

ENVIRONMENTAL OCCURRENCE, ANALYSIS AND TOXICOLOGY OF TOXAPHENE COMPOUNDS

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TOXAPHENE: OCCURRENCE, ANALYSIS AND TOXICOLOGY

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ABBREVIATIONS

AchE	Acetyl cholinesterase
ADI	Acceptable daily intake
BCF	Bioconcentration factor
BF	Bottom fraction
CAT	Chloramphenicol acetyltransferase
CHB	Chlorinated bornane
ChE	Cholinesterase
CTT	Compound of technical toxaphene
DDE	4,4-Dichlorodiphenyl,2,2-dichloroethene
DDT	4,4-Dichlorodiphenyl,2,2,2-trichloroethane
ECD	Electron capture detector
EI	Electron impact
EF	Enantiomer fraction
ER	Enantiomer ratios
ERE	Estrogen-responsive element
E-RmNASF	Estrogen-regulated mRNA stabilising 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
i.p.	Intra peritoneal
i.v.	Intra venous
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 ionisation
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
USEPA	United States environmental protection agency
VLDL	Very low density lipoproteins

SUMMARY

Toxaphene production, in quantities similar to those of PCBs, has resulted in high toxaphene levels in fish from the Great Lakes and in Arctic marine mammals (up to 10 and 16 $\mu\text{g g}^{-1}$ lipid). The total toxaphene data suffer from a large variability. Consequently, little can reliably be said about trends or geographical differences in toxaphene concentrations. New developments in mass spectrometric detection, using either negative chemical ionisation or electron impact modes as well as in multi-dimensional gas chromatography have recently led to congener specific approaches.

Recently, several nomenclature systems have been developed for toxaphene compounds. Although all systems have some specific advantages and limitations, it is suggested that an international body like IUPAC make a decision on this subject to obtain uniformity in the literature.

Toxicological information on individual chlorobornanes is scarce but some first reports have recently appeared. Neurotoxic effects of toxaphene exposure, such as effects on behaviour and learning have been reported to occur. Technical toxaphene and some individual congeners were found to be weakly oestrogenic *in vitro* test systems, *in vivo* no evidence for endocrine effects has been reported. *In vitro*, technical toxaphene and toxaphene congeners have been shown to be mutagenic. However, *in vivo* studies have not shown genotoxicity, rather a non-genotoxic mechanism is proposed. Nevertheless, toxaphene is regarded to present a potential carcinogenic risk to humans.

A legal tolerance level for toxaphene (0.1 mg kg^{-1} wet weight for fish) has until now only been established in Germany.

INTRODUCTION

Toxaphene, a complex mixture of polychlorinated camphenes, was first introduced in 1945 by Hercules Co. 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 in various water systems (1). The lipophilic, persistent, and volatile nature of toxaphene, have contributed to its global dispersion throughout the fresh water and marine environment. It has even been found in remote areas such as the Arctic (2) where the pesticide was never used. In addition to bioaccumulation in biota inhabiting these regions, it is also been detected in humans (3-10). Toxaphene was banned by the US Environmental Protection Agency in 1982, this example was followed by many countries. However, in the early 1990s the detection of toxaphene in marine fish in Europe caused concern with regard to human health in relation to fish consumption. Attention to toxaphene has increased, both in the analytical and the toxicological field. A major impulse to research in this field was initiated by the synthesis of individual compounds of toxaphene and the commercial availability of them (11,12). By using individual standards it is possible to gain more insight into the transport, fate and toxicological effects of toxaphene in the environment. The determination of individual congeners provides more detailed information but this also leads to more complicated analyses. Another problem lies in the nomenclature of individual compounds. Some proposals for simpler codes than the systematic nomenclature have been published recently and will be discussed.

In 1997 a European research project entitled "Investigation into the monitoring, analysis and toxicity of toxaphene" (MATT) started. As part of this project an update of available knowledge on the developments in toxaphene analysis, new environmental data and toxicology is presented. 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 company in the USA (1). The process consists of the extraction of crude α -pinene from pine stumps, using methylisobutylketone, heat and pressure. Isomerisation of the α -pinene produces camphene, bornylene and α -terpineol. The camphene was subsequently chlorinated under UV light to produce toxaphene. The average chlorine content is 67-69% (13). The structure of the main components of toxaphene is given in figure 1.

Toxaphene is a yellow, waxy solid with a mild terpene odour, softening in the range of 343 to 363 K. While 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). Fingerling et al. (14) state that toxaphene consists of at least 180-190 components mostly with the formula $C_{10}H_{18-n}Cl_n$ or $C_{10}H_{16-n}Cl_n$ where n is 6-10. Buser et al. (15) report that polychlorobornanes ($C_{10}H_{18-n}Cl_n$ where $n = 5-12$) 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 on prolonged exposure to sunlight, alkali, or temperatures above 393 K (16). Saleh (1) states 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 seem to affect toxaphene composition. When adsorbed on silica, however, technical toxaphene is completely mineralised to CO_2 and HCl at 230 nm (19).

A specific gravity of 1.6 kg l^{-1} has been reported for technical toxaphene (20). The vapour pressure and the log octanol-water partition coefficient (K_{ow}) value have been

estimated to be comparable to that of hexachlorobenzene (HCB), $1.73 \cdot 10^{-3}$ Pa at 298 K (21), and a log K_{ow} of 5.5 (22). Howard (23) and Sullivan and Armstrong (24) have 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 PCB mixtures but higher than those of p,p'-DDT and its metabolites, suggesting that the bioconcentration of toxaphene would be high. These data are difficult to compare due to the variation 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 predicted from the log K_{ow} . Based on their vapour pressure calculations Wania and Mackay (27) have suggested that toxaphene changes its characteristic of being a chemical which is mostly in the gas-phase, to one which is largely aerosol-absorbed within the range of global environmental temperatures. At 298 K less than 10% is adsorbed to aerosols, at 253 K almost 90% is adsorbed. This implies that with this change of temperature, almost all of the toxaphene in air condenses onto particles present in the atmosphere and thus becomes subject to wet and dry deposition. Essentially at low temperatures toxaphene is more rapidly transferred from the atmosphere to soil and water.

The water solubility of toxaphene has been reported with an equally broad spectrum of values, these range from 0.4 mg l^{-1} at 298 K (28), and 0.55 to 3.3 mg kg^{-1} at 293 to 298 K (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 being $0.62 \text{ Pa m}^3 \text{ mol}^{-1}$ at 293 K. Using fugacity-based equations (22,30), the direction and magnitude of the flux can be calculated, as was done by McConnel et al. (31), who assumed that the temperature slope of Tateya et al. (32) for PCBs is valid for toxaphene as well. With the insertion of the measured H, 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 have suggested that up to 2 kg of material will be deposited on Lake Baikal per month by gas exchange, this process being further enhanced by the low water temperature of the lake (32). More accurate congener specific H values are required to improve these estimations. 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 ten to twenty times smaller than those from gas absorption.

Most chlorinated bornanes contain at least one chlorine atom at C2 and C10, while the bridging carbons, C1 and C4, are non-chlorinated (35). Technical toxaphene, as it is synthesized by photoinduction, will have a high percentage of components containing a dichloro group in the C2 position (36).

Toxaphene congeners demonstrate a different stability to 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) according to Ref. (37) and Nomenclature section) 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 nonachlorobornanes > octachlorobornane > heptachlorobornanes.

Fingerling et al. (14) also found that during irradiation in solvents, the bornane structure is generally preserved and that 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, such as 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), according to Ref. (37) and Nomenclature section), have been found to be extremely photostable.

SOURCES

The Hercules Company first introduced toxaphene as an insecticide in the late 1940's (1). In the ensuing years it had a variety of uses until it was ultimately banned by the United States Environmental Protection Agency (USEPA) in 1982 because it was suspected of being a probable human carcinogen and persistent hazardous compound to non target 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 and Table 1).

In 1989 there were 168 registered uses in the USA (40), and more than 277 worldwide for agricultural commodities and crops to control 167 major insect pests. Its use in livestock dips as a miticide, and in lakes as a piscicide to control rough fish populations have all been widely reported (1). The interpolated total global use between 1950 to 1993 was $1,330 \times 10^6$ kg and from 1970 to 1993 was 670×10^6 kg (41). This estimation was based on literature data and contacts with international agencies and researchers, its quality varies and shows large spatial and temporal gaps.

The USA (42), the Central American states, and the former Soviet states have had the highest recorded usage. This may be, because more detailed information on usage was received from these countries whereas information is not kept or is confidential in others (41).

El-Sebae et al. (28) report that toxaphene continues to be used in African countries especially Ethiopia, Sudan, Tanzania and Uganda where field run-off eventually leads to the Nile and ultimately the Mediterranean Sea. These could act an additional reservoir for future contamination. Information is lacking for other African countries.

In 1970 toxaphene was used in a formulation called 'polydophen' comprising 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) stated that application likely continues in Soviet states, Mexico, Romania, Hungary, Poland and the Indian Subcontinent in addition to many African Nations, Nicaragua and Mexico.

The most recent data available on toxaphene usage from the FAO (as reported by Swackhamer et al. (45)) indicates that Korea and Mexico were the only countries using toxaphene into the 1980's, Mexico reported usage of 600 tonnes in 1985.

Toxaphene is currently banned in many countries world-wide. Argentina and Mexico however allow restricted use. Toxaphene was only used in small quantities in Sweden and has been banned since 1956 (46).

In 1971 the Soviet government restricted the use of toxaphene, it is thought to be still in use as an insecticide for sugar beet, peas, potatoes, mustard, rape and perennial herbs in the following formulation; 50% active ingredient, 30% oil, 15% amalgamate at 1.6-3.0 kg ha⁻¹ during sprout stage (47). Voldner and Li (41) state that 1 x 10⁸ kg has been used since 1970 in the former Soviet Union.

In 1956 toxaphene was recommended for nation-wide use in Egypt as a protective insecticide against cotton leafworm, pink bollworm and spiny bollworm on cotton fields. Field

efficacy was the only consideration for the use. It was applied as a formulated emulsifiable concentrate of toxaphene (60% chlorinated camphene) used in four successive sprays during the cotton season. This method of administration caused a maximum contamination of soil and can result in up to 20% being lost to air, 20-50% lost to soil and 20-50% lost to water systems. This can ultimately lead to air and groundwater pollution and soil contamination. A concentration of 10 ppm has been reported in Egyptian soil, biota and water (1). Although the dosage applied doubled between 1956 and 1961, major crop losses were experienced as efficacy decreased and resistance helped by the removal of the insect's natural enemies became a problem. Egypt alone used 54×10^3 kg toxaphene between 1956-61, which was estimated at 25% of the Non-US usage of toxaphene (28). The level of resistance conferred to cotton leafworm was 26-fold compared to the laboratory controls. This led to the banning in Egypt of toxaphene in 1961, not for its environmental impact but for its poor efficacy factor.

It was previously thought that chlorohydrocarbons were produced in chloro-bleaching from the residual monoterpenes in the wood pulp industry. However, no indication of compounds identical to the main congeners in commercial toxaphene was found. This proved that toxaphene in fish did not originate from chlorobleaching of pulp (48). However, chlorinated camphenes are present in pulp mill recipients (49). Chlorine bleaching of wood pulp produces chlorinated compounds that are 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, a so far 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), according to Ref. (37) and Nomenclature section), respectively (52,15), were detected in

samples close to pulp mill. These chlorobornanes were probably due to aerial transport and degradation in the sediment. Additional chlorobornanes (in particular hexachloro congeners) were observed in some samples that were closer to the pulp mill, (4 km as opposed to 150 km.).

Toxaphene usage in the Great Lakes of North America

Much of the research on toxaphene has been carried out in the Great Lakes of North America with some conflicting data on the sources of the pollutant. These range from its use as a piscicide to the contribution of the wood pulp industry in addition to atmospheric sources. Swackhamer et al. (53) state that approximately 1% or less of the US use was used in the Great Lakes basin (54). The rate of use in the basin was ca. 1×10^6 kg year⁻¹ between 1970-77, and would have peaked around 1977. Thus the presence of toxaphene in the Great Lakes has been largely attributed to long-range atmospheric transport from the southern US or Central America, followed by wet and dry deposition to the lakes (55,56).

Historical investigation of the records of Lake Michigan showed that 224×10^3 kg of toxaphene was used in the Green Bay watershed between 1950-80, with most being used as a pesticide on cropland but some on livestock and in lakes as a piscicide. It has been stated that even if there were only a 1% run-off 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, revolatilisation and new transport along a decreasing temperature system will result in accumulation of

toxaphene in sediments as the vapour pressure becomes so low that it restricts the atmospheric transport.

In the 1960's several lakes in Wisconsin were treated with toxaphene to kill rough fish. Kidd et al. (9) reported that the concentration of toxaphene in fish in Laberge, Canada was entirely due to atmospheric input followed by long food chain bioaccumulation giving hazardous fish concentrations. Kidd et al. (9) provides further information on possible sources and reports 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 source of some toxaphene in the lake with atmospheric deposition being important also.

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

NOMENCLATURE

For many years it was assumed that the pesticide toxaphene primarily consisted 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 ionisation mass spectrometry

(NCI/MS) studies. Mass spectra with fragments 2 or 4 amu below the $[M-Cl]^-$ ions of bornanes, were interpreted as bornenes and bornadienes. However, new insights in synthetic pathways of technical toxaphene indicate the formation of camphenes and dihydrocamphenes (63). Therefore, the observed mass spectra should probably mainly be attributed to chlorinated camphenes and dihydrocamphenes. According to Saleh (62), the technical mixture also consists of small amounts of other chlorinated hydrocarbons and non-chlorinated 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.

Based on structural considerations, Hainzl et al. calculated that 138 bornane and 59 camphene congeners may be found at significant concentrations worth mentioning, using an a-chiral separation (66). 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, while they found only 76 partially resolved peaks with possible co-elution in a total ion chromatogram (150-500 amu) when the mixture was not pre-separated, GC-NCI/MS detection being utilised in both instances. De Boer et al. found 246 compound peaks in technical toxaphene using two-dimensional heart-cut GC using ECD while only 107 peaks were found using a single column GC-ECD set-up (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 camphanes (PCCs) (72), polychlorinated terpenes (25) and chlorinated bornanes (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 use of trivial names of some of the compound classes referred to in Table 1, complicate the field of nomenclature. Names that are no longer supported by IUPAC, like 'norbornanes' and 'camphanes' for bornanes and 'iso-camphanes' for dihydrocamphanes compound the problem.

Systematic names

For all groups of compounds mentioned above (Table 1) it is a complex task to formulate systematic names, which are in agreement with 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 downwards are in the endo position and substituents that point upwards 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 C5 - C6 is C8.
- The lowest possible numbering should be applied:

The carbon neighbouring C1 is decisive for the direction of numbering. If both carbons neighbouring C1 bear the same number of chlorine atoms, then the substitution of the next

carbon in the ring is decisive in deciding 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 additional 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.

For chlorinated camphenes (Figure 1D) and dihydrocamphenes (Figure 1E) the systematic names are even more complicated than for the chlorinated bornanes, bornenes and bornadienes. Coelhan and Parlar state that the systematic name for non-substituted 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 (75). 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, regarding chlorinated camphenes and dihydrocamphenes as chlorinated 8,9,10-trinorbornanes, creates even longer systematic names. Moreover, such names would be strongly dependent on the substituents present.

Systematic names for chlorinated camphenes and dihydrocamphenes can also be regarded as being derived from bicyclo(2.2.1)heptane as the basic structure (64,75). The non-substituted 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)heptane and

2,2,3-trimethyl-bicyclo(2.2.1) heptane, respectively. Chlorinated camphenes and dihydrocamphenes will receive very long systematic names, which easily leads to confusion.

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

Nomenclature systems

Because the chlorinated bornanes are the most abundant compounds in technical toxaphene, most attention has been devoted to them, both with regard to analytical method development and monitoring, and nomenclature. In the past the systematic nomenclature of the bornane skeleton has been non-uniform because several authors 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 presented the definitive numbering of 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 indicated by more simple names, such as 'T12', 'Toxicant A', 'Toxicant Ac', 'Toxicant B', 'TOX8' and 'TOX9', however, a clear nomenclature system was absent. Several authors have proposed and used more systematic nomenclatures in an attempt to remedy this situation. Table 3 gives an overview of these nomenclatures which will be discussed below.

The nomenclature used by Parlar et al. is based on gas chromatographic retention on a certain stationary phase (11,77). The nomenclature simply consists of a two-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 has to consider that (i) with such large numbers of congeners, coelution cannot be excluded (69), Parlar No. 42, for example, represents at least two different chlorobornanes (77); (ii) the chlorinated bornanes, camphenes and dihydrocamphenes will be part of the same coding system without any class distinction; (iii) the theoretical number of chlorinated bornanes, camphenes and dihydrocamphenes will require many thousands of codes and will become at least four-digit. At the present time 17 chlorinated bornanes and five chlorinated camphenes have been reported as 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 the 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, as will be evident from the above, five out of the maximum number of eighteen substitution positions had to be fixed. This was done 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. Because of this assumption the code can be limited to four digits instead of six, but cannot be used for all the theoretically possible congeners. Applying the nomenclature to 2,2,5-endo,6-exo,8,9,10-heptachloroborane (see Figure 2A) results in the code HpCB-6452, where the 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. The IUPAC rule for systematic numbering is to obtain the lowest possible numbering, and, therefore, the code HpCB-3188 would be selected, while that

represents the incorrect systematic name. The most important limitation is that it is not very easy to convert binary and decimal codes into each other and the help of a computer is desirable for rapid conversion. The general code becomes:

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

Oehme and Kallenborn (79) also proposed a nomenclature for chlorinated bornanes based on a binary number representing all possible chlorination positions, followed by conversion into a decimal code. The positions at the six-membered ring and at the three methyl groups are numbered separately, which results in two three-digit decimal numbers at maximum, separated by a hyphen. This was done in order to prevent a code with a maximum of six digits, which would be necessary if all possible positions are included in a single code. For example, the enantiomers of Figure 2 are represented by the code 195-421, however, a distinction between the enantiomers is absent. The advantage of this system over the previous one is that related structures have similar codes. However, the basic bornane structure used by the authors 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 nine-digit number should be read from the right to the left starting with 2-exo. This was done incorrectly in Ref. (81), which, subsequently, was cited in Refs. (16,37). Thus, decoding of the three-digit number to reveal the chemical structure is not simple, structural information therefore is not directly available. The general code becomes:

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

$2^0*8a + 2^1*8b + 2^2*8c$ (first digit second part)

$2^0*9a + 2^1*9b + 2^2*9c$ (second digit second part)

$2^0*10a + 2^1*10b + 2^2*10c$ (third digit second part)

Andrews and Vetter proposed a systematic nomenclature for chlorinated bornanes by listing them in order of preference according to IUPAC rules (74). The congeners were split into series of homologues to restrict the code length to less than five digits, which would be necessary if all possible congeners are tabulated in the same way as is done for chlorinated biphenyls (82). In this way the maximum number of digits is four. 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 shown in Figure 2 are coded B7-515 and are distinguished by an 'a' or 'b', but 'b' coded enantiomers correspond to incorrect structural names (71), if IUPAC rules are applied. The disadvantage of the method is that structural information can only be obtained after consultation of extensive tables or by using a computer programme which, until now, are available for chlorinated bornanes only.

Wester et al. (37) proposed a nomenclature which can be regarded as a mixture of the nomenclatures mentioned above, with the advantages that structural information can be directly deduced, and that the nomenclature is applicable to chlorinated bornanes as well as chlorinated bornenes and bornadienes. The proposed nomenclature yields a code consisting of two parts. The digits in the first part of the code reflect the degree of chlorination of carbons C2 - C6, presented according to the rules of Table 4. C4, i.e. the third digit, can only have code 0 or 1. In the second part the digits simply indicate the number of chlorine atoms of C8 - C10. 'B' precedes the 8-digit number in the case of bornanes. For example, 2-endo,3-exo,5-

endo,6-exo,8,8,9,10,10-nonachlorobornane will be presented as B[12012]-(212). The code for the conformation of the enantiomer would be B[21021]-(122), this clockwise numbering is arrived at by simply reversing the first part of the code and exchanging the digits for the C8 and C9 positions. Hence, because of the IUPAC rules, B[12012]-(212) is the only correct representation for both enantiomers. To differentiate both enantiomers 'r' is proposed for clock-wise numbering of the six-membered ring (Figure 2A: B[30012]-(111)r) and 's' for anti-clockwise 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 nomenclature is the simplicity in establishing whether a congener has an enantiomer or not. 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 and exchanging the digits representing the number of chlorine atoms at the C8 and C9 position, gives the same code.

The nomenclature proposed by Wester et al. (37) was extended to include bornenes and bornadienes which can easily be done because of structural similarity. Only one chlorine atom can be attached to a carbon atom participating in a double bond. To such a carbon, only '0' or '1' can be assigned. For example, 2,5-endo,6-exo,8,9,9,10,10-octachloroborn-2-ene will receive the code E[10012]-(122), and 2,3,5,8,8,9,10-heptachloroborna-2,5-diene will be coded D[11010]-(211). For enantiomers, the r/s nomenclature can be used, and is left out in the case of a racemic mixture or if there are no enantiomers.

The structures of polychlorinated camphenes and dihydrocamphenes could not be represented by codes based on the system described above because their structures differ too much. However, Wester et al. developed a coding system based on the same logic (80). The

numbering of the carbon atoms in the skeleton is the same as proposed by Hainzl (83); however, the 'a' and 'b' indication of the substituents at C10 (63) have been replaced by 'E' (trans) and 'Z' (cis) respectively (Figure 2A). As can be seen from Figure 1, there is a strong resemblance with the clockwise numbering of the bornane carbon skeleton (37).

Figure 1D shows that there is no need to consider carbons C5 and C6, because they cannot be chlorinated. The first part of the code deals with the substituents at carbons C1 - C4, C7 and C10. Carbons C1 and C4 can only have one chlorine substituent and these will be invariably 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 have to 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 by C for camphenes (74), with enantiomers being distinguished by an 'r' or 's' according to Ref. (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 as was used above, can be applied to the dihydrocamphenes (Figure 1E). There is no need to consider carbon C5, since chlorination cannot occur. As regards the first part of the code: for carbons C1 - C4 and C7 the same rules are applied as for the chlorinated camphenes. Carbon C6 can only have one substituent which can be in the endo or exo position and the rules of table 2 were applied. However, if C6 is not chlorinated, it will not be clear in which position the hydrogen atom is. A subscript, selected according to the rules of table 2, is then used to denote the endo or exo position of the hydrogen atom. The second part

of the code deals with C8 - 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 Ref. (37). The general code then becomes:

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

ANALYTICAL METHODS

The determination of 'total toxaphene' as is mostly performed may involve a large over- or under-estimate of the true concentration since the peak pattern of the sample under study does not resemble that of the standard. In the environment peak patterns may be considerably altered (84,85) but there also exists a large difference between the commercially available technical toxaphene standards. Using various technical standards Carlin and Hoffman (86) found variations between 19 and 131% compared to their laboratory standard. Furthermore, the detector response is in general not equal for all congeners. However, 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 towards congener-specific approaches, which are possible after the first isolation and synthesis of individual compounds (87,88). About thirty individual congeners are commercially available so far. In general it can be said that for comparison of monitoring results it is important that authors report the full analytical procedure used, because different methods can cause large variations in the results and ultimately lead to incorrect conclusions being recorded.

Extraction

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

Pre-separation / 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 & C18) can also be used to isolate B[12012]-(202) and B[12012]-(212) from environmental samples.

Silica gel pre-separation can be used to obtain a separation of technical toxaphene over a wide range (68). In that reference it is also mentioned that B[12012]-(202) can be detected free from co-eluting compounds after the pre-separation. 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 a 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 optimisation (69,98). De Boer et al. (69) used columns of 2.5 g SiO₂.2% H₂O (w/w), the bulk 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 which contained most PCBs. Only 1-2% 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 80-96% for total toxaphene, 84-100% 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 85-95%. In a collaborative study for the determination of four bornane congeners in fish oil, gel permeation was used followed by adsorption chromatography on silica gel (99). This silica gel clean-up was carried out using 1.0 g silica deactivated with 1.5% water. The toxaphene compounds were collected together with PCBs and some organochlorine pesticides in the first fraction 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 would have preferred a clean-up in which the toxaphene compounds and PCBs are separated as was done by 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 this method by using 8.0 g activated silica. The sample is eluted with 48 ml of hexane to remove PCBs followed by 50 ml hexane/toluene (65:35, v/v) in which the toxaphene compounds were recovered.

Injection

Alder et al. state that the injector temperature should not exceed 513 K because severe decomposition of compounds may take place (100). Bartha et al. (101) recommend an injector

temperature below 523 K. It should be mentioned here that care should be taken with active sites in the liner and the injector. Since there is a large variability of injector geometry it is recommended to verify the optimal temperature by a series of simple tests. Alawi et al. (102) showed that the response factors using splitless injection are lower than those using on-column injection.

Bartha et al. (101) reported the use of pressure pulse injection (PPI) at 498 K, which resulted in response factors 4 times higher than those obtained with splitless injection. This was especially significant for compounds with a low vapour pressure and long retention times (e.g. B[30030]-(122)) (101). Using this technique, the residence time of the compounds in the injector is short and, therefore, the chance of degradation within it smaller.

Gas chromatographic separation

Table 5 gives the elution order of toxaphene compounds on various stationary phases. The relative non-polar stationary phase, 5% diphenyl, 95% dimethylpolysiloxane (DB-5, Sil-8, Ultra-2) column with lengths of 30 to 60 m and diameters of 0.15 to 0.32 mm I.D. is most frequently used. However, more polar columns are often used to validate the results e.g., 14% cyanopropylphenyl 86% dimethylpolysiloxaan (DB-1701, Sil-19) (107), 6% cyanopropylphenyl 94% dimethylpolysiloxaan (DB-1301) (99).

Krock et al. obtained a relatively good separation using a very non-polar 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 up to a temperature as high as 563 K, although the supplier advised a maximum temperature of 473 K. No alteration of retention times was observed after several hundred analysis on this phase (101). By 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 an alternating endo-exo substitution elute earlier than compounds with two chlorines at both 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 the degree 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 co-elution 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) phase, in addition to a polyethylene glycol terephthalic acid ester (FFAP and a 10% cyanopropyl, 90% biscyanopropyl polysiloxane (Rtx-2330) phase for further separation (a multidimensional set-up) (69), it was observed that a large number of peaks were to be found in the secondary chromatograms indicating that the resolution offered by one single column is insufficient and can easily lead to false-positive results, especially when the non-selective ECD is used for quantification. There were no large

differences between the column combinations but the DB-5 - Rtx-2330 combination was preferred because of its somewhat better separation but mainly because of its low bleed. However, Baycan-Keller and Oehme (109) found degradation of B[32012]-(111), B[30012]-(211), B[30012]-(121), B[30012]-(212), B[30030]-(122), B[12012]-(212), B[32030]-(112) on the Rtx-2330 phase. Re-evaluation 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 together with the analyte peak) as described by Baycan-Keller and Oehme, and indeed this could have been caused by decomposition on the stationary phase. However, for B[12012]-(202), B[30012]-(111) and B[30030]-(122) normal peak shapes were obtained. Karlsson and Oehme also mention that there is a possibility that the low response of B[30030]-(122) is due to losses on the polar Rtx-2330 phase (107).

Alder et al. found that B[12012]-(202) and B[30030]-(122) were decomposed to a great extent on the highly polar DX-4 phase (100). In the study of de Boer et al. (69) degradation effects were not observed on this phase, this was also evident after re-evaluation of data and further experiments with this stationary phase (Figure 3). This can, at least partly, be due to the fact that a shorter column was used (15 m instead of 30 m) which limits the exposure time of the components to a high temperature, which was 493 K.

It is extremely time consuming to analyse several compounds in a complex sample using a multidimensional set-up, even when a system with several parallel traps for storage of heart-cuts is available (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. From the secondary chromatograms the complete two-dimensional chromatogram can be constructed, much like thin layer chromatography. A method capable of doing this 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 (are not correlated). For a method to be comprehensive it is necessary that the first dimension is sampled at least every peak width by the second one. 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

The use of MS detection would overcome some of the problems of co-elution, in addition to those caused by compound class or the degree of congener chlorination. In the electron impact (EI) mode co-elution with compounds having similar fragmentation patterns may well occur 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, since ECD is less selective than MS detection, an even more efficient separation will be necessary.

Flame ionisation detection of toxaphene shows little difference to the profiles obtained with full scan EI/MS and has 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 shows a completely different peak profile, which 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 due to 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 ionisation method to obtain a total chlorinated bornane result without interference from other compounds. However, this approach is less sensitive than the NCI mode and it does not distinguish between homologue

groups. NCI offers both selectivity and sensitivity for bornane congeners (114), but does not offer the possibility of structure elucidation.

NCI is most widely used MS detection method for toxaphene, but is insensitive to lower chlorinated congeners. The EI mode is relatively more sensitive to lower chlorinated congeners and, consequently, an additional 25 peaks from lower chlorinated compounds were found with EI compared to NCI (68). Often both the M^- and $(M-Cl)^-$ ions are monitored (67,101). Problems with GC-NCI/MS in the SIM mode include the formation of $(M-OCl)^-$ fragments of PCBs, false positive signals may, in part, be caused by chlordanes and the appearance of higher chlorinated bornane congeners (67,73,115). Krock et al. do 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 used tandem MS/MS with EI to identify B[12012]-(202) and B[12012]-(202) in penguin and harbour seal samples (15). Most toxaphene congeners produce fragments with $m/z = 125$ under EI conditions, this ion, together with ions at $m/z = 159$, 195 and 231, are considered to be characteristic of toxaphene congeners (62). In contrast with quadrupole or double focussing 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 in the MS/MS mode. Therefore, the ion at $m/z = 89$ (dechlorinated monochlorotropylium ion), which originates from

the $m/z = 125$ ion, would be more useful for quantification of toxaphene congeners (117). However, co-elution of compounds which produce this ion cannot be observed. Furthermore, the response factors with this method will vary considerably for individual congeners ((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 the fact that 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 $1600 \mu\text{g kg}^{-1}$. In contrast with this result, Fowler et al. (118) determined a value of $5410 \mu\text{g kg}^{-1}$ in the same sample using GC-NCI/MS. Alder and Vieth reanalysed the sample then using NCI/MS and found a value of $5210 \mu\text{g kg}^{-1}$, which is close to the value reported by Fowler et al. They concluded that this large difference is caused by the large difference in response factors between congeners with NCI which gives a positive bias to the results when compared with ECD which has a smaller difference between response factors. Rantio et al. (48) also showed that NCI/MS gave in general higher results than ECD. However, the results showed a linear relationship between the two detection techniques, which made it possible to compare the obtained results. The higher response of NCI/MS was also reported by Wideqvist et al. (46), especially when the degree of chlorination is higher. In contrast with this, Xu et al. (119) found that GC-ECD gave identical results to GC-NCI/MS for the quantification of individual chlorobornanes in fish samples. A possible explanation for these, at first view, contradictory observations could be the use of different standards in combination with the detector used, which can influence the end result to a large extent, as was shown by Carlin and Hoffman (86). For example, with one standard the same result for GC-ECD and GC-NCI/MS can be obtained while with another largely differing results can be

found. Another explanation can be differences between the MS configuration used in the studies.

ECD determination of total toxaphene has insufficient selectivity, while NCI/MS suffers from variable response factors. To obtain precise and comparable data the use of indicator compounds as a basis for calculation of total concentration was advised (92). However, this approach can only successfully be used when the indicator compounds do not co-elute with other compounds. Co-elution of suggested indicator compounds was shown by heart-cut multidimensional gas chromatography (69). Depending on the sample type, up to ten 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%, while that of B[12012]-(212) was 85 to 95%. Therefore, only B[12012]-(212) can reliably be determined after a one-dimensional separation. However, it must be noted that most samples analysed were from organisms placed 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 equals zero) when a compound is not found, but this can lead

to very high or low numbers. In addition, due to the reciprocal-like scale, ERs larger than unity 'seem' 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 proportional to the sum of the two (126). This enantiomer fraction (EF) is 50% if both enantiomers have the same abundance. The advantages of using EFs are an understandable linear scale, dividing by zero or very high and low values do not occur and similar enantiomer proportions are distinguished easier. 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 whether a specific biological 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 biological persistence (124). The comparison of the EFs of different congeners in combination with their molecular structures can help to gain an insight into the metabolism of these compounds.

When determining EFs of chlorinated bornanes in biota, it cannot be excluded that the values found are not merely due to metabolism in the species studied, since 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 will 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 P-450 dependent monooxygenase enzyme systems which are involved in enantioselective and non-enantioselective

biotransformation. Boon et al. (127,128) successfully used such an approach to study the achiral biotransformation of toxaphene congeners by microsomes from harbour seal, whitebeaked dolphin, sperm whale and laysan albatross.

For the determination of EFs the separation should be enantioselective as well as isomer-specific, unfortunately, this doubles the number of peaks that have 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, the enantiomer separation of bornane congeners is still a rather empirical task and the selection of a convenient stationary phase is primarily determined by trial and error (131). It has been shown that columns based on heptakis(2,3,6-O-tert.butyldimethylsilyl)- β -cyclodextrins (TBDM-CD) 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 is dependent on the column oven temperature profile. It was found that this phase can be used up to a temperature of 535 K 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 1 K resulted in much better separations compared to 10 K. 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 a low concentration have to 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) states that all parent compounds in toxaphene occur as racemates. Buser and Müller showed that some compounds are present in the technical formulation (Melipax) in non-racemic compositions (140). 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 $55.8 \pm 0.6\%$. Furthermore, the authors showed that the EF of this compound was 50.0% in a cod liver extract from the Baltic. When a synthesised standard with a racemic composition would have been used, the conclusion that no enantioselective process has taken place might be drawn, which shows the importance of carefully choosing the standard. On the other hand deviation of this compound from the racemic value should also be shown in other technical formulations since 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) present EFs of several chlorinated bornanes in Cod liver oil, Herring, Halibut, Caviar and Redfish samples obtained from Refs. (107,124,136). The EFs show little variation; $50.2 \pm 1.2\%$ and therefore, Parlar et al conclude that no significant degradation of toxaphene enantiomers takes place in fish. Unfortunately, the Refs. (124,136). present other EFs (Table 6) and Ref. (107). does not present EFs. The data in Table 6 are only for B[12012]-(202) and B[12012]-(212), the mean EF is $53.1 \pm 2.7\%$, which is not a large deviation from the racemic value. In a recent study (138), Parlar et al. 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 closely around 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 cynomolgus 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 other species (fish).

Interlaboratory study.

A German collaborative study with a contaminated milk fat undertaken in the mid eighties demonstrated the analytical difficulties and uncertainties in the analysis of technical (total) toxaphene by packed column GC-ECD (142). Andrews found that in many laboratories only about 15-30% of toxaphene components were eluted from silica or Florisil columns with a non-polar solvent. This was thought to be the main source of the large variation between labs (98). In a German inter-calibration 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 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, at present little is known about acute and chronic toxicity of individual congeners to mammals. Occurrence determines, in combination with toxicity, whether a compound is important or not. Stereochemistry may play an important role since the biological disposition of enantiomers varies (Ref. (125) and Table 7). Boon et al. (128) showed that

B[12012]-(202) and B[12012]-(212) did not give a positive response in a mutatox test, while technical toxaphene and B[30012]-(111) did. The latter compound is only detected in low concentrations in wildlife samples (69,99,117).

Next to these parameters the analytical 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 the analytical convenience dictate the choice of compounds, a situation similar with that of PCBs. The concentrations of B[12012]-(202), B[12012]-(212) and B[30030]-(122) are in the 0.05 - 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 to use them as indicator compounds. To be able to distinguish between recent contamination (e.g., recent use of the pesticide) or from persistent congeners still present in the environment), an indicator compound can be included which is not stable in the environment. B[30012]-(111) was suggested for this purpose (92). Xu et al. (119) proposed a second compound B[30032]-(122) for the same purpose. This compound was found to degrade easily in the detector, and is only present in minor amounts in technical formulations, therefore, it is not very useful as indicator compound (92). Measuring the individual indicator compounds on a single GC-column has the problem that several compounds may be present in one peak as was shown 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 the technical mixture (as a result of environmental and metabolic modification) (e.g., 88,113), values for total toxaphene should only be considered 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 literature are from freshwater systems in Canada and the U.S.A. where it was one of the most dominant organochlorine residues (161). Toxaphene concentrations plateaued after a period of steady increase through the 1970's, but its incidence continued to increase: residues were present at 88% of the stations sampled in 1980-81 (162). In 1978-79, toxaphene concentrations were highest in lake trout (*Salvelinus namaycush*) samples from Lakes Michigan and Superior with typical concentrations of 5-10 $\mu\text{g g}^{-1}$ lipid (163). In 1980-81 concentrations were generally lower, 2-5 $\mu\text{g g}^{-1}$ lipid. Concentrations of toxaphene declined in trout and smelt from the Great Lakes between 1982 and 1992, with the exception of fish from Lake Superior (159).

Little toxaphene has been used in the Great Lakes basin itself, the main input is thought to be through atmospheric transport from the southern US 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 through the atmosphere from the site of application and its accumulation is widespread in freshwater and marine fish (e.g., 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 results for Arctic char are consistent with the movement of organochlorines in air masses from south/central America in a northeasterly direction. Declines in concentrations of several organochlorines were also found for burbot (*Lota lota*) along with increasing northern latitude (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 from lakes in the Yukon Territory which were collected between 1990-92 and found that the levels varied considerably between lakes probably due to 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 five- to ten-fold higher than for DDT or PCB (146). Musial and Uthe (84) found that levels of CHBs in Arctic cod liver were about two-fold lower than those of Atlantic cod (*Gadus morhua*). Bidleman et al. (2) reported levels of toxaphene to be equivalent to 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 wt basis) to PCB but generally higher than DDT

(84). Toxaphene was not detected in deep-sea (Canadian waters) scallop (*Placopecten magellanicus*) (84).

High levels of toxaphene have been reported for white-beaked dolphins (*Lagenorhynchus albirostris*) and pilot whales (*Globicephala malaene*) collected during 1980-82 from the coast of Newfoundland (152). This was explained with the increased use of toxaphene during the 1970s. Toxaphene levels were higher than for other organochlorines measured (PCB, DDT, etc.). The majority of the peaks in the toxaphene standard were not present in dolphin blubber, indicating 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 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 (E. Hudson Bay, Cumberland Sound, W. Hudson Bay, Beaufort Sea and Jones Sound). Geographic comparisons of toxaphene levels for belugas are difficult because they 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 north coast of Alaska contained higher toxaphene concentrations in blubber (151), than for PCBs, DDTs and chlordanes in the same samples. Males had higher concentrations than the females and the oldest male had a higher concentration than the younger male. Transplacental transfer to the foetus in addition to lactation is the most probable cause of the lower levels in females compared to males. Stern et al. (165) identified the two major recalcitrant toxaphene congeners in aquatic biota from beluga blubber; B[12012]-(202) and B[12012]-(212). Their sum constituted 28-34% of total toxaphene in arctic char, 53% in burbot and 81-89% in beluga whale blubber from Canadian Arctic.

Toxaphene were the dominating organochlorines in narwal (*Monodon monoseros*) collected 1982-83 from northern Baffin Island in the Canadian Arctic (94). The toxaphene consisted of two major components, an octachloro- and a nonachlorobornane. The pattern of organochlorines in narwal tissue suggests they are exposed to proportionally more volatile compounds, and may have a lesser capacity to metabolize some of these compounds, relative to odontocetes living closer to sources of these contaminants.

Toxaphene was measured in landlocked Arctic char and ringed seal (*Phoca hispida*) from Greenland (145). The char showed levels that were significantly higher on the east coast compared to areas of the west coast, however overall the levels of toxaphene in muscle were low. Seals displayed no significant geographical variance, presumably due to relatively high biotransformation capacity for toxaphene (128).

Zell and Ballschmiter (4) analysed fish from different regions to characterize organohalogens in pristine aquatic environments. They found toxaphene in spawn of Arctic char (*Salvelinus alpinus*) from a lake in the Tyrolian Alps, pike (*Esox lucius*) from northwest Ireland, sturgeon (*Acispenser stellatus*) from the Caspian Sea, salmon (*Salmo salar*) from Ireland and Alaska, and Antarctic cod (*Dissostichus eleginoides*) (liver) from South Georgia. They indicated that the global pollution by toxaphene could be as important or even outrange the global spreading of compounds like the DDT and the PCB-group. The pattern of toxaphene was modified to a variable extent compared to that of technical mixtures. The samples from the North Atlantic Ocean and the Caspian Sea contained about 10-fold higher levels compared to samples from the 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 - 2.3 $\mu\text{g g}^{-1}$ lipid in sculpin and seal respectively, indicating little fish to seal biomagnification of toxaphene. The toxaphene pattern in the seal was degraded to a greater extent relative to the fish but retained several prominent congeners. These results are in agreement with degradation studies by Boon et al. (128).

The levels of toxaphene and other organochlorine pesticides have been analysed in tilapia (*Sarotherodon mossambicus*) and guapote (*Cichlasoma manag,ense*) collected in 1991 from Lake Xolotlán in Nicaragua (157). The carnivorous tilapia contained 4-5 higher toxaphene concentrations than the omnivorous guapote. Location of a factory on the shore producing toxaphene may have played a role for some of the relatively high values found.

Jansson et al. (149) reported total toxaphene residues in Arctic Char (*Salvelinus fontinalis*) from lake Vättern in southern Sweden, and grey seal (*Halichoerus gryphus*) and herring (*Clupea harengus*) from the Baltic Sea. The fish from the different areas gave similar chromatograms indicating a widespread input to the whole region via the atmosphere. This was supported by Paasivirta and Rantio (166) which 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 pesticide in Scandinavia. Andersson and Wartanian (72) analysed toxaphene in blubber samples from different seal species collected from the Baltic and the west coast of Sweden. Toxaphene levels in Baltic seals were higher than those found in animals on the west coast of Sweden. Comparison of the data for adult and juvenile seals showed one major species dependant in addition to an age-related variation in contamination, i.e. the toxaphene levels in adult Baltic ringed seal were significantly higher compared to the adult grey seal from the same region, and 5-10 times higher than in juvenile ringed seal from the same region. Andersson et al. (148) reported no geographical differences in concentrations of toxaphene from animals in the Arctic region with corresponding species in the Baltic.

Several reports on levels of toxaphene in fish and fish products from Europe show the ubiquitous presence in all types of fish (e.g., 89,167,168). High residues of toxaphene in fish and fish products from Europe was reported by Müller et al. (167) showing 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 Shetland Islands exceeded the German tolerance level (which was 0.1 mg kg⁻¹ on lipid basis or 0.01 mg kg⁻¹ w.w. at that time). Van der Valk and Wester (89) carried out a study in fish from northern Europe. The highest toxaphene concentrations were found in herring oil from the Baltic (7 µg g⁻¹ lipid). Toxaphene in cod liver showed an upward trend from southern to northern North Sea, increasing from 0.4 to 1 µg g⁻¹ lipid. This was somewhat unexpected since the northern North Sea usually is considered less polluted than southern North Sea. De Boer and Wester (7) state that in Western Europe toxaphene has practically never been used. Accumulation of toxaphene in North Eastern Atlantic waters may be brought about by aerial transport from the American continent. They also reported that Baltic herring oil contained a high toxaphene concentration probably due to the continued use of toxaphene in the East European countries.

Concentrations of individual congeners

Gooch and Matsumura (158) suggested that since the environmentally derived toxaphene is extensively altered in comparison to the technical material, measurements of 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 the fish were 0.26 and 0.1 µg g⁻¹ w.w., respectively, approximately one order of magnitude less than the estimated concentration of total toxaphene.

Hainzl et al. (88) analysed 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 while B[12012]-(202) and B[30032]-(122) were below detection or at very low concentrations ($<0.1 \text{ ng g}^{-1}$) in all samples analysed (Table 9). Icelandic cod liver contained the highest concentrations (Table 9). Toxaphene in hake liver from west of Ireland, herring muscle and dolphin blubber from the North Sea were all studied by de Boer et al. (170) who found that B[12012]-(202) and B[12012]-(212) were dominant compounds while B[30012]-(111) was not detected in most samples.

Alawi et al. (102) analysed B[30012]-(111), B[12012]-(212), B[30030]-(122), B[12012]-(202) and B[30032]-(122) in samples of marine fish by different analytical techniques. 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 at concentrations similar to important PCB congeners and cyclodiene insecticides in most of the fish samples, especially from the North Atlantic. 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 8-12% of total toxaphene. B[30012]-(111) was found in only a few samples and at very low concentrations. B[30032]-(122) was below detection in all samples analysed. 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) analysed 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, e.g. halibut, herring, redfish and mackerel. The sum of the indicator compounds in sardines and in fish with lean muscle tissue (Alaska pollock, saithe, hake and cod) levels 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 ca. 5 - 50 ng g⁻¹ fat and are in agreement with previously reported results for mackerel, eel, salmon and herring from Skagerak, whereas they are 3-8 times those that were reported for herring from the Baltic Sea (100). However, fish, especially herring are not stationary and differences might be influenced by migration.

Conclusion

The literature shows that toxaphene has a global distribution and can be found in both fresh water and marine biota all over the world. Also at remote areas at long distance from the sources the levels in biota can be quite high. This illustrates the importance of long range transport probably through the atmosphere for 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 and hence pose a treat to human (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 prevented successful re-stocking of treated lakes with desirable fish (178,179). However, experimental information on the depuration of toxaphene in fish and their residue kinetics is scarce. 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 intra peritoneal (i.p.) injection of technical toxaphene ($7 \mu\text{g g}^{-1}$ for white suckers and 3.5 and $7 \mu\text{g g}^{-1}$ for lake trout). The estimated half-lives for total toxaphene were found to be 524 days in white suckers and 232 (high dose) and 322 (low dose) days in 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-live was 716

days. From 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 (B[12012]-(202) and B[12012]-(212)) of toxaphene is different within a given species.

Mohammed et al. (181) studied the role of plasma lipoprotein in the transport and tissue accumulation of toxaphene. ¹⁴C-radiolabeled toxaphene in the absence or presence of either low density lipoproteins (LDL) or high density lipoproteins (HDL), was injected intravenous (i.v.) into normo- and hypolipidemic mice. In normolipidemic mice, most of the radioactivity was initially 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, a lower amount of radioactivity was found after 20 min in mice injected with toxaphene in combination with HDL compared to ¹⁴C-toxaphene-LDL injected mice, suggesting a more efficient metabolism and disposal of toxaphene when HDL was used as carrier. In hypolipidemic mice, Mohammed and co-workers initially found lesser label in the liver and adrenals and more in the kidney and heart (181). ¹⁴C-toxaphene was redistributed mainly to the liver and only in small amounts to adipose tissue 4 h after injection. According to the authors, these results indicate that changes in the lipid pattern may influence the tissue distribution of toxaphene. Mohammed et al. (181) also studied the distribution of ¹⁴C-radiolabeled toxaphene among lipoprotein fractions *in vitro* and *in vivo* using human and rat plasma. In rat 37-52% of radioactivity was recovered in the HDL fraction, while 18-52% was associated to the albumin-rich bottom fraction (BF) both *in vivo* and *in vitro*. In contrast to rat, the *in vitro* distribution of ¹⁴C-toxaphene among human lipoprotein fractions was shown to be relatively homogenous. 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 was recovered, respectively.

Reductive dechlorination or dehydrochlorination and in some cases oxidation have been shown to be the major mechanisms by which toxaphene is metabolised 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 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 under anaerobic conditions. All of the individual bornanes were transformed by reductive dechlorination, generally starting with the removal of a chlorine atom from the geminal dichlorogroup in the C-2 position. The dechlorination rate was shown to depend on the chlorination stage (nonachlorobornanes > octachlorobornanes > heptachlorobornanes). Two stable end-metabolites formed from all of the 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 individual bornanes tested. In addition, Fingerling et al. (36) showed that none of the components tested was 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 characterisation of oxygen containing products is scarce. Fingerling and Parlar (183) for the first time isolated and characterised an oxygen-containing product as conversion product of the toxaphene components B[30012]-(211), B[30012]-(121), and B[30012]-(111) under anaerobic conditions. This cyclo ketone (7b,8c,9c-trichlorocamphene-2-one) is most probably not formed from one of the two main hexachlorobornane products (36), but possible

formed from the dehydrochlorination product B[21001]-(111) which is formed as a small byproduct from B[30012]-(111) as well as from B[30012]-(211) and B[30012]-(121).

As was reviewed by Saleh (1) hepatic microsomal mixed function oxidases play the most important role in toxaphene metabolism in mammals, followed by glutathione-S-transferases. Chandra and Durairaj (184) showed that in addition to induction of cytochrome P-450 and aniline hydroxylase activity in the liver, toxaphene also induces these enzymes in the kidney. Therefore, the authors speculate that toxaphene itself might be metabolised in the liver as well as 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 harbour seal, whitebeaked dolphin, harbour porpoise, and the 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 metabolised by hepatic microsomes isolated from the four wildlife species. It was also found that harbour seal hepatic microsomes only metabolised B[30030]-(122). Neither toxaphene nor the four congeners were metabolised *in vitro* using hepatic microsomes of the sperm whale. Interestingly, their results showed that the *in vitro* capacity of microsomes derived from the different species to metabolise the technical toxaphene, reflects the decreasing number of peaks in the toxaphene residues of wildlife extracts.

Aquatic toxicity

Toxaphene is highly toxic for aquatic organisms. It was found that in general saltwater fish are more sensitive to toxaphene than freshwater fish (mean acute toxicity values of $0.07 \mu\text{g l}^{-1}$ and $1.6 \mu\text{g l}^{-1}$, respectively) (1). Keller (185) studied the acute toxicity of several pesticides, among

which toxaphene, to freshwater mussels (*Anodonta imbecilis*) and compared their sensitivities to common test organisms such as *Daphnia Magna*, *Ceriodaphnia dubia* and fathead minnow (*Pimephales promelas*). The 96-h LC₅₀ for *A. imbecilis* exposed to toxaphene was 0.74 mg l⁻¹. Compared to the other organisms tested, *A. imbecilis* is less sensitive to toxaphene. The acute toxicity for most aquatic organisms range from 1-40 µg l⁻¹ for toxaphene (1). Interestingly, addition of sediment to the test chambers drastically reduced the toxicity of toxaphene to *A. imbecilis*, indicating differences in susceptibility of *A. imbecilis* to either sediment-bound or aqueous concentrations of toxaphene.

The application of toxaphene as a piscicide to lakes has caused direct as well as indirect damage to the ecosystem. Direct damage includes: the disappearance of target as well as nontarget organisms inhabiting toxaphene exposed waters. Indirectly, application of toxaphene resulted in some cases in the replacement of native organisms by a new population of organisms thereby modifying the structure of the ecosystem. Miskimmin and Schindler (186) examined the response to toxaphene application and stocking with a non-native fish species on total chironomids, *Chaoborus* spp., and 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 a lake that had not been treated (Peanut Lake, south basin). They studied some members of invertebrates prior to application of toxaphene during 1961-62 and examined the recovery of the community in the following 30 years by analysing sediment cores from the lakes. They found that as a result of toxaphene application to Chatwin Lake (0.0184 ppm), planktonic Cladocera decreased in abundance and dominance changes from small- to large-bodied types. Short-term effects in sediment cores from the treated Peanut Lake (0.0075 ppm) were not detected. 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 caused low levels of *Chaoborus* spp. throughout the

1960s. The long-term changes in invertebrates of both lakes was most probably a result of the manipulation of fish communities rather than residual toxicity.

Mammalian toxicity

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

General toxicity

The majority of acute toxicity studies of toxaphene in mammals were conducted between 1950 and 1980. As reviewed by Saleh (1), the acute LD₅₀ of toxaphene to laboratory mammals ranged from 5 to 1075 mg kg⁻¹, depending on the species studied and the route of exposure used. In addition, female rats appeared to be somewhat more sensitive to toxaphene exposure than male rats. Among the most prominent symptoms observed in acutely intoxicated laboratory animals by toxaphene are generalised epileptic-like convulsions, starting with excessive saliva production, followed by vomiting and muscle spasms. In the course of time, the frequency of convulsions increased. Finally, animals became exhausted and died because of respiratory failure (173). Pathological changes upon toxaphene exposure may include degeneration of the brain and spinal cord, pulmonary oedema (1).

Combination toxicity

Since toxaphene was widely used as a pesticide in addition to other pesticides, the toxicity of toxaphene alone as well as in combination to 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 body weight as well as increases in liver/body weight ratios were observed in mice exposed to toxaphene and toxaphene containing mixtures. Optically, no pathological 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. Co-treatment of mice with toxaphene and parathion resulted in higher levels of inhibition of serum cholinesterase (serum ChE) activity than 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 co-treated with toxaphene and 2,4-D as compared to toxaphene alone. The phenobarbital-induced sleeping time was reduced in mice exposed to toxaphene and toxaphene containing mixtures, whereas in mice exposed to either one of the other pesticides no reduction was observed. This was probably caused by the capability of toxaphene containing exposures to induce the hepatic mixed-function oxygenase (MFO) system. From these studies it can not be concluded that the combinatorial toxicity of toxaphene and other pesticides is synergistic or antagonistic in nature, but rather a resultant of the effects showed by their components individually.

Neurotoxicity

Neurotoxic effects of toxaphene exposure, such as effects on behaviour 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 brain, e.g. hypoxic (disorganisation) 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 exposure to the high 5 mg kg⁻¹ day⁻¹ dose resulted in disfigurement of myelin in the brain. 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 in guinea pig. In the brain, an increase in neutral lipids and cholesterol and a reduction of phospholipids was observed. The individual phosphoglycerides phosphatidylinositol, sphingomyelin and phosphatidic acid increased in both the acute and subacutely intoxicated guinea pig brain. From their studies, Chandra and Durairaj

(197) postulated that the observed effects of toxaphene on lipid contents in brain, liver and kidney brought about membrane damage. In addition, alterations in phospholipids and cholesterol 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 since an increase in sphingomyelin inhibits the permeability of the membrane to small molecules and ions.

Chandra and Durairaj (184) also observed reduced ATPase and acetyl cholinesterase (AChE) activities in the brain upon 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 acetyl cholinesterase can result in neural and neuromuscular disorders. In addition, respiratory failure, leading to hypoxic and anoxic changes, would eventually result in decreased phosphorylation and ATP production, as evidenced by inhibition of ATPases. *In vitro*, toxaphene has been shown to inhibit brain and kidney ATPases in mammals as well as in fish and insects (1). In contrast to the observed effects on brain acetyl cholinesterase activity in the guinea pig, little effects on brain cholinesterase 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 pathological changes in brain and liver at the light microscopic level.

Table 11 gives an overview of neurological, reproductive, and endocrine effects caused by exposure to toxaphene.

Nephrotoxicity

In a number of studies, the effects on the kidney of mammals exposed to toxaphene were observed. In the 1992 study by Chandra and Durairaj (194), a single administration of 300 mg toxaphene kg⁻¹ b.w. to guinea pigs did not result in observable changes in the ultrastructure of

the kidney, 72 h after exposure. In a subacute exposure study, administration of 2 or 6 mg toxaphene $\text{kg}^{-1} \text{day}^{-1}$ administered for 60 days revealed vacuolisation in cells of the collecting system and glomerulus, degeneration of cortical tubular cells, vacuolisation 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 of the guinea pig to cope with a disturbance in membrane associated glycoproteins and glycolipid metabolism in liver and kidney. Studying 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 which were accompanied with a decrease in cardiolipin and sphingomyelin content. However, no alterations in other phosphoglyceride contents were found. Both acute and subacute exposure of guinea pig resulted in reduced ATPase and AchE activities in the kidney (184). This study also indicated that in addition to the liver, toxaphene may be metabolised in the kidney since an enhanced cytochrome P-450 content and induced aniline hydroxylase activity was found in the kidney upon toxaphene exposure.

Hepatotoxicity

A number of studies have shown that toxaphene or toxaphene containing mixtures are capable of inducing a number of hepatic biotransformation enzymes. Toxaphene as well as 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 were also shown to decrease the phenobarbital induced sleeping time in mice, suggesting an effect of toxaphene on CYP2B

type metabolizing enzymes (195). These studies shown that the toxaphene induced increase of appropriate biotransformation enzymes, among which cytochrome P-450, potentially stimulates the metabolism of a number of other xenobiotics and as a consequence might even reduce their toxicity.

In guinea pig, a single dose of 300 mg toxaphene kg^{-1} b.w. did not result in histopathological 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 effects 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 cholesterol levels (197). Markedly, in this study the acute dose of 300 mg kg^{-1} b.w. resulted in piloerection, sedation, crouching, clonic-tonic convulsions and death within 72 h. The changes observed in lipid profile were thought to be an adaptive mechanism to cope with stress due to toxaphene intoxication. In a similar experiment, toxaphene was also shown to reduce hepatic ATPase and acetyl cholinesterase activities and interfere with collagen and calcium metabolism (184).

Reproductive effects

Little data is available on the reproductive effects of toxaphene in mammals and fish. In mammals little or no effects were found indicating interference of toxaphene with reproduction (203-205). Recently, the reproductive effect of toxaphene in sexually mature female zebrafish was studied after being fed toxaphene contaminated food (0.02, 0.23 and 2.2 $\mu\text{g g}^{-1}$ fish day⁻¹) for two weeks (206). In the highest dose group, all fish died within 24 h and 9 out of 14 fish died in the group exposed to 0.23 $\mu\text{g g}^{-1}$ fish day⁻¹ between day 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 non-significant

decrease in mean total number of eggs spawned was observed. No differences in reproductive success were observed as assessed by percentage of fertilised eggs 24 h after fertilisation, percentage of embryo mortality and percentage hatching at 72 h after fertilisation. In contrast, toxaphene produced a dose related decrease of the percentage of oviposition of female zebrafish. Hence, it was concluded that dietary exposure of zebrafish to toxaphene affects the reproductive process.

Endocrine toxicity

One of the recent concerns of many environmental pollutants is that they might have endocrine-like properties. Environmental xenobiotics that mimic steroidal hormones have been implicated in the increasing 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, 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. In contrast to some DDT metabolites, toxaphene did not interact with the estrogen receptor. Interestingly, a combination of toxaphene with other chemicals decreased [³H]17 β -estradiol binding in a greater than additive way.

Possible estrogenic or antiestrogenic potencies of toxaphene either alone or in combination with other pesticides were however mainly 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 (CYP19) activity was not decreased as a result of toxaphene exposure. The expression of estrogen-regulated mRNA stabilising 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 was also shown to inhibit the binding of progesterone, dexamethasone and testosterone to their respective receptor (IC_{20} values of 68.4, 4.2, and 3.5 μ M respectively), isolated from eggshell gland mucosa of the domestic owl (213).

In contrast to the antiestrogenic potencies, weakly estrogenic potencies of toxaphene was observed in a number of other in vitro test systems. In the human E-screen test, 10 μ M toxaphene showed 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). Using transient gene expression studies employing a chimeric reporter construct containing one estrogen-responsive element (ERE) controlling the expression of the chloramphenicol acetyltransferase (CAT) gene in human breast cancer cells, technical toxaphene (10 μ M) as well as the toxaphene congener B[12012]-(212) (10 μ M) were shown to act as antiestrogens which exert their effects by blocking the action of estrogens by inhibiting the ER:ERE activated gene transcription (216).

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 not 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 toxaphene (10^{-8} - 10^{-5} M) or co-treated with toxaphene and dieldrin (equimolar concentrations, 10^{-5} M). Furthermore, no estrogenic response in 21-day-old female B6C3F1 mouse uterus was observed after oral exposure to toxaphene (2.5-275 μ mol kg^{-1} body weight) or toxaphene in combination with equimolar concentrations of dieldrin. In contrast to the above described systems, the same authors observed a slight estrogenic effect in an estrogen-responsive reporter system in yeast

expressing mouse estrogen receptor, 2.5 h after treatment with toxaphene ($2.5 \cdot 10^{-5}$ M) or mixtures of toxaphene with endosulfan, dieldrin or chlordane. The latter treatments were not synergistic of nature. In contrast, no estrogenic effect was observed in yeast expressing human estrogen receptor treated with toxaphene alone or in combination with other pesticides.

Carcinogenicity

Table 12 gives an overview of carcinogenic and mutagenic data of toxaphene presented in the literature.

In the past, much effort has been put on studying the carcinogenic properties of toxaphene. It was shown that toxaphene is highly carcinogenic in rat and mice inducing malignant liver tumours, reticulum cell sarcomas, sarcomas in the uterus, neoplasms in the reproductive system and/or mammary gland and neoplasms in the pituitary, adrenal gland and the thyroid gland (1,177). The study in which neoplasms were found in the thyroid gland of the rat were conducted by the National Cancer Institute (218). In order to investigate whether the increased incidence in thyroid tumours seen in the rat in the National Cancer Institute bioassay of toxaphene may have had a nongenotoxic aetiology, Waritz et al. (219) studied the thyroid function and thyroid tumours in male Crl:CD BR (Sprague-Dawley derived) rats orally exposed to 75 mg toxaphene $\text{kg}^{-1} \text{day}^{-1}$ for 28 days (the first 4 days, 100 mg toxaphene $\text{kg}^{-1} \text{day}^{-1}$ was administered). Rats were sacrificed at day 0, 7, 14 and 28 of exposure. A significant time-dependent increase in serum TSH levels was found whereas serum levels of T3, T4, rT3 and corrected rT3 did not change. They observed a time-dependent increase in thyroid follicular cell hypertrophy and intrafollicular hyperplasia and a decrease in thyroid follicular cell colloid stores, characteristic of a hyperactive thyroid. Considering the fact that toxaphene has the characteristics of a PB-type inducer of the cytochrome P-450 enzyme system, the authors concluded that the increase in thyroid follicular neoplasia in toxaphene treated rats was most

probably caused by a nongenotoxic mechanism such as proposed for thyroid tumour increases in rats chronically treated with PB. Since this type of mechanism for thyroid neoplasia is not known to occur in humans, the authors also state 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. (224) investigated two potential mechanisms: peroxisomal proliferation which has been invoked as a non-genotoxic mechanism of hepatocarcinogenicity, and DNA adduct formation. After oral treatment of CD/1 mice for seven days with toxaphene (0-100 mg kg⁻¹), no increases in immunodetectable levels of CYP4A1 were detected, suggesting that peroxisomal proliferation is not involved in toxaphene's toxicity. Furthermore, no evidence for DNA adduct formation in the liver of toxaphene-treated mice was found. Based on this study, the authors suggest that the hepatocarcinogenic properties of toxaphene may be exerted via a non-genotoxic or promotional mechanism rather than 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. (220) 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 TA 100 using a validated microsuspension procedure instead of the usual plate incorporated procedure. Toxaphene was found to be mutagenic only in the TA100 at concentration of 2500, 5000, and 10000 µg ml⁻¹. In contrast, toxaphene was also mutagenic to strain TA98 at a concentration of 10000 µg plate⁻¹ when using the plate incorporated assay. Using the microsuspension method, all four tested toxaphene congeners did not show mutagenic activity in strain TA100 at any of the

concentrations tested (maximum concentration: 10000 $\mu\text{g ml}^{-1}$). A dose-dependent (10-10,000 $\mu\text{g plate}^{-1}$) 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 harbour 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 $\mu\text{g ml}^{-1}$) dose-dependent SCE induction at the HGPRT gene locus in V79 cells.

Since cell-cycle delay may interfere with the expression of genotoxicity, Steinel et al. (221) 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 a dose- and treatment time-relationship was 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 the recommendations for prolonged incubation times in SCE assays affected by mitotic delay.

To study promotional mechanism rather than a genetic mechanism for toxaphene-induced tumor formation, Kang et al. (222) studied the inhibition of gap junctional intercellular communication (GJIC) by toxaphene. Non-cytotoxic concentrations of toxaphene (0-10 $\mu\text{g ml}^{-1}$) inhibited GJIC in normal human breast epithelial cells reversibly in a dose-dependent fashion after 90 min of exposure. In an attempt to determine how toxaphene inhibited GJIC, Kang and co-workers examined Cx43 protein in cells treated with toxaphene (222). A reduction in the

number of gap junctional plaques and a induction of hypophosphorylation of Cx43 in normal human breast epithelial cells was observed at toxaphene concentrations that affected GJIC. In addition, these studies also shown that toxaphene inhibit GJIC via a nonestrogen receptor mechanism since the cells used in these studies do not express the estrogen receptor. An alternative working hypothesis has been proposed for skin-tumour promotion, suggesting a central role for protein kinase C (PKC) (227). Moser and Smart (223) 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. From this result, it is premature however to conclude that stimulation of PKC activity is involved in toxaphene induced hepatic-tumour promotion.

Toxaphene, a human risk factor

As mentioned earlier, toxaphene is carcinogenic in rats and mice and has been proven to be mutagenic as well (1,178). Such findings led to the assumption that toxaphene poses a risk as a human carcinogen. Human exposure to toxaphene mainly occurs through the consumption of contaminated fish or occupational exposure. Data on the risk to humans associated with toxaphene exposure is scarce (1). Brown et al. (228) and Cantor et al. (229) further evaluated the association between elevated risk of leukemia and non-Hodgkin's lymphoma (NHL) among farmers and exposure to pesticides and other agricultural chemicals. Their results point to an elevated risk of NHL amongst farmers. Risk increased in cases where farmers had personally handled, mixed, or applied pesticides, did not use protecting clothes, and when more specific definitions of pesticide exposure was used. With regard to the latter, 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 concerning the toxicity of toxaphene to humans, difficulties arise in the interpretation of the human risk. In a IARC evaluation of the carcinogenic risk of toxaphene to humans, toxaphene was regarded as if it presented a carcinogenic risk to humans based on the evidence that toxaphene is carcinogenic in rat and mice and despite the lack of adequate data in humans (230). However, so far most studies on carcinogenicity of toxaphene have been performed with technical toxaphene mixtures. Exposure of humans is however mainly through consumption of toxaphene contaminated fish. The composition of the toxaphene mixture is changed from the original technical mixture due to weathering conditions and internal metabolism. The real exposure of humans is therefore to another mixture than technical toxaphene. It is not known what the toxic and carcinogenic properties are of those fish-born residues of toxaphene. In the framework of an EU-funded project "MATT", our laboratories are involved in a joint effort to

produce and isolate fish (cod)-based toxaphene residues which are chemically characterised and toxicologically evaluated, particularly for genotoxicity (*in vitro*) and tumour promotion capacity in a semi-chronic exposure study.

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), which had to be integrated in the national food laws of all member states. At that time toxaphene was still used as pesticide. In 1982 the European Maximum residue limit (MRL) for fruits and vegetables was extended to some food from 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. Based on toxicological concerns as 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} wet weight (233), equal to the limit of determination (LOD) of common residue analysis methods. Thus residues of toxaphene should not be found in these foods. In 1994 during the implementation of this regulation into the German MRL Ordinance, this strict MRL was extended to all food from 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 at 0.01 mg kg^{-1} wet weight.

In general the above mentioned regulations were based on total toxaphene levels. Just in the beginning of the nineties a sensitive residue analysis method by GC-ECD and GC-NCI/MS using 3 individual chlorinated bornane congeners (B[12012]-(202), B[12012]-(212) and B[30030]-(122)) was developed in Germany (11,92), applied in the routine analysis of many German laboratories and validated by an interlaboratory exercise (99,235). First reports

indicated relatively high concentrations of these toxaphene congeners were to be found in some fish from the North Atlantic which is an area from which much of Germany's fish stocks are derived (100). It was obvious that some of the consumer relevant fish would exceed this low MRL. Therefore, the new regulation for fish, fish products and mussels was suspended till the end of 1996 (234,236) to give the legislator the possibility to check, which MRL has to be established that takes into account the question of an acceptable level of consumer protection and on the other hand the necessary supply of the population with fish and fish products. In the interim much data about the contamination of all for the consumer relevant fish by the 3 indicator congeners was collected and evaluated in order to calculate the average toxaphene intake by fish consumption ($0.22 \mu\text{g person}^{-1} \text{ day}^{-1}$) (237). At present there exists no acceptable daily intake (ADI) value for toxaphene for carrying out a risk assessment. Therefore, the average toxaphene intake was compared with the lowest no observed adverse effect level (NOAEL) considering a sufficient high safety factor (ca. 25,000-50,000). In 1997 the German MRL for toxaphene in food from animal origin was then established with a new concept. The MRL for fish and fish products was set at 0.1 mg kg^{-1} wet weight on the basis of the sum of the 3 indicator congeners (238), for all other food from animal origin at 0.1 mg kg^{-1} on the basis of total toxaphene. It is the intention of the German government to fix also this latter MRL in future on the basis of the toxaphene indicator congener concept. It is the first national MRL for fish on the basis of toxaphene congeners.

Tolerance levels for toxaphene with regard to food consumption by humans in other countries are only known from the USA and Canada. The USA tolerance level was 5 mg kg^{-1} wet weight, however, this USA tolerance level has been withdrawn a few years ago. In Canada instead of a tolerance level an ADI value of $0.2 \mu\text{g kg}^{-1}$ body weight is used. The calculated daily intake values from the results of Alder et al. (100) stay below this Canadian ADI.

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