Toxaphene - recent developments in analysis and biomonitoring

Jacob de Boer
DLO-Netherlands Institute for Fisheries Research, P.O. Box 68, 1970 AB IJmuiden, The Netherlands.

Abstract
Recent developments in the analysis of toxaphene now enable a congener-specific analysis of toxaphene. However, the comparability of laboratory results still needs considerable improvement. Because of the complexity of the chromatograms multidimensional gas chromatographic techniques are recommended for a congener-specific determination of toxaphene congeners. Mass spectrometry with electron capture negative ionisation is a very sensitive detection technique for toxaphene congeners, but attention should be paid to variations in response. Fish consumption is supposed to be the main source of toxaphene exposure for man. Therefore, there is a need for more information on concentrations of toxaphene congeners in fish and shellfish, together with more information on the toxicological meaning of such data.

Introduction
The insecticide toxaphene is a complex mixture primarily consisting of chlorinated bornanes (CHBs) with an average elemental composition of $C_{10}H_{10}Cl_8$[1-3]. Prior to its ban in 1982 by the US Environmental Protection Agency[1], toxaphene was the most extensively used pesticide in the USA and many other parts of the world. The cumulative global production of toxaphene is estimated to be 1.3 megatons[4], which is higher than that of polychlorinated biphenyls (PCBs). Considerable amounts may still be placed in storage, although exact data are difficult to obtain[5]. Toxaphene has been detected as a contaminant in various environmental compartments and has a widespread distribution[6-10]. It is highly mutagenic[11]. Some studies have indicated that toxaphene is also potentially carcinogenic[12,13]. Due to aerial transport toxaphene concentrations were detected even in very remote areas[14,15]. Condensation at low temperatures is supposed to result in elevated concentrations in polar regions[16]. It is supposed that toxaphene concentrations in North-East Atlantic fish are also due to this cold condensation process taking place after aerial transport of toxaphene from more southern and western latitudes in Central and North-America[8].

Different chlorine substitution can theoretically lead to 32,768 possible congeners[17], of which a number also shows chiral activity[15]. This large number of congeners will, however, by far not be found, neither in the technical mixture, nor in environmental samples. Technical toxaphene, also known as strobane, phenatox or toxin 63, mainly consists of Cl_7 and Cl_8 congeners and can therefore contain not more than 6,840 congeners. However, a number of these CHBs is unlikely to be present because of unfavourable substitution positions on ring and bridge carbon atoms[19]. Jansson and Wideqvist have separated 670 different CHBs from a technical toxaphene mixture[20]. In environmental samples the total number of CHBs will be smaller due to degradation in the environment (weathering effects) and biotransformation, but nevertheless the remaining toxaphene patterns are still complex and require highly sophisticated techniques for their analysis.

Nomenclature
Toxaphene mixtures consist of polychlorinated bornanes (ca. 75 %), bornenes, bornadienes, chlorinated camphenes, dihydrocamphenes, other chlorinated hydrocarbons, and non-chlorinated hydrocarbons[21]. At a Workshop on Toxaphene, held at Burlington, Ontario, Canada, 1993, it was agreed that for the time being and to avoid the use of many different names for the same subject the term chlorobornanes (CHBs) would be used for toxaphene compounds[22]. This term is also used in this paper. Meanwhile there have been several proposals on a proper nomenclature for toxaphene[23-25]. A number of congeners were numbered by Parlar[23], based on gas chromatographic retention

and are not structure-related. The advantage is that the two digits are easy to use, but the system will be incomplete as soon as more congeners will be identified and become available. Other systems provide structural information, but are either incomplete[24], or are complex to handle, needing two conversions to obtain structural information[25] or needing large tables or a computer programme[26]. Recently a new system was introduced by Wester et al.[21], which directly provides structural information, is complete including distinguishing enantiomers and is relatively easy to use. It uses nine digits. The Parlar no. 50 is for example named B[12012]-(212)[21]. It is desirable that an authoritative body will make a final slection of one of the nomenclatures systems available.

Analysis

The production process of the Hercules company in the USA, one of the main producers of toxaphene[1], started with extraction of crude α-pinene from pine stumps, using methyl isobutylketone, heat and pressure. Isomerisation of the α-pinene produced camphene, bornylene and α-terpineol. The camphene was subsequently chlorinated to produce toxaphene. Since chlorination of camphene can take place to varying levels and on various sites, thousands of congeners can be theoretically be formed. It has been shown that at least 670 congener exist[1,20,27].

The degree of complexity of toxaphene is ca. 4-fold higher than that of polychlorinated biphenyls (PCBs) of which 209 congeners are theoretically possible and ca. 150 occur in the technical mixtures[28]. Although the sensitivity to weathering effects and metabolism is higher for toxaphene than for PCBs, also in biota the number of CHBs present is higher than that of PCBs in similar samples. In average fish samples the number of PCB congeners present will be between 50 and 100[29], whereas 100-200 toxaphene congeners may be expected[30]. It is generally agreed upon that it is not possible to separate all PCB congeners present in biota samples by single-column high resolution gas chromatography with electron caption detection (ECD)[31-33]. Karlsson and Oehme stated that it will be very difficult to find a stationary phase able to separate most of the major, not to mention all toxaphene congeners in fish samples[34]. The chances for CHB separation are better in marine mammals because of a more efficient biotransformation. However, because strong differences in biotransformation exist between different species of marine mammals, this chance on a reliable separation will be species-dependent[35].

Summarising it can be concluded that it will be very difficult to carry out a reliable congener-specific CHB determination by single-column GC/ECD. This means that there are two ways left open for the determination of toxaphene, (i) a determination of the total toxaphene concentration without attention to individual CHBs, and (ii) a congener-specific CHB determination by using more advanced analytical techniques.

Determination of total-toxaphene

Due to the analytical difficulties of a congener-specific approach and the lack of analytical standards of individual CHBs, most determinations of toxaphene carried out until now were based on the measurement of total-toxaphene. Obviously, there are a number of drawbacks attached to this approach. In the first place, due to weathering effects and biotransformation, the toxaphene patterns in the biological samples are considerably different from that in the technical mixture. This difference causes an error in the final result, because normally the total area of the peaks in the chromatograms of the technical mixture and the sample are measured and compared to each other, assuming equal response factors for all CHBs. The latter will be certainly not be true, although Alder et al.[36] showed that the differences in response of a range of CHBs are smaller for ECD than for electron capture negative ionisation (ECNI). The error is unknown, but is expected to be considerable[37]. In addition, the ECNI response varies between different types of mass spectrometers[36]. More sophisticated approaches, in which groups of isomers were measured[38], did not result in more accurate data. Additionally, with this approach it is not possible to obtain any information on the concentration and behaviour of more toxic or more persistent congeners.

The extraction and clean-up for a total toxaphene determination are fairly similar to that used for a PCB determination[39]. Most toxaphene compounds normally elute in the second fraction after elution over silica gel columns, together with most organochlorine pesticides.

Until now two rounds of an international interlaboratory study on the determination of total toxaphene in a fish oil were carried out[40,41]. The participants were allowed to use their own clean-up methods and detection systems. The mean, standard deviation and range of results in a sample cod liver oil in the first round were 3.99±1.98 mg/kg, 0.79-6.8 mg/kg (n=17), respectively. In the second round results did not improve much and the mean, standard deviation and range were

4.28±2.01, 1.67-9.1 mg/kg (n=13), respectively, also in cod liver oil[42]. The results obtained by GC/MS, using ECNI were slightly better than those using GC/ECD. The ECD response for the toxpahene compounds, which have an aliphatic character, is less good than for chlorinated compounds with an aromatic character such as PCBs. Several participants had difficulties with recoveries of the clean-up procedure, mainly due to the use of florisil or silica gel columns, which caused losses due to absorption of specific CHBs. Another source of variation may be the use of splitless injection. It has been shown that some CHBs a re not stable during splitless injection at high temperatures[43]. More information on this subject is required. On-column injection may be a suitable alternative. The broad range of results shows that most laboratories still have serious difficulties in analysing individual toxaphene congeners.

It can be concluded that the determination of total toxaphene may be used to obtain a rough estimation of the toxaphene concentration in biological samples. The uncertainty in this determination will in principle be larger than in that of individual compounds, particularly in samples from biota with a high biotransformation rate for toxaphene such as marine mammals[35], and in human milk[8]. Improvement of the comparability of laboratory results is desired.

Congener specific determination of toxaphene

For a reliable congener-specific analysis of CHBs a higher resolution is required than can be obtained with single-column GC/ECD[34]. This can be obtained by refining the detection system, for example by applying high resolution mass spectrometry (HRMS). The GC resolution remains similar to that of GC/ECD, but separation of co-eluting congeners is obtained by separating mass fragments with small mass differences. HRMS can be applied with two ionisation techniques, electron impact (EI) and ECNI. HREIMS offers a higher selectivity due to a broad fragmentation pattern, but is not very sensitive (ca. 10 to 100-fold less sensitive than ECD). HRECNI-MS combines selectivity with a high sensitivity (ca. 10-fold better than ECD)[44,45]. The selectivity is less good than in HREIMS due to less fragmentation. Both HRMS techniques do not always offer a solution for the determination of co-eluting CHB-homologues (isomers), although HREIMS may offer better possibilities in this respect than HRECNIMS. Another MS technique which has been applied for a congener-specific CHB analysis is EIMS/MS. Buser and Müller used this technique to characterise a CHB in whale blubber heptachlorobornanes in penguin and harbour seal extracts[46]. The application of ECNIMS/MS for CHB analysis is presently unknown.

A better resolution, needed for a reliable congener-specific CHB analysis, can be obtained by a more advanced GC technique such as multidimensional GC (MDGC). Two capillary GC columns, preferably installed in two independently controllable ovens, are used in this technique. Heart-cuts of target CHBs can be transferred by pressure switching from the first to the second column. By selecting a column combination with different characters the resolution can be highly enhanced. Separation of all PCB congeners was easily possible with MDGC/ECD[47,48]. Also for toxaphene promising results have been obtained[30,39,49]. A congener-specific CHB determination was possible in various biota sample. Only in the very complex technical toxaphene mixture overlap of peaks was still observed in the chromatograms of the heart-cuts. A combination of MDGC with ECD offers in principle sufficient sensitivity and selectivity for a reliable congener specific CHB determination. A combination of MDGC with ECNI-MS would be ideal because of the high sensitivity and the extremely high selectivity. Until now such a combination has not been applied for the analysis of CHBs.

A drawback of MDGC may be a relatively long analysis time and, consequently, less sample throughput, compared to single-column GC/ECD. The heart-cut technique offers a reliable determination of two or three specific CHBs per GC run. If heart-cuts of more CHBs would be combined per GC run, there is an increasing risk on co-elution because peaks in the heart-cuts would start to overlap again on the second column. A repeated run to enable the determination of for example four or maybe five CHBs solves this problem, but is obviously time consuming. It is very likely that a new technique called comprehensive multidimensional GC (CMDGC) will solve these problems[50,51]. With CMDGC, using a thermal modulator, all peaks of the first column are transferred to the second column without causing peak overlap. Until now this technique has been combined with flame ionisation detection (FID) and MS, but combinations with other detectors are being studied. It is essential that the detector cell is small enough to enable the detector to respond to the rapid througput of analytes. Besides a very high selectivity, also a high sensitivity is expected because peaks can be considerably narrowed by using the modulation technique.

Despite the poor perspective for a reliable congener-specific CHB analysis by single-column GC, such a method has been developed in Germany and is now used by several German laboratories[52,53]. This method, in which the sum of the congeners 26, 50 and 62 (Parlar nrs.[23]) is determined, consists of a Bligh and Dyer fat extraction and a clean-up with Bio beads SX-3, 2.5 x 40 cm, 200-400 mesh, cyclohexane/ethylacetate (1:1, v/v), 5 ml/min. The CHBs eluted together with the PCBs, chlordanes and other chlorinated pesticides between 95 and 150 ml. After removal of the solvent the sample was dissolved in iso-octane and eluted over an 1 g silica gel column, with 1 cm sodiumsulphate on top. The PCBs and p,p'-DDE were fractionated by elution with 8 ml hexane. This fraction also contained CHB 26. The other CHBs, chlordanes and organochlorine pesticides were collected in the second fraction, consisting of 8 ml hexane/toluene (65:35, v/v). The early elution of CHB 26 was also reported by de Boer et al.[54]. Krock et al.[54] developed a fractionation procedure in which CHB 26 was collected together with the other CHBs, but the separation was still incomplete and the solvent volumes needed were relatively large. An interlaboratory study between German laboratories, all using the same clean-up method but different methods for the final determination, resulted in coefficients of variation between 10 and 50%[53]. Because of the risk of co-elution, the use of this method may lead to positively biased results, and consequently, to a higher chance on exceeding tolerance levels. In the second round of the international interlaboratory study on toxaphene organised by Andrews[42] participants were asked to determine four toxaphene congeners (26, 32, 50 and 62) in cod liver oil using their own methods for clean-up and final determination. All results showed a broad range with the following means and standard deviations: CHB 26 0.30±0.28 mg/kg (n=13), CHB 32 0.03±0.04 mg/kg (n=10), CHB 50 0.43±0.36 mg/kg (n=13) and CHB 62 0. 22±0.15 mg/kg (n=9). A new world-wide interlaboratory study on the determination of CHBs is currently being organised under the QUASIMEME (Quality Assurance of Information for Marine Environmental Monitoring in Europe) Programme[55]. This will be a stepwise-designed study, in which the CHB determination will firstly be studied in standard solution, followed by a study in cleaned-up and uncleaned extracts of biological samples and finally in biological tissues.

Many CHBs show a chiral activity[15]. Progress has been made in the study of chiral toxaphene congeners[56]. Information on enantiomeric ratios can give insight in the degradation process of CHBs. Recently, a study on toxaphene enantiomers has been carried out using a combination of an apolar GC column and a chiral column in heart-cut MDGC[57].

Analytical standards of a number of CHBs, including some of the most persistent congeners, are now available. Toxicological information is needed to identify whether or not these congeners are the most relevant ones to determine. There is also a need for isotopically labelled standard to assure an accurate quantification of CHBs by GC/MS. Certified reference materials are not available yet, neither for total toxaphene, nor for CHBs.

Distribution of toxaphene in the aquatic environment

The octanol-water partition coefficient (log K_{ow}) of toxaphene is estimated to be 6.44[11], which is somewhat lower than that of technical PCB mixtures, but higher than those of p,p'-DDT and its metabolites. This means that bioconcentration of toxaphene is expected to be relatively high. Indeed high levels of toxaphene have been found in aquatic organisms from all over the world. High toxaphene levels in fish up to 28 mg/kg have been found in fish from the St. Lawrence river (Canada) and in Canadian cod liver oil[58,59]. Baltic fish contained toxaphene concentrations up to 6 mg/kg lipid weight[60]. Toxaphene concentrations in North Sea fish are at least 10-fold lower than in fish from Arctic and Canadian waters and vary from 1 to 600 µg/kg wet weight[8]. Glassmeyer et al.[27] showed decreasing total toxaphene concentrations in lake trout and smelt from the Great Lakes, in trout from 25-30 mg/kg lipid weight in 1982 to ca. 15 mg/kg lipid weight in 1992. In Lake Superior toxaphene levels were constant at a level of 30-35 mg/kg on a lipid weight basis. Biomagnification of toxaphene in some food chains in the Great Lakes was found, but depended on the type and length of the food chain present[27]. Recently an extensive study on toxaphene levels in fish from the North Sea, Baltic, English Channel, Bay of Biscay and from waters south of Ireland, west of Norway, around Iceland and east of Canada was carried out by Alder et al.[61]. In this study only the sum of the CHBs 26, 50 and 62 was reported, determined by single-column GC/ECD. In addition confirmation with MS was carried out in some samples. A significant relationship between the toxaphene level and the fishing ground could not be established, this in contrast with the indicative study carried out by de Boer and Wester[8], in which higher toxaphene levels in the northern North Sea were found, compared to the southern North sea. Concentrations

of 1-34 µg/kg wet weight were found in herring samples for the sum of the three CHBs[61]. Concentrations of these compounds in halibut, redfish, sardine and mackerel, and in farmed fish such as salmon, trout and eel were comparable to those in herring, while concentrations in lean fishes such as plaice were lower, between not detectable and 1 µg/kg. The daily intake of the total toxaphene, calculated from the results of this study, based on a daily consumption of 20 g fish/person, was estimated between 2.8 and 5.6 ng/kg bodyweight.

Very high toxaphene concentrations have been found in marine mammals, such as 23 mg/kg in St. Lawrence Beluga whales[62] and 19 mg/kg in dolphin blubber from the central North Sea[8]. Toxaphene has also been determined in human milk from Sweden, Finland and the Netherlands (0.05-0.7 mg/kg lipid weight) and from Nicaragua (up to 68 mg/kg lipid weight)[8,59,63,64]. Fish consumption is supposed to be the main source of exposure for man[61].

Toxaphene and fish consumption

Tolerance levels for toxaphene with regard to fish consumption by humans are only known from the USA and Germany. The USA tolerance level is 5 mg/kg wet weight and the German tolerance level is 0.1 mg/kg lipid weight for fatty fish (> 10% fat) and 0.1 mg/kg wet weight for lean fish (< 10% fat). There seems to be no sound toxicological basis for both standards. The German tolerance level will presumably be changed into 0.1 mg/kg on a wet weight basis for the sum of the CHBs 26, 50 and 62 for all types of fish. According to the study of Alder et al.[61] most fish samples analysed do not exceed this new German tolerance level. Other studies show, however, that several fish species from the North Sea and from the Baltic may exceed this level. In Canada instead of a tolerance level an acceptable daily intake (ADI) value of 0.2 mg/kg is used. The calculated daily intake values from the results of Alder et al.[61] stay far below this Canadian ADI.

Conclusions

With regard to discussions on tolerance levels for toxaphene, which in Germany and in Europe tend to go in the direction of congener-based tolerance levels, it is desirable that concentrations of individual CHBs are determined. Developments in analytical techniques for the determination of toxaphene congeners now enable a congener-specific determination of CHBs. The use of MDGC/ECD offers sufficient resolution for a reliable determination of CHBs. GC/MS techniques, particularly those using HRECNIMS, may be used as well, but attention should be paid to response variations under different conditions. Other promising techniques may be developed in the future, such as GC/ECNI-MS/MS, MDGC/MS, and CMDGC/ECD. Methods based on single-column GC/ECD should be critically evaluated with regard to false-positive results due to possible peak-overlap in the chromatograms. The use of splitless injection should also be critically be evaluated with regard to the possible decomposition of some CHBs. There is a need for further improvements in the pre-fractionation of toxaphene. There is also a need for certified reference materials and isotopically labelled CHB standards.

It will be essential to improve the quality of analytical data on toxaphene. The coming QUASIMEME interlaboratory study may be helpful in this respect. The use of one system for the nomenclature is desirable to avoid confusion in the literature due to the use of different systems.

The information on toxaphene concentrations should be combined with information on the toxicity of toxaphene.

References

(1) Saleh, M.A. *Rev. Environ. Contam. Toxicol.* **1991**, 118, 1-85.
(2) Casida, J.E.; Holmstead, R.L.; Khalifa, S.; Knox, J.R.; Oshawa, T.; Palmer, K.J.; Wong, R.Y. *Science* **1974**, 183, 520-521.
(3) Cairns, T.; Siegmund, E.G.; Froberg, J.E. *Mass. Spectrom.* **1981**, 8, 569.
(4) Voldner, E.C.; Li, Y.F. *Chemosphere* **1993**, 27, 2073-2078.
(5) Voldner, E.C.; Li, Y.F. *Sci. Total Environ.* **1995**, 160/161, 201-210.
(6) Muir, D.C.G.; de Boer, J. *Trends Anal. Chem.* **1995**, 14, 56-66.
(7) Wideqvist, U.; Jansson, B.; Olsson, M.; Odsjö, T.; Reutergårdh, L.; Uvemo, U.B. *Chemosphere* **1993**, 27, 187- 201.
(8) Boer, J. de; Wester, P.G. *Chemosphere* **1993**, 27, 1879-1890.
(9) Hargrave, B.T.; Muir, D.C.G. ; Bidleman, T.F. *Chemosphere* **1993**, 27, 1949-1963.
(10) Ballschmiter, K.; Zell, M. *Intern. J. Environ. Anal. Chem.* **1980**, 8, 15-35.
(11) Hooper, N.R.; Ames, B.N.; Saleh, M.A., Casida J.E. *Science* **1978**, 205, 591- 593.
(12) Reuber, M.D. *J. Toxicol. Environ. Health* **1979**, 5, 729-748.

(13) Innes, J.R.M.; Ulland, B.M.; Velerico, M.G.; Petrucelli, L.; Hart, E.R.; Arlotta, A.J.; Bates, R.R.; Falk, H.L.; Gart, J.J.; Klein, M.; Mitchell, I.; Peters, J. *J. Natl. Cancer Inst.* **1969**, 52, 1101-1114.
(14) Zell, M. ; Ballschmiter, K. *Fresenius Z. Anal. Chem.* **1980**, 300, 387-402.
(15) Bidleman, T.F.; Falconer, R.L; Walla, M.D. *Sci. Total Environ.* **1995**, 160/161, 55-63.
(16) Wania, F.; Mackay, D. *Sci. Total. Environ.* **1995**, 160/161, 211-232.
(17) Vetter, W.*Chemosphere* **1993**, 26, 1079-1084.
(18) Kallenborn, R.; Oehme, M.; Vetter, W.; Parlar, H. *Chemosphere* **1994**, 28, 89-98.
(19) Hainzl, D.; Burhenne, J.; Parlar, H. *Chemosphere* **1994**, 28, 237-243.
(20) Jansson, B. ; Wideqvist, U. *Intern. J. Environ. Anal. Chem.* **1993**, 13, 309-321.
(21) Wester, P.G.; de Geus, H.-J.; de Boer, J.; Brinkman, U.A.Th. *Chemosphere* **1997**, in press.
(22) Muir, D.C.G.; de Boer, J. *Chemosphere* **1993**, 27, 1827-1834.
(23) Burhenne, J.; Hainzl, D.; Xu, L.; Vieth, B.; Alder, L.; Parlar, H. *Fresenius J. Anal. Chem.* **1993**, 346, 779-785.
(24) Tribulovic, V.G.; Nikiforov; V.A., Karavan; V.S., Miltsov; S.A., Bolshakov; S. *Organohalogen Compounds* **1994**, 19, 94-107.
(25) Oehme, M.; Kallenborn, R. *Chemosphere* **1995**, 30, 1739-1750.
(26) Andrews, P.; Vetter, W., *Chemosphere* **1996**, 31, 3879-3886.
(27) Glassmeyer, S.T.; Myers, T.L.; De Voult, D.S.; Hites, R.A. *Environ. Sci. Technol.* **1997**, 31, 84-88.
(28) Mullin, M.D.; Pochini, C.; Mc Grindle, S.; Rankes, M.; Safe, S.; Safe, L. *Environ. Sci. Technol.* **1984**, 18, 468-476.
(29) Boer, J. de ; Dao, Q.T. *J. High Resolt. Chromatogr.* **1989**, 12, 755-759.
(30) Boer, J. de; de Geus, H.-J.; Brinkman, U.A.Th. *Environ. Sci. Technol.* **1997**, 31, 873-879.
(31) Duinker, J.C.; Schulz, D.E.; Petrick, G. *Anal. Chem.* **1988**, 60, 478-482.
(32) Larsen, B.; Bøwadt, S. *Proceed. 15th Intern. Symp. Capill. Chromatogr.*, Riva del Garda, Italy, **1993**, I, 503-510.
(33) Boer, J. de; van der Meer, J.; Brinkman, U.A.Th. *J. Assoc. Off. Anal. Chem.* **1996**, 79, 83-96.
(34) Karlsson, H.; Oehme, M. *Organohalogen Compounds* **1996**, 28, 369-374.
(35) Boon, J.P.; Helle, M.; Dekker, M.; Sleiderink, H.M.; Klamer, H.J.; Govers, B.; Wester, P.G.; de Boer, J. *Organohalogen Compounds* **1996**, 28, 389-394.
(36) Wester, P.G.; de Boer, J.; Brinkman, U.A.Th. *Environ. Sci. Technol.* **1996**, 30, 473-480.
(37) Alder, L.; Palavinskas, R.; Nikiforov, V.A.; Trribulovich, V.G. *Organohalogen Compounds* **1996**, 28, 423-428.
(38) Swackhammer, D.L.; Hites, C.M.J. *Anal. Chem.* **1987**, 59, 913-917.
(39) Boer, J. de; de Geus, H.-J. (1995). *Organohalogen Compounds* 26, 345-350.
(40) Andrews, P.; Headrick, K.; Pilar, J.-C.; Lau, B; Weber, D. *Chemosphere* **1995**, 31, 4393-4402.
(41) Andrews, P.; Newsome, W.H., Boyle, M.; Collins, P.*Chemosphere* **1993**, 27, 1865-1872.
(42) Andrews, P. **1996**. Draft report second round robin study on toxaphene. Dept. Health Canada, Ottawa, Canada.
(43) Alawi, M.; Barlas, M.H.; Hainzl, D.; Burhenne, J.; Coelhan, M.; Parlar, H. *Fresenius Z. Anal. Chem.* **1994**, 350, 3-9.
(44) Barrie, L.,; Bidleman, T.F.; Dougherty, D.; Fellin, P.; Grift, N.; Muir, D.C.G.; Rosenberg, R.; Stern, G.; Toan, D. *Chemosphere* **1993**, 27, 2037-2046.
(45) Stern, G.A.; Muir, D.C.G; Westmore, J.B.; Buckannon, W.D. *Biol. Mass Spectrom.* **1993**, 22, 19-24.
(46) Buser, H-R.; Müller, M.D. *Environ. Sci. Technol.* **1994**, 28, 119-128.
(47) Boer, J. de; Dao, Q.T. *J. High Resolut. Chromatogr.* **1991**, 14, 593-596.
(48) Boer, J. de; Dao, Q.T; Wester, P.G.; Bøwadt, S.; Brinkman, U.A.Th. *Anal. Chem. Acta* **1995**, 300, 155-165.
(49) Boer, J. de; de Geus, H.-J.; Brinkman, U.A.Th.*Organohalogen Compounds* **1996**, 28, 363-368.
(50) Phillips, J.B.;Xu, J. *J. Chromatogr. A* **1995**, 703, 327-334.
(51) Geus, H-J. de; de Boer, J; Brinkman, U.A.Th. *Trends Anal. Chem.* **1996**, 15, 398-408.
(52) Alder, L.Beck, H.; Khander, S.; Karl, H.; Lehman, I. *Organohalogen Compounds* **1995**, 26, 323-328.
(53) Alder, L.; Bache, K.; Beck, H.; Parlar, H. *Organohalogen Compounds* **1995**, 26, 369-374.
(54) Krock, B.; Vetter, W.; Luckas B. *Chemosphere* **1997**, in press.
(55) The QUASIMEME II International Laboratory Performance Studies, Year 2. Marine Laboratory, Aberdeen, UK.
(56) Karlsson, H.; Oehme, M; Müller, L. *Organohalogen Compounds* **1996**, 28, 405-409.
(57) Geus, H.-J.; Baycan-Keller, R.; de Boer, J.; Oehme, M.; Brinkman, U.A.Th., in preparation.
(58) Müller, R.; Lach, G.; Parlar, H. *Chemosphere* **1988**, 17, 2289-2298.
(59) Musial, C.J. ; Uthe, J.F. *Intern. J. Environ. Anal. Chem.* **1983**, 14, 117-126.
(60) Andersson, Ö.; Linden, C.-E.; Olsson, M.; Reutergårdh, L.; Uvemo, U.B.; Wideqvist, U. *Arch. Environ. Contam. Toxicol* **1988**, 17, 755-765.
(61) Alder, L.; Beck, H.; Khandker, S.; Karl, H.; Lehmann, I. *Chemosphere*, **1997**, in press.
(62) Muir, D.C.G.; Ford, C.A.; Steward, R.E.A.; Smith, T.G.; Addison, R.F.; Zurich, M.T.; Béland, P. *Can. Bull. Fish. Aquat. Sci.* **1990**, 224, 165-190.
(63) Pyysalo, H.; Antervo, H. *Chemosphere* **1985**, 14, 1723-1728.
(64) Vaz, R.; Blomkvist, G. *Chemosphere* **1985**, 14, 223-231.