Environmental Occurrence, Analysis, and Toxicology of Toxaphene Compounds

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Toxaphene production, in quantities similar to those of polychlorinated biphenyls, has resulted in high toxaphene levels in fish from the Great Lakes and in Arctic marine mammals (up to 10 and 16 μg g^{-1} lipid). Because of the large variabiliity in total toxaphene data, few reliable conclusions can be drawn about trends or geographic differences in toxaphene concentrations. New developments in mass spectrometric detection using either negative chemical ionization or electron impact modes as well as in multidimensional gas chromatography recently have led researchers to suggest congener-specific approaches. Recently, several nomenclature systems have been developed for toxaphene compounds. Although all systems have specific advantages and limitations, it is suggested that an international body such as the International Union of Pure and Applied Chemistry make an attempt to obtain uniformity in the literature. Toxicologic information on individual chlorobornanes is scarce, but some reports have recently appeared. Neurotoxic effects of toxaphene exposure such as those on behavior and learning have been reported. Technical toxaphene and some individual congeners were found to be weakly estrogenic in *in vitro* test systems; no evidence for endocrine effects in vivo has been reported. *In vitro* studies show technical toxaphene and toxaphene congeners to be mutagenic. However, *in vivo* studies have not shown genotoxicity; therefore, a nongenotoxic mechanism is proposed. Nevertheless, toxaphene is believed to present a potential carcinogenic risk to humans. Until now, only Germany has established a legal tolerance level for toxaphene—0.1 mg kg^{-1} wet weight for fish. — *Environ Health Perspect* 107(Suppl 1):115–144 (1999). *http://ehpnet1.niehs.nih.gov/docs/1999/Suppl-1/115-144deGeus/abstract.html*

Key words: toxaphene, chlorinated bornanes, camphenes, occurrence, individual congeners, nomenclature, analysis, toxicology, legislation

Toxaphene, a complex mixture of polychlorinated camphenes, was first introduced in 1945 by Hercules Co. (Wilmington, DE) as Hercules 3965. Until the mid 1980s, it was mass produced and widely used as an insecticide, particularly in the cotton-growing industry. It was also used as a piscicide to control rough fish (undesired stock) in various water systems (*1*). The lipophilic, persistent, and volatile natures of toxaphene have contributed to its global dispersion throughout freshwater and marine environments. Traces of toxaphene have even been found in remote areas such as the Arctic (*2*) where the pesticide was never used. In addition to bioaccumulating in biota inhabiting these regions, toxaphene also has been detected in humans (*3–10*). Toxaphene was banned by the U.S. Environmental Protection Agency (U.S. EPA) in 1982, an example that was followed by many countries. However, in the early 1990s toxaphene detected in marine fish in Europe caused concern about the relationship of human health and fish consumption. Therefore, increased attention has been focused on toxaphene, both in the analytic and toxicologic fields. Research in this field has received further impetus with the synthesis of individual compounds of toxaphene and their increasing commercial availability (*11,12*). Using individual standards, we can gain more insight into the transport, fate, and toxicological effects of toxaphene in the environment. Although identification of individual congeners provides more detailed information, it also leads to more complicated analyses. Another problem lies in the nomenclature of individual compounds. Proposals have been published recently for codes simpler than the systematic nomenclature now in use. These proposals will be discussed in this review.

In 1997 a European research project titled "Investigation into the Monitoring, Analysis and Toxicity of Toxaphene" (MATT) was initiated. As part of the project, an update of available knowledge on the developments in toxaphene analysis, new environmental data, and toxicology was prepared. To avoid duplication of the extensive review on toxaphene published by Saleh in 1991 (*1*), this review concentrates on developments since 1990.

Physical and Chemical Properties

Toxaphene (CAS No. 8001-35-2) was one of the main products produced by the Hercules Co. in the United States (*1*). The process of producing toxaphene consists of extracting crude α-pinene from pine

Manuscript received at *EHP* 30 July 1998; accepted 20 October 1998.

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Abbreviations used: 2,4-D, 2,4-dichlorophenoxyacetic acid; AChE, acetylcholinesterase; ADI, acceptable daily intake; BCF, bioconcentration factor; BF, bottom fraction; CAT, chloramphenicol acetyltransferase; CHB, chlorinated bornane; ChE, cholinesterase; CTT, compound of technical toxaphene; DDE, 1,1-dichloro-2,2-*bis*(*p*-chlorophenyl)ethene; DDT, 1,1,1-trichloro-2,2-*bis*(*p*-chlorophenyl)ethane; ECD, electron capture detector; EI, electron impact; EF, enantiomer fraction; ER, enantiomer ratios; ERE, estrogen-responsive element; E-RmRNASF, estrogen-regulated mRNA stabilizing factor; FAO, Food and Agriculture Organization of the United Nations; GC, gas chromatography; GJIC, gap junctional intercellular communication; GPC, gel permeation chromatography; H, Henry's law constant; HCB, hexachlorobenzene; HDL, high-density lipoproteins; HPLC, high performance liquid chromatography; IUPAC, International Union of Pure and Applied Chemistry; K_{ow}, octanol–water partition coefficient; LDL, low-density lipoproteins; LOD, limit of determination; MATT, investigation into the Monitoring, Analysis and Toxicity of Toxaphene; MDGC, multidimensional gas chromatography; MFO, mixed-function oxygenase; MRL, maximum residue limit; MS, mass spectrometry; NCI, negative chemical ionization; NHL, non-Hodgkin's lymphoma; NOAEL, no-observed adverse effect level; PCB, polychlorinated biphenyl; PCC, polychlorinated camphene; PKC, protein kinase C; PPI, pressure pulse injection; RI, retention indices; SCE, sister chromatid exchange; SIM, selected ion monitoring; U.S. EPA, U. S. Environmental Protection Agency; VLDL, very low-density lipoproteins.

stumps, using methylisobutylketone, heat, and pressure. Isomerization of the α-pinene produces camphene, bornylene, and α-terpineol. The camphene is then subsequently chlorinated under ultraviolet (UV) light to produce toxaphene. The average chlorine content is 67 to 69% (*13*). Structures of the main components of toxaphene are shown in Figure 1.

Toxaphene is a yellow, waxy solid and has a mild terpene odor, with softening occurring at a temperature range of 343 to 363K. Although readily soluble in most organic solvents, it is more soluble in aromatic than aliphatic hydrocarbons. Its average elemental composition is $C_{10}H_{10}Cl_8$ (*1*). Toxaphene comprises at least 180 to 190 components, most with the formula $C_{10}H_{18-n}Cl_n$ or $C_{10}H_{16-n}Cl_n$, where *n* is 6 to 10 (*14*). Buser et al. (*15*) report that polychlorobornanes ($C_{10}H_{18-n}Cl_n$, n = 5–12) are formed as the main components in a Wagner-Meerwin-type rearrangement reaction. The peak area percentage of all components identified, measured using the electron capture detector (ECD), amounts to 50% of the total toxaphene area (*1*).

The commercial product is relatively stable but may be degraded by losing HCl or Cl_2 with prolonged exposure to sunlight, alkali, or temperatures above 393K (*16*). Saleh (*1*) found that technical toxaphene does not undergo a serious change when exposed to normal sunlight. Saleh and Casida (*17*) and Parlar et al. (*18*) reported that irradiation at wavelengths below 290 nm results in reductive dechlorination and dehydrochlorination; radiation above 290 nm does not appear to affect toxaphene composition. When adsorbed on silica, however, technical toxaphene is completely mineralized to CO_2 and HCl at 230 nm (*19*).

A specific gravity of 1.6 kg liter^{-1} has been reported for technical toxaphene (*20*). Vapor pressure and the log octanol–water partition coefficient (K_{ow}) value have been estimated to be comparable to that of hexachlorobenzene (HCB), 1.73×10^{-3} Pa at 298K (*21*), and a log K_{ow} of 5.5 (*22*). Howard (*23*) and Sullivan and Armstrong (*24*) recorded K_{ow} values of 4.82 to 6.4, respectively. A log K_{ow} value of 6.44 was recorded by Hooper et al. (*25*). This is somewhat lower than that of technical polychlorinated biphenyl (PCB) mixtures but higher than those of *p,p'*-DDT and its metabolites, suggesting that the bioconcentration of toxaphene is high. These data are difficult to compare because of the variety of mixtures used. Bioconcentration factors (BCFs) of 2×10^6 have been observed by Kucklick et al. (*26*) for toxaphene in Arctic cod. This value is higher than that predicted from the log K_{ow}. On the basis of their vapor pressure calculations, Wania and Mackay (*27*) suggested that toxaphene changes its chemical characteristic from gas phase to largely aerosol absorbed within the range of global environmental temperatures. At 298K, less than 10% is adsorbed to aerosols; at 253K, almost 90% is adsorbed. This implies that with a change in temperature, most toxaphene in the air condenses onto particles present in the atmosphere and thus becomes subject to wet and dry deposition. Toxaphene is transferred more rapidly from the atmosphere to soil and water at low temperatures.

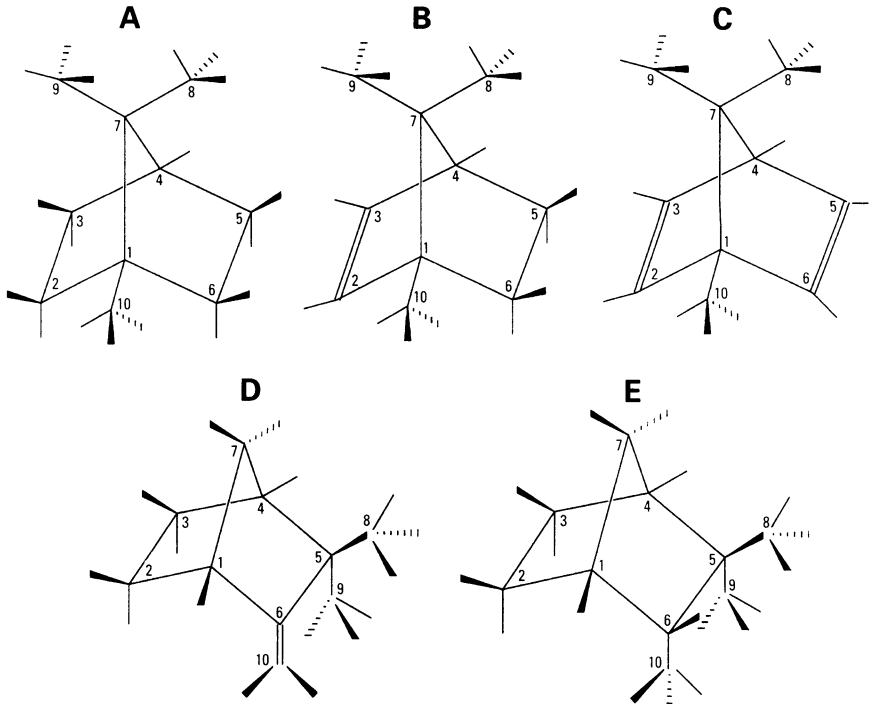

Figure 1. Carbon skeletons of (*A*) bornane, (*B*) bornene, (*C*) bornadiene, (*D*) camphene, and (*E*) dihydrocamphene. The numbering of skeletons *D* and *E* is as proposed by Hainzl (*63*).

Water solubility values of toxaphene have been reported with an equally broad spectrum and range from 0.4 mg liter^{-1} at 298K (*28*) to 0.55 to 3.3 mg kg^{-1} at 293 to 298K (*24*).

The most important factor determining the flux between the air–water interface is the Henry's law constant (H). Murphy et al. (*29*) measured H for a technical mixture of toxaphene congeners as 0.62 Pa m^3 mole^{-1} at 293K. Using fugacity-based equations (*22,30*), the direction and magnitude of the flux can be calculated according to McConnell et al. (*31*), who assumed that the temperature slope determined by Tateya et al. (*32*) for PCBs is also valid for toxaphene. Using the H measurement, a toxaphene-specific intercept can be determined and from that a temperature-corrected H can be obtained. This value allows the direction of the flux to be calculated. Such calculations suggest that up to 2 kg of material would be deposited in Lake Baikal, Russia, per month by gas exchange; the process is further enhanced by the low water temperatures of the lake (*32*). More accurate congener-specific H values are required to improve these estimates. This flux direction of air to water has also been recorded by Bidleman et al. (*33*). Hoff et al. (*34*) report that additional inputs via precipitation and particle deposition are likely to be 10 to 20 times less than those from gas absorption.

Most chlorinated bornanes contain at least one chlorine atom at C2 and C10, whereas the bridging carbons, C1 and C4, are nonchlorinated (*35*). Technical toxaphene, as synthesized by photoinduction, has a high percentage of components containing a dichloro group in the C2 position (*36*).

Toxaphene congeners demonstrate different stabilities under UV light, acid, and alkaline treatment. Fingerling et al.

(*14,36*) demonstrated that in soil 2,2,5-*endo*,6-*exo*,8,9,10-heptachlorobornane, 2,2,5-*endo*,6-*exo*,8,8,9,10-octachlorobornane, 2,2,5-*endo*,6-*exo*,8,9,9,10-octachlorobornane, 2,2,3-*exo*,5-*endo*,6-*exo*,8,9,9,10-nonachlorobornane, 2,2,5-*endo*,6-*exo*,8,8,9,10,10-nonachlorobornane, and 2,2,5-*endo*,6-*exo*,8,9,9,10,10-nonachlorobornane (B[30012]-(111), B[30012]-(211), B[30012]-(121), B[32012]-(121), B[30012]-(212), and B[30012]-(122) [(*37*) and as discussed in "Nomenclature"] were all dechlorinated by reductive removal of one chlorine atom from each geminal dichloro group beginning with that in the C2 position, which is the most labile under anaerobic conditions. The authors suggest that dechlorination also occurs during photodegradation and that the dechlorination rate is nonachlorobornanes > octachlorobornane > heptachlorobornanes.

Fingerling et al. (*14*) also found that during irradiation in solvents, the bornane structure is generally preserved and photoability seems to depend on the presence of a geminal dichloro group in C2 position. The dechlorination rate is enhanced by an additional chlorine atom in the C3 position but not by a dichloro group in C5 position. Components with only a single chlorine atom at each secondary ring atom in alternating orientation, for example, 2-*endo*,3-*exo*,5-*endo*,6-*exo*,8,8,10,10-octachlorobornane, 2-*endo*,3-*exo*,5-*endo*,6-*exo*,8,8,9,10,10-nonachlorobornane, or 2-*endo*,3-*exo*,5-*endo*,6-*exo*,8,9,9,10,10-nonachlorobornane (B[12012]-(202), B[12012]-(212), or B[12012]-(212) [(*37*) and as discussed in "Nomenclature"], were found to be extremely photostable.

Sources

The Hercules Company first introduced toxaphene as an insecticide in the late 1940s (*1*). In the ensuing years toxaphene had a variety of uses until it was banned by the U.S. EPA in 1982 because it was suspected of being a human carcinogen and a persistent hazardous compound to nontarget organisms. A stipulation existed that stocks could be used through 1986, as reported by Voldner and Smith (*38*) and Rapaport and Eisenreich (*39*). By that year usage had dropped from a reported 45×10^6 kg year^{-1} to 7.20×10^6 kg year^{-1}. Over 180 companies are reported to have produced toxaphene since 1947 with various product names (*1*) (Table 1).

Table 1. General aspects of the reviewed classes of compounds.

Class of compounds	General formula	Number of congeners
Chlorinated bornanes	$C_{10}H_{18-x}Cl_x$	32,767
Chlorinated bornenes	$C_{10}H_{16-x}Cl_x$	8,191
Chlorinated bornadienes	$C_{10}H_{14-x}Cl_x$	2,047
Chlorinated camphenes	$C_{10}H_{16-x}Cl_x$	16,383
Chlorinated dihydrocamphenes	$C_{10}H_{18-x}Cl_x$	65,534

In 1989 there were 168 registered uses of toxaphene in the United States (*40*) and more than 277 worldwide to control 167 major insect pests encountered in the production of agricultural commodities and crops. Its use in livestock dips as a miticide and in lakes as a piscicide to control rough fish populations has been widely reported (*1*). The interpolated total global use between 1950 to 1993 was 1330×10^6 kg and from 1970 to 1993, 670×10^6 kg (*41*). This estimation was based on data from the literature and on contacts with international agencies and researchers; data quality varies and shows large spatial and temporal gaps.

The United States (*42*), the Central American states, and the former Soviet states have recorded the highest usage of toxaphene. This may be because more detailed information on usage was received from these countries, whereas in other countries information often is not recorded or is kept confidential (*41*). El-Sebae et al. (*28*) report that toxaphene continues to be used in African countries, especially Ethiopia, Sudan, Tanzania, and Uganda where field runoff eventually flows into the Nile and ultimately into the Mediterranean Sea. These runoffs could be a source of future contamination. Information is lacking for other African countries.

In 1970 toxaphene was used in a formulation called polydophen, which was composed of 20% DDT and 40% toxaphene in a diesel fuel oil solvent. This was recommended as a substitute for DDT in Central Asia (*31*). Bidleman et al. (*43*) and Voldner and Schroeder (*44*) suggested that toxaphene application likely continues in the Soviet states, Mexico, Romania, Hungary, Poland, and the Indian subcontinent as well as many African nations, Nicaragua, and Mexico.

The most recent data available from the Food and Agricultural Organization of the United Nations (FAO) on toxaphene usage [as reported by Swackhamer et al. (*45*)] indicates that Korea and Mexico were the only countries using toxaphene into the 1980s; Mexico reported using 600 tonnes in 1985.

Although toxaphene is currently banned in many countries, Argentina and Mexico allow restricted use. Toxaphene was used only in small quantities in Sweden and has been banned since 1956 (*46*).

The Soviet government restricted the use of toxaphene in 1971. It is thought to still be in use as an insecticide for sugar beets, peas, potatoes, mustard, rape seed, and perennial herbs in the following formulation: 50% active ingredient, 30% oil, 15% amalgamate at 1.6 to 3.0 kg ha^{-1} during sprout stage (47). Voldner and Li (*41*) report that 1×10^8 kg of toxaphene has been used since 1970 in the former Soviet Union.

In 1956 toxaphene was recommended for nationwide use in Egypt as an insecticide to protect against cotton leafworm, pink bollworm, and spiny bollworm in cotton fields. Field efficacy was the only consideration for the use. It was applied as a formulated emulsifiable concentrate of toxaphene (60% chlorinated camphene) and used in four successive sprays during the cotton season. This method of administering the chemical caused maximum contamination of soil and can result in up to 20% being released into air, 20 to 50% into soil, and 20 to 50% into water systems. This can ultimately lead to air and groundwater pollution and to soil contamination. A concentration of 10 ppm has been reported in Egyptian soil, biota, and water (*1*). Although insecticide application doubled between 1956 and 1961, major crop losses were experienced as efficacy decreased and insect resistance increased as a result of removal of the insect's natural enemies. Egypt alone used 54×10^3 kg toxaphene between 1956 and 1961, an estimated 25% of the non-U.S. toxaphene use (*28*). The resistance level of the cotton leafworm was 26-fold that of the laboratory controls. This led to Egypt banning toxaphene in 1961, not because of its environmental impact but because of the poor efficacy factor.

It was previously thought that chlorohydrocarbons were produced in the wood pulp industry from residual monoterpenes during the chlorobleaching process. However, no evidence has been found of compounds identical to the main congeners in commercial toxaphene. This indicated

that toxaphene in fish did not come from chlorobleaching of pulp (*48*). However, chlorinated camphenes are present in pulp mill recipients (bleached Kraft pulp mill discharged organic matter in lake sediment) (*49*). Chlorine bleaching of wood pulp produces chlorinated compounds similar in composition to toxaphene but with lower chlorine content (*50*).

Rappe et al. (*51*) reported that the main chlorobornanes Tox9, TC1, TC2, TC6, and TC7, which are most likely 2-*endo*,3-*exo*,5-*endo*,6-*exo*,8,8,10,10-octachlorobornane, 2-*endo*,3-*exo*,5-*endo*,6-*exo*,8,9,10-heptachlorobornane, an as yet unknown heptachlorobornane, 2-*exo*,3-*endo*,5-*exo*,8,9,9,10,10-octachlorobornane, and 2-*exo*,5,5,8,9,9,10,10-octachlorobornane (B[12012]-(212), B[12012]-(111), ?, B[21020]-(122), and B[20030]-(122), respectively [(*37*); see discussion in "Nomenclature") (*15,52*), were detected in samples obtained close to pulp mills. These chlorobornanes were probably present because of aerial transport and/or degradation in the sediment. Additional chlorobornanes (in particular, hexachloro congeners) were observed in several of the samples that were closer to the pulp mill (4 km as opposed to 150 km).

Toxaphene Use in the Great Lakes of North America

Much of the research on toxaphene has been conducted in the Great Lakes of North America, with conflicting data on the sources of pollution. These range from the use of toxaphene as a piscicide to the contribution of the wood pulp industry in addition to atmospheric sources. Swackhamer et al. (*53*) report that approximately 1% or less of U.S. toxaphene use was in the Great Lakes basin (*54*). The rate of use in the basin was approximately 1×10^6 kg year^{-1} between 1970 and 1977 and peaked around 1977. Thus, the presence of toxaphene in the Great Lakes has been largely attributed to long-range atmospheric transport from the southern United States or from Central America followed by wet and dry deposition to the lakes (*55,56*).

Historical investigation of records on Lake Michigan revealed that 224×10^3 kg of toxaphene was used in the Green Bay watershed between 1950 and 1980, with most used as a pesticide on cropland but small amounts on livestock and in lakes as a piscicide. It has been noted that even if there were only a 1% runoff into Lake Michigan, this would represent a large fraction of the estimated inventory of toxaphene in the lake, i.e., 11×10^3 kg (*57*).

Inputs from the atmosphere to water surfaces such as the Great Lakes include dry fallout of particulate-associated contaminants, washout of gas phase and particulate phase contaminants by precipitation events, and gas transfer across the air–water interface (*58*). Oehme et al. (*10*) reported that the continual process of transport, deposition, revolatilization, and new transport along a decreasing temperature system results in accumulation of toxaphene in sediments because vapor pressure becomes so low that it restricts atmospheric transport.

In the 1960s several lakes in Wisconsin were treated with toxaphene to kill rough fish. Kidd et al. (*9*) reported that concentrations of toxaphene in fish in Laberge, Canada, were entirely due to atmospheric input followed by long food chain bioaccumulation giving rise to hazardous concentrations in fish. Kidd et al. (*9*) provide further information on possible sources and report that some contamination of Lake Ontario was due to surreptitious dumping.

Howdeshell and Hites (*59*) claim that the Niagara River is the main riverine source of sediment and water to Lake Ontario and therefore likely to be a source of some toxaphene in the lake; atmospheric deposition is also important.

Scheel (*60*) reports that toxaphenelike contaminants found in Michigan sport lakes may not be completely due to the presence of toxaphene compounds. It was suggested that they may be due to a mixture of chlorinated bicyclic monoterpenes, including the chlorinated pinenes, occurring as unwanted byproducts from chlorination of naturally occurring plant-derived product materials. Results suggest that not all chlorinated bicyclic monoterpenes found in fish tissue are the result of chlorinated camphenes or camphanes from toxaphene but may be from other sources such as the natural product family of bicyclic monoterpenes including pinene and borneol (*61*).

Nomenclature

For many years it was assumed that the pesticide toxaphene consisted primarily of chlorinated bornanes in addition to small amounts of chlorinated bornenes and even smaller amounts of chlorinated bornadienes (*62*). The existence of bornenes and bornadienes was based on data obtained with gas chromatography (GC) with negative chemical ionization mass spectrometry (NCI/MS) studies. Mass spectra with fragments 2 or 4 amu below the $[M\text{-}Cl]^-$ ions of bornanes were interpreted as bornenes and bornadienes. However, new insights into synthetic pathways of technical toxaphene indicate the formation of camphenes and dihydrocamphenes (*63*). Therefore, the observed mass spectra probably should be attributed mainly to chlorinated camphenes and dihydrocamphenes. According to Saleh (*62*), the technical mixture also consists of small amounts of other chlorinated hydrocarbons and nonchlorinated hydrocarbons.

As can be seen from Table 1, the total number of theoretical congeners calculated from the formula from Vetter (*64*) from all five classes of compounds is extensive. At present, 61 compounds of technical toxaphene have been identified (*65*); these chlorinated compounds consist of 48 bornanes, 6 bornenes, 1 bornadiene, 5 camphenes, and 1 dihydrocamphene. Nevertheless, the number of congeners can easily lead to confusion in systematic names and nomenclatures, especially because many of them are enantiomers.

On the basis of structural considerations, Hainzl et al. (*66*), using an a-chiral separation, calculated that 138 bornane and 59 camphene congeners may be found at significant concentrations. Jansson and Wideqvist (*67*) reported the separation of 670 individual components in technical toxaphene. Zhu et al. (*68*) recorded more than 300 penta- to decachlorobornanes and bornene/camphene isomers after collection of five fractions from high performance liquid chromatography (HPLC) on silica gel, whereas they found only 76 partially resolved peaks with possible coelution in a total ion chromatogram (150–500 amu) when the mixture was not preseparated; GC–NCI/MS detection was used in both instances. De Boer et al. found 246 compound peaks in technical toxaphene using two-dimensional heart-cut GC–ECD, whereas they found only 107 peaks using a single-column GC–ECD setup (*69*).

Trade Names, Names of Classes of Compounds

Although toxaphene is the most commonly used name, a wide variety of trade names exists (Table 2) (*65,70,71*). Other names used for toxaphene are compounds of technical toxaphene (CTTs) (*71*), polychlorinated monoterpenes (*71*), polychlorinated camphenes (PCCs) (*72*), polychlorinated terpenes (*25*), and chlorinated bornanes

Table 2. Common names and trade names of toxaphene.[a]

Trade names			Common names
Allotox	Geniphene	Synthetic 3956	Toxaphene
Attac	Gy-phene	Toxadust	Camphechlor
Chem-Phene	Hercules 3956	Toxadust 10	Chlorinated camphene
Chlor Chem T-590	Huileux	Toxakil	Octachlorocamphene
Chlorter	Melipax	Toxyphen	Polychlorocamphene
Cristoxo-90	Morox	Toxaspra	
Dark	Penphene	Toxon 63	
Delicia Fribal	Phenacide	Toxyphen	
Estonox	Phenatox	Vapotone	
Fasco Terpene	Strobane-T		

[a]Compiled from Coelhan et al (*65*), Sergeant and Onusaka (*70*), and Krock et al. (*71*).

(CHBs) (*73*), where several of these names only contain one of the groups present in the technical mixture.

The variety of trade and common names used for toxaphene, in addition to the trivial names of various compound classes referred to in Table 1, complicate any nomenclature system. Names no longer supported by International Union of Pure and Applied Chemistry (IUPAC), such as norbornanes and camphanes for bornanes and iso-camphanes for dihydrocamphenes, compound the naming problem.

Systematic Names

It is a complex task to formulate systematic names for all groups of compounds mentioned above (Table 1) that conform to IUPAC rules. The structure of these compounds is given in Figure 1. The generally accepted systematic nomenclature for bornanes, according to IUPAC rules, is based on the following rules and agreements (Figure 1A) (*74,75*):

- Numbering of the carbon atoms, as shown in Figure 1A (as presented by IUPAC).
- Substituents on the six-membered ring that point downward are in the *endo* position and substituents that point upward are in the *exo* position (the bridging carbon, C7, is above the ring).
- The carbon atom above the C2—C3 bond is C9, the carbon above the C5—C6 bond is C8.
- The lowest possible numbering should be applied. The carbon neighboring C1 is decisive for the direction of numbering. If both carbons next to C1 bear the same number of chlorine atoms, substitution of the next carbon in the ring is decisive in determining the direction of numbering. If these are also equivalent, the first carbon with an *endo* chlorine determines the direction of numbering.
- Enantiomers receive the same systematic nomenclature.

For bornenes (Figure 1B) and bornadienes (Figure 1C) the following agreements and additonal rules should be applied:

- If one double bond is present, the carbon atoms at this bond are numbered C2 and C3.
- If two double bonds are present, the numbering of the six-membered ring should result in the lowest possible numbers, as with the bornanes.
- As with the bornanes, the C9 carbon should be positioned over the C2–C3 double bond.

The systematic names are even more complicated for chlorinated camphenes (Figure 1D) and dihydrocamphenes (Figure 1E) than for the chlorinated bornanes, bornenes, and bornadienes. Coelhan and Parlar (*75*) suggest that the systematic name for nonsubstituted camphene should be 2,2-dimethyl-3-methylene-8,9,10-trinorbornane and that other substituted camphenes should be regarded as derivatives of 8,9,10-trinorbornane. However, according to Vetter (*76*), IUPAC has abolished the name norbornane, which was used to indicate the bornane structure without C8, C9, and C10. In addition, considering chlorinated camphenes and dihydrocamphenes to be chlorinated 8,9,10-trinorbornanes creates even longer systematic names. Moreover, such names would strongly depend on the substituents present.

Systematic names for chlorinated camphenes and dihydrocamphenes can also be regarded to have bicyclo(2.2.1) heptane as their basic structure (*64,75*). The nonsubstituted bornane would then become 1.7.7-trimethyl-bicyclo(2.2.1) heptane. Camphene and dihydrocamphene would be referred to as 2,2-dimethyl-3-methylene-bicyclo(2.2.1)hepta ne and 2,2,3-trimethyl-bicyclo(2.2.1) heptane, respectively. Chlorinated camphenes and dihydrocamphenes will receive very long systematic names, which can easily lead to confusion.

Hainzl (*63*) proposed systematic names based on a fixed numbering of the camphene skeleton (Figure 1D,E). This approach is quite straightforward, resembles the bornane nomenclature, and is more user friendly. However, IUPAC does not yet support assigning these fixed numbers and, in addition, there still are no IUPAC rules for designating C8 and C9 orientations in camphene (*76*).

Nomenclature Systems

Because chlorinated bornanes are the most abundant compounds in technical toxaphene, most attention has been devoted to them, both with regard to analytic method development and monitoring, and nomenclature. In the past systematic nomenclature of the bornane skeleton has been nonuniform because several authors have cited the IUPAC nomenclature incorrectly, particularly the C8 and C9 positions (Figure 1) (*74*). Difficulties in formulating the correct systematic names for chlorinated bornanes were solved when IUPAC assigned definitive numbering for the carbon skeleton.

Because of the extensive systematic names for chlorinated bornanes (e.g., 2-endo,3-*exo*,5-*endo*,6-*exo*,8,9,9,10,10-nonachlorobornane), isolated congeners were often designated by simpler names such as T12, Toxicant A, Toxicant Ac, Toxicant B, TOX8, and TOX9; however, a clear nomenclature system was lacking. Several authors proposed and used more systematic nomenclatures in attempts to remedy this situation. Table 3 gives an overview of these nomenclatures, which will be discussed below.

The nomenclature used by Burhenne et al. (*11*) and Hainzl et al. (*77*) is based on GC retention on a certain stationary phase. Consisting simply of a 2-digit code representing a peak in the technical mixture, the nomenclature can be applied to chlorinated bornanes, camphenes, and dihydrocamphenes.

To indicate enantiomers, an additional code, "+" or "–", is proposed. However, apart from the fact that the code does not provide any structural information, one also must consider that *a*) with such large numbers of congeners, coelution cannot be excluded (*69*); Parlar no. 42, for example, represents at least two different chlorobornanes (*77*); *b*) the chlorinated bornanes, camphenes, and dihydrocamphenes

Table 3. Systematic names of compounds present in toxaphene and their codes of alternative nomenclatures presented in the literature.

Systematic name	Parlar nos. (*11,77*)	Nikiforov et al. (*78*)	Oehme and Kallenborn[a] (*79*)	Andrews and Vetter (*74*)	Wester et al. (*37,80*)
2-*exo*,3-*endo*,6-*endo*,8,9,10-hexachlorobornane		HxCB-3156	265-111	B6-913	B[21001]-(111)
2-*exo*,3-*endo*,6-*exo*,8,9,10-hexachlorobornane		HxCB-3124	137-111	B6-923	B[30030]-(012)
2,2,5,5,9,10,10-heptachlorobornane	21	HpCB-6533	99-043	B7-499	B[30030]-(012)
2,2,5-*endo*,6-*exo*,8,9,10-heptachlorobornane	32	HpCB-6452	195-421	B7-515	B[30012]-(111)
2,2,5-*exo*,8,9,10,10-heptachlorobornane		HpCB-6293	35-113	B7-560	B[30020]-(112)
2-*endo*,3-*exo*,5-*exo*,6-*exo*,8,10,10-heptachlorobornane		HpCB-4785	166-103	B7-1026	B[12022]-(102)
2-*endo*,3-*exo*,6-*exo*,8,9,10,10-heptachlorobornane		HpCB-4661	134-113	B7-1059	B[12002]-(112)
2-*exo*,3-*endo*,5-*exo*,8,9,10,10-heptachlorobornane		HpCB-3221	41-113	B7-1450	B[21020]-(112)
2-*exo*,3-*endo*,5-*exo*,9,9,10,10-heptachlorobornane		HpCB-3207	41-033	B7-1453	B[21020]-(022)
2-*exo*,3-*endo*,6-*endo*,8,9,10,10-heptachlorobornane		HpCB-3157	265-113	B7-1462	B[21001]-(112)
2-*endo*,3-*endo*,5-*exo*,6-*exo*,9,10,10-heptachlorobornane		HpCB-5285	170-013	B7-1572	B[11022]-(012)
2-*exo*,3-*exo*,5-*endo*,8,9,10,10-heptachlorobornane		HpCB-2837	69-113	B7-1584	B[22010]-(112)
2-*endo*,3-*exo*,5-*exo*,6-*exo*,9,10,10-heptachlorobornane		HpCB-4773	166-013	B7-1592	B[12022]-(012)
2-*exo*,5,5,8,9,10,10-heptachlorobornane		HpCB-2453	97-463	B7-1712	B[20030]-(112)
2-*exo*,5,5,9,9,10,10-heptachlorobornane		HpCB-2439	97-063	B7-1715	B[20030]-(022)
2,2,3-*exo*,5-*endo*,6-*exo*,8,9,10-octachlorobornane	39	OCB-6964	199-421	B8-531	B[32012]-(111)
2,2,5,5,6-*endo*,8,9,10-octachlorobornane		OCB-6612	355-111	B8-763	B[30031]-(111)
2,2,5,5,8,9,10,10-octachlorobornane	51	OCB-6549	99-423	B8-786	B[30030]-(112)
2,2,5,5,9,9,10,10-octachlorobornane	38	OCB-6535	99-063	B8-789	B[30030]-(022)
2,2,5-*endo*,6-*exo*,8,8,9,10-octachlorobornane	42a	OCB-6460	195-641	B8-806	B[30012]-(211)
2,2,5-*endo*,6-*exo*,8,9,9,10-octachlorobornane	42b	OCB-6454	195-461	B8-809	B[30012]-(121)
2,2,5-*endo*,6-*exo*,8,9,10,10-octachlorobornane		OCB-6453	195-423	B8-810	B[30012]-(112)
2-*endo*,3,3,5-*exo*,6-*exo*,9,10,10-octachlorobornane		OCB-5797	174-013	B8-1058	B[13022]-(012)
2-*endo*,3-*endo*,5-*exo*,6-*exo*,8,9,10,10-octachlorobornane		OCB-5301	170-113	B8-1252	B[11022]-(112)
2-*endo*,3-*exo*,5-*endo*,6-*exo*,8,8,10,10-octachlorobornane	26	OCB-4921	198-605	B8-1413	B[12012]-(202)
2-*endo*,3-*exo*,5-*endo*,6-*exo*,8,9,10,10-octachlorobornane	40	OCB-4917	198-245	B8-1414	B[12012]-(112)
2-*endo*,3-*exo*,5-*exo*,6-*exo*,8,9,10,10-octachlorobornane		OCB-4789	166-113	B8-1440	B[12022]-(112)
2-*exo*,3-*endo*,5-*exo*,8,9,9,10,10-octachlorobornane	41	OCB-3223	41-463	B8-1945	B[21020]-(122)
2-*exo*,3-*exo*,5,5,8,8,10,10-octachlorobornane		OCB-2969	101-303	B8-2075	B[22030]-(202)
2-*exo*,3-*exo*,5,5,8,9,10,10-octachlorobornane		OCB-2965	101-113	B8-2078	B[22030]-(112)
2-*exo*,5,5,8,9,9,10,10-octachlorobornane	44	OCB-2455	97-463	B8-2229	B[20030]-(122)
2,2,3-*exo*,5,5,9,9,10,10-nonachlorobornane		NCB-7047	103-033	B9-718	B[32030]-(022)
2,2,3-*exo*,5-*endo*,6-*exo*,8,9,9,10-nonachlorobornane		NCB-6966	199-461	B9-742	B[32012]-(121)
2,2,3-*exo*,5-*endo*,6-*exo*,8,9,10,10-nonachlorobornane		NCB-6965	199-113	B9-743	B[32012]-(112)
2,2,5,5,6-*exo*,8,9,9,10-nonachlorobornane		NCB-6582	227-461	B9-1011	B[30032]-(121)
2,2,5,5,8,9,9,10,10-nonachlorobornane	62	NCB-6551	99-133	B9-1025	B[30030]-(122)
2,2,5-*endo*,6-*exo*,8,8,9,10,10-nonachlorobornane	56	NCB-6461	195-645	B9-1046	B[30012]-(212)
2,2,5-*endo*,6-*exo*,8,9,9,10,10-nonachlorobornane	59	NCB-6455	195-463	B9-1049	B[30012]-(122)
2-*endo*,3,3,5-*exo*,6-*exo*,8,9,10,10-nonachlorobornane		NCB-5813	174-113	B9-1327	B[13022]-(112)
2-*endo*,3-*exo*,5-*endo*,6-*exo*,8,8,9,10,10-nonachlorobornane	50	NCB-4925	198-643	B9-1679	B[12012]-(212)
2-*endo*,3-*exo*,5-*endo*,6-*exo*,8,9,9,10,10-nonachlorobornane		NCB-4919	198-133	B9-2200	B[12012]-(122)
2-*exo*,3-*endo*,5-*exo*,6-*exo*,8,8,9,10,10-nonachlorobornane	63	NCB-3261	169-643	B9-2206	B[21022]-(212)
2,2,3-*exo*,5,5,8,9,9,10,10-decachlorobornane		DCB-7063	103-463	B10-831	B[32030]-(122)
2,2,3-*exo*,5-*endo*,6-*exo*,8,9,9,10,10-decachlorobornane		DCB-6967	199-463	B10-860	B[32012]-(122)
2,2,5,5,6-*exo*,8,9,9,10,10-decachlorobornane	69	DCB-6583	227-463	B10-1110	B[30032]-(122)
2-*endo*,3,3,5-*endo*,6-*endo*,8,9,9,10,10-decachlorobornane		DCB-5975	334-133	B10-1361	B[13011]-(122)
2-*exo*,3,3,5-*exo*,6-*endo*,8,9,9,10,10-decachlorobornane		DCB-3799	301-133	B10-1993	B[23021]-(122)
2,5-*endo*,6-*exo*,8,9,10-hexachloroborn-2-ene					E[10012]-(111)
3,6,6,8,9,10-hexachloroborn-2-ene					E[01003]-(111)
3,5-*exo*,6,6,8,9,10-heptachloroborn-2-ene					E[01023]-(111)
2,3,5-*exo*,6-*exo*,9,10,10-heptachloroborn-2-ene					E[11022]-(012)
2,3,5-*exo*,6-*exo*,8,9,10,10-octachloroborn-2-ene					E[11022]-(112)
2,5-*endo*,6-*exo*,8,9,9,10,10-octachloroborn-2-ene					E[10012]-(122)
2,3,5,8,9,10-hexachloroborn-2,5-diene					D[11010]-(111)
2,2,3-*exo*,8,9,10(E)-hexachlorocamphene	11				C[032001]-(11)
2-*exo*,3-*endo*,8,8,9,10(E)-hexachlorocamphene	12				C[021001]-(21)
2-*exo*,3-*endo*,7a,8,9,10(E)-hexachlorocamphene					C[021011]-(11)
2,2,3-*exo*,8,8,9,10(E)-heptachlorocamphene					C[032001]-(21)
2,2,3-*exo*,8,8,9,9,10(E)-octachlorocamphene					C[032001]-(22)
2-*exo*,3-*exo*,6-*exo*,8,9,10,10-heptachlorodihydrocamphene					DC[022020]-(112)

[a]The rules to determine the code according to Oehme and Kallenborn (*79*) are applied on the systematic names in the table; in the original reference, C8 and C9 positions were swapped. Decoding the first part of this code gives a 9-digit binary number, which should be read from right to the left starting with the *exo* position! The code for the C8 to C10 substitution was calculated according to Burhenne et al. (*11*) and Hainzl et al. (*77*). When the the chlorine substitution positions at these carbons were not given, the lowest possible number was chosen.

will be part of the same coding system without any class distinction; *c*) the theoretical number of chlorinated bornanes, camphenes, and dihydrocamphenes will require many thousands of codes, which will be at least 4 digits. Presently, 17 chlorinated bornanes and 5 chlorinated camphenes have been assigned Parlar numbers (Table 3).

A binary coding system for chlorinated bornanes was proposed by Nikiforov et al. (*78*). The possible chlorination positions were ordered according to IUPAC order of preference into a 13-digit binary number. A "1" is assigned if a chlorine is present and a "0", if not. This binary number is then converted into a short 4-digit decimal number. However, 5 of the maximum number of 18 substitution positions had to be fixed. This was determined on the basis of the assumption that environmentally important congeners have at least one chlorine at C10, not more than two chlorines at C8, C9, and C10, and no chlorine at C4. Using this assumption, the code can be limited to 4 digits instead of 6 but cannot be used for all the theoretically possible congeners. Applying the nomenclature to 2,2,5-*endo*,6-*exo*,8,9,10-heptachloroborane (Figure 2A) results in the code HpCB-6452; these characters are the acronym for heptachloroborane. However, the enantiomer given in Figure 2B, which is chlorinated at positions 2-*exo*,3-*endo*,6,6,8,9,10, would have the code HpCB-3188. Because the goal of the IUPAC rule for systematic numbering is to obtain the lowest possible number, the code HpCB-3188 would be selected; this is an incorrect systematic name. The most important limitation of Nikiforov's suggested system is that it is difficult to convert between binary and decimal codes; a computer is needed for rapid conversion. In this case, the general code becomes:

$$2^{12}\times 2\text{-}endo + 2^{11}\times 2\text{-}exo + 2^{10}\times 3\text{-}endo + 2^{9}\times 3\text{-}exo + 2^{8}\times 5\text{-}endo + 2^{7}\times 5\text{-}exo + 2^{6}\times 6\text{-}endo + 2^{5}\times 6\text{-}exo + 2^{4}\times 8\text{a} + 2^{3}\times 8\text{b} + 2^{2}\times 9\text{a} + 2^{1}\times 9\text{b} + 2^{0}\times (\text{second chlorine substituent to C10})$$

Oehme and Kallenborn (*79*) also proposed a nomenclature for chlorinated bornanes based on a binary number representing all possible chlorination positions, which was then converted to decimal code. The positions at the six-membered ring and at the three methyl groups are numbered separately, which results in two 3-digit decimal numbers at maximum, separated by a hyphen. This was to prevent generation of a code with a maximum of 6 digits, which would be necessary if all possible positions were included in a single code. For example, the enantiomers in Figure 2 are represented by the code 195-421; however, there is no distinction between the enantiomers. The advantage of this system over Nikiforov's (*78*) is that related structures in Oehme and Kallenborn's system have similar codes. However, the basic bornane structure used by Oehme and Kallenborn is not according to IUPAC rules because the C8 and C9 atoms are reversed (*74*). Care should be taken when decoding the first part of the code: the binary 9-digit number must be read from the right to the left starting with 2-*exo*. This was noted incorrectly by Lau et al. (*81*), who were subsequently cited in Nordic Council of Ministers (*16*) and Wester et al. (*37*). Thus, decoding of the 3-digit number to reveal the chemical structure is not simple, and structural information therefore is not directly available. The general code becomes

$$2^{0}\times 2\text{-}exo + 2^{1}\times 2\text{-}endo + 2^{2}\times 3\text{-}exo + 2^{3}\times 3\text{-}endo + 2^{4}\times 4 + 2^{5}\times 5\text{-}exo + 2^{6}\times 5\text{-}endo + 2^{7}\times 6\text{-}exo + 2^{8}\times 6\text{-}endo \quad \text{(first part)}$$

$$2^{0}\times 8\text{a} + 2^{1}\times 8\text{b} + 2^{2}\times 8\text{c} \quad \text{(first digit second part)}$$

$$2^{0}\times 9\text{a} + 2^{1}\times 9\text{b} + 22\times 9\text{c} \quad \text{(second digit second part)}$$

$$2^{0}\times 10\text{a} + 2^{1}\times 10\text{b} + 2^{2}\times 10\text{c} \quad \text{(third digit second part)}$$

Andrews and Vetter (*74*) proposed a systematic nomenclature for chlorinated bornanes by listing them in order of preference according to IUPAC rules. The congeners were split into a series of homologs to restrict the code length to less than 5 digits, which is necessary if all possible congeners are tabulated by the method used for chlorinated biphenyls (*82*). With this method the maximum number of digits is 4. The code is preceded by a character to indicate whether the compound is a bornane (B), camphene (C), bornadiene (D), or bornene (E), and a number denoting the degree of chlorination (1–18). For example, the enantiomers in Figure 2 are coded B7-515 and are distinguished by "a"- or "b"-, but b-coded enantiomers correspond to incorrect structural names (*71*) if IUPAC rules are applied. The disadvantage of this method is that structural information can only be obtained after consulting extensive tables or by using a computer program that currently are only available for chlorinated bornanes.

Wester et al. (*37*) proposed a nomenclature system that is a mixture of the systems previously mentioned, with advantages that the structural information can be directly deduced and that the nomenclature is applicable to chlorinated bornanes as well as to chlorinated bornenes and bornadienes. The proposed system yields a code consisting of two parts. The digits in the first part of the code reflect the degree of chlorination of carbons C2 to C6, presented according to the rules listed in Table 4. C4, i.e., the third digit, can only have a code of 0 or 1. The digits in the second part indicate the number of chlorine atoms of C8 to C10. The letter "B"precedes the 8-digit number in the case of bornanes. For example, the code for 2-*endo*,3-*exo*,5-*endo*,6-*exo*,8,8,9,10,10-nonachlorobornane is B[12012]-(212). The code for the conformation of

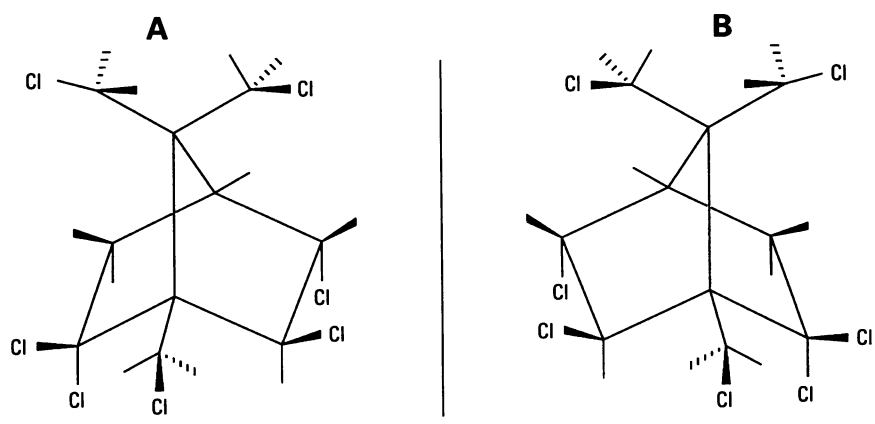

Figure 2. (*A*) and (*B*) show the two enantiomers of 2,2,5-*endo*,6-*exo*,8,9,10-heptachlorobornane.

Table 4. Codes indicating chlorination of C2 to C6 atoms of chlorinated bornanes.

Endo	*Exo*	Code
0	0	0
1	0	1
0	1	2
1	1	3

the enantiomer would be B[21021]-(122); this clockwise numbering is arrived at by reversing the first part of the code and exchanging the digits for the C8 and C9 positions. Hence, according to the IUPAC rules, B[12012]-(212) is the only correct representation for both enantiomers. To differentiate both enantiomers "r" is proposed for clockwise numbering of the six-membered ring (Figure 2A; B[30012]-(111)r) and "s" for counterclockwise numbering (Figure 2B; B[30012]-(111)s), provided the bridging carbon atom, C7, is above the ring. The advantage of this notation is that it is related to the generally accepted notation for chirality (R/S), and enantiomers receive the same code. For racemates, the r/s notation can be left out.

Another advantage of this system of nomenclature is the simplicity of establishing whether a congener has an enantiomer. For example, 2,2,3-*endo*,5-*endo*,6,6,8,9,10-nonachlorobornane, coded as B[31013]-(111), has no enantiomer. Reversing the first part of the notation and exchanging the digits representing the number of chlorine atoms at the C8 and C9 positions gives the same code.

Wester et al. (*37*) extended their nomenclature to include bornenes and bornadienes, which is easily done because of structural similarity. Only one chlorine atom can be attached to a carbon atom participating in a double bond. Only a "0" or "1" can be assigned to such a carbon. For example, 2,5-endo,6-exo,8,9,9,10,10-octachloroborn-2-ene will be coded E[10012]-(122) and 2,3,5,8,8,9,10-heptachloroborna-2,5-diene will be coded D[11010]-(211). The r/s nomenclature can be used if there are enantiomers; it is not used in the case of a racemic mixture or if there are no enantiomers.

Polychlorinated camphene and dihydrocamphene structures could not be represented by codes based on the system previously described because of the large differences among structures. However, Wester et al. (*80*) developed a coding system analog to their system for bornanes, bornenes, and bornadienes (*37*). The numbering of the carbon atoms in the skeleton is the same as that proposed by Hainzl (*83*); however, the "a" and "b" indications of the substituents at C10 (*63*) have been replaced by "E" (*trans*) and "Z" (*cis*), respectively (Figure 2A). As seen in Figure 1, this method bears a strong resemblance to the clockwise numbering of the bornane carbon skeleton (*37*).

Figure 1D illustrates that it is not necessary to consider carbons C5 and C6 because they cannot be chlorinated. The first part of the code concerns the substituents at carbons C1 to C4, C7, and C10. Carbons C1 and C4 can only have one chlorine substituent and these invariably will be in the *endo* position. Substitution at carbons C2 and C3 can be denoted according to the rules of Table 2. For carbon C7, the positions of the substituents must be defined. A "0" is assigned for no substitution, "1" for substitution in the "a" position, "2" for substitution in the "b" position, and "3" for two substituents. For C10 the known "E"(*trans*) nomenclature corresponds with code 1 and "Z" (*cis*) with code 2. Carbons C8 and C9 are dealt with in the second part of the proposed code, which merely reflects the number of chlorine substituents at C8 and C9. Finally, the code is preceded with a "C" for camphenes (*74*), with enantiomers being distinguished by an "r" or "s"according to Wester (*37*). The general code then becomes

C[code C1, code C2, code C3, code C4, code C7, code C10]-(code C8, code C9)r/s.

The same logic used previously can be applied to the dihydrocamphenes (Figure 1E). There is no need to consider carbon C5, as chlorination cannot occur. For the first part of the code, the same rules are applied for carbons C1 to C4 and C7 as for the chlorinated camphenes. Carbon C6 can only have one substituent, which can be in the *endo* or *exo* position, and the rules in Table 2 were applied. However, if C6 is not chlorinated, the position of its hydrogen atom is unclear, in which case a subscript selected according to the rules of Table 2 is used to denote the *endo* or *exo* position of the hydrogen atom. The second part of the code deals with C8 to C10, with the code reflecting the number of chlorine substituents. Finally, the code is preceded by DC (dihydrocamphenes) and enantiomers are distinguished by adding an "r" or "s" according to Wester (*37*). The general code then becomes

DC[code C1, code C2, code C3, code C4, code $C6_{H6}$, code C7]-(code C8, code C9, code C10)r/s.

Analytical Methods

Mostly toxaphene levels are determined that may lead to a large over- or underestimatation of the true concentration, as the peak pattern of the sample under study does not resemble that of the standard. Peak patterns may be considerably altered in the environment (*84,85*), but there also are large differences among standards for the commercially available technical toxaphene. Using various technical standards, Carlin and Hoffman (*86*) found variations between 19 and 131% compared to their laboratory standard. Furthermore, detector response generally is not equal for all congeners. The most relevant question may be: What does a total concentration imply when the composition is unknown? Because of this the trend at present is toward using congener-specific approaches, which is possible after the first isolation and synthesis of individual compounds (*87,88*). Currently, about 30 individual congeners are commercially available. For comparison of monitoring results, it is important that authors report the full analytical procedure used, as different methods can yield large variations in results, which could lead to incorrect conclusions.

Extraction

Little attention has been paid to the efficiency of extraction procedures. However, it is thought that extraction procedures suitable for related compounds such as PCBs, DDT, and chlordanes could also be used for toxaphene compounds because of lipophilic and structural similarities (*73*).

Pre-Separation and Clean-Up

Several stationary phases have been used in the sample preparation for residue analysis. Aluminium oxide (*89*) and gel permeation chromatography (GPC) (*90*) or a combination of the two (*91–93*) can be used to remove lipids from the sample. Florisil (*48,90,94*) and silica gel (*7,89,95*) can be used for further fractionation of the extract. Reversed-phase chromatography (C8 and C18) can also be used to isolate B[12012]-(202) and B[12012]-(212) from environmental samples.

Zu et al. (*68*) noted that silica gel preparation can be used to obtain separation of technical toxaphene over a wide range and that B[12012]-(202) can be

detected free from coeluting compounds after the preparation. The elution order of individual chlorinated bornane congeners on silica gel is B[12012]-(202) << B[21020]-(022) < B[12012]-(212) << B[20030]-(122) < B[12012]-(112) << B[21020]-(122) < B[30030]-(122) << B[32012]-(111) << B[30012]-(211) + B[30012]-(121) << B[30030]-(111) (*96*). The elution order of several individual toxaphene congeners on reversed-phase HPLC was recently presented (*97*).

When silica fractionation is used, individual congeners should be used to establish the volume range of the toxaphene fractions and to evaluate recoveries; low recoveries for certain congeners may occur when only a technical mixture is used for optimization (*69,98*). de Boer et al. (*69*) used columns of 2.5 g $SiO_2 \cdot 2\%$ H_2O (w/w); most of the toxaphene compounds, including the most relevant congeners, were eluted in a second fraction of 12 ml diethyl ether/iso-octane (20:80, v/v) after a first fraction of 13 ml iso-octane that contained mostly PCBs. Only 1 to 2% PCBs were present in the toxaphene fraction, which did not seriously interfere with the toxaphene quantification using ECD. The entire clean-up procedure resulted in recoveries of 80 to 96% for total toxaphene, and 84 to 100% for B[30012]-(111), B[12012]-(212), and B[30030]-(122). B[12012]-(202) was divided over the two fractions (about 40% in the first fraction and 60% in the second fraction), with an overall recovery of 85 to 95%. In a collaborative study to determine four bornane congeners in fish oil, gel permeation was used followed by adsorption chromatography on silica gel (*99*). The silica gel clean-up was performed using 1.0 g silica deactivated with 1.5% water. The toxaphene compounds were collected with the PCBs and some organochlorine pesticides in the first fraction and eluted with 8 ml hexane/toluene (65:35, v/v). Although the results of this study were obtained using GC–ECD, the recoveries were 77 to 100%, and the relative standard deviations of reproducibility were 18 ± 4, 24 ± 5, 29 ± 19, and 21 ± 5% for B[12012]-(202), B[12012-(212), B[30030]-(122), and B[30012]-(111), respectively. Some participants preferred a clean-up in which the toxaphene compounds and PCBs are separated according to Alder and Vieth (*92*). They eluted the silica column before the hexane/toluene fraction with 8 ml hexane in which the PCBs and *p,p'*-DDE were recovered. Some chlordane/nonachlor and *p,p'*-DDT and B[12012]-(202) were also found in that fraction. Krock et al. (*96*) improved on this method by using 8.0 g activated silica. The sample was eluted with 48 ml hexane to remove PCBs. This was followed by elution with 50 ml hexane/toluene (65:35, v/v) in which the toxaphene compounds were recovered.

Injection

Alder et al. (*100*) reported that injector temperature should not exceed 513K because severe decomposition of compounds may take place. Bartha et al. (*101*) recommend an injector temperature below 523K. Care should be taken with active sites in the liner and the injector. It is recommended that the optimal temperature be verified by a series of simple tests, as there is much variation in injector geometry. Alawi et al. (*102*) showed that response factors obtained using splitless injection are lower than those obtained using on-column injection.

Bartha et al. (*101*) reported that using pressure pulse injection (PPI) at 498K resulted in response factors 4 times that of those obtained with splitless injection. This was especially significant for compounds with a low vapor pressure and long retention times [e.g., B[30030]-(122)] (*101*). With this technique, the time the compounds spend in the injector is short, so there is less chance of degradation.

Gas Chromatographic Separation

Table 5 gives the elution order of toxaphene compounds for various stationary phases. The relative nonpolar stationary phase, 5% diphenyl, 95% dimethylpolysiloxane (DB-5 [J&W Scientific, Folsom, CA; Sil-8 [Chrompack, Middleburg, The Netherlands]; Ultra-2 [Hewlett Packard, Palo Alto, CA]), column with lengths of 30 to 60 m and diameters of 0.15 to 0.32 mm ID is most frequently used. However, more polar columns are often used to validate the results, e.g., 14% cyanopropylphenyl, 86% dimethylpolysiloxaan (DB-1701 [J&W Scientific]; Sil-19 [Chrompack]) (*107*), and 6% cyanopropylphenyl, 94% dimethylpolysiloxaan (DB-1301 [J&W Scientific]) (*99*).

Krock et al. obtained a relatively good separation using a very nonpolar Sil-2 stationary phase (comparable to squalene) (*96*), the same elution order as on the more polar DB-5 columns was found (*103*). The CP-Sil 2 phase was successfully used to a temperature as high as 563K, although the supplier advised a maximum temperature of 473K. No alteration of retention times was observed after analysis of several hundred toxaphene compounds on this phase (*101*). After comparing the retention times of B[12012]-(202) with B[12012]-(112) and B[30030]-(022) with B[30030]-(112), it was suggested that compounds with one chlorine on both C8 and C9 elute much later from this phase than compounds with two chlorines on one of these carbons. Furthermore, by comparing B[12012]-(202) with B[03003]-(202) and B[12012]-(112) with B[30030]-(112), it was found that compounds with alternating *endo–exo* substitution elute earlier than compounds with two chlorines at C2 and C5 (*103*).

Nikiforov et al. (*106*) split the bornane skeleton into two parts, the six-membered ring, "Ring," and the three methyl groups, "Metil." By comparing available retention indices (RI) to those from a DB-5-type phase with the substitution of these two parts of the molecule, several correlations were found and the following conclusions were drawn:

1. The RI of compounds with either the same Ring or Metil increases with increasing degrees of chlorination of the other.
2. For all Metil substitutions the RI increases with the following Ring substitution: [12012] < [30012] < [30030] < [21022].
3. For all Ring substitutions the RI increases with the following Metil substitution: (202) < (112) and (211) < (112).

The use of heart-cut multidimensional gas chromatography (MDGC) (*108*) offers a possibility to overcome coelution problems due to the large amount of congeners. By transferring heart-cuts from a separation performed on a DB-5-type phase to a 15% dimethylsilicone, 85% polyethylene glycol (DX-4; J&W Scientific) phase, in addition to a polyethylene glycol terephthalic acid ester (FFAP; Hewlett Packard) and a 10% cyanopropyl, 90% biscyanopropyl polysiloxane (Rtx-2330; Restek Corp., Bellefonte, PA) phase for further separation (a multidimensional set-up) (*69*), a large number of peaks were observed in the secondary chromatograms, which indicated that the resolution offered by a single column is insufficient and can easily contribute to false-positive results, especially when nonselective ECD is used for quantification. There were no large differences between the column combinations; the DB-5–Rtx-2330

Table 5. Elution order reported in the literature for several stationary phases.[a]

Sil-2[b]	DB-5, Sil-8, Ultra-2[c,d]	Ultra-2[e]	Smectic[c]	Rtx-2330[c,f]	Chiral[c]	DX-4[f]	FFAP[f]
	C[032001]-(11)		B[30030]-(012)	B[12012]-(202)	B[12012]-(202)	B[12012]-(202)	B[12012]-(202)
	C[021001]-(21)		B[12012]-(202)	B[30030]-(012)	C[032001]-(21)	B[12012]-(212)	B[12012]-(212)
	C[021011]-(11)	B[20030]-(022)	C[032001]-(21)	+ B[32012]-(111)	C[032001]-(22)	B[30012]-(111)	B[30012]-(111)
	B[30030]-(012)	B[30030]-(012)	C[032001]-(22)	B[32030]-(112)	B[21020]-(122)	B[30030]-(122)	B[30030]-(122)
B[21020]-(022)	C[032001]-(21)		+ B[30030]-(022)	B[30030]-(022)	B[30030]-(012)		
B[12012]-(202)	B[12012]-(202)	B[12012]-(202)	B[30012]-(111)	C[032001]-(22)	B[32012]-(111)		
B[30012]-(111)	B[30012]-(111)	B[30012]-(111)	B[32012]-(111)	B[12012]-(212)	+ B[12012]-(112)		
B[30030]-(022)	+ C[032001]-(22)	B[21022]-(111)	B[20030]-(122)	C[032001]-(21)	B[12012]-(212)		
		B[12002]-(112)	B[21020]-(122)	B[20030]-(122)	B[30012]-(111)		
		B[22002]-(112)	+ B[30012]-(121)	B[30012]-(212)	B[30012]-(211)		
		B[21002]-(112)	+ B[30012]-(211)	B[21020]-(122)	B[20030]-(122)		
		B[30030]-(022)	B[12012]-(212)	B[30012]-(111)	B[30012]-(121)		
B[32012]-(111)	B[32012]-(111)	B[32012]-(111)	B[12012]-(112)	B[30030]-(122)	B[30030]-(022)		
B[12012]-(112)	B[12012]-(112)	B[12012]-(112)	B[32030]-(112)	B[30032]-(122)	B[32030]-(112)		
B[21020]-(122)	B[21020]-(122)	B[21020]-(122)	B[30012]-(212)	B[12012]-(112)	B[30012]-(212)		
B[30012]-(211)	B[30012]-(211)	B[30012]-(211)	B[30030]-(112)	B[30030]-(112)	B[30030]-(112)		
+ B[30012]-(121)	+ B[30012]-(121)	B[30012]-(121)	B[30030]-(122)		B[30030]-(122)		
B[20030]-(122)	B[20030]-(122)	B[20030]-(122)					
		B[30012]-(112)					
B[12012]-(212)	B[12012]-(212)	B[12012]-(212)					
		B[23021]-(202)					
	B[30030]-(112)	B[30030]-(112)					
		B[32030]-(022)					
		B[32012]-(121)					
		B[32012]-(112)					
		B[23021]-(112)					
	B[30012]-(212)	B[30012]-(212)					
	B[32030]-(112)	B[32030]-(112)					
	B[30012]-(122)	B[30012]-(122)					
B[30030]-(122)	B[30030]-(122)	B[30030]-(122)					
B[21022]-(212)	B[21022]-(212)	B[22012]-(212)					
		B[30032]-(121)					
		B[32012]-(122)					
		B[23021]-(122)					
	B[30032]-(122)	B[30032]-(122)					
		B[32030]-(122)					

[a]Sil-2, similar to squalene; DB-5, 5% diphenyl, 95% dimethylpolysiloxane; Smectic, *N,N'*-bis(*p*-butoxy-benzylidene)-α,α'-bis-*p*-toluidine; Rtx-2330, 10% phenylcyanopropyl 90% biscyanopropyl polysiloxane; Chiral, 10% heptakis(2,3,6-*O*-*t*-butyl-dimethylsilyl-β-cyclodextrin in OV-1701-OH; DX-4, 15% dimethylsilicone, 85% polyethylene glycol; FFAP, polyethylene glycol terephthalic acid ester. [b]Data from Vetter et al. (*103*). [c]Data from Baycan-Keller and Oehme (*104*). [d]Data from Coelhan et al. (*105*). [e]Data from Nikirov et al. (*106*). [f]Based on the measurements of de Boer et al. (*69*).

combination was preferred because of its somewhat better separation but mainly because of its low bleed. However, Baycan-Keller and Oehme (*109*) observed degradation of B[32012]-(111), B[30012]-(211), B[30012]-(121), B[30012]-(212), B[30030]-(122), B[12012]-(212), and B[32030]-(112) on the Rtx-2330 phase. Reevaluation of the multidimensional heart-cut data of de Boer et al. (*69*) showed that the standard of B[12012]-(212) has the same profile (a broad hump eluting with the analyte peak) as that described by Baycan-Keller and Oehme; this could have been caused by decomposition on the stationary phase. However, normal peak shapes were obtained for B[12012]-(202), B[30012]-(111), and B[30030]-(122). Karlsson and Oehme (*107*) also mention the possibility that the low response of B[30030]-(122) is due to losses on the polar Rtx-2330 phase.

Alder et al. (*100*) found that B[12012]-(202) and B[30030]-(122) were decomposed to a great extent on the highly polar DX-4 phase. de Boer et al. (*69*) did not observe degradation effects on this phase; this was also true after reevaluation of data and further experiments with this stationary phase (Figure 3). This may be partly because a shorter column was used (15 m instead of 30 m), which limits exposure time of the components to a high temperature, 493K.

It is extremely time consuming to analyze several compounds in a complex sample using a multidimensional set-up, even when a system is available that has several parallel traps for storage of heart-cuts (*108*). If the speed of the secondary separation is high enough to separate a cut from the first dimension while the next cut is being collected, it will then be possible to record a connecting set of secondary chromatograms. The complete two-dimensional chromatogram can be constructed from the secondary chromatograms, similar to that in thin-layer chromatography. A method with this capability is called comprehensive (*110*). A comprehensive separation uses the whole two-dimensional separation space to generate resolution provided that the individual separations are based on different interactions (i.e., are not correlated). For a method to be comprehensive, it is necessary that the first dimension be sampled at least every peak width by the second separation dimension. The first dimension can then be constructed from the secondary chromatograms (*111,112*). Research to make this powerful separation technique available for toxaphene analysis is currently under investigation.

Detection

Using MS detection would overcome some problems of coelution, in addition to those

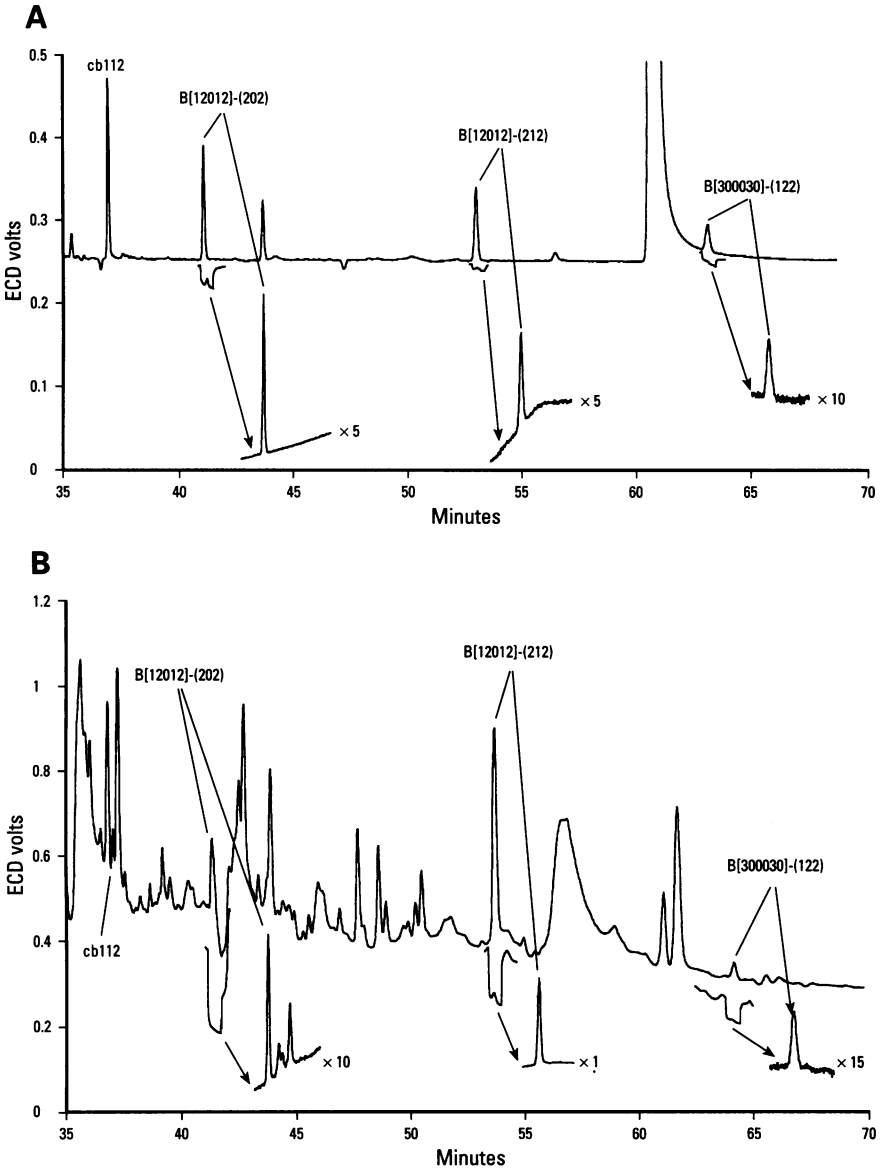

Figure 3. Heart-cuts of chlorobornanes B[12012]-(202), B[12012]-(212), and B[30030]-(122) in (*A*) a commercial standard and (*B*) a Hake liver extract. Upper traces show separation on the first column (DB-5); lower traces show separation of the heart-cut peaks on the second column (DX-4).

caused by compound class or the degree of congener chlorination. Coelution with compounds having similar fragmentation patterns may well occur in the electron impact (EI) mode and will lead to false positive results. Structural information is, of course, much more limited in the NCI mode. The ECD is an attractive alternative detector, however, and as ECD is less selective than MS detection, an even more efficient separation will be necessary.

The profiles obtained with flame ionization detection are similar to those obtained with full-scan EI/MS and have a low response dependency on the chlorine substitution pattern; however, only the latter technique has the selectivity and sensitivity necessary for residual analysis (*92*). NCI/MS has a completely different peak profile that is probably caused by the higher variation in response factors for individual congeners (*11*).

When using the ECD, removal of interfering compounds is a prerequisite. PCBs, for example, are present at high concentrations in most environmental samples, which may also contain toxaphene compounds. In addition, PCBs have higher response factors because of their aromatic character. Andrews et al. (*113*) used high resolution MS in the selected ion monitoring (SIM) mode at m/z 158.8768 and 160.9739 with EI as the ionization method used to obtain a total chlorinated bornane result without interference from other compounds. However, this approach is less sensitive than the NCI mode and does not distinguish between homolog groups. NCI offers both selectivity and sensitivity for bornane congeners (*114*) but does not offer the possibility of structure elucidation.

NCI is the most widely used MS detection method for toxaphene, but it is insensitive to lower chlorinated congeners. The EI mode is more sensitive to lower chlorinated congeners and, consequently, an additional 25 peaks from lower chlorinated compounds were found with EI than with NCI (*68*). Often both the M^- and $(M\text{-}Cl)^-$ ions are monitored (*67,101*). Problems with GC–NCI/MS in the SIM mode include the formation of $(M\text{-}OCl)^-$ fragments of PCBs; false-positive signals may be caused in part by chlordanes and the appearance of higher chlorinated bornane congeners (*67,73,115*). Krock et al. (*96*) did not observe the interfering oxygen adducts of PCBs, which have only been reported to occur when small leakages are present in the MS (*96*). Good linearity over four orders of magnitude for five chlorinated bornane congeners was obtained using NCI/MS (*102*). It was tentatively found that a 2,2,5,5 substitution of chlorobornane congeners ([30030]) had a negative effect on the NCI/MS response (*81,116*).

Buser and Müller (*15*) used tandem MS/MS with EI to identify B[12012]-(202) and B[12012]-(202) in penguin and harbor seal samples. Most toxaphene congeners produce fragments with m/z = 125 under EI conditions; this ion, together with ions at m/z = 159, 195, and 231, is considered to be characteristic of toxaphene congeners (*62*). In contrast with quadrupole or double focusing MS/MS in which tandem mass spectrometry is accomplished through space, Saturn 4D MS/MS uses the time dimension to accomplish MS/MS. The isolation of precursor ions and further dissociation takes place in the same chamber (m/z locking) but at a different time. This reduces loss of precursor ions and hence provides better sensitivity. The major ion in the daughter spectrum of m/z = 159 is a fragment at m/z = 125. However, PCBs and some organochlorine compounds also

produce this fragment in the MS/MS mode. Therefore, it would appear that the ion at m/z = 89 (dechlorinated monochlorotropylium ion), which orginates from the m/z = 125 ion, would be more useful for quantification of toxaphene congeners (*117*). However, coelution of compounds that produce this ion cannot be observed. Furthermore, the response factors with this method vary considerably for individual cogeners [B[12012]-(202) 2.3; B[30012]-(111) 3.2; B[12012]-(212) 0.7; B[30030]-(122) 0.7; technical mixture 1.7)]. The authors suggest that because their results were all lower than the average, results in a round robin test in which they took part (*98*) could be explained by the specificity of their method.

Alder and Vieth (*92*) determined the toxaphene concentration in a standard reference sample (SRM 1588) on the basis of three indicator congeners using GC–ECD. They found a total toxaphene concentration of about 1600 μg kg^{-1}. In contrast to this result, Fowler et al. (*118*) obtained a value of 5410 μg kg^{-1} in the same sample using GC–NCI/MS. Alder and Vieth then reanalyzed the sample using NCI/MS and obtained a value of 5210 μg kg^{-1}, which is close to the value reported by Fowler et al. (*118*). They concluded that this large difference between the concentrations found is caused by the large difference in response factors between congeners with NCI, which gives a positive bias to the results when they are compared with those using ECD, which has a smaller difference between response factors. Rantio et al. (*48*) also showed that NCI/MS gave generally higher results than ECD. However, the results demonstrated a linear relationship between the two detection techniques, which made it possible to compare the result obtained. A higher response for NCI/MS was also reported by Wideqvist et al. (*46*), especially when the degree of chlorination was higher. In contrast, Xu et al. (*119*) found that GC–ECD gave results identical to those for GC–NCI/MS for quantification of individual chlorobornanes in fish samples. A possible explanation for these, which at first are contradictory observations, could be different standards used in combination with the detector, which could influence the result to a large extent, as shown by Carlin and Hoffman (*86*). For example, it is possible to obtain the same results for GC–ECD and GC–NCI/MS with one standard, but largely differing results with another. Another explanation can be differences between the MS configuration used in the studies.

ECD determination of total toxaphene is subject to insufficient selectivity, whereas NCI/MS is subject to variable response factors. Using indicator compounds as a basis for calculation of total concentration was suggested as a way to obtain precise and comparable data (*92*). However, this approach can only be used successfully when the indicator compounds do not coelute with other compounds. Coelution of suggested indicator compounds was shown by heart-cut multidimensional gas chromatography (*69*). Depending on the sample type, up to 10 peaks were found when the analyte peak was further separated on a second, different column. For B[12012]-(202) and B[30030]-(122), the area fraction of the compound in its first dimensional peak was between 20 and 85%, whereas that for B[12012]-(212) was 85 to 95%. Therefore, only B[12012]-(212) can be determined reliably after a one-dimensional separation. However, it must be noted that most samples analyzed were from organisms relatively high in the food chain.

Enantiomers

Usually enantiomer ratios (ERs) are used to express the ratio in which the enantiomers are present. The peak area/height of the (+)-enantiomer is divided by that of the (–)-enantiomer (*120–123*). When the conformation of the enantiomers eluting from a chromatographic system is not known, as with enantiomers present in toxaphene, the ER is often expressed as the peak area/height of the first eluting enantiomer divided by that of the second (*124*). Using the quotient of the two enantiomers gives an undefined result when the second enantiomer is not detected. de Geus et al. (*125*) observed this and therefore divided the second enantiomer by the first. Of course, this approach only shifts the problem. It would be better to divide by the detection limit (which does not equal zero) when a compound is not found, but this can lead to very high or low numbers. In addition, because of the reciprocal-like scale, ERs larger than unity appear to deviate more than ERs smaller than unity (e.g., 6.7 and 5.0 vs 0.15 and 0.20). To avoid these disadvantages, the (+)-enantiomer or the first eluting enantiomer can be expressed as a proportion of the sum of the two (*126*). This enantiomer fraction (EF) is 50% if both enantiomers have the same abundance. Advantages of using EFs are an understandable linear scale, dividing by zero or very high and low values does not occur, and similar enantiomer proportions are distinguished more easily. Deviations from the racemic value have the same magnitude in both directions.

Most of the compounds in toxaphene are chiral. Since bioaccumulation and metabolism in biota are often different for enantiomers, a change in the EF can be expected during disposition in the food chain. Furthermore, enantiomers often have different toxic properties. The determination of EFs in biota can give an indication about whether a specific biologic mechanism changes the ratio in the course of disposition in the body. A significant deviation from the EF value present in the technical mixture (usually 50%) suggests a specific metabolic transformation of one of the enantiomers. On the other hand, an equal EF points to biologic persistence (*124*). Comparison of the EFs of different congeners in combination with their molecular structures can help us gain insight into the metabolism of these compounds.

When determining EFs of chlorinated bornanes in biota, the possibility cannot be excluded that the values found are not merely due to metabolism in the species studied because a change during previous disposition in the food chain is also possible. Feeding studies in which the species of interest is exposed to (racemic) mixtures of known composition would eliminate this problem. As an alternative, *in vitro* assays can be used in which microsomes are incubated with the compounds of interest. The microsomes contain the cytochrome P450-dependent monooxygenase enzyme systems involved in enantioselective and nonenantioselective biotransformation. Boon et al. (*127,128*) successfully used such an approach to study the a-chiral biotransformation of toxaphene congeners by microsomes from harbor seal, whitebeaked dolphin, sperm whale, and laysan albatross.

Separation should be enantioselective as well as isomer specific to determine EFs. Unfortunately, this doubles the number of peaks to be separated (*125*). A *tert*-butyldimethylsilylated β-cyclodextrin phase, introduced by Blum and Aichholz (*129*), has been shown to give a good enantiomer separation of toxaphene compounds (*15,104,124,125,130*). However, enantiomer separation of bornane congeners is still a rather empirical task and the selection of a convenient stationary phase is determined primarily by trial and error

(*131*). It has been shown that columns based on heptakis(2,3,6-*O*-*tert*-butyldimethylsilyl)-β-cyclodextrins are especially suitable for the separation of polychlorinated bornane enantiomers (*15,124, 132,133*). Unfortunately, this stationary phase is not very well defined and batch-to-batch differences have been observed (*134*). Vetter et al. (*135*) compares several enantioselective phases for the separation of toxaphene compounds.

The obtainable enantiomer resolution depends on the column oven temperature profile. It was found that this phase can be used up to a temperature of 535K in a programmed run. However, at lower temperatures the obtained resolution is much higher (*130*). Baycan-Keller and Oehme (*104*) showed that a temperature ramp of 1K resulted in much better separations compared to one of 10K. This was also found by de Geus et al. (*125*). Unfortunately, slow temperature programs lead to very long run times, which can be a problem when compounds with low concentrations must be detected.

Most attention has been devoted to measuring the EFs of B[12012]-(202) and B[12012]-(212) (Table 6) (*15,137*). However, studies by Vetter et al. (*130*) and de Geus et al. (*125*) show that other compounds can be much more interesting because they show more enantioselective activity (Table 7).

Parlar et al. (*139*) report that all parent compounds in toxaphene occur as racemates. Buser and Müller (*140*) showed that some compounds are present in the technical formulation (Melipax) in nonracemic compositions. However, interferences from other compounds cannot be excluded, even in the MS/MS method they used. Vetter et al. (*130*) isolated the compound B[21020]-(022) from Melipax. The mass spectrum of this compound showed no significant impurities; however, the first eluting enantiomer was significantly more abundant than the second. The EF was 55.8 ± 0.6%. Furthermore, the authors showed that the EF of this compound was 50.0% in a cod liver extract from the Baltic. If a synthesized standard with a racemic composition were used, it might be concluded that no enantioselective process took place. This demonstrates the importance of carefully choosing the standard. On the other hand, deviation of this compound from the racemic value should also appear in other technical formulations because Melipax accounts for only 5% of the global toxaphene production (*141*) and B[21020]-(022) and the contribution of B[21020]-(022) to Melipax is < 1% (*130*).

Parlar et al. (*139*) document EFs of several chlorinated bornanes in cod liver oil, herring, halibut, caviar, and redfish samples obtained from Karlson and Oehme (*107*), Kallenborn et al. (*124*), and Alder et al. (*136*). The EFs show little variation—50.2 ± 1.2%; therefore, Parlar et al. conclude that no significant degradation of toxaphene enantiomers takes place in

Table 6. Enantiomer fractions.[a] Percentage of several chlorinated bornanes in seal blubber[b] and several other species.[c]

Sample	Compound	
	B[12012]-(202)	B[12012]-(112)
Seal blubber (n = 10)	50.59 ± 0.56	51.43 ± 0.78
Herring (Baltic Sea)	48.7 ± 0.8	51.9 ± 0.7
Herring (North Sea)	51.5	51.9 ± 0.7
Mackerel (North Sea)	53.0 ± 2.6	52.6 ± 0.7
Mackerel (North Atlantic)	49.5 ± 0.8	51.9 ± 0.7
Halibut (North Atlantic)	47.6 ± 2.2	51.9 ± 0.7
Redfish (North Atlantic)	52.4 ± 2.3	51.9 ± 0.7
Saithe (North Atlantic)	51.9 ± 0.7	51.7 ± 0.7
Farmed salmon (Norway)	50.5 ± 1.5	53.0 ± 0.9
Monkey adipose tissue	56.3 ± 1.9	59.0 ± 0.8
Monkey adipose tissue[d]	56.7 ± 0.6	57.4 ± 0.6
Human milk A	56.1 ± 1.7	51.5 ± 0.7
Human milk B	56.1 ± 0.8	53.9 ± 0.6
Human milk C	53.9 ± 2.1	55.2 ± 1.2
Human milk D	55.2 ± 2.0	54.8 ± 0.8
Human milk (pooled sample)	51.7 ± 1.2	57.1 ± 0.6

[a]Enantiomer fraction is the abundance of the first eluting enantiomer relative to the abundance of the sum of both enantiomers (*126*). [b]Data from Kallenborn et al. (*124*). [c]Data from Alder et al. (*136*). [d]Identical extract determined with GC–ECD.

Table 7. Enantiomer fractions.[a] Percentage reported in the literature.

Sample	Compound						
	B[12012]-(202)	B[12012]-(212)	B[20030]-(022)	B[20030]-(022)	B[21001]-(112)	B[30030]-(022)	B[30030]-(122)
Data from Vetter et al. (*130*)							
Weddell seal (female, subadult)	52	55		77			
Weddell seal (male, adult)	55	57		72			
Weddell seal (male, adult)	52	57		74			
Leopard seal (unkown, adult)	57	62		81			
Data from de Geus et al. (*125*)							
Hake liver (pooled, male and female, 1989–1991, Ireland)	53.5	52.9	ND		ND	87.7	74.1
Dolphin blubber (40-year-old male, 1990, southern North Sea)		51.6	100		ND	>95	76.9
Dolphin blubber (10-year-old male, 1990, southern North Sea)	51.0				ND	>95	80.0
Data from Parler et al. (*138*)[b]							
Cod liver oil	50.0–58.5	50.0–52.6		52.4–56.1			50.2–52.2
Fish oil	50.0–52.2	50.2–50.5		54.6–56.1			63.6–65.4

ND, not detected. [a]Enantiomer fraction is the abundance of the first eluting enantiomer relative to the abundance of the sum of both enantiomers (*126*). [b]Samples were obtained from different countries.

fish. Unfortunately, Kallenborn et al. (*124*) and Alder et al. (*136*) present other EFs (Table 6) and Karlsson and Oehme (*107*) do not present EFs. The data in Table 6 are only for B[12012]-(202) and B[12012]-(212); the mean EF is 53.1 ± 2.7%, which is not a large deviation from the racemic value. In a recent study Parlar et al. (*138*) show that B[20030]-(122) and B[30030]-(122) show EFs deviating from the racemic value in cod liver oil and fish oil (Table 7). B[12012]-(202) and B[12012]-(212) have EFs near the racemic value, but in one cod liver oil sample a deviating value is found for B[12012]-(202), 58.5%. That sample is subject to further study.

The results of Alder et al. (*136*) show that the EF value of these compounds in warm-blooded species (human milk and cynomologus monkey adipose) deviate from unity. This is in accordance with the observed EF of 57.3% for B[12012]-(212) in Antarctic penguins by Buser and Müller (*140*). This could indicate a more efficient metabolism present in these species compared to that in other species (fish).

Interlaboratory Study

A German collaborative study (*142*) with contaminated milk fat undertaken in the mid-1980s demonstrated the analytic difficulties and uncertainties in analysis of technical (total) toxaphene by packed column GC–ECD. Andrews found that in many laboratories only about 15 to 30% of toxaphene components were eluted from silica or Florisil columns with a nonpolar solvent. This was thought to be the main source of the large variation between laboratories (*98*). In a German intercalibration experiment, recoveries of 77 to 100% with a relative standard deviation of reproducibility of 23 (9.2–50.5%) were found for four indicator compounds in a fatty matrix. On the basis of these results, the method was recommended for routine analysis in food inspection in Germany (*99*). In a recent QUASIMEME (Quality Assurance of Information for Marine Environmental Monitoring in Europe) laboratory performance study with four toxaphene congeners in standard solutions, most of the 15 participants reported satisfactory results (*143*).

Indicator Compounds

Ideally, toxicity should play a major role in the selection of indicator compounds. Unfortunately, little is currently known about acute and chronic toxicity of individual congeners to mammals. Occurrence determines, in combination with toxicity, whether a compound is important. Stereochemistry may play an important role since the biologic disposition of enantiomers varies (*125*) (Table 7). Boon et al. (*128*) showed that B[12012]-(202) and B[12012]-(212) did not yield a positive response in a mutatox test, whereas technical toxaphene and B[30012]-(111) did yield a positive response. The latter compound is only detected in low concentrations in wildlife samples (*69,99,117*).

Next to these parameters, analytic convenience is important. The compounds should be detectable without the interference of other compounds when common extraction, clean-up, and separation/detection procedures are used. The compounds should also be commercially available (*92*).

In practice, the availability of standards and analytic convenience dictate the choice of compounds, a situation similar to that with PCBs. The concentrations of B[12012]-(202), B[12012]-(202), and B[30030]-(122) are in the 0.05 to 0.08 mg kg^{-1} (fat basis) range in fish and other foodstuffs and their peaks represent about 50% of the total toxaphene ECD response. Because it is presumed that these congeners are also dominant in human toxaphene intake, Alder and Vieth (*92*) suggested their use as indicator compounds. To distinguish between recent contamination (e.g., recent use of the pesticide) and persistent congeners still present in the environment, an indicator compound that is not stable in the environment can be concluded. B[30012]-(111) was suggested for this purpose (*92*). Xu et al. (*119*) proposed a second compound, B[30032]-(122), for the same purpose. Because this compound was found to degrade easily in the detector and is only present in minor amounts in technical formulations, it is not useful as an indicator compound (*92*). Measuring the individual indicator compounds on a single GC column presents a problem in that several compounds may be present in one peak, as demonstrated by heart-cut multidimensional GC (*69*).

Instead of measuring the toxaphene compounds in all fish for consumption, samples from important species and fishing areas can be selected to answer the question of human intake of these compounds, as was done in a large study by Alder et al. (*100*). As an alternative, Alder and Vieth (*92*) chose to mix edible parts of relevant fish samples (*97*), before extraction and residue analysis. The fish samples, however, were not prepared in the way they are generally consumed.

Levels in Biota

Total Toxaphene

Most of the available information about toxaphene concentrations in biota is referred to in terms of total toxaphene. However, since the number and pattern of congeners in environmental samples is substantially different from those in the technical mixture (as a result of environmental and metabolic modification) (*88,113*), values for total toxaphene should be considered only indicative. Table 8 gives an overview of the total toxaphene levels in biota samples as reported in the literature.

Much of the information about total toxaphene levels in biota described in the literature is from studies of freshwater systems in Canada and the United States, where toxaphene was one of the most dominant organochlorine residues (*161*). Toxaphene concentrations plateaued after a period of steady increase through the 1970s, but its incidence continued to increase; residues were present at 88% of the stations sampled from 1980 to 1981 (*162*). From 1978 to 1979, toxaphene concentrations were highest in lake trout (*Salvelinus namaycush*) samples from Lakes Michigan and Superior, with typical concentrations of 5 to 10 μg g^{-1} lipid (*163*). From 1980 to 1981, concentrations were generally lower, 2 to 5 μg g^{-1} lipid. Concentrations of toxaphene declined in trout and smelt from the Great Lakes between 1982 and 1992 except for fish from Lake Superior (*159*).

Little toxaphene has been used in the Great Lakes basin alone. The main input is thought to be through atmospheric transport from the southern United States or Central America, followed by wet and dry deposition (*53,115*). Atmospheric transport was probably also responsible for residues detected in fish from lakes in Alaska. Several other reports conclude that toxaphene is carried in the atmosphere from the site of application and its accumulation is widespread in freshwater and marine fish (*90*).

Geographic variation in toxaphene and other organochlorine pesticides within the Canadian Arctic has been examined in several studies. In Arctic char (*Salvelinus alpinus*) residues of toxaphene generally increased from west to east, with generally higher levels in samples from Baffin Island and Hudson Bay (*164*). The

Table 8. Total toxaphene in biota.

Species	Location	Year	Tissue	*n*	Sex	Total toxaphene, µg/g ww	Total toxaphene, µg/g lipid	Reference
Marine invertebrates								
Zooplankton	Ice Island	1986	Whole	Pool			0.024	(*2*)
	810N,1010 W	1987	Whole	Pool			0.16	
Zooplankton	64N,169W	1988	Whole	Pool		0.002		(*144*)
Benthic amphipods	Ice Island	1986	Whole	Pool			1.5	(*2*)
(*Anoxy sarsi* and	810N,1010 W	1987	Whole	Pool			8.3	
Tmetonyx cicada)								
Scallops	Georges Bank	1979	Muscle	Pool		ND		(*84*)
(*Placopecten magellanicus*)								
Blue mussel (*Mytilus edulis*)	Greenland	1994–1995	Muscle	Pool		ND		(*145*)
Marine fish								
Arctic cod	Arctic Bay	1984	Muscle	Pool		0.023		(*146*)
(*Boreogadus saida*)	Resolute Bay	1984	Muscle	Pool		0.014		
	Pangnirtung	1984	Muscle	Pool		0.046		
Arctic cod	Greenland	1994–1995	Liver	Pool		ND		(*145*)
Antarctic cod	South Georgia	1977	Liver	1		0.22	0.68	(*4*)
(*Dissostichus eleginoides*)								
Atlantic cod (*Gadus morhua*)	Gulf St. Lawrence	1979	Liver	Pool		1.1	2.4	(*84*)
Atlantic cod	Gulf of Finland	1988		Pool			0.64	(*49*)
	Vester Tana	1989		Pool			0.54	
Atlantic cod	Southern North Sea	1989	Liver	Pool			0.4	
	Central North Sea	1989	Liver	Pool			0.6	
	Northern North Sea	1989	Liver	Pool			1	
Atlantic cod	Germany	1993(?)	Liver oil	Pool			2.45	(*102*)
Atlantic cod		1993(?)	Liver oil	Pool			2.73	
Atlantic cod	North Sea	?	Liver	Pool		0.3		(*147*)
Atlantic salmon (*Salmo salar*)	Alaska	1979	Spawn	1		0.041	0.285	(*4*)
	Galway, Ireland	1979	Spawn	1		0.26	3.5	
Atlantic salmon	W. Greenland	1979	Whole	2		0.3		(*148*)
	Labrador Sea	1979	Whole	2		0.084		
Atlantic salmon	Teno R., Arctic	1988	Muscle	Pool			2.87	(*49*)
		1990	Muscle	Pool			0.89	
	Simo R., Bothnian Bay	1988	Muscle	Pool			2.58	
		1991	Muscle	Pool			0.91	
	Bothnian Sea	1988	Muscle	Pool			2.01	
	Baltic Proper	1988	Muscle	Pool			0.78	
	L. Saimaa	1988	Muscle	Pool			6.99	
Atlantic salmon	Norway	1993(?)	Oil	Pool			1.1	(*102*)
	Norway	1993(?)	Oil	Pool			0.54	
Greenland cod (*Gadus ogac*)	Frobisher Bay	1984	Whole	2		0.006		(*146*)
	Victoria Island	1984	Muscle	Pool		<0.002		
Hake (*Merluccius merluccius*)	Ireland	?	Liver			0.9		(*147*)
Herring (*Clupea harengus*)	Baltic	1978	Muscle	Pool			13	(*149*)
Herring	Gulf St. Lawrence	1979	Muscle	Pool		1	12	(*84*)
	Halifax	1979	Muscle	Pool		0.4	4.4	
Herring	Southern North Sea	1989	Muscle	Pool			0.4	(*89*)
Herring	Skagerrak	?	Muscle	Pool		0.04		(*147*)
Inconnu (*Stenodus leucichthys*)	Tuktoyaktuk Harbour	1984	Muscle	1		0.024		(*146*)
Pacific herring	Tuktoyaktuk Harbour	1984	Muscle	Pool		0.074		(*146*)
(*Clupea havengus*)								
Plaice (*Pleuronectes platessa*)	German Bight	1989	Liver	Pool			0.2	(*89*)
	Skagerrak	1989	Muscle	Pool			0.1	
Pollack (*Gadus pollachius*)	Bering	1988	?	?		0.013		(*144*)
Shorthorn sculpins	Greenland	1994–1995	Liver	Pool		ND		(*145*)
(*Myoxocephalus scorpius*)								
Sturgeon (*Acispencer stellatus*)	Caspian Sea	1978–1979	Spawn	Pool		0.304	1.625	(*4*)
Trout (*Salmo trutta*)	Bothnian Bay	1990–1991	Muscle	Pool			1.12	(*49*)
	Bothnian Sea	1990–1991	Muscle	Pool			0.52	
Twait shad (*Alosa fallax*)	North Sea	?	Muscle	Pool			0.02	(*147*)
Marine mammals								
Beluga whale	S. Beaufort Sea	1983–1987	Blubber	10	M	3.83 ± 1.16		(*150*)
(*Delphinapterus leucas*)				2	F	1.38		
	Jones Sound	1984		8	M	4.25 ± 1.02		
				7	F	3.74 ± 2.12		

(*Continued*)

Table 8. *Continued.*

Species	Location	Year	Tissue	*n*	Sex	Total toxaphene, µg/g ww	Total toxaphene, µg/g lipid	Reference
	W. Hudson Bay	1986		4	M	5.10 ± 0.42		
				4	F	1.77 ± 1.41		
	Cumberland Sound	1983		6	M	5.78 ± 5.39		
				6	F	1.77 ± 1.76		
Beluga whale	Alaska	1992	Blubber	7			5.18 ± 4.77	(*151*)
Narwhal	W. Baffin Bay	1982–1983	Blubber	15	M	9.16 ± 2.35		(*94*)
(*Monodon monoceros*)				6	F	2.44 ± 2.84		
White-beaked dolphin	Newfoundland	1980–1982	Blubber	9	M	46.0 ± 22.1	83.6 ± 75.4	(*152*)
(*Lagenorhynchus albirostris*)				13	F	38.2 ± 10.7	53.3 ± 11.7	
White-beaked dolphin	North Sea	?	Blubber	1(?)		19		(*147*)
Pilot whale	Newfoundland	1980–1982	Blubber	5	M	11.7 ± 7.05	16.0 ± 9.73	
(*Globicephala melaena*)				9	F	2.22 ± 2.29	3.41 ± 3.26	
Walrus (*Odobenus rosmarus divergens*)	N. Bering Sea	1981–1984	Blubber	53	M&F	<0.1		(*153*)
Grey seal (*Halichoerus gryphus*)	Baltic	1974–1977	Blubber	Pool			11	(*149*)
Harbor seal (*Phoca vitulina*)	Skagerrak	1988	Blubber	Pool			0.35	(*72*)
	Baltic	1988	Blubber	Pool			1.5	
Ringed seal (*Phoca hispida*)	Greenland	1994–1995	Blubber	Pool		0.26		(*145*)
Freshwater fish								
Arctic char (*Salvelinus alpinus*)	Drachensee	1978	Spawn			0.017	0.125	(*4*)
Arctic char	W. Davies Strait	1985	Whole	6		0.157 ± 0.067		(*154*)
Arctic char	Greenland	1994–1995	Muscle	Pool		0.013		(*145*)
Burbot (*Lota lota*)	L625 ELA	1985–1986	Liver	12			1.73	(*155*)
	Lake Winnipeg	1985–1986	Liver	14			0.807	
	Trout Lake	1985–1986	Liver	7			2.34	
	South Indian Lake	1985–1986	Liver	14			1.47	
	Mackenzie River, Ft. Simpson	1985–1986	Liver	5			1.13	
	Mackenzie River, Ft. Good Hope	1985–1986	Liver	8		1.57		
	Mackenzie River, Arctic Red River	1985–1986	Liver	4		1.7		
	Mackenzie River, Ft. McPherson	1985–1986	Liver	4		0.93		
Burbot	Yukon River, Laberge Lake	1990–1992	Liver	Pool		2.81		(*156*)
	Yukon River, Kluane Lake	1990–1992	Liver	Pool		54		
Carp	Saginaw Bay	?	?	Pool		0.51		(*115*)
Eel (*Anguilla anguilla*)	River Rhine	1989	Muscle	Pool			0.3	(*89*)
	Lake Yssel	1989	Muscle	Pool			0.09	
Guapote (*Cichlasoma managuense*)	Lake Xolotlán, Monotombo	1991	Muscle	19		0.051		(*157*)
Lake trout (*Salvelinus namaycush*)	Lake Michigan	1982	Belly	4		4.3 ± 3.7		(*158*)
Lake trout	Lake Michigan	1982	Whole	Pool		5	27	(*159*)
		1992–1994	Whole	Pool		1.5	7.6	
	Lake Superior	1982	Whole	Pool		4.9	28	
		1992–1994	Whole	Pool		6.7	35	
	Lake Huron	1982	Whole	Pool		5.2	30	
		1992–1994	Whole	Pool		2.4	13	
	Lake Ontario	1982	Whole	Pool		4.5	24	
		1992–1994	Whole	Pool		0.54	2.8	
Lake trout	Yukon River, Laberge Lake (?)	1990–1992	Muscle	Pool		0.657		(*156*)
	Yukon River, Watson Lake (?)	1990–1992	Muscle	Pool		<0.01		
Lake trout	Siskiwit	?	Whole	Pool		0.29		(*115*)
Pike (*Esox lucius*)	Lake Carigellen	1979	Spawn	1		0.03	0.24	(*4*)
Rainbow trout	Peanut Lake	1993	Muscle	3		0.008	0.71	(*160*)
(*Salmo gairdnerii*)	Chatwin Lake	1993	Muscle	3		0.068	1.04	
Rainbow smelt	Lake Superior	1982	Whole	Pool		0.41	10	(*159*)
(*Osmerus mordax*)		1992–1994	Whole	Pool		0.16	3.1	
	Lake Michigan	1982	Whole	Pool		0.74	10	
		1992–1994	Whole	Pool		0.059	1.1	
	Lake Ontario	1982	Whole	Pool		0.72	11	
		1992–1994	Whole	Pool		0.067	1.1	
Sculpin (*Comephorus dybowskii*)	Lake Baikal	1990	Whole	Pool			1.6–2.1	(*26*)
Tilapia (*Sarotherodos mossambicus*)	Lake Xolotlàn, Monotombo	1991	Muscle	14		0.04		(*157*)

(*Continued*)

Table 8. *Continued.*

Species	Location	Year	Tissue	*n*	Sex	Total toxaphene, µg/g ww	Total toxaphene, µg/g lipid	Reference
Walleye (*Stizostedion vitreum vitreum*)	Lake Erie	1982	Whole	Pool		0.25	2.2	(*159*)
		1992–1994	Whole	Pool		0.13	1.4	
White fish (*Corogunus autumnalis*)	Lake Baikal	1991	Whole	2			0.93–1.3	(*26*)
White fish (*Corogunus clupeaformis*)	Yukon River, Laberge Lake	1990–1992	Muscle	Pool		0.04		(*156*)
	Yukon River, Watson Lake	1990–1992	Muscle	Pool		<0.01		
White fish	Siskiwit	?	?	Pool		0.22		(*115*)

Abbreviations: M, male; F, female; ?, unknown.

results for Arctic char are consistent with the movement of organochlorines in air masses from South/Central America in a northeasterly direction. As northern latitude increases, concentrations of several organochlorines were also found to decrease for burbot (*Lota lota*) (*155*). Toxaphene was the predominant organochlorine residue in northern fish samples. The peak pattern of toxaphene in the chromatograms showed extensive transformation compared to technical toxaphene mixtures. Levels of toxaphene were not significantly correlated with age or weight of the fish. Kidd et al. (*156*) studied the spatial variability of toxaphene in fish collected between 1990 and 1992 from lakes in the Yukon Territory and found that the levels varied considerably between lakes probably because of differences in the food chains of the lakes.

Toxaphene was also the major organochlorine residue in Canadian Arctic marine invertebrates and fish. Arctic cod (*Boreogadus saida*) in three eastern Arctic locations had concentrations of toxaphene 5- to 10-fold higher than those for DDT or PCB (*146*). Musial and Uthe (*84*) found that levels of CHBs in Arctic cod liver were about 2-fold lower than those in Atlantic cod (*Gadus morhua*). Bidleman et al. (*2*) reported levels of toxaphene to be equivalent to those of PCBs in zooplankton and in amphipodes collected from an ice island in the Arctic Ocean. Other organochlorines had lower concentrations. Toxaphene was found to be a major contaminant in Atlantic cod liver and herring (*Clupea harengus*) muscle from eastern Canadian waters, with levels similar (lipid weight basis) to those for PCB but generally higher than those for DDT (*84*). Toxaphene was not detected in deep-sea (Canadian waters) scallop (*Placopecten magellianicus*) (*84*).

High levels of toxaphene were reported for white-beaked dolphins (*Lagenorhynchus albirostris*) and pilot whales (*Globicephala malaene*) collected from 1980 to 1982 from the coast of Newfoundland (*152*). This was explained by the increased use of toxaphene during the 1970s. Toxaphene levels were higher than those for other organochlorines such as PCBs and DDT Most of the peaks in the toxaphene standard were not present in dolphin blubber, an indication of the dolphin's considerable metabolism and/or selective accumulation of some isomers and/or metabolites. Two peaks accounted for about 50% of the toxaphene peaks (probably GC–EI/MS). Toxaphene also was the major organochlorine contaminant detected in blubber of Arctic belugas (*Delphinapterus leucas*) (*150*). Little geographic variation in the concentration of toxaphene was observed in five different areas (East Hudson Bay, Cumberland Sound, West Hudson Bay, Beaufort Sea, and Jones Sound). Geographic comparisons of toxaphene levels for belugas are difficult because belugas migrate over relatively long distances and spend most of the year at the ice edge rather than at the locations where they were sampled. Belugas collected from the north coast of Alaska had higher toxaphene concentrations in blubber (*151*) than PCBs, DDTs, and chlordanes in the same samples. Males had higher concentrations of toxaphene than females and the oldest male had a higher concentration than the youngest male. Transplacental transfer to the fetus and through lactation to the nursing pups are the most probable causes of the lower toxaphene levels in females compared to males. Stern et al. (165) identified the two major recalcitrant toxaphene congeners in aquatic biota from beluga blubber as B[12012]-(202) and B[12012]-(212). Their sum constituted 28 to 34% of total toxaphene in arctic char, 53% in burbot, and 81 to 89% in beluga whale blubber from the Canadian Arctic.

Toxaphenes were the dominating organochlorines in narwals (*Monodon monoseros*) collected from 1982 to 1983 from northern Baffin Island in the Canadian Arctic (*94*) and was composed of two major components, an octachloroborane and a nonachlorobornane. The pattern of organochlorines in tissue suggests that narwals are exposed proportionally to more volatile compounds and may be less able to metabolize some of these compounds than odontocetes living closer to sources of these contaminants.

Toxaphene was measured in landlocked Arctic char and ringed seal (*Phoca hispida*) from Greenland (*145*). Char from the east coast of Greenland had toxaphene levels that were significantly higher than those in char from areas of the west coast. However, overall levels of toxaphene in muscle were low. Seals displayed no significant geographic variation in toxaphene levels, presumably because of their relatively high biotransformation capacity for toxaphene (*128*).

Zell and Ballschmiter (*4*) analyzed fish from different regions to characterize organohalogens in pristine aquatic environments. They found toxaphene in spawn of Arctic char (*S. alpinus*) from a lake in the Tyrolean Alps, pike (*Esox lucius*) from northwest Ireland, sturgeon (*Acispenser stellatus*) from the Caspian Sea, salmon (*Salmo salar*) from Ireland and Alaska, and in the livers of Antarctic cod (*Dissostichus eleginoides*) from South Georgia. They indicated that the global pollution by toxaphene could be as widespread or more so than compounds like those in the DDT and PCB groups. The pattern of toxaphene spread was modified to a variable extent compared to that of technical mixtures. Samples from the North Atlantic Ocean and the Caspian Sea contained levels about 10-fold higher than those from samples of other areas.

Few investigations of toxaphene in biota have been carried out in the lakes of Asia. Kucklick et al. (*26*) studied organochlorines in the food chain of Lake Baikal in central Siberia. Baikal seals (*Phoca siberica*) occupy the top trophic level,

feeding primarily on the endemic whitefish or omul (*Coregonus autumnalis*) and planktivorous sculpin (*Comephorus dybowskii*). Toxaphene in biota ranged from 1.1 to 2.3 μg g^{-1} lipid in sculpin and seal, respectively, indicating little biomagnification of toxaphene from fish to seal. Toxaphene patterns in seals were degraded to a greater extent than those in fish but retained several prominent congeners. These results are in agreement with degradation studies by Boon et al. (*128*).

Levels of toxaphene and other organochlorine pesticides have been analyzed in tilapia (*Sarotherodon mossambicus*) and guapote (*Cichlasoma managuense*) collected in 1991 from Lake Xolotlán in Nicaragua (*157*). The carnivorous tilapia contained concentrations of toxaphene 4 to 5 times that in the omnivorous guapote. Located on the shore of the lake was a factory producing toxaphene, which may have contributed to some of the high levels of toxaphene.

Jansson et al. (*149*) reported total toxaphene residues in Arctic Char (*S. fontinalis*) from lake Vättern in southern Sweden, and in grey seal (*Halichoerus gryphus*) and herring (*C. harengus*) from the Baltic Sea. Fish from the different areas gave similar chromatograms, indicating widespread input of toxaphene to the whole region through the atmosphere. This finding was supported by Paasivirta and Rantio (*166*), who compared toxaphene levels in salmon from the Arctic and the Baltic and found no significant difference. Similarly, levels of toxaphene in cod liver did not differ. Toxaphene has not been used as a pesticide in Scandinavia. Andersson and Wartanian (*72*) analyzed toxaphene in blubber samples from various seal species collected from the Baltic and the west coast of Sweden. Toxaphene levels in Baltic seals were higher than those in animals from the west coast of Sweden. Comparison of the data for adult and juvenile seals revealed, in addition, to age-related variation in contamination, i.e., toxaphene levels in adult Baltic ringed seals were significantly higher than those in adult grey seals from the same region and 5 to 10 times than those in juvenile ringed seals from the same region. Andersson et al. (*148*) reported no geographic differences in concentrations of toxaphene from animals in the Arctic region with those in corresponding species in the Baltic.

Several reports on levels of toxaphene in fish and fish products from Europe show the ubiquitous presence of toxaphene in all types of fish (*89,167,168*). High residues of toxaphene in fish and fish products from Europe were reported by Müller et al. (*167*), who showed that toxaphene concentrations in herring and mackerel (*Scomber scombrus*) from the North Sea and the relatively remote waters west and northwest and of Ireland and the Shetland Islands exceeded the German tolerance level, which was 0.1 mg kg^{-1} on a lipid basis or 0.01 mg kg^{-1} wet weight (ww) at that time. van der Valk and Wester (*89*) conducted a study in fish from northern Europe. Highest toxaphene concentrations were found in herring oil from the Baltic (7 μg g^{-1} lipid). Toxaphene levels in cod liver showed an upward trend from the southern to the northern North Sea, increasing from 0.4 to 1 μg g^{-1} lipid. This finding was somewhat unexpected, as the northern North Sea usually is considered less polluted than the southern North Sea. de Boer and Wester (*7*) report that toxaphene has almost never been used in Western Europe. Accumulation of toxaphene in northeastern Atlantic waters may be attributable to aerial transport from the American continent. The authors also reported that Baltic herring oil probably contained high toxaphene concentrations because of continued use of toxaphene in East European countries.

Concentrations of Individual Congeners

Gooch and Matsumura (*158*) suggested that since environmentally derived toxaphene is extensively altered compared to the technical material, measuring only the toxic congeners would be environmentally relevant. They reported mean levels of Toxicant A [a mixture of B[30030]-(211) and B[30030]-(121)] and B[30012]-(111) in fish from Lake Michigan were 0.26 and 0.1 μg g^{-1} ww, respectively, approximately one order of magnitude less than the estimated concentration of total toxaphene.

Hainzl et al. (*88*) analyzed individual toxaphene compounds in fish and caviar from several European countries. B[30012]-(111), B[12012]-(212), and B[30030]-(122) were the most prominent compounds, whereas concentrations of B[12012]-(202) and B[30032]-(122) were below detection levels or very low (< 0.1 ng g^{-1}) in all samples analyzed (Table 9). Icelandic cod liver contained the highest concentrations (Table 9). Toxaphene in the livers of hake from west of Ireland and herring muscle and dolphin blubber from the North Sea were all studied by de Boer et al. (*170*), who found B[12012]-(202) and B[12012]-(212) to be dominant compounds, whereas B[30012]-(111) was not detected in most samples.

Alawi et al. (*102*), using different analytical techniques, analyzed B[30012]-(111), B[12012]-(212), B[30030]-(122), B[12012]-(202), and B[30032]-(122) in samples of marine fish. The fish and fish products were obtained from Iceland, Greenland, Japan, Norway, and Germany. The compounds B[12012]-(202), B[12012]-(212), and B[30030]-(122) were present in most of the fish samples (especially those from the North Atlantic) in concentrations similar to those for important PCB congeners and cyclodiene. These three chlorinated bornanes constituted the major portion of the toxaphene residues in cod liver oil (25–30%). In fresh fish and caviar these substances amount to approximately 8 to 12% of total toxaphene. B[30012]-(111) was found in only a few samples and at very low concentrations. B[30032]-(122) was below detection levels in all samples analyzed. Cod liver oil and salmon oil from the North Atlantic contained higher levels of toxaphene than red fish and halibut (Table 9).

Alder et al. (*100,136*) analyzed three indicator compounds, B[12012]-(202), B[12012]-(212), and B[30030]-(122), in different samples of fish from the North Atlantic Ocean, North Sea, Baltic Sea, and a few other locations (Table 9). Highest residue concentrations were found in marine fish with moderate-to-high fat content such as halibut, herring, redfish, and mackerel. The sum of the indicator compounds in sardines and in fish with lean muscle tissue levels (Alaska pollock, saithe, hake, and cod) were low. Farmed salmon from Chile showed lower levels of the three compounds than salmon from the northern hemisphere. Eel from the Baltic contained relatively low levels. Fromberg et al. (*171*) determined the three indicator compounds in several fish samples from Danish waters. Their concentrations ranged from approximately 5 to 50 ng g^{-1} fat and are in agreement with previously reported results for mackerel, eel, salmon, and herring from Skagerak; however, these concentrations are 3 to 8 times that of those reported for herring from the Baltic Sea (*100*). Fish, especially herring, are not stationary, so these differences could be attributable to migration.

Conclusion

The literature shows that toxaphene has a global distribution and can be found in

Table 9. Amounts of individual chlorinated bornane congeners in biota.

Sample/species	Location	B[12012]-(202), ng g^{-1} ww	B[12012]-(212), ng g^{-1} ww	B[30030]-(122), ng g^{-1} ww	Reference
Arctic char, whole fish	North Quebec (Ungava)	44–74	83–138	—	(*165*)
Pollock muscle	Northwest Pacific	0.1	0.1	ND	(*100*)
	Arctic Sea	ND	0.1	ND	
Caviar substitute	Iceland	24–58	33–110	7–33	(*102*)
	Germany	14–33	18–94	5–18	
Cod liver oil	Germany	100–245	140–550	75–171	
Cod muscle	Greenland	30–50	35–145	14–58	
Cod muscle	Iceland	ND	0.1	ND	(*100*)
Cod liver	Barents Sea	19–70	37–122	11–51	(*169*)
Eel muscle	France	<0.1–0.2	19–20	5.7–6.4	(*119*)
	Spain	ND	18–19	1.3–1.7	
	Germany	ND-0.2	10	3.7	
	Italy	ND	7.9–8.5	0.45–0.60	
Eel muscle	Baltic Sea	1.8–2.8	ND–3.7	ND–2.8	(*100*)
Hake	Southeast Pacific	ND	0.1	ND	
Halibut muscle	Iceland	5.4–23.4	10.0–27.8	8.4–29.2	
	Norway, west coast	5.4–11.8	9.4–19.6	7.2–13.3	
Halibut muscle	Germany	2–5	8–34	12–98	(*102*)
Hake liver	Atlantic Ocean	30–100	7–30	<1–1	(*170*)
Herring muscle	North Sea	<1–2	1–3	<1–2	
Herring muscle	Baltic Sea	2.9–5.7	3.4–7.2	1.6–4.3	(*100*)
	Ireland	1.6–2.6	3.4–5.0	1.6–3.8	
	English Channel	1.5–4.3	3.3–8.4	2.1–6.3	
	North Sea, Fladen Ground	1.1–4.2	1.5–8.0	ND–5.5	
	North Sea, Skagerrak	ND–1.5	1.0–2.0	ND	
	North Sea, Bressay Ground	7.1–13.9	4.1–4.2	8.7–10.2	
	West of British Isles	4.3	8.4	6.5	
	Norway, west coast	4.7–6.8	8.2–16.0	6.4–10.8	
	Canada, east coast	1.2–2.6	1.4–3.2	1.0–2.0	
	West of Shetland	5.8	12.0	9.3	
Mackerel muscle	West of Shetland	2.5–5.6	3.0–6.0	1.9–3.0	
	North of Shetland	1.7	3.4	3.6	
	North Sea	ND–2.5	2.3–5.0	ND–3.9	
Plaice muscle	North Sea	ND	ND	ND	
Polar cod liver	Barents Sea	23	50	26	(*169*)
Red fish muscle	Greenland	2–9	8–34	1–17	(*102*)
Redfish muscle	Iceland	1.2–6.9	2.3–9.7	2.2–15.2	(*100*)
	Norway	1.2–2.5	2.2–4.9	1.7–3.8	
Saithe muscle	Iceland	0.4	0.5	0.2	
	North Sea	0.1–0.2	0.2–0.5	0.1	
Salmon oil	Norway	7–28	20–105	1–27	(*102*)
	Norway	2.8–8.0	3.5–13.4	2.4–8.2	(*100*)
	Iceland	6.5	9.0	6.5	
	Ireland	1.6	3.0	1.8	
	Scotland	2.4	6.3	4.9	
	Chile	0.6	0.9	ND	
Sardine	Bay of Biscay	0.2–1.1	0.4–1.9	ND–0.8	
Trout muscle	Germany	0.5–1.2	0.7–2.1	ND–1.5	

ND, not determined.

both fresh water and marine biota all over the world. Also, at remote areas long distances from toxaphene sources, the levels in biota can be quite high. These findings illustrate the importance of long-range transport, perhaps through the atmosphere, in the global spreading of this group of contaminants.

Toxicology

Since the late 1940s, reports have been published addressing the toxicity of the chlorinated camphenes to fish, birds, and mammals (*172–176*). In addition, toxaphene was found to elicit mutagenic and carcinogenic properties in mammalian test systems, thereby posing a threat to humans (*25,177*).

Toxicokinetics and Biotransformation

The use of toxaphene as a piscicide was discontinued after the discovery that toxaphene was persistent in the aquatic environment and its presence prevented successful restocking of treated lakes with desirable fish (*178,179*). However, experimental information is scarce on the depuration of toxaphene in fish and their residue kinetics. Delorme et al. (*180*) studied the elimination rate of toxaphene and two of the more persistent congeners, B[12012]-(202) and B[12012]-(212), in lake trout and white suckers in a natural ecosystem following intraperitoneal injection of technical toxaphene (7 μg g^{-1} for white suckers; 3.5 and 7 μg g^{-1} for lake trout). The estimated half-lives for total toxaphene were 524 days for white suckers and 232 (high dose) and 322 (low dose) days for lake trout. Half-lives for the two congeners in trout were 294 and 376 days (high dose) and 316 and 367 days (low dose), respectively. In white suckers, only B[12012]-(202) was detected and its half-life was 716 days. On the basis of these results, the authors concluded that under natural living conditions these species differ in elimination rates of toxaphene and that elimination of two different chlorobornane components of toxaphene, B[12012]-(202) and B[12012]-(212), is different within a given species.

Mohammed et al. (*181*) studied the role of plasma lipoprotein in the transport and tissue accumulation of toxaphene. ^{14}C-Radiolabeled toxaphene in the absence or presence of either low-density lipoproteins (LDL) or high-density lipoproteins (HDL) was injected intravenously into normo- and hypolipidemic mice. In normolipidemic mice, most of the radioactivity initially was found in the liver and adrenals either in the absence or presence of LDL or HDL. Four hours after application, the radioactivity was redistributed into the adipose tissue. Notably, lower amounts of radioactivity were found 20 min after mice were injected with toxaphene in combination with HDL than in mice injected with ^{14}C-toxaphene–LDL, suggesting a more efficient metabolism and disposal of toxaphene when HDL was used as a carrier. Mohammed and co-workers initially found less ^{14}C-radiolabeled toxaphene in the liver and adrenals and more in the kidney and heart of hypolipidemic mice (*181*). ^{14}C-Toxaphene was redistributed mainly to the liver and only in small amounts to adipose tissue 4 hr after injection. According to the authors, these results indicate that changes in the lipid pattern may influence tissue distribution of toxaphene. Mohammed et al. (*181*) also studied the distribution of ^{14}C-radiolabeled toxaphene among lipoprotein fractions *in vitro* and *in vivo* using

human and rat plasma. In rat 37 to 52% of radioactivity was recovered in the HDL fraction, whereas 18 to 52% was associated with the albumin-rich bottom fraction (BF) both *in vivo* and *in vitro*. In contrast to distribution in the rat, the *in vitro* distribution of ^{14}C-toxaphene among human lipoprotein fractions is relatively homogeneous. In the BF, 26% of radioactivity was found, whereas in the HDL, LDL, and very low-density lipoproteins (VLDL) fractions, 27, 29, and 18% of radioactivity were recovered, respectively.

Reductive dechlorination or dehydrochlorination and, in some cases, oxidation, have been shown to be the major mechanisms by which toxaphene is metabolized in microorganisms as well as in insects, birds, aquatic organisms, and mammals (*1,182*). Degradation of toxaphene in the soil proceeds rather slowly under aerobic conditions, whereas under anaerobic conditions toxaphene is more easily degraded (*139*). Fingerling et al. (*36*) investigated the degradation under anaerobic conditions of six polychlorinated bornanes, B[30012]-(111), B[30012]-(211), B[30012]-(121), B[30012]-(112), B[30012]-(212), and B[30012]-(122), isolated from technical toxaphene as well as the technical mixture in a loamy silt. All individual bornanes were transformed by reductive dechlorination, usually starting with the removal of a chlorine atom from the geminal dichlorogroup in the C-2 position. The dechlorination rate depends on the chlorination stage (nonachlorobornanes > octachlorobornanes > heptachlorobornanes). Two stable end metabolites formed from all six bornanes were isolated and identified as B[21002]-(111) and B[21001]-(111). Interestingly, the major degradation product of technical toxaphene was shown to be B[20012]-(111), one of the two end-metabolites of the six bornanes tested. In addition, Fingerling et al. (*36*) showed that none of the components tested were degraded in autoclaved soil, indicating that degradation is mediated primarily by microorganisms.

In contrast to the identification of dechlorination products formed from toxaphene components as well as technical toxaphene under anaerobic conditions in soil, reports on the isolation and characterization of oxygen-containing products is scarce. Fingerling and Parlar (*183*) for the first time isolated and characterized under anaerobic conditions an oxygen-containing product as a conversion product of the toxaphene components B[30012]-(211), B[30012]-(121), and B[30012]-(111). This cyclo ketone (7b,8c,9c-trichlorocamphene-2-one) probably is not formed from one of the two main hexachlorobornane products (*36*); possibly it is formed from the dehydrochlorination product B[21001]-(111), which is formed as a small byproduct of B[30012]-(111), B[30012]-(211), and B[30012]-(121).

According to Saleh (*1*), hepatic microsomal mixed-function oxidases are most important in toxaphene metabolism in mammals, followed by glutathione *S*-transferases. Chandra and Durairaj (*184*) showed that in addition to inducing cytochrome P450 and aniline hydroxylase activity in the liver, toxaphene also induces activity of these enzymes in the kidney. Therefore, the authors speculate that toxaphene alone may be metabolized in the liver as well as in the kidney.

In an attempt to evaluate the role of phase I biotransformation in the bioaccumulation process of toxaphene, Boon et al. (*128*) demonstrated *in vitro* metabolism of toxaphene using hepatic microsomes from harbor seal, whitebeaked dolphin, harbor porpoise, and albatross sampled shortly after death. In addition to toxaphene, the *in vitro* metabolism of four individual chlorobornane congeners was tested. B[12012]-(202) and B[12012]-(212) were persistent in all assays, whereas B[30012]-(111) was metabolized by hepatic microsomes isolated from the four wildlife species. It was also found that harbor seal hepatic microsomes only metabolized B[30030]-(122). Neither toxaphene nor the four congeners were metabolized *in vitro* using hepatic microsomes of the sperm whale. Interestingly, the authors' results showed that the *in vitro* capacity of microsomes derived from the different species to metabolize technical toxaphene, reflects the decreasing number of peaks in the toxaphene residues of wildlife extracts.

Aquatic Toxicity

Toxaphene is highly toxic to aquatic organisms. It was found that in general saltwater fish are more sensitive to toxaphene then freshwater fish (mean acute toxicity values of 0.07 µg liter^{-1} and 1.6 µg liter^{-1}, respectively) (*1*). Keller (*185*) studied the acute toxicity of several pesticides, including toxaphene in freshwater mussels (*Anodonta imbecilis*), and compared their sensitivities to those in common test organisms such as *Daphnia magna, Cerio dubia,* and fathead minnow (*Pimephales promelas*). The 96-hr LC_{50} for *A. imbecilis* exposed to toxaphene was 0.74 mg liter^{-1}. Compared to the other organisms tested, *A. imbecilis* is less sensitive to toxaphene. The acute toxicity levels for toxaphene in most aquatic organisms range from 1 to 40 µg liter^{-1} (*1*). Interestingly, addition of sediment to the test chambers drastically reduced the toxicity of toxaphene to *A. imbecilis*. Thus, susceptibility of *A. imbecilis* to toxaphene toxicity appeared to vary depending on whether concentrations were sediment- or aqueous-bound.

Application of toxaphene to lakes as a piscicide has caused direct as well as indirect damage to the ecosystem. Direct damage includes disappearance of target as well as nontarget organisms inhabiting toxaphene-exposed waters. Indirect damage occurred when application of toxaphene resulted in some cases in replacement of native organisms by a new population of organisms, which modified the structure of the ecosystem. Miskimmin and Schindler (*186*) examined the response to toxaphene application and stocking with a nonnative fish species on total chironomids, *Chaoborus* spp., planktonic Cladocera in a mesotrophic lake (Peanut Lake, north basin), and a eutrophic lake (Chatwin Lake) in central Alberta, Canada. The response in these lakes was compared to that in a lake that had not been treated (Peanut Lake, south basin). The authors studied some invertebrates prior to application of toxaphene during 1961 to 1962 and examined recovery of the community in the following 30 years by analyzing sediment cores from the lakes. They found that as a result of toxaphene application (0.0184 ppm) to Chatwin Lake, planktonic Cladocreans decreased in abundance and dominance changed from small- to large-body types. No short-term effects were detected by examining sediment cores from the toxaphene-treated Peanut Lake (0.0075 ppm). In the absence of native fish and during trout stocking, large invertebrates became dominant in both treated lakes. Residual toxicity and/or predation by stocked fish in both lakes probably resulted in low population levels of *Chaoborus* spp. throughout the 1960s. Long-term changes in invertebrates in both lakes probably were a result of the manipulation of fish communities rather than effects of residual toxicity.

Mammalian Toxicity

Table 10 is an overview of the acute effects caused by exposure to toxaphene.

General Toxicity. Most acute toxicity studies of toxaphene in mammals were

Table 10. Acute effects of toxaphene exposure.

Species	Route/Duration	Dose, mg kg^{-1} day^{-1}	Type of effect	Effect	Reference
Rat	Oral/once	90 male	Death	LD_{50}	(*175*)
		80 female	Death	LD_{50}	
Mouse	Oral/once	120	Death	LD_{50}	(*187*)
Guinea pig	Oral/once	270	Death	LD_{50}	(*187*)
Cat	Oral/once	25–40	Death	LD_{50}	(*187*)
Dog	Oral/once	49	Death	LD_{50}	(*187*)
Pheasant	Oral/once	40	Death	LD_{50}	(*188*)
Quail	Oral/once	80–100	Death	LD_{50}	(*189*)
Rainbow trout		10.6 (μg $liter^{-1}$)	Death	LC_{50}	(*190*)
Coho salmon		8 (μg $liter^{-1}$)	Death	LC_{50}	(*190*)
Striped bass		4.4 (μg $liter^{-1}$)	Death	LC_{50}	(*191*)
Sheepshead minnow		1.1 (μg $liter^{-1}$)	Death	LC_{50}	(*191*)
Rat	Oral/gestation days 6–15, 1×/day	32	Systemic	50% reduction in body weight	(*192*)
Rat	Ad lib/14 days	10	Systemic	No body weight gain (NOAEL)	(*193*)
Rat	Ad lib/14 days	10	Systemic	20% increase in liver weight	(*193*)
Guinea pig	Oral/once	300	Systemic	13% increase in liver weight	(*194*)
Guinea pig	Oral/once	300	Pathologic	Hypoxic and anoxic changes and disfigurement of myelin in brain	(*194*)
Guinea pig	Oral/60 days	2–6	Pathologic	Vacuolization in cells of collecting system and glomerulus, degeneration of corticol tubular cells	(*194*)
Mice	Oral/14 days	50	Pathologic	Dilatation of hepatic ER	(*195*)

conducted between 1950 and 1980. As reviewed by Saleh (*1*), the acute LD_{50} of toxaphene to laboratory mammals ranged from 5 to 1075 mg kg^{-1}, depending on the species studied and the route of exposure. In addition, female rats appeared to be somewhat more sensitive to toxaphene exposure than male rats. Among the most prominent symptoms observed in laboratory animals acutely intoxicated by toxaphene are generalized epilepticlike convulsions starting with excessive saliva production followed by vomiting and muscle spasms. In time, the frequency of convulsions increased. Finally, animals became exhausted and died from respiratory failure (*173*). Pathologic changes upon toxaphene exposure may include degeneration of the brain, spinal cord, and pulmonary edema (*1*).

Combination Toxicity. Because toxaphene was widely used as a pesticide, in addition to other pesticides, the toxicity of toxaphene alone as well as in combination with other widely used pesticides was evaluated in ICR mice after 14 days of oral administration or 90 days in drinking water (*195,196*). Overall, decreases in body weight as well as increases in liver to body weight ratios were observed in mice exposed to toxaphene and toxaphene-containing mixtures. Visually, no pathologic changes were observed in tissues from treated animals. However, proliferation along with dilatation and fragmentation of the endoplasmatic reticulum and scattering of ribosomes in the liver were pronounced. Cotreatment of mice with toxaphene and parathion resulted in higher levels of inhibition of serum cholinesterase (serum ChE) activity than did treatment of mice with toxaphene alone for up to 3 days after initial exposure. In contrast, an increase of serum ChE activity was observed in mice cotreated with toxaphene and 2,4-dichlorophenoxyacetic acid (2,4-D) compared to mice treated with toxaphene alone. Phenobarbital-induced sleeping time was reduced in mice exposed to toxaphene and toxaphene-containing mixtures, whereas no reduction was observed in mice exposed to either parathion or 2,4-D This was probably because exposure to toxaphene-containing mixtures induces the hepatic mixed-function oxygenase (MFO) system. It cannot be determined from these studies whether the combination of toxicity of toxaphene and other pesticides is synergistic or antagonistic in nature or the result of effects manifested by their components individually.

Neurotoxicity. Neurotoxic effects of toxaphene exposure such as effects on behavior and learning have been reported to occur (1). The mechanisms underlying neurotoxicity, however, are little understood. In guinea pig, Chandra and Durairaj (*194*) observed histological changes in the guinea pig brain, e.g., hypoxic (disorganization) and anoxic (enlargement) changes in the neurones, upon exposure to toxaphene. Depletion of cytoplasmic organelles in the oligodendritic cells of the cerebrum was observed in guinea pigs exposed to 2 mg kg^{-1} toxaphene, whereas disfigurement of myelin in the brain occurred when they were exposed to the high 5 mg kg^{-1} day^{-1} dose. In a subsequent study, Chandra and Durairaj (*197*) investigated the impact of acute and subacute toxicity of toxaphene on the lipid profile in brain, liver, and kidney of guinea pig. An increase in neutral lipids and cholesterol and a reduction of phospholipids was observed in the brain. The individual phosphoglycerides phosphatidylinositol, sphingomyelin, and phosphatidic acid increased in both the acute and subacutely intoxicated guinea pig brain. On the basis of their studies, Chandra and Durairaj (*197*) postulated that the observed effects of toxaphene on lipid contents in brain, liver, and kidney led to membrane damage. In addition, alterations in phospholipids and cholestrol content were thought to be an adaptive mechanism to cope with the stress due to toxaphene intoxication. Furthermore, they argued that the increase of sphingomyelin in the brain might be related to neurotoxic symptoms, as an increase in sphingomyelin inhibits the permeability of the membrane to small molecules and ions.

Chandra and Durairaj (*184*) also observed reduced ATPase and acetylcholinesterase (AChE) activities in the brain on acute and subacute exposure of guinea pigs to similar concentrations of toxaphene. Addressing the mode of action of the neurotoxic effects of toxaphene, Chandra and Durairaj discussed that inhibition of AChE can result in neural and neuromuscular disorders. In addition,

respiratory failure, which leads to hypoxic and anoxic changes, would eventually result in decreased phosphorylation and ATP production, as evidenced by inhibition of ATPases. Toxaphene *in vitro* inhibits brain and kidney ATPases in mammals, as well as in fish, and insects (*1*). In contrast to the observed effects on brain AChE activity in the guinea pig, little effects on brain ChE activity were observed in mice treated with toxaphene and toxaphene-containing mixtures (*195*). The exposure of mice to toxaphene or a toxaphene-containing mixture did not result in pathologic changes in brain and liver at the light microscopic level. Table 11 gives an overview of neurologic, reproductive, and *endo*crine effects caused by exposure to toxaphene.

Nephrotoxicity. The effects of toxaphene exposure on the kidney of mammals were observed in a number of studies. In the 1992 study by Chandra and Durairaj (*194*), a single administration of 300 mg toxaphene kg^{-1} bw to guinea pigs resulted in no observable changes in the ultrastructure of the kidney 72 hr after exposure. In a subacute exposure study, 2 or 6 mg toxaphene kg^{-1} day^{-1} administered for 60 days led to vacuolization in cells of the collecting system and glomerulus, degeneration of corticol tubular cells, vacuolization, and an increase in the number of mitochondria of tubular epithelial. From this study, the authors evaluated the toxaphene-induced nephrotic changes as an adaptive mechanism in the guinea pig to cope with a disturbance in membrane-associated glycoproteins and glycolipid metabolism in liver and kidney. In a study on the impact of acute and subacute effects of toxaphene on the lipid profile in kidney, Chandra and Durairaj (*197*) observed an increase in phosphatidylcholine, phosphatidylinositol, and phosphatidic acid levels accompanied by a decrease in cardiolipin and sphingomyelin contents. However, no alterations in other phosphoglyceride contents were found. Both acute and subacute exposure of the guinea pig to toxaphene resulted in reduced ATPase and AChE activities in the kidney (*184*). This study also indicated that toxaphene may be metabolized in the kidney in addition to the liver, as an enhanced cytochrome P450 content and induced aniline hydroxylase activity were found in the kidney when exposed to toxaphene.

Hepatotoxicity. Several studies have shown that toxaphene or toxaphene-containing mixtures induce a number of hepatic biotransformation enzymes. Toxaphene and combinations of toxaphene with parathion (5 mg kg^{-1}) and/or 2,4-D (50 mg kg^{-1}) induced hepatic enzymes such as cytochrome P450, benzo[*a*]pyrene hydroxylase, and aliesterase in mice after 7 days of oral exposure. Furthermore, the *in vitro* biotransformation of parathion and paraoxon was effectively enhanced using hepatic 9000 g supernatant from mice exposed to toxaphene (*202*). Toxaphene and toxaphene-containing mixtures also decrease the phenobarbital-induced sleeping time in mice, suggesting an effect of toxaphene on CYP2B-type metabolizing enzymes (*195*). These studies show that the toxaphene-induced increase of appropriate biotransformation enzymes, including cytochrome P450, potentially stimulates the metabolism of a number of other xenobiotics and consequently may even reduce their toxicity.

A single dose of 300 mg toxaphene kg^{-1} bw in guinea pig did not result in histopathologic or ultrastructural changes of the liver, whereas administration of 2 or 5 mg kg^{-1} day^{-1} for 60 days led to a relative increase in liver weight, chronic venous congestion, mononuclear infiltration, and fatty changes in hepatocytes (*194*). The effect of subacute toxicity of 2 and 5 mg toxaphene kg^{-1} day^{-1} on the hepatic lipid profile was a decrease of phospholipids without significant alterations in glycolipid, neutral lipids, and cholestrol levels (*197*). Notably, in this study the acute dose of 300 mg kg^{-1} bw resulted in piloerection, sedation, crouching, clonic–tonic convulsions, and death within 72 hr. The changes observed in the lipid profile were thought to be an adaptive mechanism to cope with stress associated with toxaphene intoxication. In a similar experiment, toxaphene also reduced hepatic ATPase and AChE activities and interfered with collagen and calcium metabolism (*184*).

Reproductive Effects. Few data are available on the effects of toxaphene on reproduction in mammals and fish. Few or no effects were found in mammals to indicate interference of toxaphene with reproduction (*203–205*). Recently, the effects of toxaphene on reproduction were studied in sexually mature female zebrafish after being fed toxaphene-contaminated food (0.02, 0.23, and 2.2 μg g^{-1} fish day^{-1}) for 2 weeks (*206*). In the highest dose group, all fish died within 24 hr; 9 of 14 fish died in the group exposed to 0.23 μg g^{-1} fish day^{-1} between days 8 and 12. Other toxic effects observed in the parent fish were skin discoloration, subcutaneous hemorrhages, and curved backbones in the vertical plane. With regard to reproductive success, a nonsignificant decrease in mean total number of eggs spawned was observed. No differences in reproductive success were observed, as assessed by percentage of viable eggs 24 hr after fertilization, percentage of embryo mortality, and percentage of eggs hatching 72 hr after fertilization. In contrast, toxaphene produced a dose-related decrease in the percentage of oviposition for female zebrafish. Hence, it was concluded that dietary exposure of zebrafish to toxaphene affects their reproductive process.

Endocrine Toxicity. A recent concern about many environmental pollutants is that they might have endocrinelike properties. Environmental xenobiotics that mimic steroidal hormones have been implicated in the increasingly high incidence of breast cancer and other gender-specific disorders (*207–209*). To determine whether environmental chemicals act as exogenous hormones in the American alligator,

Table 11. Neurologic and developmental effects of toxaphene exposure.

In vivo species	Route/Duration	Dose, mg kg^{-1} day^{-1}	Type of effect	Effect	Reference
Rat	Oral/3 days, 1×/day	25	Neurologic	Tremors, nervousness	(*198*)
Guinea pig	Oral/once	300	Neurologic	10% decreased brain weight	(*194*)
Dog	Oral/2 days	10	Neurologic	Convulsions, salivation, vomiting	(*199*)
		5	Neurologic	NOAEL	
Rat	Ad lib/14 days	10	Developmental	NOAEL	(*193*)
Rat	Oral/gestation day 7–16, 1×/day	12.5	Developmental	Decreased fetal renal protein	(*200*)
Mouse	Oral/gestation day 7–16, 1×/day	35	Developmental	NOAEL	(*201*)

Vonier et al. (*210*) examined the ability of chemicals to bind to the estrogen receptor and progesterone receptor in a protein extract prepared from the oviduct of the alligator. Unlike some DDT metabolites, toxaphene did not interact with the estrogen receptor. Interestingly, toxaphene combined with other chemicals decreased [^{3}H]17β-estradiol binding in a greater than additive way.

Possible estrogenic or antiestrogenic potencies of toxaphene either alone or in combination with other pesticides were studied in a number of *in vitro* systems by other authors. The effect of toxaphene on the aromatase enzyme complex, which converts androgenic to estrogenic enzymes, was studied by Drenth et al. (*211*) in the human choriocarcinoma cell line JEG-3. Aromatase activity did not decrease as a result of toxaphene exposure. The expression of estrogen-regulated mRNA-stabilizing factor (E-RmRNASF) in toxaphene-treated leghorn rooster liver was studied by determining the stability of apolipoprotein II (apoII) mRNA *in vitro*. It was shown that toxaphene prevented estrogen stimulation of E-RmRNASF expression, acting as an antiestrogen (*212*). Toxaphene also inhibits the binding of progesterone, dexamethasone, and testosterone to their respective receptors (IC_{20} values of 68.4, 4.2, and 3.5 μM, respectively) isolated from eggshell gland mucosa of the domestic owl (*213*).

In contrast to the antiestrogenic potencies, weakly estrogenic potencies of toxaphene were observed in a number of other *in vitro* test systems. In the human E-screen test, 10 μm of toxaphene was shown to be weakly estrogenic (0.0001, as potent as estradiol). Interestingly, a more-than-additive estrogenic response was observed in the human E-screen test after administration of a mixture of 10 estrogenic chemicals including toxaphene (*214,215*). Bonefeld-Jørgenson et al. (*216*) conducted transient gene expression studies using a chimeric reporter construct containing one estrogen-responsive element (ERE) to expression of the chloramphenicol acetyltransferase (CAT) gene in human breast cancer cells. They found that technical toxaphene (10 μM), as well as the toxaphene congener B[12012]-(212) (10 μM), acted as an antiestrogen that blocked the action of estrogens by inhibiting the ER:ERE-activated gene transcription.

In a study by Ramamoorthy et al. (*217*), minimal estrogenic potencies of toxaphene and no synergistic effects of combinations of toxaphene and other pesticides were observed. Induction of CAT activity was not observed in MCF-7 human breast cancer cells transiently transfected with plasmids containing estrogen-responsive 5′-promotor regions from either rat creatine kinase B or human cathepsin D genes after treatment with a combination of toxaphene ($10^{-8}-10^{-5}$ M) or cotreated with toxaphene and dieldrin (equimolar concentrations, 10^{-5} M). Furthermore, no estrogenic response was found in the uterus of a 21-day-old female B6C3F$_1$ mouse after oral exposure to toxaphene (2.5–275 μmole kg^{-1} bw) or to toxaphene in combination with equimolar concentrations of dieldrin. In contrast to the results obtained with the systems previously mentioned, Ramamoorthy et al. (*217*) observed a slight estrogenic effect in an estrogen-responsive reporter system in yeast-expressing mouse estrogen receptor 2.5 hr after treatment with toxaphene (2.5 10^{-5} M) or mixtures of toxaphene with endosulfan, dieldrin, or chlordane. The latter treatments were not synergistic. In contrast, no estrogenic effect was observed in yeast-expressing human estrogen receptor treated with toxaphene alone or in combination with other pesticides.

Carcinogenicity

In the past, much effort has been expended on studying the carcinogenic properties of toxaphene. Table 12 gives an overview of carcinogenic and mutagenic data of toxaphene presented in the literature. Toxaphene was found to be highly carcinogenic in rat and mice and induced malignant liver tumors, reticulum cell sarcomas, sarcomas in the uterus, neoplasms in the reproductive system and/or mammary gland, and neoplasms in the pituitary, adrenal, and thyroid glands (*1,177*). The National Cancer Institute conducted a study in which neoplasms were found in the thyroid gland of the rat (*218*). To investigate whether the increased incidence of thyroid tumors observed in the rat in the National Cancer Institute bioassay of toxaphene had a nongenotoxic etiology, Waritz et al. (*219*) studied the thyroid function and thyroid tumors in male Crl:Cd BR (Sprague-Dawley-derived) rats orally exposed to 75 mg toxaphene kg^{-1} day^{-1} for 28 days (100 mg toxaphene kg^{-1} day^{-1} was administered for the first

Table 12. Carcinogenicity and mutagenicity data of toxaphene.

In vivo species	Route/Duration	Dose, mg kg^{-1} day^{-1}	Effect	Reference
Rat	Ad lib/80 weeks	55.6 (males) 54 (females)	Follicular-cell carcinomas, thyroid adenomas	(*218*)
Mouse	Ad lib/80 weeks	12.9 males 25.7 females	Hepatocellular carcinoma Hepatocellular carcinoma	(*218*)
Rat	Oral/29 days maximum	100 for 3 days, 75 remaining days	Thyroid follicularepithelial hyperplasia, hypertrophy	(*219*)

In vitro test system	Dose	Response	Reference
Ames test *Salmonella* strains TA1535, TA1537, TA1538, TA98, and TA100	0–3000 μg/plate	Toxaphene mixture mutagenic TA98 in *Salmonella* strains and TA100 in presence or absence of liver S9. Heptachlorobornane-I not mutagenic in *Salmonella* strains TA1535, TA1537, TA1538, TA98, and TA100, either with or without liver S9.	(*25*)
Ames test Microsuspension procedure *Salmonella* strain TA98 and TA100)	0–10,000 μg/ml	Toxaphene mutagenic in *Salmonella* strain TA100 (2500, 5000, and 10000 μg/ml). Parlar 26, 50, 62, and 32 not mutagenic in *Salmonella* strain TA100 (strain TA98 not tested).	(*219*)
SCE induction Chinese hamster lung (Don) cells	0–20 μg/ml; incubation time up to 28.5 hr	Dose- and time-dependent induction of SCEs. Dose-dependent induction of cell-cycle delay.	(*220*)
GJIC Normal human breast epithelial cells (HBEC)	0–10 μg/ml; incubation time up to 24 hr	Dose- and time-dependent inhibition of GJIC. Reduction of phosporylated Cx43 levels.	(*221*)
Brain PKC activity (mouse)	200 μM	Induction of mouse brain PKC activity.	(*222*)

Abbreviations: GJIC, gap junctional intercellular communication; HBEC, human breast epithelial cells.

4 days). Rats were sacrificed at days 0, 7, 14, and 28 of exposure. A significant time-dependent increase in serum thyroid-stimulating hormone levels was found, whereas there were no changes in serum levels of T3, T4, rT3, and corrected rT3. They observed a time-dependent increase in thyroid follicular cell hypertrophy and intrafollicular hyperplasia and a decrease in thyroid follicular cell colloid stores, both characteristic of a hyperactive thyroid. Considering that toxaphene has the characteristics of a phenobarbital-type inducer of the cytochrome P450 enzyme system, the authors concluded that the increase in thyroid follicular neoplasia in toxaphene-treated rats probably was caused by a nongenotoxic mechanism such as that believed to be responsible for thyroid tumor increases in rats chronically treated with phenobarbital. Because this type of mechanism for thyroid neoplasia is not known to occur in humans, the authors also conclude that it becomes increasingly unlikely that toxaphene presents a hazard as a thyroid carcinogen for humans.

In an attempt to further elucidate the mechanism of toxaphene-induced hepatocarcinogenicity, Hedli et al. (*223*) investigated two potential mechanisms: peroxisomal proliferation, which has been invoked as a nongenotoxic mechanism of hepatocarcinogenicity, and DNA adduct formation. After oral treatment of CD/1 mice for 7 days with toxaphene (0–100 mg kg^{-1} day^{-1}), no increases in immunodetectable levels of CYP4A1 were detected, suggesting that peroxisomal proliferation is not involved in the toxicity of toxaphene. Furthermore, no evidence was found for DNA adduct formation in the liver of toxaphene-treated mice. On the basis of this study, the authors suggest that the hepatocarcinogenic properties of toxaphene may be exerted through a nongenotoxic or promotional mechanism rather than through a genetic mechanism.

Although *in vivo* no evidence for a genetic mechanism for toxaphene-induced tumor formation was found, *in vitro* studies showed that toxaphene is genotoxic in mammalian cell systems and mutagenic in the Ames *Salmonella* test without requiring metabolic activation by liver homogenates (*1*). More recently, Steinberg et al. (*224*) tested toxaphene and four toxaphene congeners, B[12012]-(202), B[12012]-(212), B[30030]-(122), and B[30012]-(111), for mutagenic activity in *Salmonella typhimurium* strains TA98 and TA100 using a validated microsuspension procedure instead of the usual plate-incorporated procedure. Toxaphene was mutagenic only in the TA100 strain at concentrations of 2,500, 5,000, and 10,000 µg ml^{-1}. In contrast, toxaphene was also mutagenic to strain TA98 at a concentration of 10,000 µg $plate^{-1}$ when using the plate-incorporated assay. Using the microsuspension method, none of the four tested toxaphene congeners showed mutagenic activity in strain TA100 at any of the concentrations tested (maximum concentration: 10,000 µg ml^{-1}). A dose-dependent (10–10,000 kg $plate^{-1}$) increase in His revertants was also observed in strains TA97, TA98, TA100, TA102, and TA104 by Schrader et al. (*225*) in the absence of S9 metabolic activation. Genotoxicity of the technical toxaphene, as well as B[30012]-(111), but not B[12012]-(202), B[12012]-(212), and B[30030]-(122), was also demonstrated by Boon et al. (*128*) using the Mutatox assay. Addition of rat S9 fraction or microsomes of harbor seal and albatross decreased the genotoxic potential of the tested congeners and toxaphene. More *in vitro* evidence for genotoxicity was found by Sobti et al. (*226*) showing toxaphene-induced sister-chromatid exchange (SCE) in cultured lymphoblasts. In contrast, Schrader et al. (*225*) could not demonstrate convincing evidence of a toxaphene-induced (1–10 µg ml^{-1}) dose-dependent SCE induction at the HGPRT gene locus in V79 cells.

Knowing that cell-cycle delay may interfere with the expression of genotoxicity, Steinel et al. (*220*) studied the effect of cell-cycle delay on the induction of SCE by toxaphene in Chinese hamster lung (Don) cells. They found that toxaphene exhibited a dose- and time-dependent decrease in cell-cycle progression. At similar concentrations of toxaphene, higher numbers of SCEs were observed and dose and treatment time relationships were demonstrated. Hence, SCE induced by toxaphene was not masked by mitotic delay and longer toxaphene treatment times were not necessary in Don Chinese hamster cells. Nevertheless, the authors support recommendations for prolonged incubation times in SCE assays affected by mitotic delay.

To study a promotional mechanism rather than a genetic mechanism for toxaphene-induced tumor formation, Kang et al. (*221*) studied the inhibition of gap junctional intercellular communication (GJIC) by toxaphene. Noncytotoxic concentrations of toxaphene (0–10 µg ml^{-1}) inhibited GJIC in normal human breast epithelial cells reversibly in a dose-dependent manner after 90 min of exposure. In an attempt to determine how toxaphene inhibited GJIC, Kang and co-workers (*221*) examined Cx43 protein in cells treated with toxaphene. A reduction in the number of gap junctional plaques and induction of hypophosphorylation of Cx43 in normal human breast epithelial cells were observed at toxaphene concentrations that affected GJIC. In addition, these studies also showed that toxaphene inhibits GJIC through a nonestrogen receptor mechanism, as the cells used in these studies do not express the estrogen receptor. An alternative working hypothesis suggesting a central role for protein kinase C (PKC) has been proposed for skin tumor promotion (*227*). Moser and Smart (*222*) examined the potency of some hepatocarcinogenic organochlorine pesticides to stimulate PKC *in vitro* in mouse brain, hepatic, and epidermal homogenates. Two hundred µM toxaphene increased brain PKC 469-fold. The induction was phospholipid and calcium dependent. It is premature to conclude from this result, however, that stimulation of PKC activity is involved in toxaphene-induced hepatic tumor promotion.

Toxaphene, a Human Risk Factor

As mentioned previously, toxaphene is carcinogenic in rats and mice and also has been proven to be mutagenic (*1,178*). Such findings have led to the assumption that toxaphene poses a risk as a human carcinogen. Human exposure to toxaphene occurs mainly through the consumption of contaminated fish or by occupational exposure. Data are scarce on the risk to humans from toxaphene exposure (*1*). Brown et al. (*228*) and Cantor et al. (*229*) evaluated the association between elevated risk of leukemia and non-Hodgkin's lymphoma (NHL) among farmers and exposure to pesticides and other agricultural chemicals and concluded that there is an elevated risk of NHL among farmers. Risk increased in cases in which farmers personally handled, mixed, or applied pesticides, did not use protective clothing, and when more specific active mixures of pesticide exposure were used. Chemicals most strongly associated with risk of NHL were carbaryl, chlordane, DDT, diazinon, dichlorvos, lindane, malathion, nicotine, and toxaphene.

Although studies like these contribute to our knowledge about the toxicity of toxaphene for humans, difficulties arise in the interpretation of human risk. In an

International Agency for Research on Cancer evaluation of the carcinogenic risk of toxaphene to humans, toxaphene was regarded a carcinogenic risk to humans on the basis of evidence that toxaphene is carcinogenic in rats and mice and, despite the lack of adequate data, humans (*230*). To date most studies on carcinogenicity of toxaphene have been conducted using technical toxaphene mixtures. Human exposure, however, is mainly through consumption of toxaphene-contaminated fish. Composition of toxaphene mixtures is changed from original technical mixtures through weathering conditions and internal metabolism. Human exposure, therefore, is to a mixture other than technical toxaphene. The toxic and carcinogenic properties of fishborne residues of toxaphene are unknown. Under the auspices of the European Union (EU)-funded project MATT, our laboratories are involved in a semichronic exposure study in a joint effort to produce and isolate fish (cod)-based toxaphene residues that are chemically characterized and toxicologically evaluated, particularly for genotoxicity (*in vitro*) and tumor promotion capacity.

Legislation of Toxaphene in Food

In 1976 a European directive regulating residues of toxaphene in fruits and vegetables (0.4 mg kg^{-1}) was issued (*231*) that was integrated into the national food laws of all member states in the EU. At that time toxaphene was still used as a pesticide. In 1982 the European maximum residue limit (MRL) for fruits and vegetables was extended to some food of animal origin such as meat and meat products, milk and milk products, and animal edible fat in the German MRL ordinance (*232*). During that period no reports or data about toxaphene residues were published. On the basis of growing toxicologic concerns when toxaphene was internationally classified as a compound possibly carcinogenic to humans (*1*), the European MRL for fruits and vegetables was further reduced in 1993 to 0.1 mg kg^{-1} ww (*233*), equal to the limit of determination of common residue analysis methods. Thus, residues of toxaphene should not be found in these foods. In 1994 during implementation of this regulation into the German MRL Ordinance, this strict MRL was extended to all food of animal origin (*234*) including fish and fish products. For fatty fish (lipid content > 10%) the MRL was set at 0.1 mg kg^{-1} lipid weight, for lean fish with a lipid content > 10%, the MRL was set as at 0.01 mg kg^{-1} ww. In general the previously mentioned regulations were based on total toxaphene levels. At the beginning of the 1990s a sensitive residue analysis method by GC–ECD and GC–NCI/MS using three individual chlorinated bornane congeners, B[12012]-(202), B[12012]-(212), and B[30030]-(122), was developed in Germany (*11,92*). The method was applied in routine analyses of many German laboratories and validated by an interlaboratory exercise (*99,235*). First reports indicated that relatively high concentrations of these toxaphene congeners were in some fish from the North Atlantic, an area from which much of Germany's fish stock is derived (*100*). It was obvious that some edible fish would exceed this low MRL. Therefore, the new regulation for fish, fish products, and mussels was suspended until the end of 1996 (*234,236*) to give legislators time to determine the level at which the MRL should be established to take into account the questions of, on the one hand, an acceptable level of consumer protection and, on the other hand, the necessary supply of fish and fish products. In the interim, data about the contamination of all edible fish by the three indicator congeners were collected and evaluated in order to calculate the average toxaphene intake through consumption of fish (0.22 μg $person^{-1}$ day^{-1}) (*237*). At present there is no acceptable daily intake (ADI) value for toxaphene for use in conducting a risk assessment study. Therefore, the average toxaphene intake was compared with the lowest no-observed adverse effect level (NOAEL) considering a sufficient high safety factor (~25,000–50,000). In 1997 a new concept was incorporated into the German MRL for toxaphene in food of animal origin. The MRL for fish and fish products was set at 0.1 mg kg^{-1} ww on the basis of the sum of the three indicator congeners (*238*); the MRL for all other food of animal origin was set at 0.1 mg kg^{-1} on the basis of total toxaphene. The German government plans to adjust the MRL in the future on the basis of the toxaphene indicator congener concept. The German ordinance is the first national MRL for fish on the basis of toxaphene congeners.

The United States and Canada are the only countries to have established tolerance levels for toxaphene in food consumed by humans. The U.S. tolerance level was set at 5 mg kg^{-1} ww; however, this was withdrawn in the early 1990s. Instead of using a tolerance level, Canada uses an ADI value of 0.2 μg kg^{-1} bw. The calculated daily intake values from the results of Alder et al. (*100*) stay below this Canadian acceptable daily intake.

ACKNOWLEDGMENT: The preparation of this review was made possible by grant no. FAIR PL.96.3131 of the European Commission, DG XIV, FAIR Programme.

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