

TOXAPHENE

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

1. Exposure Data

1.1 Chemical and physical data

1.1.1 Nomenclature

Chem. Abstr. Serv. Reg. No.: 8001-35-2

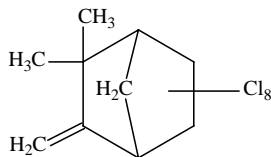
Deleted CAS Reg. Nos: 8022-04-6; 12687-42-2; 12698-98-5; 12770-20-6;
37226-11-2; 56645-28-4

Chem. Abstr. Name: Toxaphene

IUPAC Systematic Name: Toxaphene

Synonyms: Camphechlor; chlorinated camphene; PCC; polychlorocamphene

1.1.2 Structural and molecular formulae and relative molecular mass



$C_{10}H_{10}Cl_8$ (approximately)

Relative molecular mass: 414 (average)

[Note: Structure representative of the predominant chlorinated camphene compounds present in technical-grade toxaphene]

1.1.3 *Chemical and physical properties of the pure substance*

From Budavari (2000)

- (a) *Description*: Yellow waxy solid
- (b) *Melting-point*: 65–90 °C
- (c) *Solubility*: Very slightly soluble in water (0.003 g/L); freely soluble in aromatic hydrocarbons
- (d) *Stability*: Dehydrochlorinates in the presence of alkali, prolonged exposure to sunlight and at temperatures of about 155 °C
- (e) *Octanol/water partition coefficient (P)*: log P, 6.44

1.1.4 *Technical products and impurities*

In the past, toxaphene was available as dust formulations, emulsifiable concentrates, granules and wettable powders (FAO/UNEP, 1999). Trade names for toxaphene include Alltox, Anatox, Camphochlor, Canfeclor, Estonox, Geniphene, Hercules 3956, Kamfochlor, M 5055, Melipax, Motox, PChK, Phenacide, Phenatox, PKhF, Strobane-T, Toxakil, Toxaphen and Toxyphen.

1.1.5 *Analysis*

Methods for the analysis of toxaphene in various media are summarized in Table 1.

1.2 **Production**

Toxaphene is a very complex, but fairly reproducible mixture of at least 177 C₁₀ polychloro derivatives, having an approximate overall empirical formula of C₁₀H₁₀Cl₈. Toxaphene is produced by chlorination of camphene to 67–69% chlorine by weight and is made up mainly of compounds of C₁₀H₈Cl₁₀, C₁₀H_{18-n}Cl_n (mostly polychlorobornanes) and C₁₀H_{16-n}Cl_n (polychlorobornenes and/or polychlorotricyclenes) with *n* = 6–9 (Budavari, 2000). Annual production of toxaphene in the USA in 1976 was about 19 000 t (Agency for Toxic Substances and Disease Registry, 1998).

Information available in 2000 indicated that toxaphene was manufactured by one company in the USA (CIS Information Services, 2000).

1.3 **Use**

Toxaphene (chlorinated camphene) was used as a broad-spectrum, nonsystemic contact and stomach insecticide, with some acaricidal action. It was often used in combination with other pesticides. Its primary usage was on agricultural crops, mainly cotton, but also corn, fruit, vegetables and small grains. It has been used as an insecticide to control armyworms, boll weevils, bollworms, cotton aphids, cotton fleahoppers, cotton leafworms, grasshoppers and others. It was also used to control livestock ectoparasites such as lice, flies, ticks, mange and scab mites.

Table 1. Methods for the analysis of toxaphene

Sample matrix	Sample preparation	Assay procedure	Limit of detection	Reference
Air	Collect sample in prefilter and ethylene glycol; dilute with water; extract with hexane; extract; prefilter with hexane; pool extracts before drying; concentrate	GC/ECD	1–10 ng/m ³	Kutz <i>et al.</i> (1976)
	Adsorb onto polyurethane foam; extract with hexane; reduce volume	GC/ECD; GC/MS	1.6 pg/m ³ (11 300 m ³ sample)	Barrie <i>et al.</i> (1993)
	Collect vapours on cellulose ester membrane; desorb with petroleum ether	GC/ECD	0.14 µg/ sample	Eller (1994) [Method 5039]
	Collect vapours on polyurethane foam (low or high volume); extract with 5–10% diethyl ether in hexane	GC/ECD	NR	Environmental Protection Agency (1999a) [Method TO-04A]
Drinking-water	Extract with hexane; inject extract	GC/ECD	1.0 µg/L	Environmental Protection Agency (1995a) [Method 505]
	Extract with dichloromethane; isolate extract; dry; concentrate with methyl <i>tert</i> -butyl ether (capillary column)	GC/ECD	0.03 µg/L	Environmental Protection Agency (1995b) [Method 508.1]
	Extract by passing sample through liquid–solid extractor; elute with dichloromethane; concentrate by evaporation (capillary column)	GC/MS	1.0–1.7 µg/L	Environmental Protection Agency (1995c) [Method 525.2]
Tapwater, groundwater, river water	Isolate compounds from water on C ₁₈ SPE followed by recovery of adsorbed analytes with supercritical carbon dioxide containing acetone	GC/ion trap MS	7.4 µg/L (w/v)	Ho <i>et al.</i> (1995)
Liquid and solid wastes	Extract with dichloromethane (liquid); hexane:acetone (1:1) or dichloromethane:acetone (1:1) (solid); clean-up	GC/ECD or GC/ELCD	NR	Environmental Protection Agency (1996a) [Method 8081A]

Table 1 (contd)

Sample matrix	Sample preparation	Assay procedure	Limit of detection	Reference
Liquid and solid wastes (contd)	Mix with anhydrous sodium sulfate; extract by Soxhlet or sonication process; clean-up on Florisil or gel-permeation (capillary column)	GC/MS	NR	Environmental Protection Agency (1996b) [Method 8270C]
	Extract with dichloromethane	Tandem MS	5 µg/sample	Hunt <i>et al.</i> (1985)
	Extract with dichloromethane; dry; exchange to hexane; clean-up on Florisil	GC/ECD	0.24 µg/L	Environmental Protection Agency (1999b) [Method 608]
	Extract with dichloromethane; dry; concentrate (packed column)	GC/MS	NR	Environmental Protection Agency (1999c) [Method 625]
Municipal and industrial wastewater	Extract with dichloromethane; dry; concentrate (packed column)	GC/ECD	NR	Environmental Protection Agency (1993a) [Method 617]
	Adjust to pH 11; extract with dichloromethane; dry; concentrate	GC/MS	NR	APHA/AWWA/WEF (1999a)
	Extract with diethyl ether: hexane or dichloromethane: hexane; concentrate; clean-up by column adsorption chromatography	GC/ECD	NR	APHA/AWWA/WEF (1999b) [Method 6630B]
	Extract with dichloromethane; solvent exchange to hexane; clean-up with magnesia-silica gel; concentrate	GC/ECD	0.24 µg/L	APHA/AWWA/WEF (1999c)

Table 1 (contd)

Sample matrix	Sample preparation	Assay procedure	Limit of detection	Reference
Municipal and industrial waste water, sludges	If solids < 1%, extract with dichloromethane; for non-sludges with solids 1–30%, dilute to 1% and extract with dichloromethane; if solids > 30%, sonicate with dichloromethane:acetone; for sludges: if solids < 30%, treat as 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	0.91 µg/L	Environmental Protection Agency (1993b) [Method 1656]
Soil	Add water; extract with methanol:toluene (1:1); concentrate; add methanolic KOH solution and reflux; extract with hexane; clean-up on Florisil	GC/MS and HPLC	50 µg/kg	Crist <i>et al.</i> (1980)
	Soxhlet extract with dichloromethane or sonicate with dichloromethane:acetone (1:1, v/v); clean-up with GPC or SPE	GC/EC-NIMS	100 µg/kg	Brumley <i>et al.</i> (1993)
	Extract with dichloromethane:acetone (1:1) with sonication; remove water with sodium sulfate; solvent exchange to isooctane; clean-up on Florisil	GC/NCIMS	50 µg/kg (w/w)	Onuska <i>et al.</i> (1994)
	Extract with methanol; add aliquot and enzyme conjugate reagent to immobilized antibody; compare colour produced to reference reaction	Immuno-assay	500 µg/kg	Environmental Protection Agency (1996c) [Method 4040]
Sediment and mussel tissue	Extract with hexane; elute from alumina column; concentrate	HPLC followed by GC/FID or GC/ECD	< 1 µg/kg	Petrick <i>et al.</i> (1988)

Table 1 (contd)

Sample matrix	Sample preparation	Assay procedure	Limit of detection	Reference
Pesticide formulations	Extract with methanolic KOH; elute with diethyl ether from Florisil	GC/ECD	1 ng/sample	Gomes (1977)
	Remove solvent (xylene) from pesticide sample by reduced pressure; extract with hexane	GC and GC/TLC	NR	Saleh & Casida (1977)
	Extraction with hexane	TLC	1 µg/sample	Ismail & Bonner (1974)
	Dissolve in hexane and load onto alumina column; elute with hexane, dichloromethane in benzene then methanol	GC/ECD or GC/FID	NR	Seiber <i>et al.</i> (1975)
	Extract with acetone; filter or centrifuge	TLC	NR	AOAC International (2000) [Method 972.05]
Non-fatty foods	Extract with acetone; partition or remove water; clean-up on Florisil; elute with dichloromethane	GC/ECD or GC/ELCD	NR	Food and Drug Administration (1999) [Method 302]
	Extract with acetonitrile or water:acetonitrile; partition into petroleum ether; clean-up on Florisil	GC/ECD or GC/ELCD	NR	Food and Drug Administration (1999)
Various products	Extract with acetonitrile; filter; add salt to affect phase separation; evaporate to near dryness; reconstitute in benzene	GC/ECD	2 mg/kg	Hsu <i>et al.</i> (1991)
Fruits and vegetables	Extract with acetone; filter extract with petroleum ether:dichloromethane; evaporate solvent; dissolve in acetone	GC/ECD	NR	WHO (1984)
Molasses	Dilute with water; extract with hexane:isopropanol	GC/ECD	0.03 mg/kg	WHO (1984)
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]

Table 1 (contd)

Sample matrix	Sample preparation	Assay procedure	Limit of detection	Reference
Meat	Blend with ethyl acetate; dry (Na_2SO_4) and filter; treat with KOH and heat; extract with hexane; clean-up on Florisil	GC/ECD	NR	Boshoff & Pretorius (1979)
Bovine defibrinated whole blood	Dilute with water; extract with hexane	GC/ECD	0.58 mg/L	Maiorino <i>et al.</i> (1980)
	Add sample to formic acid and shake; extract with hexane; extract with potassium carbonate; reduce volume	GC/ECD	0.47 mg/L	Maiorino <i>et al.</i> (1980)
	Add sample to formic acid; mix and load onto Florisil column; elute with diethyl ether in petroleum ether; reduce volume; wash with hexane	GC/ECD	0.03 mg/L	Maiorino <i>et al.</i> (1980)
Lard	Extract with petroleum ether; centrifuge; remove water with anhydrous Na_2SO_4 ; reduce volume	GC/ECD	1.4 mg/kg	Head & Burse (1987)
Poultry fat	Render fat; direct analysis	GC/ECD	0.48 mg/kg	Ault & Spurgeon (1984)
Milk fat	Centrifuge; fractionate on Florisil	GC/ECD and GC/MS	< 10 $\mu\text{g}/\text{kg}$ (ECD) 7 $\mu\text{g}/\text{kg}$ (MS)	Cairns <i>et al.</i> (1981)
Milk and butter	Add to KOH; heat; extract with hexane; centrifuge; clean-up on Florisil	GC/ECD	NR	Boshoff & Pretorius (1979)
Fish (whole)	Blend frozen sample with dry ice and anhydrous Na_2SO_4 ; extract with hexane:acetone (1:1), followed by methanol	GC/NCIMS	75 pg/sample	Swackhamer <i>et al.</i> (1987)
Fish tissues	Extract with hexane:acetone; extract with hexane:diethyl ether; evaporate; dissolve in hexane; shake with H_2SO_4 to remove lipid	GC/NCIMS	NR	Jansson <i>et al.</i> (1991)

Table 1 (contd)

Sample matrix	Sample preparation	Assay procedure	Limit of detection	Reference
Fish tissues (contd)	Homogenize sample with hexane:acetone (1:2.5) under acid conditions; extract twice more with diethyl ether in hexane; treat with concentrated H ₂ SO ₄ , clean-up with GPC and silica gel	GC/NCIMS	NR	Jansson <i>et al.</i> (1991)
	Homogenize sample; extract with hexane:acetone; add internal standards; clean-up with GPC and Florisil	GC/HRMS (SIM)	10 µg/kg (wet weight)	Andrews <i>et al.</i> (1993)
	Pulverize tissue with anhydrous sodium sulfate; extract with acetone; solvent exchange to hexane; reduce volume; clean-up on Florisil and silica gel	GC/MS (SIM)	0.1 µg/kg	Jarnuzi & Wakimoto (1991)
Human tissues (toxaphene and some metabolites)	Macerate tissue; add anhydrous Na ₂ SO ₄ and acetone; filter; extract with chloroform; add KOH; extract with water; remove water (Na ₂ SO ₄); evaporate; dissolve in acetone	TLC	1 µg/sample	Tewari & Sharma (1977)
Human tissues	Grind sample; extract with dichloromethane:hexane (1:1), reduce volume; clean-up with GPC and Florisil	GC/NCIMS	~ 10 µg/kg	Fowler <i>et al.</i> (1993)
Human breast milk	Centrifuge; freeze-dry fat concentrate; dissolve in acetone; re-dissolve in hexane; shake with concentrated H ₂ SO ₄ ; clean-up with silica gel	GC/ECD and GC/NCIMS	100 µg/L	Vaz & Blomkvist (1985)
Human breast fat	Homogenize; extract with petroleum ether; remove water with anhydrous Na ₂ SO ₄ ; reduce volume	GC/ECD	NR	Head & Burse (1987)
Stomach washings and urine (toxaphene and some metabolites)	Filter sample; wash with water; add saturated solution of Na ₂ SO ₄ ; extract with hexane; filter through anhydrous Na ₂ SO ₄ ; evaporate to dryness; dissolve in acetone	TLC	1 µg/sample	Tewari & Sharma (1977)

Table 1 (contd)

Sample matrix	Sample preparation	Assay procedure	Limit of detection	Reference
Human blood	Add H ₂ SO ₄ to blood sample; extract with hexane:acetone (9:1); centrifuge and evaporate to dryness; dissolve in hexane	GC/ECD or GC/MCD	NR 10–40 µg/L	Griffith & Blanke (1974)
	Add to dilute H ₂ SO ₄ and 10% sodium tungstate solution; filter and wash with water; remove water with Na ₂ SO ₄ ; extract with hexane; filter through anhydrous Na ₂ SO ₄ ; evaporate to dryness; dissolve in acetone	TLC	1 µg/sample	Tewari & Sharma (1977)

NR, not reported; APHA/AWWA/WEF, American Public Health Association/American Water Works Association/Water Environment Federation; ECD, electron capture detection; EC-NIMS, electron capture–negative-ion mass spectrometry; ELCD, electrolytic conductivity detection; FID, flame ionization detection; FTIR, Fourier transform infrared spectroscopy; GC, gas chromatography; GPC, gel permeation chromatography; HPLC, high-performance liquid chromatography; HRMS, high-resolution mass spectrometry; MCD, microcoulometry detection; MS, mass spectrometry; NCIMS, negative chemical ionization mass spectrometry; SIM, selected ion monitoring; SPE, solid-phase extraction; TLC, thin-layer chromatography

Introduced in 1948, toxaphene was the most heavily used insecticide in the USA in the 1960s and 1970s, having replaced many of the agricultural applications of the banned DDT (Blair & Hoar Zahn, 1993; Agency for Toxic Substances and Disease Registry, 1998; FAO/UNEP, 1999).

1.4 Occurrence

1.4.1 Occupational exposure

The concentrations of toxaphene in the air of manufacturing plants in the former USSR were found to exceed the permissible level of 0.2 mg/m³ by five to six times. By the end of a work shift, the concentrations on uncovered skin of employees were 30–1000 mg/m²; covered skin areas had toxaphene concentrations of up to 40 mg/m² (Ashirova, 1971).

1.4.2 Environmental occurrence

Toxaphene is a persistent pesticide, the use of which has diminished substantially over the past two decades. This compound has low volatility and is only slightly soluble in water. Its biodegradation in soil is very slow, with a half-time measured in

decades. Hence, toxaphene persists in the environment and can be expected to accumulate in the sediment long after application has ceased.

The environmental occurrence of toxaphene has been reviewed (IARC, 1979; WHO, 1984; Agency for Toxic Substances and Disease Registry, 1998).

The concentrations in the air in Bermuda and in the USA measured in the 1970s ranged from $< 0.02 \text{ ng/m}^3$ to 1540 ng/m^3 (IARC, 1979). A more recent study in Canada (Shoeib *et al.*, 1999) reported airborne toxaphene concentrations of 0.9–10.1 pg/m^3 between 1995 and 1997.

In the 1970s, the concentrations of toxaphene in rainwater in the USA ranged from 44 to 280 ng/L (Munson, 1976). A more recent study in the USA showed concentrations in water ranging from 0.17 ng/L in Lake Ontario to 1.12 ng/L in Lake Superior (Swackhamer *et al.*, 1998).

In the 1970s, the concentrations of toxaphene in soil in the USA were found to range from 7.7 to 33.4 mg/kg (Carey *et al.*, 1976). Soil samples taken in the USA in 1969 had concentrations ranging from 0.1 to 53 mg/kg (Wiersma *et al.*, 1972). More recently, the concentration of toxaphene in Great Lakes sediments were found to be 15 ± 4 (SD) $\mu\text{g/kg}$ dry weight (Swackhamer *et al.*, 1998).

Toxaphene present in sediments can continue to enter the food chain by uptake by small organisms in direct contact with the sediment. The log of the bioaccumulation factor (organism concentration/water concentration) ranged from 5.8 to 7.0 for a series of biota including phytoplankton, zoo plankton, *Mysis*, *Bythotrephes*, sculpin and lake trout (Swackhamer *et al.*, 1998).

In the USA, the concentrations in wildlife ranged from 1.7 to 88.9 mg/kg in adult animals (Causey *et al.*, 1972) and from 0.12 to 0.58 mg/kg in pelican eggs (Blus *et al.*, 1975). A study in 1976–86 by the National Contamination Biomonitoring Program in the USA of various freshwater fish across the country showed annual geometric mean concentrations of toxaphene ranging from 0.066 to 0.178 mg/kg (Schmitt *et al.*, 1999). Musial and Uthe (1983) reported that the concentration of toxaphene in Canadian East Coast marine fish tissues was 0.4–1.1 mg/kg on a wet weight basis.

Saleh (1991) reviewed the concentrations of toxaphene in the environment before 1990. Those in birds' eggs ranged from 0.03 (osprey) to 10 (vulture) mg/kg , while the concentrations in adult birds ranged from 0.02 to 4.0 mg/kg on a wet weight basis. The maximum concentration in tertiary consumer species in the Mississippi River (Louisiana, USA), such as herons, was 24.0 mg/kg , indicating substantial biomagnification of toxaphene. The concentrations in bats were $\leq 2 \text{ mg/kg}$. In fish, the concentrations of toxaphene were 5–7 mg/kg in Lake Superior trout, 5–10 mg/kg in Lake Michigan trout, 9 mg/kg in Lake Huron trout, 0.068 mg/kg of lipid in South Atlantic cod, 9 mg/kg of lipid in Arctic char in Sweden and 13 mg/kg of lipid in Atlantic herring from the Baltic Sea. Datta *et al.* (1999) reported a concentration of 0.154 mg/kg in a trout from Lake Tahoe (USA).

The arithmetic mean concentrations of toxaphene in traditional foods in northern and Arctic Canada, e.g., marine mammal meat and blubber and terrestrial mammal

meat and organs ranged from < 0.001 to 3.89 mg/kg wet weight (Chan, 1998). In another study, the concentration of toxaphene in beluga whale blubber ranged from 2.38 to 3.54 mg/kg lipid in females and 4.06 and 15.94 mg/kg in two males. A single fetal specimen contained 3.71 mg/kg, which was comparable to the mother's value of 3.20 mg/kg (Wade *et al.*, 1997).

Concentrations of 2.3–18 mg/L were reported in the milk of cows fed toxaphene-treated hay in Finland (Bateman *et al.*, 1953).

The estimate average dietary intake of toxaphene in the USA during the period 1986–91 ranged from 0.0057 to 0.0224 µg/kg bw per day, with age- and sex-specific differences (Agency for Toxic Substances and Disease Registry, 1998).

The median concentrations in breast milk from various populations in northern and southern Canada in 1986, 1992 and 1996 ranged from 4.94 to 56.4 ng/g of extractable lipid, the concentrations observed in northern Canada being substantially higher than those observed elsewhere (Newsome & Ryan, 1999). In the Nordic countries, the mean toxaphene concentration in 1985 in pooled breast milk was 0.1 mg/kg of milk fat in Uppsala and Stockholm, Sweden, and 1–10 µg/L as a fraction of whole milk in Finland (Saleh, 1991). Adipose tissue samples taken in Finland in 1985 showed toxaphene concentrations of 0.01–0.1 mg/kg, but the result was strongly dependent on the diet.

1.5 Regulations and guidelines

Occupational exposure limits for toxaphene in several countries are presented in Table 2. The use of toxaphene has been banned or product registrations have been cancelled or withdrawn in many countries since 1970, because of concerns about risks to human health and the environment. 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 19 countries have reported that use of toxaphene had been banned or severely restricted (FAO/UNEP, 1999).

Toxaphene is one of 12 persistent organic pollutants being considered for international action to reduce or eliminate their releases under a global convention (FAO/UNEP, 1999). At negotiations in September 1999, the participating governments agreed to phase out use of toxaphene and two other chlorinated pesticides (aldrin and endrin). As of December 2000, three other chlorinated pesticides had been phased out (chlordane, heptachlor and hexachlorobenzene) (Hogue, 2000).

No maximum residue limit or acceptable daily intake values have been allocated to toxaphene by the FAO/WHO Joint Meeting on Pesticide Residues (FAO/UNEP, 1999).

The Environmental Protection Agency (2000) set a maximum contaminant level for toxaphene in drinking-water of 0.003 mg/L and a 'maximum contaminant level goal' of zero.

Table 2. Occupational exposure limits and guidelines for toxaphene

Country	Year	Concentration (mg/m ³)	Interpretation
Australia	1993	0.5 (sk) 1	TWA STEL
Austria	1993	0.5 (sk)	TWA
Belgium	1993	0.5 (sk) 1	TWA STEL
Denmark	1993	0.5 (sk)	TWA
Egypt	1993	0.5 (sk)	TWA
Finland	1998	0.5 (sk) 1.5	TWA STEL
France	1993	0.5 (sk)	TWA
Germany	2000	carcinogen-2	
Netherlands	1999	0.5 (sk)	TWA
Philippines	1993	0.5 (sk)	TWA
Switzerland	1993	0.5 (sk)	TWA
Thailand	1993	0.5	TWA
Turkey	1993	0.5 (sk)	TWA
USA			
ACGIH (TLV)	2000	0.05 (A3, sk) 1	TWA STEL
NIOSH (REL)	1997	(Ca, lfc, sk)	
OSHA (PEL)	1999	0.5 (sk)	TWA

From Ministry of Social Affairs and Health (1998); American Conference of Governmental Industrial Hygienists (ACGIH) (2000); Deutsche Forschungsgemeinschaft (2000)

A3, confirmed animal carcinogen with unknown relevance to humans; Ca, carcinogen; lfc, lowest feasible concentration; carcinogen-2, substances which are considered to be carcinogenic for man because sufficient data from long-term animal studies or limited evidence from animal substantiated by evidence from epidemiological studies indicate that they can make a significant contribution to cancer risk; sk, danger of cutaneous absorption; TWA, time-weighted average; STEL, short-term exposure limit; REL, recommended exposure limit; PEL, permissible exposure limit; NIOSH, National Institute for Occupational Safety and Health; OSHA, Occupational Safety and Health Administration

2. Studies of Cancer in Humans

2.1 Case-control studies

In a case-control study on non-Hodgkin lymphoma in the USA, cases were identified through the Iowa State Health Registry and 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 of the cases were confirmed to be non-Hodgkin lymphoma after the review. The 1245 controls were frequency matched to cases by age, residence and vital status. Living subjects were selected by random-digit dialling (age < 65 years) and from Medicare rosters (age ≥ 65); death certificate files were used to select deceased controls. 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 men who had never worked or lived on a farm as adults (266 cases and 547 controls). Eight patients and 19 controls had ever handled toxaphene as an animal insecticide (odds ratio, 0.8; 95% confidence interval [CI], 0.3–2.0), and 10 patients and 13 controls had used it as crop insecticide (odds ratio, 1.5; 0.6–3.5). When the analysis was limited to those who had handled it prior before 1965, the odds ratio for use on crops was 2.4 (0.7–8.2).

In a study of leukaemia parallel to that of non-Hodgkin lymphoma conducted in Iowa and Minnesota (Cantor *et al.*, 1992) described above, 578 men with leukaemia (340 living and 238 deceased) and 1245 controls (820 living and 425 deceased) were included (Brown *et al.*, 1990). Farmers who reported use of toxaphene on animals had an odds ratio of 1.4 (95% CI, 0.6–3.1), which was higher for those who had handled it at least 20 years before interview (or at least 16 years before diagnosis) (2.6; 0.8–8.8).

3. Studies of Cancer in Experimental Animals

3.1 Oral administration

Mouse: Groups of 50 male and 50 female B6C3F₁ mice, 5 weeks of age, were fed a diet containing 160 or 320 mg/kg toxaphene for 19 weeks and 80 or 160 mg/kg of diet for a further 61 weeks, followed by a toxaphene-free diet for 10–11 weeks. A group of 10 mice of each sex served as matched controls and received normal diet for 90–91 weeks; 50 male and 50 female pooled controls from other experiments were used to provide further control data. Survival was not significantly affected by toxaphene: by 52 weeks, 49/50 males at the low dose and 46/50 at the high dose and 46/50 females at both doses were still alive. The incidences of hepatocellular carcinoma in males were 0/10 matched controls, 4/48 (8%) pooled controls, 34/49 (69%) at the low dose and 45/46 (98%) at the high dose ($p < 0.001$, dose-related response). In females, the incidences were 0/9 matched controls, 0/48 pooled controls, 5/49 (10%) at the low dose and 34/49 (69%) at the high dose ($p < 0.001$, dose-related response). ‘Neoplastic nodules’ (authors’ terminology) of the liver were found in 2/10 (20%) matched control males, 6/49 (12%) males at the low dose, 0/46 males at the high dose, 0/9 matched control females, 13/49 (26%) females at the low dose and 6/49 (12%) females at the high dose (National Cancer Institute, 1979). The liver tumours in this study were re-evaluated by a pathology working group, which reclassified most of the hepatocellular carcinomas as adenomas and the adenomas as hepatocellular foci. This analysis indicated no statistically significant increase in the incidence of carcinomas at any dose, but the incidences of adenomas and total tumours remained statistically significantly increased in both male and female mice at the high dose. The revised incidences of carcinomas in males were 3/48 pooled controls, 8/50 at the low dose and 5/47 at the high dose, and those in females were 0/50 pooled controls, 0/49 at the low dose and 3/47 at the high dose. The revised incidences of adenomas in males were 5/48 pooled controls, 30/50 at the low dose and 42/47 at the high dose, and those in females were 1/50 pooled controls, 11/49 at the low dose and 37/47 at the high dose (Goodman *et al.*, 2000).

A study conducted by Litton Bionetics in 1978, but not published by that organization, was later reviewed by Goodman *et al.* (2000). Groups of 55 male and 55 female B6C3F₁ mice [age unspecified] were fed a diet containing 0, 7, 20 or 50 mg/kg toxaphene [purity unspecified] for 18 months, followed by untreated diet for a further 6 months. Survival was not affected by treatment, and no clinical signs were observed. No significant difference in the incidence of liver adenomas or carcinomas was observed between the treated and untreated groups when evaluated separately, although a significant difference was observed in the total number of liver tumours in males at the high dose and controls (18/51 versus 10/53; $p < 0.05$). No such difference was observed in females. [The Working Group noted that the original report was not available and that the slides of liver lesions were not available for reclassification by modern histological criteria].

Rat: Groups of 50 male and 50 female Osborne-Mendel rats, 5 weeks of age, were fed a diet containing toxaphene for 80 weeks and were then observed for 28 (males) or 30 (females) weeks. The dose regimen for high-dose males was 2560 mg/kg of diet for 2 weeks, 1280 mg/kg of diet for 53 weeks and 640 mg/kg of diet for a further 25 weeks; that for high-dose females was 1280 mg/kg of diet for 55 weeks followed by 640 mg/kg of diet for 25 weeks; that for low-dose males was 1280 mg/kg of diet for 2 weeks, 640 mg/kg of diet for 53 weeks and 320 mg/kg of diet for 25 weeks; and that for low-dose females was 640 mg/kg of diet for 55 weeks, followed by 320 mg/kg diet for 25 weeks. Matched control groups of 10 untreated rats of each sex were given toxaphene-free diet for 108–109 weeks; 55 untreated males and 55 untreated females from other bioassays served as pooled controls. Survival was not significantly affected, as 90–92% of rats were alive at week 52 of the study, even at the high dose. The incidences of thyroid follicular-cell tumours (adenomas and carcinomas) were 7/41 (17%) and 9/35 (26%) in male rats at the low and high doses, respectively, in comparison with 1/7 (14%) matched controls and 2/44 (5%) pooled controls. In females, the incidences were 1/43 (2%) at the low dose and 7/42 (17%) at the high dose in comparison with 0/6 (0%) matched controls and 1/46 (2%) pooled controls. In male rats, the incidence of thyroid tumours (adenomas and carcinomas) was dose-related ($p = 0.007$) in comparison with pooled controls. In female rats, the incidence of thyroid follicular-cell adenomas was dose-related in comparison with either matched ($p = 0.022$) or pooled ($p = 0.008$) controls. Follicular-cell carcinomas were found in two males at the high dose, while all the remaining thyroid tumours were follicular-cell adenomas. Hyperplasia of thyroid follicular cells was observed only in treated males (low-dose, 3/41; high-dose, 3/35) and females (low-dose, 5/43; high-dose, 3/42). In female rats, pituitary tumours (mainly chromophobe adenomas) occurred in 15/41 at the low dose and 23/39 at the high dose, the incidence being statistically significantly increased in comparison with either the matched (3/8; $p = 0.046$) or pooled (17/51; $p = 0.012$) controls. In male rats, the incidence of hepatocellular adenomas at the low dose (6/44) was higher than that in pooled controls (1/52; $p = 0.034$), but animals at the high dose did not show a significantly higher incidence (4/45) than that in either control group (National Cancer Institute, 1979).

Hamster: A study conducted by Litton Bionetics in 1978, but not published by that organization, was later reviewed by Goodman *et al.* (2000). Groups of 51 male and 51 female ARS golden Syrian hamsters [age unspecified] were fed diets containing 0, 100, 300 or 1000 mg/kg toxaphene for 21.5 (males) or 18 (females) months. Treatment-related effects were observed only in males and included decreased body weight and the presence of megahepatocytes in the liver at the high dose. Treatment with toxaphene was not associated with tumours of any type. [The Working Group noted that the original report was not available and that the slides of liver lesions were not available for reclassification by modern histological criteria.]

3.2 Administration with known carcinogens

Mouse: To investigate the effects of toxaphene on benzo[*a*]pyrene-induced lung adenoma development, groups of female A/J mice, 9 weeks of age, were fed diets containing 0, 100 or 200 mg/kg toxaphene [purity not specified] in corn oil for 12 or 20 weeks, having been intubated with 0 or 3 mg of benzo[*a*]pyrene on day 7 and day 21 of the experiment. Toxaphene administered for 12 weeks had no effect on the induction of forestomach tumours by benzo[*a*]pyrene although at 200 mg/kg of diet there appeared to be a slight but significant decrease in the number of forestomach tumours per mouse (4.18 ± 0.34 (control group) versus 3.14 ± 0.34 ; $p < 0.05$) [no significant change in the incidence of tumours as stated by the authors; 100% in all groups]. Toxaphene fed at 100 mg/kg of diet for 12 weeks resulted in a small but significant reduction in the incidence (25% versus 8.1%; p value not given) and number of lung tumours per mouse induced by benzo[*a*]pyrene (1.17 ± 0.11 versus 1.00 ± 0.00 ; $p < 0.05$). At 200 mg/kg of diet for 20 weeks, toxaphene markedly decreased the incidence of lung tumours (100% versus 67%) and the mean number of lung tumours (7.2 ± 0.8 versus 1.6 ± 0.3 ; $p < 0.001$) per mouse. Groups fed toxaphene only did not develop lung tumours at either dose (Triolo *et al.*, 1982). [The Working Group noted that the incidences of forestomach tumours at 20 weeks were not reported.]

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

The chemistry, biochemistry, toxicity and environmental fate of toxaphene have been reviewed (Saleh, 1991).

4.1 Absorption, distribution, metabolism and excretion

4.1.1 *Humans*

Toxaphene has been found in human milk collected in Finland and Sweden (Mussalo-Rauhamaa *et al.*, 1988; Saleh, 1991; see also section 1.4.2). No other information was available to the Working Group on the absorption, distribution metabolism and excretion of toxaphene in humans.

4.1.2 *Experimental systems*

In mice and rats, toxaphene is absorbed through the skin and gastrointestinal tract, at a rate depending on the vehicle used for its administration. Of a single oral dose of 20 mg/kg bw technical-grade [^{36}Cl]toxaphene administered by gavage in 0.5 mL

peanut oil/acacia gum to rats, about 52% was excreted within 9 days, with 15% in the urine, mainly as ^{36}Cl ion, and 37% in the faeces (Crowder & Dindal, 1974).

Approximately 3% of an oral dose of [^{14}C]toxaphene was excreted unchanged in the faeces of rats after 14 days. More than 5% of the administered dose was excreted in the urine and faeces as completely dechlorinated metabolites and 27% as partially dechlorinated metabolites; 1.2% of the label was found in expired air, probably as $^{14}\text{CO}_2$. The concentrations of radiolabel associated with toxaphene or its metabolites 14 days after administration of 8.5 mg/kg bw [^{14}C]toxaphene were 0.52 mg/kg in fat, 0.17 mg/kg in kidney, 0.14 mg/L in blood, 0.12 mg/kg in liver and 0.02–0.09 mg/kg each in bone, brain, heart, lung, muscle, spleen and testis. After administration of [^{36}Cl]toxaphene, 50% of the activity was excreted as ^{36}Cl ion in the urine (Ohsawa *et al.*, 1975).

Toxaphene was analysed in tissues 72 h after administration of about 13 mg/kg bw by gavage to female white Leghorn chickens, male rabbits, Swiss-Webster mice, Sprague-Dawley rats, Hartley guinea-pigs, hamsters and long-tailed monkeys (*Macaca fascicularis*). Analysis of acetone extracts of fat by capillary gas chromatography showed similar peaks in all species. In the faeces, the peaks were similar, except for that of the monkeys, which contained three metabolites of heptachlorobornane: two hexachlorobornane isomers and hexachlorobornene (Saleh *et al.*, 1979).

Toxaphene is metabolized not only to reductive dechlorination and dehydrochlorination products but also to polar hydroxyl and acidic compounds and water-soluble conjugates by the NADPH-dependent mixed-function oxidase system in rats. Thus, male Sprague-Dawley rats given radiolabelled toxaphene and rat liver microsomal preparations *in vitro* treated with radiolabelled toxaphene showed the metabolites 2-*endo*,3,3,5,6-*exo*,8,9,10,10-nonachlorobornane and 2,2,5-*endo*,6-*exo*,8,9,10-heptachlorobornane (Chandurkar & Matsumura, 1979a,b).

When pregnant Sprague-Dawley rats were given [^{14}C]toxaphene orally, 28.3% of the activity was excreted in the faeces and 22.0% in the urine within 5 days. The fetuses contained the lowest concentration of radiolabel of all tissues tested at 5 days (28 $\mu\text{g}/\text{kg}$), and maternal fat contained the highest concentration (7476 mg/kg). A comparison of the activity in the fetuses with that in the dams' fat showed slight differences, indicating the presence of more polar compounds (perhaps metabolites) in the fetal tissue (Pollock & Hillstrand, 1982).

Autoradiographic studies in virgin and pregnant albino mice given [^{14}C]toxaphene at 16 mg/kg bw intravenously showed that, after initial accumulation in the liver, brown fat, lung, brain, kidney and corpora lutea, gradual redistribution to the white fat occurred within 4 h. The labelling then decreased rapidly and only very small amounts of radiolabel were present in adipose tissue after 32 days. In the fetus, only the liver and adrenals showed distinct labelling. Specific, persistent accumulation of the label was detected in some zones of the adrenal cortex. In hypolipidaemic mice, less label accumulated initially in the liver and adrenals and more in the kidneys and heart, with much less subsequent distribution to the adipose tissue (Mohammed *et al.*, 1983).

In cultured adrenocortical cells, toxaphene inhibited adrenocorticotrophic hormone-stimulated corticosterone synthesis at a median inhibitory concentration of about 12 µg/mL. When female rats [strain not specified] were given a diet containing 1.2 mg/kg toxaphene for 5 weeks, adrenocorticotrophic hormone-stimulated corticosterone synthesis was also found to be decreased in isolated adrenocortical cells (Mohammed *et al.*, 1983, 1985, 1990).

4.2 Toxic effects

4.2.1 Humans

The acute lethal dose of toxaphene for humans has been estimated to be 2–7 g/person (Conley, 1952).

A 9-month-old child poisoned with a 2:1 mixture of toxaphene:DDT died after convulsions and respiratory arrest. The ratio of toxaphene:DDT in the brain and liver was 10:1, and that in the kidneys was 3:1 (Haun & Cueto, 1967). Four other cases of acute poisoning in children, three of which were fatal, have been reported (McGee *et al.*, 1952).

4.2.2 Experimental systems

In Sherman rats, the oral LD₅₀ of technical-grade toxaphene was 90 mg/kg bw for males and 80 mg/kg bw for females (Gaines, 1960). The oral LD₅₀ of technical-grade toxaphene was 80 mg/kg bw in male albino Wistar rats fed a protein-deficient diet (3.5% casein), 293 mg/kg bw in those fed a 26% casein diet and 220 mg/kg bw in those fed standard laboratory diet. In rats that died after ingesting toxaphene, renal tubular damage and fatty degeneration of the liver with necrosis were observed (Boyd & Taylor, 1971).

In fasted dogs, the oral LD₅₀ of toxaphene was reported to be approximately 25 mg/kg bw (Lackey, 1949). In male mice, the intraperitoneal LD₅₀ of technical-grade toxaphene was 42 mg/kg bw. The intraperitoneal LD₅₀ values of two toxic fractions were 3.1 and 6.6 mg/kg bw (Khalifa *et al.*, 1974); the first was identified by nuclear magnetic resonance spectroscopy as a mixture of 2,2,5-*endo*, 6-*exo*, 8,8,9,10-octachlorobornane and 2,2,5-*endo*, 6-*exo*, 8,9,9,10-octachlorobornane (Turner *et al.*, 1975) and the second as 2,2,5-*endo*, 6-*exo*, 8,9,10-heptachlorobornane (Casida *et al.*, 1974).

In Sherman rats fed diets containing 50 or 200 mg/kg toxaphene for 2–9 months, centrilobular hypertrophy of liver cells was observed in 3 of 11 and 6 of 12 animals, respectively (Ortega *et al.*, 1957); however, no effects on liver-cell histology were observed by Clapp *et al.* (1971) in rats fed a diet containing up to 189 mg/kg for 12 weeks.

Administration of a diet containing 5, 50 or 500 mg/kg toxaphene to quail for up to 4 months produced hypertrophy of the thyroid, with increased uptake of ¹³¹I and adrenal hypertrophy (Hurst *et al.*, 1974).

Toxaphene fed to female weanling Swiss-Webster mice in the diet at a concentration of 10, 100 or 200 mg/kg for 8 weeks depressed immunoglobulin G antibody formation at the two higher doses, but cell-mediated immune responses were not affected. In another experiment, mature female mice fed diets containing the same amounts of toxaphene were mated 3 weeks after feeding began and were maintained on the diets until 3 weeks after parturition, at which time the pups were weaned onto the control diet. Assays performed on the offspring 8 weeks after birth revealed suppressed antibody formation in the offspring of dams given 100 mg/kg of diet toxaphene and enhanced antibody formation in those of dams given 200 mg/kg of diet. The cell-mediated immune response was suppressed only at the intermediate concentration. The phagocytic capacity of macrophages was significantly reduced in all treated groups, but to a greater extent in the offspring of the mice that consumed toxaphene at 100 mg/kg of diet (Allen *et al.*, 1983).

In estrogen-sensitive MCF7 human breast cancer cells, toxaphene increased cell proliferation only at a concentration of 10 $\mu\text{mol/L}$ and not at lower concentrations. In comparison, estradiol caused proliferation at 10 pmol/L (Soto *et al.*, 1994).

In MCF-7 human breast cancer cells treated with 10 $\mu\text{mol/L}$ toxaphene for 48 h, approximately 60% and 80% inhibition of constitutive and 17 β -estradiol-induced estrogen receptor-dependent transactivation, respectively, were observed. The involvement of the estrogen receptor in the ability of toxaphene to block estrogen activity was verified by cotransfection studies with estrogen receptor-negative MDA-MB-231 cells. The interference of toxaphene with the estrogen receptor-mediated responses was confirmed by the observation of significant suppression of endogenously expressed pS2 RNA and decreased secretion of pS2 protein. These results indicate that toxaphene disturbed hormonal signals mediated by the estrogen receptor (Bonefeld Jørgensen *et al.*, 1997).

In transfection experiments, toxaphene antagonized estrogen-related receptor α -1 (ERR α -1) expression of the reporter chloramphenicol acetyltransferase activity in SK-BR-3 breast cancer cells. ERR α -1 is a member of the orphan nuclear receptor family, since its ligand has not been identified. Toxaphene was also found to suppress aromatase activity through an ERR α -1-mediated mechanism (Yang & Chen, 1999).

Toxaphene was active in a variety of in-vitro assay systems with the androgen receptor (Schrader & Cooke, 2000) and estrogen receptors (Yang & Chen, 1999). Toxaphene and two congeners prevalent in humans can stimulate proliferation of MCF7-E3 human breast cancer cells (Stelzer & Chan, 1999), but this result was not found consistently: Arcaro *et al.* (2000) reported that toxaphene was weakly anti-estrogenic in the MCF7 focus assay and that it did not stimulate cell proliferation.

Groups of 10 male and 10 female Sprague-Dawley rats were fed a diet containing 0, 4, 20, 100 or 500 mg/kg toxaphene for 13 weeks, corresponding to intakes of 0.35–45.9 mg/kg bw per day for males and 0.50–63 mg/kg bw per day for females. No clinical signs of toxicity, such as effects on weight gain or food consumption, or deaths were observed, but effects on the liver (see section 4.4.2) were seen in males

and females at the highest dietary concentration, at which toxaphene also caused kidney enlargement in male but not female rats and dose-dependent histological changes in the kidney, thyroid and liver. The changes in the liver and thyroid were considered to be adaptative, but the injury in the proximal tubules of the kidney was focally severe. The changes in the thyroid included increased epithelial height with multifocal papillary proliferation and reduced colloid density. These treatment-related changes were observed at concentrations ≥ 20 mg/kg of diet in males but only at 500 mg/kg of diet in females, and were considered to be more severe in the males. The kidneys of males at 20 mg/kg of diet had large eosinophilic inclusions in the proximal tubules. In the kidneys of rats at 100 or 500 mg/kg of diet, these inclusions were smaller, more refractive in appearance and more prevalent, occupying 50% of the tubular area. The changes were accompanied by anisokaryosis and focal tubular necrosis. In the females, only mild changes were found (Chu *et al.*, 1986).

Groups of six male and six female beagle dogs were given toxaphene in gelatin capsules at 0, 0.2, 2 or 5 mg/kg bw per day for 13 weeks. Food consumption and growth rate were not affected, and all animals survived the treatment period. No clinical signs of toxicity were observed. The liver:body weight ratio and serum alkaline phosphatase activity were increased in both males and females at the highest dose. Mild-to-moderate, dose-dependent histological changes were observed in the liver and thyroid. The effects in the thyroid were similar to those seen in rats. Toxaphene accumulated in a dose-dependent manner in the fat and liver of both dogs and rats. On the basis of these findings, the no-observed-adverse-effect levels of the pesticide were considered to be 0.35 mg/kg bw per day for rats and 0.2 mg/kg bw per day for dogs (Chu *et al.*, 1986).

Forty male Sprague-Dawley Crl:CD BR rats were given 100 mg/kg bw per day technical-grade toxaphene in corn oil by gavage for 3 days, at which time the dose was reduced to 75 mg/kg bw per day because of toxicity. The lower dose was administered daily for 25 days. Another group of 40 male rats were given equivalent volumes of corn oil. A blood sample was obtained on days 0, 7, 14 and 28 from each of 10 treated and 10 vehicle-control animals to determine the serum concentrations of thyroid-stimulating hormone (TSH), thyroxine (T4), triiodothyronine (T3) and reverse T3 (rT3) (see Figure 1 in General Remarks). Significant, time-related increases in serum TSH concentration were found on days 7, 14 and 28, and, at the last two times, the concentration was increased about two- and threefold. The serum concentrations of T3, T4 and rT3 and the thyroid gland weights and the thyroid:brain weight ratios in the treated group were not significantly different from those of controls at any time. The degree of thyroid follicular-cell hypertrophy and intrafollicular hyperplasia increased and the thyroid follicular-cell colloid stores decreased with duration of treatment with toxaphene (Waritz *et al.*, 1996).

The effects of organochlorine pesticide exposure on the chemotactic functions of rhesus monkey (*Macaca mulatta*) neutrophils and monocytes were 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 toward RANTES (Miyagi *et al.*, 1998).

4.3 Reproductive and developmental effects

4.3.1 *Humans*

No data were available to the Working Group.

4.3.2 *Experimental systems*

Administration of a diet containing 25 mg/kg toxaphene to mice through five generations caused no embryotoxic or teratogenic effects (Keplinger *et al.*, 1968). In a three-generation study of reproductive toxicity, Sprague-Dawley rats received a diet containing either 25 or 100 mg/kg toxaphene; no effects on litter size, pup survival, weanling body weights or reproductive capacity were observed (Kennedy *et al.*, 1973). In a standard, two-generation study of reproductive toxicity in Sprague-Dawley rats, administration of a diet containing toxaphene at a concentration of 0, 4, 20, 100 or 500 mg/kg [estimated intake, 0.29–49 mg/kg bw per day] did not affect litter size, pup weight or weight gain, fertility, gestation or neonatal survival. Toxic effects were seen in adult animals exposed for 2 weeks to the two highest concentrations, and the highest concentration also decreased weight gain, but did not affect food intake. The liver weights of F₀ and F₁ animals were increased. No effects were seen on the reproductive tissues of F₀ animals. Morphological changes were observed in the thyroid, liver and kidney, and groups at all concentrations had reduced follicle size and other histological changes; however, the authors noted the absence of a dose-dependent effect for many of these observations [the data were not adequate to evaluate this conclusion]. Follicular hyperplasia was described in one F₁ female and two F₁ male rats and an adenoma in one F₀ male at the highest concentration (Chu *et al.*, 1988).

Toxaphene was administered by oral intubation to CD-1 mice and CD rats during the period of embryonic organogenesis (days 7–16 of gestation) at a dose of 0, 15, 25 or 35 mg/kg bw per day. The highest dose produced caused maternal mortality in rats (31%) and mice (8%) and an increase in the incidence of encephaloceles among the offspring of the mice. The fetal mortality rate was slightly increased in mice at all three doses. Small decreases in fetal body weight and in the number of sternal and caudal ossification centres were seen in rats, mostly in the group receiving 25 mg/kg bw per day (Chernoff & Carver, 1976).

Sprague-Dawley rats ($n = 25$) were treated with 32 mg/kg bw per day toxaphene by gavage on days 6–15 of gestation. This high dose caused the deaths of 50% of the

dams before parturition but was chosen deliberately to test the hypothesis that developmental toxicity would be observed with a compound known to be toxic to the dam. Treatment reduced the weight gain of dams during gestation, although they had gained weight similarly to controls by the time of parturition. Only six of the toxaphene-treated animals delivered litters, in which a significant increase in the incidence of supernumerary ribs were found as compared with controls (Chernoff *et al.*, 1990).

Injection of 1.5 mg/egg toxaphene had no effect on the hatchability rates of the eggs of chickens (Smith *et al.*, 1970). In a similar study, no embryotoxicity was observed in chicken embryos hatched from eggs previously injected with 400 or 500 mg/kg toxaphene in acetone; although when it was dissolved in corn oil embryotoxicity was seen at 300–400 mg/kg (Dunachie & Fletcher, 1969).

Toxaphene did not induce sex reversal in a temperature-dependent test in the slider turtle (*Trachemys scripta elegans*) (Willingham & Crews, 1999).

In a study of the behavioural effects of pre- and postnatal exposure to toxaphene, Holtzman rats were fed a diet providing a dose of 0.05 mg/kg bw per day. Retarded neurodevelopment on days 7–17 and impaired performance in learning and retaining in a symmetrical maze test were found (Olson *et al.*, 1980).

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

In early experiments, alterations in serum alkaline phosphatase and acid phosphatase activity, indicating liver damage, were observed in rats fed toxaphene (Grebnyuk, 1970; Gertig & Nowaczyk, 1975). Toxaphene induces various hepatic microsomal enzymes, such as *O*- and *N*-demethylases (Kinoshita *et al.*, 1966) and androgen hydroxylase (Peakall, 1976); it also stimulates estrone metabolism in rats (Welch *et al.*, 1971). Phenobarbital sleeping times were reduced in rats given toxaphene orally by gavage (Schwabe & Wendling, 1967).

Adult male Sprague-Dawley rats were fed diets containing 0, 50, 100, 150 or 200 mg/kg toxaphene for 14 days. There were no signs of toxicity, but the liver weight was significantly increased and the thymus weight was decreased in all treated groups (Trottman & Desaiyah, 1980). A similar effect on relative liver weight was found in young (70 g) Sprague-Dawley rats given an intraperitoneal injection of toxaphene at a dose of 0, 5, 25 or 100 mg/kg bw per day for 5 days. All doses increased the liver:body weight ratios, cytochrome P450 (CYP) enzyme activity, aminopyrine demethylation and aldrin epoxidation. The latter activity was increased nearly sevenfold at the highest dose (Pollock *et al.*, 1983).

Groups of 10 male and 10 female Sprague-Dawley rats were fed a diet containing 0, 4, 20, 100 or 500 mg/kg toxaphene for 13 weeks, corresponding to intakes of 0.35–45.9 mg/kg bw per day for males and 0.50–63 mg/kg bw per day for females. The liver:body weight ratio and the activities of hepatic microsomal enzymes (phenobarbital type) were increased in both males and females at the highest dietary concentration (Chu *et al.*, 1986).

The activities of pentobarbital hydroxylase and aniline hydroxylase were significantly enhanced in rats exposed to toxaphene, and that of ethylmorphine-*N*-demethylase was elevated. Enhanced hydroxylation of pentobarbital was evident from the decreased sleeping time seen after administration of the two compounds. Exposure to toxaphene increased the activities of CYP isozymes, NADPH-cytochrome c-reductase and dehydrogenase in hepatic microsomal fractions (Trottman & Desaiah, 1980). Toxaphene also induced UDP glucuronosyl transferase and aryl hydrocarbon hydroxylase activity in Sprague-Dawley rats given 20 mg/kg bw orally twice a week for 2 weeks (Thunberg *et al.*, 1984).

Male CD-1 mice given 10, 25, 50 or 100 mg/kg bw per day toxaphene by gavage for 7 days also showed increased liver weight, liver:body weight ratio, total hepatic CYP content and cytochrome *b*₅. No increase in the activity of immunodetectable CYP4A1 was found, in contrast to the high levels of this enzyme found in clofibrate-exposed mice. However, increases in CYP2B activity were found, indicating induction by toxaphene of phenobarbital-inducible CYP enzymes. No DNA adducts were found by ³²P-postlabelling methods in this study (Hedli *et al.*, 1998).

Toxaphene at 200 µmol/L stimulated mouse brain protein kinase C activity in the 10⁵ × *g* supernatant of brain tissue to a maximum velocity almost equal to that obtained when the enzyme was maximally stimulated with the skin tumour-promoting phorbol ester, 12-*O*-tetradecanoylphorbol-13-acetate (Moser & Smart, 1989).

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 (concentration, 10–50 µmol/L) on retinoblastoma (*Rb*) gene expression. The lowest concentration of toxaphene tested reduced *Rb* protein levels. Analysis of *Rb* mRNA revealed no detectable difference over the same concentration range, suggesting that *Rb* expression is down-regulated at the post-transcriptional level (Rought *et al.*, 1999).

4.5 Genetic and related effects

The genetic toxicity of toxaphene has been reviewed (Saleh, 1991).

4.5.1 Humans

Cultured peripheral blood lymphocytes were examined from eight women working in an area that had been sprayed from aircraft with toxaphene at 2 kg/ha and an

unspecified number of control individuals. The incidence of chromosomal aberrations (acentric fragments and chromosomal exchanges) was 13.1% in the exposed group and 1.6% in the controls (Samosh, 1974).

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

Toxaphene was shown to induce gene mutations in several studies with *Salmonella typhimurium* strains with and without metabolic activation. It also induced prophage lambda, but not alkali-labile sites in an *Escherichia coli* plasmid assay. It induced reverse mutation in a photoluminescence assay. In cultured mammalian cells, it did not induce gene mutations at the *Hprt* locus or sister chromatid exchange in Chinese hamster lung V79 cells in single studies, whereas it did induce sister chromatid exchange in Chinese hamster lung Don cells and human lymphoid LAZ-007 cells and micronuclei in beluga whale skin fibroblasts. Toxaphene also inhibited gap-junctional intercellular communication in Chinese hamster lung V79 cells and human primary breast cancer cells.

Toxaphene administered to mice *in vivo* did not bind covalently to liver-cell DNA or induce dominant lethal mutation in males.

4.6 Mechanistic considerations

The results of some tests for genotoxicity with toxaphene were positive. Toxaphene is also a well-known inducer of hepatic microsomal enzymes in rodents, especially phenobarbital-type CYP2B and UDP-glucuronosyl transferase. Administration of toxaphene by gavage to male rats at a dose somewhat higher than the thyroid tumorigenic dose but for a shorter time resulted in increased concentrations of TSH and changes in the thyroid gland including hypertrophy, diffuse hyperplasia and decreased colloid. No effects on T4 or T3 were found in this study. These findings are consistent with the hypothesis that thyroid tumours are produced in rats secondary to increased turnover of thyroid hormones and increased trophic stimulation by TSH secondary to induction of UDP-glucuronosyl transferase, resulting in increasing hepatic disposition of thyroid hormone. No definitive conclusion could be reached about the mechanism of tumour production, in view of the results of the assays for genotoxicity.

Toxaphene has been shown to produce hypertrophy of liver cells without effects on their histological appearance, but the relationship of this finding to the production of liver tumours in mice has not been established.

The results of the tests for genetic toxicity conducted with toxaphene do not provide strong evidence that induction of genetic damage is important in its carcinogenicity, but the possibility cannot be excluded. An important gap in the database is the results of a test for chromosomal aberrations in rodent cells *in vitro* or *in vivo*. Deterioration in gap-junctional intercellular communication could play some role in the carcinogenic process.

Table 3. Genetic and related effects of toxaphene

Test system	Result ^a		Dose ^b (LED/HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
<i>Escherichia coli</i> WP-2, prophage ϕ induction	+	+	40 ^c	Houk & DeMarini (1987)
<i>Escherichia coli</i> plasmid ColE1 DNA strand breaks or alkali-labile sites	-	NT	100	Griffin & Hill (1978)
<i>Salmonella typhimurium</i> TA100, TA98, reverse mutation	+	+	500 μ g/plate	Hooper <i>et al.</i> (1979)
<i>Salmonella typhimurium</i> TA100, reverse mutation	+	+	100 μ g/plate	Mortelmans <i>et al.</i> (1986)
<i>Salmonella typhimurium</i> TA98, reverse mutation	+	+	333 μ g/plate	Mortelmans <i>et al.</i> (1986)
<i>Salmonella typhimurium</i> TA100, TA98, reverse mutation	+	+	500 μ g/plate	Schrader <i>et al.</i> (1998)
<i>Salmonella typhimurium</i> TA104, TA97, reverse mutation	+	(+)	1000 μ g/plate	Schrader <i>et al.</i> (1998)
<i>Salmonella typhimurium</i> TA102, reverse mutation	(+)	(+)	10 000 μ g/plate	Schrader <i>et al.</i> (1998)
<i>Salmonella typhimurium</i> TA1535, TA1537, reverse mutation	-	-	1000 μ g/plate	Mortelmans <i>et al.</i> (1986)
<i>Vibrio fischeri</i> dim variant, reverse mutation	+	-	3150	Boon <i>et al.</i> (1998)
Gene mutation, Chinese hamster lung V79 cells, <i>Hprt</i> locus <i>in vitro</i>	-	- ^d	10	Schrader <i>et al.</i> (1998)
Sister chromatid exchange, Chinese hamster lung V79 cells <i>in vitro</i>	-	- ^d	10	Schrader <i>et al.</i> (1998)
Sister chromatid exchange, Chinese hamster lung Don cells <i>in vitro</i>	+	NT	5	Steinel <i>et al.</i> (1990)
Micronucleus formation, beluga whale skin fibroblasts <i>in vitro</i>	+	? ^e	0.05	Gauthier <i>et al.</i> (1999)
Sister chromatid exchange, human lymphoid LAZ-007 cells <i>in vitro</i>	+	+	4	Sobti <i>et al.</i> (1983)
Inhibition of intercellular communication, Chinese hamster lung V79 cells <i>in vitro</i>	+	NT	3	Trosko <i>et al.</i> (1987)
Inhibition of intercellular communication (dye transfer), human primary breast epithelial cells <i>in vitro</i>	+	NT	5	Kang <i>et al.</i> (1996)

TOXAPHENE

Table 3 (contd)

Test system	Result ^a		Dose ^b (LED/HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Dominant lethal mutation, ICR/Ha mice <i>in vivo</i>	–		180 ip × 1 or 80 po × 5	Epstein <i>et al.</i> (1972)
Covalent binding to DNA (³² P-postlabelling) male CD-1 (Swiss) mouse liver <i>in vivo</i>	–		100 po × 7	Hedli <i>et al.</i> (1998)

^a +, positive; (+), weak positive; –, negative; NT, not tested; ?, inconclusive

^b LED, lowest effective dose; HID, highest ineffective dose; in-vitro tests, µg/mL; in-vivo tests, mg/kg bw per day; ip, intraperitoneal; po, orally

^c With an exogenous metabolic activation system from 9000 × g supernatant of rodent liver (S9), active only at ≥ 4-fold higher dose

^d Metabolic activation provided by co-cultured HepG2 human hepatoma cells

5. Summary of Data Reported and Evaluation

5.1 Exposure data

Toxaphene is a complex mixture of chlorinated hydrocarbons produced by the chlorination of camphene. Toxaphene was widely used from the late 1940s as an insecticide on crops and to control parasites on livestock. The use of toxaphene is presently banned or restricted in many countries. Occupational exposure to toxaphene has occurred during its production and application. Human exposure to toxaphene is still possible owing to its persistence in the environment and its consequent continuing occurrence in fish, milk and other foodstuffs. In those countries in which its use has been banned, dietary intake has probably decreased in recent years.

5.2 Human carcinogenicity data

One case-control study of non-Hodgkin lymphoma and one of leukaemia not otherwise specified in the same populations showed no significant increase in risk associated with exposure to toxaphene.

5.3 Animal carcinogenicity data

Toxaphene has been tested for carcinogenicity by oral administration in one study in mice and one study in rats. It increased the incidence of hepatocellular adenomas and carcinomas combined in male and female mice. In rats, it produced thyroid follicular-cell adenomas and carcinomas in both males and females and pituitary adenomas in females.

5.4 Other relevant data

Toxaphene is lipid-soluble and accumulates in animals. It is metabolized by dechlorination and excreted into the bile. Toxaphene is a well-known microsomal enzyme inducer that increases phase I and II drug-metabolizing enzymes, consistent with a phenobarbital-like effect. It also increases the size of the thyroid gland and thyroid-stimulating hormone concentrations.

Toxaphene produced hepatotoxicity and immunotoxicity in experimental animals.

No reproductive or developmental effects were seen in three multigeneration studies in rats.

An increased frequency of chromosomal aberrations was observed in the lymphocytes of workers exposed to toxaphene in one study. In mammalian cells *in vivo*, toxaphene did not bind to DNA or produce dominant lethal mutations. *In vitro*, toxaphene was mutagenic to bacteria but did not induce mutations in mammalian cells. It induced micronuclei in the only assay for this end-point performed in mammalian cells.

It also induced sister chromatid exchange and inhibited gap-junctional intercellular communication in cultured mammalian cells.

5.5 Evaluation

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

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

Overall evaluation

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

6. References

- Agency for Toxic Substances and Disease Registry (1998) *Toxicological Profile for Toxaphene* (Report 95/10; US NTIS PB97-121057), Atlanta GA, Department of Health and Human Services
- Allen, A.L., Koller, L.D. & Pollock, G.A. (1983) Effect of toxaphene exposure on immune responses in mice. *J. Toxicol. environ. Health*, **11**, 61–69
- American Conference of Governmental Industrial Hygienists (2000) *TLVs and Other Occupational Exposure Values — 2000 CD-ROM*, Cincinnati, OH
- 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., Washington DC
- 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., Washington DC
- 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., Washington DC
- Andrews, P., Newsome, W.H., Boyle, M. & Collins, P. (1993) High resolution selective ion monitoring GC–MS determination of toxaphene in Great Lakes fish. *Chemosphere*, **27**, 1865–1872
- 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]
- Arcaro, K.F., Yang, Y., Vakharia, D.D. & Gierthy, J.F. (2000) Toxaphene is antiestrogenic in a human breast-cancer cell assay. *J. Toxicol. environ. Health*, **59**, 197–210

- Ashirova, S.A. (1971) [Work hygiene and effectiveness of health measures in the manufacture of chlorinated terpenes.] *Nauch. Tr. Leningrad Gos. Inst. Usoversh. Vrach.*, **98**, 26–30 (in Russian)
- Ault, J.A. & Spurgeon, T.E. (1984) Multiresidue gas chromatographic method for determining organochlorine pesticides in poultry fat: Collaborative study. *J. Assoc. off. anal. Chem.*, **67**, 284–289
- Barrie, L., Bidleman, T., Dougherty, D., Fellin, P., Grift, N., Muir, D., Rosenberg, B., Stern, G. & Toom, D. (1993) Atmospheric toxaphene in the high Arctic. *Chemosphere*, **27**, 2037–2046
- Bateman, G.Q., Biddulph, C., Harris, J.R., Greenwood, D.A. & Harris, L.E. (1953) Transmission studies of milk of dairy cows fed toxaphene-treated hay. *J. agric. Food Chem.*, **1**, 322–324
- Blair, A. & Hoar Zahm, S. (1993) Patterns of pesticide use among farmers: Implications for epidemiologic Research. *Epidemiology*, **4**, 55–62
- Blus, L.J., Joanen, T., Belisle, A.A. & Prouty, R.M. (1975) The brown pelican and certain environmental pollutants in Louisiana. *Bull. environ. Contam. Toxicol.*, **13**, 646–655
- Bonefeld Jørgensen, E.C., Autrup, H. & Hansen, J.C. (1997) Effect of toxaphene on estrogen receptor functions in human breast cancer cells. *Carcinogenesis*, **18**, 1651–1654
- Boon, J.P., Sleiderink, H.M., Helle, M.S., Dekker, M., van Schanke, A., Roex, E., Hillebrand, M.T.J., Klamer, H.J.C., Govers, B., Pastor, D., Morse, D., Wester, P.G. & de Boer, J. (1998) The use of a microsomal in vitro assay to study phase I biotransformation of chlorobornanes (toxaphene®) in marine mammals and birds. *Comp. Biochem. Physiol. Part C*, **121**, 385–403
- Boshoff, P.R. & Pretorius, V. (1979) Determination of toxaphene in milk, butter and meat. *Bull. environ. Contam. Toxicol.*, **22**, 405–412
- Boyd, E.M. & Taylor, F.I. (1971) Toxaphene toxicity in protein-deficient rats. *Toxicol. appl. Pharmacol.*, **18**, 158–167
- 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
- Brumley, W.C., Brownrigg, C.M. & Grange, A.H. (1993) Determination of toxaphene in soil by electron-capture negative-ion mass-spectrometry after fractionation by high-performance gel-permeation chromatography. *J. Chromatogr.*, **633**, 177–183
- Budavari, S., ed. (2000) *The Merck Index*, 12th Ed., Version 12:3, Whitehouse Station, NJ, Merck & Co. & Boca Raton, FL, Chapman & Hall/CRC [CD-ROM]
- Cairns, T., Siegmund, E.G. & Froberg, J.E. (1981) Chemical ionization mass spectrometric examination of metabolized toxaphene from milk fat. *Biomed. Mass Spectrom.*, **8**, 569–574
- 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
- Carey, A.E., Wiersma, G.B. & Tai, H. (1976) Pesticide residues in urban soils from 14 United States cities, 1970. *Pestic. Monit. J.*, **10**, 54–60
- Casida, J.E., Holmstead, R.L., Khalifa, S., Knox, J.R., Ohsawa, T., Palmer, K.J. & Wong, R.Y. (1974) Toxaphene insecticide: A complex biodegradable mixture. *Science*, **183**, 520–521
- Causey, K., McIntyre, S.C., Jr & Richburg, R.W. (1972) Organochlorine insecticide residues in quail, rabbits, and deer from selected Alabama soybean fields. *J. agric. Food Chem.*, **20**, 1205–1209

- Chan, H.M. (1998) A database for environmental contaminants in traditional foods in northern and Arctic Canada: Development and applications. *Food. Addit. Contam.*, **15**, 127–134
- Chandurkar, P.S. & Matsumura, F. (1979a) Metabolism of toxaphene components in rats. *Arch. environ. Contam. Toxicol.*, **8**, 1–24
- Chandurkar, P.S. & Matsumura, F. (1979b) Metabolism of toxicant B and toxicant C of toxaphene in rats. *Bull. environ. Contam. Toxicol.*, **21**, 539–547
- Chernoff, N. & Carver, B.D. (1976) Fetal toxicity of toxaphene in rats and mice. *Bull. environ. Contam. Toxicol.*, **15**, 660–664
- Chernoff, N., Setzer, R.W., Miller, D.B., Rosen, M.B. & Rogers, J.M. (1990) Effects of chemically induced maternal toxicity on prenatal development in the rat. *Teratology*, **42**, 651–658
- Chu, I., Villeneuve, D.C., Sun, C.-W., Secours, V., Procter, B., Arnold, E., Clegg, D., Reynolds, L. & Valli, V.E. (1986) Toxicity of toxaphene in the rat and beagle dog. *Fundam. appl. Toxicol.*, **7**, 406–418
- Chu, I., Secours, V., Villeneuve, D.C., Valli, V.E., Nakamura, A., Colin, D., Clegg, D.J. & Arnold, E.P. (1988) Reproduction study of toxaphene in the rat. *J. environ. Sci. Health*, **B23**, 101–126
- CIS Information Services (2000) *Directory of World Chemical Producers (Version 2000.1)*, Dallas, TX [CD-ROM]
- Clapp, K.L., Nelson, D.M., Bell, J.T. & Rousek, E.J. (1971) Effect of toxaphene on the hepatic cells of rats. In: *Proceedings of Annual Meeting, Western Section, American Society of Animal Science*, Vol. 22, Fresno, CA, Fresno State College, pp. 313–323
- Conley, B.E. (1952) Pharmacologic properties of toxaphene, a chlorinated hydrocarbon insecticide. *J. Am. med. Assoc.*, **149**, 1135–1137
- Crist, H.L., Harless, R.L., Moseman, R.F. & Callis, M.H. (1980) Application of dehydrochlorination to the determination of toxaphene in soil and identification of the major gas chromatographic peak. *Bull. environ. Contam. Toxicol.*, **24**, 231–237
- Crowder, L.A. & Dindal, E.F. (1974) Fate of ³⁶Cl-toxaphene in rats. *Bull. environ. Contam. Toxicol.*, **12**, 320–327
- Datta, S., Ohyama, K., Dunlap, D.Y. & Matsumura, F. (1999) Evidence of organochlorine contamination in tissues of salmonids in Lake Tahoe. *Ecotoxicol. environ. Saf.*, **42**, 94–101
- Deutsche Forschungsgemeinschaft (2000) *List of MAK and BAT Values 1999* (Report No. 36), Weinheim, Wiley-VCH Verlag GmbH, p. 37
- Dunachie, J.F. & Fletcher, W.W. (1969) An investigation of the toxicity of insecticides to birds' eggs using the egg-injection technique. *Ann. appl. Biol.*, **64**, 409–423
- Eller, P.M., ed. (1994) Method 5039. 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 (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 organohalide 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 (1996a) Method 8081A. Organochlorine pesticides by gas chromatography [Rev. 1]. In: *Test Methods for Evaluating Solid Waste — Physical/Chemical Methods* (US EPA 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* (US EPA 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* (US EPA 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/multidetector 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) 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 (1999c) 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., 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

- FAO/UNEP (1999) *Prior Informed Consent Decision Guidance Documents: Binapacryl and Toxaphene*, Rotterdam Convention on the Prior Informed Consent Procedure for Certain Hazardous Chemicals and Pesticides in International Trade, Geneva
- 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., Management Methods Branch, Washington DC
- Fowler, B., Hoover, D. & Hamilton, M.C. (1993) The quantification of toxaphene in environmental samples. *Chemosphere*, **27**, 1891–1905
- Gaines, T.B. (1960) The acute toxicity of pesticides to rats. *Toxicol. appl. Pharmacol.*, **2**, 88–99
- 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
- Gertig, H. & Nowaczyk, W. (1975) The influence of carathane and toxaphene on the activity of some enzymes in rat's tissues in the studies *in vivo*. *Pol. J. Pharmacol. Pharm.*, **27**, 357–364
- Gomes, E.D. (1977) Determination of toxaphene by basic alcoholic hydrolysis and Florisil separation. *Bull. environ. Contam. Toxicol.*, **17**, 456–462
- Goodman, J.I., Brusick, D.J., Busey, W.M., Cohen, S.M., Lamb, J.C. & Starr, T.B. (2000) Reevaluation of the cancer potency factor of toxaphene: Recommendations from a peer review panel. *Toxicol. Sci.*, **55**, 3–16
- Grebenyuk, S.S. (1970) [Effect of polychlorocamphene on liver functions.] *Gig. Primen. Toksikol. Pestits. Klin. Otravlenii*, **8**, 166–169 (in Russian)
- Griffin, D.E., III & Hill, W.E. (1978) In vitro breakage of plasmid DNA by mutagens and pesticides. *Mutat. Res.*, **52**, 161–169
- Griffith, F.D., Jr & Blanke, R.V. (1974) Microcoulometric determination of organochlorine pesticides in human blood. *J. Assoc. off. anal. Chem.*, **57**, 595–603
- Haun, E.C. & Cueto, C., Jr (1967) Fatal toxaphene poisoning in a 9-month-old-infant. *Am. J. Dis. Child.*, **113**, 616–618
- Head, S.L. & Burse, V.W. (1987) Organochlorine recovery from small adipose samples with the universal trace residue extractor (Unitrex). *Bull. environ. Contam. Toxicol.*, **39**, 848–856
- Hedli, C.C., Snyder, R., Kinoshita, F.K. & Steinberg, M. (1998) Investigation of hepatic cytochrome P-450 enzyme induction and DNA adduct formation in male CD1 mice following oral administration of toxaphene. *J. appl. Toxicol.*, **18**, 173–178
- Ho, J.S., Tang, P.H., Eichelberg, J.W. & Budde, W.L. (1995) Liquid–solid disk extraction followed by SFE and GC–ion-trap MS for the determination of trace organic pollutants in water. *J. chromatogr. Sci.*, **33**, 1–8
- Hogue, C. (2000) Dioxins to be major issue at treaty negotiations. *Chem. Eng. News*, **20 March**, 35
- Hooper, N.K., Ames, B.N., Saleh, M.A. & Casida, J.E. (1979) Toxaphene, a complex mixture of polychloroterpenes and a major insecticide, is mutagenic. *Science*, **205**, 591–593
- Houk, V.S. & DeMarini, D.M. (1987) Induction of prophase lambda by chlorinated pesticides. *Mutat. Res.*, **182**, 193–201
- Hsu, J.P., Schattenberg, H.J. & Garza, M.M. (1991) Fast turnaround multiresidue screen for pesticides in produce? *J. Assoc. off. anal. Chem.*, **74**, 886–892
- Hunt, D.F., Shabanowitz, J., Harvey, T.M. & Coates, M. (1985) Scheme for the direct analysis of organics in the environment by tandem mass spectrometry. *Anal. Chem.*, **57**, 525–537

- Hurst, J.G., Newcomer, W.S. & Morrison, J.A. (1974) Some effects of DDT, toxaphene and polychlorinated biphenyl on thyroid function in bobwhite quail. *Poultry Sci.*, **53**, 125–133
- IARC (1979) *IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans*, Vol. 20, *Some Halogenated Hydrocarbons*, IARC Press, Lyon, pp. 327–348
- IARC (1987) *IARC Monographs on the Evaluation of Carcinogenic Risks to Humans*, Suppl. 7, *Overall Evaluations of Carcinogenicity: An Updating of IARC Monographs Volumes 1–42*, IARC Press, Lyon, p. 72
- Ismail, R.J. & Bonner, F.L. (1974) New, improved thin layer chromatography for polychlorinated biphenyls, toxaphene, and chlordane components. *J. Assoc. off. anal. Chem.*, **57**, 1026–1032.
- Jansson, B., Andersson, R., Asplund, L., Bergman, A., Litzén, K., Nylund, K., Reutergrårdh, L., Sellström, U., Uvemo, U.-B., Wahlberg, C. & Wideqvist, U. (1991) Multiresidue method for the gas-chromatographic analysis of some polychlorinated and polybrominated pollutants in biological samples. *Fresenius J. anal. Chem.*, **340**, 439–445
- Jarnuzi, G. & Wakimoto, T. (1991) Determination of chlorinated pinene originated from pulp mill. *Analyt. Sci.*, **7** (Suppl.), 1177–1180
- Kang, K.-S., Wilson, M.R., Hayashi, T., Chang, C.-C. & Trosko, J.E. (1996) Inhibition of gap junctional intercellular communication in normal human breast epithelial cells after treatment with pesticides, PCBs, and PBBs, alone or in mixtures. *Environ. Health Perspectives*, **104**, 192–200
- Kennedy, G.L., Jr, Frawley, J.P. & Calandra, J.C. (1973) Multigeneration reproductive effects of three pesticides in rats. *Toxicol. appl. Pharmacol.*, **25**, 589–596
- Keplinger, M.L., Deichmann, W.B. & Sala, F. (1968) Effects of combinations of pesticides on reproduction in mice. *Ind. Med. Surg.*, **37**, 525
- Khalifa S., Mon, T.R., Engel, J.L. & Casida, J.E. (1974) Isolation of 2,2,5-endo,6-exo,8,9,10-heptachlorobornane and an octachloro toxicant from technical toxaphene. *J. agric. Food Chem.*, **22**, 653–657
- Kinoshita, F.K., Frawley, J.P. & DuBois, K.P. (1966) Quantitative measurement of induction of hepatic microsomal enzymes by various dietary levels of DDT and toxaphene in rats. *Toxicol. appl. Pharmacol.*, **9**, 505–513
- Kutz, F.W., Yobs, A.R. & Yang, H.S.C. (1976) National pesticide monitoring programs. In: Lee, R.I., ed., *Air Pollution from Pesticide and Agricultural Processes*, Cleveland, OH, CRC Press, pp. 95–137 [cited in Agency for Toxic Substances and Disease Registry (1998)]
- Lackey, R.W. (1949) Observations on the acute and chronic toxicity of toxaphene in the dog. *J. ind. Hyg. Toxicol.* **31**, 117–120
- Maiorino, R.M., Whiting, F.M., Brown, W.H., Reid, B.L. & Stull, J.W. (1980) Quantitative determination of toxaphene in blood by Florisil extraction and gas chromatography. *J. anal. Toxicol.*, **4**, 192–198
- McGee, L.C., Reed, H.L. & Fleming, J.P. (1952) Accidental poisoning by toxaphene. Review of toxicology and case reports. *J. Am. med. Assoc.*, **149**, 1124–1126
- Ministry of Social Affairs and Health (1998) *Finnish Occupational Exposure Limits 1998. Concentrations Known to be Harmful*, Helsinki
- 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

- Mohammed, A., Andersson, Ö., Biessmann, A. & Slanina, P. (1983) Fate and specific tissue retention of toxaphene in mice. *Arch. Toxicol.*, **54**, 311–321
- Mohammed, A., Hallberg, E., Rydström, J. & Slanina, P. (1985) Toxaphene: Accumulation in the adrenal cortex and effect on ACTH-stimulated corticosteroid synthesis in the rat. *Toxicol. Lett.*, **24**, 137–143
- Mohammed, A., Eklund, A., Östlund-Lindqvist, A.-M. & Slanina, P. (1990) Tissue accumulation of lipoprotein associated toxaphene in normo- and hypolipidemic mice. *Arch. Toxicol.*, **64**, 38–42
- 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
- Munson, R.O. (1976) A note on toxaphene in environmental samples from the Chesapeake Bay region. *Bull. environ. Contam. Toxicol.*, **16**, 491–494
- Musial, C.J. & Uthe, J.F. (1983) Widespread occurrence of the pesticide toxaphene in Canadian East Coast marine fish. *Int. J. environ. anal. Chem.*, **14**, 117–126
- Mussalo-Rauhamaa, H., Pyysalo, H. & Antervo, K. (1988) Relation between the content of organochlorine compounds in Finnish human milk and characteristics of the mothers. *J. Toxicol. environ. Health*, **25**, 1–19
- National Cancer Institute (1979) *Bioassay of Toxaphene for Possible Carcinogenicity* (Technical Report Series No. 37), Bethesda, MD, Department of Health, Education, and Welfare
- 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
- Ohsawa, T., Knox, J.R., Khalifa, S. & Casida, J.E. (1975) Metabolic dechlorination of toxaphene in rats. *J. agric. Food Chem.*, **23**, 98–106
- Olson, K.L., Matsumura, F. & Boush, G.M. (1980) Behavioral effects on juvenile rats from perinatal exposure to low levels of toxaphene, and its toxic components, toxicant A, and toxicant B. *Arch. environ. Contam. Toxicol.*, **9**, 247–257
- Onuska, F.L., Terry, K.A., Seech, A. & Antonic, M. (1994) Determination of toxaphene in soil by electron-capture negative-ion mass spectrometry and capillary column gas chromatography. *J. Chromatogr.*, **A665**, 125–132
- Ortega, O., Hayes, W.J., Jr & Durham, W.F. (1957) Pathologic changes in the liver of rats after feeding low levels of various insecticides. *Arch. Pathol.*, **64**, 614–622
- Peakall, D.B. (1976) Effects of toxaphene on hepatic enzyme induction and circulating steroid levels in the rat. *Environ. Health Perspect.*, **13**, 117–120
- Petrick, G., Schulz, D.E. & Duinker, J.C. (1988) Clean-up of environment samples by high-performance liquid chromatography for analysis of organochlorine compounds by gas chromatography with electron-capture detection. *J. Chromatogr.*, **435**, 241–248
- Pollock, G.A. & Hillstrand, R. (1982) The elimination, distribution, and metabolism of ¹⁴C-toxaphene in the pregnant rat. *J. environ. Sci. Health*, **B17**, 635–648
- Pollock, G.A., Krasnec, J.P. & Niemann, B.R. (1983) Rat hepatic microsomal enzyme induction by pretreatment with toxaphene and toxaphene fractions. *J. Toxicol. environ. Health*, **11**, 355–363

- 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
- Saleh, M.A. (1991) Toxaphene: Chemistry, biochemistry, toxicity and environmental fate. *Rev. environ. Contam. Toxicol.*, **118**, 1–85
- Saleh, M.A. & Casida, J.E. (1977) Consistency of toxaphene composition analyzed by open tubular column gas–liquid chromatography. *J. agric. Food Chem.*, **25**, 63–68
- Saleh, M.A., Skinner, R.F. & Casida, J.E. (1979) Comparative metabolism of 2,2,5-*endo*,6-*exo*,8,9,10-heptachlorobornane and toxaphene in six mammalian species and chickens. *J. agric. Food Chem.*, **27**, 731–737
- Samosh, L.V. (1974) Chromosome aberrations and character of satellite associations after accidental exposure of the human body to polychlorocamphene. *Cytol. Genet.*, **8**, 23–27
- 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
- Schrader, T.J. & Cooke, G.M. (2000) Examination of selected food additives and organochlorine food contaminants for androgenic activity *in vitro*. *Toxicol. Sci.*, **53**, 278–288
- Schrader, T.J., Boyes, B.G., Matula, T.I., Héroux-Metcalf, C., Langlois, I. & Downie, R.H. (1998) *In vitro* investigation of toxaphene genotoxicity in *S. typhimurium* and Chinese hamster V79 lung fibroblasts. *Mutat. Res.*, **413**, 159–168
- Schwabe, U. & Wendling, I. (1967) [Stimulation of drug metabolism by low doses of DDT and other chlorinated hydrocarbon insecticides.] *Arzneimittel-Forsch.*, **17**, 614–618 (in German)
- Seiber, J.N., Landrum, P.F., Madden, S.C., Nugent, K.D. & Winterlin, W.L. (1975) Isolation and gas chromatographic characterization of some toxaphene components. *J. Chromatogr.*, **114**, 361–368
- Shoeib, M., Brice, K.A. & Hoff, R.M. (1999) Airborne concentrations of toxaphene congeners at Point Petre (Ontario) using gas-chromatography–electron capture negative ion mass spectrometry (GC–ECNIMS). *Chemosphere*, **39**, 849–871
- Smith, S.I., Weber, C.W. & Reid, B.L. (1970) The effect of injection of chlorinated hydrocarbon pesticides on hatchability of eggs. *Toxicol. appl. Pharmacol.*, **16**, 179–185
- 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
- Soto, A.M., Chung, K.L. & Sonnenschein, C. (1994) The pesticides endosulfan, toxaphene, and dieldrin have estrogenic effects on human estrogen-sensitive cells. *Environ. Health Perspectives*, **102**, 380–383
- Steinel, H.H., Arlauskas, A. & Baker, R.S.U. (1990) SCE induction and cell-cycle delay by toxaphene. *Mutat. Res.*, **230**, 29–33
- Stelzer, A. & Chan, H.M. (1999) The relative estrogenic activity of technical toxaphene mixture and two individual congeners. *Toxicology*, **138**, 69–80
- Swackhamer, D.L., Charles, M.J. & Hites, R.A. (1987) Quantitation of toxaphene in environmental samples using negative ion chemical ionization mass spectrometry. *Anal. Chem.*, **59**, 913–917
- Swackhamer, D.L., Pearson, R.F. & Schlotter, S.P. (1998) Toxaphene in the Great Lakes. *Chemosphere*, **37**, 2545–2561

- Tewari, S.N. & Sharma, J.C. (1977) Isolation and determination of chlorinated organic pesticides by thin-layer chromatography and the application to toxicological analysis. *J. Chromatogr.*, **131**, 275–284
- Thunberg, T., Ahlberg, U.G. & Wahlstrom, B. (1984) Comparison between the effects of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin and six other compounds on the vitamin A storage, the UDP-glucuronosyltransferase and the aryl hydrocarbon hydroxylase activity in the rat liver. *Arch. Toxicol.*, **55**, 16–19
- Triolo, A.J., Lang, W.R., Coon, J.M., Lindstrom, D. & Herr, D.L. (1982) Effect of the insecticides toxaphene and carbaryl on induction of lung tumors by benzo[*a*]pyrene in the mouse. *J. Toxicol. environ. Health*, **9**, 637–649
- Trosko, J.E., Jone, C. & Chang, C.C. (1987) Inhibition of gap junctional-mediated intercellular communication *in vitro* by aldrin, dieldrin, and toxaphene: A possible cellular mechanism for their tumor-promoting and neurotoxic effects. *Mol. Toxicol.*, **1**, 83–93
- Trottman, C.H. & Desai, D. (1980) Induction of rat hepatic microsomal enzymes by toxaphene pretreatment. *J. environ. Sci. Health*, **B15**, 121–134
- Turner, W.V., Khalifa, S. & Casida, J.E. (1975) Toxaphene toxicant A. Mixture of 2,2,5-*endo*,6-*exo*,8,8,9,10-octachlorobornane and 2,2,5-*endo*,6-*exo*,8,9,9,10-octachlorobornane. *J. agric. Food Chem.*, **23**, 991–994
- Vaz, R. & Blomkvist, G. (1985) Traces of toxaphene components in Swedish breast milk analyzed by capillary GC using ECD, electron impact and negative ion chemical ionization MS. *Chemosphere*, **14**, 223–231
- Wade, T.L., Chambers, L., Gardinali, P.R., Sericano, J.L., Jackson, T.J., Tarpley, R.J. & Suydam, R. (1997) Toxaphene, PCB, DDT, and chlordane analyses of beluga whale blubber. *Chemosphere*, **34**, 1351–1357
- Waritz, R.S., Steinberg, M., Kinoshita, F.K., Kelly, C.M. & Richter, W.R. (1996) Thyroid function and thyroid tumors in toxaphene-treated rats. *Regul. Toxicol. Pharmacol.*, **24**, 184–192
- Welch, R.M., Levin, W., Kuntzman, R., Jacobson, M. & Conney, A.H. (1971) Effect of halogenated hydrocarbon insecticides on the metabolism and uterotrophic action of estrogens in rats and mice. *Toxicol. appl. Pharmacol.*, **19**, 234–246
- Wiersma, G.B., Tai, H. & Sand, P.F. (1972) Pesticide residues in soil from eight cities — 1969. *Pestic. Monit. J.*, **6**, 126–129
- Willingham, E. & Crews, D. (1999) Sex reversal effects of environmentally relevant xenobiotic concentrations on the red-eared slider turtle, a species with temperature-dependent sex determination. *Gen. Comp. Endocrinol.*, **113**, 429–435
- WHO (1984) *Campechlor* (Environmental Health Criteria No. 45), Geneva
- Yang, C. & Chen, S. (1999) Two organochlorine pesticides, toxaphene and chlordane, are antagonists for estrogen-related receptor α -1 orphan receptor. *Cancer Res.*, **59**, 4519–4524