Phthalate esters and their effect on the liver. *Hepatology*, 4: 541–547. doi:10.1002/hep.1840040331 PMID:6373551

Ganning AE, Brunk U, Edlund C *et al.* (1987). Effects of prolonged administration of phthalate ester on the liver. *Environ Health Perspect*, 73: 251–258. doi:10.1289/ehp.8773251 PMID:3665868

Ganning AE, Olsson MJ, Brunk U, Dallner G (1990). Effects of prolonged treatment with phthalate ester on rat liver. *Pharmacol Toxicol*, 67: 392–401. doi:10.1111/j.1600-0773.1990.tb00851.x PMID:1965743

Garner RC, Campbell J (1985). *Tests for the induction of mutations to ouabain or 6-thioguanine resistance in mouse lymphoma L5178Y cells*. In: *Evaluation of Short-term Tests for Carcinogens: Report of the International Programme on Chemical Safety's Collaborative Study on In Vitro Assays*. Ashby J, de Serres FJ, Draper M *et al.*, editors. Amsterdam: Elsevier, pp. 525–529.

Garrido Frenich A, de las Nieves Barco Bonilla M, López Martínez JC *et al.* (2009). Determination of di-(2-ethyl-hexyl)phthalate in environmental samples by liquid chromatography coupled with mass spectrometry. *J Sep Sci*, 32: 1383–1389. doi:10.1002/jssc.200900020 PMID:19330790

Gasperi J, Garnaud S, Rocher V, Moilleron R (2009). Priority pollutants in surface waters and settleable particles within a densely urbanized area: case study of Paris (France). *Sci Total Environ*, 407: 2900–2908. doi:10.1016/j.scitotenv.2009.01.024 PMID:19223062

Gaudin R, Marsan P, Ndaw S *et al.* (2010). Biological monitoring of exposure to di(2-ethylhexyl) phthalate in six French factories: a field study *Int Arch Occup Environ Health*,

Gaunt IF & Butterworth KR (1982). Autoradiographic study of orally administered di-(2-ethylhexyl) phthalate in the mouse. *Food Chem Toxicol*, 20: 215–217. doi:10.1016/S0278-6915(82)80252-4 PMID:7200940

Ge RS, Chen GR, Dong Q *et al.* (2007). Biphasic effects of postnatal exposure to diethylhexylphthalate on the timing of puberty in male rats. *J Androl*, 28: 513–520. doi:10.2164/jandrol.106.001909 PMID:17287459

Gee P, Sommers CH, Melick AS *et al.* (1998). Comparison of responses of base-specific Salmonella tester strains with the traditional strains for identifying mutagens: the results of a validation study. *Mutat Res*, 412: 115–130. PMID:9539966

Geiss O, Tirendi S, Barrero-Moreno J, Kotzias D (2009). Investigation of volatile organic compounds and phthalates present in the cabin air of used private cars. *Environ Int*, 35: 1188–1195. doi:10.1016/j.envint.2009.07.016 PMID:19729200

Gervois P, Torra IP, Chinetti G *et al.* (1999). A truncated human peroxisome proliferator-activated receptor alpha splice variant with dominant negative activity. *Mol Endocrinol*, 13: 1535–1549. doi:10.1210/me.13.9.1535 PMID:10478844

Ghassemi M, Quinlivan S, Bachmaier J (1984). Characterization of leachates from hazardous waste landfills. *J Environ Sci Health A*, 19: 579–620. doi:10.1080/10934528409375180

Ghisari M & Bonefeld-Jorgensen EC (2009). Effects of plasticizers and their mixtures on estrogen receptor and thyroid hormone functions. *Toxicol Lett*, 189: 67–77. doi:10.1016/j.toxlet.2009.05.004 PMID:19463926

Ghosh J, Das J, Manna P, Sil PC (2010). Hepatotoxicity of di-(2-ethylhexyl)phthalate is attributed to calcium aggravation, ROS-mediated mitochondrial depolarization, and ERK/NF-κB pathway activation. *Free Radic Biol Med*, 49: 1779–1791. doi:10.1016/j.freeradbiomed.2010.09.011 PMID:20854900

Giam CS & Atlas E (1980). Accumulation of phthalate ester plasticizers in Lake Constance sediment. *Naturwissenschaften*, 67: 508–510. doi:10.1007/BF01047632

Giam CS, Atlas E, Chan HS, Neff GS (1980). Phthalate esters, PCB and DDT residues in the Gulf of Mexico atmosphere. *Atmos Environ*, 14: 65–69. doi:10.1016/0004-6981(80)90108-0

Giam CS, Atlas E, Powers MA Jr, Leonard JE (1984). *Phthalic acid esters*. In: *The Handbook of Environmental Chemistry*, Vol. 3, Part C, *Anthropogenic Compounds*. Hutzinger O, editor. Berlin: Springer Verlag, pp. 67–142.

Giam CS, Chan HS, Neff GS, Atlas EL (1978). Phthalate ester plasticizers: a new class of marine pollutant. *Science*, 199: 419–421. PMID:413194

Giam CS & Wong MK (1987). Plasticizers in food. *J Food Prot*, 50: 769–782.

Gilbert J (1994). The fate of environmental contaminants in the food chain. *Sci Total Environ*, 143: 103–111. doi:10.1016/0048-9697(94)90536-3 PMID:8202695

Glue C, Platzer MH, Larsen ST *et al.* (2005). Phthalates potentiate the response of allergic effector cells. *Basic Clin Pharmacol Toxicol*, 96: 140–142. doi:10.1111/j.1742-7843.2005.pto960208.x PMID:15679477

Goll V, Alexandre E, Viollon-Abadie C *et al.* (1999). Comparison of the effects of various peroxisome proliferators on peroxisomal enzyme activities, DNA synthesis, and apoptosis in rat and human hepatocyte cultures. *Toxicol Appl Pharmacol*, 160: 21–32. doi:10.1006/taap.1999.8737 PMID:10502499

Goll V, Viollon-Abadie C, Nicod L, Richert L (2000). Peroxisome proliferators induce apoptosis and decrease DNA synthesis in hepatoma cell lines. *Hum Exp*

Toxicol, 19: 193–202. doi:10.1191/096032700678827753 PMID:10889518

Gollamudi R, Prasanna HR, Rao RH *et al.* (1983). Impaired metabolism of di(2-ethylhexyl) phthalate (DEHP) in old rats–an in vitro study. *J Toxicol Environ Health*, 12: 623–632. doi:10.1080/15287398309530454 PMID:6668612

Gomez-Rico MF, Font R, Aracil I, Fullana A (2007). Analysis of organic pollutants in sewage sludges from the Valencian community (Spain). *Arch Environ Contam Toxicol*, 52: 306–316. doi:10.1007/s00244-006-0081-8 PMID:17384980

González MC, Corton JC, Cattley RC *et al.* (2009). Peroxisome proliferator-activated receptor alpha (PPARalpha) agonists down-regulate alpha2-macroglobulin expression by a PPARalpha-dependent mechanism. *Biochimie*, 91: 1029–1035. doi:10.1016/j.biochi.2009.05.007 PMID:19497347

Göttlicher M, Widmark E, Li Q, Gustafsson JA (1992). Fatty acids activate a chimera of the clofibric acid-activated receptor and the glucocorticoid receptor. *Proc Natl Acad Sci USA*, 89: 4653–4657. doi:10.1073/pnas.89.10.4653 PMID:1316614

Grande SW, Andrade AJ, Talsness CE *et al.* (2007). A dose-response study following in utero and lactational exposure to di-(2-ethylhexyl) phthalate (DEHP): reproductive effects on adult female offspring rats. *Toxicology*, 229: 114–122. doi:10.1016/j.tox.2006.10.005 PMID:17098345

Gray LE Jr, Ostby J, Furr J *et al.* (2000). Perinatal exposure to the phthalates DEHP, BBP, and DINP, but not DEP, DMP, or DOTP, alters sexual differentiation of the male rat. *Toxicol Sci*, 58: 350–365. doi:10.1093/toxsci/58.2.350 PMID:11099647

Gray TJ, Beamand JA, Lake BG *et al.* (1982). Peroxisome proliferation in cultured rat hepatocytes produced by clofibrate and phthalate ester metabolites. *Toxicol Lett*, 10: 273–279. doi:10.1016/0378-4274(82)90087-X PMID:7080097

Gray TJ, Butterworth KR, Gaunt IF *et al.* (1977). Short-term toxicity study of di-(2-ethylhexyl) phthalate in rats. *Food Cosmet Toxicol*, 15: 389–399. doi:10.1016/S0015-6264(77)80003-5 PMID:598790

Gray TJ, Lake BG, Beamand JA *et al.* (1983). Peroxisomal effects of phthalate esters in primary cultures of rat hepatocytes. *Toxicology*, 28: 167–179. doi:10.1016/0300-483X(83)90115-4 PMID:6636199

Gray TJB & Butterworth KR (1980). Testicular atrophy produced by phthalate esters. *Arch Toxicol Suppl*, 4: 452–455. PMID:6776936

Green R, Hauser R, Calafat AM *et al.* (2005). Use of di(2-ethylhexyl) phthalate-containing medical products and urinary levels of mono(2-ethylhexyl) phthalate in neonatal intensive care unit infants. *Environ Health Perspect*, 113: 1222–1225. doi:10.1289/ehp.7932 PMID:16140631

Greener Y, Gillies B, Wienckowski D *et al.* (1987). Assessment of the safety of chemicals administered intravenously in the neonatal rat. *Teratology*, 35: 187–194. doi:10.1002/tera.1420350204 PMID:3603403

Grolier P & Elcombe CR (1993). *In vitro* inhibition of carnitine acyltransferase activity in mitochondria from rat and mouse liver by a diethylhexylphthalate metabolite. *Biochem Pharmacol*, 45: 827–832. doi:10.1016/0006-2952(93)90165-S PMID:8452557

Gruber L, Wolz G, Piringer O (1998). [Analysis of phthalates in baby foods.]*Dtsch Lebensmitt Rundsch*, 94: 177–179.

Guidotti M, Colasanti G, Chinzari M *et al.* (1998). Investigation on the presence of aromatic hydrocarbons, polycyclic aromatic hydrocarbons, persistent organochlorine compounds, phthalates and the breathable fraction of atmospheric particulates in the air of Rieti urban area. *Ann Chim*, 88: 419–427.

Gulati DK, Sabharwal PS, Shelby MD (1985). *Tests for the induction of chromosomal aberrations and sister chromatid exchanges in cultured Chinese hamster ovary (CHO) cells.* In: *Evaluation of Short-term Tests for Carcinogens: Report of the International Programme on Chemical Safety's Collaborative Study on In Vitro Assays.* Ashby J, de Serres FJ, Draper M *et al.*, editors. Amsterdam: Elsevier, pp. 413–426.

Gunz D, Shephard SE, Lutz WK (1993). Can nongenotoxic carcinogens be detected with the lacI transgenic mouse mutation assay? *Environ Mol Mutagen*, 21: 209–211. doi:10.1002/em.2850210302 PMID:8462524

Guo Z, Wei D, Wang M, Wang S (2010). Determination of six phthalic acid esters in orange juice packaged by PVC bottle using SPE and HPLC-UV: application to the migration study. *J Chromatogr Sci*, 48: 760–765. PMID:20875239

Gupta RC, Goel SK, Earley K *et al.* (1985). 32P-Postlabeling analysis of peroxisome proliferator-DNA adduct formation in rat liver in vivo and hepatocytes in vitro. *Carcinogenesis*, 6: 933–936. doi:10.1093/carcin/6.6.933 PMID:4006083

Hagiwara A, Tamano S, Ogiso T *et al.* (1990). Promoting effect of the peroxisome proliferator, clofibrate, but not di(2-ethylhexyl)phthalate, on urinary bladder carcinogenesis in F344 rats initiated by N-butyl-N-(4-hydroxybutyl)nitrosamine. *Jpn J Cancer Res*, 81: 1232–1238. PMID:2125992

Hagmar L, Akesson B, Nielsen J *et al.* (1990). Mortality and cancer morbidity in workers exposed to low levels of vinyl chloride monomer at a polyvinyl chloride processing plant. *Am J Ind Med*, 17: 553–565. doi:10.1002/ajim.4700170502 PMID:2337081

Hamosh M (1990). Lingual and gastric lipases. *Nutrition*, 6: 421–428. PMID:2134569

Hanselman JC, Vartanian MA, Koester BP *et al.* (2001). Expression of the mRNA encoding truncated PPAR alpha does not correlate with hepatic insensitivity to

peroxisome proliferators. *Mol Cell Biochem*, 217: 91–97. doi:10.1023/A:1007248007372 PMID:11269670

Hansen J (1999). Risk for testicular cancer after occupational exposure to plastics. *Int J Cancer*, 82: 911–912. doi:10.1002/(SICI)1097-0215(19990909)82:6<911::AID-IJC23>3.0.CO;2-O PMID:10446462

Hansen JS, Larsen ST, Nielsen GD (2008). Comment to "Di-(2-ethylhexyl) phthalate is without adjuvant effect in mice on ovalbumin" [Toxicology 244 (2008) 231–241]. [Toxicology 244 (2008) 231–241]*Toxicology*, 247: 162–165. doi:10.1016/j.tox.2008.02.010 PMID:18417265

Hardell L, Malmqvist N, Ohlson CG *et al.* (2004). Testicular cancer and occupational exposure to polyvinyl chloride plastics: a case-control study. *Int J Cancer*, 109: 425–429. doi:10.1002/ijc.11709 PMID:14961582

Hardell L, Ohlson CG, Fredrikson M (1997). Occupational exposure to polyvinyl chloride as a risk factor for testicular cancer evaluated in a case-control study. *Int J Cancer*, 73: 828–830. doi:10.1002/(SICI)1097-0215(19971210)73:6<828::AID-IJC10>3.0.CO;2-0 PMID:9399660

Hasmall S, Orphanides G, James N *et al.* (2002). Downregulation of lactoferrin by PPARalpha ligands: role in perturbation of hepatocyte proliferation and apoptosis. *Toxicol Sci*, 68: 304–313. doi:10.1093/toxsci/68.2.304 PMID:12151626

Hasmall SC, James NH, Macdonald N *et al.* (1999). Suppression of apoptosis and induction of DNA synthesis in vitro by the phthalate plasticizers monoethylhexylphthalate (MEHP) and diisononylphthalate (DINP): a comparison of rat and human hepatocytes in vitro. *Arch Toxicol*, 73: 451–456. doi:10.1007/s002040050634 PMID:10650916

Hasmall SC, James NH, Macdonald N *et al.* (2000a). Suppression of mouse hepatocyte apoptosis by peroxisome proliferators: role of PPARalpha and TNFalpha. *Mutat Res*, 448: 193–200. PMID:10725472

Hasmall SC, James NH, Macdonald N *et al.* (2000b). Species differences in response to diethylhexylphthalate: suppression of apoptosis, induction of DNA synthesis and peroxisome proliferator activated receptor alpha-mediated gene expression. *Arch Toxicol*, 74: 85–91. doi:10.1007/s002040050657 PMID:10839475

Hasmall SC & Roberts RA (1997). Hepatic ploidy, nuclearity, and distribution of DNA synthesis: a comparison of nongenotoxic hepatocarcinogens with noncarcinogenic liver mitogens. *Toxicol Appl Pharmacol*, 144: 287–293. doi:10.1006/taap.1997.8133 PMID:2134569

Hasmall SC & Roberts RA (2000). The nongenotoxic hepatocarcinogens diethylhexylphthalate and methylclofenapate induce DNA synthesis preferentially in octoploid rat hepatocytes. *Toxicol Pathol*, 28: 503–509. doi:10.1177/019262330002800401 PMID:10930035

Hatch GG, Anderson TM (1985). *Assays for enhanced DNA viral transformation of primary Syrian hamster embryo (SHE) cells*. In: *Evaluation of Short-term Tests for Carcinogens: Report of the International Programme on Chemical Safety's Collaborative Study on In Vitro Assays*. Ashby J, de Serres FJ, Draper M *et al.*, editors. Amsterdam: Elsevier, pp. 629–638.

Hauser R, Meeker JD, Duty S *et al.* (2006). Altered semen quality in relation to urinary concentrations of phthalate monoester and oxidative metabolites. *Epidemiology*, 17: 682–691. doi:10.1097/01.ede.0000235996.89953.d7 PMID:17003688

Hauser R, Meeker JD, Park S *et al.* (2004). Temporal variability of urinary phthalate metabolite levels in men of reproductive age. *Environ Health Perspect*, 112: 1734–1740. doi:10.1289/ehp.7212 PMID:15579421

Hauser R, Meeker JD, Singh NP *et al.* (2007). DNA damage in human sperm is related to urinary levels of phthalate monoester and oxidative metabolites. *Hum Reprod*, 22: 688–695. doi:10.1093/humrep/del428 PMID:17090632

Hays T, Rusyn I, Burns AM *et al.* (2005). Role of peroxisome proliferator-activated receptor-alpha (PPARalpha) in bezafibrate-induced hepatocarcinogenesis and cholestasis. *Carcinogenesis*, 26: 219–227. doi:10.1093/carcin/bgh285 PMID:15447978

He H, Hu GJ, Sun C *et al.* (2011). Trace analysis of persistent toxic substances in the main stream of Jiangsu section of the Yangtze River, China. *Environ Sci Pollut Res Int*, 18: 638–648. doi:10.1007/s11356-010-0414-z PMID:21063796

Heindel JJ & Powell CJ (1992). Phthalate ester effects on rat Sertoli cell function in vitro: effects of phthalate side chain and age of animal. *Toxicol Appl Pharmacol*, 115: 116–123. doi:10.1016/0041-008X(92)90374-2 PMID:1321518

Heineman EF, Olsen JH, Pottern LM *et al.* (1992). Occupational risk factors for multiple myeloma among Danish men. *Cancer Causes Control*, 3: 555–568. doi:10.1007/BF00052753 PMID:1420859

Hellwig J, Freudenberger H, Jäckh R (1997). Differential prenatal toxicity of branched phthalate esters in rats. *Food Chem Toxicol*, 35: 501–512. doi:10.1016/S0278-6915(97)00008-2 PMID:9216749

Helm D (2007). Correlation between production amounts of DEHP and daily intake. *Sci Total Environ*, 388: 389–391. doi:10.1016/j.scitotenv.2007.07.009 PMID:17688915

Herr C, zur Nieden A, Koch HM *et al.* (2009). Urinary di(2-ethylhexyl)phthalate (DEHP)–metabolites and male human markers of reproductive function. *Int J Hyg Environ Health*, 212: 648–653. doi:10.1016/j.ijheh.2009.08.001 PMID:19733116

Hines CJ, Hopf NB, Deddens JA *et al.* (2011). Estimated daily intake of phthalates in occupationally exposed groups. *J Expo Sci Environ Epidemiol*, 21: 133–141. doi:10.1038/jes.2009.62 PMID:19018275

Hines CJ, Nilsen Hopf NB, Deddens JA *et al.* (2009). Urinary phthalate metabolite concentrations among

workers in selected industries: a pilot biomonitoring study. *Ann Occup Hyg*, 53: 1–17. doi:10.1093/annhyg/men066 PMID:18948546

Hinton RH, Mitchell FE, Mann A *et al.* (1986). Effects of phthalic acid esters on the liver and thyroid. *Environ Health Perspect*, 70: 195–210. doi:10.1289/ehp.8670195 PMID:3830106

Hirayama K, Tanaka H, Kawana K, Nakazawa H (2001). Analysis of plasticizers in cap-sealing resins for bottled foods. *Food Addit Contam*, 18: 357–362. doi:10.1080/02652030119099 PMID:11339270

Högberg J, Hanberg A, Berglund M *et al.* (2008). Phthalate diesters and their metabolites in human breast milk, blood or serum, and urine as biomarkers of exposure in vulnerable populations. *Environ Health Perspect*, 116: 334–339. PMID:18335100

Hollyfield S & Sharma VK (1995). Organic contaminants and characteristics of sediments from Oso Bay, South Texas, USA. *Environ. Geol*, 25: 137–140. doi:10.1007/BF00767870

Hoppin JA, Ulmer R, London SJ (2004). Phthalate exposure and pulmonary function. *Environ Health Perspect*, 112: 571–574. doi:10.1289/ehp.6564 PMID:15064163

Hosokawa M, Hirata K, Nakata F *et al.* (1994). Species differences in the induction of hepatic microsomal carboxylesterases caused by dietary exposure to di(2-ethylhexyl)phthalate, a peroxisome proliferator. *Drug Metab Dispos*, 22: 889–894. PMID:7895606

Howroyd P, Swanson C, Dunn C *et al.* (2004). Decreased longevity and enhancement of age-dependent lesions in mice lacking the nuclear receptor peroxisome proliferator-activated receptor alpha (PPARalpha). *Toxicol Pathol*, 32: 591–599. doi:10.1080/01926230490515283 PMID:15603543

HSDB (2010). *Hazardous Substances Data Bank*. Available at: http://toxnet.nlm.nih.gov/

Hu XY, Wen B, Shan XQ (2003). Survey of phthalate pollution in arable soils in China. *J Environ Monit*, 5: 649–653. doi:10.1039/b304669a PMID:12948243

Huang PC, Kuo PL, Chou YY *et al.* (2009). Association between prenatal exposure to phthalates and the health of newborns. *Environ Int*, 35: 14–20. doi:10.1016/j.envint.2008.05.012 PMID:18640725

Huang P-C, Tien C-J, Sun Y-M *et al.* (2008). Occurrence of phthalates in sediment and biota: relationship to aquatic factors and the biota-sediment accumulation factor. *Chemosphere*, 73: 539–544. doi:10.1016/j.chemosphere.2008.06.019 PMID:18687453

Huber WW, Grasl-Kraupp B, Schulte-Hermann R (1996). Hepatocarcinogenic potential of di(2-ethylhexyl)phthalate in rodents and its implications on human risk. *Crit Rev Toxicol*, 26: 365–481. doi:10.3109/10408449609048302 PMID:8817083

Hurst CH & Waxman DJ (2003). Activation of PPARalpha and PPARgamma by environmental phthalate monoesters. *Toxicol Sci*, 74: 297–308. doi:10.1093/toxsci/kfg145 PMID:12805656

Hurst CH & Waxman DJ (2004). Environmental phthalate monoesters activate pregnane X receptor-mediated transcription. *Toxicol Appl Pharmacol*, 199: 266–274. doi:10.1016/j.taap.2003.11.028 PMID:15364542

Hwang H-M, Green PG, Young TM (2006). Tidal salt marsh sediment in California, USA. Part 1: occurrence and sources of organic contaminants. *Chemosphere*, 64: 1383–1392. doi:10.1016/j.chemosphere.2005.12.024 PMID:16442586

IARC (1982). Some industrial chemicals and dyestuffs. *IARC Monogr Eval Carcinog Risk Chem Hum*, 29: 1–398. PMID:6957379

IARC (1987). Overall evaluations of carcinogenicity: an updating of IARC Monographs volumes 1 to 42. *IARC Monogr Eval Carcinog Risks Hum Suppl*, 7: 1–440. PMID:3482203

IARC (2000). Some industrial chemicals. *IARC Monogr Eval Carcinog Risks Hum*, 77: 1–529. PMID:11236796

Ikeda GJ, Sapienza PP, Couvillion JL *et al.* (1980). Comparative distribution, excretion and metabolism of di-(2-ethylhexyl) phthalate in rats, dogs and miniature pigs. *Food Cosmet Toxicol*, 18: 637–642. doi:10.1016/S0015-6264(80)80012-5 PMID:7203310

Inge-Vechtomov SG, Pavlov YI, Noskov VN *et al.* (1985). *Tests for genetic activity in the yeast Saccharomyces cerevisiae: study of forward and reverse mutation, mitotic recombination and illegitimate mating induction.* In: *Evaluation of Short-term Tests for Carcinogens: Report of the International Programme on Chemical Safety's Collaborative Study on In Vitro Assays.* Ashby J, de Serres FJ, Draper M *et al.*, editors. Amsterdam: Elsevier, pp. 243–255.

Inoue K, Kawaguchi M, Yamanaka R *et al.* (2005). Evaluation and analysis of exposure levels of di(2-ethylhexyl) phthalate from blood bags. *Clin Chim Acta*, 358: 159–166. doi:10.1016/j.cccn.2005.02.019 PMID:15893743

Isenberg JS, Kamendulis LM, Ackley DC *et al.* (2001). Reversibility and persistence of di-2-ethylhexyl phthalate (DEHP)- and phenobarbital-induced hepatocellular changes in rodents. *Toxicol Sci*, 64: 192–199. doi:10.1093/toxsci/64.2.192 PMID:11719701

Isenberg JS, Kamendulis LM, Smith JH *et al.* (2000). Effects of di-2-ethylhexyl phthalate (DEHP) on gap-junctional intercellular communication (GJIC), DNA synthesis, and peroxisomal beta oxidation (PBOX) in rat, mouse, and hamster liver. *Toxicol Sci*, 56: 73–85. doi:10.1093/toxsci/56.1.73 PMID:10869455

Ishidate M Jr & Odashima S (1977). Chromosome tests with 134 compounds on Chinese hamster cells in vitro–a screening for chemical carcinogens. *Mutat Res*, 48: 337–353. PMID:876270

Ishidate M, Sofuni T (1985). *The in vitro chromosomal aberration test using Chinese hamster lung (CHL) fibroblast*

cells in culture. In: *Evaluation of Short-term Tests for Carcinogens: Report of the International Programme on Chemical Safety's Collaborative Study on In Vitro Assays.* Ashby J, de Serres FJ, Draper M *et al.*, editors. Amsterdam: Elsevier, pp. 427–432.

Issemann I & Green S (1990). Activation of a member of the steroid hormone receptor superfamily by peroxisome proliferators. *Nature*, 347: 645–650. doi:10.1038/347645a0 PMID:2129546

Ito Y, Yamanoshita O, Asaeda N *et al.* (2007a). Di(2-ethylhexyl)phthalate induces hepatic tumorigenesis through a peroxisome proliferator-activated receptor alpha-independent pathway. *J Occup Health*, 49: 172–182. doi:10.1539/joh.49.172 PMID:17575397

Ito Y, Yamanoshita O, Kurata Y *et al.* (2007b). Induction of peroxisome proliferator-activated receptor alpha (PPARalpha)-related enzymes by di(2-ethylhexyl) phthalate (DEHP) treatment in mice and rats, but not marmosets. *Arch Toxicol*, 81: 219–226. doi:10.1007/s00204-006-0141-x PMID:16937134

Ito Y, Yokota H, Wang R *et al.* (2005). Species differences in the metabolism of di(2-ethylhexyl) phthalate (DEHP) in several organs of mice, rats, and marmosets. *Arch Toxicol*, 79: 147–154. doi:10.1007/s00204-004-0615-7 PMID:15798888

Itoh H, Yoshida K, Masunaga S (2007). Quantitative identification of unknown exposure pathways of phthalates based on measuring their metabolites in human urine. *Environ Sci Technol*, 41: 4542–4547. doi:10.1021/es062926y PMID:17695894

IUCLID DataBase (2000). *Bis (2-ethylhexyl) Phthalate.* Bruxelles: European Commission, European Chemicals Bureau. Available at: http://esis.jrc.ec.europa.eu/doc/IUCLID/data_sheets/117817.pdf

Jaakkola JJ & Knight TL (2008). The role of exposure to phthalates from polyvinyl chloride products in the development of asthma and allergies: a systematic review and meta-analysis. *Environ Health Perspect*, 116: 845–853. doi:10.1289/ehp.10846 PMID:18629304

Jacobson MS, Kevy SV, Grand RJ (1977). Effects of a plasticizer leached from polyvinyl chloride on the subhuman primate: a consequence of chronic transfusion therapy. *J Lab Clin Med*, 89: 1066–1079. PMID:404371

Jaeger RJ & Rubin RJ (1970). Plasticizers from plastic devices extraction, metabolism, and accumulation by biological systems. *Science*, 170: 460–462. doi:10.1126/science.170.3956.460 PMID:5460077

Jaeger RJ & Rubin RJ (1972). Migration of a phthalate ester plasticizer from polyvinyl chloride blood bags into stored human blood and its localization in human tissues. *N Engl J Med*, 287: 1114–1118. doi:10.1056/NEJM197211302872203 PMID:5082191

Jain GC & Joshi SC (1991). Effects of plasticizer di-(2-ethyl hexyl) phthalate (DEHP) on reproductive function of mice. *Z Angew Zool*, 4: 465–470.

James NH, Gill JH, Brindle R *et al.* (1998b). Peroxisome proliferator-activated receptor (PPAR) alpha-regulated growth responses and their importance to hepatocarcinogenesis. *Toxicol Lett*, 102-103: 91–96. doi:10.1016/S0378-4274(98)00291-4 PMID:10022238

James NH, Soames AR, Roberts RA (1998a). Suppression of hepatocyte apoptosis and induction of DNA synthesis by the rat and mouse hepatocarcinogen diethylhexylphlathate (DEHP) and the mouse hepatocarcinogen 1,4-dichlorobenzene (DCB). *Arch Toxicol*, 72: 784–790. doi:10.1007/s002040050574 PMID:9950075

Jeng W-L (1986). Phthalate esters in marine sediments around Taiwan. *Acta Oceanogr Taiwanica*, 17: 61–68.

Jepsen KF, Abildtrup A, Larsen ST (2004). Monophthalates promote IL-6 and IL-8 production in the human epithelial cell line A549. *Toxicol In Vitro*, 18: 265–269. doi:10.1016/j.tiv.2003.09.008 PMID:15046772

Jönsson BA, Richthoff J, Rylander L *et al.* (2005). Urinary phthalate metabolites and biomarkers of reproductive function in young men. *Epidemiology*, 16: 487–493. doi:10.1097/01.ede.0000164555.19041.01 PMID:15951666

Kang SC & Lee BM (2005). DNA methylation of estrogen receptor alpha gene by phthalates. *J Toxicol Environ Health A*, 68: 1995–2003. doi:10.1080/15287390491008913 PMID:16326419

Kanki K, Nishikawa A, Masumura K *et al.* (2005). In vivo mutational analysis of liver DNA in gpt delta transgenic rats treated with the hepatocarcinogens N-nitrosopyrrolidine, 2-amino-3-methylimidazo[4,5-f]quinoline, and di(2-ethylhexyl)phthalate. *Mol Carcinog*, 42: 9–17. doi:10.1002/mc.20061 PMID:15486947

Karle VA, Short BL, Martin GR *et al.* (1997). Extracorporeal membrane oxygenation exposes infants to the plasticizer, di(2-ethylhexyl)phthalate. *Crit Care Med*, 25: 696–703. doi:10.1097/00003246-199704000-00023 PMID:9142038

Kato K, Silva MJ, Reidy JA *et al.* (2004). Mono(2-ethyl-5-hydroxyhexyl) phthalate and mono-(2-ethyl-5-oxohexyl) phthalate as biomarkers for human exposure assessment to di-(2-ethylhexyl) phthalate. *Environ Health Perspect*, 112: 327–330. doi:10.1289/ehp.6663 PMID:14998748

Kavlock R, Barr D, Boekelheide K *et al.* (2006). NTP-CERHR Expert Panel Update on the Reproductive and Developmental Toxicity of di(2-ethylhexyl) phthalate. *Reprod Toxicol*, 22: 291–399. doi:10.1016/j.reprotox.2006.04.007 PMID:17068859

Kavlock R, Boekelheide K, Chapin R *et al.* (2002). NTP Center for the Evaluation of Risks to Human Reproduction: phthalates expert panel report on the reproductive and developmental toxicity of di(2-ethylhexyl) phthalate. *Reprod Toxicol*, 16: 529–653. doi:10.1016/S0890-6238(02)00032-1 PMID:12406494

Kawai K (1998). Enhancement of the DNA damaging activity of N-nitrosodimethylamine by di-(2-ethylhexyl)phthalate in somatic cells in vivo of Drosophila melanogaster. *Biol Pharm Bull*, 21: 579–582. PMID:9657041

Kelly TJ, Mukund R, Spicer CW, Pollack AJ (1994). Concentrations and transformations of hazardous air pollutants. *Environ Sci Technol*, 28: 378A–387A. doi:10.1021/es00057a003 PMID:22662899

Kessler W, Numtip W, Grote K *et al.* (2004). Blood burden of di(2-ethylhexyl) phthalate and its primary metabolite mono(2-ethylhexyl) phthalate in pregnant and nonpregnant rats and marmosets. *Toxicol Appl Pharmacol*, 195: 142–153. doi:10.1016/j.taap.2003.11.014 PMID:14998681

Kevy SV & Jacobson MS (1982). Hepatic effects of a phthalate ester plasticizer leached from poly(vinyl chloride) blood bags following transfusion. *Environ Health Perspect*, 45: 57–64. doi:10.1289/ehp.824557 PMID:7140697

Keys DA, Wallace DG, Kepler TB, Conolly RB (1999). Quantitative evaluation of alternative mechanisms of blood and testes disposition of di(2-ethylhexyl) phthalate and mono(2-ethylhexyl) phthalate in rats. *Toxicol Sci*, 49: 172–185. doi:10.1093/toxsci/49.2.172 PMID:10416263

Kim HS, Ishizuka M, Kazusaka A, Fujita S (2004a). Alterations of activities of cytosolic phospholipase A2 and arachidonic acid-metabolizing enzymes in di-(2-ethylhexyl)phthalate-induced testicular atrophy. *J Vet Med Sci*, 66: 1119–1124. doi:10.1292/jvms.66.1119 PMID:15472477

Kim IY, Han SY, Moon A (2004b). Phthalates inhibit tamoxifen-induced apoptosis in MCF-7 human breast cancer cells. *J Toxicol Environ Health A*, 67: 2025–2035. doi:10.1080/15287390490514750 PMID:15513900

Kim NY, Kim TH, Lee E *et al.* (2010). Functional role of phospholipase D (PLD) in di(2-ethylhexyl) phthalate-induced hepatotoxicity in Sprague-Dawley rats. *J Toxicol Environ Health A*, 73: 1560–1569. doi:10.1080/15287394.2010.511582 PMID:20954081

Kim SH, Chun S, Jang JY *et al.* (2011). Increased plasma levels of phthalate esters in women with advanced-stage endometriosis: a prospective case-control study. *Fertil Steril*, 95: 357–359. doi:10.1016/j.fertnstert.2010.07.1059 PMID:20797718

Kirby PE, Pizzarello RF, Lawlor TE *et al.* (1983). Evaluation of di-(2-ethylhexyl)phthalate and its major metabolites in the Ames test and L5178Y mouse lymphoma mutagenicity assay. *Environ Mutagen*, 5: 657–663. doi:10.1002/em.2860050504 PMID:6352251

Kleinsasser NH, Harréus UA, Kastenbauer ER *et al.* (2004b). Mono(2-ethylhexyl)phthalate exhibits genotoxic effects in human lymphocytes and mucosal cells of the upper aerodigestive tract in the comet assay. *Toxicol Lett*, 148: 83–90. doi:10.1016/j.toxlet.2003.12.013 PMID:15019091

Kleinsasser NH, Juchhoff J, Wallner BC *et al.* (2004a). The use of mini-organ cultures of human upper aerodigestive tract epithelia in ecogenotoxicology. *Mutat Res*, 561: 63–73. PMID:15238231

Klimisch HJ, Gamer AO, Hellwig J *et al.* (1992). Di-(2-ethylhexyl) phthalate: a short-term repeated inhalation toxicity study including fertility assessment. *Food Chem Toxicol*, 30: 915–919. doi:10.1016/0278-6915(92)90175-K PMID:1473784

Kluwe WM, Huff JE, Matthews HB *et al.* (1985). Comparative chronic toxicities and carcinogenic potentials of 2-ethylhexyl-containing compounds in rats and mice. *Carcinogenesis*, 6: 1577–1583. doi:10.1093/carcin/6.11.1577 PMID:4053278

Kluwe WM, McConnell EE, Huff JE *et al.* (1982). Carcinogenicity testing of phthalate esters and related compounds by the National Toxicology Program and the National Cancer Institute. *Environ Health Perspect*, 45: 129–133. doi:10.2307/3429396 PMID:7140685

Koch HM, Angerer J, Drexler H *et al.* (2005a). Di(2-ethylhexyl)phthalate (DEHP) exposure of voluntary plasma and platelet donors. *Int J Hyg Environ Health*, 208: 489–498. doi:10.1016/j.ijheh.2005.07.001 PMID:16325559

Koch HM, Bolt HM, Angerer J (2004b). Di(2-ethylhexyl) phthalate (DEHP) metabolites in human urine and serum after a single oral dose of deuterium-labelled DEHP. *Arch Toxicol*, 78: 123–130. doi:10.1007/s00204-003-0522-3 PMID:14576974

Koch HM, Bolt HM, Preuss R, Angerer J (2005b). New metabolites of di(2-ethylhexyl)phthalate (DEHP) in human urine and serum after single oral doses of deuterium-labelled DEHP. *Arch Toxicol*, 79: 367–376. doi:10.1007/s00204-004-0642-4 PMID:15700144

Koch HM, Drexler H, Angerer J (2003a). An estimation of the daily intake of di(2-ethylhexyl)phthalate (DEHP) and other phthalates in the general population. *Int J Hyg Environ Health*, 206: 77–83. doi:10.1078/1438-4639-00205 PMID:12708228

Koch HM, Drexler H, Angerer J (2004a). Internal exposure of nursery-school children and their parents and teachers to di(2-ethylhexyl)phthalate (DEHP). *Int J Hyg Environ Health*, 207: 15–22. doi:10.1078/1438-4639-00270 PMID:14762970

Koch HM, Preuss R, Angerer J (2006). Di(2-ethylhexyl) phthalate (DEHP): human metabolism and internal exposure– an update and latest results. *Int J Androl*, 29: 155–165, discussion 181–185. doi:10.1111/j.1365-2605.2005.00607.x PMID:16466535

Koch HM, Rossbach B, Drexler H, Angerer J (2003b). Internal exposure of the general population to DEHP and other phthalates–determination of secondary and primary phthalate monoester metabolites in

urine. *Environ Res*, 93: 177–185. doi:10.1016/S0013-9351(03)00083-5 PMID:12963402

Koo HJ & Lee BM (2007). Toxicokinetic relationship between di(2-ethylhexyl) phthalate (DEHP) and mono(2-ethylhexyl) phthalate in rats. *J Toxicol Environ Health A*, 70: 383–387. doi:10.1080/15287390600882150 PMID:17454563

Kornbrust DJ, Barfknecht TR, Ingram P, Shelburne JD (1984). Effect of di(2-ethylhexyl) phthalate on DNA repair and lipid peroxidation in rat hepatocytes and on metabolic cooperation in Chinese hamster V-79 cells. *J Toxicol Environ Health*, 13: 99–116. doi:10.1080/15287398409530484 PMID:6716514

Krüger T, Long M, Bonefeld-Jørgensen EC (2008). Plastic components affect the activation of the aryl hydrocarbon and the androgen receptor. *Toxicology*, 246: 112–123. doi:10.1016/j.tox.2007.12.028 PMID:18294747

Kurata Y, Kidachi F, Yokoyama M *et al.* (1998). Subchronic toxicity of Di(2-ethylhexyl)phthalate in common marmosets: lack of hepatic peroxisome proliferation, testicular atrophy, or pancreatic acinar cell hyperplasia. *Toxicol Sci*, 42: 49–56. PMID:9538047

Kurokawa Y, Takamura N, Matushima Y *et al.* (1988). Promoting effect of peroxisome proliferators in two-stage rat renal tumorigenesis. *Cancer Lett*, 43: 145–149. doi:10.1016/0304-3835(88)90227-3 PMID:3203323

Lacquemant C, Lepretre F, Pineda Torra I *et al.* (2000). Mutation screening of the PPARalpha gene in type 2 diabetes associated with coronary heart disease. *Diabetes Metab*, 26: 393–401. PMID:11119019

Lake BG, Kozlen SL, Evans JG *et al.* (1987). Effect of prolonged administration of clofibric acid and di-(2-ethylhexyl)phthalate on hepatic enzyme activities and lipid peroxidation in the rat. *Toxicology*, 44: 213–228. doi:10.1016/0300-483X(87)90151-X PMID:3564055

Lake BG, Phillips JC, Linnell JC, Gangolli SD (1977). The in vitro hydrolysis of some phthalate diesters by hepatic and intestinal preparations from various species. *Toxicol Appl Pharmacol*, 39: 239–248. doi:10.1016/0041-008X(77)90157-0 PMID:403636

Lamb JC 4th, Chapin RE, Teague J *et al.* (1987). Reproductive effects of four phthalic acid esters in the mouse. *Toxicol Appl Pharmacol*, 88: 255–269. doi:10.1016/0041-008X(87)90011-1 PMID:3564043

Lampen A, Zimnik S, Nau H (2003). Teratogenic phthalate esters and metabolites activate the nuclear receptors PPARs and induce differentiation of F9 cells. *Toxicol Appl Pharmacol*, 188: 14–23. doi:10.1016/S0041-008X(03)00014-0 PMID:12668118

Lapinskas PJ, Brown S, Leesnitzer LM *et al.* (2005). Role of PPARalpha in mediating the effects of phthalates and metabolites in the liver. *Toxicology*, 207: 149–163. doi:10.1016/j.tox.2004.09.008 PMID:15590130

Larsen ST, Hansen JS, Hammer M *et al.* (2004). Effects of mono-2-ethylhexyl phthalate on the respiratory tract in BALB/c mice. *Hum Exp Toxicol*, 23: 537–545. doi:10.1191/0960327104ht486oa PMID:15625780

Larsen ST, Hansen JS, Thygesen P *et al.* (2001). Adjuvant and immuno-suppressive effect of six monophthalates in a subcutaneous injection model with BALB/c mice. *Toxicology*, 169: 37–51. doi:10.1016/S0300-483X(01)00484-X PMID:11696408

Larsen ST, Lund RM, Nielsen GD *et al.* (2002). Adjuvant effect of di-n-butyl-, di-n-octyl-, di-iso-nonyl- and di-iso-decyl phthalate in a subcutaneous injection model using BALB/c mice. *Pharmacol Toxicol*, 91: 264–272. doi:10.1034/j.1600-0773.2002.910508.x PMID:12570034

Larsen ST & Nielsen GD (2007). The adjuvant effect of di-(2-ethylhexyl) phthalate is mediated through a PPARalpha-independent mechanism. *Toxicol Lett*, 170: 223–228. doi:10.1016/j.toxlet.2007.03.009 PMID:17462839

Latini G, De Felice C, Presta G *et al.* (2003). In utero exposure to di-(2-ethylhexyl)phthalate and duration of human pregnancy. *Environ Health Perspect*, 111: 1783–1785. doi:10.1289/ehp.6202 PMID:14594632

Latini G, Wittassek M, Del Vecchio A *et al.* (2009). Lactational exposure to phthalates in Southern Italy. *Environ Int*, 35: 236–239. doi:10.1016/j.envint.2008.06.002 PMID:18684505

Lawrence N, McGregor DB (1985). *Assays for the induction of morphological transformation in C3H/10T–1/2 cells in culture with and without S9-mediated metabolic activation*. In: *Evaluation of Short-term Tests for Carcinogens: Report of the International Programme on Chemical Safety's Collaborative Study on In Vitro Assays.* Ashby J, de Serres FJ, Draper M *et al.*, editors. Amsterdam: Elsevier, pp. 651–658.

Lawrence WH, Malik M, Turner JE *et al.* (1975). A toxicological investigation of some acute, short-term, and chronic effects of administering di-2-ethylhexyl phthalate (DEHP) and other phthalate esters. *Environ Res*, 9: 1–11. doi:10.1016/0013-9351(75)90043-2 PMID:1122902

Lee J, Richburg JH, Younkin SC, Boekelheide K (1997). The Fas system is a key regulator of germ cell apoptosis in the testis. *Endocrinology*, 138: 2081–2088. doi:10.1210/en.138.5.2081 PMID:9112408

Liber HL (1985). *Mutation tests with Salmonella using 8-azaguanine resistance as the genetic marker*. In: *Evaluation of Short-term Tests for Carcinogens: Report of the International Programme on Chemical Safety's Collaborative Study on In Vitro Assays.* Ashby J, de Serres FJ, Draper M *et al.*, editors. Amsterdam: Elsevier, pp. 213–216.

Lide DR, editor (2010). *CRC Handbook of Chemistry and Physics*, 91st ed. Boca Raton, FL: CRC Press, pp. 3–186.

Lindahl-Kiessling K, Karlberg I, Olofsson AM (1989). Induction of sister-chromatid exchanges by direct and indirect mutagens in human lymphocytes, co-cultured with intact rat liver cells. Effect of enzyme induction

and preservation of the liver cells by freezing in liquid nitrogen. *Mutat Res*, 211: 77–87. PMID:2922003

Lindgren A, Lindquist NG, Lydén A *et al.* (1982). A whole body autoradiographic study on the distribution of 14C-labelled di-(2-ethylhexyl)phthalate in mice. *Toxicology*, 23: 149–158. doi:10.1016/0300-483X(82)90094-4 PMID:6810506

Liss GM, Albro PW, Hartle RW, Stringer WT (1985). Urine phthalate determinations as an index of occupational exposure to phthalic anhydride and di(2-ethylhexyl) phthalate. *Scand J Work Environ Health*, 11: 381–387. doi:10.5271/sjweh.2209 PMID:4071004

Liss GM, Hartle RW (1983). *Health Hazard Evaluation Report, Badische Corporation, Kearny, New Jersey* (Report No. HETA 82–032–1384). Cincinnati, OH: National Institute for Occupational Safety and Health.

Liu MH, Li J, Shen P *et al.* (2008). A natural polymorphism in peroxisome proliferator-activated receptor-alpha hinge region attenuates transcription due to defective release of nuclear receptor corepressor from chromatin. *Mol Endocrinol*, 22: 1078–1092. doi:10.1210/me.2007-0547 PMID:18292238

Ljungvall K, Tienpont B, David F *et al.* (2004). Kinetics of orally administered di(2-ethylhexyl) phthalate and its metabolite, mono(2-ethylhexyl) phthalate, in male pigs. *Arch Toxicol*, 78: 384–389. doi:10.1007/s00204-004-0558-z PMID:15022035

Løkke H & Rasmussen L (1983). Phytotoxicological effects of di-(2-ethylhexyl)-phthalate and di-n-butyl-phthalate on higher plants in laboratory and field experiments. *Environ Pollut*, 32: 179–199. doi:10.1016/0143-1471(83)90035-1

López-Carrillo L, Hernández-Ramírez RU, Calafat AM *et al.* (2010). Exposure to phthalates and breast cancer risk in northern Mexico. [PMID:20368132]*Environ Health Perspect*, 118: 539–544. doi:10.1289/ehp.0901091 PMID:20368132

Loprieno N, Boncristiani G, Forster R *et al.* (1985). *Assays for forward mutation in Schizosaccharomyces pombe strain P1.* In: *Evaluation of Short-term Tests for Carcinogens: Report of the International Programme on Chemical Safety's Collaborative Study on In Vitro Assays.* Ashby J, de Serres FJ, Draper M *et al.*, editors. Amsterdam: Elsevier, pp. 297–306.

Lutz WK (1986). Investigation of the potential for binding of di(2-ethylhexyl) phthalate (DEHP) to rat liver DNA in vivo. *Environ Health Perspect*, 65: 267–269. doi:10.2307/3430193 PMID:3709452

Lyche JL, Gutleb AC, Bergman A *et al.* (2009). Reproductive and developmental toxicity of phthalates. *J Toxicol Environ Health B Crit Rev*, 12: 225–249. PMID:20183522

Main KM, Mortensen GK, Kaleva MM *et al.* (2006). Human breast milk contamination with phthalates and alterations of endogenous reproductive hormones in infants three months of age. *Environ Health Perspect*, 114: 270–276. doi:10.1289/ehp.8075 PMID:16451866

Malcolm AR & Mills LJ (1989). Inhibition of gap-junctional intercellular communication between Chinese hamster lung fibroblasts by di(2-ethylhexyl) phthalate (DEHP) and trisodium nitrilotriacetate monohydrate (NTA). *Cell Biol Toxicol*, 5: 145–153. doi:10.1007/BF00122649 PMID:2766028

Malcolm AR, Mills LJ, McKenna EJ (1983). Inhibition of metabolic cooperation between Chinese hamster V79 cells by tumor promoters and other chemicals. *Ann N Y Acad Sci*, 407: 1 Cellular Syst448–450. doi:10.1111/j.1749-6632.1983.tb47859.x

Mallette FS & Von Haam E (1952). Studies on the toxicity and skin effects of compounds used in the rubber and plastics industries. II. Plasticizers. *A M A Arch Ind Hyg Occup Med*, 6: 231–236. PMID:14952047

Maloney EK & Waxman DJ (1999). Trans-activation of PPARalpha and PPARgamma by structurally diverse environmental chemicals. *Toxicol Appl Pharmacol*, 161: 209–218. doi:10.1006/taap.1999.8809 PMID:10581215

Marsman DS, Cattley RC, Conway JG, Popp JA (1988). Relationship of hepatic peroxisome proliferation and replicative DNA synthesis to the hepatocarcinogenicity of the peroxisome proliferators di(2-ethylhexyl)phthalate and [4-chloro-6-(2,3-xylidino)-2-pyrimidinylthio] acetic acid (Wy-14,643) in rats. *Cancer Res*, 48: 6739–6744. PMID:3180084

Martin MT, Dix DJ, Judson RS *et al.* (2010). Impact of environmental chemicals on key transcription regulators and correlation to toxicity end points within EPA's ToxCast program. *Chem Res Toxicol*, 23: 578–590. doi:10.1021/tx900325g PMID:20143881

Martinasso G, Maggiora M, Trombetta A *et al.* (2006). Effects of di(2-ethylhexyl) phthalate, a widely used peroxisome proliferator and plasticizer, on cell growth in the human keratinocyte cell line NCTC 2544. *J Toxicol Environ Health A*, 69: 353–365. doi:10.1080/15287390500227522 PMID:16455614

Matsushima T, Muramatsu M, Haresaku M (1985). *Mutation tests on Salmonella typhimurium by the preincubation method.* In: *Evaluation of Short-term Tests for Carcinogens: Report of the International Programme on Chemical Safety's Collaborative Study on In Vitro Assays.* Ashby J, de Serres FJ, Draper M *et al.*, editors. Amsterdam: Elsevier, pp. 181–186.

Matthews EJ, DelBalzo T, Rundell JO (1985). *Assays for morphological transformation and mutation to ouabain resistance of Balb/c-3T3 cells in culture.* In: *Evaluation of Short-term Tests for Carcinogens: Report of the International Programme on Chemical Safety's Collaborative Study on In Vitro Assays.* Ashby J, de Serres FJ, Draper M *et al.*, editors. Amsterdam: Elsevier, pp. 639–650.

McKee RH; Toxicology Research Task Group, Phthalate Esters Panel American Chemistry Council (2004). Phthalate exposure and early thelarche. *Environ Health*

Perspect, 112: A541–A543. doi:10.1289/ehp.112-a541b PMID:15238295

Meek ME & Chan PKL (1994). Bis(2-ethylhexyl)phthalate: evaluation of risks to health from environmental exposure in Canada. *Environ. Carcinog. Ecotoxicol. Rev*, C12: 179–194. doi:10.1080/10590509409373439

Meek ME, Newhook R, Liteplo RG, Armstrong VC (1994). Approach to assessment of risk to human health for priority substances under the Canadian Environmental Protection Act. *Environ. Carcinog. Ecotoxicol. Rev*, C12: 105–134. doi:10.1080/10590509409373434

Meeker JD, Calafat AM, Hauser R (2007). Di(2-ethylhexyl) phthalate metabolites may alter thyroid hormone levels in men. *Environ Health Perspect*, 115: 1029–1034. doi:10.1289/ehp.9852 PMID:17637918

Meeker JD, Calafat AM, Hauser R (2009b). Urinary metabolites of di(2-ethylhexyl) phthalate are associated with decreased steroid hormone levels in adult men. *J Androl*, 30: 287–297. doi:10.2164/jandrol.108.006403 PMID:19059903

Meeker JD, Hu H, Cantonwine DE *et al.* (2009a). Urinary phthalate metabolites in relation to preterm birth in Mexico city. *Environ Health Perspect*, 117: 1587–1592. PMID:20019910

Mehta RD & von Borstel RC (1985). Tests for genetic activity in the yeast Saccharomyces cerevisiae using strains D7–144, XV185–14C and RM52. *Progress in Mutation Research*, 5: 271–284.

Merkle J, Klimisch HJ, Jäckh R (1988). Developmental toxicity in rats after inhalation exposure of di-2-ethylhexylphthalate (DEHP). *Toxicol Lett*, 42: 215–223. doi:10.1016/0378-4274(88)90080-X PMID:3406961

Mettang T, Thomas S, Kiefer T *et al.* (1996a). The fate of leached di(2-ethylhexyl)phthalate in patients undergoing CAPD treatment. *Perit Dial Int*, 16: 58–62. PMID:8616175

Mettang T, Thomas S, Kiefer T *et al.* (1996b). Uraemic pruritus and exposure to di(2-ethylhexyl) phthalate (DEHP) in haemodialysis patients. *Nephrol Dial Transplant*, 11: 2439–2443. PMID:9017619

Mikalsen SO, Holen I, Sanner T (1990). Morphological transformation and catalase activity of Syrian hamster embryo cells treated with hepatic peroxisome proliferators, TPA and nickel sulphate. *Cell Biol Toxicol*, 6: 1–13. doi:10.1007/BF00135022 PMID:2334865

Mikalsen SO & Sanner T (1993). Intercellular communication in colonies of Syrian hamster embryo cells and the susceptibility for morphological transformation. *Carcinogenesis*, 14: 251–257. doi:10.1093/carcin/14.2.251 PMID:8435866

Mitchell FE, Price SC, Hinton RH *et al.* (1985). Time and dose-response study of the effects on rats of the plasticizer di(2-ethylhexyl) phthalate. *Toxicol Appl Pharmacol*, 81: 371–392. doi:10.1016/0041-008X(85)90409-0 PMID:2867621

Miura Y, Naito M, Ablake M *et al.* (2007). Short-term effects of di-(2-ethylhexyl) phthalate on testes, liver, kidneys and pancreas in mice. *Asian J Androl*, 9: 199–205. doi:10.1111/j.1745-7262.2007.00220.x PMID:16855774

Mnif W, Pascussi JM, Pillon A *et al.* (2007). Estrogens and antiestrogens activate hPXR. *Toxicol Lett*, 170: 19–29. doi:10.1016/j.toxlet.2006.11.016 PMID:17379461

Moore RW, Rudy TA, Lin TM *et al.* (2001). Abnormalities of sexual development in male rats with in utero and lactational exposure to the antiandrogenic plasticizer Di(2-ethylhexyl) phthalate. *Environ Health Perspect*, 109: 229–237. doi:10.1289/ehp.01109229 PMID:11333183

Morimura K, Cheung C, Ward JM *et al.* (2006). Differential susceptibility of mice humanized for peroxisome proliferator-activated receptor alpha to Wy-14,643-induced liver tumorigenesis. *Carcinogenesis*, 27: 1074–1080. doi:10.1093/carcin/bgi329 PMID:16377806

Mortensen A, Bertram M, Aarup V, Sørensen IK (2002). Assessment of carcinogenicity of di(2-ethylhexyl) phthalate in a short-term assay using Xpa-/- and Xpa-/-/p53+/- mice. *Toxicol Pathol*, 30: 188–199. doi:10.1080/019262302753559524 PMID:11950162

Moser VC, Cheek BM, MacPhail RC (1995). A multi-disciplinary approach to toxicological screening: III. Neurobehavioral toxicity. *J Toxicol Environ Health*, 45: 173–210. doi:10.1080/15287399509531988 PMID:7783252

Mukherjee R, Jow L, Noonan D, McDonnell DP (1994). Human and rat peroxisome proliferator activated receptors (PPARs) demonstrate similar tissue distribution but different responsiveness to PPAR activators. *J Steroid Biochem Mol Biol*, 51: 157–166. doi:10.1016/0960-0760(94)90089-2 PMID:7981125

Müller-Tegethoff K, Kasper P, Müller L (1995). Evaluation studies on the in vitro rat hepatocyte micronucleus assay. *Mutat Res*, 335: 293–307. PMID:8524345

Murray HE, Ray LE, Giam CS (1981). Phthalic acid esters, total DDTs, and polychlorinated biphenyls in marine samples from Galveston Bay, Texas. *Bull Environ Contam Toxicol*, 26: 769–774. doi:10.1007/BF01622169 PMID:6789916

Musial CJ, Uthe JF, Sirota GR *et al.* (1981). Di-*n*-hexyl phthalate (DHP), a newly identified contaminant in Atlantic herring (*Clupea harengus larengus*) and Atlantic mackerel (*Scomber scombus*). *Can J Fish Aquat Sci*, 38: 856–859. doi:10.1139/f81-113

Myhr B, Bowers L, Caspary WJ (1985). *Assays for the induction of gene mutations at the thymidine kinase locus in L5178Y mouse lymphoma cells in culture*. In: *Evaluation of Short-term Tests for Carcinogens: Report of the International Programme on Chemical Safety's Collaborative Study on In Vitro Assays*. Ashby J, de Serres FJ, Draper M *et al.*, editors. Amsterdam: Elsevier, pp. 555–568.

Nair KG, Deepadevi KV, Arun P *et al.* (1998). Toxic effect of systemic administration of low doses of the

plasticizer di-(2-ethyl hexyl) phthalate [DEHP] in rats. *Indian J Exp Biol*, 36: 264–272. PMID:9754059

Nakamura R, Teshima R, Sawada J (2002). Effect of dialkyl phthalates on the degranulation and Ca2+ response of RBL-2H3 mast cells. *Immunol Lett*, 80: 119–124. doi:10.1016/S0165-2478(01)00318-2 PMID:11750043

Narotsky MG & Kavlock RJ (1995). A multidisciplinary approach to toxicological screening: II. Developmental toxicity. *J Toxicol Environ Health*, 45: 145–171. doi:10.1080/15287399509531987 PMID:7783251

Nässberger L, Arbin A, Östelius J (1987). Exposure of patients to phthalates from polyvinyl chloride tubes and bags during dialysis. *Nephron*, 45: 286–290. doi:10.1159/000184165 PMID:3587468

National Library of Medicine (2011). *Toxic Chemical Release Inventory (TRI) Databases*. Available at: http://toxnet.nlm.nih.gov/cgi-bin/sis/htmlgen?TRI

Nielsen J, Åkesson B, Skerfving S (1985). Phthalate ester exposure–air levels and health of workers processing polyvinylchloride. *Am Ind Hyg Assoc J*, 46: 643–647. doi:10.1080/15298668591395463 PMID:4072908

NIOSH (2003). *Manual of Analytical Methods (NMAM) Di(2-ethylhexyl) Phthalate. Method 5020*. DHHS (NIOSH) Pub. No. 2003–154. Schlect PC, O'Connor PF, editors. Cincinnati, OH: National Institute for Occupational Safety and Health.

NOES (1999). *National Occupational Exposure Survey 1981–83*. Unpublished data as of July 1999. Cincinnati, OH: Department of Health and Human Services, Public Health Service, Centers for Disease Control, National Institute for Occupational Safety and Health.

Nohmi T, Miyata R, Yoshikawa K, Ishidate M Jr (1985). [Mutagenicity tests on organic chemical contaminants in city water and related compounds. I. Bacterial mutagenicity tests] *Eisei Shikenjo Hokoku*, 103: 6O–4. PMID:3830314

NTIS (1988). *Reproduction and Fertility Evaluation of Diethylhexyl Phthalate in CD-1 Mice Exposed during Gestation*. Springfield, VA.

NTP (1982). Carcinogenesis Bioassay of Di(2-ethylhexyl) phthalate (CAS No. 117–81–7) in F344 Rats and B6C3F$_1$ Mice (Feed Studies). *Natl Toxicol Program Tech Rep Ser*, 217: 1–127. PMID:12778218

NTP (1997). Diethylhexylphthalate. *Environ Health Perspect*, 105: 241–242. doi:10.2307/3433425 PMID:9114305

Obe G, Hille A, Jonas R *et al.* (1985). *Tests for the induction of sister-chromatid exchanges in human peripheral lymphocytes in culture*. In: *Evaluation of Short-term Tests for Carcinogens: Report of the International Programme on Chemical Safety's Collaborative Study on In Vitro Assays*. Ashby J, de Serres FJ, Draper M *et al.*, editors. Amsterdam: Elsevier, pp. 439–442.

Oberly TJ, Bewsey BJ, Probst GS (1985). *Tests for the induction of forward mutation at the thymidine kinase locus of L5178Y mouse lymphoma cells in culture*. In: *Evaluation of Short-term Tests for Carcinogens: Report of the International Programme on Chemical Safety's Collaborative Study on In Vitro Assays*. Ashby J, de Serres FJ, Draper M *et al.*, editors. Amsterdam: Elsevier, pp. 569–582.

Ochs JB, Baldwin LA, Leonard DA, Calabrese EJ (1992). Effects of joint exposures to selected peroxisome proliferators on hepatic acyl-CoA oxidase activity in male B6C3F$_1$ mice. *Hum Exp Toxicol*, 11: 83–88. doi:10.1177/096032719201100204 PMID:1349224

Ohlson CG & Hardell L (2000). Testicular cancer and occupational exposures with a focus on xenoestrogens in polyvinyl chloride plastics. *Chemosphere*, 40: 1277–1282. doi:10.1016/S0045-6535(99)00380-X PMID:10739073

Ohta M, Kitamura J, Fukuwatari T *et al.* (2004). Effects of dietary di(2-ethylhexyl)phthalate on the metabolism of tryptophan to niacin in mice. *Exp Anim*, 53: 57–60. doi:10.1538/expanim.53.57 PMID:14993743

Øie L, Hersoug L-G, Madsen JØ (1997). Residential exposure to plasticizers and its possible role in the pathogenesis of asthma. *Environ Health Perspect*, 105: 972–978. doi:10.1289/ehp.97105972 PMID:9374082

Oishi S (1985). Reversibility of testicular atrophy induced by di(2-ethylhexyl) phthalate in rats. *Environ Res*, 36: 160–169. doi:10.1016/0013-9351(85)90014-3 PMID:3967638

Okai Y & Higashi-Okai K (2000). Enhancing effect of a plastic plasticizer, di-2-ethylhexyl phthalate on umu C gene expression in Salmonella typhimurium (TA 1535/pSK 1002). *J UOEH*, 22: 305–315. PMID:11132519

Ono H, Saito Y, Imai K, Kato M (2004). Subcellular distribution of di-(2-ethylhexyl)phthalate in rat testis. *J Toxicol Sci*, 29: 113–124. doi:10.2131/jts.29.113 PMID:15206579

Page BD & Lacroix GM (1992). Studies into the transfer and migration of phthalate esters from aluminium foil-paper laminates to butter and margarine. *Food Addit Contam*, 9: 197–212. doi:10.1080/02652039209374064 PMID:1397395

Page BD & Lacroix GM (1995). The occurrence of phthalate ester and di-2-ethylhexyl adipate plasticizers in Canadian packaging and food sampled in 1985–1989: a survey. *Food Addit Contam*, 12: 129–151. doi:10.1080/02652039509374287 PMID:7758627

Palmer CN, Hsu MH, Griffin KJ *et al.* (1998). Peroxisome proliferator activated receptor-alpha expression in human liver. *Mol Pharmacol*, 53: 14–22. PMID:9443928

Pan G, Hanaoka T, Yoshimura M *et al.* (2006). Decreased serum free testosterone in workers exposed to high levels of di-n-butyl phthalate (DBP) and di-2-ethylhexyl phthalate (DEHP): a cross-sectional study in China. *Environ Health Perspect*, 114: 1643–1648. PMID:17107847

Pant K, Sly JE, Bruce SW *et al.* (2010). Syrian Hamster Embryo (SHE) cell transformation assay with and

without X-ray irradiation of feeder cells using di(2-ethylhexyl)phthalate (DEHP) and N-nitroso-N-methylnitroguanidine (MNNG). *Mutat Res*, 698: 6–10. PMID:20226874

Park K & Kwak IS (2008). Characterization of heat shock protein 40 and 90 in Chironomus riparius larvae: effects of di(2-ethylhexyl) phthalate exposure on gene expressions and mouthpart deformities. *Chemosphere*, 74: 89–95. doi:10.1016/j.chemosphere.2008.09.041 PMID:18977013

Park SY & Choi J (2007). Cytotoxicity, genotoxicity and ecotoxicity assay using human cell and environmental species for the screening of the risk from pollutant exposure. *Environ Int*, 33: 817–822. doi:10.1016/j.envint.2007.03.014 PMID:17499852

Parks LG, Ostby JS, Lambright CR *et al.* (2000). The plasticizer diethylhexyl phthalate induces malformations by decreasing fetal testosterone synthesis during sexual differentiation in the male rat. *Toxicol Sci*, 58: 339–349. doi:10.1093/toxsci/58.2.339 PMID:11099646

Parmar D, Srivastava SP, Seth PK (1986). Effect of di(2-ethylhexyl)phthalate (DEHP) on spermatogenesis in adult rats. *Toxicology*, 42: 47–55. doi:10.1016/0300-483X(86)90091-0 PMID:2879365

Parmar D, Srivastava SP, Seth PK (1988). Effect of di(2-ethylhexyl)phthalate (DEHP) on hepatic mixed function oxidases in different animal species. *Toxicol Lett*, 40: 209–217. doi:10.1016/0378-4274(88)90043-4 PMID:3354005

Parmar D, Srivastava SP, Srivastava SP, Seth PK (1985). Hepatic mixed function oxidases and cytochrome P-450 contents in rat pups exposed to di-(2-ethylhexyl) phthalate through mother's milk. *Drug Metab Dispos*, 13: 368–370. PMID:2861998

Parry EM (1985). *Tests for the effects on mitosis and the mitotic spindle in Chinese hamster primary liver cells (CH1-L) in culture*. In: *Evaluation of Short-term Tests for Carcinogens: Report of the International Programme on Chemical Safety's Collaborative Study on In Vitro Assays*. Ashby J, de Serres FJ, Draper M *et al.*, editors. Amsterdam: Elsevier, pp. 479–485.

Parry JM & Eckardt F (1985). The detection of mitogenic gene conversion, point mutation and mitotic segregation using the yeast Saccharomyces cervisiae strain D7. *Progress in Mutation Research*, 5: 261–269.

Peakall DB (1975). Phthalate esters: occurrence and biological effects. *Residue Rev*, 54: 1–41. PMID:1093245

Peck CC & Albro PW (1982). Toxic potential of the plasticizer di(2-ethylhexyl) phthalate in the context of its disposition and metabolism in primates and man. *Environ Health Perspect*, 45: 11–17. doi:10.2307/3429378 PMID:7140682

Peck CC, Albro PW, Hass JR *et al.* (1978). Metabolism and excretion of the plasticizer di-(2-ethylhexyl) phthalate in man. *Clin Res*, 25: 101A

Persson PE, Penttinen H, Nuorteva P (1978). DEHP in the vicinity of an industrial area in Finland. *Environ Pollut*, 16: 163–166. doi:10.1016/0013-9327(78)90130-1

Peters JM, Taubeneck MW, Keen CL, Gonzalez FJ (1997). Di(2-ethylhexyl) phthalate induces a functional zinc deficiency during pregnancy and teratogenesis that is independent of peroxisome proliferator-activated receptor-alpha. *Teratology*, 56: 311–316. doi:10.1002/(SICI)1096-9926(199711)56:5<311::AID-TERA4>3.0.CO;2-# PMID:9451755

Petersen JH (1991). Survey of di-(2-ethylhexyl) phthalate plasticizer contamination of retail Danish milks. *Food Addit Contam*, 8: 701–705. doi:10.1080/02652039109374027 PMID:1812016

Petersen JH & Breindahl T (2000). Plasticizers in total diet samples, baby food and infant formulae. *Food Addit Contam*, 17: 133–141. doi:10.1080/026520300283487 PMID:10793844

Peterson JC & Freeman DH (1982). Phthalate ester concentration variations in dated sediment cores from the Chesapeake Bay. *Environ Sci Technol*, 16: 464–469. doi:10.1021/es00102a007

Phillips BJ, James TE, Gangolli SD (1982). Genotoxicity studies of di(2-ethylhexyl)phthalate and its metabolites in CHO cells. *Mutat Res*, 102: 297–304. doi:10.1016/0165-1218(82)90139-2 PMID:6890626

Plonait SL, Nau H, Maier RF *et al.* (1993). Exposure of newborn infants to di-(2-ethylhexyl)-phthalate and 2-ethylhexanoic acid following exchange transfusion with polyvinylchloride catheters. *Transfusion*, 33: 598–605. doi:10.1046/j.1537-2995.1993.33793325058.x PMID:8333024

Pogribny IP, Tryndyak VP, Boureiko A *et al.* (2008). Mechanisms of peroxisome proliferator-induced DNA hypomethylation in rat liver. *Mutat Res*, 644: 17–23. PMID:18639561

Pollack GM, Buchanan JF, Slaughter RL *et al.* (1985). Circulating concentrations of di(2-ethylhexyl) phthalate and its de-esterified phthalic acid products following plasticizer exposure in patients receiving hemodialysis. *Toxicol Appl Pharmacol*, 79: 257–267. doi:10.1016/0041-008X(85)90347-3 PMID:4002227

Poole M, Bridgers K, Alexson SE, Corton JC (2001). Altered expression of the carboxylesterases ES-4 and ES-10 by peroxisome proliferator chemicals. *Toxicology*, 165: 109–119. doi:10.1016/S0300-483X(01)00416-4 PMID:11522369

Poon R, Lecavalier P, Mueller R *et al.* (1997). Subchronic oral toxicity of di-n-octyl phthalate and di(2-ethylhexyl) phthalate in the rat. *Food Chem Toxicol*, 35: 225–239. doi:10.1016/S0278-6915(96)00064-6 PMID:9146736

Popp JA, Garvey LK, Hamm TE Jr, Swenberg JA (1985). Lack of hepatic promotional activity by the peroxisomal proliferating hepatocarcinogen di(2-ethylhexyl) phthalate. *Carcinogenesis*, 6: 141–144. doi:10.1093/carcin/6.1.141 PMID:2857115

Preston MR & Al-Omran LA (1989). Phthalate ester speciation in estuarine water, suspended particulates and sediments. *Environ Pollut*, 62: 183–193. doi:10.1016/0269-7491(89)90186-3 PMID:15092344

Priston RA, Dean BJ (1985). *Tests for the induction of chromosome aberrations, polyploidy and sister-chromatic exchanges in rat liver (RL4) cells*. In: *Evaluation of Short-term Tests for Carcinogens: Report of the International Programme on Chemical Safety's Collaborative Study on In Vitro Assays*. Ashby J, de Serres FJ, Draper M *et al.*, editors. Amsterdam: Elsevier, pp. 387–395.

Probst GS, Hill LE (1985). *Tests for the induction of DNA-repair synthesis in primary cultures of adult rat hepatocytes*. In: *Evaluation of Short-term Tests for Carcinogens: Report of the International Programme on Chemical Safety's Collaborative Study on In Vitro Assays*. Ashby J, de Serres FJ, Draper M *et al.*, editors. Amsterdam: Elsevier, pp. 381–386.

Pugh G Jr, Isenberg JS, Kamendulis LM *et al.* (2000). Effects of di-isononyl phthalate, di-2-ethylhexyl phthalate, and clofibrate in cynomolgus monkeys. *Toxicol Sci*, 56: 181–188. doi:10.1093/toxsci/56.1.181 PMID:10869467

Putman DL, Moore WA, Schechtman LM, Hodgson JR (1983). Cytogenetic evaluation of di-(2-ethylhexyl) phthalate and its major metabolites in Fischer 344 rats. *Environ Mutagen*, 5: 227–231. doi:10.1002/em.2860050211 PMID:6861726

Rael LT, Bar-Or R, Ambruso DR *et al.* (2009). Phthalate esters used as plasticizers in packed red blood cell storage bags may lead to progressive toxin exposure and the release of pro-inflammatory cytokines. *Oxid Med Cell Longev*, 2: 166–171. doi:10.4161/oxim.2.3.8608 PMID:20592772

Rais-Bahrami K, Nunez S, Revenis ME *et al.* (2004). Follow-up study of adolescents exposed to di(2-ethylhexyl) phthalate (DEHP) as neonates on extracorporeal membrane oxygenation (ECMO) support. *Environ Health Perspect*, 112: 1339–1340. doi:10.1289/ehp.6901 PMID:15345350

Rank J, Lopez LC, Nielsen MH, Moretton J (2002). Genotoxicity of maleic hydrazide, acridine and DEHP in Allium cepa root cells performed by two different laboratories. *Hereditas*, 136: 13–18. doi:10.1034/j.1601-5223.2002.1360103.x PMID:12184484

Rao MS, Lalwani ND, Scarpelli DG, Reddy JK (1982). The absence of gamma-glutamyl transpeptidase activity in putative preneoplastic lesions and in hepatocellular carcinomas induced in rats by the hypolipidemic peroxisome proliferator Wy-14,643. *Carcinogenesis*, 3: 1231–1233. doi:10.1093/carcin/3.10.1231 PMID:6129072

Rao MS, Usuda N, Subbarao V, Reddy JK (1987). Absence of gamma-glutamyl transpeptidase activity in neoplastic lesions induced in the liver of male F-344 rats by di-(2-ethylhexyl)phthalate, a peroxisome proliferator. *Carcinogenesis*, 8: 1347–1350. doi:10.1093/carcin/8.9.1347 PMID:2887302

Ray LE, Murray HE, Giam CS (1983). Analysis of water and sediment from the Nueces Estuary/ Corpus Christi Bay (Texas) for selected organic pollutants. *Chemosphere*, 12: 1039–1045. doi:10.1016/0045-6535(83)90256-4

Reddy BS, Rozati R, Reddy BV, Raman NV (2006). Association of phthalate esters with endometriosis in Indian women. *BJOG*, 113: 515–520. doi:10.1111/j.1471-0528.2006.00925.x PMID:16637895

Reddy JK, Reddy MK, Usman MI *et al.* (1986). Comparison of hepatic peroxisome proliferative effect and its implication for hepatocarcinogenicity of phthalate esters, di(2-ethylhexyl) phthalate, and di(2-ethylhexyl) adipate with a hypolipidemic drug. *Environ Health Perspect*, 65: 317–327. doi:10.2307/3430199 PMID:3709457

Ren H, Aleksunes LM, Wood C *et al.* (2010). Characterization of peroxisome proliferator-activated receptor alpha–independent effects of PPARalpha activators in the rodent liver: di-(2-ethylhexyl) phthalate also activates the constitutive-activated receptor. *Toxicol Sci*, 113: 45–59. doi:10.1093/toxsci/kfp251 PMID:19850644

Rexroat MA, Probst GS (1985). *Mutation tests with Salmonella using the plateincorporation assay*. In: *Evaluation of Short-term Tests for Carcinogens: Report of the International Programme on Chemical Safety's Collaborative Study on In Vitro Assays*. Ashby J, de Serres FJ, Draper M *et al.*, editors. Amsterdam: Elsevier, pp. 201–212.

Rhind SM, Kyle CE, Mackie C, McDonald L (2009). Accumulation of endocrine disrupting compounds in sheep fetal and maternal liver tissue following exposure to pastures treated with sewage sludge. *J Environ Monit*, 11: 1469–1476. doi:10.1039/b902085c PMID:19657530

Rhodes C, Orton TC, Pratt IS *et al.* (1986). Comparative pharmacokinetics and subacute toxicity of di(2-ethylhexyl) phthalate (DEHP) in rats and marmosets: extrapolation of effects in rodents to man. *Environ Health Perspect*, 65: 299–307. doi:10.2307/3430197 PMID:3086078

Richburg JH & Boekelheide K (1996). Mono-(2-ethylhexyl) phthalate rapidly alters both Sertoli cell vimentin filaments and germ cell apoptosis in young rat testes. *Toxicol Appl Pharmacol*, 137: 42–50. doi:10.1006/taap.1996.0055 PMID:8607140

Ritsema R, Cofino WP, Frintrop PCM, Brinkman UAT (1989). Trace-level analysis of phthalate esters in surface water and suspended particulate matter by means of capillary gas chromatography with electron-capture and mass-selective detection. *Chemosphere*, 18: 2161–2175.

Ritter EJ, Scott WJ Jr, Randall JL, Ritter JM (1987). Teratogenicity of di(2-ethylhexyl) phthalate, 2-ethylhexanol, 2-ethylhexanoic acid, and valproic acid, and potentiation by caffeine. *Teratology*, 35: 41–46. doi:10.1002/tera.1420350107 PMID:3105103

Roberts DR (1983). *Summary Report NIOSH-EPA Interagency Agreement for the Assessment of Human Health Effects from Exposure to Di-2-(Ethylhexyl) Phthalate*. Cincinnati, OH: National Institute for Occupational Safety and Health.

Roberts RA (1999). Peroxisome proliferators: mechanisms of adverse effects in rodents and molecular basis for species differences. *Arch Toxicol*, 73: 413–418. doi:10.1007/s002040050629 PMID:10650911

Roberts RA, James NH, Hasmall SC *et al.* (2000). Apoptosis and proliferation in nongenotoxic carcinogenesis: species differences and role of PPARalpha. *Toxicol Lett*, 112-113: 49–57. doi:10.1016/S0378-4274(99)00243-X PMID:10720712

Robertson IG, Sivarajah K, Eling TE, Zeiger E (1983). Activation of some aromatic amines to mutagenic products by prostaglandin endoperoxide synthetase. *Cancer Res*, 43: 476–480. PMID:6336656

Rock G, Labow RS, Franklin C *et al.* (1987). Hypotension and cardiac arrest in rats after infusion of mono(2-ethylhexyl) phthalate (MEHP), a contaminant of stored blood. *N Engl J Med*, 316: 1218–1219. doi:10.1056/NEJM198705073161915 PMID:3574376

Rock G, Labow RS, Tocchi M (1986). Distribution of di(2-ethylhexyl) phthalate and products in blood and blood components. *Environ Health Perspect*, 65: 309–316. PMID:3709456

Rock G, Secours VE, Franklin CA *et al.* (1978). The accumulation of mono-2-ethylhexylphthalate (MEHP) during storage of whole blood and plasma. *Transfusion*, 18: 553–558. doi:10.1046/j.1537-2995.1978.18579036383.x PMID:705860

Rose ML, Rivera CA, Bradford BU *et al.* (1999). Kupffer cell oxidant production is central to the mechanism of peroxisome proliferators. *Carcinogenesis*, 20: 27–33. doi:10.1093/carcin/20.1.27 PMID:9934846

Rosen MB, Abbott BD, Wolf DC *et al.* (2008). Gene profiling in the livers of wild-type and PPARalpha-null mice exposed to perfluorooctanoic acid. *Toxicol Pathol*, 36: 592–607. doi:10.1177/0192623308318208 PMID:18467677

Roth B, Herkenrath P, Lehmann H-J *et al.* (1988). Di-(2-ethylhexyl)-phthalate as plasticizer in PVC respiratory tubing systems: indications of hazardous effects on pulmonary function in mechanically ventilated, preterm infants. *Eur J Pediatr*, 147: 41–46. doi:10.1007/BF00442609 PMID:3422189

Rotroff DM, Beam AL, Dix DJ *et al.* (2010). Xenobiotic-metabolizing enzyme and transporter gene expression in primary cultures of human hepatocytes modulated by ToxCast chemicals. *J Toxicol Environ Health B Crit Rev*, 13: 329–346. doi:10.1080/10937404.2010.483949 PMID:20574906

Rozati R, Reddy PP, Reddanna P, Mujtaba R (2002). Role of environmental estrogens in the deterioration of male factor fertility. *Fertil Steril*, 78: 1187–1194. doi:10.1016/S0015-0282(02)04389-3 PMID:12477510

RTECS (Registry of Toxic Effects of Chemical Substances Database) (2009). *Phthalic Acid, Bis (2 - ethylhexyl) Ester*. (RTECS: TI0350000) CAS: 117-81-7

Russell DJ & McDuffie B (1983). Analysis for phthalate esters in environmental samples: separation from PCBs and pesticides using dual column liquid chromatography. *Int J Environ Anal Chem*, 15: 165–183. doi:10.1080/03067318308071916

Rusyn I, Denissenko MF, Wong VA *et al.* (2000). Expression of base excision repair enzymes in rat and mouse liver is induced by peroxisome proliferators and is dependent upon carcinogenic potency. *Carcinogenesis*, 21: 2141–2145. doi:10.1093/carcin/21.12.2141 PMID:11133801

Rusyn I, Kadiiska MB, Dikalova A *et al.* (2001). Phthalates rapidly increase production of reactive oxygen species in vivo: role of Kupffer cells. *Mol Pharmacol*, 59: 744–750. PMID:11259618

Sanchez JH, Abernethy DJ, Boreiko CJ (1987). Lack of di-(2-ethylhexyl) phthalate activity in the C3H 10T 1 2 cell transformation system. *Toxicol In Vitro*, 1: 49–53. doi:10.1016/0887-2333(87)90038-5 PMID:20702379

Sanner T & Rivedal E (1985). Tests with the Syrian hamster embryo (SHE) cell transformation assay. *Progress in Mutation Research*, 5: 665–671.

Sapone A, Peters JM, Sakai S *et al.* (2000). The human peroxisome proliferator-activated receptor alpha gene: identification and functional characterization of two natural allelic variants. *Pharmacogenetics*, 10: 321–333. doi:10.1097/00008571-200006000-00006 PMID:10862523

Sasakawa S & Mitomi Y (1978). Di-2-ethylhexylphthalate (DEHP) content of blood or blood components stored in plastic bags. *Vox Sang*, 34: 81–86. doi:10.1111/j.1423-0410.1978.tb03727.x PMID:622821

Schmezer P, Pool BL, Klein RG *et al.* (1988). Various short-term assays and two long-term studies with the plasticizer di(2-ethylhexyl)phthalate in the Syrian golden hamster. *Carcinogenesis*, 9: 37–43. doi:10.1093/carcin/9.1.37 PMID:3335045

Schmid P & Schlatter C (1985). Excretion and metabolism of di(2-ethylhexyl)phthalate in man. *Xenobiotica*, 15: 251–256. doi:10.3109/00498258509045356 PMID:4024660

Schulz CO, Rubin RJ, Hutchins GM (1975). Acute lung toxicity and sudden death in rats following the intravenous administration of the plasticizer, di(2-ethylhexyl) phthalate, solubilized with Tween surfactants. *Toxicol Appl Pharmacol*, 33: 514–525. doi:10.1016/0041-008X(75)90077-0 PMID:1188948

Schwartz HE, Anzion CJM, Van Vliet HPM *et al.* (1979). Analysis of phthalate in sediments from Dutch rivers by means of high performance liquid chromatography. *Int J Environ Anal Chem*, 6: 133–144. doi:10.1080/03067317908071167

SciFinder (2010). *SciFinder Databases: Registry, Chemcats 143*. American Chemical Society.

Selenskas S, Teta MJ, Vitale JN (1995). Pancreatic cancer among workers processing synthetic resins. *Am J Ind Med*, 28: 385–398. doi:10.1002/ajim.4700280308 PMID:7485192

Seo KW, Kim KB, Kim YJ *et al.* (2004). Comparison of oxidative stress and changes of xenobiotic metabolizing enzymes induced by phthalates in rats. *Food Chem Toxicol*, 42: 107–114. doi:10.1016/j.fct.2003.08.010 PMID:14630134

Sérée E, Villard PH, Pascussi JM *et al.* (2004). Evidence for a new human CYP1A1 regulation pathway involving PPAR-alpha and 2 PPRE sites. *Gastroenterology*, 127: 1436–1445. doi:10.1053/j.gastro.2004.08.023 PMID:15521013

Shaban Z, El-Shazly S, Ishizuka M *et al.* (2004). PPARalpha-dependent modulation of hepatic CYP1A by clofibric acid in rats. *Arch Toxicol*, 78: 496–507. doi:10.1007/s00204-004-0569-9 PMID:15127182

Shaffer CB, Carpenter CP, Smyth HF Jr (1945). Acute and subacute toxicity of di(2-ethylhexyl)phthalate with note upon its metabolism. *J Ind Hyg Toxicol*, 27: 130–135.

Sharman M, Read WA, Castle L, Gilbert J (1994). Levels of di-(2-ethylhexyl)phthalate and total phthalate esters in milk, cream, butter and cheese. *Food Addit Contam*, 11: 375–385. doi:10.1080/02652039409374236 PMID:7926171

Sher T, Yi HF, McBride OW, Gonzalez FJ (1993). cDNA cloning, chromosomal mapping, and functional characterization of the human peroxisome proliferator activated receptor. *Biochemistry*, 32: 5598–5604. doi:10.1021/bi00072a015 PMID:7684926

Shiota K & Mima S (1985). Assessment of the teratogenicity of di(2-ethylhexyl)phthalate and mono(2-ethylhexyl)phthalate in mice. *Arch Toxicol*, 56: 263–266. doi:10.1007/BF00295165 PMID:3994510

Shiota K & Nishimura H (1982). Teratogenicity of di(2-ethylhexyl) phthalate (DEHP) and di-n-butyl phthalate (DBP) in mice. *Environ Health Perspect*, 45: 65–70. doi:10.1289/ehp.824565 PMID:7140698

Shirota M, Saito Y, Imai K *et al.* (2005). Influence of di-(2-ethylhexyl)phthalate on fetal testicular development by oral administration to pregnant rats. *J Toxicol Sci*, 30: 175–194. doi:10.2131/jts.30.175 PMID:16141652

Shneider B, Schena J, Truog R *et al.* (1989). Exposure to di(2-ethylhexyl)phthalate in infants receiving extracorporeal membrane oxygenation. *N Engl J Med*, 320: 1563 doi:10.1056/NEJM198906083202322 PMID:2725593

Short RD, Robinson EC, Lington AW, Chin AE (1987). Metabolic and peroxisome proliferation studies with di(2-ethylhexyl)phthalate in rats and monkeys. *Toxicol Ind Health*, 3: 185–195. PMID:3617067

Siddiqui A & Srivastava SP (1992). Effect of di(2-ethylhexyl)phthalate administration on rat sperm count and on sperm metabolic enzymes. *Bull Environ Contam Toxicol*, 48: 115–119. doi:10.1007/BF00197492 PMID:1581667

Silva MJ, Reidy JA, Herbert AR *et al.* (2004). Detection of phthalate metabolites in human amniotic fluid. *Bull Environ Contam Toxicol*, 72: 1226–1231. doi:10.1007/s00128-004-0374-4 PMID:15362453

Silva MJ, Samandar E, Preau JL Jr *et al.* (2006). Urinary oxidative metabolites of di(2-ethylhexyl) phthalate in humans. *Toxicology*, 219: 22–32. doi:10.1016/j.tox.2005.10.018 PMID:16332407

Singh AR, Lawrence WH, Autian J (1972). Teratogenicity of phthalate esters in rats. *J Pharm Sci*, 61: 51–55. doi:10.1002/jps.2600610107 PMID:5058645

Singh AR, Lawrence WH, Autian J (1974). Mutagenic and antifertility sensitivities of mice to di-2-ethylhexyl phthalate (DEHP) and dimethoxyethyl phthalate (DMEP). *Toxicol Appl Pharmacol*, 29: 35–46. doi:10.1016/0041-008X(74)90159-8 PMID:4283679

Sjöberg P, Bondesson U, Gray TJ, Plöen L (1986a). Effects of di-(2-ethylhexyl) phthalate and five of its metabolites on rat testis in vivo and in in vitro. *Acta Pharmacol Toxicol (Copenh)*, 58: 225–233. doi:10.1111/j.1600-0773.1986.tb00098.x PMID:3716815

Sjöberg P, Bondesson U, Kjellen L *et al.* (1985c). Kinetics of di-(2-ethylhexyl) phthalate in immature and mature rats and effect on testis. *Acta Pharmacol Toxicol (Copenh)*, 56: 30–37. doi:10.1111/j.1600-0773.1985.tb01249.x PMID:3976401

Sjöberg P, Bondesson U, Sedin G, Gustafsson J (1985b). Dispositions of di- and mono-(2-ethylhexyl) phthalate in newborn infants subjected to exchange transfusions. *Eur J Clin Invest*, 15: 430–436. doi:10.1111/j.1365-2362.1985.tb00297.x PMID:3938415

Sjöberg P, Lindqvist NG, Plöen L (1986b). Age-dependent response of the rat testes to di(2-ethylhexyl) phthalate. *Environ Health Perspect*, 65: 237–242. doi:10.2307/3430188 PMID:3709447

Sjöberg POJ, Bondesson UG, Sedin EG, Gustafsson JP (1985a). Exposure of newborn infants to plasticizers. Plasma levels of di-(2-ethylhexyl) phthalate and mono-(2-ethylhexyl) phthalate during exchange transfusion. *Transfusion*, 25: 424–428. doi:10.1046/j.1537-2995.1985.25586020115.x PMID:4049487

Smith-Oliver T & Butterworth BE (1987). Correlation of the carcinogenic potential of di(2-ethylhexyl) phthalate (DEHP) with induced hyperplasia rather than with genotoxic activity. *Mutat Res*, 188: 21–28. doi:10.1016/0165-1218(87)90110-8 PMID:3574334

Stanley LA, Horsburgh BC, Ross J *et al.* (2006). PXR and CAR: nuclear receptors which play a pivotal role in drug disposition and chemical toxicity. *Drug Metab Rev*, 38: 515–597. doi:10.1080/03602530600786232 PMID:16877263

Staples CA, Peterson DR, Parkerton TF, Adams WJ (1997). The environmental fate of phthalate esters:

a literature review. *Chemosphere*, 35: 667–749. doi:10.1016/S0045-6535(97)00195-1

Staples CA, Werner AF, Hoogheem TJ (1985). Assessment of priority pollutant concentrations in the United States using STORET data base. *Environ Toxicol Chem*, 4: 131–142. doi:10.1002/etc.5620040202

Stefanini S, Serafini B, Nardacci R *et al.* (1995). Morphometric analysis of liver and kidney peroxisomes in lactating rats and their pups after treatment with the peroxisomal proliferator di-(2-ethylhexyl) phthalate. *Biol Cell*, 85: 167–176. doi:10.1016/0248-4900(96)85277-4 PMID:8785518

Steiner I, Scharf L, Fiala F, Washüttl J (1998). Migration of di-(2-ethylhexyl) phthalate from PVC child articles into saliva and saliva simulant. *Food Addit Contam*, 15: 812–817. doi:10.1080/02652039809374715 PMID:10211190

Stenchever MA, Allen MA, Jerominski L, Petersen RV (1976). Effects of bis(2-ethylhexyl) phthalate on chromosomes of human leukocytes and human fetal lung cells. *J Pharm Sci*, 65: 1648–1651. doi:10.1002/jps.2600651121 PMID:993999

Styles JA, Clay P, Cross MF (1985). *Assays for the induction of gene mutations at the thymidine kinase and the Na+/K+ ATPase loci in two different mouse lymphoma cell lines in culture*. In: *Evaluation of Short-term Tests for Carcinogens: Report of the International Programme on Chemical Safety's Collaborative Study on In Vitro Assays*. Ashby J, de Serres FJ, Draper M *et al.*, editors. Amsterdam: Elsevier, pp. 587–596.

Suk WA, Humphreys JE (1985). *Assay for the carcinogenicity of chemical agents using enhancement of anchorage-independent survival of retrovirus-infected Fischer rat embryo cells*. In: *Progress in Mutation Research Evaluation of Short-term Tests for Carcinogens: Report of the International Programme on Chemical Safety's Collaborative Study on In Vitro Assays*. Ashby J, de Serres FJ, Draper M *et al.*, editors. Amsterdam: Elsevier, pp. 673–683.

Suzuki H, Ikeda N, Kobayashi K *et al.* (2005). Evaluation of liver and peripheral blood micronucleus assays with 9 chemicals using young rats. A study by the Collaborative Study Group for the Micronucleus Test (CSGMT)/Japanese Environmental Mutagen Society (JEMS)-Mammalian Mutagenicity Study Group (MMS). *Mutat Res*, 583: 133–145. PMID:15899588

Suzuki Y, Niwa M, Yoshinaga J *et al.* (2010). Prenatal exposure to phthalate esters and PAHs and birth outcomes. *Environ Int*, 36: 699–704. doi:10.1016/j.envint.2010.05.003 PMID:20605637

Swan SH, Main KM, Liu F *et al.*Study for Future Families Research Team (2005). Decrease in anogenital distance among male infants with prenatal phthalate exposure. *Environ Health Perspect*, 113: 1056–1061. doi:10.1289/ehp.8100 PMID:16079079

Swedish Environmental Protection Agency (1996). *Disposal of PVC Waste. Report on a Government Assignment* (No. 4594), Stockholm.

Tai ES, Demissie S, Cupples LA *et al.* (2002). Association between the PPARA L162V polymorphism and plasma lipid levels: the Framingham Offspring Study. *Arterioscler Thromb Vasc Biol*, 22: 805–810. doi:10.1161/01.ATV.0000012302.11991.42 PMID:12006394

Takagi A, Sai K, Umemura T *et al.* (1990a). Relationship between hepatic peroxisome proliferation and 8-hydroxydeoxyguanosine formation in liver DNA of rats following long-term exposure to three peroxisome proliferators; di(2-ethylhexyl) phthalate, aluminium clofibrate and simfibrate. *Cancer Lett*, 53: 33–38. doi:10.1016/0304-3835(90)90007-K PMID:2397479

Takagi A, Sai K, Umemura T *et al.* (1990b). Significant increase of 8-hydroxydeoxyguanosine in liver DNA of rats following short-term exposure to the peroxisome proliferators di(2-ethylhexyl)phthalate and di(2-ethylhexyl)adipate. *Jpn J Cancer Res*, 81: 213–215. PMID:2112523

Takahashi Y, Shibata T, Sasaki Y *et al.* (2008). Di(2-ethylhexyl) phthalate exposure during cardiopulmonary bypass. *Asian Cardiovasc Thorac Ann*, 16: 4–6. PMID:18245696

Takashima K, Ito Y, Gonzalez FJ, Nakajima T (2008). Different mechanisms of DEHP-induced hepatocellular adenoma tumorigenesis in wild-type and Ppar alpha-null mice. *J Occup Health*, 50: 169–180. doi:10.1539/joh.L7105 PMID:18403868

Takeshita A, Inagaki K, Igarashi-Migitaka J *et al.* (2006). The endocrine disrupting chemical, diethylhexyl phthalate, activates MDR1 gene expression in human colon cancer LS174T cells. *J Endocrinol*, 190: 897–902. doi:10.1677/joe.1.06664 PMID:17003290

Takeshita A, Koibuchi N, Oka J *et al.* (2001). Bisphenol-A, an environmental estrogen, activates the human orphan nuclear receptor, steroid and xenobiotic receptor-mediated transcription. *Eur J Endocrinol*, 145: 513–517. doi:10.1530/eje.0.1450513 PMID:11581012

Takeuchi S, Iida M, Kobayashi S *et al.* (2005). Differential effects of phthalate esters on transcriptional activities via human estrogen receptors alpha and beta, and androgen receptor. *Toxicology*, 210: 223–233. doi:10.1016/j.tox.2005.02.002 PMID:15840436

Tamura H, Iida T, Watanabe T, Suga T (1991). Lack of induction of hepatic DNA damage on long-term administration of peroxisome proliferators in male F-344 rats. *Toxicology*, 69: 55–62. doi:10.1016/0300-483X(91)90153-R PMID:1926155

Tan GH (1995). Residue levels of phthalate esters in water and sediment samples from the Klang River basin. *Bull Environ Contam Toxicol*, 54: 171–176. doi:10.1007/BF00197427 PMID:7742623

Tandon R., Chowdhary SR, Seth PK, Sricastava SP (1990). Altered development of testis of rat exposed to di(2-ethylhexyl) phthalate (DEHP) during lactation *J Environ Biol*, 11: 345–354.

Tandon R, Seth PK, Srivastava SP (1991). Effect of in utero exposure to di(2-ethylhexyl)phthalate on rat testes. *Indian J Exp Biol*, 29: 1044–1046. PMID:1816082

Thiess AM, Frentzel-Beyme R, Wieland R (1978). [*Mortality study in workers exposed to di-2-ethylhexyl phthalate (DOP)]. In: Möglichkeiten und Grenzen des Biological Monitoring. Arbeitsmedizinische Probleme des Dienstleistungsgewerbes.*] Arbeitsmedizinisches Kolloquium, Stuttgart, AW Gentner, pp. 155–164.

Thor Larsen S, My Lund R, Damgård Nielsen G *et al.* (2001). Di-(2-ethylhexyl) phthalate possesses an adjuvant effect in a subcutaneous injection model with BALB/c mice. *Toxicol Lett*, 125: 11–18. doi:10.1016/S0378-4274(01)00419-2 PMID:11701218

Thurén A (1986). Determination of phthalates in aquatic environments. *Bull Environ Contam Toxicol*, 36: 33–40. doi:10.1007/BF01623471 PMID:3940564

Thurén A & Larsson P (1990). Phthalate esters in the Swedish atmosphere. *Environ Sci Technol*, 24: 554–559. doi:10.1021/es00074a015

Timsit YE & Negishi M (2007). CAR and PXR: the xenobiotic-sensing receptors. *Steroids*, 72: 231–246. doi:10.1016/j.steroids.2006.12.006 PMID:17284330

Tomaszewski KE, Heindel SW, Jenkins WL, Melnick RL (1990). Induction of peroxisomal acyl CoA oxidase activity and lipid peroxidation in primary rat hepatocyte cultures. *Toxicology*, 65: 49–60. doi:10.1016/0300-483X(90)90078-U PMID:2274969

Tomita I, Nakamura Y, Aoki N, Inui N (1982a). Mutagenic/carcinogenic potential of DEHP and MEHP. *Environ Health Perspect*, 45: 119–125. doi:10.2307/3429394 PMID:6814903

Tomita I, Nakamura Y, Yagi Y, Tutikawa K (1982b). Teratogenicity/fetotoxicity of DEHP in mice. *Environ Health Perspect*, 45: 71–75. doi:10.1289/ehp.824571 PMID:7140699

Tomonari Y, Kurata Y, David RM *et al.* (2006). Effect of di(2-ethylhexyl) phthalate (DEHP) on genital organs from juvenile common marmosets: I. Morphological and biochemical investigation in 65-week toxicity study. *J Toxicol Environ Health A*, 69: 1651–1672. doi:10.1080/15287390600630054 PMID:16854791

Towae FK, Enke W, Jäckh R, Bhargava N (1992). Phthalic acid and derivatives. In: *Ullmann's Encyclopedia of Industrial Chemistry*, Vol. A20, 5th rev. Elvers B, Hawkins S, Schulz G, editors. New York: VCH Publishers, pp. 181–211.

Toxics Use Reduction Institute (2005). *Five Chemicals Alternatives Reduction Study, Final report, Chapter 7, DEHP*. Lowell, MA: University of Massachusetts Lowel. Available at: http://www.turi.org/library/turi_publications/five_chemicals_study

Toyosawa K, Okimoto K, Kobayashi I *et al.* (2001). Di(2-ethylhexyl)phthalate induces hepatocellular adenoma in transgenic mice carrying a human prototype c-Ha-ras gene in a 26-week carcinogenicity study. *Toxicol Pathol*, 29: 458–466. doi:10.1080/01926230152499944 PMID:11560251

Tsuchiya K & Hattori K (1976). Chromosomal study on human leucocytes cultures treated with phthalate acid ester. *Rep Hokkaido Inst Public Health*, 26: 114

Tsutsui T, Watanabe E, Barrett JC (1993). Ability of peroxisome proliferators to induce cell transformation, chromosome aberrations and peroxisome proliferation in cultured Syrian hamster embryo cells. *Carcinogenesis*, 14: 611–618. doi:10.1093/carcin/14.4.611 PMID:8472324

Tugwood JD, Aldridge TC, Lambe KG *et al.* (1996). Peroxisome proliferator-activated receptors: structures and function. *Ann N Y Acad Sci*, 804: 1 Peroxisomes252–265. doi:10.1111/j.1749-6632.1996.tb18620.x PMID:8993548

Turan N, Cartwright LS, Waring RH, Ramsden DB (2008). Wide-ranging genomic effects of plasticisers and related compounds. *Curr Drug Metab*, 9: 285–303. doi:10.2174/138920008784220655 PMID:18473747

Turner JH, Petricciani JC, Crouch ML, Wenger S (1974). An evaluation of the effects of diethylhexyl phthalate (DEHP) on mitotically capable cells in blood packs. *Transfusion*, 14: 560–566. doi:10.1111/j.1537-2995.1974.tb04577.x PMID:4432261

Tyl RW, Price CJ, Marr MC, Kimmel CA (1988). Developmental toxicity evaluation of dietary di(2-ethylhexyl)phthalate in Fischer 344 rats and CD-1 mice. *Fundam Appl Toxicol*, 10: 395–412. doi:10.1016/0272-0590(88)90286-2 PMID:3371580

Usui T, Mutai M, Hisada S *et al.* (2001). CB6F1-rasH2 mouse: overview of available data. *Toxicol Pathol*, 29: Suppl90–108. doi:10.1080/019262301753178500 PMID:11695565

Vainiotalo S & Pfäffli P (1990). Air impurities in the PVC plastics processing industry. *Ann Occup Hyg*, 34: 585–590. doi:10.1093/annhyg/34.6.585 PMID:2291582

van Ravenzwaay B, Coelho-Palermo Cunha G, Strauss V *et al.* (2010). The individual and combined metabolite profiles (metabolomics) of dibutylphthalate and di(2-ethylhexyl)phthalate following a 28-day dietary exposure in rats. *Toxicol Lett*, 198: 159–170. doi:10.1016/j.toxlet.2010.06.009 PMID:20600714

Vitali M, Leoni V, Chiavarini S, Cremisini C (1993). Determination of 2-ethyl-1-hexanol as contaminant in drinking water. *J AOAC Int*, 76: 1133–1137. PMID:8241817

Vogel EW (1985). *The Drosophila somatic recombination and mutation assay (SRM) using the white-coral somatic eye color system*. In: *Evaluation of Short-term Tests for Carcinogens: Report of the International Programme on Chemical Safety's Collaborative Study on In Vitro*

Assays. Ashby J, de Serres FJ, Draper M *et al.*, editors. Amsterdam: Elsevier, pp. 313–317.

von Däniken A, Lutz WK, Jäckh R, Schlatter C (1984). Investigation of the potential for binding of di(2-ethylhexyl) phthalate (DEHP) and di(2-ethylhexyl) adipate (DEHA) to liver DNA in vivo. *Toxicol Appl Pharmacol*, 73: 373–387. doi:10.1016/0041-008X(84)90089-9 PMID:6719458

von Rettberg H, Hannman T, Subotic U *et al.* (2009). Use of di(2-ethylhexyl)phthalate-containing infusion systems increases the risk for cholestasis. *Pediatrics*, 124: 710–716. doi:10.1542/peds.2008-1765 PMID:19651587

Voskoboinik I, Ooi SG, Drew R, Ahokas JT (1997). Peroxisome proliferators increase the formation of BPDE-DNA adducts in isolated rat hepatocytes. *Toxicology*, 122: 81–91. doi:10.1016/S0300-483X(97)00083-8 PMID:9274804

Voss C, Zerban H, Bannasch P, Berger MR (2005). Lifelong exposure to di-(2-ethylhexyl)-phthalate induces tumors in liver and testes of Sprague-Dawley rats. *Toxicology*, 206: 359–371. doi:10.1016/j.tox.2004.07.016 PMID:15588926

Walgren JE, Kurtz DT, McMillan JM (2000). Expression of PPAR(alpha) in human hepatocytes and activation by trichloroacetate and dichloroacetate. *Res Commun Mol Pathol Pharmacol*, 108: 116–132. PMID:11758968

Wams TJ (1987). Diethylhexylphthalate as an environmental contaminant–a review. *Sci Total Environ*, 66: 1–16. doi:10.1016/0048-9697(87)90072-6 PMID:3317819

Wang T, Uezato T, Miura N (2001). Inhibition effects of di(2-ethylhexyl)phthalate on mouse-liver lysosomal vacuolar H(+)-ATPase. *J Cell Biochem*, 81: 295–303. doi:10.1002/1097-4644(20010501)81:2<295::AID-JCB1044>3.0.CO;2-6 PMID:11241669

Ward JM, Diwan BA, Ohshima M *et al.* (1986). Tumor-initiating and promoting activities of di(2-ethylhexyl) phthalate in vivo and in vitro. *Environ Health Perspect*, 65: 279–291. doi:10.2307/3430195 PMID:3709454

Ward JM, Ohshima M, Lynch P, Riggs C (1984). Di(2-ethylhexyl)phthalate but not phenobarbital promotes N-nitrosodiethylamine-initiated hepatocellular proliferative lesions after short-term exposure in male B6C3F₁ mice. *Cancer Lett*, 24: 49–55. doi:10.1016/0304-3835(84)90079-X PMID:6498799

Ward JM, Peters JM, Perella CM, Gonzalez FJ (1998). Receptor and nonreceptor-mediated organ-specific toxicity of di(2-ethylhexyl)phthalate (DEHP) in peroxisome proliferator-activated receptor alpha-null mice. *Toxicol Pathol*, 26: 240–246. doi:10.1177/019262339802600208 PMID:9547862

Weglarz TC & Sandgren EP (2004). Cell cross-talk mediates PPARalpha null hepatocyte proliferation after peroxisome proliferator exposure. *Carcinogenesis*, 25: 107–112. doi:10.1093/carcin/bgg180 PMID:14514660

Westberg HB, Hardell LO, Malmqvist N *et al.* (2005). On the use of different measures of exposure-experiences from a case-control study on testicular cancer and PVC exposure. *J Occup Environ Hyg*, 2: 351–356. doi:10.1080/15459620590969046 PMID:16020098

Weuve J, Hauser R, Calafat AM *et al.* (2010). Association of exposure to phthalates with endometriosis and uterine leiomyomata: findings from NHANES, 1999–2004. *Environ Health Perspect*, 118: 825–832. doi:10.1289/ehp.0901543 PMID:20185384

Weuve J, Sánchez BN, Calafat AM *et al.* (2006). Exposure to phthalates in neonatal intensive care unit infants: urinary concentrations of monoesters and oxidative metabolites. *Environ Health Perspect*, 114: 1424–1431. doi:10.1289/ehp.8926 PMID:16966100

WHO (1992). *Diethylhexyl Phthalate* (Environmental Health Criteria 131). Geneva: International Programme on Chemical Safety.

WHO (2008). *Guidelines for Drinking-water Quality.* 3rd edition, incorporating the first and second addenda. Volume 1 – Recommendations. Geneva: World Health Organization

Wilkinson CF & Lamb JC 4th (1999). The potential health effects of phthalate esters in children's toys: a review and risk assessment. *Regul Toxicol Pharmacol*, 30: 140–155. doi:10.1006/rtph.1999.1338 PMID:10536109

Williams DT & Blanchfield BJ (1974). Retention, excretion and metabolism of di-(2-ethylhexyl) phthalate administered orally to the rat. *Bull Environ Contam Toxicol*, 11: 371–378. doi:10.1007/BF01684945 PMID:4433824

Williams GM, Maruyama H, Tanaka T (1987). Lack of rapid initiating, promoting or sequential syncarcinogenic effects of di(2-ethylhexyl)phthalate in rat liver carcinogenesis. *Carcinogenesis*, 8: 875–880. doi:10.1093/carcin/8.7.875 PMID:3594721

Williams GM, Tong C, Ved Brat S (1985). *Tests with the rat hepatocyte primary culture/DNA-repair test.* In: *Evaluation of Short-term Tests for Carcinogens: Report of the International Programme on Chemical Safety's Collaborative Study on In Vitro Assays.* Ashby J, de Serres FJ, Draper M *et al.*, editors. Amsterdam: Elsevier, pp. 341–345.

Wittassek M & Angerer J (2008). Phthalates: metabolism and exposure. *Int J Androl*, 31: 131–138. doi:10.1111/j.1365-2605.2007.00837.x PMID:18070048

Wittassek M, Heger W, Koch HM *et al.* (2007b). Daily intake of di(2-ethylhexyl)phthalate (DEHP) by German children – A comparison of two estimation models based on urinary DEHP metabolite levels. *Int J Hyg Environ Health*, 210: 35–42. doi:10.1016/j.ijheh.2006.11.009 PMID:17185035

Wittassek M, Wiesmüller GA, Koch HM *et al.* (2007a). Internal phthalate exposure over the last two decades–a retrospective human biomonitoring study. *Int J Hyg Environ Health*, 210: 319–333. doi:10.1016/j.ijheh.2007.01.037 PMID:17400024

Wolf C Jr, Lambright C, Mann P *et al.* (1999). Administration of potentially antiandrogenic pesticides

(procymidone, linuron, iprodione, chlozolinate, p,p'-DDE, and ketoconazole) and toxic substances (dibutyl- and diethylhexyl phthalate, PCB 169, and ethane dimethane sulphonate) during sexual differentiation produces diverse profiles of reproductive malformations in the male rat. *Toxicol Ind Health*, 15: 94–118. doi:10.1177/074823379901500109 PMID:10188194

Wong JS & Gill SS (2002). Gene expression changes induced in mouse liver by di(2-ethylhexyl) phthalate. *Toxicol Appl Pharmacol*, 185: 180–196. doi:10.1006/taap.2002.9540 PMID:12498735

Woods CG, Burns AM, Maki A *et al.* (2007). Sustained formation of alpha-(4-pyridyl-1-oxide)-N-tert-butyl-nitrone radical adducts in mouse liver by peroxisome proliferators is dependent upon peroxisome proliferator-activated receptor-alpha, but not NADPH oxidase. *Free Radic Biol Med*, 42: 335–342. doi:10.1016/j.freeradbiomed.2006.10.053 PMID:17210446

Wu S, Zhu J, Li Y *et al.* (2010). Dynamic epigenetic changes involved in testicular toxicity induced by di-2-(ethylhexyl) phthalate in mice. *Basic Clin Pharmacol Toxicol*, 106: 118–123. doi:10.1111/j.1742-7843.2009.00483.x PMID:19912166

Würgler FE, Graf U, Frei H (1985). *Somatic mutation and recombination test in wings of Drosophila melanogaster.* In: *Evaluation of Short-term Tests for Carcinogens: Report of the International Programme on Chemical Safety's Collaborative Study on In Vitro Assays.* Ashby J, de Serres FJ, Draper M *et al.*, editors. Amsterdam: Elsevier, pp. 325–340.

Xu Y, Agrawal S, Cook TJ, Knipp GT (2008). Maternal di-(2-ethylhexyl)-phthalate exposure influences essential fatty acid homeostasis in rat placenta. *Placenta*, 29: 962–969. doi:10.1016/j.placenta.2008.08.011 PMID:18829104

Yagi Y, Nakamura Y, Tomita I *et al.* (1980). Teratogenic potential of di- and mono-(2-ethylhexyl)phthalate in mice. *J Environ Pathol Toxicol*, 4: 533–544. PMID:7462917

Yamakawa-Kobayashi K, Ishiguro H, Arinami T *et al.* (2002). A Val227Ala polymorphism in the peroxisome proliferator activated receptor alpha (PPARalpha) gene is associated with variations in serum lipid levels. *J Med Genet*, 39: 189–191. doi:10.1136/jmg.39.3.189 PMID:11897821

Yamazaki T, Hirose A, Sakamoto T *et al.* (2009). Peroxisome proliferators attenuate free arachidonic acid pool in the kidney through inducing lysophospholipid acyltransferases. *J Pharmacol Sci*, 111: 201–210. doi:10.1254/jphs.09162FP PMID:19809218

Yang Q, Ito S, Gonzalez FJ (2007). Hepatocyte-restricted constitutive activation of PPAR alpha induces hepatoproliferation but not hepatocarcinogenesis. *Carcinogenesis*, 28: 1171–1177. doi:10.1093/carcin/bgm046 PMID:17331954

Yokoyama Y, Okubo T, Kano I *et al.* (2003). Induction of apoptosis by mono(2-ethylhexyl)phthalate (MEHP) in U937 cells. *Toxicol Lett*, 144: 371–381. doi:10.1016/S0378-4274(03)00256-X PMID:12927354

Yoon JS, Mason JM, Valencia R *et al.* (1985). Chemical mutagenesis testing in Drosophila. IV. Results of 45 coded compounds tested for the National Toxicology Program. *Environ Mutagen*, 7: 349–367. doi:10.1002/em.2860070310 PMID:3930235

Yoshikawa K, Tanaka A, Yamaha T, Kurata H (1983). Mutagenicity study of nine monoalkyl phthalates and a dialkyl phthalate using Salmonella typhimurium and Escherichia coli. *Food Chem Toxicol*, 21: 221–223. doi:10.1016/0278-6915(83)90239-9 PMID:6339336

Zacharewski TR, Meek MD, Clemons JH *et al.* (1998). Examination of the in vitro and in vivo estrogenic activities of eight commercial phthalate esters. *Toxicol Sci*, 46: 282–293. PMID:10048131

Zeiger E, Haworth S (1985). *Tests with a preincubation modification of the Salmonella/microsome assay.* In: *Evaluation of Short-term Tests for Carcinogens: Report of the International Programme on Chemical Safety's Collaborative Study on In Vitro Assays.* Ashby J, de Serres FJ, Draper M *et al.*, editors. Amsterdam: Elsevier, pp. 187–199.

Zeiger E, Haworth S, Mortelmans K, Speck W (1985). Mutagenicity testing of di(2-ethylhexyl)phthalate and related chemicals in Salmonella. *Environ Mutagen*, 7: 213–232. doi:10.1002/em.2860070209 PMID:3971959

Zhang Y, Lin L, Cao Y *et al.* (2009). Phthalate levels and low birth weight: a nested case-control study of Chinese newborns. *J Pediatr*, 155: 500–504. doi:10.1016/j.jpeds.2009.04.007 PMID:19555962

Zhang YH, Zheng LX, Chen BH (2006). Phthalate exposure and human semen quality in Shanghai: a cross-sectional study. *Biomed Environ Sci*, 19: 205–209. PMID:16944777

Zhu H, Zheng J, Xiao X *et al.* (2010). Environmental endocrine disruptors promote invasion and metastasis of SK-N-SH human neuroblastoma cells. *Oncol Rep*, 23: 129–139. PMID:19956873

Zhu J, Phillips SP, Feng Y-L, Yang X (2006). Phthalate esters in human milk: concentration variations over a 6-month postpartum time. *Environ Sci Technol*, 40: 5276–5281. doi:10.1021/es060356w PMID:16999099

Zimmering S, Mason JM, Valencia R (1989). Chemical mutagenesis testing in Drosophila. VII. Results of 22 coded compounds tested in larval feeding experiments. *Environ Mol Mutagen*, 14: 245–251. doi:10.1002/em.2850140406 PMID:2583131