CHLORDANE AND HEPTACHLOR

Chlordane and heptachlor were considered together because of their close structural similarity and because technical-grade products each contain about 10–20% of the other compound.

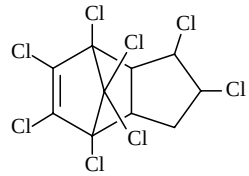
These substances were considered by previous working groups, in 1978 (IARC, 1979), 1987 (IARC, 1987) and 1990 (IARC, 1991). Since that time, new data have become available, and these have been incorporated into the monograph and taken into consideration in the present evaluation.

1. Exposure Data

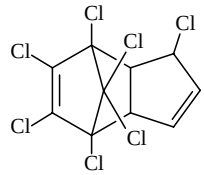
1.1 Chemical and physical data

1.1.1 *Synonyms, structural and molecular data*

Chemical Abstract Services Registry numbers, names and synonyms of chlordane and heptachlor and its epoxide are given in Table 1.



$C_{10}H_6Cl_8$ Chlordane Relative molecular mass: 409.8

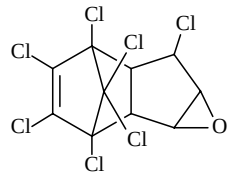


$C_{10}H_5Cl_7$ Heptachlor Relative molecular mass: 373.5

Table 1. Chemical Abstract Services Registry numbers, names and synonyms of chlordane, heptachlor and its epoxide

Name	CAS Reg. Nos[a]	Chem. Abstr. names[b] and synonyms
Chlordane	57-74-9 (39400-80-1); 53637-13-1)	ENT 9932; **1,2,4,5,6,7,8,8-octachloro-2,3,3a,4,7,7a-hexahydro-4,7-methano-1*H*-indene;** 1,2,4,5,6,7,8,8-octachloro-2,3,3a,4,7,7a-hexahydro-4,7-methanoindene (IUPAC); octachloro-4,7-methanotetrahydroindane; 1,2,4,5,6,7,8,8-octachloro-3a,4,7,7a-tetrahydro-4,7-methanoindan; OMS 1437
Technical-grade chlordane	12789-03-6	
cis-Chlordane	5103-71-9 (152322-29-7; 22212-52-8; 26703-86-6; 28140-46-7)	α-Chlordan; α-chlordane; *cis*-chlordan; **(1α,2α,3aα,4β,7β,7aα)-1,2,4,5,6,7,8,8-octachloro-2,3,3a,4,7,7a-hexahydro-4,7-methano-1*H*-indene;** 1α,2α,4β,5,6,7β,8,8-octachloro-3aα,4,7,7aα-tetrahydro-4,7-methanoindan
trans-Chlordane[c]	5103-74-2 (152322-27-5; 17436-70-3; 28181-89-7)	β-Chlordan; β-chlordane; γ-chlordane; *trans*-chlordan; **(1α,2β,3aα,4β,7β,7aα)-1,2,4,5,6,7,8,8-octachloro-2,3,3a,4,7,7a-hexahydro-4,7-methano-1*H*-indene;** 1β,2α,4α,5,6,7α,8,8-octachloro-3aβ,4,7,7aβ-tetrahydro-4,7-methanoindan
'γ-Chlordane'[c]	5566-34-7[c]	γ -Chlordan; **2,2,4,5,6,7,8,8-octachloro-2,3,3a,4,7,7a-hexahydro-4,7-methano-1*H*-indene;** 2,2,4,5,6,7,8,8-octachloro-3a,4,7,7a-tetrahydro-4,7-methanoindan stereoisomer
Heptachlor	76-44-8 (23720-59-4; 37229-06-4)	3-Chlorochlordene; E 3314; ENT 15 152; **1,4,5,6,7,8,8-heptachloro-3a,4,7,7a-tetrahydro-4,7-methano-1*H*-indene;** 1,4,5,6,7,8,8-heptachloro-3a,4,7,7a-tetrahydro-4,7-methanoindene (IUPAC); OMS 193
Heptachlor epoxide	1024-57-3 (4067-30-5; 24699-42-1; 24717-72-4; 28044-82-8; 66429-35-4; 66240-71-9)	ENT 25584; epoxyheptachlor; 1,4,5,6,7,8,8-heptachloro-2,3-epoxy-3a,4,7,7a-tetrahydro-4,7-methanoindan; **(1aα,1bβ,2α,5α,5aβ,6β,6aα)-2,3,4,5,6,7,7-heptachloro-1a,1b,5,5a,6,6a-hexahydro-2,5-methano-2*H*-indeno(1,2-b)-oxirene;** heptachlor *cis*-oxide

[a] Deleted CAS Registry number(s) in parentheses
[b] In bold
[c] In most of the published literature, authors have used the term 'γ-chlordane' as a synonym for *trans*-chlordane, although the Chemical Abstracts Service has indexed such references with the 5566-34-7 CAS Registry Number. See also section 1.1.3.



$C_{10}H_5Cl_7O$ Heptachlor epoxide Relative molecular mass: 389.4

1.1.2 *Chemical and physical properties*

Chlordane

(*a*) *Description*: Light-yellow to amber-coloured, viscous liquid (technical product) (WHO, 1988a)
(*b*) *Boiling-point*: 175 °C at 1 mm Hg [0.13 kPa] (pure material) (Royal Society of Chemistry, 1989; Tomlin, 1999)
(*c*) *Melting-point*: 106–107 °C (*cis*-isomer); 104–105 °C (*trans*-isomer) (WHO, 1988a)
(*d*) *Spectroscopy data*: Infrared (prism [534]; grating [41094P]) spectroscopy data have been reported (Sadtler Research Laboratories, 1980).
(*e*) *Solubility*: Practically insoluble in water (1.0×10^{-4} g/L at 25 °C) but soluble in most organic solvents (e.g., acetone, cyclohexanone, ethanol, isopropanol, kerosene, trichloroethylene) (Worthing & Walker, 1987; Tomlin, 1999)
(*f*) *Stability*: Decomposed by alkalis, with loss of chlorine; ultraviolet irradiation induces a change in the skeletal structure and of the chlorine content; corrosive to iron, zinc and various protective coatings (Royal Society of Chemistry, 1989; Tomlin, 1999)

Heptachlor

(*a*) *Description*: White crystalline solid (Worthing & Walker, 1987; WHO, 1988b; Tomlin, 1999)
(*b*) *Boiling-point*: 135–145 °C at 1–1.5 mm Hg [0.13–0.20 kPa] (Tomlin, 1999)
(*c*) *Melting-point*: 95–96 °C (pure compound) (Worthing & Walker, 1987; Tomlin, 1999)
(*d*) *Spectroscopy data*: Infrared (prism/grating [74915]) and nuclear magnetic resonance (proton [47772]) spectral data have been reported (Sadtler Research Laboratories, 1990).
(*e*) *Solubility*: Practically insoluble in water (5.6×10^{-8} g/L at 25–29 °C); fairly soluble in organic solvents: acetone, benzene, ethanol and xylene (WHO, 1988b)
(*f*) *Stability*: Stable in daylight, air, moisture and moderate heat (160 °C); corrosive to metals; susceptible to epoxidation; slowly loses hydrogen chloride in alkaline media (WHO, 1988b; Royal Society of Chemistry, 1989; Tomlin, 1999)

Heptachlor epoxide

(*a*) *Description*: Solid (Agency for Toxic Substances and Disease Registry, 1989a)
(*b*) *Melting-point*: 160–161.5 °C (Environmental Protection Agency, 1987a)
(*c*) *Spectroscopy data*: Infrared (prism/grating [74932]) and nuclear magnetic resonance (proton [47783]) spectral data have been reported (Sadtler Research Laboratories, 1990).
(*d*) *Solubility*: Practically insoluble in water (3.5×10^{-4} g/L at 25 °C) (Environmental Protection Agency, 1987a)

1.1.3 *Trade names, technical products and impurities*

Examples of trade names for chlordane are Aspon, Belt, CD 68, Chlordan, Chlorindan, Chlor-Kil, Chlorotox, Corodane, Cortilan-neu, Dowchlor, Gold Crest C-100, HCS 3260, Intox, Kilex, Kypchlor, M-140, Niran, Octachlor, Oktaterr, Ortho-Klor, Penticklor, Prentox, Starchlor, Sydane, Synklor, Tat Chlor 4, Termex, Topichlor, Toxichlor, Unexan-Koeder and Velsicol 1068.

Examples of trade names for heptachlor are Aahepta, Agroceres, Arbinex 30TN, Basaklor, Biarbinex, Cupincida, Drinox, Fennotox, GPKh, Heptachlorane, Heptaf, Heptagran, Heptagranox, Heptamak, Heptamul, Heptasol, Heptox, Rhodiachlor, Soleptax and Velsicol 104. The trade names for heptachlor epoxide include GPKh epoxide, HCE, Hepox, Heptepoxide and Velsicol 53-CS-17.

The term 'chlordane' commonly refers to a complex mixture of chlordane isomers, other chlorinated hydrocarbons and by-products (WHO, 1988a). Technical-grade chlordane contains more than 140 components, consisting mainly of C_{10} alicyclic chlorinated hydrocarbons, the most abundant being *cis*- and *trans*-chlordane (Royal Society of Chemistry, 1989; Dearth & Hites, 1991; Tomlin, 1999). The nomenclature of *cis*- and *trans*-chlordane used in the literature has been confused. The *cis*-isomer, often referred to as 'α-chlordane', is described above under [5103-71-9]; the *trans*-isomer [5103-74-2], also usually known as 'γ-chlordane', is occasionally referred to as 'β-chlordane' (the term 'γ-chlordane' has also been assigned by the Chemical Abstracts Service to the 2,2,4,5,6,7,8,8-octachloroisomer [5566-34-7]). However, as the α/*cis* and γ/*trans* relationships have been reversed in some cases, particularly in the older literature, the α,β and γ nomenclature should be avoided (Buchert *et al.*, 1989). One description of the approximate composition of technical chlordane is as follows: *trans*-chlordane, 24%; *cis*-chlordane, 19%; chlordene isomers, 21.5%; heptachlor, 10%; nonachlor, 7% (Rostad, 1997); octachlorocyclopentene, 1%; hexachlorocyclopentadiene, 1%; other, 16.5% (Brooks, 1974). Several reviews give details of the composition of technical-grade chlordane (Cochrane & Greenhalgh, 1976; Sovocool *et al.*, 1977; Miyazaki *et al.*, 1985; Buchert *et al.*, 1989).

Chlordane has been available in various formulations, including 5–30% granules, oil solutions containing 2–300 g/L chlordane and emulsifiable concentrates containing

400–900 g/L (Royal Society of Chemistry, 1986; Worthing & Walker, 1987; WHO,1988a).

Technical-grade heptachlor contains about 72% heptachlor and 28% related compounds (20–22% *trans*-chlordane and 4–8% nonachlor). Formulations have included emulsifiable concentrates, wettable powders, dusts and granules containing various concentrations of active material (National Cancer Institute, 1977a; Izmerov, 1982; Worthing & Walker, 1987; WHO, 1988b; Tomlin, 1999). Two Finnish products which were used as components of plywood glues contained 17–25% heptachlor and 6–9% chlordanes. One of these products also contained 40% tetrachlorophenol and 1.5% tributyltin oxide (Mussalo-Rauhamaa *et al.*, 1991).

1.1.4 *Analysis*

Determination of chlordane residues is difficult because of the complex nature of the components and the fact that each component degrades independently. The resulting residues may bear little relation to the proportions in the technical product. Extraction from crops, other plant products, dairy products, plants and oils has been achieved with an 80–100% efficiency with the use of acetonitrile for extraction, petroleum ether for partitioning and clean-up on a Florisil column. Gel-permeation chromatography can also be used for clean-up, particularly of human adipose tissue. The method of choice for the qualitative and quantitative estimation of chlordane isomers and heptachlor is gas chromatography with electron-capture detection. Gas chromatographic analyses can be confirmed by gas chromatography–mass spectrometry, a method that can also provide good determination of some of the components, such as heptachlor epoxide. Analysis for total organically bound chlorine remains the preferred method for determination of technical-grade chlordane and heptachlor and of the active ingredient in formulations (WHO, 1988a,b).

Selected methods for the analysis of chlordane, heptachlor and heptachlor epoxide in various matrices are summarized in Table 2. Several reviews are available on the analysis of chlordane, heptachlor and heptachlor epoxide in technical products, formulations and as residues in various matrices. The methods include titrimetric, colorimetric, spectrophotometric, infrared spectroscopic and gas chromatographic methods (Bowery, 1964; Raw, 1970; Izmerov, 1982; WHO, 1984a,b; Williams, 1984a,b; Anon., 1985; Worthing & Walker, 1987; Agency for Toxic Substances and Disease Registry, 1989a,b; Royal Society of Chemistry, 1989; Fendick *et al.*, 1990; Tomlin, 1999).

1.2 Production

The chemistry and uses of chlordane and heptachlor and the problems associated with their technical-grade products have been reviewed (Brooks, 1974).

Table 2. Methods for the analysis of chlordane, heptachlor and heptachlor epoxide

Sample matrix	Sample preparation	Assay procedure	Limit of detection[a]	Reference
Air	Collect vapours on polyurethane foam (low or high volume); extract with 5–10% diethyl ether in hexane	GC/ECD	NR	Environmental Protection Agency (1999a,b) [Methods TO-04A, TO-10A]
	Collect vapours on Chromosorb 102; desorb with toluene	GC/ECD	0.1 µg/sample	Eller (1994) [Method 5510]
Water	Extract with hexane; inject extract	GC/ECD	0.14 (0.006, 0.012 µg/L)[b], 0.003, 0.004 µg/L	Environmental Protection Agency (1995a) [Method 505]
	Extract with dichloromethane; isolate extract; dry; concentrate with methyl *tert*-butyl ether (capillary column)	GC/ECD	0.0015[b], 0.01, 0.015 µg/L	Environmental Protection Agency (1995b) [Method 508.1]; AOAC International (2000) [Method 990.06]
	Extract by passing sample through liquid–solid extractor; elute with dichloromethane; concentrate by evaporation (capillary column)	GC/MS	Varies[c]	Environmental Protection Agency (1995c) [Method 525.2]
	Extract with methyl *tert*-butyl ether or pentane	GC/ECD	NA, 0.002–0.08, 0.002–0.2 µg/L	Environmental Protection Agency (1995d) [Method 551.1]
Municipal & industrial wastewater	Extract with dichloromethane; dry; concentrate; optional clean-up (acetonitrile partition or Florisil)	GC/ECD	NR, 0.004, 0.003 µg/L	Environmental Protection Agency (1993a) [Method 617]
	(1) If solids < 1%, extract with dichloromethane; (2) for nonsludges with solids 1–30%, dilute to 1% and extract with dichloromethane; if solids > 30%, sonicate with dichloromethane:acetone; (3) for sludges with solids < 30%, treat as in (2) above; if solids > 30%, sonicate with acetonitrile then dichloromethane; back extract with sodium sulfate; concentrate; clean-up	GC/ECD or GC/MCD or GC/ELCD	NR (8, 9 ng/L)[b], 5, 12 ng/L	US Environmental Protection Agency (1993b) [Method 1656]

Table 2 (contd)

Sample matrix	Sample preparation	Assay procedure	Limit of detection[a]	Reference
Municipal & industrial wastewater (contd)	Adjust to pH 11; extract with dichloromethane; dry; concentrate	GC/MS	NR, 1.9, 2.2 µg/L	APHA/AWWA/WEF (1999a) [Method 6410B]
	Extract with diethyl ether:hexane or dichloromethane:hexane; concentrate; clean-up with column adsorption chromatography	GC/ECD	NR	APHA/AWWA/WEF (1999b) [Method 6630B]
	Extract with dichloromethane; dry; exchange to hexane; clean-up with magnesia–silica gel; concentrate	GC/ECD	0.014, 0.003 0.083 µg/L	APHA/AWWA/WEF (1999c) [Method 6630C]
Liquid & solid waste	Extract with dichloromethane; dry; exchange to hexane; clean-up on Florisil	GC/ECD	0.014, 0.003, 0.083 µg/L	Environmental Protection Agency (1999c) [Method 608]
	Extract with dichloromethane; dry; concentrate (packed column)	GC/MS	NR, 1.9, 2.2 µg/L	Environmental Protection Agency (1999d) [Method 625]
	Extract with dichloromethane (liquid); hexane:acetone (1:1) or dichloromethane:acetone (1:1) (solid); clean-up	GC/ECD	groundwater: 1.5/1.8[b], 1.3, 1.5 µg/L; wastewater: 0.58[b], 0.56, 0.34 µg/L	Environmental Protection Agency (1996a) [Method 8081A]
	Mix with anhydrous sodium sulfate; extract with Soxhlet or sonication process; clean-up with Florisil or gel-permeation (capillary column)	GC/MS	NR	Environmental Protection Agency (1996b) [Method 8270C]
Soil	Extract with methanol; add aliquot and enzyme conjugate reagent to immobilized antibody; compare colour produced to reference reaction	Immuno-assay	20 µg/kg	Environmental Protection Agency (1996c) [Method 4041]

Table 2 (contd)

Sample matrix	Sample preparation	Assay procedure	Limit of detection[a]	Reference
Formulations (chlordane)	Dissolve in toluene or benzene, then toluene; extract with 0.1N silver nitrate solution	TCM	NR	AOAC International (2000) [Method 962.05]
	Dissolve in methanol:benzene or extract with pentane; add Davidow reagent[d], boil; cool; dilute with methanol; read absorbance at 550 nm	Colorimetry	NR	AOAC International (2000) [Method 965.14]
	Extract with acetone; filter or centrifuge	TLC	NR	AOAC International (2000) [Method 972.05]
	Dissolve and dilute with carbon disulfide; read absorbance at 13.3–14.1 μm for α-chlordane; at 7.19–7.75 μm for γ-chlordane	IRS	NR	AOAC International (2000) [Methods 973.15, 973.16]
Formulations (heptachlor)	Dissolve in acetic acid; add silver nitrate or extract with pentane; dissolve	ACM	NR	AOAC International (2000) [Method 962.07]
	Dissolve in carbon disulfide or extract with pentane; dissolve	GC/FID	NR	AOAC International (2000) [Methods 968.04, 973.17]
Selected vegetables	Extract with pentane; clean-up on Florex column; evaporate to dryness; react with Polen-Silver-man reagent[e]; read absorbance at 560 nm for heptachlor and at 410 nm for heptachlor oxide	Colorimetry	NR, 0.02, 0.02–0.04 mg/kg	Food and Drug Administration (1989)
Nonfatty 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]

Table 2 (contd)

Sample matrix	Sample preparation	Assay procedure	Limit of detection[a]	Reference
Fatty foods	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 with acetonitrile; extract fat; dilute with water; extract residue into petroleum ether; clean-up on Florisil; elute with petroleum:ethyl ethers	GC/ECD-TD	NR	AOAC International (2000) [Method 970.52]
Fish tissue	Extract with petroleum ether; clean-up on Florisil	GC/ECD	NR	AOAC International (2000) [Method 983.21]

APHA/AWWA/WEF, American Public health Association/American Water Works Association/Water Environment Federation; ACM, active chlorine method; ECD, electron capture detection; ELCD, electrolytic conductivity detection; FID, flame ionization detection; GC, gas chromatography; IRS, infrared spectroscopy; MCD, microcoulometry detection; MS, mass spectrometry; TCM, total chlorine method; TD, thermionic detection; TLC, thin-layer chromatography

[a] The limits of detection are presented for chlordane, heptachlor and heptachlor epoxide, respectively; NA, not applicable; NR, not reported

[b] Detection limit(s) for *cis*-/*trans*-chlordane

[c] Limits of detection vary with extraction technique (cartridge or disc) and mass spectrometer (quadrupole or ion trap) from 0.061 to 0.17 µg/L for *cis*-chlordane; from 0.050 to 0.16 µg/L for *trans*-chlordane; from 0.059 to 0.15 µg/L for heptachlor; and from 0.048 to 0.13 µg/L for heptachlor epoxide

[d] Diethanolamine–potassium hydroxide solution

[e] Prepared by dissolving potassium hydroxide in distilled water, cooling to room temperature, adding butyl Cellosolve and monoethanolamine and diluting to 1 L with butyl Cellosolve. This solution, after standing for several days, is decanted from any sediment and diluted with an equal volume of benzene.

Chlordane was first produced commercially in the USA in 1947. In 1974, production in the USA amounted to 9500 tonnes (WHO, 1988a); the Environmental Protection Agency estimated that approximately 1600–1800 tonnes of chlordane were used in 1986. From 1 July 1983, the only use of chlordane approved in the USA was in the control of underground termites. This use was prohibited in April 1988. The amounts of chlordane both produced and used have decreased considerably (Environmental Protection Agency, 1987b; Agency for Toxic Substances and Disease Registry, 1989b, 1994).

Heptachlor was isolated from technical-grade chlordane in 1946. Production of heptachlor in the USA was 2700 tonnes in 1971, 900 tonnes in 1974, 590 tonnes in 1978, 180 tonnes in 1980 and 45 tonnes in 1982. Sales of heptachlor in the USA were voluntarily stopped by the sole local producer in August 1987, and, since April 1988, heptachlor can no longer be used for the underground control of termites in the USA (WHO, 1988b; Agency for Toxic Substances and Disease Registry, 1989a, 1993).

Chlordene, the starting material for the synthesis of both chlordane and heptachlor, is prepared by Diels-Alder condensation of hexachlorocyclopentadiene with cyclopentadiene (Agency for Toxic Substances and Disease Registry, 1989b). Chlordane is prepared by the Lewis acid-catalysed addition of chlorine to chlordene (WHO, 1984a), whereas heptachlor is prepared by free-radical chlorination of chlordene (Sittig, 1980).

Heptachlor epoxide can be prepared from heptachlor in a one-step oxidation. It is a metabolite as well as an environmental oxidation product of heptachlor (Anon., 1985).

Information available in 2000 indicated that chlordane is manufactured by two companies in India and one company in Argentina. The same source indicated that heptachlor is manufactured by one company each in India and Japan (CIS Information Services, 2000).

1.3 Use

Chlordane has been used as an insecticide since the 1950s. It is a versatile, broad-spectrum, contact insecticide and has been used mainly for non-agricultural purposes (primarily for the protection of structures, but also on lawns and turf, ornamental trees and drainage ditches). It has also been used on maize, potatoes and livestock (WHO, 1984a). The use pattern for chlordane in the USA in the mid-1970s was as follows: 35% by pest control operators, mostly on termites; 28% on agricultural crops, including maize and citrus; 30% for home lawn and garden use; and 7% on turf and ornamental plants (Agency for Toxic Substances and Disease Registry,1989b). Since the mid-1970s, the use of chlordane has been increasingly restricted in many countries (WHO, 1988a). By 1980, less than 4500 t of chlordane were being used yearly in the USA, mostly for termite control (Esworthy, 1985). By 1986, use had been reduced to 1800 t (Environmental Protection Agency, 1987b).

Heptachlor was first introduced as a contact insecticide in the USA in 1952 for foliar, soil and structural applications. It has also been used in the control of malaria. It is a non-systemic internal and contact insecticide (WHO, 1988b). The use pattern for heptachlor in the USA in the mid-1970s was as follows: 58% on maize, 27% by pest control operators, 13% as seed treatment and 2% for miscellaneous uses, including fire ant control, use on pineapples and possibly on citrus (Environmental Protection Agency, 1976). In 1970, the use of heptachlor throughout the world was as follows: Africa, 5%; Asia, 15%; Canada and the USA, 5%; Europe, 60%; and South America, 15% (WHO, 1988b). For example, in the Republic of Korea, average use of heptachlor was about 33 t/year over the period 1962–79 (Lee, 1982). The use of heptachlor has been increasingly restricted in many countries (WHO, 1988b). By 1986, less than 340 t of heptachlor were used in the USA, mainly for termite control (Environmental Protection Agency, 1987a).

1.4 Occurrence

1.4.1 *Occupational exposure*

According to the 1981–83 National Occupational Exposure Survey (National Institute for Occupational Safety and Health, 2000), about 3800 pesticide control workers in the USA were potentially exposed to chlordane, and about 1000 workers, including electrical power installers and pesticide control workers, were potentially exposed to heptachlor. According to the Finnish Register of Employees Exposed to Carcinogens, 18 laboratory workers were exposed to chlordane in Finland in 1997 (Savela *et al*., 1999). Formerly, about 200 Finnish plywood workers were exposed to heptachlor, which was used in special glues in the production of plywood to be exported to tropical countries (Mussalo-Rauhamaa *et al*., 1991).

Pesticide applicators were exposed on average to a concentration of 17 μg/m^3 (range, 0.6–116 μg/m^3) chlordane and 33 μg/m^3 (2.0–176 μg/m^3) heptachlor during the control of subterranean termites in the USA. Dermal exposure, monitored by collection on sterile gauze pads, was estimated to be higher (2.5 μg/kg bw per h [194 μg/h] for chlordane, 1.8 μg/kg bw per h [140 μg/h] for heptachlor) than that by inhalation (0.04 μg/kg bw per h [3 μg/h] for chlordane, 0.08 μg/kg bw per h [6 μg/h] for heptachlor), for a mean body weight of 77.5 kg (Kamble *et al*., 1992). The concentrations of chlordane compounds (*trans*-nonachlor and oxychlordane) in the serum of Japanese pesticide spraymen who had been spraying chlordane formulations for < 3 and > 5 years were on average 2.4 ng/g and 5.1 ng/g, respectively (Takamiya, 1987). The concentration of heptachlor in the air of Finnish plywood mills was < 10–140 μg/m^3 during assembling, 1–50 μg/m^3 during hot pressing, 2–10 μg/m^3 during patching of veneers and 620 μg/m^3 (short-term exposure) during glue preparation (Kauppinen, 1986). The serum of these workers contained concentrations of heptachlor from below the level of detection to 0.3 ng/g heptachlor epoxide at up to 19.2 ng/g and chlordanes (oxychlordane, *trans*-nonachlor, *cis*-chlordane, *trans*-chlordane) at up to 1.3 ng/g (Mussalo-Rauhamaa *et al*., 1991).

1.4.2 *Environmental occurrence*

Chlordane and heptachlor are persistent pesticides, the use of which has diminished substantially over the last two decades. These compounds have very low volatility and are essentially insoluble in water. Their biodegradation in soil is very slow, with half-times measured in decades. These chemicals are therefore persistent in the environment and can be expected to accumulate in sediment long after application has ceased.

The environmental occurrence of chlordane and heptachlor was reviewed previously (IARC, 1991). During the period when these compounds were being used as pesticides, a number of studies were carried out to determine the concentrations of chlordane, heptachlor and related compounds in foods. Most foods were found to

contain low or undetectable concentrations of these chemicals, with the exception of meat, poultry and dairy products, in which significant concentrations were found.

The estimated dietary intake of heptachlor epoxide in the 1960s and 1970s in the USA was about 0.3–2 μg/day (Duggan & Corneliussen, 1972; Peirano, 1980; WHO, 1984b). Estimates of the intake of heptachlor epoxide in a Basque population in Spain in 1990–91 showed an average of < 0.1 μg/day (Urieta *et al*., 1996). The estimated intake of total chlordane (chlordane, chlordene, *trans*-nonachlor, oxychlordane) in various age groups in the USA in 1982–84 ranged from 2 ng/kg bw per day for 14–16-year-old girls to 6.5 ng/kg bw per day for 2-year-old children (Gunderson, 1988).

Several studies published since the last evaluation (IARC, 1991), illustrating continuing detection of chlordane and heptachlor in the environment, are summarized below.

The concentrations of chlordane (measured as the sum of *cis*- and *trans*-chlordane) in coastal Nicaragua lagoons in 1995 ranged from 0.013 to 6.29 ng/g dry weight (*trans*-nonachlor, 0.005–2.0 ng/g dry weight), and the concentrations of heptachlor were < 0.004–65.4 ng/g dry weight (Carvalho *et al.*, 1999). A study of the transport of persistent organochlorine pesticides in suspended sediment along the Mississippi River from St Louis to New Orleans, USA, in 1988–90 showed concentrations of chlordane (reported as the sum of *cis*- and *trans*-chlordane) ranging from < 7 to 263 ng/g of organic carbon; similar amounts of nonachlor (*cis* + *trans*) were found. The annual transport of chlordane in suspended sediment from the Mississippi River to the Gulf of Mexico was estimated to be approximately 110 kg (nonachlor, 100 kg) (Rostad, 1997).

Chlordane and heptachlor present in sediments continue to enter the food chain by uptake by organisms in direct contact with the sediment. The National Contamination Biomonitoring Program in the USA determined the concentrations of various organochlorine pesticides in samples of freshwater fish taken from 107 sites in the USA in 1976–86. The annual geometric mean concentration ranged from 19 (1986) to 39 ng/g (1976–79) for the sum of *cis*- and *trans*-chlordane; from 48 (1986) to 82 ng/g (1978–79) for the sum of *cis*- and *trans*-chlordane, oxychlordane and *cis*- and *trans*-nonachlor; and from 5 (1984) to 10 ng/g (1978–79) for heptachlor epoxide. The annual maximum concentrations ranged from 490 (1986) to 3070 ng/g (1978–79) for the sum of *cis*- and *trans*-chlordane; from 980 (1986) to 6690 ng/g (1978–79) for the sum of *cis*- and *trans*-chlordane, oxychlordane and *cis*- and *trans*-nonachlor; and from 100 (1986) to 1170 ng/g (1978–79) for heptachlor epoxide (Schmitt *et al.*, 1999). The concentration of chlordane in a single 6.6-kg trout taken from Lake Tahoe, USA in 1993–94 was 17.7 ng/g wet weight, measured as the sum of *cis*- and *trans*-chlordane and 78 ng/g measured as *cis*- and *trans*-chlordane plus oxychlordane plus *trans*-nonachlor (Datta *et al.*, 1999). The mean concentrations of the sum of *cis*- and *trans*-chlordane, oxychlordane and *cis*- and *trans*-nonachlor in yellowtail and winter flounder (flat fish) from off the coast of Newfoundland, Canada, at several locations in 1993 ranged from 0.35 to 6.25 ng/g wet weight (Ray *et al.*, 1998).

The concentrations of chlordane in beluga whale blubber from Alaska's north coast in 1992 ranged from 320 to 990 ng/g of fat in three females and were 2470 and 3880 ng/g of fat in two males. A single fetal specimen contained 690 ng/g of fat, comparable to the concentration in maternal fat of 620 ng/g (Wade *et al.*, 1997). A comprehensive review of the available data showed that the arithmetic mean concentrations of chlordane in traditional foods in northern and Arctic Canada, e.g. marine mammal meat, fish, birds and terrestrial animals, ranged from 2 to 34 ng/g wet weight (1160 ng/g wet weight for marine mammal blubber) (Chan, 1998).

Consumption of foods containing chlordane and heptachlor may result in measurable concentrations of these compounds in human tissues. A compilation of data from the 1960s and 1970s indicated that the mean concentration of heptachlor epoxide in adipose tissue from the general population ranged from 10 to 460 ng/g of fat (IARC, 1991). In a study in 1985–88 of 183 healthy German children, the mean concentration of heptachlor was 6 ng/g of fat (maximum, 87 ng/g of fat) and the mean concentration of heptachlor epoxide was 4 ng/g of fat (maximum, 86 ng/g of fat) (Teufel *et al.*, 1990). In Canadian newborns in 1993–95, the concentrations of *cis*- and *trans*-chlordane in cord blood from non-Inuit infants ranged from 0.01 to 0.07 μg/L, with 0.01–0.3 μg/L for *cis*-nonachlor, 0.01-0.17 μg/L for *trans*-nonachlor and 0.01-0.05 μg/L for oxychlordane, while the concentrations in cord blood from Inuit populations were 0.01–0.20 μg/L for *cis*-chlordane, 0.01–0.03 μg/L for *trans*-chlordane, 0.01–0.18 μg/L for *cis*-nonachlor, 0.01-1.13 μg/L for *trans*-nonachlor and 0.01-0.67 μg/L for oxychlordane. The concentrations in omental fat from Greenland Inuits at autopsy in 1993 were 11.6 ng/g of fat for *cis*- and *trans*-chlordane, 3.1 ng/g for *cis*-nonachlor, 1463 ng/g for *trans*-nonachlor and 862 ng/g for oxychlordane (Van Oostdam *et al.*, 1999).

The most significant source of exposure of infants to chlordane, heptachlor and their metabolites appears to be breast milk, in which the concentrations can be much higher than those in dairy milk. In a large international survey carried out in the 1970s, the mean concentrations of heptachlor and heptachlor epoxide in human breast milk ranged from 2 to 720 ng/g of fat; 2560 ng of heptachlor per gram of fat was found in a rural area in Spain (WHO, 1984b). The median concentration of heptachlor epoxide in breast milk of women in the USA reported in 1991 was 10 ng/g of fat, with a 90th percentile value of 100 ng/g of fat (Rogan *et al.*, 1991).

The concentrations of *cis*- and *trans*-chlordane in breast milk were higher in Inuit mothers from northern Quebec (3.7 ng/g of fat) than in southern Canadian residents (0.37 ng/g of fat) in 1989–92 (Van Oostdam *et al.*, 1999). The mean concentration of chlordane, measured as the sum of *cis*- and *trans*-chlordane, in 12 samples of breast milk from Arctic Canada in 1996 was 1.27 ng/g of fat, the values being 59 ng/g for oxychlordane, 4.29 ng/g for *cis*-nonachlor and 78 ng/g for *trans*-nonachlor (Newsome & Ryan, 1999).

1.5 Regulations and guidelines

The use of chlordane in agriculture has been banned or product registrations have been cancelled or withdrawn in many countries, beginning as early as 1968 and continuing through the 1970s and 1980s, because of concerns about risks to human health and the environment (WHO, 1988a; FAO/UNEP, 1996). For example, in the Joint FAO/UNEP Programme for the Operation of Prior Informed Consent for Banned or Severely Restricted Chemicals in International Trade (PIC Programme), more than 35 countries reported that the use of chlordane had been discontinued or severely restricted (e.g., to structural subterranean termite control) (FAO/UNEP, 1996).

Because of similar concerns and beginning as early as 1958, the agricultural use of heptachlor has been banned or product registrations cancelled or withdrawn in many countries (WHO, 1988b; Mussalo-Rauhamaa *et al.*, 1991; FAO/UNEP, 1996). More than 29 countries reported to the PIC Programme that the use of heptachlor had been discontinued or severely restricted.

In those countries where use is restricted but may continue, the applications are restricted to seed treatment, structural termite control or wood treatment. In tropical and subtropical countries that have retained use for seed treatment or preplanting agricultural use, chlordane/heptachlor is restricted to crops that form the edible portions above ground and, in particular, to crops with long growing seasons that undergo processing before conception (FAO/UNEP, 1996).

Chlordane and heptachlor are among the 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 use of chlordane and heptachlor and four other chlorinated pesticides, aldrin, endrin, hexachlorobenzene and toxaphene (Hogue, 2000).

In 1970, the FAO/WHO Joint Meeting on Pesticide Residues (JMPR) evaluated chlordane and established tolerances for residues in food of 0.02–0.5 mg/kg for the sum of *cis*- and *trans*-isomers of chlordane and oxychlordane (FAO/WHO, 1971). In 1986, an acceptable daily intake (ADI) in food of 0–0.0005 mg/kg bw was established (FAO/WHO, 1987). In 1994, the ADI was changed to a provisional tolerable daily intake (PTDI) value at the same level, 0.0005 mg/kg bw (WHO, 1999).

In 1966, JMPR also established an ADI in food of 0–0.0005 mg/kg bw for heptachlor/heptachlor epoxide; this value was reduced to 0–0.0001 mg/kg bw in 1991. In 1994, the ADI was changed to a PTDI value of 0.0001 mg/kg bw (WHO, 1999).

Extraneous residue limits (previously designed 'maximum residue levels' have been established by the Codex Alimentarius Commission for the sum of *cis*- and *trans*-chlordane or, in the case of animal products, the sum of *cis*- and *trans*-chlordane and 'oxychlordane' (fat-soluble residue) in or on the following commodities (in mg/kg): 0.05 for cottonseed oil (crude), linseed oil (crude), meat (fat), poultry meat (fat) and soya bean oil (crude); 0.02 for almonds, eggs, fruit, hazelnuts, maize, oats, pecan, rice (polished),

rye, sorghum, soya bean oil (refined), cottonseed oil (edible), vegetables, walnuts and wheat; and 0.002 for milk (fat soluble) (FAO/UNEP, 1996).

Extraneous residue limits were established by the Codex Alimentarius Commission (1997) for the sum of heptachlor and heptachlor epoxide (fat-soluble residue) in or on the following commodities (in mg/kg): 0.5 for soya bean oil (crude); 0.2 for carrots, meat (fat) and poultry meat (fat); 0.05 for eggs and vegetables (except carrots, soya beans, sugar beets and tomatoes); 0.02 for cereal grains, cottonseed, tomatoes, soya beans (immature seeds) and soya bean oil (refined); 0.01 for citrus fruit and pineapples; and 0.006 for milk (fat soluble) (FAO/UNEP, 1996).

WHO (1993) recommended guideline values of 0.2 μg/L for chlordane (all isomers) and 0.03 μg/L for heptachlor and heptachlor epoxide in drinking-water. The Environmental Protection Agency (2000) in the USA has set maximum contaminant levels for chlordane, heptachlor and heptachlor epoxide in drinking-water of 0.002, 0.0004 and 0.0002 mg/L, respectively, and a goal of zero for all three chemicals. In Mexico, the maximum permissible concentrations of chlordane in ambient water are 0.002 mg/L for coastal and estuarine waters and 0.003 mg/L for water treated for drinking; those of heptachlor in ambient water are 0.2 μg/L for coastal waters, 0.002 mg/L for estuarine waters and 0.018 mg/L for water treated for drinking (WHO, 1988a,b). The Environmental Protection Agency in the USA has established a national ambient water quality criterion for heptachlor of 0.28 μg/L (Agency for Toxic Substances and Disease Registry, 1989a).

National and regional pesticide residue limits for chlordane, heptachlor and heptachlor epoxide in foods were compiled by the Food and Drug Administration (1990), Health and Welfare Canada (1990) and IARC (1991). Tables 3 and 4 present occupational exposure limits and guidelines for chlordane and heptachlor in several countries.

2. Studies of Cancer in Humans

2.1 Cohort studies

Deaths among workers at two plants in the USA, one producing chlordane and the other producing heptachlor, were analysed in a series of studies with slightly different inclusion criteria (Wang & MacMahon, 1979a; Ditraglia *et al*., 1981; Shindell & Ulrich, 1986; Infante & Freeman, 1987; Shindell, 1987; Brown, 1992). Pesticide production started in 1946 in the first plant and in 1951 in the other. Exposures to other chemicals, including chlorine and dicyclopentadiene (in the chlordane plant) and to endrin, chlorine, chlorendic anhydride, hexachlorocyclopentadiene and vinyl chloride (in the heptachlor plant), were also reported. As the bases of these studies overlap substantially, they do not provide independent information on the carcinogenicity of chlordane/heptachlor. Only the results of the most recent analysis with the longest follow-up are summarized below. In some (Wang & MacMahon, 1979a; Ditraglia *et al.*, 1981) but not all of the previous reports, a non-significant excess of lung cancer, of 20–30%, was reported.

Table 3. Occupational exposure limits and guidelines for chlordane

Country	Year	Concentration (mg/m^3)	Interpretation
Australia	1993	0.5 (Ca, sk)	TWA
		2	STEL
Austria	1993	0.5 (sk)	TWA
Belgium	1993	0.5 (sk)	TWA
		2	STEL
Denmark	1993	0.5 (Ca, sk)	TWA
Egypt	1993	0.5 (sk)	TWA
Finland	1993	(Ca)	TWA
France	1993	0.5 (sk)	TWA
Germany	2000	0.5 (IF, 3B, sk)	TWA
India	1993	0.5 (sk)	TWA
		2	STEL
Ireland	1997	0.5 (sk)	TWA
		2	STEL
Netherlands	1999	0.5 (sk)	TWA
Philippines	1993	0.5 (sk)	TWA
Russian Federation	1993	0.01 (sk)	STEL
Switzerland	1993	0.5 (sk)	TWA
Thailand	1993	0.5	TWA
Turkey	1993	0.5 (sk)	TWA
USA			
ACGIH (TLV)	2000	0.5 (A3, sk)	TWA
NIOSH (REL)	2000	0.5 (Ca, sk)	TWA
OSHA (PEL)	2000	0.5 (sk)	TWA

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

sk, danger of cutaneous absorption; TWA, time-weighted average; Ca, carcinogen; STEL, short-term exposure limit; IF, inhalable fraction of aerosol; 3B, substances for which in-vitro or animal studies have yielded evidence of carcinogenic effects that is not sufficient for classification of the substance in one of the other categories; further studies are required before a final decision can be made; a maximum acceptable concentration (MAC) value can be established provided no genotoxic effects have been detected; TLV, threshold limit value; A3, confirmed animal carcinogen with unknown relevance to humans; NIOSH, National Institute for Occupational Safety and Health; OSHA, Occupational Safety and Health Administration; REL, recommended exposure limit; PEL, permissible exposure limit

The most recent investigation included white men employed in the two plants for at least 6 months before 1965, with follow-up to the end of 1987 (Brown, 1992). Most workers in non-production jobs were excluded from this analysis. There were 405 workers in the chlordane manufacturing plant, one (0.25%) of whom was lost to follow-up; in the heptachlor/endrin production plant, there were 305 men, with one

Table 4. Occupational exposure limits and guidelines for heptachlor

Country	Year	Concentration (mg/m^3)	Interpretation
Australia	1993	0.5 (sk)	TWA
Belgium	1993	0.5 (sk)	TWA
Denmark	1993	0.5 (sk)	TWA
Finland	1993	0.5 (sk)	TWA
		1.5	STEL
Germany	2000	0.5 (IF, 3B, sk)	TWA
Ireland	1997	0.5 (sk)	TWA
		2	STEL
Netherlands	1999	0.5 (sk)	TWA
Philippines	1993	0.5 (sk)	TWA
Russian Federation	1993	0.01	STEL
Switzerland	1993	0.5 (sk)	TWA
Turkey	1993	1 (sk)	TWA
USA			
ACGIH (TLV)	2000	0.05 (A3, sk)[a]	TWA
NIOSH (REL)	2000	0.5 (Ca, sk)	TWA
OSHA (PEL)	2000	0.5 (sk)	TWA

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

sk, danger of cutaneous absorption; TWA, time-weighted average; Ca, carcinogen; STEL, short-term exposure limit; IF, inhalable fraction of aerosol; 3B, substances for which in-vitro or animal studies have yielded evidence of carcinogenic effects that is not sufficient for classification of the substance in one of the other categories; further studies are required before a final decision can be made; a maximum acceptable concentration (MAC) value can be established provided no genotoxic effects have been detected; TLV, threshold limit value; A3, confirmed animal carcinogen with unknown relevance to humans; NIOSH, National Institute for Occupational Safety and Health; OSHA, Occupational Safety and Health Administration; REL, recommended exposure limit; PEL, permissible exposure limit

[a] Heptachlor and heptachlor epoxide

(0.33%) lost to follow-up. The expected numbers of deaths were calculated from the mortality rates of white males in the USA. The results for cancers at all sites and for specific sites at which at least two cases were observed are shown in Table 5, including the results for the haematopoietic system.

Cancer risks were also evaluated among cohorts of pesticide applicators engaged in termite control, in which chlordane has until recently been the most widely used chemical. As pesticide applicators are exposed to several different pesticides, it is difficult to disentangle the effects of chlordane from those of the others.

A study by Wang and MacMahon (1979b) of deaths in a cohort of over 16 000 urban pesticide applicators was extended (MacMahon *et al*., 1988) to give a maximal period

of follow-up of 18 years (from 1967 to 1984). For the 8% of deaths for which a certificate was not located, the numbers of deaths attributable to cancer at specific sites were estimated on the basis of the proportion among observed causes of death. A significant excess of lung cancer based on 108 estimated deaths (standardized mortality ratio [SMR], 1.4; 90% confidence interval [CI], 1.1–1.6) was observed, with nonsignificant excesses for cancers of the skin (1.3; 0.65–2.2) and urinary bladder (1.2; 0.50–2.5). No excess was observed for cancers of the digestive organs and peritoneum, with 45 esti-

Table 5. Studies on cohorts of workers exposed to chlordane/heptachlor in the USA

Reference	Cancer	No. of cases	SMR	95% CI	Comments
Brown (1992)	*Chlordane manufacturers*				Workers were also exposed to several other chemicals
	All sites	35	0.87	0.61–1.2	
	Stomach	4	2.1	0.57–5.4	
	Pancreas	2	0.93	0.11–3.4	
	Respiratory system	19	1.3	0.80–2.1	
	Lymphatic/ haematopoietic	4	1.1	0.30–2.8	
	Heptachlor/endrin manufacturers				
	All sites	18	1.0	0.60–1.6	
	Stomach	2	2.8	0.34–10	
	Respiratory system	6	0.88	0.32–1.9	
	Bladder	3	7.1	1.5–21	
	Lymphatic/ haematopoietic	1	0.58	0.01–3.2	
MacMahon *et al*. (1988)	*Entire cohort of applicators*				Pesticide applicators; chlordane and heptachlor were among the components used. 90% CI
	Lung	108	1.4	1.1–1.6	
	Skin	9	1.3	0.65–2.2	
	Bladder	5	1.2	0.50–2.5	
	Lymphatic/ haematopoietic	25	0.97	0.67–1.4	
	Termite control operators only				
	Lung	30	0.97	0.7–1.3	Chlordane and heptachlor were the main components used. 90% CI
	Skin	3	1.2	[0.4–2.9]	
	Bladder	2	1.3	[0.3–3.9]	
	Buccal cavity and pharynx	5	1.4	0.5–3.3	
	Stomach	5	1.1	0.4–2.5	
	Colon	11	1.1	0.6–2.0	
	Liver	2	1.1	0.1–4.0	
	Pancreas	6	1.0	0.4–2.2	
	Larynx	4	2.4	0.7–6.2	

Table 5 (contd)

Reference	Cancer	No. of cases	SMR	95% CI	Comments
Blair *et al.* (1983); Pesatori *et al.* (1994) (Florida)	Lung	54	1.4	(1.0–1.8)	Pesticide applicators; chlordane and heptachlor were among the components used.
	Skin	2	0.9	0.1–3.1	
	Prostate	5	0.8	0.2–1.8	
	Testis	2	2.9	0.3–11	
	Bladder	3	1.0	0.2–3.0	
	Kidney	2	0.7	0.1–2.5	
	Brain	8	2.2	0.9–4.4	
	Lymphatic/ haematopoietic	9	0.8	0.4–1.5	
	Leukaemia	4	1.2	0.4–2.7	
	Nested case–control study of lung cancer	9	1.2	0.4–3.8	Exposure to chlordane Dead controls
			0.5	0.2–1.3	Living controls

mated deaths (0.84; 0.64–1.1), nor of the lymphohaematopoietic system, with 25 estimated deaths (0.97; 0.67–1.4). When the analyses were restricted to termite control operators, who have a higher probability of exposure to chlordane and heptachlor, no excess of lung cancer deaths (30) was observed, with 31 estimated deaths (0.97; 0.7–1.3), while the SMRs for skin (1.2) and bladder (1.3) cancer were comparable with those of the whole cohort. The risk for lung cancer did not rise with increasing duration of employment. No thyroid cancers were reported.

In an investigation of 3827 white male pesticide applicators in Florida, USA, licensed during 1965–66 (Blair *et al*., 1983), whose follow-up was subsequently extended until 1 January 1982, an overall excess of lung cancer (SMR,1.4 [95% CI,1.0–1.8]) was found (Pesatori *et al*., 1994). Small numbers of deaths also occurred from leukaemia (1.2; 0.4–2.7), oral cancer (1.4; 0.5–3.3) and cancers of the larynx (2.4; 0.7–6.2), testis (2.9; 0.3–11) and brain (2.2; 0.9–4.4), but the nonsignificant excesses of skin and urinary bladder cancer observed in the first report were no longer present. For other lymphatic/haematopoietic cancers, the SMRs were below 1.0. In a nested case–control study conducted for lung cancer, surrogate responders were identified for 65 (83%) of the 78 lung cancer cases (some of which occurred after the closing date of the cohort). Five controls were randomly matched by age to each case: three living at the time of death of the case and two who died in the same year. For dead controls, next-of-kin were interviewed, and for living controls information up to the time of death of the case was used. Interviews were obtained for 122 (80%) of the 152 selected deceased controls and 172 (75%) of the 229 living controls. When information on specific pesticides reported by the surrogate responders was considered, the age- and smoking-adjusted odds ratio for lung cancer associated with use of chlordane

was 1.2 (95% CI, 0.4–3.8) in comparison with deceased controls and 0.5 (0.2–1.3) in comparison with living controls.

2.2 Case–control studies

As chlordane and heptachlor accumulate 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.

The case–control studies that were considered valid are summarized in Table 6.

2.2.1 *Lymphohaematopoietic system*

A case–control study was conducted in western Washington State, USA, on 128 men with soft-tissue sarcoma and 576 men with non-Hodgkin lymphoma aged 20–79 years at diagnosis during 1981–84 and identified between 1983 and 1985 through a population-based tumour registry (Woods *et al*., 1987). Of the 150 eligible patients with soft-tissue sarcoma who were alive at the time of interview, 97 (65%) were included in the study; of the 56 eligible deceased cases, 31 (55%) were included. The corresponding figures for non-Hodgkin lymphoma were 402 (76%) of 527 eligible living patients and 174 (79%) of 219 eligible deceased cases. Living controls were selected by random-digit dialling (for ages 20–64) or from the Health Care Financing Administration (ages 65–79), while deceased controls were identified from death certificates showing a cause other than cancer for residents of the same area. Men whose cause of death was suicide or homicide were not eligible. Included in the analysis were 475 (76%) of the 622 living controls identified as eligible and 219 (76%) of the 288 deceased controls. The age-adjusted odds ratio for men reporting exposure to chlordane was 1.6 for non-Hodgkin lymphoma (95% CI, 0.7–3.8) and 0.96 for soft-tissue sarcoma (95% CI, 0.2–4.8). Adjustment for exposure to other selected chemicals by regression analysis did not change the risk estimates substantially. In an additional report from the same study in which the analysis was restricted to farmers (Woods & Polissar, 1989), the odds ratio for non-Hodgkin lymphoma in relation to exposure to chlordane was again 1.6 (0.5–5.1). [The Working Group noted that the number of proxy interviews with living subjects was not reported.]

In a case–control study of non-Hodgkin lymphoma in the USA, cases were identified through the Iowa State Health Registry and a special surveillance of Minnesota hospital and pathology laboratory records (Cantor *et al*., 1992). Men were eligible as cases if they had been aged 30 years or more at the time of diagnosis, their lymphoma had been diagnosed between March 1981 and October 1983 in Iowa and between October 1980 and September 1982 in Minnesota, and they were resident in the state, excluding, for Minnesota, the cities of Minneapolis, St Paul, Duluth and Rochester. The diagnoses were reviewed by a panel of four experienced regional pathologists. Of the 780 identified patients, 694 (89%) were interviewed, and 622 were confirmed in the

Table 6. Case–control studies of exposure to chlordane/heptachlor

Reference and location	No of cases : controls (types of controls)	Exposure assessment	Exposure categories	Odds ratio (95% CI) or *p* value	Comments
Non-Hodgkin lymphoma					
Woods *et al.* (1987); Woods & Polissar (1989) Washington State, USA	576 : 694 (population) (men)	Self-reported exposure to chlordane	No Yes Among farmers: No Yes	1 1.6 (0.7–3.8) 1 1.6 (0.5–5.1)	1.6% of study population exposed to chlordane
Cantor *et al.* (1992) Iowa and Minnesota, USA	622 : 1245 (population) (men)	Self-reported exposure to chlordane	Non-farmers Ever handled on animals Handled on animals prior to 1965 Ever handled on crops Handled on crops prior to 1965 Ever handled on animals without protective equipment Ever handled on crops without protective equipment	1 1.7 (1.0–2.9) 2.2 (1.2–4.2) 1.7 (0.9–3.2) 1.6 (0.7–3.6) 2.2 (1.2–4.2) 2.1 (1.1–4.3)	Proxy interviews for deceased cases and controls
Hardell *et al.* (1996, 1997) Sweden	27 : 17 (surgical patients) (men and women)	Self-reported exposure to heptachlor Chlordane metabolites in abdominal wall adipose tissue	Ever handled on animals Sum of chlordane metabolites < 119 ng/g lipid > 119 ng/g lipid	1.3 (0.7–2.2) 1 3.3 (0.7–16)	 Adjusted

Table 6 (contd)

Reference and location	No of cases : controls (types of controls)	Exposure assessment	Exposure categories	Odds ratio (95% CI) or *p* value	Comments
Hoar-Zahm *et al.* (1988) Nebraska, USA	385 : 1432 (population) (men and women)	Self-reported exposure to chlordane	No Yes	1 2.1	Abstract
Leukaemia					
Brown *et al.* (1990) Iowa and Minnesota, USA	578 : 1245 (population) (men)	Self-reported exposure to chlordane and heptachlor	Non-farmers *Chlordane* Ever handled on crops Ever handled on animals Handled on animals at least 20 years previously Days/year of use on crops 1–4 5–9 ≥ 10 Days/year of use on animals 1–4 5–9 ≥ 10 *Heptachlor* Ever handled on crops Days/year of use on crops 1–4 5–9 ≥ 10	1 0.7 (0.3–1.6) 1.3 (0.7–2.3) 1.5 (0.7–3.1) 0.3 (0.0–2.5) 1.5 (0.1–18) 0.3 (0.0–2.5) 1.1 (0.4–2.8) 0 3.2 (0.9–11) 0.9 (0.5–1.7) 1.2 (0.4–3.3) 1.0 (0.3–3.2) 0.2 (0.0–1.8)	Proxy interviews for deceased cases and controls

Table 6 (contd)

Reference and location	No of cases : controls (types of controls)	Exposure assessment	Exposure categories	Odds ratio (95% CI) or *p* value	Comments
Hairy-cell leukaemia					
Nordström *et al.* (2000) Sweden	54 : 54 (population)	Chlordane metabolites in blood samples (ng/g of lipid)	≤ 44 > 44	1 1.4 (0.5–4.1)	Blood samples obtained from patients a median of 7.1 years after diagnosis
		Epstein-Barr virus early antigen immunoglobulin G titre (EBV)	EBV < 40, chlordane ≤ 44 EBV > 40, chlordane ≥ 44	1 16 (2.8-111)	
Multiple myeloma					
Brown *et al.* (1993) Iowa, USA	173 : 650 (population)	Self-reported exposure to chlordane	Non-farmers Ever used on animals	1 1.6 (0.7–3.6)	
Soft-tissue sarcoma					
Woods *et al.* (1987) Washington State, USA	128 : 694 (population) (men)	Self-reported exposure to chlordane	No Yes	1 0.96 (0.2–4.8)	
Breast cancer					
Falck *et al.* (1992) Connecticut, USA	20 (carcinoma) : 20 (benign breast disease)	Heptachlor epoxide, oxychlordane and *trans*-nonachlor in breast fat (wet weight) (ng/g)	Carcinoma [116 ± 50] Benign disease [97 ± 49]	*p* = 0.22	Mean heptachlor epoxide and oxychlordane
			Carcinoma [87 ± 37] Benign disease [96 ± 80]	*p* = 0.65	Mean *trans*-nonachlor

Table 6 (contd)

Reference and location	No of cases : controls (types of controls)	Exposure assessment	Exposure categories	Odds ratio (95% CI) or *p* value	Comments
Dewailly *et al.* (1994) Canada	20 : 17 (benign breast disease)	Oxychlordane and *trans*-nonachlor in breast fat (ng/g)	Controls [31 ± 12]		Mean oxychlordane
			Cases ER-negative [27 ± 7]	*p* = 0.59	
			Cases ER-positive [39 ± 14]	*p* = 0.12	
			Controls [42 ± 18]		Mean *trans*-nonachlor
			Cases ER-negative [35 ± 8]	*p* = 0.37	
			Cases ER-positive [50 ± 11]	*p* = 0.07	
Høyer *et al.* (1998) Denmark	240 : 477 (population)	Several chlordane metabolites in serum		No association found [data not shown]	Samples collected prospectively
Dorgan *et al.* (1999) Missouri, USA	105 : 208 (population)	Several chlordane metabolites in serum	% above detection limit *Oxychlordane* Controls 13.0%		Samples collected prospectively
			Cases 16.2%	*p* = 0.55	
			trans-*Nonachlor* Controls 42.8%		
			Cases 49.5%	*p* = 0.22	
			Heptachlor Controls 19.2%		
			Cases 20.0%	*p* = 0.90	

Table 6 (contd)

Reference and location	No of cases : controls (types of controls)	Exposure assessment	Exposure categories	Odds ratio (95% CI) or *p* value	Comments
Aronson *et al*. (2000) Canada	217 : 213 (benign breast disease)	Several chlordane metabolites in breast fat (ng/g)	cis-*Nonachlor*		Results were similar for pre- and post-menopausal women
			≤ 4.3	1	
			4.4–6.5	0.81 (0.47–1.4)	
			6.6–10	0.48 (0.27–0.86)	
			≥ 11	0.80 (0.41–1.5)	
			trans-*Nonachlor*		
			≤ 31	1	
			32–43	0.93 (0.54–1.6)	
			44–64	0.69 (0.39–1.2)	
			≥ 65	0.78 (0.40–1.5)	
			Oxychlordane		
			≤ 24	1	
			25–32	0.68 (0.40–1.2)	
			33–46	0.61 (0.35–1.1)	
			≥ 47	0.59 (0.31–1.2)	
Demers *et al*. (2000) Canada	315 : 219 (hospital), 307 (population)	Several chlordane metabolites in serum (ng/g)	*Oxychlordane*		No effect on tumour size and lymph-node involvment
			Hospital controls		
			< 8.4	1	
			8.4–< 10.6	1.1 (0.58–2.1)	
			10.6–< 12.6	0.96 (0.49–1.9)	
			12.6–< 16.3	0.81 (0.41–1.6)	
			≥ 16.3	0.55 (0.27–1.1)	
			Population controls		
			< 8.4	1	
			8.4–< 10.6	1.1 (0.65–1.8)	
			10.6–< 12.6	1.0 (0.59–1.7)	
			12.6–< 16.3	1.3 (0.74–2.2)	
			≥ 16.3	1.5 (0.83–2.6)	

Table 6 (contd)

Reference and location	No of cases : controls (types of controls)	Exposure assessment	Exposure categories	Odds ratio (95% CI) or *p* value	Comments
Demers *et al.* (2000) (contd)			trans-*Nonachlor*		
			Hospital controls		
			< 10.6	1	
			10.6–< 13.5	1.3 (0.64–2.4)	
			13.5–< 16.9	1.5 (0.77–2.8)	
			16.9–< 20.7	0.59 (0.29–1.2)	
			≥ 20.7	0.74 (0.38–1.5)	
			Population controls		
			< 10.6	1	
			10.6–< 13.5	0.82 (0.49–1.4)	
			13.5–< 16.9	1.5 (0.91–2.6)	
			16.9–< 20.7	0.69 (0.39–1.2)	
			≥ 20.7	1.2 (0.68–2.1)	
Zheng *et al.* (2000) Connecticut, USA	304 : 186 (benign breast disease)	*trans*-Nonachlor and oxychlordane in breast fat (ng/g)	*Oxychlordane*		
			< 26.0	1	
			26.0–33.6	0.7 (0.4–1.2)	
			33.7–47.5	0.7 (0.4–1.2)	
			≥ 47.6	0.7 (0.4–1.3)	
			trans-*Nonachlor*		
			< 36.4	1	
			36.4–53.1	1.2 (0.7–2.1)	
			53.2–71.0	0.7 (0.4–1.3)	
			≥ 71.1	1.1 (0.6–1.9)	

Table 6 (contd)

Reference and location	No of cases : controls (types of controls)	Exposure assessment	Exposure categories	Odds ratio (95% CI) or *p* value	Comments
Endometrial cancer					
Weiderpass *et al.* (2000) Sweden	154 : 205 (population)	Oxychlordane and *trans*-nonachlor in serum (quartiles)	*Oxychlordane* 1 (low) 2 3 4 (high)	 1 1.1 (0.6–2.2) 1.0 (0.5–2.0) 1.4 (0.7–2.8) *p* for trend, 0.33	
			trans-*Nonachlor* 1 (low) 2 3 4 (high)	 1 1.2 (0.6–2.3) 1.3 (0.7–2.7) 1.2 (0.6–2.5) *p* for trend, 0.56	
Pancreatic cancer					
Hoppin *et al.* (2000) California, USA	108 : 82 (population)	*trans*-Nonachlor in serum (ng/g)	0 (below detection limit) 0.1–75 > 75	1 0.9 (0.5–1.9) 1.7 (0.8–3.5)	

Table 6 (contd)

Reference and location	No of cases : controls (types of controls)	Exposure assessment	Exposure categories	Odds ratio (95% CI) or *p* value	Comments
Childhood brain cancer					
Davis *et al*. (1993) Missouri, USA	45 : 85 (friends of cases), 108 (cancer controls)	Reported by parents	*Termite treatment of home within 1 year before residence*		
			Yes versus no, friend controls	2.6 (0.9–7.5)	
			Yes versus no, cancer controls	2.3 (0.8–6.4)	
			Termiticide used between 7 months of age and diagnosis		
			Yes versus no, friend controls	1.4 (0.5–3.9)	
			Yes versus no, cancer controls	1.4 (0.5–3.8)	
			Any termite treatment		
			Yes versus no, friend controls	2.9 (1.3–7.1)	
			Yes versus no, cancer controls	3.0 (1.3–7.4)	
			Chlordane used for termite treatment		
			Yes versus no, friend controls	1.5 (0.5–4.9)	
			Yes versus no, cancer controls	1.5 (0.5–5.1)	

ER, estrogen receptor

review to have a non-Hodgkin lymphoma. The 1245 controls were frequency matched to cases by age, residence and vital status. Living subjects aged less than 65 years were selected by random-digit dialling or from Medicare rosters for those aged over 65, and deceased men were selected from death certificate files. The response rates for the various groups of controls were 77–79%. Proxy interviews were conducted for deceased or incompetent men (184 cases and 425 controls). A detailed history of farming and pesticide use was obtained by an interviewer from all subjects who had worked on a farm for at least 6 months since the age of 18 by means of a questionnaire to the participating subjects or proxy responders. Odds ratios were estimated by unconditional multiple logistic regression, allowing for the matching variables plus other potential risk factors; the reference category was those who had never worked or lived on a farm as adults (266 cases, 547 controls). Thirty-one patients and 38 controls had ever handled chlordane as an animal insecticide (odds ratio, 1.7; 95% CI, 1.0–2.9), and 21 patients and 26 controls had used it as crop insecticide (1.7; 0.9–3.2); 25 patients and 43 controls had ever handled heptachlor as an animal insecticide (1.3; 0.7–2.2). When the analysis was limited to those who had handled chlordane before 1965, the odds ratios became 2.2 (1.2–4.2) for use on animals and 1.6 (0.7–3.6) for use on crops; the results for heptachlor were similar to those for any handling. The corresponding figures for those who had handled chlordane without protective equipment were 2.2 (1.2–4.2) for use on animals and 2.1 (1.1–4.3) for use on crops. The odds ratios were similar in the two study areas for use on animals; but for use on crops, the odds ratios were 1.3 (0.5–3.3) for Iowa and 3.1 (0.7–15) for Minnesota [The Working Group noted that part of the excess risk may have been due to the fact that people who were not farmers were used as the reference category.]

In a study in Sweden, 27 consecutive patients in whom non-Hodgkin lymphoma (17 men and 10 women) was diagnosed between 1994 and 1995 and living in the Uppsala–Örebro region were compared with 17 surgical controls (nine men and eight women) without a history of malignancy (Hardell *et al.*, 1996). None of the patients or controls reported use of or occupational exposure to chlordane. Six chlordane metabolites were identified in adipose tissue obtained from the abdominal wall of the patients, while two others were not detected. The mean sum of the metabolites was 180 ng/g of lipid for the cases (range, 48–680 ng/g) and 93 ng/g of lipid (37–160 ng/g) for the control group ($p = 0.002$). For 17 cases and five controls, the sum of the metabolites was above the median for the whole study population (119 ng/g lipid), giving a crude odds ratio of 4.1 (95% CI, 1.1–15). These estimates were not adjusted for age. As reported in a letter published subsequently (Hardell *et al.*, 1997), the odds ratio adjusted for age and sex by multiple logistic regression was 3.3 (0.7–16). The authors reported that the age distribution of cases and controls was similar.

A population-based case–control study of non-Hodgkin lymphoma conducted in eastern Nebraska, USA, on 385 histologically confirmed non-Hodgkin lymphoma cases (201 men and 184 women) and 1432 controls (725 men and 707 women) was presented in an abstract (Hoar Zahm *et al.*, 1988). The odds ratio associated with

chlordane use was 2.1, but no further information on the association was given. A subsequent publication on the relation between non-Hodgkin lymphoma in men and exposure to 2,4-D gives more details of the study design, with no mention however of chlordane (Hoar Zahm *et al*., 1990).

In a study of leukaemia in men parallel to that of non-Hodgkin lymphoma conducted in Iowa and Minnesota, described above (Cantor *et al.*, 1992), 578 cases of leukaemia (340 living and 238 deceased) and 1245 controls (820 living and 425 deceased) were included (Brown *et al*., 1990). The odds ratios for farmers were 0.7 (95% CI, 0.3–1.6) for those who reported use of chlordane on crops and 0.9 (0.5–1.7) for those who had used heptachlor on crops. Those who reported use of chlordane on animals had an odds ratio of 1.3 (0.7–2.3), while that for those who had handled it at least 20 years before diagnosis was 1.5 (0.7–3.1). Among farmers using chlordane on animals, the risks rose inconsistently with frequency of use, from an odds ratio of 1.1 (0.4–2.8) for fewer than 5 days per year, to no exposed case and five exposed controls for use on 5–9 days per year and to an odds ratio of 3.2 (0.9–11) for use ≥ 10 days per year. The risk estimates were not adjusted for other agricultural exposures. There was no evidence of increasing risk with increasing frequency of use of chlordane or heptachlor on crops.

In a study conducted in Sweden, 121 cases of hairy-cell leukaemia, a rare lymphohaematopoietic malignancy, that were diagnosed between 1987 and 1992 were identified from the Swedish Cancer Registry, and 484 controls were drawn from the national population registry and matched to the cases on age, sex and county (Nordström *et al*., 2000). Of these, 111 patients (91%) and 400 controls (83%) answered the mailed questionnaire. Blood samples were taken a median of 7.1 years after diagnosis from 71 cases and from 186 controls; owing to refusal and other technical problems, chlordane and other pesticides were measured in only 54 (76%) cases and 54 (29%) controls. The odds ratio for having a blood concentration of chlordane above the median of controls (44 ng/g) was 1.4 (95% CI, 0.5–4.1), estimated from logistic regression with adjustment for several confounding factors. Antibodies to Epstein-Barr virus early antigen immunoglobulin G_1 in blood were also measured, and the effect of the interaction with chlordane on the risk for hairy-cell leukaemia was evaluated. The odds ratios were 4.3 (1.1–19) for patients with antibody titres above the median of 40, 1.3 (0.4–5.2) for those with chlordane concentrations above the median (of 44 ng/g) and 16 (2.8–111) for those with both measures above the median. [The Working Group noted that the blood samples were taken after heavy treatment with immunosuppressive drugs.]

In a study on multiple myeloma conducted in men in Iowa in parallel to the studies of non-Hodgkin lymphoma and leukaemia among men in Iowa and Minnesota, described above (Brown *et al.*, 1990; Cantor *et al*., 1992), 173 patients (101 alive, 72 deceased) and 650 controls (452 alive, 198 deceased) were included in the analysis (Brown *et al*., 1993). Logistic regression was used to obtain adjusted odds ratios for (self-reported) mixing, handling or application of specific pesticides, relative to the rates of men who were not farmers. Nine cases and 29 controls had used chlordane as animal insecticides, and the estimated odds ratio was 1.6 (95% CI, 0.7–3.6). [The Working

Group noted that part of the excess may have been due to the fact that men who were not farmers were used as the reference category.]

2.2.2 *Breast and female genital tract*

Fat tissue samples from mastectomy or biopsy specimens were obtained from 50 white women with a palpable breast mass or mammographic abnormalities at Hartford Hospital, Connecticut, USA, between May and September 1987 (Falck *et al*., 1992). Histological examination revealed that 23 women had a mammary carcinoma, while the remaining 27 had benign disease. Twenty samples were selected from women in each group (mean age of cases, 63, range 36–86 years; mean age of controls, 59, range, 45–76 years) for analysis of several pesticides, including three metabolites of chlordane (heptachlor epoxide, oxychlordane and *trans*-nonachlor). The mean value (wet weight basis ± standard deviation) of the sum of heptachlor epoxide and oxychlordane was 116 ± 50 ng/g for women with breast carcinoma and 97 ± 49 ng/g for those with benign disease (*t* test, $p = 0.22$). For *trans*-nonachlor, the corresponding figures were 87 ± 37 ng/g and 96 ± 80 ng/g ($p = 0.65$).

Between November 1991 and May 1992, adipose tissue was collected from 41 women aged 40–69 who had undergone a biopsy in a hospital in Québec City (Canada), and the organochlorine content was determined (Dewailly *et al*., 1994). Twenty women had breast cancer (mean age, 54.1 years), and 17 had adenomas or lipomas (mean age, 51.2 years); four women with other diseases were excluded. The mean oxychlordane concentration was 31 ± 12 ng/g in breast adipose tissue from the 17 subjects with benign disease, 27 ± 7 ng/g (Student *t* test, $p = 0.59$) in the nine estrogen receptor-negative cases and 39 ± 14 ng/g ($p = 0.12$) in the nine estrogen receptor-positive cases. For *trans*-nonachlor, the mean concentrations in the three groups were 42 ± 18, 35 ± 8 ($p = 0.37$) and 50 ± 11 ng/g ($p = 0.07$), respectively. Estrogen receptor status was not determined for two cases. [The Working Group noted that estrogen receptor-negative cancers of the breast are more frequent among younger women.]

In 1976, baseline information and blood samples were obtained from 7712 women enrolled in the Copenhagen City Heart Study, who were randomly selected through the Civil Registration System (Høyer *et al*., 1998). According to the Danish Cancer Registry, 268 of the women developed breast cancer between 1976 and 1993. For each case, two women free of breast cancer and matched for age, date of examination and vital status at the time of diagnosis were randomly selected as controls. Blood samples were available in 1995 for 240 cases and 477 controls. Heptachlor, heptachlor epoxide, α- and γ-chlordane, oxychlordane and *trans*-nonachlor were among the compounds measured. Conditional logistic regression was used to estimate odds ratios for categories of pesticide concentrations. The authors did not report the concentrations of chlordane metabolites but indicated that no association was found.

A total of 7224 women donated blood to the Columbia, Missouri, breast cancer serum bank (USA) between 1977 and 1987 (over 90% in 1980 or earlier) (Dorgan *et al*.,

1999). Although active postal follow-up continued until 1989, 70% of the cohort was last contacted in 1982–83. A histologically confirmed breast cancer was diagnosed in 105 of the 6426 women for whom at least 4 mL of serum remained in the bank and who had had no history of cancer at the time of blood collection. Two controls were selected for each case, who were alive and free of cancer and matched to the cases by age, date of blood sample collection and history of benign breast disease at enrolment. Since the serum samples of two controls could not be analysed, 208 controls were included in the analysis. Among the compounds measured by gas chromatography were heptachlor, heptachlor epoxide, *cis*- and *trans*-chlordane, oxychlordane and *trans*-nonachlor. None of the samples contained *cis*- or *trans*-chlordane or heptachlor epoxide at concentrations above the limit of detection. The concentrations of oxychlordane were above the limit of detection for 16.2% of cases and 13.0 % of controls ($p = 0.55$). The corresponding figures were 20.0% and 19.2 % ($p = 0.90$) for heptachlor and 49.5% and 42.8% ($p = 0.22$) for *trans*-nonachlor.

Of 824 women who were under the age of 80, were scheduled for biopsy in two hospitals in Toronto and Kingston (Canada) between July 1995 and June 1997, had no history of cancer, had not participated in tamoxifen trials, had not had a breast implant and were not too ill to participate, 735 (89%) agreed to participate in a case–control study and 663 (81%) completed a questionnaire by telephone or mail (Aronson *et al*., 2000). Organochlorine compounds were determined in benign tissue taken during biopsy from 217 women with in-situ or invasive breast cancer and in 213 women matched for age and study site whose biopsy samples showed no malignancy but most of whom had a diagnosis of some form of benign breast disease. Over 30% of the women had undetectable levels of α- and γ-chlordane. For other compounds, the women were divided into four categories according to the tissue concentration. For *cis*-nonachlor, the odds ratios estimated by logistic regression and adjusted for several potential confounders were 0.81 (95% CI, 0.47–1.4), 0.48 (0.27–0.86) and 0.80 (0.41–1.5) for women in the second (4.4–6.5 μg/kg tissue), third (6.6–10 μg/kg) and fourth (≥ 11 μg/kg) categories, respectively, as compared with the first (≤ 4.3 μg/kg). The corresponding odds ratios were 0.93 (0.54–1.6), 0.69 (0.39–1.2) and 0.78 (0.40–1.5) for *trans*-nonachlor (with concentration ranges of 32–43 μg/kg, 44–64 μg/kg and ≥ 65 μg/kg in the second, third and fourth categories, respectively, in comparison with ≤ 31 μg/kg in the first category) and 0.68 (0.40–1.2), 0.61 (0.35–1.1) and 0.59 (0.31–1.2) for oxychlordane (with concentration ranges of 25–32 μg/kg, 33–46 μg/kg and ≥ 47 μg/kg in the second, third and fourth categories, respectively, in comparison with ≤ 24 μg/kg in the first category). When analyses were conducted separately for pre- and postmenopausal women, the results were similar in the two groups.

A study conducted in Québec, Canada, between 1994 and 1997 included 315 women aged 30–70 years and residing in the Québec City area with histologically confirmed breast cancer, 219 controls recruited in four hospitals of the study area and free of gynaecological diseases and 307 controls selected from the general population (Demers *et al*., 2000). The participation rates were 91% for cases, 89% for hospital controls and

47% for population controls. Blood samples were obtained before therapy, and *cis*- and *trans*-chlordane, *cis*-nonachlor, *trans*-nonachlor and oxychlordane were measured. As *cis*- and *trans*-chlordane and *cis*-nonachlor were detected in less than 70% of the blood samples, they were excluded from further analysis. In comparison with the first quintile of oxychlordane serum concentration, the adjusted odds ratios for women with concentrations in subsequent quintiles were 1.1 (0.58–2.1), 0.96 (0.49–1.9), 0.81 (0.41–1.6) and 0.55 (0.27–1.1) in relation to hospital controls and 1.1 (0.65–1.8), 1.0 (0.59–1.7), 1.3 (0.74–2.2) and 1.5 (0.83–2.6) in relation to population controls. For *trans*-nonachlor, the corresponding figures were 1.3 (0.64–2.4), 1.5 (0.77–2.8), 0.59 (0.29–1.2) and 0.74 (0.38–1.5) in relation to hospital controls and 0.82 (0.49–1.4), 1.5 (0.91–2.6), 0.69 (0.39–1.2) and 1.2 (0.68–2.1) in relation to population controls. The concentrations of oxychlordane and *trans*-nonachlor in blood were associated with the extent of disease.

Between 1994 and 1997, women aged 40–79 without a previous diagnosis of cancer, who had undergone breast-related surgery at the Yale-New Haven Hospital, New Haven, Connecticut, USA, and whose breast specimen was suitable for chemical analysis were asked to participate in a case–control study (Zheng *et al*., 2000). Of the 490 women enrolled, 304 had histologically confirmed breast cancer and 186 had histologically confirmed benign breast disease (excluding atypical hyperplasia). The participation rate was 79% for cases and 74% for controls. The age- and lipid-adjusted geometric mean adipose tissue concentrations of oxychlordane and *trans*-nonachlor were similar for cases and controls. In comparison with the lowest quartile of concentration, the odds ratios adjusted for several covariates were 0.7 (0.4–1.2), 0.7 (0.4–1.2) and 0.7 (0.4–1.3) for those with subsequent quartiles of oxychlordane and 1.2 (0.7–2.1), 0.7 (0.4–1.3) and 1.1 (0.6–1.9) for *trans*-nonachlor.

A case–control study of endometrial cancer was conducted between 1996 and 1997 in 12 Swedish counties, which included 288 (73%) of the 396 cases of histologically confirmed endometrial cancer identified through a network of personnel at the departments of gynaecology and gynaecological oncology in the study area (Weiderpass *et al*., 2000). An additional 134 women were excluded since they had used hormone replacement therapy, thus leaving 154 cases. Of the 742 women selected as controls from population registers and frequency matched to cases by 5-year age group, 205 were included in the study; the others were excluded because they had undergone hysterectomy, had used hormone replacement therapy or refused to participate. Serum samples were taken from all participants and analysed for organochlorine compounds. The odds ratios were obtained by logistic regression and adjusted for age and body mass index. In comparison with women in the first quartile of serum concentrations of chlordane metabolites, the odds ratios were 1.1 (0.6–2.2), 1.0 (0.5–2.0) and 1.4 (0.7–2.8) for subsequent quartiles of oxychlordane (p for trend = 0.33) and 1.2 (0.6–2.3), 1.3 (0.7–2.7) and 1.2 (0.6–2.5) for *trans*-nonachlor (p for trend = 0.56).

2.2.3 *Other cancers*

A study was conducted between 1996 and 1998 on cases of exocrine pancreatic cancer diagnosed among persons aged 21–85 in hospitals of the San Francisco Bay Area (USA) (Hoppin *et al.*, 2000). Only 113 of 611 potential cases were included in the study (108 cases included in the analysis), the most common cause of exclusion being death of the patient (55%). Age- and sex-matched controls were selected by random-digit dialling (age, < 65) or from the Health Care Financing Administration lists (age ≥ 65). Eighty-two of the selected controls agreed to provide a blood sample, giving a participation rate of 78% for people < 65 and 65% for those ≥ 65. Cases had significantly higher median concentrations of *trans*-nonachlor than controls. The adjusted odds ratios relative to individuals with concentrations below the detection limit were 0.9 (0.5–1.9) for a blood concentration between 0.1 and 75 ng/g and 1.7 (0.8–3.5) for a blood concentration > 75 ng/g of lipid.

The relationship between pesticide use in the home and childhood brain cancer was examined in a case–control study of 45 white children with brain cancer diagnosed between 1985 and 1989, resident in Missouri (USA) and identified through the Missouri Cancer Registry (Davis *et al.*, 1993). The participation rate was 73%, and histological confirmation was made for 89%. Two groups of controls were selected: the first consisted of friends of the children with brain cancer or of children with acute lymphocytic leukaemia (85 children; participation rate, 94%), and the second consisted of 108 children with cancer (mostly of the lymphohaematopoietic system; 71 children; participation rate, 78%). The adjusted odds ratio associated with living in a home that had been treated for termites within 1 year before residence or during residence from pregnancy to diagnosis was 2.9 (95% CI, 1.3–7.1) when friends were used as controls and 3.0 (1.3–7.4) with cancer controls. Of the 21 patients who reported any termite control treatment, only seven reported specific use of chlordane, giving an odds ratio of 1.5 (0.5–4.9) for friend controls and 1.5 (0.5–5.1) for cancer controls.

3. Studies of Cancer in Experimental Animals

3.1 Oral administration

Mouse: A study on chlordane conducted by the International Research and Development Corporation in 1973 but not published by that organization was later reported by Epstein (1976). Groups of 100 male and 100 female CD-1 mice, 6 weeks of age, were fed a diet containing 0 (control), 5, 25 or 50 mg/kg technical-grade chlordane [purity unspecified] for 18 months. When 10 animals from each group that were killed for interim study at 6 months were excluded, the mortality rate at 18 months was 27–49%, with the exception of males and females receiving 50 mg/kg of diet, in which the rates were 86 and 76%, respectively. In addition, a relatively large number of animals were

lost because of autolysis. A review of the histopathology of liver samples from this study by a panel of the National Academy of Sciences (1977) indicated a significant increase in the incidence of hepatocellular carcinomas in males at the intermediate dietary concentration and in females at the two higher concentrations (Table 7). [The Working Group noted that the original report and data were not available.]

Groups of 50 male and 50 female B6C3F_1 hybrid mice, 5 weeks of age, were fed a diet containing analytical-grade chlordane (71.7% *cis*-chlordane, 23.1% *trans*-chlordane, 0.3% heptachlor, 0.6% nonachlor, 1.1% hexachlorocyclopentadiene, 0.25% chlordene isomers and other chlorinated compounds) for 80 weeks. Males received an initial concentration of 20 or 40 mg/kg of diet and females received 40 or 80 mg/kg of diet; the time-weighted average dietary concentrations were 30 and 56 mg/kg for males and 30 and 64 mg/kg for females. There were 20 male and 20 female matched controls. The survivors were killed at 90–91 weeks. The survival rates in all groups was relatively high, being > 60% of treated males, > 80% of treated females and > 90% of male and female controls (National Cancer Institute, 1977b). A review of the histopathology of liver samples from this study by the panel of the National Academy of Sciences (1977) indicated a significant increase in the incidence of hepatocellular carcinomas by linear trend analysis in males and females and a significant increase in the combined incidence of hepatocellular carcinomas and 'nodular changes' in males and females at the higher concentration (Table 8).

Groups of 210 male B6C3F_1 and 160 male B6D2F_1 mice, 9 weeks of age, were fed diets containing 55 mg/kg technical-grade chlordane. A 'stop group' of 75 B6C3F_1 mice was returned to normal diet when they were 70 weeks (491 days) of age. Groups of 100 male B6C3F_1 and 50 male B6D2F_1 mice were used as untreated controls. When the treated mice were about 8 months of age, the concentration in the diet was increased to 60 mg/kg. From 408 days of age, groups of 5–33 mice were killed for pathological examination. In B6C3F_1 mice, the prevalence of hepatocellular adenomas in continuously treated animals exceeded 99% by 530 days, and the prevalence of hepatocellular carcinomas was 89% at terminal killing at 568 days. In B6D2F_1 mice, the prevalence of hepatocellular adenomas reached 91% and that of hepatocellular carcinomas 86% at the terminal killing, although there was a lag of more than 100 days for tumour development in this strain. The prevalence of hepatocellular tumours in controls was less than 22% in B6C3F_1 males and 9% in B6D2F_1 males throughout the study. In the 'stop group', the prevalence of adenomas decreased from 100% to 93% between 548 and 568 days and that of carcinomas from 80% to 54% between 526 and 568 days (Malarkey *et al*., 1995).

A study on heptachlor and its epoxide by the Food and Drug Administration in the USA carried out in 1965 but not published by that organization, was later summarized by Epstein (1976). Three groups of 100 male and 100 female C3H mice [age unspecified] were fed diets containing 0 (control) or 10 mg/kg heptachlor or 10 mg/kg heptachlor epoxide [purity unspecified] for 24 months. A review of the histopathology of liver samples from this study by the panel of the National Academy of Sciences

Table 7. Tumour incidence in CD-1 mice treated with chlordane

Concentration (mg/kg of diet)	Males		Females	
	Hepatocellular carcinomas	Hepatocellular carcinomas and nodules	Hepatocellular carcinomas	Hepatocellular carcinomas and nodules
0 (controls)	1/33	4/33	0/44	1/44
5	1/55	11/55	0/61	0/61
25	11/51 ($p = 0.015$)	30/51 ($p < 0.001$)	11/51 ($p < 0.001$)	23/51 ($p < 0.001$)
50	7/44	25/44 ($p < 0.001$)	6/40 ($p < 0.001$)	22/40 ($p < 0.001$)

From National Academy of Sciences (1977)

Table 8. Tumour incidence in B6C3F$_1$ mice treated with analytical-grade chlordane or technical-grade heptachlor

Treatment	Males		Females	
	Hepatocellular carcinomas	Hepatocellular carcinomas and nodules	Hepatocellular carcinomas	Hepatocellular carcinomas and nodules
Controls	2/20	5/20	1/19	1/19
Chlordane (low dose)	5/45	16/45	0/46	2/46
Chlordane (high dose)	12/46 ($p = 0.031$)[a]	30/46 ($p = 0.003$)	7/47 ($p = 0.018$)[a]	20/47 ($p = 0.002$)
Controls	2/19	5/19	0/10	1/10
Heptachlor (low dose)	3/45	14/45	0/44	3/44
Heptachlor (high dose)	2/45	24/45 ($p = 0.042$)	2/42	21/42 ($p = 0.022$)

From National Academy of Sciences (1977)
[a] Armitage's test for linear trend

(1977) indicated a significant increase in the incidence of hepatocellular carcinomas in females but not in males given heptachlor and in both males and females given heptachlor epoxide (Table 9). [The Working Group noted that the original report and data were not available.]

Table 9. Tumour incidence in C3H mice treated with heptachlor or heptachlor epoxide

Treatment	Males		Females	
	Hepatocellular carcinomas	Hepatocellular carcinomas and nodules	Hepatocellular carcinomas	Hepatocellular carcinomas and nodules
Control	29/77	48/77	5/53	11/53
Heptachlor (10 mg/kg of diet)	35/85	72/85 ($p = 0.001$)	18/80 ($p = 0.04$)	61/80 ($p < 0.001$)
Heptachlor epoxide (10 mg/kg of diet)	42/78 ($p = 0.031$)	71/78 ($p < 0.001$)	34/83 ($p < 0.001$)	75/83 ($p < 0.001$)

From National Academy of Sciences (1977)

A study on heptachlor and its epoxide conducted by the International Research and Development Corporation in 1973 but not published by that organization, was later reported by Epstein (1976). Groups of 100 male and 100 female CD-1 mice, 7 weeks of age, were fed diets containing a mixture of 75% heptachlor epoxide and 25% heptachlor [purity unspecified] at a concentration of 0 (control), 1, 5 or 10 mg/kg for 18 months. After exclusion of 10 animals from each group that were killed for interim study at 6 months, the mortality rate at 18 months was 34–49%, with the exception of males and females receiving the 10 mg/kg concentration, for which the rate was approximately 70%. In addition, comparatively large numbers of animals from all groups were lost because of autolysis. A review of the histopathology of liver samples from this study by the panel of the National Academy of Sciences (1977) indicated a significant increase in the combined incidence of hepatocellular carcinomas and nodules in the groups at the high concentration (Table 10). [The Working Group noted that the original report and data were not available.]

Groups of 50 male and 50 female B6C3F_1 hybrid mice, 5 weeks of age, were fed a diet containing technical-grade heptachlor (72 ± 3% heptachlor, 18% *trans*-chlordane, 2% *cis*-chlordane, 2% nonachlor, 1% chlordene, 0.2% hexachlorobutadiene and 10–15 other compounds) for 80 weeks. Males received an initial dietary concentration of 10 or 20 mg/kg and time-weighted average concentrations of 6 and 14 mg/kg; females received an initial concentration of 20 or 40 mg/kg of diet and time-weighted average concentrations of 9 and 18 mg/kg of diet. These concentrations were reduced during the

Table 10. Tumour incidence in CD-1 mice treated with a mixture of heptachlor and heptachlor epoxide (25%:75%)

Concentration (mg/kg of diet)	Males		Females	
	Hepatocellular carcinomas	Hepatocellular carcinomas and nodules	Hepatocellular carcinomas	Hepatocellular carcinomas and nodules
0 (controls)	1/59	2/59	1/74	1/74
1	1/58	1/58	0/71	0/71
5	2/66	4/66	1/65	3/65
10	1/73	27/73 ($p < 0.001$)	4/52	16/52 ($p < 0.001$)

From National Academy of Sciences (1977)

experiment because of adverse toxic effects. Matched controls consisted of 20 males and 20 females. The survivors were killed at 90–91 weeks. The survival rates in all groups were relatively high, with > 70% of treated and control males and 60% of treated and control females still alive at 90 weeks. The survival of treated female mice showed a significant decreasing trend in comparison with controls (National Cancer Institute, 1977a). A review of the histopathology of liver samples from this study by the panel of the National Academy of Sciences (1977) indicated a significant increase in the combined incidence of hepatocellular carcinomas and 'nodular changes' ($p < 0.05$) in males and females receiving the higher concentration (Table 8).

Rat: Groups of 50 male and 50 female Osborne-Mendel rats, 5 weeks of age, were fed diets containing analytical-grade chlordane (71.7% *cis*-chlordane, 23.1% *trans*-chlordane, 0.3% heptachlor, 0.6% nonachlor, 1.1% hexachlorocyclopentadiene, 0.25% chlordene isomers and other chlorinated compounds) for 80 weeks at an initial concentration of 400 or 800 mg/kg for males and 200 or 400 mg/kg of diet for females. These concentrations were reduced during the experiment because of adverse toxic effects, and the time-weighted average dietary concentrations were 204 and 407 mg/kg for males and 121 and 242 mg/kg for females. There were 10 male and 10 female matched controls and 60 male and 60 female pooled controls from similar bioassays of other compounds. The survivors were killed at 109 weeks, at which time approximately 50% of treated and control males, 60% of treated females and 90% of control females were still alive. Treated females showed a marginal increase in the incidence of thyroid follicular-cell neoplasms: 6/32 at the higher dietary concentration (four adenomas, two carcinomas; $p < 0.05$), 4/43 at the lower concentration and 3/58 in pooled controls ($p = 0.03$, trend test). There was also a marginal increase in the incidence of malignant fibrous histiocytomas [site unspecified] in treated males: 7/44 at the higher concentration ($p < 0.05$), 1/44 at the lower concentration and 2/58 in pooled controls (National Cancer Institute, 1977b).

Groups of 80 male and 80 female Fischer 344 rats, 5 weeks of age, were fed diets containing 0 (control), 1, 5 or 25 mg/kg technical-grade chlordane (containing unspecified amounts of *cis*- and *trans*-chlordane, isomers of chlordene, heptachlor and nonachlor) for 130 weeks. Eight males and nine females in each group were killed for evaluation at 26 and 52 weeks. The survival rate in all groups was > 65% at 104 weeks. Combined evaluations by the original pathologist and a panel of seven other pathologists indicated incidences of hepatocellular adenomas in males of 2/64 in controls, 4/64 at the low concentration, 2/64 at 5 mg/kg of diet and 7/64 at 25 mg/kg ($p = 0.018$ trend test) (Khasawinah & Grutsch, 1989a).

Epstein (1976) reported on a study conducted by the Kettering Laboratories in 1959, but not published by that organization. Groups of 25 female CFN rats, 7 weeks of age, were fed diets containing heptachlor epoxide [purity unspecified] (added by spraying alcoholic solutions on chow pellets) at a concentration of 0.5, 2.5, 5.0, 7.5 or 10 mg/kg for 108 weeks. The survival rate at that time was > 45% in both treated and control groups. A review of the histopathology of liver samples from this study by the panel of the National Academy of Sciences (1977) found no increase in the incidence of liver tumours in treated animals. [The Working Group noted that the original report and data were not available and also noted the small number of animals and the uncertain concentrations in the feed.]

Groups of 50 male and 50 female Osborne-Mendel rats, 5 weeks of age, were fed diets containing technical-grade heptachlor (71.7% *cis*-chlordane, 23.1% *trans*-chlordane, 0.3% heptachlor, 0.6% nonachlor, 1.1% hexachlorocyclopentadiene, 0.25% chlordene isomers and other chlorinated compounds) for 80 weeks. Males received an initial dietary concentration of 80 or 160 mg/kg and a time-weighted average concentration of 39 or 78 mg/kg of diet; females received an initial concentration of 40 or 80 mg/kg of diet and a time-weighted average concentration of 26 or 51 mg/kg of diet. Matched controls consisted of 10 males and 10 females; and pooled controls consisted of 60 males and 60 females. At 111 weeks, 55–75% of all treated and control groups were still alive. Thyroid follicular-cell neoplasms (10 adenomas, five carcinomas) occurred in 14/38 females at the higher concentration ($p < 0.01$), 3/43 females at the lower concentration and 3/58 controls. Follicular-cell neoplasms were found in 9/38 (seven adenomas, four carcinomas) males at the lower concentration ($p < 0.05$), 3/38 males at the higher concentration and 4/51 controls. The incidence of follicular-cell hyperplasia was not significantly increased in treated animals (National Cancer Institute, 1977a).

3.2 Administration with known carcinogens

Mouse: Groups of male B6C3F$_1$ mice, 8 weeks of age, were given drinking-water containing 0 (control) or 20 mg/L *N*-nitrosodiethylamine for 14 weeks. After 4 weeks with no treatment, mice received diets containing 0 (control), 25 or 50 mg/kg technical chlordane or 5 or 10 mg/kg technical heptachlor for 25 weeks. All surviving animals were killed at 43 weeks; five mice from each group were killed after 8 and 16 weeks

of administration of chlordane or heptachlor. Both agents significantly increased the incidence of hepatocellular adenomas and carcinomas combined over that with *N*-nitrosodiethylamine alone (Table 11) (Williams & Numoto, 1984).

To study the tumour-promoting activity of chlordane on mouse skin, groups of male and female CD-1 mice, 7–8 weeks of age, were treated dermally with 0.2 μmol of 7,12-dimethylbenz[*a*]anthracene, followed 1 week later by 0 (control) or 2 μmol (820 μg) of chlordane in 200 μL acetone three times per week for 20 weeks. No increase in the incidence of skin tumours was reported (Moser *et al*., 1993). [The Working Group noted that numerical data were not presented.]

Table 11. Preneoplastic and neoplastic liver lesions in B6C3F$_1$ mice treated with chlordane or heptachlor after initiation with *N*-nitrosodiethylamine (NDEA)

Exposure	Foci, G6Pase-deficient		Liver-cell neoplasms		
	No./cm^2	Area (mm^2/cm^2)	Incidence	No. of adenomas	No. of carcinomas
Control	0.04 ± 0.11	21 ± 0	3/28	2	1
NDEA	1.27 ± 1.07	10.2 ± 12.5	8/20	11	2
NDEA + 25 mg/kg diet chlordane	3.01 ± 1.28	15.5 ± 15.9	17/21[a]	27	16
NDEA + 50 mg/kg diet chlordane	3.83 ± 2.07	30.1 ± 22.1	18/22[a]	42	10
NDEA + 5 mg/kg diet heptachlor	1.81 ± 1.14	27.3 ± 40.7	16/21[b]	24	9
NDEA + 10 mg/kg diet heptachlor	2.29 ± 1.70	31.0 ± 38.7	20/26[b]	34	9

From Williams & Numoto (1984); G6Pase, glucose-6-phosphatase
[a] Significantly different from group given NDEA alone at $p < 0.01$
[b] Significantly different from group given NDEA alone at $p < 0.05$

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

The toxicology of chlordane and heptachlor has been reviewed (FAO/WHO, 1964, 1965, 1967, 1968, 1971, 1978, 1983; WHO, 1984a,b; FAO/WHO, 1987; Public Health Service, 1989a,b).

4.1 Absorption, distribution, metabolism and excretion

4.1.1 *Humans*

Technical-grade chlordane contains nonachlor, heptachlor as well as *cis*- and *trans*-chlordane, and is metabolized into epoxides (Cassidy *et al*., 1994), and these are compounds to which humans are exposed from the continued use and environmental presence of chlordane. Thus, information on nonachlor and heptachlor epoxide is included here. Heptachlor epoxide was the first of the materials derived from technical-

grade chlordane to be analysed routinely in human adipose tissue. Residues result from its use in agriculture and in households. Studies on the storage of heptachlor epoxide and oxychlordane in the adipose tissue of the general population in various countries are summarized in section 1. *trans*-Nonachlor has also been identified in the adipose tissue of people representative of the general population of the USA (Sovocool & Lewis, 1975). Components of technical-grade chlordane — chlordane, heptachlor and *trans*-nonachlor — have been identified in human blood after a variety of exposures, indicating that all are absorbed (Saito *et al*., 1986). As these compounds are lipophilic, they stored mainly in the adipose tissue. *trans*- and *cis*-Chlordane are metabolized to oxychlordane, and heptachlor is metabolized to heptachlor epoxide. Elimination takes place via both urine (Curley & Garrettson, 1969) and faeces (Garrettson *et al.*, 1985). Breast milk is a supplementary excretory route in lactating women (WHO, 1984a,b). Components of technical-grade chlordane and their metabolites are excreted in human milk in quantities that vary with agricultural and household use, dietary habits, individual phenotype and time of milk sampling. Chlordane and its metabolites are accumulated over time (Hirasawa & Takizawa, 1989) and have been found in the blood of pest control operators and in indoor air of houses treated with chlordane (Saito *et al*., 1986; Menconi *et al*., 1988). The use of chlordane for termite control is reported to result in detectable levels of chlordane in breast milk of women living in treated houses (Taguchi & Yakushiji, 1988).

The mean blood concentrations of total chlordane (*trans*-nonachlor, oxychlordane and heptachlor epoxide) of pest control operators were correlated with the conditions under which they sprayed technical-grade chlordane, including the total amount of chlordane sprayed ($r = 0.68$) and the number of days on which they had sprayed within the past year ($r = 0.78$), particularly, within the past 3 months ($r = 0.81$) (Saito *et al.*, 1986). Analysis of blood for chlordane metabolites showed their presence in the descending order *trans*-nonachlor, oxychlordane, heptachlorepoxide and *cis*-nonachlor. Serum concentrations of triglycerides and the activities of creatine phosphokinase and lactate dehydrogenase (LDH) were also found to be higher in pest-control operators (Ogata & Izushi, 1991).

The presence of heptachlor epoxide in the adipose tissue of stillborn infants (Wassermann *et al.*, 1974) and in the cord blood of newborns (D'Ercole *et al.*, 1976) demonstrates placental transfer of heptachlor and/or heptachlor epoxide.

Human liver preparations had little capacity to convert *trans*-nonachlor (a minor component of technical-grade chlordane) to *trans*-chlordane in comparison with rat liver prepared similarly (Tashiro & Matsumura, 1978). In liver microsomes, heptachlor epoxide constituted 85.8% of the metabolized heptachlor in those from rats but only 20.4% in those from human liver. Other metabolites identified in the human liver microsome system were 1-hydroxy-2,3-epoxychlordene (5%), 1-hydroxychlordene (4.8%) and 1,2-dihydroxydihydrochlordene (0.1%); 68.6% was unmetabolized heptachlor (Tashiro & Matsumura, 1978).

4.1.2 *Experimental systems*

(*a*) *Chlordane*

The metabolism of chlordane (WHO 1984a; Nomeir & Hajjar, 1987) and of heptachlor (WHO, 1984b; Fendick *et al.*, 1990) in experimental animals has been reviewed.

(i) *Absorption and distribution*

Chlordane is readily absorbed from the gastrointestinal tract of rats and mice (Barnett & Dorough, 1974; Tashiro & Matsumura, 1977; Ewing *et al.*, 1985), from the skin of rats (Ambrose *et al.*, 1953) and from the respiratory system of rats (Nye & Dorough, 1976).

Chlordane absorbed after oral administration to rats was rapidly distributed, with the highest concentrations in fat and lower concentrations in other organs, in the order liver, kidney, brain and muscle. Treatment with *trans*-chlordane resulted in slightly higher tissue concentrations than with *cis*-chlordane. The patterns of distribution were similar after single and repeated oral dosing (Barnett & Dorough, 1974).

(ii) *Metabolism and elimination*

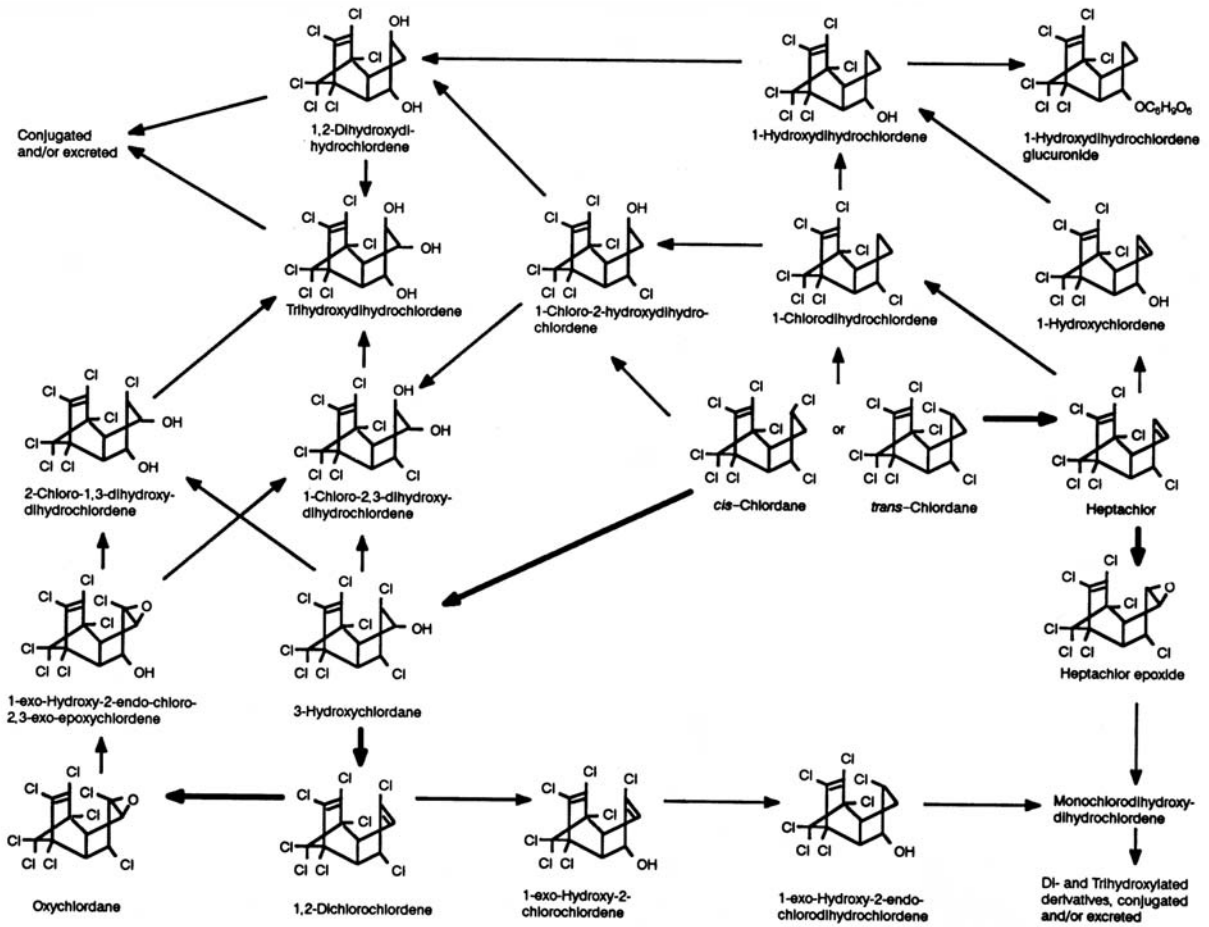
The major route of metabolism of chlordane in treated animals is via oxychlordane. Heptachlor is a minor metabolite of both optical isomers of chlordane. *cis*- and *trans*-Chlordane give rise qualitatively to the same metabolites (Tashiro & Matsumura, 1977). Four metabolic pathways for the metabolism of chlordane have been proposed (Nomeir & Hajjar, 1987, Figure 1):

- hydroxylation to form 3-hydroxychlordane, followed by dehydration to form the postulated precursor of oxychlordane, 1,2-dichlorochlordene;
- dehydrochlorination to form heptachlor, with subsequent formation of heptachlor epoxide and various hydroxylation products;
- dechlorination to monochlorodihydrochlordene;
- replacement of chlorine atoms by hydroxyl groups, with formation of mono-, di- and trihydroxy metabolites which are excreted or conjugated with glucuronic acid.

Rats and mice eliminated 80–≥ 90% of a single oral dose of [^{14}C]chlordane within 7 days (Barnett & Dorough, 1974; Tashiro & Matsumura, 1977; Ewing *et al.*, 1985). Most of the radiolabel was eliminated in faeces. C57BL/6JX mice showed two distinct excretory patterns: the vast majority were high excretors, their elimination rate during the first day after dosing being 20 times faster than that of the low excretors (Ewing *et al.*, 1985).

Mice were given repeated doses of technical-grade chlordane (containing 5.88% *trans*- and 1.45% *cis*-nonachlor) in olive oil by gavage at 0.48 mg/mouse every other day for 29 days. No increase in the body burden of *trans*- or *cis*-chlordane was noted; rather, the levels decreased continuously, indicating that chlordane induced its own metabolism. The concentrations of *trans*- and *cis*-nonachlor and of oxychlordane, however, increased throughout the study period (Hirasawa & Takizawa, 1989).

Figure 1. Metabolic pathways of chlordane



From Nomeir and Hajjar (1987)

(*b*) *Heptachlor*

(i) *Absorption and distribution*

Heptachlor is readily absorbed after intake by most routes and is readily metabolized to heptachlor epoxide by mammals (Public Health Service, 1989a; Fendick *et al.*, 1990). Heptachlor epoxide is stored mainly in fat but also in liver, kidney and muscle in rats and dogs. In rats fed 30 mg/kg of diet heptachlor for 12 weeks, the maximal concentrations of heptachlor epoxide were found in fat within 2–4 weeks; 12 weeks after cessation of exposure, heptachlor epoxide had completely disappeared from the adipose tissue (Radomski & Davidow, 1953). Heptachlor is also stored in fat as heptachlor epoxide in steers (Bovard *et al.*, 1971) and laying hens (Kan & Tuinstra, 1976). Heptachlor epoxide and a hydrophilic metabolite, 1-*exo*-hydroxy-2,3-epoxychlordene, were excreted in the faeces and urine of rat and rabbits treated with heptachlor (Klein *et al.*, 1968). Another metabolite, a dehydrogenated derivative of 1-hydroxy-2,3-epoxychlordene, was isolated from rat faeces (Matsumura & Nelson, 1971).

(ii) *Metabolism*

Phenobarbital pretreatment significantly enhanced the metabolism of heptachlor in rats, causing a 6–11-fold increase in the formation of heptachlor epoxide in liver (Miranda *et al.*, 1973).

4.1.3 *Comparison of human and animal data*

In humans, *trans*- and *cis*-chlordane are metabolized to oxychlordane; heptachlor is metabolized to its epoxide. In experimental animals, the metabolism has been studied more extensively but is similar.

4.2 Toxic effects

4.2.1 *Humans*

Case reports and epidemiological studies of poisoning with technical-grade chlordane and heptachlor after occupational exposures and due to exposure of the general population are summarized in Table 12.

Sublethal exposure to chlordane has not been found to cause delayed neurotoxic effects (Grutsch & Khasawinah, 1991).

4.2.2 *Experimental systems*

(*a*) *Chlordane*

The toxic effects of chlordane have been reviewed (WHO, 1984a).

The oral LD_{50} of chlordane in peanut oil was 335 (299–375) mg/kg bw for male and 430 (391–473) mg/kg bw for female Sherman rats (Gaines, 1960). The oral LD_{50} values

Table 12. Case reports, health surveys and epidemiological studies of cases of poisoning with technical-grade chlordane and technical-grade heptachlor

Population	Clinical features	Reference
22 workers manufacturing and formulating chlordane, aldrin, dieldrin	No evidence of adverse health effects	Princi & Spurbeck (1951)
24 workers employed for 2 months to 5 years in a plant manufacturing chlordane	No evidence of adverse health effects	Alvarez & Hyman (1953)
A female worker spilled a mixture of pesticides including chlordane on her clothing	Confusion, generalized convulsions, death; congestion of brain, lung and stomach mucosa	Derbes *et al*. (1955)
Suicide of a 32-year-old woman who ingested a 5% chlordane talc formulation; estimated ingested dose of chlordane, 6 g (104 mg/kg bw)	Vomiting, dry cough, agitation and restlessness, haemorrhagic gastritis, bronchopneumonia, muscle twitching, convulsions, death after 9.5 days	Derbes *et al*. (1955)
15 workers exposed for 1–15 years to chlordane during manufacture	No evidence of adverse health effects	Fishbein *et al*. (1964)
A 20-month-old boy drank an unknown quantity of a 74% technical-grade chlordane formulation	Vomiting and seizures 45 min after ingestion; serum alkaline phosphatase activity and thymal turbidity slightly elevated after 3 months	Curley & Garrettson (1969)
A 4-year-old girl ingested an unknown amount of 45% chlordane	Convulsions, increased excitability, loss or coordination, dyspnoea, tachycardia	Aldrich & Holmes (1969)
A segment of municipal water system in Chattanooga, TN (USA), 1976, was contaminated with chlordane, initially up to 1200 mg/L. Of 105 residents in affected houses, 71 reported contact with contaminated water	13/71 (18%) described mild symptoms (gastro-intestinal and/or neurological) compatible with chlordane exposure	Harrington *et al*. (1978)

Table 12 (contd)

Population	Clinical features	Reference
Case reports of blood dyscrasias associated with exposure to chlordane or heptachlor alone or in combination with other agents	25 previously reported and six new cases included 22 of aplastic anaemia, three of acute leukaemia, two of leukopenia and one each of hypoplastic anaemia, haemolytic anaemia, megaloblastic anaemia and thrombocytopenia	Infante *et al*. (1978)
Workers employed for more than 3 months in the manufacture of chlordane and heptachlor, 1946–76 [study population overlaps with that of Shindell & Ulrich (1986)]	17 deaths from cerebrovascular disease observed versus 9.3 expected	Wang & MacMahon (1979a)
Cohort of 16 126 men employed as pesticide applicators for ≥ 3 months in 1967, 1968 and 1976, including group of 6734 termite control operators	All deaths, 311 (SMR, 84); cerebrovascular disease among termite control operators (SMR, 39)	Wang & MacMahon (1979b)
A 62-year-old man accidentally ingested ~ 300 mL of 75% chlordane.	Unresponsive to verbal commands, generalized tonic seizures, profuse diarrhoea, transient increase in liver enzymes; recovery by 2 months	Olanoff *et al*. (1983)
A 59-year-old man with a history of Alzheimer disease inadvertently drank from a bottle containing a chlordane formulation	Rapid occurrence of convulsions, death despite cardiopulmonary resuscitation and treatment	Kutz *et al*. (1983)
A 30-year-old woman exposed to chlordane through excessive household use	Numbness around mouth and nose and in arm used for spraying; nausea, vomiting, persistent fatigue and anorexia, menometrorrhagia	Garrettson *et al*. (1985)
Workers employed in the manufacture of chlordane for ≥ 3 months, in 1946–85 [study population overlaps with that of Wang & MacMahon (1979a)]	20 deaths from cerebrovascular disease observed versus 11.7 expected	Shindell & Ulrich (1986)

Table 12 (contd)

Population	Clinical features	Reference
45 members of dairy-farm families who consumed milk and milk products contaminated with heptachlor metabolites	No heptachlor-related metabolic effects observed in routine liver function tests or specific assays for hepatic enzyme induction	Stehr-Green *et al*. (1986)
25 case reports of blood dyscrasias associated with exposure to chlordane and heptachlor; of 16 cases for which exposure data available, 75% involved home and garden applications and 25% professional applications	Aplastic anaemia, thrombocytopenic purpura, leukaemia, pernicious anaemia, megaloblastic anaemia	Epstein & Ozonoff (1987)
261 residents of 85 households treated with chlordane for termite control	Headache in 22% of cases; sore throat and respiratory infections in 16%; fatigue, 14%; sleeping difficulties, blurred vision and fainting also frequent	Menconi *et al*. (1988)

SMR, standardized mortality ratio

for *cis*- and *trans*-chlordane were reported to be similar (392 and 327 mg/kg bw, respectively). The oral LD_{50} of the major metabolite, oxychlordane, in rats was reported to be 19.1 mg/kg bw; the values for several other metabolites were > 4600 mg/kg bw. The signs associated with acute chlordane poisoning include ataxia, convulsions, respiratory failure and cyanosis, followed by death (WHO, 1984a).

An oral dose of chlordane of 50 mg/kg bw per day for 15 days resulted in convulsions and death in rats, whereas a dose of 25 mg/kg bw per day had no such effect (Ambrose *et al.*, 1953).

Cumulative autonomic, neuromuscular and sensorimotor neurotoxic effects were reported in female Fischer 344 rats at doses of up to 156 mg/kg bw of chlordane (Moser *et al.*, 1995). The neurotoxic effects of heptachlor occurred at much lower doses, but did not include neuromuscular or sensorimotor effects.

Mice treated with chlordane at doses of 0.1–8 mg/kg bw for 14 days showed a dose-related increase in cell-mediated immunity, as evaluated *in vitro*. Expression of delayed hypersensitivity and the antibody response to sheep red blood cells *in vivo* were unaltered (Johnson *et al.*, 1986).

(i) *Studies related to liver toxicity*

During the first week of long-term studies (see section 3), chlordane caused tremor in female rats fed the high dietary concentration of 25 mg/kg. Later in the study, decreased body-weight gain and increased liver weights were observed in these animals (National Cancer Institute, 1977b; Khasawinah & Grutsch, 1989a). In mice, the liver was the target organ for non-neoplastic toxicity; the serum activities of aspartate and alanine aminotransferases were elevated in animals of each sex, and the liver weight was increased in males at 12.5 mg/kg of diet. Increased liver-cell volume was seen in males and females at 5 or 12.5 mg/kg of diet, and hepatocyte degeneration and necrosis were seen only in treated males (Khasawinah & Grutsch, 1989b).

Rats were given 100 mg/kg bw per day chlordane by stomach tube or 50 mg/kg bw per day chlordane by intraperitoneal injection once a day for 4 days. The total cholesterol and serum triglycerides concentrations and the activities of creatinine phosphokinase and LDH were increased by chlordane treatment. The isoenzyme patterns suggested that the increase in enzyme activities was related to skeletal muscle. Furthermore, significant increases in liver weight, liver water content and total lipid, triglyceride and phospholipid concentrations were recorded. Chlordane induced lipid peroxidation in the liver, with a dose–response relationship. Although no appreciable effect on mitochondrial function and latent ATPase activity was observed, 2,4-dinitrophenol-stimulated ATPase activity was inhibited. Histological examination of the liver confirmed fatty infiltration (Ogata & Izushi, 1991).

Administration of a single dose of chlordane at 120 mg/kg bw by gavage to female Sprague-Dawley rats resulted in significant increases in hepatic lipid peroxidation, measured as thiobarbituric acid-reactive substances (Hassoun *et al.*, 1993).

Chlordane was administered orally at two 0.25 LD_{50} doses to female Sprague-Dawley rats at 0 h and 21 h, and the animals were killed at 24 h. A threefold increase in hepatic lipid peroxidation was observed, while an increase in lipid peroxidation (measured as thiobarbituric acid-reactive substances) of 2.1-fold was observed in brain homogenates. After incubation of hepatic and brain tissues with 1 nmol/mL of chlordane *in vitro*, maximum increases in chemiluminescence, a measure of the generation of reactive oxygen species, occurred within 4–7 min of incubation and persisted for over 10 min. Increases of 2.7-fold were observed in chemiluminescence after incubation of the liver homogenates with chlordane, while an increase of 1.8-fold was observed in the brain homogenates. An increase of 2.3-fold was observed in the chemiluminescence responses in the liver homogenates from animals treated with chlordane, while an increase of twofold was observed in the brain homogenates. In an experiment in which cultured neuroactive PC-12 cells were incubated with chlordane, the release of LDH into the medium was used as an indicator of cell damage and cytotoxicity. Maximal release of LDH from cultured PC-12 cells was observed at a concentration of 100 nmol/L of the pesticide. An increase of 2.5-fold was observed in LDH leakage after incubation of the PC-12 cells with chlordane (Bagchi *et al.*, 1995).

(ii) *Studies related to thyroid function and liver toxicity*

The effect of chlordane on the concentration of radiolabel from [^{125}I]thyroxine in plasma was studied in male CD rats given an intraperitoneal injection of 25 or 75 mg/kg bw per day chlordane for 5 days and 24 h later an injection of [^{125}I]thyroxine–[^{131}I]albumin; the animals were bled 35 min later. The concentration of radiolabel in plasma was statistically significantly decreased ($p < 0.001$), and increased uptake in the liver was found. The results at the two doses did not differ significantly. Similar effects were found when phenobarbital (see monograph in this volume) was administered at a dose of 100 mg/kg bw (Bernstein *et al.*, 1968).

Wistar rats and cynomolgus monkeys (*Macaca fascicularis*) were exposed to chlordane by inhalation at a concentration close to 0.1, 1 or 10 mg/m^3 for 8 h/day on 5 days per week for 90 days. In rats, the liver was the main target organ, and the liver weights were significantly increased in animals of each sex exposed to 10 mg/m^3. Histopathological changes, such as centrilobular hepatocyte enlargement, were observed in males and females at 1 and 10 mg/m^3. In male rats, increased height of the follicular thyroid epithelium was observed in 11/35 animals at 10 mg/m^3. A dose-related increase in cytochrome P450 and microsomal protein content was evident in animals of each sex. Essentially all of the observed changes were reversed within 90 days after cessation of exposure. No significant finding was made in male or female cynomolgus monkeys; however, cytochrome P450 and microsomal protein were not measured (Khasawinah *et al.*, 1989).

The time course over 6 months of liver and thyroid cell proliferation was studied in C57BL/10J mice fed 50 mg/kg of diet chlordane and killed on day 4, 5, 8, 15, 29, 99 or 190 after the start of dosing. Groups were withdrawn from treatment during days

29–99 and days 190–247. Replicating cells were labelled with bromodeoxyuridine delivered by an osmotic minipump for 3 days before necropsy. The peak labelling index was seen in the thyroid on day 5 (5.99 ± 2.90% versus 1.00 ± 20% in controls) and in the liver on day 8 (9.0 ± 1.6% versus 0.5 ± 0.4% in controls). Both organs showed an elevated labelling index during the first month of dosing, but while that in thyroid follicular cells was not statistically significantly increased at 190 days, that in liver cells was significantly elevated at all times, except in the withdrawal groups (Barrass *et al.*, 1993).

Chlordane at 200 μmol/L stimulated protein kinase C activity in preparations from mouse brains, liver and epidermis *in vitro*. The stimulation was calcium- and phospholipid-dependent and could be inhibited by quercetin, a known inhibitor of protein kinase C activity (Moser & Smart, 1989).

(*b*) *Heptachlor*

The toxic effects of heptachlor have been reviewed (WHO, 1984b; Fendick *et al.*, 1990).

The acute oral LD_{50} of heptachlor in peanut oil was 100 (74–135) mg/kg bw for male and 162 (140–188) mg/kg bw for female Sherman rats (Gaines, 1960). The signs associated with acute heptachlor poisoning include hyperexcitability, tremors, convulsions and paralysis. Liver damage may occur as a late manifestation (WHO, 1984b). Administration of a single dose of 23 mg/kg bw heptachlor by gavage to female Fischer 344 rats resulted in necrotic lymphocytes in the spleen and thymus (Berman *et al.*, 1995).

Heptachlor epoxide is more acutely toxic than the parent compound, e.g. the oral LD_{50} of the epoxide in rats was 62 mg/kg bw (Sperling & Ewinike, 1969), and the intravenous LD_{100} values for heptachlor and heptachlor epoxide in mice were 40 and 10 mg/kg bw, respectively (WHO, 1984b).

Oral doses of pure heptachlor at 50 and 100 mg/kg bw per day were lethal to rats after 10 days. In animals given 5 mg/kg bw, hyperreflexia, dyspnoea and convulsions occurred, and pathological changes were observed in the liver, kidney and spleen (Pelikan *et al.*, 1968).

Dogs given 5 mg/kg bw heptachlor per day orally died within 21 days (Lehman, 1952).

Mink (*Mustela vison*) were fed diets that contained heptachlor at a concentration of 0, 12.5, 25, 50 or 100 mg/kg (as the technical-grade formulation) for 28 days, followed by a 7-day observation period. Concentrations ≥ 25 mg/kg resulted in a significant decrease in feed consumption, while concentrations ≥ 50 mg/kg caused a significant reduction in body weight. Deaths (37.5%) occurred only in the group at 100 mg/kg. Animals at the highest concentration also had reduced relative weights of the spleen and kidney and an increased relative weight of the adrenal glands when necropsied at the time of death or at termination of the study (Aulerich *et al.*, 1990).

Groups of mice were given heptachlor by intraperitoneal injection (50 mg/kg bw per day for 3 days), by gavage (10 mg/kg bw twice a week for 92 days) or in the diet (30 mg/kg of diet for 180 days). All groups showed increased activity of serum alanine aminotransferase; only the group that received heptachlor in the diet showed decreased serum cholinesterase activity. Serum creatine phosphokinase activity was increased significantly in the groups that received heptachlor by intraperitoneal injection or in the diet. Significant differences in serum lipid concentrations from those of controls were seen in all treated groups, as heptachlor has a known effect on lipid metabolism. Except in the group that received heptachlor orally, lipid peroxidation in the liver, expressed as malondialdehyde concentration, was also increased significantly (Izushi & Ogata, 1990).

Addition of heptachlor at a final concentration of 50 μmol/L to rat liver mitochondria, with succinate as the substrate, decreased the respiratory control index by markedly inhibiting state 3 respiration and slightly inhibiting state 4 respiration. Heptachlor at 100 μmol/L, with succinate as the substrate, suppressed states 3 and 4 respiration almost completely. In contrast, heptachlor at a final concentration of 50–100 μmol/L with β-hydroxybutylate as the substrate slightly decreased the respiratory control index, and use of ascorbate plus *N*,*N*,*N*′,*N*′-tetramethylphenylene diamine as the substrate decreased the index hardly at all. Heptachlor at a concentration of 50 μmol/L in the presence of succinate also decreased the ADP:oxygen ratio of mitochondria. The mode of inhibition of succinate oxidation by heptachlor was apparently non-competitive, as seen in a Lineweaver-Burk plot (Meguro *et al.*, 1990).

Cell death was observed when ML-1 cells were treated with heptachlor at concentrations > 80 μmol/L. At lower concentrations, heptachlor induced cell adherence and formation of extended cytoplasmic pseudopodia. At 80 μmol/L, there was cell differentiation to monocyte or macrophage types (Chuang & Chuang, 1991).

The effects of exposure to organochlorine pesticides on the chemotactic functions of rhesus monkey (*Macaca mulatta*) neutrophils and monocytes was investigated with a 48-well chemotaxis chamber. The chemokines interleukin-8 and RANTES (the natural ligand for the CC chemokine receptor 5) were used as the chemoattractants to induce chemotaxis. When the neutrophils and monocytes were treated with heptachlor, chlordane or toxaphene for 1 h at 37 °C, inhibition of chemotaxis was seen in all samples at concentrations as low as 10^{-14} to 10^{-5} mol/L. Toxaphene was the least effective of the three compounds in preventing monocytes from migrating towards RANTES (Miyagi *et al.*, 1998).

Administration of approximately 2 mg/kg bw heptachlor to male albino rats [strain not specified] by gavage daily for 21 days did not alter serum thyroxine, triiodothyronine or thyroid-stimulating hormone concentrations (Akhtar *et al.*, 1996). [The Working Group noted the low dose of heptachlor used in this experiment.]

4.3 Reproductive and developmental effects

4.3.1 *Humans*

An ecological study was carried out to compare the incidence rates of 37 congenital malformations in Hawaii with those in the USA as a whole (Le Marchand *et al*., 1986), after contamination of milk on Oahu Island with heptachlor between the autumn of 1980 and December 1982, which was traced to contaminated foliage of pineapple plants used as cattle feed. Data on birth defects were obtained from the Birth Defects Monitoring Program, which covers 62–76% of all births in Hawaii. Temporal and geographical comparisons were made (Table 13). Increased incidence rates were reported on Oahu for cardiovascular malformations and hip dislocation: in 1978–80, the incidence of vascular malformations was 63.2/10 000 births on Oahu Island and 24.9 on the other Hawaian islands; in 1981–83, these rates were 76.2 and 24.4, respectively. For hip dislocation, the only increase occurred in 1981–83: the rates were 42.2/10 000 on Oahu and 22.4 on the other islands. All the increased rates on Oahu were statistically significant ($p < 0.01$). The authors noted that the increase in the incidence of cardiovascular malformations and hip dislocation began in 1978–80, which included only the first few months of contamination. [The Working Group noted that the incidence rates for hip dislocation were unstable.]

Table 13. Incidence rates per 10 000 births of cardiovascular malformations and hip dislocation on Oahu Island and on the other Hawaiian islands, 1970–83

Defect	Oahu				Other islands			
	1970–74	1975–77	1978–80	1981–83	1970–74	1975–77	1978–80	1981–83
Cardiovascular malformations	38.3	33.6	63.2	76.2	21.3	23.4	24.9	24.4
Hip dislocation	12.6	8.8	29.3	42.2	9.9	30.8	31.1	22.4

From Le Marchand *et al*. (1986)

4.3.2 *Experimental systems*

(*a*) *Chlordane*

Chlordane has a range of effects on the reproductive system, including effects on circulating concentrations of hormones and gonadotropins and reductions in uterine and seminal vesicle weights (reviewed by Cassidy *et al*., 1994). A thorough study of the developmental toxicity of chlordane was conducted by Cassidy *et al*. (1994), in which Sprague-Dawley rats were given technical-grade chlordane in peanut butter at a concentration resulting in a dose of 100, 500 or 5000 µg/kg bw per day. The dams were dosed from gestation day 4 to postnatal day 21 (i.e., through lactation). The pups were then exposed from 22 to 80 days of age. The lowest dose was designed to generate serum

concentrations of heptachlor epoxide and oxychlordane similar to those found at the 99th percentile of exposure in the population of the USA. The end-points investigated included testosterone concentration in pups; general toxicity; neurobehavioural effects, in tests for learning (water maze), sex-specific reproductive behaviour, open-field activity and response to auditory startle; and a neurochemical parameter (γ-amino butyric acid-stimulated synaptosomal chloride uptake) in the whole brain. Exposure of the dams resulted in measurable concentrations of heptachlor and other metabolites in the offspring and in the dams' milk. Pre- and postnatal chlordane treatment lowered the concentrations of testosterone to 40% of the control level in female, but not in male, offspring in a dose-dependent fashion. These exposures also affected male mating behaviour, reducing the latency to intromission and increasing the total number of intromissions. In females, exposure to chlordane improved performance in the water maze, reducing the time for completing trials and reducing error rates; in males, no effects on maze behaviour was observed. Open-field behaviour was not changed. In tests of acoustic startle, there was some increase in maximum response but not in latency. There was a significant decrease in γ-amino butyric acid-mediated chloride uptake by synaptosomes from male rats exposed to the highest dose of chlordane; it should be noted that chlordane decreases chloride uptake *in vitro*, as has been reported for other cyclodiene pesticides (Gant *et al*., 1987).

(*b*) *Heptachlor*

A study of developmental toxicity was conducted to test interactions of chemical mixtures, including heptachlor, trichloroethylene and diethylhexylphthalate (Narotsky & Kavlock, 1995; Narotsky *et al*., 1995). Fischer 344 rats were given 0, 5.1, 6.8, 9.0 or 12 mg/kg bw per day heptachlor (99% pure) by gavage on gestational days 6–15. At the two higher doses, heptachlor decreased maternal weight gain and increased postnatal loss. Heptachlor interacted with diethylhexylphthalate in terms of increasing maternal death and decreasing pup weights on postnatal days 1 and 6. No terata were observed in heptachlor-exposed offspring (Amita-Rani & Krishnakumari, 1995). Other studies indicated that chlordane can affect testicular tissues in mice (Balash *et al*., 1987; Al-Omar *et al.*, 2000) and gonadal production of progesterone and estradiol measured *ex vivo* after exposure of rats to 5–30 mg/kg bw heptachlor by subcutaneous injection every other day for 18 days (Oduma *et al*., 1995). Oduma *et al*. (1995) also reported that exposure of female Sprague-Dawley rats to 5 or 20 mg/kg bw heptachlor (99% pure) by subcutaneous injection every other day for 18 days affected cyclicity, maternal body-weight gain, gestational length and litter size. [The Working Group noted that the data on cyclicity were not analysed statistically, but visual inspection indicated some disruptions at the higher dose.] Gestational length was increased in three of the 10 dams at the highest dose, and their litter size was reduced. No effects on pup survival were observed. Amita-Rani and Krishnakumari (1995) also reported that exposure of either female rats to a total dose of 25 or 50 mg/kg bw heptachlor (technical-grade) over 14 days by gavage or

males to a total dose of 45.25 or 90.5 mg/kg bw over 90 days increased the number of resorptions.

In a study of the effects of heptachlor on reproductive function in mink (*Mustela vison*), the animals were given diets containing heptachlor (purity, 72%) at 6.25, 12.5 or 25 mg/kg from 6 weeks before mating for a total of 181 days. The highest concentration, stated to be equivalent to 3.1 mg/kg bw per day, was lethal to all animals, causing seizures and ataxia before death, which followed cessation of feeding. At a dietary concentration of 6.25 mg/kg, no effects were observed on gestational length or litter size, or on sperm motility or morphology in male mink. However, postnatal growth and pup survival were significantly reduced at 12.5 mg/kg of diet (Crum *et al.*, 1993).

The offspring of pregnant BALB/c mice given an oral dose of 0.16, 2, 4 or 8 mg/kg bw per day technical-grade chlordane in peanut butter for 19 days had enhanced overall resistance to influenza virus after infection at 38 days of life (Menna *et al.*, 1985). Other studies on the effects of prenatal exposure to chlordane on immune function followed the work of Spyker-Cranmer *et al*. (1982), who reported that exposure of mice *in utero* to analytical-grade chlordane at 0.16 or 8 mg/kg bw per day throughout gestation depressed cell-mediated immunity in adulthood.

The more recent studies showed that prenatal exposure of BALB/c mice to 4 or 8 mg/kg bw per day chlordane impaired contact hypersensitivity and delayed-type hypersensitivity reactions (see Blaylock *et al*., 1995). Effects have also been reported on macrophage function, myeloid stem and progenitor cells and colony-forming unit (CFU) responses in adulthood. Theus *et al*. (1992) reported that BALB/c mice exposed *in utero* by feeding dams 8 mg/kg bw per day chlordane in peanut butter throughout gestation had significantly decreased functioning of inflammatory macrophages. Blyler *et al*. (1994) exposed BALB/c mice orally to 8 mg/kg bw per day chlordane (analytical-grade) on days 1–18 of gestation. At 6 weeks of postnatal life, bone-marrow cells were harvested and assayed for CFU. Sex-specific effects were observed, with significant increases in bone-marrow cellularity in males and females but a depression of CFU number only in females. Similar effects of the same treatment were reported on fetal liver-colony formation (Barnett *et al*., 1998). Exposure of pregnant BALB/c mice to a diet containing chlordane at a concentration resulting in a dose of 8 mg/kg bw per day during the first 18 days of gestation had no effect on circulating T lymphocyte responses in either male or female offspring, but a small increase in natural killer cell activity was seen at 100 days of age (Blaylock *et al*., 1990).

Male Sprague-Dawley rats were injected subcutaneously with 0, 5, 10, 15, 20 or 25 mg/kg bw heptachlor every other day for 2 weeks. At all doses, significantly suppressed plasma testosterone concentration ($p < 0.05$) and significantly increased plasma lutineizing hormone ($p < 0.01$) and cortisol ($p < 0.02$) concentrations were seen in treated rats as compared with corn oil-treated controls. Luteinizing hormone and testosterone concentrations were strongly correlated ($r = 0.69$; $p < 0.05$). The

testes of rats treated with 25 mg/kg bw heptachlor showed some pathological changes (Wango *et al.*, 1997).

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*

(*a*) *Studies of liver*

Chlordane induced hepatic drug-metabolizing enzymes in experimental animals (WHO, 1984a; Fendick *et al.*, 1990) and enhanced estrone metabolism in rats and mice (Welch *et al.*, 1971). Studies of liver cytosol and microsomes from male Holtzman rats showed induction of hexobarbital and aminopyrine by chlordane; the stimulation was maximal 8 days after a single intraperitoneal injection of 10 mg/kg bw (Hart *et al.*, 1963). Chlordane has been classified as an inducer of cytochrome P450 (CYP) isozymes of the phenobarbital type (Okey, 1990).

Heptachlor blocks the cell cycle by preventing progression into S phase; this is associated with deactivation in cyclin-dependent kinase cdk2 and dephosphorylation of cdc2 (Chuang *et al*., 1999).

Administration of a diet containing 2 mg/kg heptachlor for 2 weeks induced aniline hydroxylase and aminopyrine demethylase in rats (Den Tonkelaar & Van Esch, 1974). Heptachlor inhibited oxidative phosphorylation in rat liver mitochondria (Nelson, 1975) and (at 200 μmol/L) stimulated protein kinase C activity in preparations from mouse brain (Moser & Smart, 1989).

No H- or K-*ras* mutations were detected in chlordane-induced hepatocellular tumours in B6C3F_1 mice (15 adenomas and 15 carcinomas) or B6D2F_1 mice (10 adenomas and 10 carcinomas) obtained from a bioassay in which mice were exposed to 55 mg/kg of diet chlordane for up to 505 days. All acidophilic adenomas and carcinomas induced by chlordane showed increased expression of *bcl*-X_L (Malarkey *et al.*, 1995; Christensen *et al.*, 1999).

Female B6C3F_1 mice were initiated with *N*-nitrosodiethylamine (5 mg/kg bw; intraperitoneally) and were then given hepatocarcinogenic concentrations of various chemicals, including chlordane (25 mg/kg of diet), for 4 or 8 months. In the chlordane-treated animals, none of the 39 basophilic hepatic foci that were evaluated showed immunoreactivity to tumour growth factor α (TGFα), but all 63 acidophilic foci were immunoreactive. There was no significant difference in the mean hepatic labelling index, as measured by incorporation of 5-bromo-2′-deoxyuridine, between foci immunoreactive and non-immunoreactive to TGFα. The incidence of immunoreactivity to TGFα was greater in hepatocellular tumours that were predominantly of

the basophilic phenotype. A similar pattern was seen for immunoreactivity to epidermal growth factor receptor, which was lacking in basophilic foci (0/16, 0%) and basophilic hepatocellular adenomas (0/6, 0%) and present in acidophilic foci (7/30, 23%) and acidophilic adenomas (2/9, 22%), suggesting an autocrine mechanism for the development of mouse liver tumours. The increased incidence of TGFα immunoreactivity in basophilic liver tumours suggests that TGFα is a marker of tumour progression in mouse liver. Furthermore, modulations of TGFα were dependent on phenotype rather than treatment, indicating inherent differences in the expression of TGFα in basophilic and acidophilic hepatic lesions (Moser *et al.*, 1997).

(*b*) *Studies of other systems*

Expression of *ras* proto-oncogene mRNA in human myeloblastic leukaemia (ML-1) cells was analysed as a function of cDNA amplification by the polymerase chain reaction (PCR). In a pair of oligonucleotides that flank exon-2 from opposite strands (5′ and 3′) of H-*ras* cDNA for PCR amplification, ML-1 cells were found to express a 112-base pair segment of the *ras* transcript. A rapid decline in the expression of this transcript was seen in cells treated with heptachlor (80 μmol/L for up to 12 h), but addition of serum inhibited the effect of heptachlor and restored the expression of *ras* proto-oncogene mRNA. Expression of the same *ras* segment was not affected by treatment of ML-1 with the tumour promoter 12-*O*-tetradecanoylphorbol-13-acetate (Chuang & Chuang, 1991).

In peripheral blood mononuclear cells isolated form rhesus monkeys, heptachlor and chlordane affected mitogenic stimulation. At 80 μmol/L, both inhibited mitogen-induced proliferation and interleukin-2 release from the monocytes (Chuang *et al.*, 1992).

Addition of 50 μmol/L heptachlor to cultured human CEM×174 lymphocytic cells reduced the cellular levels of mitogen-activated protein kinase (MAPK) cascade proteins, including ERK1 (a 44-kDa MAPK), ERK2 (a 42-kDa MAPK), an 85-kDa and a 54-kDa MAPK, MEK1 (a 45-kDa ERK) and MEKK (a 78-kDa MEK). However, heptachlor treatment caused a marked increase in the expression of activated ERK1 and ERK2 (Thr- and Tyr-dually phosphorylated) in the cells (Chuang & Chuang, 1998).

In LLC-PK1 pig kidney cells transiently cotransfected with $(CYP3A23)_2$-tk-CAT and mouse pregnenolone X receptor, chlordane (20 μmol/L, 24 h) induced the CYP3A23 DR-3 element, and this activation required the pregnenolone X receptor (Schuetz *et al.*, 1998).

In transfection experiments, chlordane (10 μmol/L, 48 h) was found to antagonize estrogen-related receptor α-1 (ERRα-1) expression of the reporter chloramphenicol acetyltransferase activity in SK-BR-3 breast cancer cells. ERRα-1 is a member of the orphan nuclear receptor family, as its ligand has not been identified. Chlordane can suppress aromatase activity and aromatase expression by antagonizing ERRα-1 (Yang & Chen, 1999).

Experiments with CEM×174 cells, a hybrid of human T and B cells, were performed to investigate the effects of the tumour promoter heptachlor and its congeners chlordane and toxaphene on retinoblastoma (*Rb*) gene expression. Heptachlor, chlordane and toxaphene, at concentrations of 10–50 μmol/L, reduced Rb protein expression in a concentration-dependent manner. In the case of heptachlor, the reduction could be seen as early as 12 h and was time-dependent. Analysis of Rb mRNA revealed no detectable difference over the same concentration range. In a similar experiment, *p53* protein expression was decreased, with no change in that of mRNA (Rought *et al.*, 1998, 1999).

4.5 Genetic and related effects

4.5.1 *Humans*

No data were available to the Working Group.

4.5.2 *Experimental systems* (see Tables 14 and 15 for references)

Chlordane induced neither DNA damage nor point mutations in bacteria. It caused SOS repair and prophage induction in *Escherichia coli* and gene conversion in *Saccharomyces cerevisiae*. In cultured mammalian cells, it did not induce unscheduled DNA synthesis but did induce gene mutations at the *Tk* and Na^+/K^+ ATPase loci. It inhibited gap-junctional intercellular communication in cultured mammalian cells. In single studies with cultured human cells, evidence was obtained for the induction of unscheduled DNA synthesis and sister chromatid exchange, but not for the induction of gene mutations. Sister chromatid exchange was induced in intestinal cells of *Umbra limi* (mud minnow) *in vivo*. Chlordane caused micronucleus formation and chromosomal aberrations in the bone marrow of mice treated *in vivo*, and nuclear aberrations (micronucleated and apoptotic cells) in hair follicle cells of these mice. No evidence was found of adduct formation with chlordane in liver DNA of mice treated *in vivo*. No dominant lethal mutation was found in mice.

Heptachlor did not induce DNA damage or point mutations in bacteria, gene conversion in *Saccharomyces cerevisiae* or sex-linked recessive lethal mutations in *Drosophila melanogaster.* It did not induce unscheduled DNA synthesis in cultured rodent cells in the absence of metabolic activation, but did so in human fibroblasts with metabolic activation. It induced gene mutations at the *Tk* but not at the *Hprt* locus in rodent cells. Heptachlor inhibited gap-junctional intercellular communication in cultured rodent and human cells. It did not induce gene mutation in *lacI* transgenic mice or dominant lethal mutations in mice *in vivo*[1].

[1] The Working Group was aware of unpublished studies of sister chromatid exchange (positive) and chromosomal aberrations (negative) in Chinese hamster ovary cells *in vitro*.

Table 14. Genetic and related effects of chlordane

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
ColE1 plasmid DNA strand breaks (from *Escherichia coli* K12 ColE1)	–	NT	100	Griffin & Hill (1978)
Escherichia coli $WP2_s$ (λ) prophage induction	+	+	20 000	Houk & DeMarini (1987)
SOS repair (*SulA*) induction in *Escherichia coli* PQ37	+	NT	NR	Venkat *et al*. (1995)
Bacillus subtilis rec strains, differential toxicity	–	–	50	Matsui *et al*. (1989)
Salmonella typhimurium, TA1538, TA1978, differential toxicity	–	NT	2000 µg/disk	Rashid & Mumma (1986)
Escherichia coli WP2, K12, differential toxicity	–	NT	2000 µg/disk	Rashid & Mumma (1986)
Salmonella typhimurium TA100, TA1535, TA1537, TA1538, TA98, reverse mutation	NT	–	5000 µg/plate	Simmon *et al*. (1977)
Salmonella typhimurium TA100, TA1535, TA1537, TA1538, TA98, G46, C3076, D3052, reverse mutation	–	–	NR	Probst *et al*. (1981)
Salmonella typhimurium TA100, TA1535, TA1537, TA1538, TA98, reverse mutation	–	–	NR	Gentile *et al*. (1982)
Salmonella typhimurium TA100, TA1535, TA1537, TA98, reverse mutation	–	–	1000 µg/plate	Mortelmans *et al*. (1986)
Escherichia coli WP2, WP2 *uvr*A, reverse mutation	–	–	NR	Probst *et al*. (1981)
Saccharomyces cerevisiae D4, gene conversion	–	+	6.6	Gentile *et al*. (1982)
Unscheduled DNA synthesis, Fischer 344 rat, CD-1 mouse and Syrian hamster primary hepatocytes *in vitro*	–	NT	4.1	Maslansky & Williams (1981)
DNA single-strand breaks, PC-12 adrenal phaeochromocytoma cells *in vitro*	+	NT	0.041	Bagchi *et al*. (1995)
Unscheduled DNA synthesis, Fischer 344 rat primary hepatocytes *in vitro*	–	NT	41	Probst *et al*. (1981)
Unscheduled DNA synthesis, Fischer 344 rat primary hepatocytes *in vitro*	–	NT	4.1	Williams *et al*. (1989)

Table 14 (contd)

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Gene mutation, Chinese hamster lung V79 cells, *Hprt* locus *in vitro*	–	NT	1.6	Tsushimoto *et al.* (1983)
Gene mutation, Chinese hamster lung V79 cells, ouabain resistance *in vitro*	+	NT	4.1	Ahmed *et al.* (1977a)
Gene mutation, Chinese hamster lung V79 cells, diphtheria toxin resistance *in vitro*	–	NT	1.6	Tsushimoto *et al.* (1983)
Gene mutation, mouse lymphoma L5178Y cells, *Tk* locus *in vitro*	+	NT	25	McGregor *et al.* (1988)
Gene mutation, rat liver epithelial ARL cells *in vitro*, *Hprt* locus	–	NT	41	Telang *et al.* (1982)
Micronucleus formation, beluga whale skin fibroblasts *in vitro*	+	–	5	Gauthier *et al.* (1999)
Cell transformation, Syrian hamster embryo cells, focus assay	+	NT	8[c]	Bessi *et al.* (1995)
Covalent binding to DNA (^{32}P-postlabelling), Syrian hamster embryo cells	–	NT	10	Bessi *et al.* (1995)
Unscheduled DNA synthesis, human VA-4 fibroblasts *in vitro*	+	–	0.4	Ahmed *et al.* (1977b)
Gene mutation, human fibroblasts *in vitro*[d]	–	–	41	Tong *et al.* (1981)
Sister chromatid exchange, human LAZ-007 lymphocytes *in vitro*	+	+	0.41	Sobti *et al.* (1983)
Inhibition of intercellular communication, rat liver epithelial ARL cells *in vitro*	+	NT	2.1	Telang *et al.* (1982)
Inhibition of intercellular communication, V79 cells *in vitro*	(+)	NT	0.41	Tsushimoto *et al.* (1983)
Inhibition of intercellular communication, male B6C3F$_1$ mouse primary hepatocytes *in vitro*	+	NT	20.5	Ruch *et al.* (1990)
Inhibition of intercellular communication, male Fischer 344 rat primary hepatocytes *in vitro*	+	NT	20.5	Ruch *et al.* (1990)
Inhibition of intercellular communication, Syrian hamster embryo and V79 cells *in vitro*	+	NT	5	Bessi *et al.* (1995)

Table 14 (contd)

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
DNA single-strand breaks, Sprague-Dawley rat liver cells *in vivo* (12-h treatment)	+		120 po × 1	Hassoun *et al*. (1993)
DNA single-strand breaks, Sprague-Dawley rat liver cells *in vivo*	+		60 po × 2	Bagchi *et al*. (1995)
Sister chromatid exchange, *Umbra limi* intestinal cells *in vivo*	+		0.00002	Vigfusson *et al*. (1983)
Micronucleus formation, male CD-1 Swiss mouse bone-marrow cells *in vivo*	(+)		205 µmol/kg, dermal × 1	Schop *et al*. (1990)
Chromosomal aberrations, male Swiss mouse bone-marrow cells *in vivo*	+		10 po × 1	Sarkar *et al*. (1993)
Nuclear aberrations, male CD-1 Swiss mouse hair follicle cells *in vivo*	+		51 µmol/kg, dermal × 1	Schop *et al*. (1990)
Dominant lethal mutation, male ICR/Ha Swiss mice	–		240 ip × 1	Epstein *et al*. (1972)
Dominant lethal mutation, male ICR/Ha Swiss mice	–		50 po × 5	Epstein *et al*. (1972)
Dominant lethal mutation, male CD-1 mice	–		100 ip × 1	Arnold *et al*. (1977)
Dominant lethal mutation, male CD-1 mice	–		100 po × 1	Arnold *et al*. (1977)
Covalent binding to DNA (^{32}P-postlabelling), male and female B6C3F$_1$ mouse liver *in vivo*	–		50 po × 1 or 200 mg/kg of diet, 14 days	Whysner *et al*. (1998)

[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; NR, not reported; po, oral; ip, intraperitoneal
[c] Three applications were given.
[d] Mediated by rat primary hepatocytes

Table 15. Genetic and related effects of heptachlor and heptachlor epoxide

Test system	Result[a]		Dose[b] (LED/HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Heptachlor				
ColE1 plasmid DNA strand breaks (from *Escherichia coli* K12 ColE1)	–	NT	100	Griffin & Hill (1978)
Escherichia coli WP2, K12, differential toxicity	–	NT	2000 µg/disk	Rashid & Mumma (1986)
Salmonella typhimurium TA1538, TA1978, differential toxicity	–	NT	2000 µg/disk	Rashid & Mumma (1986)
Bacillus subtilis rec strains, differential toxicity	–	–	356	Matsui *et al*. (1989)
Salmonella typhimurium TA100, TA1535, TA1537, TA1538, TA98, reverse mutation	NT	–	5000 µg/plate	Simmon *et al*. (1977)
Salmonella typhimurium TA100, TA1535, TA1537, TA1538, TA98, G46, C3076, D3052, reverse mutation	–	–	NR	Probst *et al*. (1981)
Salmonella typhimurium TA100, TA1535, TA98, reverse mutation	–	(+)[c]	10 µg/plate	Gentile *et al*. (1982)
Salmonella typhimurium TA100, TA1535, TA1537, TA1538, TA98, reverse mutation	–	–	5000 µg/plate	Moriya *et al*. (1983)
Salmonella typhimurium TA100, TA1535, TA1537, TA98, reverse mutation	–	–	333 µg/plate	Zeiger *et al*. (1987)
Salmonella typhimurium TA100, TA102, TA98, TA97, reverse mutation	–	–	1000 µg/plate	Mersch-Sundermann *et al*. (1988)
Escherichia coli WP2, WP2 *uvr*A, reverse mutation	–	–	NR	Probst *et al*. (1981)
Escherichia coli WP2, reverse mutation	–	–	5000 µg/plate	Moriya *et al*. (1983)
Saccharomyces cerevisiae D4, gene conversion	–	–	NR	Gentile *et al*. (1982)
Drosophila melanogaster, sex-linked recessive lethal mutations	–		1 ng, injection	Benes & Šrám (1969)
Unscheduled DNA synthesis, rat, mouse and Syrian hamster primary hepatocytes *in vitro*	–	NT	3.7	Maslansky & Williams (1981)

Table 15 (contd)

Test system	Result[a]		Dose[b] (LED/HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Unscheduled DNA synthesis, Fischer 344 rat primary hepatocytes *in vitro*	–	NT	3.7	Probst *et al*. (1981)
Unscheduled DNA synthesis, rat primary hepatocytes *in vitro*	–	NT	3.7	Williams *et al*. (1989)
Gene mutation, mouse lymphoma L5178Y cells, *Tk* locus *in vitro*	+	NT	25	McGregor *et al*. (1988)
Gene mutation, rat liver epithelial ARL cells *in vitro*, *Hprt* locus	–	NT	37	Telang *et al*. (1982)
Unscheduled DNA synthesis, human VA-4 fibroblasts *in vitro*	–	+	37	Ahmed *et al*. (1977b)
Inhibition of intercellular communication, rat liver epithelial ARL cells *in vitro*	+	NT	0.37	Telang *et al*. (1982)
Inhibition of intercellular communication, Chinese hamster V79 cells *in vitro*	+	NT	10	Kurata *et al*. (1982)
Inhibition of intercellular communication, male Fischer 344 rat primary hepatocytes *in vitro*	+	NT	18.7	Ruch *et al*. (1990)
Inhibition of intercellular communication, male B6C3F$_1$ mouse primary hepatocytes *in vitro*	+	NT	18.7	Ruch *et al*. (1990)
Inhibition of intercellular communication, human breast epithelial cells *in vitro*	+	NT	10	Nomata *et al*. (1996)
Gene mutation, *lacI* transgenic mouse liver assay *in vivo*	–		20 mg/kg of diet, 120 days	Gunz *et al*. (1993)
Dominant lethal mutation, male ICR/Ha Swiss mice	–		24 ip × 1; 10 po × 5	Epstein *et al*. (1972)
Heptachlor epoxide				
Aspergillus nidulans, forward mutation	–	NT	10 450	Crebelli *et al*. (1986)
Aspergillus nidulans, mitotic crossing-over	–	NT	10 000	Crebelli *et al*. (1986)
Aspergillus nidulans, aneuploidy	–	NT	10 000	Crebelli *et al*. (1986)

Table 15 (contd)

Test system	Result[a]		Dose[b] (LED/HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Salmonella typhimurium TA1535, TA1536, TA1537, TA1538, reverse mutation	–	–	1000 µg/plate	Marshall *et al.* (1976)
Unscheduled DNA synthesis, human VA-4 fibroblasts *in vitro*	–	+	3.9	Ahmed *et al*. (1977b)
Inhibition of intercellular communication (dye transfer), rat liver WB F344 cells *in vitro*	+[d]	NT	10	Matesic *et al*. (1994)
Inhibition of intercellular communication, human breast epithelial cells *in vitro*	+[d]	NT	1	Nomata *et al*. (1996)

[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; NR, not reported; ip, intraperitoneal; po, oral
[c] Technical grade
[d] Loss of intercellular communication was characterized by a substantial, sustained loss of connexin 43 immunostaining within 15–60 min of treatment; at least in human cells, there was no reduction of connexin 43 mRNA.

Heptachlor epoxide did not induce forward mutation, mitotic crossing-over or aneuploidy in *Aspergillus* or reverse mutation in *Salmonella typhimurium*. It did induce unscheduled DNA synthesis in human fibroblasts *in vitro* with metabolic activation, and inhibited gap-junctional intercellular communication in rat liver and human breast epithelial cells *in vitro*, without metabolic activation.

4.6 Mechanistic considerations

Both chlordane and heptachlor have shown some potential to inhibit gap-junctional intercellular communication and to induce genetic toxicity in cultured mammalian cells. Chlordane has been reported to induce DNA damage in rat liver, but the induction of DNA repair has not been observed. While the evidence is not strong, it remains possible that such events, should they also occur *in vivo*, could play a role in the carcinogenesis induced by these compounds.

Chlordane induces hepatic microsomal metabolism and increased CYP content in rats. It also produces increased cell proliferation in the thyroid gland of mice. Chlordane administered by intraperitoneal injection has been found to lower thyroxine concentrations in rats. However, no information was available on the concentrations of thyroxine, triiodothyronine or thyroid-stimulating hormone in long-term bioassays in rodents. Therefore, while hepatic metabolism of thyroxine resulting in increased thyroid-stimulating hormone is possibly involved in the induction of thyroid tumours in rats, the other mechanisms cannot be excluded because the information is incomplete.

Chlordane is toxic to the liver in rats and mice. In mice, increased hepatocellular proliferation has been found at doses that produce hepatocellular cancer in mice. In rats, liver toxicity has been shown to be accompanied by enhanced lipid peroxidation and generation of reactive oxygen species. Chlordane has been shown to promote liver tumours in mice.

5. Summary of Data Reported and Evaluation

5.1 Exposure data

Chlordane and heptachlor are structurally related organochlorine insecticides; the technical-grade product of each contains about 10–20% of the other compound. They have been used since the 1950s for termite control, on agricultural crops, on lawns, on livestock and for other purposes. Their use has currently been banned or severely restricted in many countries. In these countries, human exposure is still possible owing to their persistence in the environment and their consequent occurrence in meat, fish and other fat-containing foodstuffs, but the mean daily intake has probably decreased.

5.2 Human carcinogenicity data

Several cohort studies, with different inclusion criteria and lengths of follow-up, have been conducted to investigate the mortality of workers at two plants in the USA, one producing chlordane and the other heptachlor and endrin. The workers were also exposed to other chemicals. Although no excess was seen in the rate of mortality from all cancers, some, but not all, of the studies showed a slight excess of lung cancer. A similar small excess of mortality from lung cancer was observed in two cohorts of pesticide applicators in the USA; however, when the analyses were limited to the workers more likely to be exposed to chlordane, the mortality rate for lung cancer was lower than in the overall cohort.

A number of case–control studies were conducted to investigate the risks for cancers of the lymphohaematopoietic system, breast and a few other sites in relation to exposure to chlordane. These studies differed widely in size and methods and in exposure assessment, which was either reported by the subjects themselves (or proxy respondents) or estimated from measures of the concentrations of chlordane metabolites in samples of fat tissue or blood. The populations studied also varied widely, some studies including higher proportions of farmers, who are occupationally exposed to pesticides, while the subjects in others (including most studies of women) had no occupational exposure to chlordane. In most studies, exposure to many other organochlorine or other types of pesticides was also assessed. Four case–control studies of non-Hodgkin lymphoma showed a consistent but modest increase in the risk associated with exposure to chlordane, although it was almost impossible to separate the effect of chlordane from those related to farming *per se* or to exposure to other pesticides. One case–control study each of hairy-cell leukaemia, leukaemia not otherwise specified, soft-tissue sarcoma and multiple myeloma yielded no notable results with respect to chlordane. No association with chlordane concentrations in blood or fat tissue was found in six of seven case–control studies of breast cancer conducted in Denmark and North America, in two of which blood samples were collected prospectively, or in one study on endometrial cancer conducted in Sweden. No clear pattern emerged in a study of pancreatic cancer in the USA, while a small study of brain cancer in children showed elevated risks associated with termite control treatment, also in comparison with children with cancer of the lymphohaematopoietic system.

5.3 Animal carcinogenicity data

Chlordane, technical-grade chlordane, heptachlor, technical-grade heptachlor, heptachlor epoxide and a mixture of heptachlor and heptachlor epoxide have been tested for carcinogenicity by oral administration in several strains of mice and rats. In the studies in mice, increased incidences of hepatocellular neoplasms (including carcinomas) were seen in both males and females. Increased incidences of thyroid follicular-cell adenomas and carcinomas were seen in one study each with chlordane and

technical-grade heptachlor in rats. In a third study in rats, technical-grade chlordane marginally increased the incidence of liver adenomas in male rats. In initiation–promotion studies in mice, administration of chlordane or heptachlor after *N*-nitrosodiethylamine resulted in increased incidences of hepatocellular tumours.

5.4 Other relevant data

Chlordane is primarily metabolized to oxychlordane and to a minor extent may also be dehydrochlorinated to heptachlor. Heptachlor, which is also a component of technical-grade chlordane, is biotransformed to its epoxide. Subsequent dechlorination reactions lead to hydroxylated compounds, which are excreted primarily as glucuronides. Minor metabolites include heptachlor and heptachlor epoxide.

Accidental or intentional exposure to chlordane has resulted in signs of neurotoxicity and, in some cases, death. In experimental animals, the toxic effects of chlordane on the liver include lipid peroxidation and cell proliferation secondary to cytotoxicity. In the thyroid, chlordane has been shown to decrease thyroxine concentrations in rats. Both chlordane and heptachlor induce hepatic and gonadal microsomal oxidative enzymes and also steroid hormone metabolism.

Chlordane and heptachlor are toxic to reproduction and development in mice, rats and mink. Pre- and postnatal exposures to chlordane affected the development of the immune system in rodents. Impaired cell-mediated immunity after prenatal exposure to chlordane has been observed in female BALB/c mice.

No data were available on the genetic and related effects of chlordane or heptachlor in humans. Both compounds inhibited gap-junctional intercellular communication and induced gene mutations in rodent cells. Likewise, both compounds induced unscheduled DNA synthesis in human fibroblasts but not in rodent hepatocytes. Chlordane induced DNA damage in liver cells of rats treated *in vivo*, but heptachlor did not induce mutations in hepatocytes of *lacI* transgenic mice treated *in vivo*. Neither chlordane nor heptachlor caused dominant lethal mutation in mice. Neither chlordane nor heptachlor was mutagenic to bacteria, and only chlordane damaged bacterial or plasmid DNA.

5.5 Evaluation

There is *inadequate evidence* in humans for the carcinogenicity of chlordane and heptachlor.

There is *sufficient evidence* in experimental animals for the carcinogenicity of chlordane and of heptachlor.

Overall evaluation

Chlordane and heptachlor are *possibly carcinogenic to humans (Group 2B)*.

6. References

Agency for Toxic Substances and Disease Registry (1989a) *Toxicological Profile for Heptachlor/Heptachlor Epoxide* (Report No. ATSDR/TP-88/16; NTIS PB89-194492), Washington DC, Public Health Service, Department of Health and Human Services

Agency for Toxic Substances and Disease Registry (1989b) *Toxicological Profile for Chlordane* (Report No. ATSDR/TP-89/06; NTIS PB90-168709), Washington DC, Public Health Service, Department of Health and Human Services

Agency for Toxic Substances and Disease Registry (1993) *Toxicological Profile for Heptachlor/Heptachlor Epoxide (Update)* (Report No. ATSDR/TP-92/11; NTIS PB93-182467), Atlanta, GA, Public Health Service, Department of Health and Human Services

Agency for Toxic Substances and Disease Registry (1994) *Toxicological Profile for Chlordane (Update)* (Report No. ATSDR/TP-93/03; NTIS PB95-100111), Atlanta, GA, Public Health Service, Department of Health and Human Services

Ahmed, F.E., Lewis, N. J. & Hart, R.W. (1977a) Pesticide induced ouabain resistant mutants in Chinese hamster V79 cells. *Chem.-biol. Interactions*, **19**, 369–374

Ahmed, F.E., Hart, R.W. & Lewis, N. J. (1977b) Pesticide induced DNA damage and its repair in cultured human cells. *Mutat. Res*., **42**, 161–173

Akhtar, N., Kayani, S.A., Ahmad, M.M. & Shahab, M. (1996) Insecticide-induced changes in secretory activity of the thyroid gland in rats. *J. appl. Toxicol.*, **16**, 397–400

Aldrich, F.D. & Holmes, J.H. (1969) Acute chlordane intoxication in a child. Case report with toxicological data. *Arch. environ. Health*, **19**, 129–132

Al-Omar, M.A., Abbas, A.K. & Al-Obaidy, S.A. (2000) Combined effect of exposure to lead and chlordane on the testicular tissues of Swiss mice. *Toxicol. Lett.*, **115**, 1–8

Alvarez, W.C. & Hyman, S. (1953) Absence of toxic manifestations in workers exposed to chlordane. *Arch. ind. Hyg. occup. Med.*, **19**, 480–483

Ambrose, A.M., Christensen, H.E., Robbins, D.J. & Rather, L.J. (1953) Toxicological and pharmacological studies on chlordane. *Arch. ind. Hyg. occup. Med*., **8**, 197–210

American Conference of Governmental Industrial Hygienists (2000) *TLVs and other Occupational Exposure Values — 2000 CD-ROM*, Cincinnati, OH, ACGIH®

Amita-Rani, B.E. & Krishnakumari, M.K. (1995) Prenatal toxicity of heptachlor in albino rats. *Pharmacol. Toxicol.*, **76**, 112–114

Anon. (1985) Epoxy heptachlor. *Dangerous Prop. ind. Mater. Rep*., **5**, 63–74

American Public Health Association/American Water Works Association/Water Environment Federation (1999a) Method 6410B. Liquid–liquid extraction gas chromatographic/mass spectrometric method. In: *Standard Methods for the Examination of Water and Wastewater*, 20th Ed., Atlanta, GA

American Public Health Association/American Water Works Association/Water Environment Federation (1999b) Method 6630B. Liquid–liquid extraction gas chromatographic method I. In: *Standard Methods for the Examination of Water and Wastewater*, 20th Ed., Atlanta, GA

American Public Health Association/American Water Works Association/Water Environment Federation (1999c) Method 6630C. Liquid–liquid extraction gas chromatographic method II. In: *Standard Methods for the Examination of Water and Wastewater*, 20th Ed., Atlanta, GA

AOAC International (2000) *Official Methods of Analysis of AOAC International*, 17th Ed., Gaithersburg, MD [CD-ROM] [Methods 962.05, 962.07, 965.14, 968.04, 970.52, 972.05, 973.15, 973.16, 973.17, 983.21, 990.06]

Arnold, D.W., Kennedy, G.L., Jr, Keplinger, M.L., Calandra, J.C. & Calo, C.J. (1977) Dominant lethal studies with technical chlordane, HCS-3260 and heptachlor: heptachlor epoxide. *J. Toxicol. environ. Health*, **2**, 547–555

Aronson, K.J., Miller, A.B., Woolcott, C.G., Sterns, E.E., McCready, D.R. & Lickley, L.A. (2000) Breast adipose tissue concentrations of polychlorinated biphenyls and other organochlorines and breast cancer risk. *Cancer Epidemiol. Biomarkers Prev.*, **9**, 55–63

Aulerich, R.J., Bursian, S.J. & Napolitano, A.C. (1990) Subacute toxicity of dietary heptachlor to mink (*Mustela vison*). *Arch. environ. Contam. Toxicol.*, **19**, 913–916

Bagchi, D., Bagchi, M., Hassoun, E.A. & Stohs, S.J. (1995) In vitro and in vivo generation of reactive oxygen species, DNA damage and lactate dehydrogenase leakage by selected pesticides. *Toxicology*, **104**, 129–140

Balash, K.J., Al-Omar, M.A. & Abdul-Latif, B.M. (1987) Effect of chlordane on testicular tissues of Swiss mice. *Bull. environ. Contam. Toxicol.*, **39**, 434–442

Barnett, J.R. & Dorough, H.W. (1974) Metabolism of chlordane in rats. *J. agric. Food Chem.*, **22**, 612–619

Barnett, J.B., Blaylock, B.L., Gandy, J., Menna, J.H., Denton, R. & Soderberg, L.S. (1998) Alteration of fetal liver colony formation by prenatal chlordane exposure. *Fundam. appl. Toxicol.*, **15**, 820–822

Barrass, N., Steward, M., Warburton, S., Aitchison, J., Jackson, D., Wadsworth, P., Marsden, A. & Orton, T. (1993) Cell proliferation in the liver and thyroid of C57B1/10J mice after dietary administration of chlordane. *Environ. Health Perspectives*, **101**, 219–224

Benes, V. & Šrám, R. (1969) Mutagenic activity of some pesticides in *Drosophila melanogaster*. *Ind. Med.*, **38**, 442–444

Berman, E., Schlicht, M., Moser, V.C. & MacPhail, R.C. (1995) A multidisciplinary approach to toxicological screening: I. Systemic toxicity. *J. Toxicol. environ. Health*, **45**, 127–143

Bernstein, G., Artz, S.A., Hasen, J. & Oppenheimer, J.H. (1968) Hepatic accumulation of ^{125}I-thyroxine in the rat: Augmentation by phenobarbital and chlordane. *Endocrinology*, **82**, 406–409

Bessi, H., Rast, C., Rether, B., Nguyen-Ba, B.G. & Vasseur, P. (1995) Synergistic effects of chlordane and TPA in multistage morphological transformation of SHE cells. *Carcinogenesis*, **16**, 237–244

Blair, A., Grauman, D.J., Lubin, J.H. & Fraumeni, J.F., Jr (1983) Lung cancer and other causes of death among licensed pesticide applicators. *J. natl Cancer Inst.*, **71**, 31–37

Blaylock, B.L., Soderberg, L.S.F., Gandy, J., Menna, J.H., Denton, R. & Barnett, J.B. (1990) Cytotoxic T-lymphocyte and NK responses in mice treated prenatally with chlordane. *Toxicol. Lett.*, **51**, 41–49

Blaylock, B.L., Newsom, K.K., Holladay, S.D., Shipp, B.K., Bartow, T.A. & Mehendale, H.M. (1995) Topical exposure to chlordane reduces the contact hypersensitivity resonse to oxazolone in BALB/c mice. *Toxicol. Lett.*, **81**, 205–211

Blyler, G., Landreth, K.S. & Barnett, J.B. (1994) Gender-specific effects of prenatal chlordane exposure on myeloid cell development. *Fundam. appl. Toxicol.*, **23**, 188–193

Bovard, K.P., Fontenot, J.P. & Priode, B.M. (1971) Accumulation and dissipation of heptachlor residues in fattening steers. *J. anim. Sci*., **33**, 127–132

Bowery, T.G. (1964) Heptachlor. In: Zweig, G., ed., *Analytical Methods for Pesticides, Plant Growth Regulators, and Food Additives*, Vol. II, *Insecticides*, New York, Academic Press, pp. 245–256

Brooks, G.T. (1974) *Chlorinated Insecticides*, Vol. 1, *Technology and Application*, Cleveland, OH, CRC Press, pp. 85–158

Brown, D.P. (1992) Mortality of workers employed at organochlorine pesticide manufacturing plants — An update. *Scand. J. Work Environ. Health*, **18**, 155–161

Brown, L.M., Blair, A., Gibson, R., Everett, G.D., Cantor, K.P., Schuman, L.M., Burmeister, L.F., Van-Lier, S.F. & Dick, F. (1990) Pesticide exposures and other agricultural risk factors for leukemia among men in Iowa and Minnesota. *Cancer Res*., **50**, 6585–6591

Brown, L.M., Burmeister, L.F., Everett, G.D. & Blair, A. (1993) Pesticide exposures and multiple myeloma in Iowa men. *Cancer Causes Control*, **4**, 153–156

Buchert, H., Class, T. & Ballschmiter, K. (1989) High resolution gas chromatography of technical chlordane with electron capture- and mass selective detection. *Fresenius Z. anal. Chem*., **333**, 211–217

Cantor, K.P., Blair, A., Everett, G., Gibson, R., Burmeister, L.F., Brown, L.M., Schuman, L. & Dick, F.R. (1992) Pesticides and other agricultural risk factors for non-Hodgkin's lymphoma among men in Iowa and Minnesota. *Cancer Res*., **52**, 2447–2455

Carvalho, F.P., Montenegro-Guillen, S., Villeneuve, J.-P., Cattini, C., Bartocci, J., Lacayo, M. & Cruz, A. (1999) Chlorinated hydrocarbons in coastal lagoons of the Pacific Coast of Nicaragua. *Arch. environ. Contam. Toxicol.*, **36**, 131–139

Cassidy, R.A., Vorhees, C.V., Minnema, D.J. & Hastings, L. (1994) The effects of chlordane exposure during pre- and postnatal periods at environmentally relevant levels on sex steroid-mediated behaviors and functions in the rat. *Toxicol. appl. Pharmacol*., **126**, 326–337

Chan, H.M. (1998) A database for environmental contaminants in traditional foods in northern and Arctic Canada: Development and applications. *Food Add. Contam.*, **15**, 127–134

Christensen, J.G., Romach, E.H., Healy, L.N., Gonzales, A.J., Anderson, S.P., Malarkey, D.E., Corton, J.C., Fox, T.R., Cattley, R.C. & Goldsworthy, T.L. (1999) Altered bcl-2 family expression during non-genotoxic hepatocarcinogenesis in mice. *Carcinogenesis*, **20**, 1583–1590

Chuang, L.F. & Chuang, R.Y. (1991) The effect of the insecticide heptachlor on ras proto-oncogene expression in human myeloblastic leukemia (ML-1) cells. *Toxicology*, **70**, 283–292

Chuang, L.F. & Chuang, R.Y. (1998) Heptachlor and the mitogen-activated protein kinase module in human lymphocytes. *Toxicology*, **128**, 17–23

Chuang, L.F., Liu, Y., Killam, K., Jr & Chuang, R.Y. (1992) Modulation by the insecticides heptachlor and chlordane of the cell-mediated immune proliferative responses of rhesus monkeys. *In Vivo*, **6**, 29–32

Chuang, L.F., Rought, S.E. & Chuang, R.Y. (1999) Differential regulation of the major cyclin-dependent kinases, cdk2 and cdc2, during cell cycle progression in human lymphocytes exposed to heptachlor. *In Vivo*, **13**, 455–461

CIS Information Services (2000) *Directory of World Chemical Producers (Version 2000.1)*, Dallas, TX [CD-ROM]

Cochrane, W.P. & Greenhalgh, R. (1976) Chemical composition of technical chlordane. *J. Assoc off. anal. Chem.*, **59**, 696–702

Codex Alimentarius Commission (1997) *Codex Maximum Limits for Pesticide Residues*, Rome, FAO

Crebelli, R., Bellincampi, D., Conti, G., Conti, L., Morpurgo, G. & Carere, A. (1986) A comparative study on selected chemical carcinogens for chromosome malsegregation, mitotic crossing-over and forward mutation induction in *Aspergillus nidulans*. *Mutat. Res.*, **172**, 139–149

Crum, J.A., Bursian, S.J., Auberich, R.J., Polin & D. & Braselton, W.E. (1993) The reproductive effects of dietary heptachlor in mink (*Mustela vison*). *Arch. environ. contam. Toxicol.*, **24**, 156–164

Curley, A. & Garrettson, L.K. (1969) Acute chlordane poisoning. Clinical and chemical studies. *Arch. environ. Health*, **18**, 211–215

Datta, S., Ohyama, K., Dunlap, D.Y. & Matsumura, F. (1999) Evidence for organochlorine contamination in tissues of salmonids in Lake Tahoe. *Ecotoxicol. environ. Saf.*, **42**, 94–101

Davis, J.R., Brownson, R.C., Garcia, R., Bentz, B.J. & Turner, A. (1993) Family pesticide use and chilhood brain cancer. *Arch. environ. Contam. Toxicol.*, **24**, 87–92

Dearth, M.A. & Hites, R.A. (1991) Complete analysis of technical chlordane using negative ionization mass spectrometry. *Environ. Sci. Technol.*, **25**, 245–254

Demers, A., Ayotte, P., Brisson, J., Dodin, S., Robert, J. & Dewailly, E. (2000) Risk and aggressiveness of breast cancer in relation to plasma organochlorine concentrations. *Cancer Epidemiol. Biomarkers Prev.*, **9**, 161–166

Den Tonkelaar, E.M. & Van Esch, G.J. (1974) No-effect levels of organochlorine pesticides based on induction of microsomal liver enzymes in short-term toxicity experiments. *Toxicology*, **2**, 371–380

Derbes, V.J., Dent, J.H., Forrest, W.W. & Johnson, M.F. (1955) Fatal chlordane poisoning. *J. Am. med. Assoc.*, **158**, 1367–1369

D'Ercole, A.J., Arthur, R.D., Cain, J.D. & Barrentine, B.F. (1976) Insecticide exposure of mothers and newborns in a rural agricultural area. *Pediatrics*, **57**, 869–874

Deutsche Forschungsgemeinschaft (2000) *List of MAK and BAT Values 2000* (Report No. 36), Weinheim, Wiley-VCH Verlag GmbH, pp. 36, 63

Dewailly, E., Dodin, S., Verreault, R., Ayotte, P., Sauve, L., Morin, J. & Brisson, J. (1994) High organochlorine body burden in women with estrogen receptor-positive breast cancer. *J. natl Cancer Inst.*, **86**, 232–234

Ditraglia, D., Brown, D.P., Namekata, T. & Iverson, N. (1981) Mortality study of workers employed at organochlorine pesticide manufacturing plants. *Scand. J. Work Environ. Health*, **7** (Suppl. 4), 140–146

Dorgan, J.F., Brock, J.W., Rothman, N., Needham, L.L., Miller, R., Stephenson, H.E, Jr, Schussler, N. & Taylor, P.R. (1999) Serum organochlorine pesticides and PCBs and breast cancer risk: Results from a prospective analysis (USA). *Cancer Causes Control*, **10**, 1–11

Duggan, R.E. & Corneliussen, P.E. (1972) Dietary intake of pesticide chemicals in the United States (III), June 1968–April 1970. *Pestic. Monit. J.*, **5**, 331–341

Eller, P.M., ed. (1994) Method 5510. In: *NIOSH Manual of Analytical Methods (DHHS (NIOSH) Publ. No. 94-113)*, 4th Ed., Cincinnati, OH, National Institute for Occupational Safety and Health

Environmental Protection Agency (1976) Velsicol Chemical Co., consolidated heptachlor/chlordane hearing. *Fed. Regist.*, **41**, 7552–7572

Environmental Protection Agency (1987a) *Heptachlor and Heptachlor Epoxide*, Washington DC, Office of Drinking Water

Environmental Protection Agency (1987b) *Chlordane, Heptachlor, Aldrin and Dieldrin* (Technical Report Document), Washington DC, Office of Pesticides and Toxic Substances

Environmental Protection Agency (1993a) Method 617. The determination of organohalide pesticides and PCBs in municipal and industrial wastewater. In: *Methods for the Determination of Nonconventional Pesticides in Municipal and Industrial Wastewater* (EPA Report No. EPA-821/R-93-010-A), Washington DC

Environmental Protection Agency (1993b) Method 1656. The determination of organo-halide pesticides in municipal and industrial wastewater. In: *Methods for the Determination of Nonconventional Pesticides in Municipal and Industrial Wastewater* (EPA Report No. EPA-821/R-93-010-A), Washington DC

Environmental Protection Agency (1995a) Method 505. Analysis of organohalide pesticides and commercial polychlorinated biphenyl (PCB) products in water by microextraction and gas chromatography [Rev. 2.1]. In: *Methods for the Determination of Organic Compounds in Drinking Water*, Supplement III (EPA Report No. EPA-600/R-95-131; US NTIS PB-216616), Cincinnati, OH, Environmental Monitoring Systems Laboratory

Environmental Protection Agency (1995b) Method 508.1. Determination of chlorinated pesticides, herbicides, and organohalides by liquid–solid extraction and electron capture gas chromatography [Rev. 2.0].]. In: *Methods for the Determination of Organic Compounds in Drinking Water*, Supplement III (EPA Report No. EPA-600/R-95-131; US NTIS PB-216616), Cincinnati, OH, Environmental Monitoring Systems Laboratory

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

Environmental Protection Agency (1995d) Method 551.1. Determination of chlorination by-products, chlorinated solvents, and halogenated pesticides/herbicides in drinking water by liquid–liquid extraction and gas chromatography with electron-capture detection [Rev. 1.0]. In: *Methods for the Determination of Organic Compounds in Drinking Water*, Supplement III (EPA Report No. EPA-600/R-95/131; US NTIS PB-216616), Cincinnati, OH, Environmental Monitoring Systems Laboratory

Environmental Protection Agency (1996a) Method 8081A. Organochlorine pesticides by gas chromatography [Rev. 1]. In: *Test Methods for Evaluating Solid Waste — Physical/Chemical Methods* (EPA Report No. SW-846), Washington DC, Office of Solid Waste

Environmental Protection Agency (1996b) Method 8270C. Semivolatile organic compounds by gas chromatography/mass spectrometry (GC/MS) [Rev. 3]. In: *Test Methods for Evaluating Solid Waste — Physical/Chemical Methods* (EPA Report No. SW-846), Washington DC, Office of Solid Waste

Environmental Protection Agency (1996c) Method 4041. Soil screening for chlordane by immunoassay [Rev. 0]. In: *Test Methods for Evaluating Solid Waste — Physical/Chemical Methods* (EPA Report No. SW-846), Washington DC, Office of Solid Waste

Environmental Protection Agency (1999a) Method TO-04A. Determination of pesticides and polychlorinated biphenyls in ambient air using high volume polyurethane foam (PUF) sampling followed by gas chromatographic/multi-detector detection (GC/MD). In: *Compendium of Methods for the Determination of Toxic Compounds in Ambient Air,* 2nd Ed. (EPA Report No. EPA-625/R-96-010b), Cincinnati, OH, Center for Environmental Research Information

Environmental Protection Agency (1999b) Method TO-10A. Determination of pesticides and polychlorinated biphenyls in ambient air using low volume polyurethane foam (PUF) sampling followed by gas chromatographic/multi-detector detection (GC/MD). In: *Compendium of Methods for the Determination of Toxic Compounds in Ambient Air,* 2nd Ed. (EPA Report No. EPA-625/R-96-010b), Cincinnati, OH, Center for Environmental Research Information

Environmental Protection Agency (1999c) Methods for organic chemical analysis of municipal and industrial wastewater. Method 608 — Organochlorine pesticides and PCBs. *US Code Fed. Regul., Title 40,* Part 136, App. A, pp. 114–134

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

Environmental Protection Agency (2000) *Drinking Water Standards and Health Advisories* (EPA Report No. EPA-882/B-00-001), Washington DC, Office of Water

Epstein, S.S. (1976) Carcinogenicity of heptachlor and chlordane. *Sci. total Environ.*, **6**, 103–154

Epstein, S.S. & Ozonoff, D. (1987) Leukemias and blood dyscrasias following exposure to chlordane and heptachlor. *Teratog. Carcinog. Mutag.*, **7**, 527–540

Epstein, S.S., Arnold, E., Andrea, J., Bass, W. & Bishop, Y. (1972) Detection of chemical mutagens by the dominant lethal assay in the mouse. *Toxicol. appl. Pharmacol.*, **23**, 288–325

Esworthy, R.F. (1985) *Preliminary Quantitative Usage Analysis of Chlordane*, Washington DC, US Environmental Protection Agency, Office of Pesticide Programs

Ewing, A.D., Kadry, A.M. & Dorough, H.W. (1985) Comparative disposition and elimination of chlordane in rats and mice. *Toxicol. Lett.*, **26**, 233–239

Falck, F., Jr, Ricci, A., Jr, Wolff, M.S., Godbold, J. & Deckers, P. (1992) Pesticides and polychlorinated biphenyl residues in human breast lipids and their relation to breast cancer. *Arch. environ. Health*, **47**, 143–146

FAO/UNEP (1996) *Prior Informed Consent Decision Guidance Documents: Chlordane/Chlordimeform/EDB/Heptachlor/Mercury Compounds*, Geneva, Rotterdam Convention on the Prior Informed Consent Procedure for Certain Hazardous Chemicals and Pesticides in International Trade (http://www.fao.org/ag/agp/agpp/pesticid/pic/dgdhome.htm)

FAO/WHO (1964) *Evaluation of the Toxicity of Pesticide Residues in Food. Report of a Joint Meeting of the FAO Committee on Pesticides in Agriculture and the WHO Expert Committee on Pesticide Residues* (FAO Meeting Report No. PL/1963/13; WHO/Food Add /23), Rome

FAO/WHO (1965) *Evaluation of the Toxicity of Pesticide Residues in Food. Report of a Joint Meeting of the FAO Committee on Pesticides in Agriculture and the WHO Expert Committee on Pesticide Residues* (FAO Meeting Report No. PL: CP/15; WHO/Food Add./67.32), Rome

FAO/WHO (1967) *Evaluation of the Toxicity of Pesticide Residues in Food. Report of a Joint Meeting of the FAO Committee on Pesticides in Agriculture and the WHO Expert Committee on Pesticide Residues* (FAO Meeting Report No. PL:CP/15; WHO/Food Add./67.32), Rome

FAO/WHO (1968) *Evaluation of the Toxicity of Pesticide Residues in Food. Report of a Joint Meeting of the FAO Committee on Pesticides in Agriculture and the WHO Expert Committee on Pesticide Residues* (FAO Meeting Report No. PL: 1967/M/11/1; WHO/Food Add./68.30), Rome

FAO/WHO (1971) *Evaluation of the Toxicity of Pesticide Residues in Food. Report of a Joint Meeting of the FAO Committee on Pesticides in Agriculture and The WHO Expert Committee on Pesticide Residues* (AGP:1970/M/12/1; WHO/Food Add. 71.42), Rome

FAO/WHO (1978) *Pesticide Residues in Food: 1977 Evaluations* (FAO Plant Production and Protection Paper 10 Supp.), Rome

FAO/WHO (1983) *Pesticide Residues in Food — 1982 Evaluations. Data and Recommendations of the Joint Meeting of the FAO Panel of Experts on Pesticide Residues in Food and the Environment and the WHO Expert Group on Pesticide Residues* (FAO Plant Production and Protection Paper 49), Rome

FAO/WHO (1987) *Pesticide Residues in Food — 1986. Report of the Joint Meeting of the FAO Panel of Experts on Pesticide Residues in Food and the Environment and a WHO Expert Group on Pesticide Residues* (FAO Plant Production and Protection Paper 77), Rome

Fendick, E.A., Mather-Mihaich, E., Houck, K.A., St Clair, M.B., Faust, J.B., Rockwell, C.H. & Owens, M. (1990) Ecological toxicology and human health effects of heptachlor. *Rev. environ. Contam. Toxicol*., **111**, 61–142

Fishbein, W.I., White, J.V. & Isaacs, H.J. (1964) Survey of workers exposed to chlordane. *Ind. Med. Surg.*, **33**, 726–727

Food and Drug Administration (1989) *Pesticide Analytical Manual*, Vol. II, *Methods Which Detect Multiple Residues*, Washington DC, Department of Health and Human Services

Food and Drug Administration (1990) Action levels for residues of certain pesticides in food and feed. *Fed. Reg.*, **55**, 14359–14363

Food and Drug Administration (1999) Methods 302, 303 and 304. In: Makovi, C.M. & McMahon, B.M., eds., *Pesticide Analytical Manual*, Vol. I*: Multiresidue Methods*, 3rd Rev. Ed., Washington DC, Management Methods Branch

Gaines, T.B. (1960) The acute toxicity of pesticides to rats. *Toxicol. appl. Pharmacol*., **2**, 88–89

Gant, D.B., Eldefrawi, M.E. & Eldefrawi, A.T. (1987) Cyclodiene insecticides inhibit GABA receptor-regulated chloride transport. *Toxicol. appl. Pharmacol*., **88**, 313–321

Garrettson, L.K., Guzelian, P.S. & Blanke, R.V. (1985) Subacute chlordane poisoning. *Clin. Toxicol*., **22**, 565–571

Gauthier, J.M., Dubeau, H. & Rassart, E. (1999) Induction of micronuclei in vitro by organochlorine compounds in beluga whale skin fibroblasts. *Mutat. Res*., **439**, 87–95

Gentile, J.M., Gentile, G.J., Bultman, J., Sechriest, R., Wagner, E.D. & Plewa, M.J. (1982) An evaluation of the genotoxic properties of insecticides following plant and animal activation. *Mutat. Res*., **101**, 19–29

Griffin, D.E., III & Hill, W.E. (1978) In vitro breakage of plasmid DNA by mutagens and pesticides. *Mutat. Res*., **52**, 161–169

Grutsch, J.F. & Khasawinah, A. (1991) Signs and mechanisms of chlordane intoxication. *Biomed. environ. Sci.*, **4**, 317–326

Gunderson, E.L. (1988) Chemical contaminants monitoring. FDA total diet study, April 1982–April 1984, dietary intakes of pesticides, selected elements, and other chemicals. *J. Assoc. off. anal. Chem.*, **71**, 1200–1209

Gunz, D., Shephard, S.E. & Lutz, W.K. (1993) Can nongenotoxic carcinogens be detected with the lacI transgenic mouse mutation assay? *Environ. mol. Mutag.*, **21**, 209–211

Hardell, L., Liljegren, G., Lindström, G., van Bavel, B., Broman, K., Fredrikson, M., Hagberg, M., Nordström, M. & Johansson, B. (1996) Increased concentrations of chlordane in adipose tissue from non-Hodgkin's lymphoma patients compared with controls without a malignant disease. *Int. J. Oncol.*, **9**, 1139–1142

Hardell, L., Liljegren, G., Lindström, G., van Bavel, B., Fredrikson, M. & Hagberg, H. (1997) Polychlorinated biphenyls, chlordanes, and the etiology of non-Hodgkin's lymphoma (Letter to the Editor). *Epidemiology*, **6**, 689

Harrington, J.M., Baker, E.L., Jr, Folland, D.S., Saucier, J.W. & Sandifer, S.H. (1978) Chlordane contamination of a municipal water system. *Environ. Res.*, **15**, 155–159

Hart, L.G., Shultice, R.W. & Fouts, J.R. (1963) Stimulatory effects of chlordane on hepatic microsomal drug metabolism in the rat. *Toxicol. appl. Pharmacol.*, **5**, 371–386

Hassoun, E., Bagchi, M., Bagchi, D. & Stohs, S.J. (1993) Comparative studies on lipid peroxidation and DNA-single strand breaks induced by lindane, DDT, chlordane and endrin in rats. *Comp. Biochem. Physiol.*, **104C**, 427–431

Health and Welfare Canada (1990) *National Pesticide Residue Limits in Foods*, Ottawa, Bureau of Chemical Safety, Food Directorate, Health Protection Branch

Hirasawa, F. & Takizawa, Y. (1989) Accumulation and declination of chlordane congeners in mice. *Toxicol. Lett.*, **47**, 109–117

Hoar Zahm, S., Weisenburger, D.D., Babbitt, P.A., Saal, R.C., Cantor, K.P. & Blair, A. (1988) A case–control study of non Hodgkin's lymphoma and agricultural factors in eastern Nebraska (Abstract). *Am. J. Epidemiol.*, **128**, 901

Hoar Zahm, S., Weisenburger, D.D., Babbitt, P.A., Saal, R.C., Vaught, J.B., Cantor, K.P. & Blair, A. (1990) A case–control study of non-Hodgkin's lymphoma and the herbicide 2,4-dichlorophenoxyacetic acid (2,4-D) in eastern Nebraska. *Epidemiology*, **1**, 349–356

Hogue, C. (2000) Dioxins to be major issue at treaty negotiations. *Chem. Eng. News*, **20 March**, 35

Hoppin, J.A., Tolbert, P.E., Holly, E.A., Brook, J.W., Korrick, S.A., Altshul, L.M., Zhang, R.H., Bracci, P.M., Burse, V.W. & Needham, L.L. (2000) Pancreatic cancer and serum organochlorine levels. *Cancer Epidemiol. Biomarkers Prev.*, **9**, 199–205

Houk, V.S. & DeMarini, D.M. (1987) Induction of prophage lambda by chlorinated pesticides. *Mutat. Res.*, **182**, 193–201

Høyer, A.P., Grandjean, P., Jorgensen, T., Brock, J.W. & Hartvig, H.B. (1998) Organochlorine exposure and risk of breast cancer. *Lancet*, **352**, 1816–1820

IARC (1979) *IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans*, Vol. 20, *Some Halogenated Hydrocarbons*, Lyon, IARC*Press*, pp. 45–65, 129–154

IARC (1987) *IARC Monographs on the Evaluation of Carcinogenic Risks to Humans*, Suppl. 7, *Overall Evaluations of Carcinogenicity: An Updating of* IARC Monographs *Volumes 1 to 42*, Lyon, IARC*Press*, pp. 146–148

IARC (1991) *IARC Monographs on the Evaluation of Carcinogenic Risks to Humans*, Vol. 53, *Occupational Exposures in Insecticide Application, and Some Pesticides*, Lyon, IARC*Press*, pp. 115–175

Infante, P.F. & Freeman, C. (1987) Cancer mortality among workers exposed to chlordane (Letter to the Editor). *J. occup. Med.*, **29**, 908–909

Infante, P.F., Epstein, S.S. & Newton, W.A., Jr (1978) Blood dyscrasias and childhood tumors and exposure to chlordane and heptachlor. *Scand. J. Work Environ. Health*, **4**, 137–150

Izmerov, N.F., ed. (1982) *International Register of Potentially Toxic Chemicals. Scientific Reviews of Soviet Literature on Toxicity and Hazards of Chemicals: Heptachlor* (Issue 3), Moscow, Centre of International Projects, United Nations Environment Programme

Izushi, F. & Ogata, M. (1990) Hepatic and muscle injuries in mice treated with heptachlor. *Toxicol. Lett.*, **54**, 47–54

Johnson, K.W., Holsapple, M.P. & Munson, A.E. (1986) An immunotoxicological evaluation of gamma-chlordane. *Fundam. appl. Toxicol.*, **6**, 317–326

Kamble, S.T., Ogg, C.L., Gold, R.E. & Vance, A.D. (1992) Exposure of applicators and residents to chlordane and heptachlor when used for subterranean termite control. *Arch. environ. Contam. Toxicol.*, **22**, 253–259

Kan, C.A. & Tuinstra, L.G.M.T. (1976) Accumulation and excretion of certain organochlorine insecticides in broiler breeder hens. *J. agric. Food Chem.*, **24**, 775–778

Kauppinen, T. (1986) Occupational exposure to chemical agents in the plywood industry. *Ann. occup. Hyg.*, **30**, 19–29

Khasawinah, A.M. & Grutsch, J.F. (1989a) Chlordane: Thirty-month tumorigenicity and chronic toxicity test in rats. *Regul. Toxicol. Pharmacol.*, **10**, 95–109

Khasawinah, A.M. & Grutsch, J.F. (1989b) Chlordane: 24-month tumorigenicity and chronic toxicity test in mice. *Regul. Toxicol. Pharmacol.*, **10**, 244–254

Khasawinah, A.M., Hardy, C.J. & Clark, G.C. (1989) Comparative inhalation toxicity of technical chlordane in rats and monkeys. *J. Toxicol. environ. Health*, **28**, 327–347

Klein, W., Korte, F., Weisgerber, I., Kaul, R., Mueller, W. & Djirsarai, A. (1968) [The metabolism of endrin, heptachlor and telodrin.] *Qual. Plant Mater. Veg.*, **15**, 225–238 (in German)

Kurata, M., Hirose, K. & Umeda, M. (1982) Inhibition of metabolic cooperation in Chinese hamster cells by organochlorine pesticides. *Jpn. J. Cancer Res. (Gann)*, **73**, 217–221

Kutz, F.W., Strassman, S.C., Sperling, J.F., Cook, B.T., Sunshine, I. & Tessari, J. (1983) A fatal chlordane poisoning. *J. Toxicol. clin. Toxicol.*, **20**, 167–174

Lee, S.-R. (1982) [Overall assessment of organochlorine insecticide residues in Korean foods.] *Korean J. Food Sci. Technol.*, **14**, 82–93 (in Korean)

Lehman, A.J. (1952) Chemicals in foods: A report to the Association of Food and Drug Officials on current developments. Part II. Pesticides. Section III: Subacute and chronic toxicity. *Q. Bull. Assoc. Food Drug 0ff. US*, **16**, 47–53

Le Marchand, L., Kolonel, L.N., Siegel, B.Z. & Dendle, W.H., III (1986) Trends in birth defects for a Hawaiian population exposed to heptachlor and for the United States. *Arch. environ. Health*, **41**, 145–148

MacMahon, B., Monson, R.R., Wang, H.H. & Zheng, T. (1988) A second follow-up of mortality in a cohort of pesticide applicators. *J. occup. Med.*, **30**, 429–432

Malarkey, D.E., Devereux, T.R., Dinse, G.E., Mann, P.C. & Maronpot, R.R. (1995) Hepatocarcinogenicity of chlordane in B6C3F1 and B6D2F1 male mice: Evidence for regression in B6C3F1 mice and carcinogenesis independent of *ras* proto-oncogene activation. *Carcinogenesis*, **16**, 2617–2625

Marshall, T.C., Dorough, H.W. & Swim, H.E. (1976) Screening of pesticides for mutagenic potential using *Salmonella typhimurium* mutants. *J. agric. Food Chem.*, **24**, 560–563

Maslansky, C.J. & Williams, G.M. (1981) Evidence for an epigenetic mode of action in organochlorine pesticide hepatocarcinogenicity: A lack of genotoxicity in rat, mouse and hamster hepatocytes. *J. Toxicol. environ. Health*, **8**, 121–130

Matesic, D.F., Rupp, H.L., Bonney, W.J., Ruch, R.J. & Trosko, J.E. (1994) Changes in gap-junction permeability, phosphorylation, and number mediated by phorbol ester and non-phorbol-ester tumor promoters in rat liver epithelial cells. *Mol. Carcinog.*, **10**, 226–236

Matsui, S., Yamamoto, R. & Yamada, H. (1989) The *Bacillus subtilis*/microsome rec-assay for the detection of DNA damaging substances which may occur in chlorinated and ozonated waters. *Water Sci. Technol.*, **21**, 875–887

Matsumura, F. & Nelson, J.O. (1971) Identification of the major metabolic product of heptachlor epoxide in rat feces. *Bull. environ. Contam. Toxicol.*, **5**, 489–492

McGregor, D.B., Brown, A., Cattanach, R., Edwards, I., McBride, D., Riach, C. & Caspary, W.J. (1988) Responses of the L5178Y tk^+/tk^- mouse lymphoma cell forward mutation assay: III. 72 coded chemicals. *Environ. mol. Mutag.*, **12**, 85–154

Meguro, T., Izushi, F. & Ogata, M. (1990) Effect of heptachlor on hepatic mitochondrial oxidative phosphorylation in rat. *Ind. Health*, **28**, 151–157

Menconi, S., Clark, J.M., Langenberg, P. & Hryhorczuk, D. (1988) A preliminary study of potential human health effects in private residences following chlordane applications for termite control. *Arch. environ. Health*, **43**, 349–352

Menna, J.H., Barnett, J.B. & Soderberg, L.S.F. (1985) Influenza type A virus infection of mice exposed in utero to chlordane: Survival and antibody studies. *Toxicol. Lett.*, **24**, 45–52

Mersch-Sundermann, V., Dickgiesser, N., Hablizel, U. & Gruber, B. (1988) [Examination of the mutagenicity of organic microcontaminants on the environment. I. The mutagenicity of selected herbicides and insecticides in the *Salmonella* microsome test (Ames test) in relation to the pathogenetic potency of contaminated ground- and drinking-water.] *Zbl. Bakt. Mikrobiol. Hyg B.*, **186**, 247–260 (in German)

Miranda, C.L., Webb, R.E. & Ritchey, S.J. (1973) Effect of dietary protein quality, phenobarbital and SKF 525-A on heptachlor metabolism in the rat. *Pestic. Biochem. Physiol.*, **3**, 456–461

Miyagi, T., Lam, K.M., Chuang, L.F. & Chuang, R.Y. (1998) Suppression of chemokine-induced chemotaxis of monkey neutrophils and monocytes by chlorinated hydrocarbon insecticides. *In Vivo*, **12**, 441–446

Miyazaki, T., Yamagishi, T. & Matsumoto, M. (1985) Isolation and structure elucidation of some components in technical grade chlordane. *Arch. environ. Contam. Toxicol.*, **14**, 475–483

Moriya, M., Ohta, T., Watanabe, K., Miyazawa, T., Kato, K. & Shirasu, Y. (1983) Further mutagenicity studies on pesticides in bacterial reversion assay systems. *Mutat. Res.*, **116**, 185–216

Mortelmans, K., Haworth, S., Lawlor, T., Speck, W., Tainer, B. & Zeiger, E. (1986) Salmonella mutagenicity tests: II. Results from the testing of 270 chemicals. *Environ. Mutag.*, **8** (Suppl. 7), 1–119

Moser, G.J. & Smart, R.C. (1989) Hepatic tumor-promoting chlorinated hydrocarbons stimulate protein kinase C activity. *Carcinogenesis*, **10**, 851–856

Moser, G.J., Robinette, C.L. & Smart, R.C. (1993) Characterization of skin tumor promotion by mirex: Structure–activity relationships, sexual dimorphism and presence of Ha-*ras* mutation. *Carcinogenesis*, **14**, 1155–1160

Moser, V.C., Cheek, B.M. & MacPhail, R.C. (1995) A multidisciplinary approach to toxicological screening: III. Neurobehavioral toxicity. *J. Toxicol. environ. Health*, **45**, 173–210

Moser, G.J., Wolf, D.C. & Goldsworthy, T.L. (1997) Quantitative relationship between transforming growth factor-alpha and hepatic focal phenotype and progression in female mouse liver. *Toxicol. Pathol*., **25**, 275–283

Mussalo-Rauhamaa, H., Pyysalo, H. & Antervo, K. (1991) Heptachlor, heptachlor epoxide, and other chlordane compounds in Finnish plywood workers. *Arch. environ. Health*, **46**, 340–346

Narotsky, M.G. & Kavlock, R.J. (1995) A multidisciplinary approach to toxicological screening: II. Developmental toxicity. *J. Toxicol. environ. Health*, **45**, 145–171

Narotsky, M.G., Weller, E.A., Chinchilli, V.M. & Kavlock, R.J. (1995) Nonadditive developmental toxicity in mixtures in trichloroethylene, di(2-ethylhexyl) phthalate, and heptachlor in a $5 \times 5 \times 5$ design. *Fundam. appl. Toxicol*., **27**, 203–216

National Academy of Sciences (1977) *An Evaluation of the Carcinogenicity of Chlordane and Heptachlor*, Washington DC

National Cancer Institute (1977a) *Bioassay of Heptachlor for Possible Carcinogenicity* (Technical Report Series No. 9; DHEW Publ. No. (NIH) 77-809), Washington DC, Department of Health, Education, and Welfare

National Cancer Institute (1977b) *Bioassay of Chlordane for Possible Carcinogenicity* (Technical Report Series No. 8; DHEW Publ. No. (NIH) 77-808), Washington DC, Department of Health, Education, and Welfare

National Institute for Occupational Safety and Health (2000) *National Occupational Exposure Survey 1981–83*, Cincinnati, OH, Department of Health and Human Services, Public Health Service

Nelson, B.D. (1975) The action of cyclodiene pesticides on oxidative phosphorylation in rat liver mitochondria. *Biochem. Pharmacol.*, **24**, 1–19

Newsome, W.H. & Ryan, J.J. (1999) Toxaphene and other chlorinated compounds in human milk from northern and southern Canada: A comparison. *Chemosphere*, **39**, 519–526

Nomata, K., Kang, K.-S., Hayashi, T., Matesic, D., Lockwood, L., Chang, C.C. & Trosko, J.E. (1996) Inhibition of gap junctional intercellular communication in heptachlor- and heptachlor epoxide-treated normal human breast epithelial cells. *Cell Biol. Toxicol*., **12**, 69–78

Nomeir, A.A. & Hajjar, N.P. (1987) Metabolism of chlordane in mammals. *Rev. environ. Contam. Toxicol.*, **100**, 1–22

Nordström, M., Hardell, L., Lindstrom, G., Wingfors, H., Hardell, K. & Linde, A. (2000) Concentrations of organochlorines related to titers to Epstein-Barr virus early antigen IgG as risk factors for hairy cell leukemia. *Environ. Health Perspectives*, **108**, 441–445

Nye, D.E. & Dorough, H.W. (1976) Fate of insecticides administered endotracheally to rats. *Bull. environ. Contam. Toxicol.*, **15**, 291–296

Oduma, J.A., Wango, E.O., Oduor-Okelo, D., Makawiti, D.W. & Odonog, H. (1995) In vivo and in vitro effects of degraded doses of the pesticide heptachlor on female sex steroid hormone production in rats. *Comp. Biochem. Physiol.*, **111C**, 191–196

Ogata, M. & Izushi, F. (1991) Effects of chlordane on parameters of liver and muscle toxicity in man and experimental animals. *Toxicol. Lett.*, **56**, 327–337

Okey, A.B. (1990) Enzyme induction in the cytochrome P-450 system. *Pharmacol. Ther.*, **45**, 241–298

Olanoff, L.S., Bristow, W.J., Colcolough, J., Jr & Reigart, J.R. (1983) Acute chlordane intoxication. *J. Toxicol clin. Toxicol.*, **20**, 291–306

Peirano, W.B. (1980) *Heptachlor — Maximum Acceptable Limit in Drinking Water*, Washington DC, Environmental Protection Agency

Pelikan, Z., Halacka, K., Polster, M. & Cerny, E. (1968) [Long-term intoxication of rats by small doses of heptachlor.] *Arch. belg. Méd. traum. Méd. Ig.*, **26**, 529–538 (in French)

Pesatori, A.C., Sontag, J.M., Lubin, J.H., Consonni, D. & Blair, A. (1994) Cohort mortality and nested case–control study of lung cancer among structural pest control workers in Florida (United States). *Cancer Causes Control*, **5**, 310–318

Princi, F. & Spurbeck, G.H. (1951) A study of workers exposed to the insecticides chlordane, aldrin, dieldrin. *Arch. ind. Hyg. occup. Med.*, **3**, 64–72

Probst, G.S., McMahon, R.E., Hill, L.E., Thompson, C.Z., Epp, J.K. & Neal, S.B. (1981) Chemically-induced unscheduled DNA synthesis in primary rat hepatocyte cultures: A comparison with bacterial mutagenicity using 218 compounds. *Environ. Mutag.*, **3**, 11–32

Public Health Service (1989a) *Agency for Toxic Substances and Disease Registry Toxicological Profile for Heptachlor/Heptachlor Epoxide* (Report No. ATSDR/TP-88/16; NTIS PB 89-194492), Washington DC

Public Health Service (1989b) *Agency for Toxic Substances and Disease Registry Toxicological Profile for Chlordane* (Report No. ATSDR/TP-89/06; NTIS PB90-168709), Washington DC

Radomski, J.L. & Davidow, B. (1953) The metabolite of heptachlor, its estimation, storage and toxicity. *J. Pharmacol. exp. Ther.*, **107**, 266–272

Rashid, K.A. & Mumma, R.O. (1986) Screening pesticides for their ability to damage bacterial DNA. *J. environ. Sci. Health*, **B21**, 319–334

Raw, G.R., ed. (1970) *CIPAC Handbook*, Vol. 1, Cambridge, Collaborative International Pesticides Analytical Council, pp. 420–427

Ray, S., Bailey, M., Paterson, G., Metcalfe, T. & Metcalfe, C. (1998) Comparative levels of organochlorine compounds in flounders from the northeast coast of Newfoundland and an offshore site. *Chemosphere*, **36**, 2201–2210

Rogan, W.J., Blanton, P.J., Portier, C.J. & Stallard, E. (1991) Should the presence of carcinogens in breast milk discourage breast feeding? *Regul. Toxicol. Pharmacol.*, **13**, 228–240

Rostad, C.E. (1997) Concentration and transport of chlordane and nonachlor associated with suspended sediment in the Mississippi River, May 1988 to June 1990. *Arch. environ. Contam. Toxicol.*, **33**, 369–377

Rought, S.E., Yau, P.M., Schnier, J.B., Chuang, L.F. & Chuang, R.Y. (1998) The effect of heptachlor, a chlorinated hydrocarbon insecticide, on p53 tumor suppressor in human lymphocytes. *Toxicol. Lett.*, **94**, 29–36

Rought, S.E., Yau, P.M., Chuang, L.F., Doi, R.H. & Chuang, R.Y. (1999) Effect of the chlorinated hydrocarbons heptachlor, chlordane, and toxaphene on retinoblastoma tumor suppressor in human lymphocytes. *Toxicol. Lett*., **104**, 127–135

Royal Society of Chemistry (1986) *European Directory of Agrochemical Products*, Vol. 3, *Insecticides, Acaricides, Nematicides*, Cambridge, pp. 112, 331–332

Royal Society of Chemistry (1989) *The Agrochemicals Handbook* [Dialog Information Services (File 306)], Cambridge

Ruch, R.J., Fransson, R., Flodstrom, S., Warngard, L. & Klaunig, J.E. (1990) Inhibition of hepatocyte gap junctional intercellular communication by endosulfan, chlordane and heptachlor. *Carcinogenesis*, **11**, 1097–1101

Sadtler Research Laboratories (1980) *The Standard Spectra, 1980, Cumulative Molecular Formula Index*, Philadelphia, PA, p. 354

Sadtler Research Laboratories (1990) *The Sadtler Standard Spectra, 1981–1990, Supplementary Index*, Philadelphia, PA

Saito, I., Kawamura, N., Uno, K., Hisanaga, N., Takeuchi, Y., Ono, Y., Iwata, M., Gotoh, M., Okutani, H., Matsumoto, T., Fukaya, Y., Yoshitomi, S. & Ohno, Y. (1986) Relationship between chlordane and its metabolites in blood of pest control operators and spraying conditions. *Int. Arch. occup. environ. Health*, **58**, 91–97

Sarkar, D., Sharma, A. & Talukder, G. (1993) Differential protection of chlorophyllin against clastogenic effects of chromium and chlordane in mouse bone marrow in vivo. *Mutat. Res*., **301**, 33–38

Savela, A., Vuorela, R. & Kauppinen, T. (1999) *ASA 1997* (Katsauksia 140), Helsinki, Finnish Institute of Occupational Health, p. 30 (in Finnish)

Schmitt, C.J., Zajicek, J.L., May, T.W. & Cowman, D.F. (1999) Organochlorine residues and elemental contaminants in US freshwater fish, 1976–1986: National contaminant biomonitoring program. *Rev. environ. Contam. Toxicol.*, **162**, 43–104

Schop, R.N., Hardy, M.H. & Goldberg, M.T. (1990) Comparison of the activity of topically applied pesticides and the herbicide 2,4-D in two short-term in vivo assays of genotoxicity in the mouse. *Fundam. appl. Toxicol*., **15**, 666–675

Schuetz, E.G., Brimer, C. & Schuetz, J.D. (1998) Environmental xenobiotics and the antihormones cyproterone acetate and spironolactone use the nuclear hormone pregnenolone X receptor to activate the *CYP*3*A*23 hormone response element. *Mol. Pharmacol.*, **54**, 1113–1117

Shindell, S. (1987) Cancer mortality among workers exposed to chlordane (Reply to a letter to the Editor). *J. occup. Med.*, **29**, 909–911

Shindell, S. & Ulrich, S. (1986) Mortality of workers employed in the manufacture of chlordane: An update. *J. occup. Med*., **28**, 497–501

Simmon, V.F., Kauhanen, K. & Tardiff, R.G. (1977) Mutagenic activity of chemicals identified in drinking water. *Dev. Toxicol. environ. Sci.*, **2**, 249–258

Sittig, M., ed. (1980) *Pesticide Manufacturing and Toxic Materials Control Encyclopedia*, Park Ridge, NJ, Noyes Data Corp., pp. 445–448

Sobti, R.C., Krishan, A. & Davies, J. (1983) Cytokinetic and cytogenetic effect of agricultural chemicals on human lymphoid cells in vitro. II. Organochlorine pesticides. *Arch. Toxicol.*, **52**, 221–231

Sovocool, G.W. & Lewis, RG. (1975) The identification of trace levels of organic pollutants in human tissues: Compounds related to chlordane/heptachlor exposure. *Trace Subst. environ. Health*, **9**, 265–280

Sovocool, G.W., Lewis, R.G., Harless, R.L., Wilson, N.K. & Zehr, R.D. (1977) Analysis of technical chlordane by gas chromatography/mass spectrometry. *Anal. Chem.*, **49**, 734–740

Sperling, F. & Ewinike, H. (1969) Changes in LD_{50} of parathion and heptachlor after turpentine pretreatment (Abstract No. 24). *Toxicol. appl. Pharmacol.*, **14**, 622

Spyker-Cranmer, J.M., Barnett, J.B., Avery, D.L. & Cranmer, M.F. (1982) Immunoteratology of chlordane: Cell-mediated and humoral immune responses in adult mice exposed in utero. *Toxicol. appl. Pharmacol*., **62**, 402–408

Stehr-Green, P.A., Schilling, R.J., Burse, V.W., Steinberg, K.K., Royce, W., Wohlleb, J.C. & Donnell, H.D. (1986) Evaluation of persons exposed to dairy products contaminated with heptachlor (Letter to the Editor). *J. Am. med. Assoc*., **256**, 3350–3351

Taguchi, S. & Yakushiji, T. (1988) Influence of termite treatment in the home on the chlordane concentration in human milk. *Arch. environ. Contam. Toxicol.*, **17**, 65–71

Takamiya, K. (1987) Residual levels of plasma oxychlordane and *trans*-nonachlor in pest control operators and some characteristics of these accumulations. *Bull. environ. Contam. Toxicol.*, **39**, 750–755

Tashiro, S. & Matsumura, F. (1977) Metabolic routes of *cis*- and *trans*-chlordane in rats. *J. agric. Food Chem.*, **25**, 872–880

Tashiro, S. & Matsumura, F. (1978) Metabolism of trans-nonachlor and related chlordane components in rat and man. *Arch. environ. Contam. Toxicol.*, **7**, 113–127

Telang, S., Tong, C. & Williams, G.M. (1982) Epigenetic membrane effects of a possible tumor promoting type on cultured liver cells by the non-genotoxic organochlorine pesticides chlordane and heptachlor. *Carcinogenesis*, **3**, 1175–1178

Teufel, M., Niessen, K.H., Sartoris, J., Brands, W., Lochbühler, H., Waag, K., Schweizer, P. & von Oelsnitz, G. (1990) Chlorinated hydrocarbons in fat tissue: Analyses of residues in healthy children, tumor patients, and malformed children. *Arch. environ. Contam. Toxicol.*, **19**, 646–652

Theus, S.A., Lau, K.A., Tabor, D.R., Soderberg, L.S. & Barnett, J.B. (1992) In vivo prenatal chlordane exposure induces development of endogenous inflammatory macrophages. *J. Leukocyte Biol*., **51**, 366–372

Tomlin, C.D.S., ed. (1999) *The e-Pesticide Manual — A World Compendium*, 11th Ed., Version 1.1, Farnham, British Crop Protection Council

Tong, C., Fazio, M. & Williams, G.M. (1981) Rat hepatocyte-mediated mutagenesis of human cells by carcinogenic polycyclic aromatic hydrocarbons but not organochlorine pesticides. *Proc. Soc. exp. Biol. Med*., **167**, 572–575

Tsushimoto, G., Chang, C.C., Trosko, J.E. & Matsumura, F. (1983) Cytotoxic, mutagenic, and cell–cell communication inhibitory properties of DDT, lindane and chlordane on Chinese hamster cells *in vitro*. *Arch. environ. Contam. Toxicol*., **12**, 721–730

Urieta, I., Jalón, M. & Eguileor, I. (1996) Food surveillance in the Basque country (Spain). II. Estimation of the dietary intake of organochlorine pesticides, heavy metals, arsenic, aflatoxin M_1, iron and zinc through the total diet study, 1990/91. *Food Addit. Contam.*, **13**, 29–52

Van Oostdam, J., Gilman, A., Dewailly, E., Usher, P., Wheatley, B., Kuhnlein, H., Neve, S., Walker, J., Tracy, B., Feeley, M., Jerome, V. & Kwavnick, B. (1999) Human health implications of environmental contaminants in Arctic Canada: A review. *Sci. total Environ.*, **230**, 1–82

Venkat, J.A., Shami, S., Davis, K., Nayak, M., Plimmer, J.R., Pfeil, R. & Nair, P.P. (1995) Relative genotoxic activities of pesticides evaluated by a modified SOS microplate assay. *Environ. mol. Mutag*., **25**, 67–76

Vigfusson, N.V, Vyse, E.R., Pernsteiner, C.A. & Dawson, R.J. (1983) In vivo induction of sister-chromatid exchange in *Umbra limi* by the insecticides endrin, chlordane, diazinon and guthion. *Mutat. Res*., **118**, 61–68

Wade, T.L., Chambers, L., Gardinall, P.R., Sericano, J.L. & Jackson, T.J. (1997) Toxaphene, PCB, DDT, and chlordane analyses of beluga whale blubber. *Chemosphere*, **34**, 1351–1357

Wang, H.H. & MacMahon, B. (1979a) Mortality of workers employed in the manufacture of chlordane and heptachlor. *J. occup. Med*., **21**, 745–748

Wang, H.H. & MacMahon, B. (1979b) Mortality of pesticide applicators. *J. occup. Med*., **21**, 741–744

Wango, E.O., Onyango, D.W., Odongo, H., Okindo, E. & Mugweru, J. (1997) In vitro production of testosterone and plasma levels of luteinising hormone, testosterone and cortisol in male rats treated with heptachlor. *Comp. Biochem. Physiol*., **118C**, 381–386

Wassermann, M., Tomatis, L., Wassermann, D., Day, N.E., Groner, Y., Lazarovici, S. & Rosenfeld, D. (1974) Epidemiology of organochlorine insecticides in the adipose tissue of Israelis. *Pestic. Monit. J*., **8**, 1–7

Weiderpass, E., Adami, H.O., Baron, J.A., Wicklund-Glynn, A., Aune, M., Atuma, S. & Persson, I. (2000) Organochlorines and endometrial cancer risk. *Cancer Epidemiol. Biomarkers Prev*., **9**, 487–493

Welch, R.M., Levin, W., Kuntzman, R., Jacobson, M. & Conney, A.H. (1971) Effect of halogenated hydrocarbon insecticides on the metabolism and uterotropic action of estrogens in rats and mice. *Toxicol appl. Pharmacol*., **19**, 234–246

WHO (1984a) *Chlordane* (Environmental Health Criteria 34), Geneva

WHO (1984b) *Heptachlor* (Environmental Health Criteria 38), Geneva

WHO (1988a) *Chlordane Health and Safety Guide* (Health and Safety Guide No. 13), Geneva

WHO (1988b) *Heptachlor Health and Safety Guide* (Health and Safety Guide No. 14), Geneva

WHO (1993) *Guidelines for Drinking-water Quality*, 2nd Ed., Vol. 1, *Recommendations*, Geneva

WHO (1999) *Inventory of IPCS and Other WHO Pesticide Evaluations and Summary of Toxicological Evaluations Performed by the Joint Meeting on Pesticide Residues [JMPR] through 1999*, 3rd Ed., Geneva, International Programme on Chemical Safety

Whysner, J., Montandon, F., McClain, R.M., Downing, J., Verna, L.K., Steward, R.E. & Williams, G.M. (1998) Absence of DNA adduct formation by phenobarbital, polychlorinated biphenyls, and chlordane in mouse liver using the ^{32}P-postlabeling assay. *Toxicol. appl. Pharmacol*., **148**, 14–23

Williams, S., ed. (1984a) *Official Methods of Analysis of the Association of Official Analytical Chemists*, 14th Ed., Washington DC, Association of Official Analytical Chemists, pp. 111–113

Williams, S., ed. (1984b) *Official Methods of Analysis of the Association of Official Analytical Chemists*, 14th Ed., Washington DC, Association of Official Analytical Chemists, pp. 533–543

Williams, G.M. & Numoto, S. (1984) Promotion of mouse liver neoplasms by the organochlorine pesticides chlordane and heptachlor in comparison to dichlorodiphenyltrichloroethane. *Carcinogenesis*, **5**, 1689–1696

Williams, G.M., Mori, H. & McQueen, C.A. (1989) Structure–activity relationships in the rat hepatocyte DNA-repair test for 300 chemicals. *Mutat. Res.*, **221**, 263–286

Woods, J.S. & Polissar, L. (1989) Non-Hodgkin's lymphoma among phenoxy herbicide-exposed farm workers in western Washington State. *Chemosphere*, **18**, 401–406

Woods, J.S., Polissar, L., Severson, R.K., Heuser, L.S. & Kulander, B.G. (1987) Soft tissue sarcoma and non-Hodgkin's lymphoma in relation to phenoxyherbicide and chlorinated phenol exposure in western Washington. *J. natl Cancer Inst.*, **78**, 899–910

Worthing, C.R. & Walker, S.B., eds (1987) *The Pesticide Manual — A World Compendium*, 8th Ed., Thornton Heath, British Crop Protection Council, pp. 145–146, 455–456

Yang, C. & Chen, S. (1999) Two organochlorine pesticides, toxaphene and chlordane, are antagonists for estrogen-related receptor alpha-1 orphan receptor. *Cancer Res.*, **59**, 4519–4524

Zeiger, E., Anderson, B., Haworth, S., Lawlor, T., Mortelmans, K. & Speck, W. (1987) *Salmonella* mutagenicity tests: III. Results from the testing of 255 chemicals. *Environ. Mutag.*, **9** (Suppl. 9), 1–110

Zheng, T., Holford, T.R., Tessari, J., Mayne, S.T., Hoar Zahm, S., Owens, P.H., Zhang, B., Ward, B., Carter, D., Zhang, Y., Zhang, W., Dubrow, R. & Boyle, P. (2000) Oxychlordane and trans-nonachlor in breast adipose tissue and risk of female breast cancer. *J. Epidemiol. Biostat.*, **5**, 153–160