

HEXACHLOROBENZENE

This substance was considered by previous working groups, in 1978 (IARC, 1979) and 1986 (IARC, 1987). Since that time, new data have become available, and these have been incorporated into the monograph and taken into consideration in the present evaluation.

1. Exposure Data

1.1 Chemical and physical data

1.1.1 Nomenclature

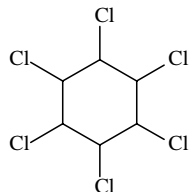
Chem. Abstr. Serv. Reg. No.: 118-74-1

Chem. Abstr. Name: Hexachlorobenzene

IUPAC Systematic Name: Hexachlorobenzene

Synonyms: HCB; pentachlorophenyl chloride; perchlorobenzene

1.1.2 Structural and molecular formulae and relative molecular mass



Relative molecular mass: 284.78

1.1.3 Chemical and physical properties of the pure substance

(a) *Description:* White needles (Lide & Milne, 1996; WHO, 1997; Budavari, 2000)

(b) *Boiling-point:* 325 °C (Lide & Milne, 1996)

(c) *Melting-point:* 231.8 °C (Lide & Milne, 1996)

- (d) *Spectroscopy data*: Infrared [prism (4545), grating (410)], ultraviolet and mass spectral data have been reported (Sadler Research Laboratories, 1980; Lide & Milne, 1996).
- (e) *Solubility*: Practically insoluble in water (5×10^{-6} g/L at 25 °C); very soluble in benzene; soluble in chloroform and diethyl ether; slightly soluble in ethanol (Lide & Milne, 1996; WHO, 1997)
- (f) *Volatility*: Vapour pressure, 1.09×10^{-5} mm Hg [1.45 mPa] at 20 °C (Budavari, 2000)
- (g) *Stability*: Flash-point, 242 °C (Budavari, 2000)
- (h) *Octanol/water partition coefficient (P)*: log P, 5.5 (WHO, 1997)

1.1.4 *Technical products and impurities*

Technical-grade hexachlorobenzene was commercially available in the past as a wetttable powder, liquid and dust. It was reported to contain about 98% hexachlorobenzene, 1.8% pentachlorobenzene and 0.2% 1,2,4,5-tetrachlorobenzene in the USA (IARC, 1979). Several other impurities have been detected, including hepta- and octachlorodibenzofurans, octachlorodibenzo-*para*-dioxin and decachlorobiphenyl (WHO, 1997).

Trade names for hexachlorobenzene include Amatin, Anticarie, Bunt-Cure, Bunt-No-More, Co-op Hexa Granox NM, HexaCB, Julin's Carbon Chloride, No Bunt, Sanocide, Smut-Go and Snieciotox.

1.1.5 *Analysis*

Methods for the analysis of hexachlorobenzene in various matrices are summarized in Table 1.

1.2 **Production**

Industrial synthesis of hexachlorobenzene involves the chlorination of benzene at 150–200 °C with a ferric chloride catalyst or distillation of residues from the production of tetrachloroethylene (WHO, 1997).

Few recent data on the quantities of hexachlorobenzene produced are available. Worldwide production of pure hexachlorobenzene was estimated to be 10 000 t/year for the years 1978–81. Hexachlorobenzene was produced or imported in the European Community at 8000 t/year in 1978, and a company in Spain reportedly produced an estimated 150 t/year. Approximately 1500 t/year of hexachlorobenzene were manufactured in Germany for the production of rubber chemicals, but this production was discontinued in 1993. Intentional production of hexachlorobenzene has declined as a result of restrictions on its use since the 1970s, but it may still be produced as an incidental by-product in some processes (see section 1.4) (WHO, 1997).

Table 1. Methods for analysis of hexachlorobenzene

Sample matrix	Sample preparation	Assay procedure	Limit of detection	Reference
Air	Trap on glass-fibre filter and XAD-2; extract with toluene	HRGC/LRMS	0.18 pg/m ³	Hippelein <i>et al.</i> (1993)
	Adsorb on polyurethane foam; extract with diethyl ether in hexane	GC/ECD	< 0.1 µg/m ³	Lewis & MacLeod (1982)
	Adsorb on polyurethane foam; extract with hexane; fractionate by HPLC	GC/ECD	Low pg/m ³ range	Oehme & Stray (1982)
	Adsorb on polyurethane foam or Tenax-GC resin; reflux with dichloromethane; reflux with hexane; clean-up by alumina chromatography	GC/ECD	NR	Billings & Bidleman (1980)
	Adsorb on Amberlite XAD-2; desorb with carbon tetrachloride	GC/PID	0.815 mg/m ³	Langhorst & Nestruck (1979)
	Collect vapours on polyurethane foam; extract with 5–10% diethyl ether in hexane	GC/ECD	NR	Environmental Protection Agency (1999a,b) [Methods TO-04A, TO-10A]
	Collect on polyurethane foam; Soxhlet extraction; concentrate	GC/ECD or GC/ECD and GC/MS	5 ng/m ³	Hsu <i>et al.</i> (1988)
Water	Extract with hexane; inject extract	GC/ECD	0.002 µg/L	Environmental Protection Agency (1995a) [Method 505]
	Extract with dichloromethane; isolate extract; dry; concentrate with methyl <i>tert</i> -butyl ether (capillary column)	GC/ECD	0.001 µg/L	Environmental Protection Agency (1995b) [Method 508.1]
	Extract by passing sample through liquid–solid extractor; elute with dichloromethane; concentrate by evaporation (capillary column)	GC/MS	0.049–0.13 µg/L ^a	Environmental Protection Agency (1995c) [Method 525.2]

Table 1 (contd)

Sample matrix	Sample preparation	Assay procedure	Limit of detection	Reference
Water (contd)	Extract with methyl <i>tert</i> -butyl ether or pentane	GC/ECD	0.001 µg/L	Environmental Protection Agency (1995d) [Method 551.1]
	Strip from water with stream of air; adsorb on activated carbon filter; extract with carbon disulfide or dichloromethane	GC/MS or GC/FID	0.001 µg/L	APHA/AWWA/WEF (1999a) [Method 6040B]
	Adjust pH; concentrate on XAD-4; clean-up on silica gel	GC/MS	0.1 µg/L	Garrison & Pellizzari (1987)
Ground-water	Solvent extraction; solvent exchange	GC/ECD	0.12 µg/L	Munch <i>et al.</i> (1990) [National Pesticide Survey Method 2]
River water	Centrifuge; digest with chromic acid; extract	GC/ECD	NR	Driscoll <i>et al.</i> (1991)
Soil, chemical waste samples	Extract with hexane	GC/ECD	10 mg/kg	DeLeon <i>et al.</i> (1980)
Municipal & industrial discharges	Extract with dichloromethane; dry; exchange to hexane; concentrate	GC/ECD	0.05 µg/L	Environmental Protection Agency (1999c) [Method 612]
	Extract with dichloromethane; dry; concentrate (packed column)	GC/MS	1.9 µg/L	Environmental Protection Agency (1999d) [Method 625]
	Adjust to pH 11; extract with dichloromethane; dry; concentrate	GC/MS	1.9 µg/L	APHA/AWWA/WEF (1999b) [Method 6410B]
	Add isotope-labelled analogue; extract with dichloromethane; dry over sodium sulfate; concentrate	GC/MS	10 µg/L	Environmental Protection Agency (1999e) [Method 1625]
Liquid & solid waste	Extract with dichloromethane (liquid); hexane:acetone (1:1) or dichloromethane:acetone (1:1) (solid); clean-up	GC/ECD	NR	Environmental Protection Agency (1996a) [Method 8081A]

Table 1 (contd)

Sample matrix	Sample preparation	Assay procedure	Limit of detection	Reference
Soil, waste & water	Extract with dichloromethane (liquid); dichloromethane:acetone (1:1) (solid); clean-up	GC/ECD	5.6 ng/L (reagent water)	Environmental Protection Agency (1994a) [Method 8121]
Air sampling media, soil, solid waste & water	Liquid-liquid extraction or Soxhlet extraction or ultrasonic extraction or waste dilution or direct injection; clean-up with Florisil or gel-permeation (capillary column)	GC/MS	10 µg/L (aqueous); 660 µg/kg (soil/sediment) (EQL)	Environmental Protection Agency (1996b) [Method 8270C]
Soil, sludge & solid waste	Thermal extraction; concentrate; thermal desorption	TE/GC/MS	0.01–0.5 mg/kg	Environmental Protection Agency (1996c) [Method 8275A]
Sediment, soil, solid waste and wastewater	Liquid-liquid extraction (water); Soxhlet or ultrasonic extraction (sediment, soil or waste)	GC/FT-IR	20 µg/L	Environmental Protection Agency (1994b) [Method 8410]
Soil	Extract with light petroleum; liquid-liquid partition; clean-up with sulfuric acid	GC/ECD	NR	Waliszewski & Szymczynski (1985)
Sediment	Extract with dichloromethane; subject to acid fractionation; subject base or neutral fraction to silica gel chromatography	GC/MS	NR	Lopez-Avila <i>et al.</i> (1983)
	Microwave; extract; centrifuge; filter	GC/ECD	NR	Onuska & Terry (1993)
Fish tissue	Grind with sodium sulfate; extract with hexane:acetone; clean-up on Na ₂ SO ₄ :alumina:silica gel:Florisil column followed by a H ₂ SO ₄ column on silica gel	GC/ECD	[~ 0.05 µg/kg]	Oliver & Nicol (1982)
	Extract with hexane:isopropanol; solvent and sulfuric acid partitioning	GC/ECD	NR	Lunde & Ofstad (1976)
	Macerate; Soxhlet extract; clean-up with sulfuric acid:silica gel	GC/ECD	5 µg/kg (lipid basis)	Rahman <i>et al.</i> (1993)

Table 1 (contd)

Sample matrix	Sample preparation	Assay procedure	Limit of detection	Reference
Fish tissue (contd)	Sulfuric acid digestion; silica gel column chromatography; methylation; alumina column chromatography	GC/ECD	10–15 µg/kg	Lamparski <i>et al.</i> (1980)
	Homogenize; Soxhlet extract; GPC fractionate; silica gel fractionate	GC/MS	12.5 µg/kg	ATSDR (1998)
Fish, aquatic biota	Homogenize with solvent; solvent exchange; clean-up on Florisil	GC/ECD	50 µg/kg wet weight	Miskiewicz & Gibbs (1994)
Aquatic organisms	Homogenize; Soxhlet extract; GPC fractionate; SPE fractionate; solvent exchange	GC/ECD	NR	Shan <i>et al.</i> (1994) [USGS method]
Oyster tissue	Extract with acetone:acetonitrile; partition into petroleum ether; clean-up with silica gel chromatography	GC/ECD	NR	Murray <i>et al.</i> (1980)
Adipose tissue (chicken)	Extract with hexane; subject to Florisil clean-up and one-fraction elution	GC/ECD	NR	Watts <i>et al.</i> (1980)
Adipose tissue	Extract; GPC clean-up; fractionate on Florisil	GC/MS	12 µg/kg	Stanley (1986)
	Soxhlet extraction; clean-up on Florisil	GC/ECD	1 µg/kg	Alawi & Ababneh (1991)
	Extract with solvent; remove bulk lipid; fractionate on Florisil	HRGC/MS	12 µg/kg	Stanley (1986)
	Extract with benzene:acetone; filter; fractionate on Florisil	GC/ECD	0.12 µg/kg	Mes (1992)
	SFE with alumina (to remove lipids); purify by column chromatography	GC/ECD	10 µg/kg	ATSDR (1998)
	Extract fat; dissolve in hexane; elute with hexane; concentrate	GC/ECD	NR	AOAC International (2000) [Method 980.22]

Table 1 (contd)

Sample matrix	Sample preparation	Assay procedure	Limit of detection	Reference
Fatty foods	SFE/SFC (on-line clean-up)	GC/ECD	4 µg/kg	Nam & King (1994)
	Extraction and pretreat; clean-up on Florisil	GC/ECD; TLC	10 µg/kg	ATSDR (1998) [DFG Method S9]
	Extract fat; partition into acetonitrile; petroleum ether; clean-up on Florisil	GC/ECD or GC/ELCD	NR	Food and Drug Administration (1999) [Method 304]
	Extract fat; clean-up on Florisil; elute with acetonitrile; separate with petroleum ether	GC/ECD	NR	AOAC International (2000) [Method 977.19]
Milk	Solvent extraction; solvent partition; solvent exchange; GPC clean-up; optional alumina clean-up	GC/ECD	0.5 µg/L	Trotter & Dickerson (1993)
Non-fatty foods	Extract with acetone; partition or remove water; clean-up on Florisil; elute with dichloromethane	GC/ECD or GC/ELCD	NR	Food and Drug Administration (1999) [Method 302]
	Extract with acetonitrile or water; acetonitrile; partition into petroleum ether; clean-up on Florisil	GC/ECD or GC/ELCD	NR	Food and Drug Administration (1999) [Method 303]
Vegetable oils, oil seeds	Sandwich-type extraction; fractionate	GC/ECD	0.1–2 µg/kg	Seidel & Linder (1993)
Fruit, vegetables	Chop and blend; blend with solvent; partition with water; dry	GC/ECD, GC/MS	2 µg/kg	Pylypiw (1993)
Crops and foods	Solvent extraction; GPC clean-up; optional silica gel clean-up	GC/ECD	NR	ATSDR (1998) [DFG Method S19]
Urine	Extract with carbon tetrachloride; clean-up on silica gel; concentrate	GC/PID	4.1 µg/L	Langhorst & Nestrick (1979)

Table 1 (contd)

Sample matrix	Sample preparation	Assay procedure	Limit of detection	Reference
Blood	Extract with carbon tetrachloride; clean-up on silica gel; concentrate	GC/PID	16 µg/L	Langhorst & Nestrick (1979)
	Extract with hexane:isopropanol	GC/ECD	NR	Lunde & Bjorseth (1977)
	Extract with hexane; concentrate	GC/ECD	0.016 µg/L	Bristol <i>et al.</i> (1982)
	Homogenize with benzene; filter; fractionate on Florisil	GC/ECD	0.01 µg/L	Mes (1992)
Serum	Extract denatured serum with solvent; fractionate on Florisil; acid treatment and clean-up on silica gel	GC/ECD	1 µg/L	Burse <i>et al.</i> (1990)
Breast milk	Separate fats; column clean-up	GC/ECD	0.4 µg/kg fat	Abraham <i>et al.</i> (1994)
	Extract with acetone:benzene; fractionate on Florisil	GC/ECD	0.033 µg/L	Mes <i>et al.</i> (1993)
Semen	Solvent extraction; clean-up on Florisil; concentrate	GC/ECD	0.3 µg/L	Stachel <i>et al.</i> (1989)
Formulations	Dissolve in toluene or methanol:toluene	GC/FID	NR	AOAC International (2000) [Method 999.04]

APHA/AWWA/WEF, American Public Health Association/American Water Works Association/Water Environment Federation; ATSDR, Agency for Toxic Substances and Disease Registry; USGS, United States Geological Survey; DFG, Deutsche Forschungsgemeinschaft; ECD, electron capture detection; ELCD, electrolytic conductivity detection; GC, gas chromatography; FID, flame ionization detection; FT-IR, Fourier transform infrared spectrometry; GPC, gel permeation chromatography; HPLC, high-performance liquid chromatography; HRGC, high-resolution gas chromatography; LRMS, low-resolution mass spectrometry; MS, mass spectrometry; NR, not reported; PID, photoionization detection; SFC, supercritical fluid chromatography; SFE, supercritical fluid extraction; TE, thermal extraction; TLC, thin-layer chromatography

^aLimit of detection varies with extraction technique (cartridge or disc) and mass spectrometer (quadrupole or ion trap).

Information available in 2000 indicated that hexachlorobenzene was manufactured by five companies in China and one company in Argentina (CIS Information Services, 2000).

1.3 Use

In the past, hexachlorobenzene had many uses in industry and agriculture. The major agricultural application was as a seed dressing for crops such as wheat, barley, oats and rye to prevent growth of fungi. The use of hexachlorobenzene in such applications was discontinued in many countries in the 1970s owing to concerns about adverse effects on the environment and human health. Hexachlorobenzene may continue to be used for this purpose in some countries; for example, hexachlorobenzene was still used in 1986 as a fungicide, seed-dressing and scabicide in sheep in Tunisia (Government of Canada, 1993; WHO, 1997).

In industry, hexachlorobenzene has been used directly in the manufacture of pyrotechnics, tracer bullets and as a fluxing agent in the manufacture of aluminium. It has also been used as a starting material in the production of pentachlorophenol, a porosity-control agent in the manufacture of graphite anodes, and as a peptizing agent in the production of nitroso and styrene rubber for tyres. It is likely that some or all of these applications have been discontinued (WHO, 1987, 1997).

1.4 Occurrence

Although hexachlorobenzene production has ceased in most countries, it may still be generated as an inadvertent by-product in the manufacture of chlorinated solvents, chlorinated aromatics and chlorinated pesticides. It was estimated in 1986 that approximately 4130 t/year of hexachlorobenzene were generated as a waste product in the USA and that nearly 77% of this was produced from the manufacture of three chlorinated solvents: carbon tetrachloride, trichloroethylene and tetrachloroethylene. The remainder was produced by the chlorinated pesticide industry (WHO, 1997). According to the Environmental Protection Agency's Toxic Chemical Release Inventory, in 1997 about 18 t of hexachlorobenzene were reported as waste product (Environmental Protection Agency, 2000). In 1977, about 300 t of hexachlorobenzene were generated in Japan as a waste by-product in the production of tetrachloroethylene, almost all of which was incinerated. It was estimated that > 5000 t/year hexachlorobenzene were produced as a by-product during tetrachloroethylene production in the former Federal Republic of Germany in 1980. Estimates from the European Chlorinated Solvent Association indicated that up to 4000 t/year of hexachlorobenzene are produced in Europe as a by-product during certain tetrachloroethylene production processes, and that over 99% of this by-product is incinerated (WHO, 1997).

While hexachlorobenzene can also be a contaminant in commercial-grade chlorinated solvents, it was not detected (detection limit, 5 mg/L) in carbon tetrachloride or tetrachloroethylene in an investigation in Canada in the late 1980s or in production lots of tri- and tetrachloroethylene produced in Europe in 1996 (detection limit, 2 µg/L solvent) (WHO, 1997).

Some chlorinated pesticides contain hexachlorobenzene as an impurity in the final product, usually at a concentration of less than 1%, although higher concentrations have been reported when inappropriate procedures were used for the synthesis and purification stages (WHO, 1997).

1.4.1 *Occupational exposure*

According to the 1981–83 National Occupational Exposure Survey (National Institute for Occupational Safety and Health, 2000), about 1000 workers in the chemical industry were potentially exposed to hexachlorobenzene in the USA. According to the Finnish Register of Employees Exposed to Carcinogens, 15 laboratory workers were exposed in Finland in 1997 (Savela *et al.*, 1999).

Hexachlorobenzene has been detected in workplace air during the production of pentachlorophenol in the Russian Federation (Melnikova *et al.*, 1975). The blood of 11 workers in a factory where chlorinated solvents were made in the USA in 1974 contained 14–233 µg/L (Burns & Miller, 1975). In 1994, the average concentration of hexachlorobenzene in the serum of 57 workers in a Spanish organochlorine compound factory was 120 µg/L. Maintenance workers had a higher average concentration (247 µg/L, n = 12) than production (105 µg/L, n = 36), laboratory (49 µg/L, n = 6) or administrative (16 µg/L, n = 3) workers (Sala *et al.*, 1999a). The concentration of hexachlorobenzene was [36 µg/L] (1–160 µg/L) in the serum of 41 workers in a Brazilian organochlorine compound plant (da Silva Augusto *et al.*, 1997). The average plasma concentration in nine Swedish aluminium foundry workers who used hexachloroethane for degassing was 313 ng/g of lipid (controls, 67 ng/g of lipid) in 1992 (Seldén *et al.*, 1997).

1.4.2 *Environmental occurrence*

Hexachlorobenzene is a persistent pesticide the use of which has diminished substantially over the past two decades. This compound has low volatility and is practically insoluble in water. Its biodegradation in soil is very slow, with a half-time measured in decades. Hence, hexachlorobenzene persists in the environment and may be expected to accumulate in sediment long after application has ceased.

Recent reviews of environmental exposure to hexachlorobenzene by WHO (1997) and by the Agency for Toxic Substances and Disease Registry (1998) in the USA offer an overview of past environmental concentrations of this chemical. During the period when it was being used as a pesticide, a number of studies were carried out to determine its concentration in food. Most foods were found to contain low or undetectable concen-

trations, with the exception of meat and dairy products, in which significant concentrations were found.

The Total Diet Study, a marketbasket survey conducted in 1985–91 in the USA, demonstrated the presence of hexachlorobenzene in several foods which may be eaten by infants or children, at concentrations of 0.1–5.0 $\mu\text{g}/\text{kg}$ (Yess *et al.*, 1993). The estimated mean dietary intake of this chemical from these studies in 1982–84 was 0.0011–0.002 $\mu\text{g}/\text{kg}$ bw per day depending on age and sex. By 1991, it was estimated that the dietary intake had fallen to 0.0002–0.0004 $\mu\text{g}/\text{kg}$ bw per day (Agency for Toxic Substances and Disease Registry, 1998).

The concentrations of hexachlorobenzene in air are generally low, although they were higher in the past. In 1976–79, air was measured for this chemical throughout the USA, and 49% of samples showed detectable concentrations, with a mean value of about 0.1 ng/m^3 . In 1977, 12% of these values exceeded 1.5 ng/m^3 . In a study in the Great Lakes region of the USA in 1981, the concentrations ranged from 0.09 to 0.28 ng/m^3 . Measurements between July 1988 and September 1989 in Egbert, Ontario (Canada), showed a wide range of concentrations in air, from 0.04 pg/m^3 to 640 pg/m^3 . The indoor concentrations in Jacksonville, FL, and Springfield, MA, in the USA in 1986–88 revealed ranges of 0.3–1.3 ng/m^3 and 0.1 ng/m^3 , respectively, whereas the values were not detected to 0.2 ng/m^3 and not detected, respectively, in paired outdoor samples, suggesting indoor sources of this chemical in Jacksonville. Measurements near chlorinated solvent and pesticide manufacturing facilities in 1974 revealed much higher concentrations in air, ranging from 24 to 23 296 ng/m^3 (Agency for Toxic Substances and Disease Registry, 1998).

In 1986–91, the average concentrations of hexachlorobenzene in precipitation samples ranged from 0.108 to 0.174 ng/L in the Great Lakes region, while drinking-water supplies in the vicinity of Lake Ontario showed a somewhat broader mean range of 0.06–0.2 ng/L . The Great Lakes themselves had average ambient concentrations of 0.02–0.10 ng/L in 1982. The St Lawrence River had concentrations ranging from not detected to 0.09 ng/L in 1991. The Niagara River, which drains a more heavily industrialized area, showed more variable and generally higher concentrations, ranging from 0.02 to 17 ng/L , in 1982. Under polluted conditions, much higher concentrations can occur (Agency for Toxic Substances and Disease Registry, 1998).

The concentrations in sediments ranged from 0.2 to 97 $\mu\text{g}/\text{kg}$ in Lake Ontario in 1982. Sediments at 1–2 cm depth had even higher concentrations, with an average of about 460 $\mu\text{g}/\text{kg}$. Previous studies suggest that the contamination was greater when hexachlorobenzene use was common. A study of agricultural sites in 37 states in the USA in 1972 indicated concentrations varying from 0.01 to 0.44 mg/kg , depending on the type of treatment the soil had experienced. Urban sites in the 1970s also had concentrations of 0.01–0.59 mg/kg . Uncontrolled hazardous waste sites established in the early 1970s are some of the most heavily contaminated areas. Concentrations as high as 20 mg/kg were found in soil at a scenic highway site near Baton Rouge, LA. Deep soil cores from the same site showed concentrations as high as 400 mg/kg (Agency for Toxic

Substances and Disease Registry, 1998). Carvalho *et al.* (1999) reported concentrations of hexachlorobenzene of 0.004–1.1 ng/g dry weight in sediment in coastal Nicaragua lagoons in 1995.

Hexachlorobenzene in sediments can enter the food chain by uptake by small organisms in direct contact with sediment. The measured concentrations in composited fish samples from the Great Lakes ranged from < 2.0 to 3470 µg/kg in 1980–81. More recent results show a decrease in concentrations in the Great Lakes, the concentrations in trout ranging from 0.22 to 9.0 µg/kg. In Galveston Bay, TX, fish, crab and oyster samples were found to contain 0.31–9.6 µg/kg wet weight in 1980–81. Hexachlorobenzene has also been found in wildlife, most notably in birds, with concentrations between 6 and 64 µg/kg in 1983–84. Hexachlorobenzene is preferentially sequestered in fat, and the typical concentration in duck muscle was only 0.4–0.9 µg/kg, whereas the concentration in fat was as high as 70 µg/kg of lipid in water fowl in 1993 (Agency for Toxic Substances and Disease Registry, 1998).

Ray *et al.* (1998) reported mean concentrations of hexachlorobenzene in yellow-tail flounder from off the coast of Newfoundland at several locations ranging from 0.09 to 0.61 µg/kg wet weight in 1993.

Van Oostdam *et al.* (1999) reported an estimated daily intake of hexachlorobenzene intake from breast milk by Inuit infants of 0.6 µg/kg bw per day. Newsome and Ryan (1999) reported a study of breast milk from various populations in northern and southern Canada in 1986, 1992 and 1996. The median and mean concentrations of hexachlorobenzene were 43.5 and 43 ng/g of lipid in northern Canada, which were substantially higher than those in southern Canada. The mean concentration in breast milk from Canadian women in a national study in 1992 was 14.5 ng/g of lipid (mean, 13 ng/g of lipid) (Newsome *et al.*, 1995).

Hexachlorobenzene is found in human tissues, including blood, with mean values of 3 µg/L in Spain (To-Figueras *et al.*, 1995) and 0.7 µg/L in Germany (Gerhard *et al.*, 1998). The concentration in cord blood of newborns ranged from 0.01 to 1.4 µg/L for non-Inuit populations and from 0.02 to 1.2 µg/L for Inuit populations (Van Oostdam *et al.*, 1999).

1.5 Regulations and guidelines

Occupational exposure limits for hexachlorobenzene in several countries are presented in Table 2.

The Joint FAO/WHO Meeting on Pesticide Residues in 1974 established a conditional acceptable daily intake for hexachlorobenzene of 0–0.0006 mg/kg bw, which was withdrawn in 1978 (WHO, 1999). WHO (1993) recommended a guideline limit value of 1 µg/L for hexachlorobenzene in drinking-water.

Because of concerns about risks to human health and the environment, the use of hexachlorobenzene has been discontinued in many countries. For example, according to voluntary national reporting to the Joint FAO/UNEP Programme for the Operation of

Table 2. Occupational exposure limits and guidelines for hexachlorobenzene

Country	Year	Concentration (mg/m ³)	Interpretation
Czech Republic	1993	1	TWA
		2	STEL
France	1993	(Ca)	
Germany	1999	4 ^a	–
Netherlands	1999	0.03	TWA
Poland	1998	0.5 (sk)	TWA
Russian Federation	1993	0.9 (sk)	STEL
USA			
ACGIH (TLV)	2000	0.002 (A3, sk)	TWA

From American Conference of Governmental Industrial Hygienists (ACGIH) (2000); Deutsche Forschungsgemeinschaft (2000)

TWA, time-weighted average; STEL, short-term exposure limit; Ca, carcinogen; sk, danger of cutaneous absorption; A3, confirmed animal carcinogen with unknown relevance to humans; 4, substances with carcinogenic effects in which genotoxicity plays little or no role.

^a Biological Tolerance Value, 150 µg/L in plasma or serum

Prior Informed Consent for Banned or Severely Restricted Chemicals in International Trade, hexachlorobenzene is banned or product registrations have been withdrawn in the Member States of the European Union and the Members associated with the European Union in the European Economic Area, effective in 1979, and in at least six other countries: Australia (1987), Japan (1979), Morocco (1984), New Zealand (1972), Switzerland (1986) and the USA (1987) (FAO/UNEP, 1997).

Hexachlorobenzene is one of 12 persistent organic pollutants being considered for international action to reduce or eliminate their releases under a global convention. As of December 2000, the participating governments had agreed to phase out hexachlorobenzene and five other chlorinated pesticides (aldrin, chlordane, endrin, heptachlor and toxaphene) Hogue, 2000).

2. Studies of Cancer in Humans

2.1 Descriptive studies

During periods of relative famine in the mid-1950s in south-eastern Turkey, seed grain treated with hexachlorobenzene (approximately 2 kg/t of seed) was diverted for bread production, resulting in numerous cases of severe intoxication and an epidemic of porphyria cutanea tarda. Of the estimated 3000–4000 people affected by the disease over

the years 1955–59, 252 patients of an average age of 36 years were traced and offered a clinical and biochemical examination between 1977 and 1987, i.e. 20–30 years after exposure (Peters *et al.*, 1982; Gocmen *et al.*, 1989). No case of malignant hepatic or thyroid tumour was found. During the examinations, 56 samples of milk were obtained from lactating women (Peters *et al.*, 1982) which had an average hexachlorobenzene content of 510 ng/g (standard deviation, 750 ng/g), whereas milk from unexposed controls contained 70 ng/g (Gocmen *et al.*, 1989). [The Working Group noted that the study subjects were relatively young and that the clinical investigation for tumours of the liver and thyroid was restricted to the subgroup of patients who survived to the date of examination, which may have resulted in an underestimation of risk.]

In a descriptive study of mortality (1984–91) and cancer incidence (1980–89) among approximately 5000 inhabitants of a small town located in the vicinity of an organochlorine-contaminating electrochemical factory in Catalonia, Spain, Grimalt *et al.* (1994) observed small clusters among men of incident cases of thyroid cancer (standard incidence ratio [SIR], 6.7; 95% confidence interval [CI], 1.6–28; two cases), soft-tissue sarcoma (5.5; 1.7–18; three cases), brain tumour (2.7; 1.0–7.2; four cases) and cancer of unknown origin (2.4; 1.25–4.4; 10 cases), while the incidence of and mortality from all types of cancers combined were found to be compatible with those expected on the basis of population-based regional incidence and mortality rates. Among the women in the population, no significant associations for equivalent sites were seen. The average concentration of hexachlorobenzene in 40 air samples taken from the area was 35 ng/m³, which was approximately 100 times higher than the average concentration measured in five samples taken in a control city (Barcelona). Similarly, the average concentration of hexachlorobenzene in sera from 13 local volunteers (26 µg/L; range 7.5–69 µg/L) was significantly higher ($p < 0.001$) than that found in samples from 13 subjects chosen in a hospital in the control city (4.8 µg/L; 1.5–15 µg/L).

In the same population, Sala *et al.* (1999b) conducted a cross-sectional study 4 years later of 1800 inhabitants, over 14 years of age, who were interviewed to obtain information on personal health, 16 selected chronic medical conditions and all diseases, cancer included, for which an association with hexachlorobenzene had previously been suspected. In addition, 608 of the 1800 study participants donated blood samples, which were analysed by gas chromatography for content of organochlorine compounds. The median concentration of hexachlorobenzene in all serum samples was 16.5 µg/L. In men, the concentrations were highest among those employed at the factory (geometric mean, 54.6–60.3 µg/L). A benign or malignant neoplasm was reported by 13% of the female participants and 4.5% of the male participants. The confirmed cancer prevalence was non-significantly higher among inhabitants employed at the electrochemical factory than among the other study participants (odds ratio, 1.9).

2.2 Case-control studies

Information on body uptake of hexachlorobenzene and other organochlorine compounds has been reported primarily in studies of breast cancer. Body uptake was measured either by cumulated concentrations of hexachlorobenzene in breast adipose tissue samples, or in serum samples, or both. The case-control studies of breast cancer are grouped according to whether biological samples were collected during diagnosis or treatment (contemporary samples) or whether they were collected from banked serum samples (archival samples). The results of these studies and of studies of cancers at other sites are summarized in Table 3. As hexachlorobenzene accumulates in fat tissue in increasing amounts with age, those studies in which the confounding effect of age was not strictly controlled for are difficult to interpret.

2.2.1 *Studies of breast cancer based on contemporary biological samples*

In a hospital-based case-control study from Helsinki, Finland, Mussalo-Rauhamaa *et al.* (1990) collected 10–20 g of breast adipose tissue from 44 patients in whom breast cancer had been diagnosed in 1985 or 1986. The tissue samples were analysed for the content of neutral organochlorine compounds and polycyclic aromatic hydrocarbons by gas chromatography, and the concentrations were compared with those seen in breast tissue samples obtained during routine post-mortem examinations of 33 accidental fatalities. A total of 41 samples from cases (93%) and all 33 samples from controls showed detectable concentrations of hexachlorobenzene, yielding a mean concentration in positive samples of 140 ng/g (standard deviation, 80) and 110 (50) ng/g of fat, respectively, with an associated *p* value of 0.48. [The Working Group noted that the criteria for selection of study subjects were not given in the report.]

In another small hospital-based case-control study, from Connecticut, USA, Falck *et al.* (1992) measured and compared the concentrations of organochlorine compound residues in 0.5-g samples of breast fat obtained during 1987 from 20 women with breast cancer (cases) and 20 women with benign breast disease (controls), who were all referred to the hospital for initial surgical evaluation. The patients' height, weight and smoking histories were obtained from the medical records or from brief telephone interviews. The mean ages were similar for the two groups (63 and 59 years for cases and controls, respectively), but more controls had a history of current or past smoking (15 of 20) than did cases (six of 20). The mean concentrations of hexachlorobenzene were 23 ng/g of fat (wet weight) in tissues of women with breast cancer and 20 ng/g in women with benign breast disease, with an associated *p* value in a test unadjusted for other variables of 0.32. [The Working Group noted that the criteria for selection of study subjects were not given in the report.]

In a further, small, hospital-based case-control study, from Québec, Canada, Dewailly *et al.* (1994) studied the content of organochlorine compounds in samples of 0.2–1 g of breast adipose tissue from 20 women with breast adenocarcinoma (cases) and

Table 3. Case-control studies of hexachlorobenzene and risk for breast cancer

Reference and location	Subjects in the analysis	Biological sample	Exposure estimates	Mean concentration (ng/g)	Odds ratio (95% CI) or <i>p</i> value	Comments
Studies of breast cancer with contemporary samples						
Mussalo-Rauhamaa <i>et al.</i> (1990) Finland	44 patients 33 controls	Breast fat	Test-positive controls (<i>n</i> = 33) Test-positive cases (<i>n</i> = 41)	110 140	<i>p</i> = 0.48	Only test-positive cases included
Falck <i>et al.</i> (1992) Connecticut, USA	20 patients 20 controls	Breast fat (wet weight)	All control samples All case samples	20 23	<i>p</i> = 0.32	No adjustment for other variables
Dewailly <i>et al.</i> (1994) Québec, Canada	20 patients 17 controls	Breast fat	All control samples Estrogen receptor-negative Estrogen receptor-positive	33 31 42	<i>p</i> = 0.53 <i>p</i> = 0.29	
Güttes <i>et al.</i> (1998) Hesse, Germany	45 patients 20 controls	Breast fat	All control samples All case samples	261 309	<i>p</i> = 0.40	Adjustment for age
Liljegren <i>et al.</i> (1998) Sweden	43 patients 35 controls	Breast fat	Any > 40 ng/g – postmenopausal cases – estrogen receptor-positive – both		1.3 (0.3–4.5) 1.9 (0.4–7.2) 2.0 (0.6–7.5) 7.1 (1.1–45)	Adjustment for age and parity
Moysich <i>et al.</i> (1998) New York, USA	154 patients 192 controls	Serum	All women – first tertile (low) – second tertile – third tertile (high) Parous; never lactated – first tertile (low) – second tertile – third tertile (high)	0–0.34 0.35–0.44 0.45–1.35 0–0.34 0.35–0.44 0.45–1.35	1 0.6 (0.3–1.0) 0.8 (0.4–1.5) 1 1.3 (0.4–4.0) 1.8 (0.6–5.4)	Adjustment for other variables; all women postmenopausal

Table 3 (contd)

Reference and location	Subjects in the analysis	Biological sample	Exposure estimates	Mean concentration (ng/g)	Odds ratio (95% CI) or <i>p</i> value	Comments
Moysich <i>et al.</i> (1998) (contd)			Parous; ever lactated – first tertile (low) – second tertile – third tertile (high)	0–0.34 0.35–0.44 0.45–1.35	1 0.3 (0.1–0.7) 0.5 (0.2–1.1)	
Zheng <i>et al.</i> ₂ (1999) Connecticut, USA	304 cases 186 controls	Breast fat	All women Premenopausal women Postmenopausal women Parous; never lactated Parous; ever lactated	< 12.5 (low) 12.5–15.9 16.0–21.0 ≥ 21.1 (high) < 12.5 (low) ≥ 19.5 (high) < 13.2 (low) ≥ 23.8 (high) > 12.4 (low) ≥ 21.1 (high) < 12.5 (low) ≥ 23.7 (high)	1 0.7 (0.4–1.3) 0.7 (0.4–1.2) 0.9 (0.5–1.6) 1 0.8 (0.3–2.0) 1 0.8 (0.4–1.8) 1 0.7 (0.3–1.7) 1 0.5 (0.2–1.4)	Adjustment for other variables; control women had benign breast disease
Mendonça <i>et al.</i> (1999) Rio de Janeiro, Brazil	162 cases 331 controls	Serum	> 0.2 ng/mL Test-positive cases (<i>n</i> = 4) Test-positive controls (<i>n</i> = 7)		[1.2 (0.3–3.9)]	Unadjusted
Aronson <i>et al.</i> (2000) Ontario, Canada	217 cases 213 controls	Breast fat	All women Premenopausal women Postmenopausal women	≤ 21 22–31 32–51 ≥ 52 ≤ 21 ≥ 32 ≤ 21 ≥ 32	1 1.0 (0.6–1.7) 0.8 (0.4–1.4) 1.2 (0.6–2.3) 1 1.0 (0.5–2.4) 1 0.6 (0.3–1.5)	Adjustment for other variables; control women had benign breast disease

Table 3 (contd)

Reference and location	Subjects in the analysis	Biological sample	Exposure estimates	Mean concentration (ng/g)	Odds ratio (95% CI) or <i>p</i> value	Comments		
Study of breast cancer with archival samples								
Dorgan <i>et al.</i> (1999) Missouri, USA	105 cases 208 controls	Serum	All women	0–62	1	Matched on age, benign breast disease diagnosis, month and year of blood collection		
				63–83	2.5 (1.2–5.3)			
				84–105	1.9 (0.9–4.3)			
				106–406	2.3 (1.0–5.0)			
					<i>p</i> = 0.38 for trend			
				Time since blood collection:				
				≤ 2.7 years				
				0–93	1			
				94–153	1.6 (0.7–3.9)			
				154–406	2.6 (1.1–6.2)			
> 2.7 years								
0–93	1							
94–153	1.9 (0.8–4.4)							
154–406	0.6 (0.2–1.7)							
Endometrial cancer								
Weiderpass <i>et al.</i> (2000) Sweden	154 cases 205 controls	Serum	All control samples	66.2	<i>p</i> = 0.08	Adjustment for lipid content in serum		
			All case samples	70.3				
			All subjects					
			– first quartile (cases/controls)	40.8/40.2 (median)	1	Multivariate, adjusted for age and body mass index		
			– second quartile		1.2 (0.6–2.2)			
			– third quartile		1.0 (0.5–1.9)			
– fourth quartile (cases/controls)	94.2/109.5 (median)	1.0 (0.5–1.9)						

Table 3 (contd)

Reference and location	Subjects in the analysis	Biological sample	Exposure estimates	Mean concentration (ng/g)	Odds ratio (95% CI) or <i>p</i> value	Comments
Pancreatic cancer						
Hoppin <i>et al.</i> (2000) San Francisco, USA	108 cases 82 controls	Serum	All control samples	22	<i>p</i> = 0.22	Adjustment for lipid content of serum
			All case samples	28		
			All subjects	0 0.1–32 > 32		
Hairy-cell leukaemia						
Nordström <i>et al.</i> (2000) Sweden	54 cases 54 controls	Serum	All control samples	45.2	<i>p</i> = 0.11	Unadjusted
			All case samples	44.7		
			Antibodies to EBV early antigen – low/low – high/high			

EBV, Epstein-Barr virus

17 women with breast adenomas or lipomas (controls). The study subjects were chosen from among 41 women who were referred to the medical centre for a biopsy because of a breast mass and who volunteered to participate in the study; four were excluded as they had lesions of borderline malignancy. The estrogen receptor status of the adenocarcinomas was determined, and fat organochlorine concentrations, including hexachlorobenzene, were measured by high-resolution gas chromatography. The mean ages of cases and controls were 54 and 51 years, respectively. The mean adipose tissue concentrations of hexachlorobenzene were 33 ng/g (standard deviation, 13) in the 17 control subjects, 42 ng/g (16) in nine estrogen receptor-positive subjects and 31 ng/g (11.5) in nine receptor-negative subjects. The associated *p* values in significance tests unadjusted for other variables were 0.29 and 0.53, respectively.

Güttes *et al.* (1998) used surgically removed breast tissue from 45 women with breast cancer (cases) and 20 women with various benign breast diseases (controls) to study the relationship between accumulated concentrations of organochlorine compounds in fat tissue and risk for breast cancer. Tissue samples of 0.5–2 g were obtained during 1993 and 1994 from two hospitals in the region of central Hesse, Germany. The mean ages of the breast cancer patients and control subjects were 61 and 50 years, respectively. Data on risk factors for breast cancer other than age were not available. The unadjusted mean concentrations of hexachlorobenzene were 343 ng/g of breast fat (range, 47–2224) in tissue samples from breast cancer patients and 206 ng/g (95–607) in samples from control subjects. However, the authors found that the concentrations of hexachlorobenzene and certain other organochlorine compounds showed a strong, positive correlation with the age of the woman at the time the sample was donated, and a regression analysis with adjustment for age differences between cases and controls gave mean concentrations of 309 and 261 ng/g, respectively, with an associated *p* value of 0.40.

Liljegren *et al.* (1998) studied 78 consecutive patients operated at one clinic in Sweden during 1993–95 for invasive breast cancer (43 cases) or a benign lesion in the breast (35 controls). A sample of approximately 10 g, free of tumour, was taken from the breast during the surgical procedure; fat was extracted, cleaned and analysed by high-resolution gas chromatography and mass spectrometry for hexachlorobenzene and other organochlorine compounds. Data on potential confounders, including parity, lactation, menopausal status, hormonal therapy, smoking habits and family history of breast cancer were assessed from a self-administered standardized questionnaire. Information on estrogen receptor status was obtained from the medical records of the breast cancer patients. The mean ages of breast cancer patients and control subjects were 58 and 54 years, respectively. The mean concentrations of hexachlorobenzene, unadjusted for age or other potential confounding factors, were 72.6 ng/g of fat (range, 12–490 ng/g) in the samples from cancer patients and 48.1 ng/g (17–400) in samples from control subjects. In a multivariate logistic regression analysis, age and parity were found to be the only potential confounders of importance. An odds ratio for breast cancer of 1.3 (95% CI, 0.3–4.5) was estimated for exposure to hexachlorobenzene after adjustment for age,

parity, familial breast cancer history and smoking. Subanalyses with inclusion only of postmenopausal women (32 cases, 21 controls) or of estrogen receptor-positive cancers (32 cases, 35 controls) yielded adjusted risk estimates of 1.9 (0.4–7.2) and 2.0 (0.6–7.5). A further analysis that included only postmenopausal women with estrogen receptor-positive cancers (23 cases, 21 controls) yielded an odds ratio of 7.1 (1.1–45) in association with exposure to hexachlorobenzene. [The Working Group noted that there was no information on the cut-off points used in the multivariate analysis for hexachlorobenzene or other organochlorine compounds in the fat tissue samples.]

Moysich *et al.* (1998) conducted a population-based case–control study of postmenopausal breast cancer in Erie and Niagara counties in western New York, USA, during the period 1986–91. Of 777 white women with histologically confirmed, postmenopausal breast cancer who were eligible for study, 439 (57%) were interviewed; of 1076 postmenopausal community controls, identified from public administrative listings, 494 (45%) agreed to participate. Information on usual diet, reproductive and medical histories and other lifestyle characteristics were obtained at a structured personal interview. Of the women who provided interviews, 262 cases (60%) and 319 controls (65%) agreed to donate a blood sample. However, patients were included only if their blood had been drawn before chemotherapy or radiation and within 3 months of surgery, leaving a total of 154 postmenopausal women with breast cancer for study. Finally, 192 of the 319 controls for whom stored blood was available were frequency matched to cases by date of blood draw (± 3 months) and age (± 3 years). The content of hexachlorobenzene and other organochlorine compounds in the sera was quantified by high-resolution gas chromatography. The mean serum concentrations, adjusted in a multivariate analysis for age and serum lipids, were 0.41 ng/mL of serum (standard deviation, 0.19) in postmenopausal women with breast cancer and 0.42 ng/mL (0.19) in female community controls. Exposure categories were examined in tertiles, on the basis of the distribution of hexachlorobenzene concentrations in the controls, and associated odds ratios for breast cancer were calculated by unconditional logistic regression. With the lower exposure category as reference, the odds ratios for breast cancer in the middle and upper exposure categories, adjusted for age, reproductive factors and other potential confounders, were 0.6 (95% CI, 0.3–1.0) and 0.8 (0.4–1.5), respectively. In order to determine any modifying effect of lactation, women were also stratified by history of breastfeeding, excluding 48 nulliparous women. Within the subgroup of 191 women who had ever breastfed, the middle and upper exposure categories for hexachlorobenzene were associated with odds ratios of 0.3 (95% CI, 0.1–0.7) and 0.5 (0.2–1.1), respectively, while similar analyses within the subgroup of 107 women who had never breastfed showed odds ratios of 1.3 (0.4–4.0) and 1.8 (0.6–5.4), respectively.

From the files of a surgical pathology department of one medical centre in Connecticut, USA, where records of newly completed breast-related surgery were kept, Zheng *et al.* (1999) identified 385 consecutive breast cancer patients aged 40–79 years who were treated surgically during 1994–97 and from whose breast specimen at least 0.4 g of residual breast adipose tissue was available. Of these, 304 (79%) agreed

to participate in the study (cases). The same files were used to identify 251 potential controls who had had breast-related surgery at the centre in whom benign breast disease was histologically diagnosed, besides fulfilling the same inclusion criteria (age, period and available fat sample) as those applied to cases. Of these, 186 (74%) agreed to participate in the study (controls). Information on major known or suspected risk factors for breast cancer was obtained at personal interviews, and the content of hexachlorobenzene in fat tissue samples was determined by gas chromatography. The cases (mean age, 56 years) were significantly older than controls (mean age, 53 years). The mean concentrations of hexachlorobenzene, adjusted in a multivariate analysis for age and sample lipid composition, were similar for breast cancer cases and benign breast disease controls overall (21 ng/g; standard deviation, 17.7 versus 19.1 ng/g; standard deviation, 15; $p = 0.21$) and by menopausal status. Cases and controls also did not differ significantly in the mean concentrations of hexachlorobenzene in adipose tissue when the cases were stratified by estrogen receptor or progesterone status. Quartiles of adipose tissue concentrations of hexachlorobenzene were formed on the basis of the frequency distribution in controls, and a linear logistic regression model was used to adjust for confounders when estimating the exposure–disease relationship. When the lower exposure quartile was used as the standard exposure category (< 12.5 ng/g), women in the second (12.5–15.9 ng/g), third (16.0–21.0 ng/g) and upper (≥ 21.1 ng/g) quartiles had adjusted odds ratios for breast cancer of 0.7 (95% CI, 0.4–1.3), 0.7 (0.4–1.2) and 0.9 (0.5–1.6), respectively. For 186 parous women who reported ever having breastfed, an odds ratio of 0.5 (0.2–1.4) was observed when the highest quartile was compared with the lowest; the equivalent risk estimate for parous women who reported never having breastfed was 0.7 (0.3–1.7).

In a case–control study in Rio de Janeiro, Brazil, Mendonça *et al.* (1999) studied 177 women with invasive breast cancer admitted to one hospital during 1995 and 1996 and 350 controls selected among female visitors to the same hospital. In addition to information obtained at a personal interview with a standardized questionnaire, 10-mL blood samples were taken. Of 162 blood samples available from cases and 331 available from controls, four (2.5%) and seven (2.1%), respectively, showed detectable hexachlorobenzene (> 0.2 ng/mL) [unadjusted odds ratio, 1.2; 95% CI, 0.3–3.9].

Aronson *et al.* (2000) conducted a hospital-based case–control study in two cities in Ontario, Canada, during 1995–97. Of 824 women eligible for study (under the age of 80, no previous diagnosis of cancer, no breast implants and not too ill) who were all scheduled for excision biopsy of a suspected breast cancer, 663 (80.5%) agreed to participate and completed a questionnaire by telephone interview or by mail. The majority of the questionnaires, providing information on known and suspected risk factors for breast cancer, were completed before the participants knew their diagnosis. After biopsy, the histological records of study subjects were reviewed: the cases were subjects in whom in-situ or invasive breast cancer was diagnosed and the controls were subjects with no malignancy (but often with some form of benign breast disease). Organochlorine compounds were determined in tissue from all case women for whom

at least 0.2 g of benign tissue was available ($n = 217$) and in tissue from a subset of control women ($n = 213$) frequency matched by age in 5-year groups and study site. The cases were on average 4 years older than the controls. The geometric mean concentrations of hexachlorobenzene, unadjusted for age, were 32 ng/g (95% CI, 29.3–34.8) in fat samples from cases and 30.1 ng/g (27.8–32.5) in samples from controls. Exposure to organochlorine compounds was examined in four categories, with the cut-point for the upper category at the 85th percentile of the control concentration, and odds ratios were assessed in an unconditional logistic regression analysis. When the lower concentration of hexachlorobenzene (≤ 21 ng/g of fat) was used as the standard exposure category, women in the second (22–31 ng/g), third (32–51 ng/g) and upper (≥ 52 ng/g) exposure categories showed odds ratios adjusted for potential confounders of 0.97 (0.6–1.7), 0.75 (0.4–1.4) and 1.2 (0.6–2.3), respectively. Similar patterns were seen after stratification of study subjects by menopausal status at diagnosis.

2.2.2 *Study of breast cancer based on archival biological samples*

In a case–control study nested in a cohort from the Columbia, Missouri Breast Cancer Serum Bank, USA, Dorgan *et al.* (1999) examined the relationship between exposure to organochlorine pesticides and polychlorinated biphenyls and breast cancer. Of 7224 women initially free of cancer who donated blood to the bank on one or more occasions between 1977 and 1987, 6426 had at least 4 mL of serum remaining in the bank and were included in the study. During up to 9.5 years of follow-up, a histologically confirmed breast cancer was diagnosed in 105 women. For each breast cancer case, two controls were selected from among the eligible women, matched to the case on age, benign breast disease diagnosis during the previous 2 years and month and year of blood collection ($n = 208$). The concentration of hexachlorobenzene was measured by gas chromatography and was corrected for the total lipid content in the sample. Information on clinical status, age, height, weight, menstrual and reproductive histories, smoking, use of medication and family history of breast cancer was obtained by initial self-reporting or medical record review. The case women tended to be better educated than the controls and were more likely to be nulliparous and to have a first-degree relative with a history of breast cancer. Smoking was significantly inversely associated with breast cancer. The percentages of case and control women with concentrations of hexachlorobenzene at or above the limit of detection of the assay were 98.1 and 95.2, respectively ($p = 0.16$). For use in the risk analysis, the women were stratified into quartiles on the basis of the concentration of hexachlorobenzene per gram of serum lipids relative to the distribution in controls. The relative risk, adjusted for potential confounders, was estimated by conditional logistic regression. When the lower exposure quartile was used as the standard exposure category (0–62 ng/g of serum lipid), the second (63–83 ng/g), third (85–105 ng/g) and upper (106–406 ng/g) quartiles showed relative risks of 2.5 (95% CI, 1.2–5.3), 1.9 (0.9–4.3) and 2.3 (1.0–5.0), with a p value of 0.38 in a test for trend. The presence of hexachlorobenzene was significantly positively

associated with the occurrence of breast cancer among women who received their diagnosis close to the time of blood collection (≤ 2.7 years), but not among women whose cancer was diagnosed later (> 2.7 years). In summary, the results of the study do not support the hypothesis that women who are exposed to organochloride pesticides are at increased risk for breast cancer.

2.2.3 *Studies of cancers at other sites*

Weiderpass *et al.* (2000) conducted a case-control study of women, 50–74 years of age in the populations of 12 coastal counties in Sweden, with incident histologically confirmed endometrial cancer diagnosed during 1996–97. The women, who were identified at departments of gynaecology and gynaecological oncology in the study area, were eligible if they were born in Sweden, had not had a hysterectomy and had never used hormone replacement therapy. Of 396 reported patients, 288 (73%) volunteered to donate blood samples and complete a questionnaire. Subsequently, 134 case women were excluded because they had used hormone replacement therapy, leaving 154 in the study. Population controls, frequency-matched to the case by 5-year age groups were randomly selected from the population registers of the study area. Of 742 control women selected, 492 (66%) responded to the questionnaire and donated blood samples. After the exclusion of 287 women because of hysterectomy or use of hormone replacement therapy, 205 control women were included in the study. The self-administered questionnaire requested information on weight, height, reproductive history, diet, hormone use, smoking, physical activity and medical history. Serum samples from the study subjects were analysed for their content of hexachlorobenzene and other organochlorine compounds in the lipid fraction by high-resolution gas chromatography. The mean serum concentrations of hexachlorobenzene, unadjusted for any potential confounder, were 70.3 ng/g of lipid in women with endometrial cancer and 66.2 ng/g in female community controls ($p = 0.08$). Exposure categories were examined in quartiles on the basis of the distribution of hexachlorobenzene concentrations in the controls, and associated odds ratios for endometrial cancer were calculated by unconditional logistic regression. With the lower exposure category as reference, the odds ratio for endometrial cancer in the second, third and fourth quartiles was 1.2 (0.6–2.2), 1.0 (0.5–1.9) and 1.0 (0.5–1.9), respectively. The data do not support the hypothesis that the exposure to the organochlorides studied increased the risk for endometrial cancer.

In a case-control study in the San Francisco Bay Area, USA, conducted during 1996–98, Hoppin *et al.* (2000) studied 108 of 611 patients with incident pancreatic cancer, diagnosed when they were aged 32–85 years, and 82 of 253 control subjects. The controls were frequency-matched to the cases on age and sex by random digit dialling and random sampling of Health Care Financing Administration lists. A personal interview was conducted in which questions on occupational exposures, tobacco use, diet and medical history were posed, and a blood sample was drawn. The serum samples

were analysed for their content of hexachlorobenzene and other organochlorine compounds in the lipid fraction by high-resolution gas chromatography. The mean concentration of hexachlorobenzene, adjusted for the lipid content of serum, was 28 ng/g of lipid in patients with pancreatic cancer and 22 ng/g in control subjects ($p = 0.22$). The exposure categories were examined in tertiles, and the associated odds ratios for pancreatic cancer were calculated by unconditional logistic regression. With the lower exposure category as reference, the odds ratio for pancreatic cancer in the middle and upper exposure categories, adjusted for potential confounders, were 0.9 (95% CI, 0.4–1.9) and 1.6 (0.8–3.4), respectively.

In a small, population-based case–control study in Sweden, Nordström *et al.* (2000) studied 54 of 121 male patients notified to the Swedish Cancer Registry with a diagnosis of hairy cell leukaemia in 1987–92 and 54 of 484 controls drawn from the national population registry and matched to the case on age, sex and county. A questionnaire mailed to study subjects requested information about previous occupations, exposure to potential risk factors for leukaemia and present height and weight. A blood sample was drawn and analysed for hexachlorobenzene and other organochlorine compounds in the serum lipid fraction by high-resolution gas chromatography. Samples were also analysed for titres of antibodies to Epstein-Barr virus early antigen immunoglobulin G. The mean concentration of hexachlorobenzene, unadjusted for the lipid content of serum, was 44.7 ng/g of lipid in patients with hairy cell leukaemia and 45.2 ng/g in control subjects ($p = 0.11$). When concentrations below 43.9 ng/g (the median concentration for controls) were used as the reference category, the odds ratio associated with higher concentrations, adjusted for age and body mass index, was 1.0 (95% CI, 0.4–2.7). A further subdivision of study subjects into those with a high titre of antibodies to Epstein-Barr virus early antigen and those with a low titre (again with the median value of controls as the cut-off) revealed a significantly increased odds ratio for hairy cell leukaemia of 11 (95% CI, 2.2–69) in the subgroup with high antibody titres and a high serum content of hexachlorobenzene. However, the estimate was based on few study subjects. [The Working Group noted that the blood samples were taken after intensive treatment with immunosuppressive drugs.]

3. Studies of Cancer in Experimental Animals

3.1 Oral administration

Mouse: Groups of 30–50 male and 30–50 female Swiss mice, 6–7 weeks of age, were fed diets containing 0 (control), 50, 100 or 200 mg/kg hexachlorobenzene (> 99.5% pure) until they were 120 weeks old, at which time all survivors were killed. A fifth group was given 300 mg/kg hexachlorobenzene for only 15 weeks. At 90 weeks of age, the percentage survival rates in males and females in the five groups were 50 and 48; 30 and 40; 27 and 30; 4 and 0; and 13 and 57%, respectively. The incidence of

lymphomas and lung tumours was not increased in treated animals. No liver-cell tumours were found in the controls or in the group receiving hexachlorobenzene at 50 mg/kg of diet. The incidences of liver-cell tumours in surviving male and female mice at the time the first liver-cell tumour was observed were 3/12, 7/29 and 1/3 in males and 3/12, 14/26 and 1/10 in females for the groups receiving 100, 200 and 300 mg/kg diet, respectively. The effective intakes of hexachlorobenzene that induced liver-cell tumours were 12–24 mg/kg bw per day (Cabral *et al.*, 1979; Cabral & Shubik, 1986).

Rat: Groups of 12 and 14 female Argus rats and four and six female MRC-Wistar rats, 5–7 weeks of age, were fed either control diet or a diet containing 100 mg/kg hexachlorobenzene (99.5% pure) for 90 weeks. The incidences of liver-cell tumours were 0/12 and 14/14 for the Argus control and treated groups, respectively, and 0/4 and 4/6 for the MRC-Wistar control and treated groups, respectively (Smith & Cabral, 1980).

Groups of 10–12 male and 10 female Fischer 334 rats, 6–7 weeks of age, were fed a diet containing 0 (control) or 200 mg/kg hexachlorobenzene for 90 weeks. Liver tumours were observed only in surviving treated female rats, the incidence in this group being 5/10 with neoplastic nodules and 5/10 with 'carcinomas' (Smith *et al.*, 1985).

Groups of 94 male and 94 female weanling Sprague-Dawley rats [age unspecified] were fed diets containing 0 (control), 75 or 150 mg/kg hexachlorobenzene (purity, > 99.5%) for up to 104 weeks. Small numbers of animals were killed at intervals for biochemical and pathological analyses. The tumour incidences at the end of the study are shown in Table 4. Increased incidences of tumours of the liver and kidney were reported. The types of liver tumours diagnosed included hepatocellular carcinomas, bile-duct adenomas and haemangiomas, while the kidney tumours were all adenomas (Ertürk *et al.*, 1986).

Table 4. Incidence of tumours in rats fed hexachlorobenzene

Tumour type	Concentration in the diet (mg/kg)					
	Control		75		150	
	Males	Females	Males	Females	Males	Females
Liver haemangioma	0/54	0/52	10/52	23/56	11/56	35/55
Hepatocellular carcinoma	0/54	0/52	3/52	36/56	4/56	48/55
Bile-duct adenoma	0/54	1/52	2/52	19/56	2/56	29/55
Renal-cell adenoma	7/54	1/52	41/52	7/56	42/56	15/55

From Ertürk *et al.* (1986)

Hamster: A total of 159 female and 157 male Syrian golden hamsters, 6 weeks of age, were given dietary concentrations of 0 (control), 50, 100 or 200 mg/kg hexachlorobenzene (> 99.5% pure) for life, equivalent to a dose of 0, 4, 8 or 16 mg/kg bw per day.

The incidences of hepatomas, liver haemangioendotheliomas and thyroid follicular-cell adenomas were increased by exposure to hexachlorobenzene. The incidences of hepatomas were 0/40, 14/30, 26/30 and 49/57 in males and 0/39, 14/30, 17/30 and 51/60 in females at 0, 50, 100 and 200 mg/kg of diet, respectively. A 'hepatoma' was first observed in a female hamster after 18 weeks of treatment. The incidences of liver haemangioendotheliomas in males and females receiving the highest concentration were 20/57 and 7/60, respectively, compared with 0/40 male and 0/39 female controls. Three of the haemangioendotheliomas metastasized [organ not specified]. A significant increase in the incidence of alveolar adenomas of the thyroid was found in treated animals, with rates of 0/40, 0/30, 1/30 and 8/57 ($p < 0.05$) in males and 0/39, 2/30, 1/30 and 3/60 in females at 0, 50, 100 and 200 mg/kg of diet, respectively (Cabral *et al.*, 1977; Cabral & Shubik, 1986).

3.2 Perinatal administration

Rat: Groups of male and female Sprague-Dawley rats were fed diets containing 0 (control), 0.32, 1.6, 8 or 40 mg/kg hexachlorobenzene. After 90 days on test, the F₀ rats were mated on a one-to-one basis within each treatment group; the F₁ pups were weaned at 21 days of age, divided into groups of 50 males and 50 females and continued on their parents' diets for up to 130 weeks. The mortality curves for control and treated rats were similar in both generations. No statistically significant increase in the incidence of thyroid follicular-cell tumours was found in the F₁ generation, but there were marginally increased incidences of tumours at other sites. In males, parathyroid adenomas were found in 2/48, 4/48, 2/48, 1/49 and 12/49 ($p < 0.05$, Fisher's exact test) at 0, 0.32, 1.6, 8 and 40 mg/kg of diet, respectively, and in females, the incidences of 'neoplastic nodules' of the liver were 0/49, 0/49, 2/50, 2/49 and 10/49 ($p < 0.01$, Fisher's exact test). The incidence of adrenal pheochromocytomas was increased in a linear trend ($p < 0.05$, Cochran-Armitage test) in both sexes: males — 10/48, 12/48, 7/48, 13/48 and 17/40; females — 2/49, 4/49, 4/50, 5/49 and 17/49 ($p < 0.01$, Fisher's exact test) (Arnold *et al.*, 1985; Arnold & Krewski, 1988).

3.3 Administration with known carcinogens or modifying factors

Mouse: Groups of 35 male ICR mice, 7 weeks of age, were fed diets containing 10 or 50 mg/kg hexachlorobenzene (99.9%), 250 or 500 mg/kg of diet polychlorinated terphenyl or 50 mg/kg of diet hexachlorobenzene combined with 250 mg/kg of diet polychlorinated terphenyl for 24 weeks. All surviving animals were killed after 40 weeks. Hexachlorobenzene alone induced no liver tumours. Nodular hyperplasia of the liver occurred in 3/28 animals given 250 mg/kg of diet polychlorinated terphenyl. When polychlorinated terphenyl was given in combination with hexachlorobenzene, 23/28 rats developed nodular hyperplasia and 8/28 developed hepatocellular carcinomas (Shirai *et al.*, 1978).

Groups of male C57BL/10ScSn and DBA/2 mice, 7–10 weeks of age, received an injection of iron dextran (600 mg/kg bw Fe) and 7 days later were fed a diet containing 0 (control) or 100 mg/kg hexachlorobenzene for up to 18 months. None of 11 surviving C57BL/10ScSn mice treated with hexachlorobenzene alone developed liver hyperplastic nodules [authors' terminology] or hepatocellular carcinomas, whereas 10/10 and 9/10 of those given iron dextran plus hexachlorobenzene developed nodules and carcinomas, respectively. All surviving DBA/2 mice were killed at 10 months; no focal hyperplasia was seen with the combined treatment (Smith *et al.*, 1989).

Rat: Groups of male and female Fischer 344/N rats, 10 weeks of age, were given drinking-water containing *N*-nitrosodiethylamine (NDEA) at a concentration of 0 (control) or 0.015% for 3 weeks. After a 2-week recovery period, the animals were fed a diet containing 0 or 200 mg/kg hexachlorobenzene for 30 weeks. No tumours or hyperplastic nodules of the liver were found in animals treated with hexachlorobenzene alone. The average numbers of visible liver tumours > 3 mm and > 10 mm in size were 10.1 and 4.4 in males receiving NDEA plus hexachlorobenzene and 4.0 and 0.6 in rats treated with NDEA alone. In females, the respective numbers were 5.1 and 1.5 compared with 1.2 and 0.3 (Stewart *et al.*, 1989).

Groups of 15–16 male Fischer 344 rats, 6 weeks of age and weighing approximately 120 g, were given a single intraperitoneal injection of 200 mg/kg bw NDEA, followed 2 weeks later by a diet containing 0 (control), 0.6, 3, 15, 75 or 150 mg/kg hexachlorobenzene. Other groups of 10 males received the diets containing hexachlorobenzene only. The numbers and areas of foci positive for glutathione-S-transferase-placental form (GST-P) in the livers were increased in a concentration-related manner, and the increase was statistically significant ($p < 0.001$) at the three highest concentrations (Cabral *et al.*, 1996). Similar results were reported by Gustafson *et al.* (2000) for male Fischer 344 rats given oral doses of hexachlorobenzene (0.1 or 0.4 mmol/kg [29 or 114 mg/kg] bw) daily for 3 weeks after initiation with NDEA.

Groups of male and female Sprague-Dawley rats weighing 175–225 g were subjected to partial hepatectomy or sham operation and 24 h later were treated by oral gavage with 0.3 mmol/kg [31 mg/kg] bw NDEA or distilled water. Four days after NDEA administration, the rats were fed a diet containing 0 or 100 mg/kg hexachlorobenzene for 45 days. One week later, all surviving animals were killed, and liver sections stained for γ -glutamyltranspeptidase (γ -GT)-positive foci. Hexachlorobenzene significantly enhanced the number of foci per cm², both with and without partial hepatectomy. Females were more sensitive than males (Pereira *et al.*, 1982).

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

4.1 Absorption, distribution, metabolism and excretion

4.1.1 *Humans*

Hepta- and octachlorodibenzodioxins and dibenzofurans have been found in technical-grade hexachlorobenzene (IARC, 1979), and there has been some dispute in the literature as to whether these dioxins play a role in the toxic effects associated with exposure to hexachlorobenzene (e.g., Jones & Chelsky, 1986).

Hexachlorobenzene is characterized by a very long half-time and high lipophilicity. The bioconcentration factor for hexachlorobenzene in humans is estimated to be 320, and estimates of the half-time in humans are between 4 and 8 years. Hexachlorobenzene crosses the placenta and is found in fetuses, cord blood, follicular fluid and breast milk. In an investigation of the concentrations of hexachlorobenzene in human placenta, maternal blood, milk and cord blood in 36 healthy pregnant women living in rural Japan, a significant linear correlation was found between the concentration of hexachlorobenzene in placenta and in cord blood and also between placenta and milk (Ando *et al.*, 1985).

Pentachlorothiophenol was initially detected and quantified in all urine samples from 40 persons in the general population with high body burdens of hexachlorobenzene (To-Figueras *et al.*, 1992). In a second study, serum and urine from 100 persons in a general population who had been heavily exposed to airborne hexachlorobenzene were analysed. Hexachlorobenzene was detected in all serum samples, at concentrations ranging between 1.1 and 953 ng/mL. Pentachlorophenol was detected in all urine samples, with values ranging between 0.58 and 13.9 µg excreted within 24 h, with a geometric mean of 2.05 µg. A sulfur derivative that, after hydrolysis, yielded pentachlorobenzenethiol was also identified and quantified in all the urine samples, with values ranging between 0.18 and 84.0 µg within 24 h and a geometric mean of 1.39 µg. The sulfur derivative assessed as pentachlorobenzenethiol appeared to be the main metabolite, its urinary concentrations surpassing those of pentachlorophenol in persons with an accumulated concentration of hexachlorobenzene in serum > 32 ng/mL. The concentrations of pentachlorobenzenethiol in urine collected over 24 h showed a strong association with the concentrations of hexachlorobenzene in serum; the association was stronger in men than in women. A weaker association was found between the concentrations of pentachlorophenol in urine and hexachlorobenzene in serum, which was statistically significant only for men. These results suggested that formation of the cysteine conjugate is a quantitatively important metabolic pathway in humans, especially in persons with high hexachlorobenzene body burdens. Moreover, pentachlorobenzenethiol is a urinary marker of the internal dose of hexachlorobenzene and of glutathione-mediated metabolism (To-Figueras *et al.*, 1997).

4.1.2 *Experimental systems*

(a) *Absorption and distribution*

Hexachlorobenzene administered orally to rats was absorbed slowly from the gut, mainly via the lymphatic system, and was stored extensively in the fat after 48 h (Iatropoulos *et al.*, 1975). In rats fed hexachlorobenzene for 4 weeks, subsequent food deprivation appeared to enhance the toxic response (liver hypertrophy), implying decreased mobilization of hexachlorobenzene residues into fat and resulting in greater accumulation of hexachlorobenzene in plasma, liver, brain and adrenal glands (Villeneuve *et al.*, 1977).

In rhesus monkeys (*Macaca mullata*) given hexachlorobenzene at a dose of 8, 32, 64 or 128 mg/kg bw per day by gavage for 60 days, body fat and bone marrow had the highest concentrations, followed by adrenal glands, liver, kidney, brain, ovaries, muscle and serum. The serum concentrations did not appear to correspond to the dose (Knauf & Hobson, 1979). After administration of a single intravenous injection of hexachlorobenzene to male beagles, the chemical was initially found primarily in the lung (2 h) but after 8 h was found primarily in the fat. Excretion in these dogs occurred essentially through the bile and faeces, urinary excretion being of less importance (Sundlof *et al.*, 1982). Absorption of hexachlorobenzene applied dermally to male Fischer 344 rats increased from 1% to 9.7% between 6 and 72 h, and the blood concentrations increased linearly with time (Koizumi, 1991).

In adult female Sprague-Dawley rats dosed with 50 mg/kg bw hexachlorobenzene by gavage, the chemical was found to concentrate primarily in the fat and also in endocrine glands with large lipid components, such as the follicular fluid of the ovary and thyroid. The concentrations of residues of hexachlorobenzene in nine rats given 50 mg/kg bw per day were significantly ($p < 0.05$) greater in the periovarian fat than in the thyroid gland and were significantly ($p < 0.05$) greater in the thyroid gland than in the adrenal gland and ovary. The concentrations of residues of hexachlorobenzene in the ovary were greater than those in the thymus, liver or lung (Foster *et al.*, 1993).

Hexachlorobenzene was found in the milk of cows given the compound (Fries & Marrow, 1976) and in the organs of 18-day-old offspring of rat dams fed a diet containing hexachlorobenzene (Mendoza *et al.*, 1975).

Toxicokinetics demonstrated that hexachlorobenzene is transferred across the placenta and into breast milk in rodents (Courtney & Andrews, 1985; Courtney *et al.*, 1985; Nakashima *et al.*, 1997). Dose-dependent increases in fetal tissues were found when CD-1 mice or CD rats received a single dose of hexachlorobenzene by gavage on day 11 or 16 of gestation or treatment on days 6–11 or 6–16 at a dose of 10, 50 or 100 mg/kg bw per day (Courtney *et al.*, 1979). A similar 6-day study of pregnant hamsters and guinea-pigs showed that the hamster fetuses had fivefold greater concentrations of hexachlorobenzene than the guinea-pig fetuses (Courtney *et al.*, 1985). In a study in which lactating rhesus monkeys were given hexachlorobenzene by gavage for 60 days at a dose of 64 mg/kg bw per day, the infant serum concentrations were two- to fivefold

higher than those in maternal serum, and their tissue concentrations were also generally higher than those of their mothers. The distribution in infants showed concentration in fat, bone marrow and adrenal glands (Bailey *et al.*, 1980). When pregnant Sprague-Dawley rats were given a diet containing hexachlorobenzene during gestation and lactation (35 nmol/100 g diet [100 µg/kg diet]), about 0.39% of the total intake during gestation was transferred to the fetuses. A large proportion of the hexachlorobenzene body burden was lost during lactation, and the concentration in the stomach contents of suckling pups was highest on day 2 after birth (Nakashima *et al.*, 1997).

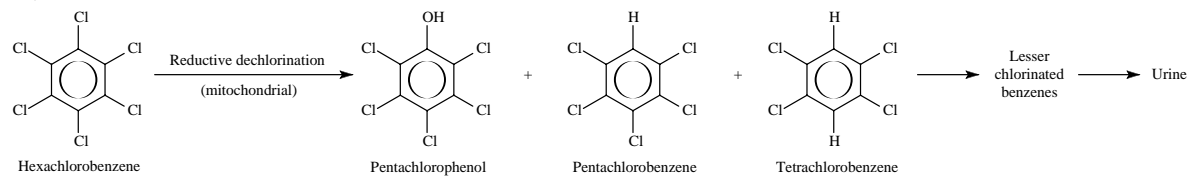
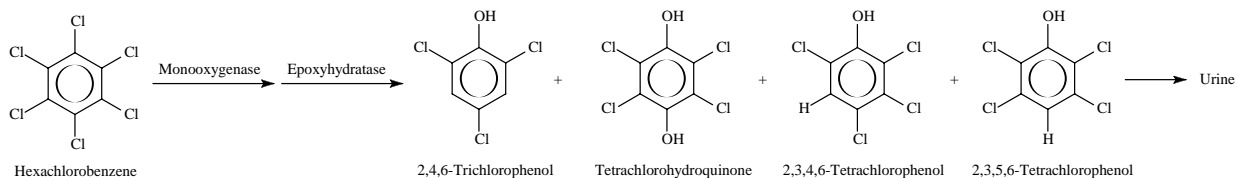
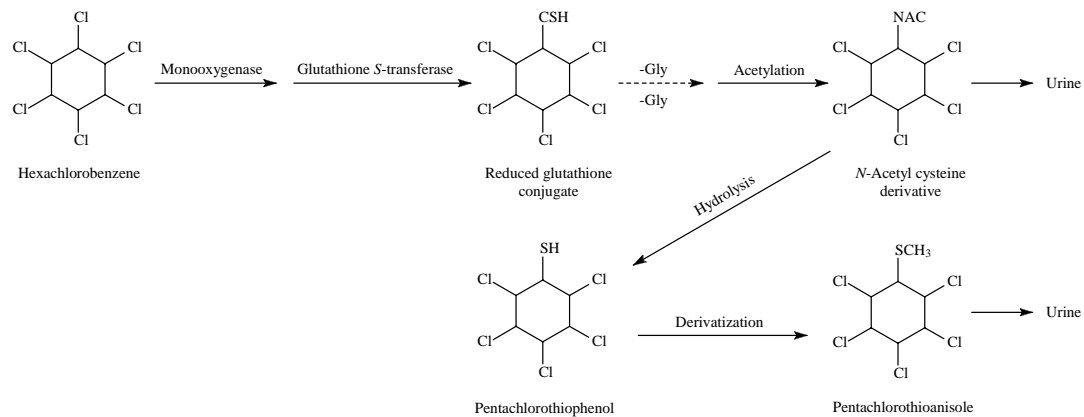
(b) *Metabolism and excretion*

Quantitative recovery of intraperitoneally and orally administered [¹⁴C]hexachlorobenzene in rats was dose-dependent, but more label was recovered from faeces than from the urine. The major urinary metabolites were pentachlorophenol, tetrachlorohydroquinone and pentachlorothiophenol. The other urinary metabolites were tetrachlorobenzene, pentachlorobenzene, 2,4,5- and 2,4,6-trichlorophenols and 2,3,4,6- and 2,3,5,6-tetrachlorophenols; 2,3,4-trichlorophenol and other tetrachlorophenols were present in traces amounts. These metabolites were excreted as conjugates or in free form in the urine. Unchanged hexachlorobenzene was found in the faeces and in fat (Mehendale *et al.*, 1975; Engst *et al.*, 1976; Koss *et al.*, 1976; Renner & Schuster, 1977) (see Figure 1).

After 110 µg/day [¹⁴C]hexachlorobenzene were given orally to *Macaca mulatta* monkeys for 11–15 months, 50% of the radiolabel found in the urine was associated with pentachlorophenol and 25% with pentachlorobenzene, the remaining being associated with unidentified metabolites and unchanged hexachlorobenzene. In the faeces, 99% of the radiolabel was attached to unchanged hexachlorobenzene. During the last 10 days of the experiment, males excreted 7.2% of the administered dose in the urine and 51.9% in the faeces and females excreted 4.6 and 42.2%, respectively (Rozman *et al.*, 1977).

Examination of 1 g of liver tissue from adult female Wistar rats given 178 µmol/kg bw [50.7 mg/kg bw] hexachlorobenzene after 9 weeks revealed the presence of 1 µmol hexachlorobenzene, 50 nmol pentachlorophenol, 5 nmol tetrachlorohydroquinone, 0.1 nmol pentachlorothiophenol and pentachlorothioanisole. The authors hypothesized that the sulfur in the latter two compounds was derived from glutathione (Koss *et al.*, 1978, 1979).

Male and female Fischer 344 rats were dosed every other day for 103 days with 50 µmol/kg bw [14.2 mg/kg bw] hexachlorobenzene by esophageal intubation. This dose produced hepatic porphyria, especially in females. Urine was collected periodically and analysed for pentachlorophenol, 2,3,5,6-tetrachlorobenzene-1,4-diol and pentachlorothiophenol. The combined urinary excretion of these metabolites was greater in females than males, especially during the first 10 weeks. Pentachlorothiophenol was present at particularly high concentrations in the urine of females. The male:female ratios for pentachlorophenol and pentachlorothiophenol in bile were identical to those

Figure 1. Urinary metabolites of hexachlorobenzene**Major metabolites****Minor metabolites****Glutathione conjugation:
formation of sulfur derivatives**

Modified from Agency for Toxic Substances and Disease Registry (1998)

for these compounds in faeces. Excretion of metabolites by both males and females was stimulated by pretreatment with diethylstilbestrol. No sex differences in metabolism were observed in immature rats (Rizzardini & Smith, 1982).

A study of the metabolism of hexachlorobenzene in isolated hepatocytes from male and female Fischer 344 adult rats showed that sex differences in metabolism did not explain the differences in porphyria development. The significant metabolites were pentachlorophenol, pentachlorothiophenol and tetrachloro-1,4-benzenedithiol. Likewise, covalent binding of [^{14}C]hexachlorobenzene to protein after incubation with hepatocytes could not account for the sex-dependent porphyrogenic activity (Stewart & Smith, 1987).

Sexually immature male and female Wistar rats given hexachlorobenzene showed initially no differences in the excretion of *N*-acetyl-*S*-(pentachlorophenyl)cysteine, but 5–8 days after weaning, the urinary concentrations of the sulfur derivative began to increase in females, until a 10-fold difference between the sexes was established. Studies *in vitro* and analysis of tissues after administration of pentachloronitrobenzene *in vivo* showed that conjugation with glutathione and hydrolysis of the conjugates to yield free pentachlorothiophenol did not differ between males and females. These findings tend to reinforce the view that an active renal secretory mechanism, probably induced by estrogens during sexual maturation, is responsible for the highly efficient excretion of sulfur derivatives of hexachlorobenzene and pentachloronitrobenzene by female rats (To-Figueras *et al.*, 1991).

The metabolism of [^{14}C]hexachlorobenzene was studied in microsomes derived from 12-week-old male Wistar rats. The metabolites formed were pentachlorophenol and tetrachlorohydroquinone. In addition, a considerable amount of covalent binding of radiolabel to protein was found: 11 pmol covalent binding per 4 mg microsomal protein in an incubation mixture containing 25 $\mu\text{mol/L}$ hexachlorobenzene. In order to establish the potential role of reductive dechlorination in the covalent binding, the anaerobic metabolism of hexachlorobenzene was investigated. Incubation at low oxygen concentrations indicated a relationship between covalent binding and microsomal oxidation of hexachlorobenzene. The finding of conversion-dependent covalent binding indicated that less than 10% of the covalent binding occurs during conversion of hexachlorobenzene to pentachlorophenol, and the remainder is produced during conversion of pentachlorophenol to tetrachlorohydroquinone, which is in redox equilibrium with the corresponding semiquinone and quinone (chloranil). The covalent binding is inhibited by addition of ascorbic acid or glutathione. These results indicate the involvement of chloranil or the semiquinone radical in covalent binding during microsomal hexachlorobenzene metabolism (van Ommen *et al.*, 1986).

In rats given diets containing either hexachlorobenzene or its metabolite pentachlorobenzene for 13 weeks, both compounds were oxidized to pentachlorophenol and tetrachlorohydroquinone, which were the only two common metabolites excreted in urine. Additional urinary metabolites of hexachlorobenzene were *N*-acetyl-*S*-(pentachlorophenyl)cysteine, which appeared to be quantitatively the most important product,

and mercaptotetrachlorothioanisole, which was excreted as a glucuronide. The biotransformation of hexachlorobenzene and pentachlorobenzene was modulated by selective inhibition of cytochrome P450 (CYP) 3A1/2 in rats by combined treatment with hexachlorobenzene or pentachlorobenzene and triacetyloleandomycin. Rats receiving this diet excreted much less pentachlorophenol and tetrachlorohydroquinone than rats fed hexachlorobenzene or pentachlorobenzene alone, indicating the involvement of CYP3A in the oxidation of both compounds (den Besten *et al.*, 1994).

Male and female Sprague-Dawley rats were given five consecutive doses of 1 g/kg bw hexachlorobenzene by gavage over 2 days. The cumulative dose produced porphyria in female but not male rats after a delay of 6 weeks. The animals were killed 0, 6, 12, 18 or 24 h after the last dose. The hepatic glutathione concentration showed a diurnal cycle in both male and female rats, which was more pronounced in males; the minimum concentration was observed 12 h after dosing. The glutathione concentration in hexachlorobenzene-treated male rats was significantly lower than that in controls at 6, 18 and 24 h, whereas no significant difference was observed in hexachlorobenzene-treated female rats. Biliary excretion of a metabolite originating from glutathione conjugation of hexachlorobenzene was higher in male than in female rats. These results suggested that hepatic glutathione conjugation of hexachlorobenzene is more important in male than in female rats, which may be related to the lower incidence of liver porphyria observed in hexachlorobenzene-treated male than female rats (D'Amour & Charbonneau, 1992).

4.2 Toxic effects

4.2.1 Humans

An epidemic of 4000 cases of porphyria cutanea tarda occurred in Turkey between 1955 and 1959 as a result of human consumption of grain that had been treated with hexachlorobenzene. The estimated intake of hexachlorobenzene was 50–200 mg/day over a relatively long period before the disease became apparent (Peters *et al.*, 1966; Mazzei & Mazzei, 1973; Peters, 1976; Peters *et al.*, 1978). The majority of the patients were children, mostly boys, aged 4–14 years (Cam & Nigogosyan, 1963). A mortality rate of 14% was reported within several years (Peters *et al.*, 1966, 1978). The exposure to hexachlorobenzene led to the development of bullae on sun-exposed areas, hyperpigmentation, hypertrichosis and porphyrinuria. The condition was known as 'kara yara' or 'black sore'. Children under the age of 4 rarely developed porphyria, but in breastfed infants a condition known as 'pink sore' was reported, with a mortality rate greater than 95% (Cam, 1960; Peters, 1976). Samples of breast milk from the mothers of these infants were shown to contain hexachlorobenzene (Peters *et al.*, 1966). Follow-up studies of 32 of the patients have shown that abnormal porphyrin metabolism and active symptomatology persisted 20 years after ingestion of hexachlorobenzene (Peters *et al.*, 1978; Cripps *et al.*, 1980).

In a later follow-up that included examination of 204 patients from this population, the following signs and symptoms were still present: weakness, paraesthesia, neuritis, myotonia, severe residual scarring from the initial bullae, hyperpigmentation and hirsutism. After exposure beginning in childhood, small stature, small hands and painless arthritis were present. Of particular note, enlarged thyroids were present in 25% of men and 60% of women in comparison with 5% of unexposed persons from this region of Turkey. Two persons died of liver failure; one was a 27-year-old man and the other a 54-year-old woman during treatment for tuberculosis (Cripps *et al.*, 1984; Peters *et al.*, 1986). In another follow-up of 252 persons with a history of porphyria after the Turkish incident 20–30 years earlier, similar findings were reported. Many of the patients had dermatological, neurological and orthopaedic symptoms and signs. The observed clinical findings include scarring of the face and hands (83.7%), hyperpigmentation (65%), hypertrichosis (44.8%), pinched facies (40.1%), painless arthritis (70.2%), small hands (66.6%), sensory shading (60.6%), myotonia (37.9%), cogwheeling (41.9%), enlarged thyroid (34.9%) and enlarged liver (4.8%). When urine and stool porphyrin concentrations were determined in all patients, 17 had an elevated concentration of at least one of the porphyrins. A total of 56 specimens of human milk obtained from mothers with porphyria were analysed for hexachlorobenzene. The average value was 0.51 mg/L in hexachlorobenzene-exposed patients and 0.07 mg/L in unexposed controls. The children of mothers with three decades of hexachlorobenzene-induced porphyria appeared to be normal. Three persons had undergone thyroidectomy, which revealed colloidal goitre. One additional person, a 47-year-old woman, had died of liver cirrhosis (Gocmen *et al.*, 1989).

A group of 52 men were exposed to hexachlorobenzene as a by-product in a chemical manufacturing plant in Brazil. The serum immunoglobulin (IgG, IgM and IgA) concentrations of these men were examined and compared with those of unexposed, age- and sex-matched individuals. At the time of testing, the exposed population had a mean concentration of hexachlorobenzene in blood of 38.4 µg/L, with a range of 1–160 µg/L. Increased IgG and IgM concentrations were found in the hexachlorobenzene-exposed workers ($p < 0.05$ and $p < 0.01$, respectively). The IgM concentrations were positively correlated with the length of exposure ($r = 0.367$) and the activities of aspartate aminotransferase ($r = 0.367$) and alanine aminotransferase ($r = 0.507$) (Queiroz *et al.*, 1998a). In 66 exposed workers from this plant, the lytic activity of neutrophils in the presence of antigens from *Candida albicans* and *C. pseudotropicalis* was found to be impaired (Queiroz *et al.*, 1998b).

The average concentration of hexachlorobenzene in the plasma of people living near a hexachlorobenzene manufacturing plant but not exposed occupationally was 3.6 µg/L; there was no evidence of porphyria, but the plasma coproporphyrin concentrations were abnormally high (Burns & Miller, 1975). Urine specimens from nine male aluminium foundry workers in smelters where aluminium was degased with hexachloroethane at six different companies, and from 18 controls, matched for sex, age, residence and socioeconomic status, were analysed for total porphyrins and porphyrin isomers.

Workers exposed to hexachlorobenzene and octachlorostyrene (thermal by-product of hexachloroethane) had a statistically significant increase in total urinary porphyrins over that in controls (mean \pm standard deviation: 13.63 ± 11.13 $\mu\text{mol/mol}$ creatinine and 6.24 ± 3.84 $\mu\text{mol/mol}$ creatinine, respectively; $p = 0.02$) (Seldén *et al.*, 1999).

In a cross-sectional study of 1800 inhabitants of a small village in the south of Catalonia, Spain, which surrounds an electrochemical factory characterized by high concentrations of hexachlorobenzene in the air, biological samples were obtained during 1994 from 615 persons. Self-reported health outcomes were validated against clinical records and cancer registry data. The serum concentrations of hexachlorobenzene were very high in men who worked in the electrochemical factory (geometric mean, 54.6 $\mu\text{g/L}$ in randomized participants) and were lower among subjects who had never worked in the electrochemical factory (women, 14.9 $\mu\text{g/L}$; men, 9.0 $\mu\text{g/L}$). Perceived health and the prevalence of self-reported common chronic conditions, porphyria cutanea tarda, thyroid disease, Parkinson disease, cancer and reproductive outcomes were within the ranges observed in other studies (Sala *et al.*, 1999b).

4.2.2 *Experimental systems*

The oral LD_{50} of hexachlorobenzene in rats varied from 3500 to 10 000 mg/kg bw (Booth & McDowell, 1975).

(a) *Effects on the thyroid*

Male Syrian hamsters were fed diets containing hexachlorobenzene at 100 mg/kg for 28 weeks, 200 mg/kg of diet for 18 or 28 weeks or 500 mg/kg of diet for 6 weeks. All the animals had an at least 2.5–3-fold increase in thyroid size, mainly due to enlargement of some follicles. Serum thyroxine (T4) concentrations were unchanged, whereas those of triiodothyronine (T3) were eventually depressed by more than 60%. The uptake of ^{131}I into the thyroid was induced approximately threefold in hamsters given 500 mg/kg of diet for 3 or 6 weeks. The effects of hexachlorobenzene in hamsters differed from those in rats, as exposure of rats to 500 mg/kg of diet for 6 weeks produced only a small increase in thyroid size (1.3-fold), only a slightly depression in serum concentrations of T3 but a 74% reduction in those of T4 (Smith *et al.*, 1986, 1987).

Administration of 1 g/kg bw hexachlorobenzene for 4 weeks to female Wistar rats resulted in a sixfold increase in T4 metabolic clearance and in the distribution space. Decreased serum T4 concentrations resulted from an increase in both deiodinative and faecal disposal. The metabolism of T3 was only slightly affected. The enhanced peripheral disposition of T4 appeared to lead to increased thyroid function, as measured by augmented thyroid-stimulating hormone (TSH) serum concentrations and ^{125}I uptake in the thyroid. Serum binding of T4 was not affected (Kleiman de Pisarev *et al.*, 1989).

When female Wistar rats were given 1 g/kg bw hexachlorobenzene by gavage daily for 1 or 8 weeks, porphyria and changes in thyroid function and thyroid hormone metabolism were seen. Serum T4 concentrations were depressed, and a 50% reduction

in protein-bound iodine was found, whereas the concentration of T3 was not depressed significantly at either treatment time. Hexachlorobenzene altered T4 metabolism in rat liver slices, increasing dehalogenation. Administration of hexachlorobenzene for 1 week inhibited porphyrinogen decarboxylase activity for uroporphyrinogen disappearance by 25% and coproporphyrinogen formation by 51%. After 8 weeks of hexachlorobenzene administration, the rats showed characteristic porphyria (Kleiman de Pisarev *et al.*, 1990).

Groups of WAG-RIJ rats received oral doses of 0–3.5 mmol/kg bw [0–1 g/kg bw] hexachlorobenzene three times per week for 2 or 4 weeks (highest dose only). Measurements of thyroid hormone status after 2 weeks showed a dose-dependent decrease in total T4 concentration, decreased free T4 concentrations and little change in total T3 concentrations. The effects on thyroid hormone status were more pronounced after 4 weeks and included increased TSH concentrations. The major metabolite, pentachlorophenol, interacted competitively with thyroid hormone-binding proteins in serum to produce a rapid, dose-dependent decrease in total and free T4 concentrations, but not in total T3 concentration, in serum. The decrease in total serum T4 concentrations was attributed to competitive interactions of pentachlorophenol with hormone serum-binding proteins and increased metabolism induced by hexachlorobenzene, to an equal degree. At lower doses and with the shorter dosing, increased metabolism of T4 was the main cause of the decrease in total serum T4 concentration. Therefore, similar effects were produced simultaneously by the parent compound and its metabolite, through different, independent mechanisms (van Raaij *et al.*, 1993a).

In WAG/MBL rats exposed to 1 g/kg bw hexachlorobenzene orally three times a week for 4 weeks, the T4 concentration was lowered by 35.5% whereas that of T3 was unchanged. Analysis of bile by high-performance liquid chromatography revealed a more than threefold increase in T4 glucuronide and a concomitant reduction in unconjugated T4. T4 UDP-glucuronosyltransferase (UGT) activity in hepatic microsomes was increased more than 4.5-fold in animals exposed to hexachlorobenzene, and *para*-nitrophenol UGT showed a comparable increase. T3 UGT activity was increased 2.5-fold by hexachlorobenzene, but androsterone UGT activity was unchanged. These results suggest that T4 is a substrate for hexachlorobenzene-inducible *para*-nitrophenol UGT and T3 for androsterone UGT. In the absence of the latter, T3 is also glucuronidated to some extent by *para*-nitrophenol UGT. Type 1 iodothyronine deiodinase activity was decreased by hexachlorobenzene treatment (van Raaij *et al.*, 1993b).

In Wistar rats treated with 1 g/kg bw hexachlorobenzene by gavage daily for 1 or 4 weeks, depletion of T4 but no change in T3 concentrations were observed in serum. In the liver, mitochondrial L-glycerolphosphate dehydrogenase activity did not change significantly, but the cytosolic enzymes, malic enzyme, glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase, were induced by hexachlorobenzene, only in animals with an intact thyroid. The absence of cytosolic enzyme induction in thyroidectomized rats treated with hexachlorobenzene indicates that this compound is

not intrinsically thyromimetic. The induction of the hepatic cytosolic enzymes in hexachlorobenzene-treated thyroidectomized rats was dependent on the presence of thyroid hormone, administered intraperitoneally at a daily dose of 100 µg/kg bw. The unchanged activity of the thyroid-regulated mitochondrial L-glycerolphosphate dehydrogenase, in contrast to the increased activities of the cytosolic enzymes, was not considered consistent with a shift in functional thyroid status after treatment with hexachlorobenzene (Kleiman de Pisarev *et al.*, 1995).

WAG/RIJ-MBL rats were given either hexachlorobenzene, pentachlorophenol or tetrachlorohydroquinone as a single equimolar intraperitoneal dose of 0.056 mmol/kg bw (i.e. 16, 15 or 14 mg/kg bw, respectively). Hexachlorobenzene did not alter serum T4 or T3 concentrations for up to 96 h after dosing, but pentachlorophenol and tetrachlorohydroquinone both reduced the serum T4 concentrations, with a maximum effect between 6 and 24 h after exposure. Tetrachlorohydroquinone was more effective in repressing T3 than T4 blood concentrations. In another experiment, rats received pentachlorophenol or tetrachlorohydroquinone intraperitoneally at various doses. The reduction in T4 concentration by pentachlorophenol was inversely related to the serum concentration of this compound, on the basis of toxicokinetics and dose-response profiles. Furthermore, the concentration of pentachlorophenol in serum after administration of hexachlorobenzene appeared to be too low to have an effect. The results of this study indicate that not hexachlorobenzene itself, but rather its metabolites pentachlorophenol and tetrachlorohydroquinone, are involved in the reduced serum thyroid hormone concentrations seen after administration of hexachlorobenzene to rats (van Raaij *et al.*, 1991a).

In a competition assay *in vitro*, pentachlorophenol was an effective competitor for the T4-binding sites of serum carriers, whereas hexachlorobenzene was ineffective. Ex-vivo experiments demonstrated occupation of T4-binding sites in sera from pentachlorophenol-exposed animals but not in sera from hexachlorobenzene- or tetrachlorohydroquinone-treated animals. Competing ability for T4-binding sites was still present in the sera of pentachlorophenol-exposed animals but was absent in hexachlorobenzene- and tetrachlorohydroquinone-exposed animals. The results suggest that thyroid hormone displacement by the major metabolite pentachlorophenol may play a role in hexachlorobenzene-induced hypothyroidism (van Raaij *et al.*, 1991b).

Serum T4 and the free T4 index were significantly ($p < 0.05$) suppressed in hexachlorobenzene-treated female Sprague-Dawley rats given 50 mg/kg bw per day by gavage when compared with a control group ($n = 8$). In contrast, no significant differences in the serum concentrations of estradiol or progesterone or in the percentage of T3 uptake were observed. In a second experiment, 16 adult female Sprague-Dawley rats were dosed as above and superovulated by subcutaneous administration of pregnant mare serum gonadotrophin (10 IU) and human chorionic gonadotrophin (20 IU). The circulating concentrations of progesterone were significantly ($p < 0.05$) higher than those in the control group ($n = 8$). The per cent uptake of T3 and the serum concentration of T4 were significantly ($p = 0.05$) suppressed when

compared with controls, with no effect on the free T4 index. These results suggest that the effects of hexachlorobenzene on thyroid parameters may be modulated by hormonal changes in female rats (Foster *et al.*, 1993).

(b) *Effects on the liver*

Hexachlorobenzene has a range of toxic effects on the liver. Those observed in rats after long-term feeding of a diet containing up to 1000 mg/kg bw technical-grade hexachlorobenzene (93–95% pure) included hepatocellular hypertrophy and necrosis, spleen enlargement and porphyria. The survival rates were 70% and 5% for males and females, respectively, at the highest concentration (Kimbrough & Linder, 1974).

Doses of hexachlorobenzene ranging from 0.05 to 50 mg/kg bw per day administered to pigs for 90 days induced porphyria. Animals given the highest dose died. Increased urinary excretion of coproporphyrin was observed in groups receiving 0.5 and 5 mg/kg bw per day after 8 weeks; in those receiving 5 mg/kg bw per day, induction of microsomal liver enzymes, accompanied by increased liver weight, was also observed (den Tonkelaar *et al.*, 1978).

Hexachlorobenzene fed in the diet at 0.1% to rats for 15 days caused marked hepatomegaly and increased microsomal CYP and protein contents. Flow-cytometric analysis revealed no changes in hepatocyte ploidy, and the changes noted were associated with increased hepatocyte size (Rizzardini *et al.*, 1990).

A great deal of research has been focused on the porphyrogenic effects of hexachlorobenzene. Rats can be made porphyrinogenic by long-term administration of 50–1000 mg/kg bw hexachlorobenzene (Koss *et al.*, 1978; Rios de Molína *et al.*, 1980; Krishnan *et al.*, 1991), and the resulting porphyria is associated with inhibition of uroporphyrinogen decarboxylase, similar to spontaneous porphyria cutanea tarda, which has been described in episodes of human poisoning (see above).

Porphyrins accumulate in the urine, liver, kidney and spleen, suggesting an effect on the activity of uroporphyrinogen decarboxylase (UROD) (Doss *et al.*, 1976; Kuiper-Goodman *et al.*, 1977; Goerz *et al.*, 1978). Dosing of female Wistar rats at 50 mg/kg bw per day resulted in increasing concentrations of porphyrins in the liver and urine and of δ -aminolaevulinic acid (ALA) and porphobilinogen in the urine (Koss *et al.*, 1978).

The course of events associated with hexachlorobenzene-induced porphyria was investigated in female Wistar rats given 0.1 g/kg bw hexachlorobenzene every other day for 6 weeks and then kept for an additional 18 months without treatment. During the first phase, the hexachlorobenzene concentration in the liver was almost constant, and the activity of UROD gradually decreased. Then, the porphyrin concentration increased, and there was complete inhibition of UROD. Next, after cessation of hexachlorobenzene administration, the porphyrin concentration continued to increase, and UROD activity continued to be inhibited. Finally, the porphyrin concentration decreased and the activity of UROD returned (Koss *et al.*, 1983).

During exposure of rats to 200 mg/kg of diet hexachlorobenzene for 4–29 weeks, some females showed elevated γ -GT activity throughout the periportal regions of the liver, but all those examined at 29 weeks had a few γ -GT-positive foci. The livers of males were not affected at 29 weeks, but after 90 weeks of feeding, males also had elevated periportal γ -GT activity and a number of γ -GT-positive foci (Manson & Smith, 1984).

In rats fed 0.3% hexachlorobenzene in the diet for 8 weeks, urinary excretion of ALA, coproporphyrin and uroporphyrin increased to reach 2.4, 3.3 and 3.8 times the control values, respectively. In the liver, an increase was observed in ALA synthetase activity and decreases in ALA dehydratase and uroporphyrinogen decarboxylase activities (Kondo & Shimizu, 1986).

Cytosol from female Wistar rat livers was incubated with uroporphyrinogen III and 1-mmol/L concentrations of chlorinated phenols, thiophenols, thioanisoles and benzenes. UROD was inhibited by tetrachlorohydroquinone, pentachlorophenol, pentachlorothiophenol and 1,2,3,5- and 1,2,4,5-tetrachlorobenzene. Other compounds including hexachlorobenzene, which was tested for comparative reasons, did not impair UROD activity, but the concentration used was 0.1 mmol/L. In the presence of tetrachlorohydroquinone, uroporphyrinogen was decarboxylated to hepta- and hexacarboxyporphyrinogen; in the presence of the four compounds with inhibitory effects, pentacarboxyporphyrinogen and coproporphyrinogen were formed in addition. Coproporphyrinogen formation was inhibited completely by tetrachlorohydroquinone, while pentachlorophenol decreased its formation by about 50% and pentachlorothiophenol and 1,2,3,5- and 1,2,4,5-tetrachlorobenzene by < 10% (Billi de Catabbi *et al.*, 1986).

In another study of the inhibitory effects of hexachlorobenzene and its metabolites on UROD, hydroxylated products including pentachlorophenol, 2,3,4,6-tetrachlorophenol and 2,4,5- or 2,4,6-trichlorophenol were active, whereas hexachlorobenzene had no effect (Rios de Molína *et al.*, 1980). In chick embryo liver cells in culture, the main metabolite did not induce porphyria but pentachlorothioanisole and tetrachlorothioanisole did. Inhibition of hepatic drug metabolism by piperonyl butoxide prevented the accumulation of porphyrins, whereas pre-incubation of liver cell cultures with β -naphthoflavone markedly enhanced porphyrin accumulation. The antioxidants ascorbic acid and vitamin E also prevented hexachlorobenzene-induced porphyria, implying a role of a pro-oxidant. Inhibition of UROD was postulated to be the primary lesion induced by hexachlorobenzene, in view of the pattern of porphyrins found in the culture (Debets *et al.*, 1980, 1981a).

Significantly higher concentrations of *N*-acetyl-*S*-(pentahalophenyl)cysteine were found in the urine of hexachlorobenzene-exposed rats than in that of hexafluorobenzene-exposed rats; furthermore, hexafluorobenzene did not cause porphyria, whereas hexachlorobenzene resulted in significantly elevated concentrations of both urinary and liver porphyrins. These results indicate that the extent of metabolism of hexahalogenated benzenes into urinary metabolites resulting from glutathione

conjugation is a better indication of their porphyrinogenic action than the extent of their metabolism to phenolic metabolites (Rietjens *et al.*, 1995).

The porphyrinogenic effects of hexachlorobenzene are stronger in female than in male Fischer and Sprague-Dawley rats (Rizzardini & Smith, 1982; Smith *et al.*, 1985; Krishnan *et al.*, 1991), and this sex-dependent susceptibility is correlated with tumorigenicity. After 90 weeks of hexachlorobenzene treatment, 100% of the surviving female Fisher 344 rats had multiple liver tumours which were strongly γ -GT-positive and classified histologically as neoplastic nodules or hepatocellular carcinomas. In contrast, only 16% of males developed tumours, which were smaller and fewer per liver than those in females. The sex difference in tumour response could not be explained by differences in hepatic hexachlorobenzene concentrations (Smith *et al.*, 1985). Female BDVI rats were given 3 mg/kg bw per day NDEA for 5 weeks by gavage beginning at 4 weeks of age. When the animals were 40 weeks old, 1 g/kg bw hexachlorobenzene was administered daily by gavage for 5 weeks to some animals, and the animals were killed between 46 and 56 weeks of age. The urinary concentration of porphyrins increased during the 5 weeks of treatment with hexachlorobenzene in both NDEA-exposed animals and controls. When the tumour tissue was compared with surrounding parenchyma and tissue from animals receiving hexachlorobenzene only, the concentrations of porphyrins in the tumours were not significantly different from those in controls, whereas there were large accumulations in hexachlorobenzene-exposed 'normal' liver. Also, the activity of ALA synthetase was increased in the latter but not the former (Wainstok de Calmanovici *et al.*, 1991).

Iron is important in the development of porphyria after treatment with hexachlorobenzene. This was demonstrated in an experiment in which female Wistar rats were fed a diet deficient in iron or given a subcutaneous injection of 25 mg/kg bw iron dextran on the first day of the experiment. On day 8, the rats received an intraperitoneal injection of about 1 g/kg bw hexachlorobenzene; all rats were killed on day 60. The uroporphyrin content of the liver of animals treated with iron and hexachlorobenzene was 10 000-fold greater than that in controls and 20-fold greater than that in animals given hexachlorobenzene alone. No uroporphyrin could be detected in the iron-deficient groups, with or without hexachlorobenzene, and iron alone had little effect on the uroporphyrin concentration. Lipid peroxidation, as measured by malondialdehyde formation, was increased, especially in the group given iron plus hexachlorobenzene and to a lesser extent in the group given iron only. Animals given hexachlorobenzene plus iron showed evidence of protein-protein cross-linking, as measured by sodium dodecyl sulfate gel electrophoresis (Alleman *et al.*, 1985).

In female AGUS and Fischer 344 rats given diets containing hexachlorobenzene for 65 weeks at 0.01 or 0.02% and pretreatment with an iron-loading subcutaneous injection of iron-dextran complex, hepatocellular necrosis and fibrin deposition in the areas of sinusoidal dilatation were more marked, and sinusoidal telangiectasis was accelerated. Control rats had none of these changes, except for an occasional macrophage with iron staining (Carthew & Smith, 1994).

Groups of 12 female Chbb THOM rats were given the iron chelator desferrioxamine by intramuscular injection of 100 mg/kg bw three times a week, hexachlorobenzene (1 g/kg bw per day) by gavage or desferrioxamine at the start of hexachlorobenzene treatment and throughout the experiment. All rats were killed after 12–14 weeks. Treatment with desferrioxamine delayed and diminished urinary excretion of precursors and porphyrins by reducing the liver iron concentration and reduced the accumulation of porphyrins in the liver induced by hexachlorobenzene by attenuating the activity of the enzymes porphyrinogen carboxylase and ALA synthetase (Wainstock de Calmanovici *et al.*, 1986).

Factors other than iron are also important. In a comparative study, female Wistar and Chbb THOM rats were given 1 g/kg bw per day hexachlorobenzene by gavage for 7 weeks. In Wistar rats, hepatic porphyrins were increased 140-fold and ALA synthetase activity fourfold, and UROD activity was inhibited by 70%. In Chbb THOM rats, these values were threefold, 1.7-fold and 22%, respectively. As the total iron content was similar in the two strains, the difference in susceptibility to porphyria was not wholly ascribable to differences in iron metabolism (Wainstock de Calmanovici *et al.*, 1989).

The involvement of CYP3A in hexachlorobenzene-induced porphyria was established in biotransformation studies with microsomes derived from male Wistar rats treated with various inducers of CYP isoenzymes, and by selective inactivation of CYP3A by triacetyloleandomycin, resulting in strong inhibition of the microsomal conversion of hexachlorobenzene and pentachlorophenol. In-vivo inactivation of CYP3A was achieved by co-administration of hexachlorobenzene and triacetyloleandomycin. Female Wistar rats treated with these compounds in the diet (hexachlorobenzene, 0.03%; triacetyloleandomycin, 0.3%) for 10 weeks showed strongly diminished urinary excretion of the major oxidative metabolites (pentachlorophenol and tetrachlorohydroquinone), as compared with rats treated with hexachlorobenzene alone. Concomitant administration of triacetyloleandomycin resulted in complexation of 70% of the total amount of hepatic microsomal CYP. The group treated with hexachlorobenzene alone had a 600-fold increase in the amount of hepatic porphyrins, whereas concomitant administration of triacetyloleandomycin almost completely inhibited this effect. A strong correlation was found between the amounts of porphyrins and oxidative metabolites excreted, as a function of length of exposure (van Ommen *et al.*, 1989).

Another study with triacetyloleandomycin confirmed the role of CYP3A in the development of porphyria. This study suggested that a putative reactive intermediate in the primary oxidative step plays a role in hexachlorobenzene-induced porphyria, since the degree of porphyria was highly correlated with the excretion of pentachlorophenol and much more weakly correlated with early excretion of tetrachlorohydroquinone (den Besten *et al.*, 1993).

Modulating thyroid function also influences hexachlorobenzene-induced porphyria. The serum T4 concentrations in female Wistar rats were depressed after 8 days of administration of 1 g/kg bw per day hexachlorobenzene by gavage, whereas the concentrations of T3 were not altered. Administration of T4 at 100 µg/kg bw per day simulta-

neously with hexachlorobenzene resulted in hyperthyroxinaemia. A significant decrease in UROD activity was found after 8 days of treatment with hexachlorobenzene in T4-treated rats, but in rats with no T4 administration and in thyroidectomized rats this decrease in UROD activity was delayed to 21 and 30 days, respectively. Therefore, thyroid hormones seemed to enhance the induction of hexachlorobenzene-induced porphyria (Sopena de Kracoff *et al.*, 1994).

Hexachlorobenzene-induced porphyria was associated with lipid peroxidation in rats with iron loading. Female Sprague-Dawley rats were fed a diet containing hexachlorobenzene at 0.2% (w/w), carbonyl iron at 1.0% (w/w) or hexachlorobenzene plus iron for 8 weeks. The total hepatic porphyrin concentration was increased 100-fold in rats receiving hexachlorobenzene or hexachlorobenzene plus iron, and there was a significant increase in mitochondrial lipid peroxidation, as measured by the concentration of conjugated dienes, in these treated rats (Feldman & Bacon, 1989).

Female Wistar rats given 1 g/kg bw per day hexachlorobenzene for 1–8 weeks showed decreased UROD activity and the presence of an inhibitor of the enzyme during the first 2 weeks. This inhibitor was isolated from a supernatant obtained after centrifugation at $11\ 000 \times g$, which was filtered through Sephadex G-25, heated for 5 min at $100\ ^\circ\text{C}$ and then centrifuged at $1000 \times g$ for 10 min. The concentration of hepatic porphyrins began to increase during the second week of treatment, became statistically significant during the third week and continued to increase over subsequent weeks. During this period, measures of lipid peroxidation malondialdehyde and conjugated dienes also increased. The phospholipid content showed an initial increase, ascribed to a proliferation of membranes, and a later decrease, ascribed to toxic effects involving membrane destruction (Billi de Catabbi *et al.*, 1997).

After 30 days of treatment of male Wistar rats with 25 mg/kg bw per day hexachlorobenzene by gavage, increases in microsomal CYP content, thiobarbituric acid-reactive substances, adrenochrome production (a marker of superoxide production), NADPH cytochrome c reductase and NADPH oxidase were observed in the liver. In plasma, increases in malondialdehyde, ascorbic acid and urinary coproporphyrin concentrations were also found. The liver showed severe lesions with vacuolization and nuclear degeneration mainly in zone 3 hepatocytes. Plasma aspartate and alanine aminotransferase activities were also increased (Almeida *et al.*, 1997).

(c) Neurotoxic effects

Adult mink (*Mustela vison*) and ferrets (*Mustela putorius furo*) were given diets containing hexachlorobenzene at a concentration of 1, 5 or 25 mg/kg for 47 weeks. The concentration of hypothalamic serotonin (5-HT) was significantly elevated at all doses in mink, and that of cerebellar 5-HT was significantly elevated at 1 mg/kg of diet in ferrets. Regional concentrations of brain biogenic amines were determined in the offspring of female mink given 1 or 5 mg/kg of diet hexachlorobenzene. The hypothalamic dopamine concentrations were significantly depressed in the kits at both concentrations. Animals receiving 125 or 625 mg/kg of diet hexachlorobenzene died

before termination of the experiment, the female ferrets at 125 mg/kg of diet displaying abnormal aggressiveness and hyperexcitability just before death (Bleavins *et al.*, 1984).

(d) *Nephrotoxicity*

Groups of male and female Sprague-Dawley rats were given hexachlorobenzene in corn oil by gavage for 15 days (on days 1–5, 8–12 and 15) or 50 mg/kg bw hexachlorobenzene in 36 doses (on 5 days/week over 50 days). Urine was collected on days 1, 8 and 15 of the 15-day treatment and on day 50 of the 50-day treatment. In both males and females in the first study, hexachlorobenzene had induced glycosuria by day 8. In males only, hexachlorobenzene induced proteinaemia in both studies. Histological examination of the kidneys of male rats revealed degenerative and regenerative cellular foci accompanied by increased accumulation of protein droplets in epithelial cells of the proximal tubules. Similar histological observations were made in male rats after 50 days of hexachlorobenzene treatment. No such histological alterations were observed in the kidneys of female rats. In male rats, the concentration of α_{2u} -globulin in kidney was increased 11-fold as compared with controls in the 15-day study; no results for this end-point were reported in the 50-day study. In addition, hexachlorobenzene was found to be bound reversibly to α_{2u} -globulin (Bouthillier *et al.*, 1991).

(e) *Effects on the immune system*

Male BALB/c mice fed a diet containing 167 mg/kg hexachlorobenzene for 6 weeks became immunosuppressed, as indicated by decreased serum globulin concentrations and a decreased response of spleen lymphocytes to sheep red blood cells (Loose *et al.*, 1977). Male Wistar rats receiving 0.5, 1 or 2 mg/kg bw per day hexachlorobenzene for 3 weeks showed increased numbers of neutrophils, monocytes and basophils and increased IgM concentrations. Hexachlorobenzene did not alter cell-mediated immunity but it altered humoral antibody responses (Vos *et al.*, 1979a). In the offspring of female rats given diets containing hexachlorobenzene at concentrations ≤ 150 mg/kg, suppression of cellular and humoral immunity was reported (Vos *et al.*, 1979b). In BALB/c mice fed a diet containing 167 mg/kg hexachlorobenzene, measures of humoral immune response were found to be decreased after 6 weeks, and increased susceptibility to *Plasmodium berghei* infection and endotoxin were found (Loose *et al.*, 1979). Some effects of hexachlorobenzene aerosols (3.5 and 35 mg/m³) on pulmonary bactericidal, macrophage phagocytic and alveolar macrophage enzyme activities were found in male Sprague-Dawley rats (Sherwood *et al.*, 1989).

Female Brown Norway, Lewis and Wistar rats were fed diets containing different doses of hexachlorobenzene (because of differences in toxicity in these strains) of 150–900 mg/kg for 4 weeks. Skin lesions were found, which were most severe in Norway and least severe in Wistar rats. The occurrence of pulmonary lesions was not strain-dependent, but immunomodulation varied somewhat by strain, Brown Norway rats showing the most splenomegaly; IgE or IgG concentrations were dose-dependent

in this strain (Michielsen *et al.*, 1997). In a study of Brown Norway and genetically euthymic and athymic WAG/Rij rats with or without depletion of T cells caused by adult thymectomy, thymus-derived T cells were not required for the production of skin and lung lesions and splenic changes by hexachlorobenzene (Michielsen *et al.*, 1999).

In a study of male Wistar rats fed diets containing 0, 500 or 1000 mg/kg hexachlorobenzene for 3 weeks, the total IgM concentration was increased, as were the weights of the spleen and lymph nodes, but the IgG concentrations were unaffected (Schielen *et al.*, 1993). The immune effects in female Wistar rats in the studies of den Besten *et al.* (1993, 1994) (described in sections 4.1.2(b) and 4.2.2(b)) were probably due to the parent compound, hexachlorobenzene, or its non-oxidative metabolites and were not dependent on oxidative metabolism, in contrast to the production of porphyria. Therefore, the dose-dependently increased weights of lymph nodes and spleen and the increased serum concentrations of IgM, IgA and autoantibody-specific IgM were not secondary to porphyria (Schielen *et al.*, 1995).

A dose-dependent increase in interleukin-2 and interferon- α mRNA levels was found in cultured spleen cells derived from male Wistar rats fed diets containing 50, 150 or 450 mg/kg hexachlorobenzene for 6 weeks (Vandebriel *et al.*, 1998).

(f) *Effects on the parathyroid*

Human exposure to hexachlorobenzene has resulted in demineralization of bone and development of osteoporosis. Experiments were undertaken to investigate the effects of hexachlorobenzene on the homeostatic mechanism of calcium metabolism. Fischer 344 rats were given an intragastric dose of 0, 0.1, 1, 10 or 25 mg/kg bw hexachlorobenzene on 5 days/week for 5 weeks. Serum cholesterol, alanine aminotransferase activity, 1,25-dihydroxyvitamin D₃ and parathyroid hormone concentrations were significantly higher than control values. The urinary calcium concentration decreased significantly with increasing dose of hexachlorobenzene, indicating conservation of calcium. In an experiment conducted for 5, 10 or 15 weeks, serum alkaline phosphatase activity was significantly decreased at the two higher doses after both 10 and 15 weeks of exposure. 1,25-Dihydroxyvitamin D₃ was measured in the group exposed for 5 weeks and was found to be significantly elevated at the three higher doses. After 5 and 15 weeks of exposure to hexachlorobenzene, the concentration of parathyroid hormone was significantly elevated at the two higher doses at both times. Wet femur density was significantly increased at the two higher doses after 10 weeks of exposure and at the three higher doses after 15 weeks. Bone strength was also significantly increased at the three higher doses (Andrews *et al.*, 1988, 1989, 1990).

(g) *Other effects*

Decreases in circulating concentrations of corticosterone were found when hexachlorobenzene was administered by gavage at 1, 10 or 100 mg/kg bw per day for 30 days to ovariectomized Sprague-Dawley rats. The serum concentrations of progesterone and aldosterone were not altered (Foster *et al.*, 1995a).

Female Wistar and Chbb THOM rats were dosed with 1 g/kg bw hexachlorobenzene by gavage on 5 days/week for up to 4 weeks. The Wistar rats showed an increased porphyrin content in the Harderian gland and changes in phospholipid metabolism, whereas the other strain showed a decrease (Cochón *et al.*, 1999).

4.3 Reproductive and developmental effects

4.3.1 Humans

Continuing occupational exposures (e.g., Grimalt *et al.*, 1994) and the accidental exposure of Turkish populations in the 1950s (Peters *et al.*, 1987) have provided extensive information on human health outcomes associated with exposure to hexachlorobenzene.

In the epidemic of poisoning that occurred in Turkey (see section 4.2.1), breastfed infants showed a condition known as 'pink-sore' and had a mortality rate > 95%. Samples of breast milk from the mothers of these infants were shown to contain hexachlorobenzene (Cam, 1960; Peters *et al.*, 1966; Peters, 1976).

Positive associations were reported between hexachlorobenzene concentrations in breast milk (> 146 µg/kg of fat) and the risk for otitis media during the first year of life among Inuit infants in Canada (Dewailly *et al.*, 2000). A case-control study of miscarriage in Germany reported a correlation between blood hexachlorobenzene concentration and small decreases in follicle-stimulating hormone concentration and decreases in immunological markers (CD8, CD4:CD8 ratio) in the women, but no apparent relationship between hexachlorobenzene concentration and the risk for miscarriage (Gerhard *et al.*, 1998).

Jarrell *et al.* (1998) reviewed the reproductive outcomes associated with the Turkish episode and reported on the reproductive effects in women who were exposed as children in the 1950s. The serum concentrations of hexachlorobenzene were measured in persons with porphyria cutanea tarda and in two control groups 40 years after the original exposure. Little difference was found. The authors also found no relationship between current hexachlorobenzene and circulating hormone concentrations, but they did find a relationship between the rate of spontaneous abortion and the serum hexachlorobenzene concentration.

4.3.2 Experimental systems

Hexachlorobenzene has been reported to affect both reproduction and development.

Prenatal exposure of CD-1 mice to 10 or 50 mg/kg bw hexachlorobenzene on days 6–17 of gestation caused significant postnatal mortality (Courtney *et al.*, 1984). Exposure to hexachlorobenzene affected the female gonad histopathologically and endocrinologically. Hexachlorobenzene induced follicular degeneration and increased atresia in rodents and primates (Sims *et al.*, 1991; Jarrell *et al.*, 1993); it affected cyclicity in both rodents and primates (Foster *et al.*, 1992a,b, 1995a,b), and it altered gonadal

steroidogenesis (Foster *et al.*, 1992a,b, 1993, 1995a,b). The highest dose of hexachlorobenzene used (10 mg/kg bw per day orally for 90 days) specifically reduced the numbers of primordial follicles in cynomolgus monkeys (*Macaca fascicularis*) (Jarrell *et al.*, 1993). The effects of hexachlorobenzene on ovarian morphology and steroidogenesis can be induced at a dose as low as 0.1 mg/kg bw per day for 90 days in cynomolgus monkeys (Foster *et al.*, 1996). Alvarez *et al.* (2000) also reported altered cyclicity and reduced ovulation in response to daily exposure of male Wistar rats by gavage to 1 g/kg bw hexachlorobenzene for 30 days. Decreased uterine estrogen receptor concentrations were also observed in these rats, but no change was found in uterine weight. No data on the histological appearance of the uterus were presented. No studies were found on the effects of hexachlorobenzene on the male reproductive system.

Hexachlorobenzene also affects development. Exposure *in utero* and during lactation caused neonatal mortality in rats fed diets containing 60–140 mg/kg hexachlorobenzene (Kitchin *et al.*, 1982) and in monkeys given 64 mg/kg bw per day by gavage for 60 days (Bailey *et al.*, 1980). After more limited exposure *in utero*, hexachlorobenzene induced structural malformations, including cleft palate, enlarged kidneys and hydronephrosis, in CD-1 mice. The latter effects were observed in the pups of dams treated with 10 or 50 mg/kg bw per day on days 6–16 of gestation (Andrews & Courtney, 1986). These malformations were strikingly similar to the terata associated with exposure to dioxins *in utero*, as noted by these authors, which raises the issue of the possible presence of traces of dioxins in technical-grade hexachlorobenzene. A dose of 10 mg/kg bw hexachlorobenzene, even at 99% purity (Andrews & Courtney, 1986) could still result in exposure to dioxin of as much as 100 µg/kg bw, which is well within the range of doses of 2,3,7,8-tetrachlorodibenzo-*para*-dioxin (TCDD) that are teratogenic (see Dienhart *et al.*, 2000).

Prenatal exposure to hexachlorobenzene causes additional developmental effects. Barnett *et al.* (1987) reported that BALB/c mice exposed daily to 0.5 or 5 mg/kg bw hexachlorobenzene throughout gestation had alterations in immunological parameters. Both doses diminished delayed-type hypersensitivity responses in offspring tested at 40 days of age; the higher dose impaired mixed lymphocyte response to allogenic spleen cells; and, in adulthood, small changes in the relative proportion of T and B cells in the spleen were observed.

Two studies were conducted to determine the effects of exposure to hexachlorobenzene during development on neurobehavioural end-points. Female Sprague-Dawley rats were exposed before gestation to hexachlorobenzene by gavage at a dose of 2.5 or 25 mg/kg bw per day for 4 days. Two weeks later, they were mated with unexposed males. This treatment did not affect either maternal or pup body weight. In assessments of behaviour during the first 20 days after birth, hexachlorobenzene-exposed pups responded more quickly to negative geotaxis tests and in olfactory discrimination tests. They were also more active than controls up to postnatal day 60, and were less reactive to acoustic startle on postnatal day 23 but more reactive on

postnatal day 90. The complexity of these effects makes it difficult to propose hypotheses about the mechanism of action, but the results demonstrate that hexachlorobenzene can affect neurobehavioural function in developing rats (Goldey & Taylor, 1992). Female albino Wistar WU rats were fed diets containing hexachlorobenzene at 4, 8 or 16 mg/kg before and during gestation and lactation. After weaning, the pups were given the same diet as their dams. No changes were found in open-field activity at postnatal day 21; however, in tests of operant learning begun at postnatal day 150, treated animals showed decreased response rates (Lilienthal *et al.*, 1996).

4.4 Effects on enzyme induction or inhibition and gene expression

4.4.1 *Humans*

No data were available to the Working Group.

4.4.2 *Experimental systems*

The porphyrinogenic effects of hexachlorobenzene are associated with induction of several hepatic CYP enzymes, as shown in early studies (Grant *et al.*, 1974; Mehendale *et al.*, 1975; Stonard, 1975).

In groups of female AGUS or Wistar rats given 50 mg/kg bw hexachlorobenzene by gavage every other day for 8 weeks, large increases in ethoxyresorufin deethylase activity were found in both strains, although the activities were higher in AGUS rats; however, AGUS rats showed less induction of GST activity (Debets *et al.*, 1981b). In adult female rats fed hexachlorobenzene at a concentration that induced porphyria, the activity of microsomal glucuronyltransferase was increased (Graef *et al.*, 1982). Both crude and purified hexachlorobenzene were found to increase the activity of liver enzymes in male and female Sprague-Dawley rats 4 days after an intraperitoneal injection of 150 mg/kg bw. The enzyme activities induced included benzphetamine *N*-dealkylation, ethoxyresorufin deethylation and ethoxycoumarin *O*-dealkylation (Franklin *et al.*, 1983).

The effect of a single dose of 200 mg/kg bw hexachlorobenzene was compared in male Syrian hamsters and male Fischer 344/N rats. Although the total CYP content of the liver was induced much more in hamsters (4.7-fold) than in rats (2.5-fold), there was little induction of either pentoxy- or ethoxyphenoxazone dealkylation in hamsters. In rats, the two enzyme activities were induced approximately 12- and fivefold, respectively (Smith *et al.*, 1987).

Hexachlorobenzene and 2,3,4,4',5-pentachlorobiphenyl induced a similar spectrum of CYP-dependent monooxygenase activities in rats, including 4-dimethylaminoantipyrine *N*-demethylase, aryl hydrocarbon (Ah) hydroxylase and ethoxyresorufin deethylase (Li *et al.*, 1986).

Hexachlorobenzene induced both of the phenobarbital-inducible forms, cytochrome P450b and P450e [CYP2B1 and CYP2B2], and the 3-methylcholanthrene-inducible

forms, cytochrome P450c and P450d [CYP1A1 and CYP1A2], in rat liver microsomes. The concentration of P450d [CYP1A2] was considerably greater than that of P450c [CYP1A1] in hexachlorobenzene-induced rat liver. Hexachlorobenzene increased the amounts of mRNAs for P450b, P450c and P450d [*CYP2B1*, *CYP1A1* and *CYP1A2*] mRNA in rat liver polysomes, suggesting that it increases the synthesis of the corresponding proteins (Goldstein *et al.*, 1986; Linko *et al.*, 1986).

Feeding 0.1% hexachlorobenzene in the diet to female Sprague-Dawley rats for 55 days resulted in a fourfold increase in urinary porphyrin excretion and a significant decrease in serum T4 concentration (< 10 versus 38 µg/L in controls) and in T3 concentration (0.49 versus 0.64 µg/L in controls). The rats were then fed normal diet for an additional 42 days. During that time, one-third of the rats died, and the urinary porphyrin concentration increased to 100 times the control level. In the surviving animals killed at the end of the experiment, the liver microsomal enzymes ethoxyresorufin deethylase, pentoxyresorufin deethylase, aminopyrine *N*-demethylase and UGT were induced 10-fold, 12-fold, < 2-fold and 2-fold, respectively. It was further noted that, whereas the animals that died began wasting 5 days after cessation of intake of hexachlorobenzene, when relatively minor disturbances in porphyrins were found, the survivors developed major porphyrin disturbances without wasting; the deaths were therefore not correlated with porphyria (Rozman *et al.*, 1986).

Microsomal cytochrome P450 was induced to a greater extent in male than in female Fischer rats, while cytochrome b₅ was induced only in males. Aminopyrine-*N*-demethylase activity doubled in animals of each sex after treatment, while that of Ah hydroxylase was 16 times the control value in females and 1.5 times the value in males. After hexachlorobenzene treatment, the phospholipid content of microsomal membranes in liver was increased, while the cholesterol content was unchanged. Analysis of the phospholipid pattern showed that hexachlorobenzene interfered with the biosynthesis of phospholipids containing choline. Hexachlorobenzene showed more pronounced features of a 'phenobarbital type' inducer in males than in females (Cantoni *et al.*, 1987).

Hexachlorobenzene at a concentration of ≥ 1 µmol/L inhibited the specific binding of [³H]TCDD (0.3 nmol/L) to the Ah receptor *in vitro*, and the inhibition was competitive, with a K_i of approximately 2.1 µmol/L. In rats fed a diet containing 3000 mg/kg hexachlorobenzene for 4 h to 7 days, the specific binding of [³H]TCDD in hepatic cytosol was reduced by up to 40%, due principally to a decrease in the number of binding sites for [³H]TCDD rather than to competition from residual hexachlorobenzene. As shown by immunoblotting and radioimmunoassay, hexachlorobenzene induced CYP1A1 and CYP1A2, which are regulated by the Ah receptor, as well as the phenobarbital-inducible isozymes CYP2B1 and CYP2B2. Taken together, these results indicate that hexachlorobenzene is a weak agonist for the Ah receptor and suggest that some of its effects are mediated by its interaction with this gene-regulatory protein (Hahn *et al.*, 1989).

A study in *Cyp1a2*(-/-) mice showed that the presence of CYP1A2 is essential for the production of the uroporphyrin caused by hexachlorobenzene and iron (and by 3-methylcholanthrene). In this experiment, male wild-type C57BL/6J mice, which had been injected with 500 mg/kg bw iron dextran 3 days earlier, were compared with mice containing the *Ahr*^b allele and injected intraperitoneally with 100 mg/kg bw hexachlorobenzene. After 42 days, the mice were injected a second time with 75 mg/kg bw hexachlorobenzene and killed 20 days later. In wild-type mice, the hepatic uroporphyrin concentration ranged from 70 to 310 nmol/g of liver, whereas in null mice it was < 1 nmol/g of liver. Exposure of another group to iron dextran and hexachlorobenzene and to ALA in drinking-water for 58 days before sacrifice resulted in a uroporphyrin concentration of about 300 nmol/g of liver in all wild-type mice, but again < 1 nmol/g of liver in the null mice. Exposure of wild-type mice to iron dextran and ALA without hexachlorobenzene for 28–31 days resulted in uroporphyrin accumulation to a mean value of 50 nmol/g of liver; the null mice were found to have the same iron liver content as the wild-type. In another experiment, CYP1A2 and uroporphyrinogen oxidation were induced twofold in wild-type mice 6 days after injection of hexachlorobenzene, but no CYP1A2 was present in null mice. This study also showed that hexachlorobenzene did not increase hepatic microsomal uroporphyrinogen oxidation in *Cyp1a2*(-/-) mice (Sinclair *et al.*, 2000).

Hexachlorobenzene was studied in two congenic strains of C57BL/6J mice that differ only at the *AhR* locus. Female B6-Ah^b mice (Ah receptor, approximately 30–70 fmol/mg of cytosolic protein) and B6-Ah^d mice (Ah receptor, undetectable) were pretreated with iron at 500 mg/kg bw (given as iron dextran) and then fed a diet containing 0 or 200 mg/kg hexachlorobenzene for up to 17 weeks. Urinary excretion of porphyrins was increased after 7 weeks of hexachlorobenzene treatment in B6-Ah^b mice, and by 15 weeks was over 200 times greater than that of mice given only iron. In B6-Ah^d mice, porphyrin excretion did not begin to increase until after 13 weeks, and after 15 weeks was only six times greater than that of controls. Similar differences were seen in the hepatic porphyrin concentrations at 15 weeks: B6-Ah^b, 1110 ± 393; B6-Ah^d, 17.6 ± 14.5; controls, approximately 0.20 nmol/g. UROD activity was diminished by 70 and 20% in B6-Ah^b and B6-Ah^d mice, respectively, after 15 weeks of treatment with hexachlorobenzene. Hexachlorobenzene induced small amounts of a protein recognized by anti-CYP1A1 in B6-Ah^b mice, but not in B6-Ah^d mice. Relatively large amounts of a protein recognized by anti-CYP1A2 were induced in both strains, but to a somewhat greater extent in the B6-Ah^b mice. The results of this experiment indicate that the Ah locus influences the susceptibility of C57BL/6J mice to hexachlorobenzene-induced porphyria and are consistent with the suggestion that sustained induction of CYP1A2 and/or CYP1A1 is a causative factor in the development of this disease (Hahn *et al.*, 1988).

The combination of a single subcutaneous dose of iron (12.5 mg/mouse) and subsequent treatment with hexachlorobenzene at 0.02% in the diet caused progressive inhibition of hepatic UROD in male C57BL/10 mice, leading to accumulation of

uroporphyrin within 4–6 weeks. The activity of the enzyme was only slightly inhibited in the absence of iron, and was not inhibited in the absence of hexachlorobenzene. Females were less sensitive than males. Comparisons of the C57BL/10, BALB/c, AKR and DBA/2 strains indicated that the susceptibility of the mice to the induction of porphyria did not completely correlate with their classification as Ah-responsive or Ah-non-responsive (Smith & Francis, 1983).

While there is a marked sex difference in hexachlorobenzene-induced porphyria (see above), the induction of oxidation of uroporphyrinogen I to uroporphyrin I by hepatic microsomes was not correlated with the sex difference in porphyria development. Ethoxyresorufin deethylase activity (associated with CYP1A1) and immunoblotting with polyclonal antibodies to CYP1A1 and CYP2A2 were significantly greater in hexachlorobenzene-exposed females. Immunocytochemical studies showed that, even after 30 weeks of exposure to hexachlorobenzene, CYP1A1 and CYP1A2 were still more highly induced in female liver, especially in the centrilobular region (Smith *et al.*, 1990).

After a single intraperitoneal administration of 200 mg/kg bw hexachlorobenzene to female rats, UROD activity was 61 and 69% of normal with and without iron loading, respectively, and the liver porphyrin concentrations were 96 and 25 mg/g, respectively. Hexachlorobenzene did not produce significant porphyric effects in male rats. Aroclor 1254 induced CYP1A to a greater extent in females than in males and to a greater extent than hexachlorobenzene, which showed a greater propensity to induce CYP2B. Overall, the correlation between decreased UROD activity and porphyrin accumulation was highest when fitted to an exponential curve, indicating the importance of extreme depression of UROD activity in evoking experimental porphyria by such chemicals (Franklin *et al.*, 1997).

Hexachlorobenzene was found to be a potent inducer of malic enzyme gene expression in the liver of female Wistar rats exposed to 1 g/kg bw by gavage for 9–15 days. No changes in T4 or T3 concentrations were found in rat liver, and the activities of other thyroid hormone-responsive enzymes were not found to be increased. In studies with H35 rat hepatoma cells exposed to 10 or 50 nmol/L hexachlorobenzene, the increase in malic enzyme mRNA was shown to occur through the thyroid response element (Loaiza-Perez *et al.*, 1999). In contrast, in brown adipose tissue of male Wistar rats given 1 g/kg bw hexachlorobenzene by gavage for 30 days, the activities of malic enzyme, glucose-6-phosphate dehydrogenase and L-glycerol-3-phosphate dehydrogenase were decreased in both euthyroid and thyriodectomized rats (Alvarez *et al.*, 1999).

Administration of hexachlorobenzene at 1 g/kg bw per day to female Wistar rats by gavage for up to 30 days resulted in time-dependent decreases in the activity of two membrane-bound enzymes, 5'-nucleotidase and Na⁺/K⁺ ATPase, and hexachlorobenzene was found to cause a significant rise in protein tyrosine kinase activity during the early stages of intoxication (day 2), followed by a significant decrease at 10 days and returning to control levels after 20 days of treatment. A stimulatory effect of

hexachlorobenzene on endogenous microsomal protein phosphorylation *in vitro* was observed from day 2 of intoxication up to 30 days of treatment. Administration of 1 g/kg bw hexachlorobenzene to rats for 10 days caused a 50% reduction in epidermal growth factor receptor–ligand binding (Randi *et al.*, 1998).

In male Fischer 344 rats exposed by gavage to 0.4 mmol/kg bw per day hexachlorobenzene [114 mg/kg bw per day] or pentachlorobenzene for 6 weeks, a distinct pattern of non-focal expression of rGSTP1-1 was observed. The expression was localized to the centrilobular region, with the most intense staining nearest the central vein. A western blot analysis revealed five- and 15-fold induction of rGSTP1-1 with pentachlorobenzene and hexachlorobenzene on an equimolar basis, respectively. Evaluation of porphyrin fluorescence also revealed centrilobular accumulation, with average concentrations of porphyrin of 0.319, 0.580 and 0.206 µg/g tissue with pentachlorobenzene, hexachlorobenzene and in corn-oil controls, respectively. In view of the role of activator protein-1 in rGSTP1-1 expression and of CYP1A2 in the pathogenesis of porphyria cutanea tarda, immunohistochemical localization of *c-jun*, *c-fos* and *CYP1A2* was also performed. Increased expression and co-localization within the liver lobule were observed for *c-jun*, *c-fos*, *CYP1A2*, rGSTP1-1 and areas of porphyrin accumulation. These observations are consistent with the results of studies that have associated the induction of GST-P with *jun*- and *fos*-related gene products (Thomas *et al.*, 1998).

In a comparative study of chlorobenzenes given to male Fischer rats by gavage daily for 6 weeks, 1,2,4,5-tetrachlorobenzene, pentachlorobenzene and hexachlorobenzene, but not 1,4-dichlorobenzene, promoted GSTP1-1-positive preneoplastic foci formation in the liver after initiation with NDEA. The induction of CYP1A2 and CYP2B1/2 correlated with both the presence and degree of promotion of GSTP1-1 foci by the four chlorobenzenes. The authors concluded that induction of CYP1A2 or CYP2B1/2 by chlorobenzene isomers is associated with promotional ability (Gustafson *et al.*, 2000; see also section 3.3).

4.5 Genetic and related effects

The genetic toxicity of hexachlorobenzene has been reviewed (Brusick, 1986).

4.5.1 Humans

Measurement of micronucleus frequency in peripheral blood lymphocytes was used to study the possible clastogenic effects of occupational exposures of workers at a chemical production factory in the State of São Paulo, Brazil, who had been exposed to a variety of chlorinated compounds but mainly carbon tetrachloride, perchloroethylene and hexachlorobenzene. The results were compared with those for 28 control workers who had not been exposed. The presence of micronuclei was investigated in peripheral blood from 41 workers of a group of 85 who were selected from a total of 130 workers

in the same company. [The Working Group noted that the basis for the selection of the 85 from 130 and the 41 from 85 was not described.] The 85 exposed workers were men of a median age of 37 years who had worked for a mean of nine years in the company and had a median serum hexachlorobenzene concentration of 4.4 µg/100 mL, with a range of 0.1–16 µg/100 mL. The 28 controls were men working in other companies in the same geographical region and of the same median age, but without detectable serum concentrations of hexachlorobenzene. It was known which subjects in both groups were current smokers. The average frequency of micronucleated lymphocytes was 0.9% (range, 0.6–4.8%) in the exposed workers and 0.25% (range, 0.0–2.8%) in the controls ($p < 0.00001$). In the exposed group, there was no correlation between the occurrence of micronuclei and age, current smoking, length of employment at the factory or hexachlorobenzene concentration in serum. The authors noted that it was not possible to identify a particular factor that might account for the difference in the frequency of micronuclei (da Silva Augusto *et al.*, 1997).

4.5.2 *Experimental systems* (see Table 5 for references)

Hexachlorobenzene did not induce mutations in *Salmonella typhimurium*, but there was a report of a small increase in mutation frequency in exposed *Saccharomyces cerevisiae*. In single studies in non-human mammalian cells, hexachlorobenzene did not induce alkali-labile sites in DNA of rat hepatocytes or chromosomal aberrations in Chinese hamster lung cells. In a study from one laboratory, hexachlorobenzene induced alkali-labile sites in human hepatocytes and micronuclei in rat and human hepatocytes *in vitro*.

Hexachlorobenzene did not induce alkali-labile sites in liver-cell DNA from rats treated *in vivo*, sister chromatid exchange in bone-marrow cells of mice treated *in vivo* or dominant lethal mutations in male rats.

Male C57BL/10ScSn mice received a subcutaneous injection of iron-dextran solution at a dose of 600 mg Fe/kg bw and were then fed a diet containing 0.01% hexachlorobenzene for up to 18 months (see section 3). A total of 23 preneoplastic and neoplastic lesions obtained from these mice were analysed for mutations of Ha-*ras* at codon 61, since these often occur at high frequency in the livers of mice treated with carcinogens. Only two mutations were found: an A → T transversion in a focus of altered cells and a C → A transversion in a trabecular-cell carcinoma (Rumsby *et al.*, 1992).

4.6 Mechanistic considerations

Neither analysis of tumours arising in hexachlorobenzene-treated mice nor the results of the small number of tests for genetic toxicity support the hypothesis that the induction of genetic damage by hexachlorobenzene plays a role in its carcinogenicity.

Table 5. Genetic and related effects of hexachlorobenzene

Test system	Result ^a		Dose ^b (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
<i>Escherichia coli</i> WP2, WP2 <i>uvrA</i> , differential toxicity	–	NT	1000 µg/disc	Siekel <i>et al.</i> (1991)
<i>Salmonella typhimurium</i> TA100, TA1535, TA1537, TA98, reverse mutation	–	–	333 µg/plate	Haworth <i>et al.</i> (1983)
<i>Salmonella typhimurium</i> TA100, TA1538, TA98, reverse mutation	–	–	1000 µg/plate	Górski <i>et al.</i> (1986)
<i>Salmonella typhimurium</i> TA100, TA98, reverse mutation	–	–	500 µg/plate	Siekel <i>et al.</i> (1991)
<i>Escherichia coli</i> WP2, WP2 <i>uvrA</i> , reverse mutation	–	–	500	Siekel <i>et al.</i> (1991)
<i>Saccharomyces cerevisiae</i> 632/4, reverse mutation	(+)	NT	100	Guerzoni <i>et al.</i> (1976)
DNA single-strand breaks and alkali-labile sites in Sprague-Dawley rat primary hepatocytes <i>in vitro</i>	–	NT	160	Canonero <i>et al.</i> (1997)
Micronucleus formation, Sprague-Dawley rat hepatocytes <i>in vitro</i>	+	NT	91	Canonero <i>et al.</i> (1997)
Chromosomal aberrations, Chinese hamster lung cells <i>in vitro</i>	–	–	12 000	Ishidate (1988)
DNA single-strand breaks and alkali-labile sites in human primary hepatocytes <i>in vitro</i>	(+)	NT	160	Canonero <i>et al.</i> (1997)
Micronucleus formation, human primary hepatocytes <i>in vitro</i>	+	NT	51	Canonero <i>et al.</i> (1997)
Chromosomal aberrations, human lymphocytes <i>in vitro</i>	–	NT	29	Siekel <i>et al.</i> (1991)
DNA single-strand breaks and alkali-labile sites in rat liver cells <i>in vivo</i>	–	–	300 ip × 1	Górski <i>et al.</i> (1986)
Sister chromatid exchange, mouse bone-marrow cells <i>in vivo</i>	–	–	400 ^c	Górski <i>et al.</i> (1986)
Dominant lethal mutation, male Wistar rats	–	–	60 po × 10	Khera (1974)
Dominant lethal mutation, male rats	–	–	221 po × 5	Simon <i>et al.</i> (1979)

^a +, positive; (+), weak positive; –, 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; po, orally; ip, intraperitoneal

^c Route not reported

4.6.1 *Thyroid tumours*

At doses much higher than those used in the bioassays for carcinogenicity, hexachlorobenzene increased the size of the thyroid, decreased the concentration of T4, with a smaller or no decrease in that of T3, and increased the concentration of TSH in rats. No thyroid tumours were reported in rats given much lower doses. The experimental results indicate that hexachlorobenzene induces hypothyroidism in rats through its main metabolite, pentachlorophenol, and through tetrachlorohydroquinone. Displacement of hormones from serum carriers by these metabolites could be a factor in the induction of the observed hypothyroidism. In addition, hexachlorobenzene increases the metabolism of T4 by inducing glucuronosyl transferase and decreases type-1 deiodinase activity. Therefore, the decreased T4 concentrations in serum of rats after exposure to hexachlorobenzene may be due to a combined effect of displacement of T4 from carriers, increased glucuronidation of T4 and enhanced bile flow.

In hamsters, hexachlorobenzene decreased the concentrations of T3 rather than T4 and increased the size of the thyroid; the concentrations of TSH have not been reported. CYP isozymes were induced, but the specific activities that are increased by hexachlorobenzene in hamsters have not been identified. The mechanism of thyroid tumour development in hamsters is probably due to effects similar to those in rats.

4.6.2 *Liver tumours*

Some reports have linked human hepatic porphyria with a risk for liver cancer, but the results vary. The hexachlorobenzene poisoning incident in Turkey demonstrated that hexachlorobenzene can produce porphyria in humans. The acquired porphyria was more frequent and more severe in women, and the effects of the exposure may be exacerbated by estrogens.

The evidence suggests that the production of hepatic tumours by hexachlorobenzene involves biotransformation, oxidative damage, CYP enzyme induction, porphyria, inhibition of UROD and effects on iron metabolism. Hexachlorobenzene produces hepatic porphyria and induces the hepatic CYP isozymes CYP1A1 and CYP1A2 in rodents. There is evidence for a role of the Ah receptor in some but not all of the effects of hexachlorobenzene in the liver. The uroporphyria produced in mice was found to be dependent on expression of CYP1A2, since *Cyp1a2*(-/-) knock-out mice did not develop uroporphyria even when exposed to 3-methylcholanthrene. A pivotal role for inhibition of UROD in the development of hexachlorobenzene-induced porphyria has been shown. Some results suggest that oxidative biotransformation may be related to the porphyrinogenic action of hexachlorobenzene. The results are consistent with the association between CYP1A isozymes and the development of uroporphyria.

Iron accumulation in subcellular organelles such as lysosomes and iron sequestration have been reported in hexachlorobenzene-exposed animals. Iron loading has

been found to significantly enhance the effects of hexachlorobenzene. The iron in hepatocyte lysosomes associated with porphyria may result in oxidative damage.

Female rats are more sensitive to the induction of porphyria than males, and mice are less sensitive than rats. Rats and mice also differ in terms of their overall sensitivity to chemical porphyrinogens.

In the absence of definitive evidence, hexachlorobenzene-induced porphyria and the other toxic end-points described above may be involved in the induction of hexachlorobenzene-induced liver tumours, but the mechanism has not been definitively established.

4.6.3 *Kidney tumours*

Hexachlorobenzene induces a male rat-specific α_{2u} -globulin nephropathy that could play a role in the induction of renal tumours in male rats. However, because female rats also develop renal adenomas, other mechanisms must play a role as well.

4.6.4 *Parathyroid tumours*

Pre- and postnatal administration of high doses of hexachlorobenzene increased the incidence of parathyroid adenomas in male and female Sprague-Dawley rats. Short-term (5-week) exposure of Fischer 344 rats to hexachlorobenzene resulted in increased circulating parathyroid hormone and 1,25-dihydroxyvitamin D₃ concentrations, decreased urinary calcium excretion and increased serum alkaline phosphatase activity. It also produced osteosclerosis. Although hexachlorobenzene appears to stimulate the parathyroid, the mechanism of parathyroid tumour development probably involves additional factors, since the parathyroid chief-cell hyperplasia commonly associated with chronic renal failure in rats is not associated with an increased incidence of parathyroid adenomas.

5. Summary of Data Reported and Evaluation

5.1 Exposure data

Hexachlorobenzene is a chlorinated hydrocarbon which may contain some higher polychlorinated dibenzofurans and dioxins as impurities. It has been used in the manufacture of industrial chemicals, including chlorinated pesticides, and as a fungicide and seed dressing in agriculture. The production and use of hexachlorobenzene have decreased since the 1970s owing to bans and restrictions on its use in many countries, but it still occurs as a by-product of the production of a number of chlorinated solvents and other industrial chemicals. Occupational exposure to hexachlorobenzene has occurred during its production and use in industry and agriculture. Hexachlorobenzene

has been detected in many foodstuffs, but dietary intake has probably decreased in recent years.

5.2 Human carcinogenicity data

The risk for breast cancer has been investigated in relation to life-long, accumulated exposure to hexachlorobenzene in nine studies.

Five small case-control studies that included fewer than 50 cases of breast cancer each showed no overall association with the concentration of hexachlorobenzene in contemporary samples of adipose breast tissue. A secondary subgroup analysis in one of the studies revealed a significant association in postmenopausal women with estrogen receptor-positive cancer, based, however, on a small number of cases.

Four large case-control studies of exposure to hexachlorobenzene have been reported, one from Canada and three from the USA. In three of these, the concentration of hexachlorobenzene was measured in biological samples (serum fat or breast fat) from the study subjects, obtained close to the time of breast cancer diagnosis. No consistent increase in the risk for breast cancer was found in women with elevated concentrations of hexachlorobenzene. In the fourth case-control study (from the USA), banked serum samples obtained before the breast cancer diagnosis were used to assess the body burden of hexachlorobenzene. The risk for breast cancer of women whose concentration of hexachlorobenzene was in the upper three quartiles was twice that of those whose samples were in the lower quartile. However, there was no evidence of a dose-response relationship, and the association was limited to women whose blood was collected close to the time of diagnosis of their breast cancer.

One case-control study each of endometrial cancer, pancreatic cancer and hairy-cell leukaemia yielded no notable results with respect to exposure to hexachlorobenzene.

5.3 Animal carcinogenicity data

Hexachlorobenzene was tested for carcinogenicity by oral administration in one study in mice, four studies in rats and one in hamsters. It produced liver-cell tumours in all three species and renal tubular tumours in rats of each sex in one study. After perinatal administration to rats, it increased the incidences of parathyroid adenomas in males and adrenal phaeochromocytomas in females. In hamsters, it also produced liver haemangioendotheliomas and thyroid follicular-cell adenomas. In several studies in which it was given with other compounds, hexachlorobenzene promoted liver carcinogenesis in mice and rats.

5.4 Other relevant data

Hexachlorobenzene is lipophilic, accumulates in humans and is excreted as a cysteine conjugate of pentachlorobenzene. In rats, hexachlorobenzene has been shown

to follow several metabolic pathways, which include the formation of pentachlorobenzene, tetrachlorobenzene and tri- and tetrachlorophenol.

Accidental consumption by humans of a large quantity of hexachlorobenzene resulted in porphyria cutanea tarda, liver toxicity, neurological effects and skin changes, which were persistent.

In experimental animals, the effects of treatment with hexachlorobenzene on the thyroid include decreased thyroid hormone concentrations due to increased glucuronidation and inhibition of type-1 deiodinase, interference with serum carrier binding of the thyroid hormones and increased thyroid-stimulating hormone concentrations. In the livers of experimental animals, hexachlorobenzene induced cytochrome P450 enzymes and inhibited uroporphyrinogen decarboxylase, iron accumulation and oxidative damage. These effects are believed to be involved in the production of hepatic tumours.

In a poisoning epidemic in Turkey, exposure to hexachlorobenzene via breast milk caused a very high rate of lethality among infants. An increased frequency of pregnancy loss was reported among women exposed to hexachlorobenzene as children. The presence of this compound in breast milk has been associated with altered immune function in Inuits. Hexachlorobenzene was teratogenic in mice, and increased mortality rates were observed among rats and monkeys exposed *in utero*. Effects on steroid hormones have also been reported in exposed female mice.

In a single study of workers exposed to a number of chlorinated solvents, including hexachlorobenzene, an increased frequency of micronucleated lymphocytes was found; there was no association with the concentrations of hexachlorobenzene in blood. Micronuclei were induced by hexachlorobenzene in human and rat primary hepatocytes *in vitro*. Otherwise, there was little evidence that hexachlorobenzene has genetic activity.

5.5 Evaluation

There is *inadequate evidence* in humans for the carcinogenicity of hexachlorobenzene.

There is *sufficient evidence* in experimental animals for the carcinogenicity of hexachlorobenzene.

Overall evaluation

Hexachlorobenzene is *possibly carcinogenic to humans (Group 2B)*.

6. References

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