The role of biotic and abiotic degradation processes during the formation of typical toxaphene peak patterns in aquatic biota

H. Parlar[1], D. Schulz-Jander[1], G. Fingerling[2], G. Koske[1], D. Angerhöfer[1], and J. Burhenne[3]

[1]Chair for Chemical Technical Analysis and Chemical Food Technology, Technical University Munich, 85350 Freising-Weihenstephan – Germany
[2]Dept. for Analytical Chemistry, University of Kassel, 34109 Kassel – Germany
[3]Dept. for Ecochemistry, University of Kassel, 37213 Witzenhausen – Germany

Introduction

Toxaphene is a complex insecticidal mixture consisting of more than 200 polychlorinated monoterpenes, predominantly bornanes. Until now, more than 60 components have been isolated and structurally identified, and more than 90% of these have a bornane skeleton [1-9]. The composition of toxaphene residues in environmental samples differs widely. Nevertheless, in some types of samples, such as fish species or fish products from North Atlantic and North Sea, the toxaphene peak patterns are remarkably similar with only 20-25 dominant congeners. Of these, 6 compounds represent the main mass of the residues. They all belong to the most stable compounds of the technical mixture and still remain in the environment when most of the other components have already been degraded. In order to find out which of the parent chlorobornanes are stable and which not, and whether the degradation pathways are biotic or abiotic, structure-degradability studies with single toxaphene components have been executed [10]. Furthermore, enantioselective residue analysis with multidimensional gas chromatography-high resolution mass spectrometry has been used to distinguish between the two different degradation pathways [11].

Materials and Methods

Technical toxaphene standard and single toxaphene components were obtained from Ehrenstorfer, Germany. Organic solvents were of purity grade for residue analysis. Na_2SO_4 and H_2SO_4 (95-97%) were from Merck, Germany. Cod liver oils and fish oils were obtained from different countries. The samples were kept under –12°C until use. The clean-up was executed as described in [9].

HRGC-ECD analysis:
Standards and samples were analyzed on a Chrompack CP 9002 GC-ECD-System. The operating conditions were as follows: column: JW Scientific 60m, id 0.32mm, film thickness 0.25µm; carrier gas: H_2; injector temperature: 220°C; detector temperature: 280°C; column temperature program: initial temperature 100°C – to 150°C at 30°/min – to 250°C at 2°/min – hold 10min.

HRGC-HRMS analysis:
Chlorobornane standards and samples were analyzed on a Hewlett-Packard 5890 Series II gaschromatograph/Finnigan 8200 high resolution mass spectrometer. The operating conditions were as follows: column: DB-5 30m, id 0.25mm, film thickness 0.25µm; carrier gas: helium;

splitless injection; emission current ca. 200 µA; injector and transfer line temperature: 280°C; detector temperature: 280°C; ion source temperature for ECNI: 100°C; column temperature program: initial temperature 140°C – hold for 1min – to 250°C at 4°/min.

Multidimensional gas chromatography: A Sichromat 2-8 with live-T-technique was used (1. column: DB5, 60m, i.d. 0.32mm, film thickness 0.25µm; temp. program: 100°C to 150°C with 30°/min, 150°C to 250°C with 2°/min; 2. column: BGB-A2 (20% BSCD); 30m, i.d. 0.32mm, film thickness 0.20µm; temp. program: 80°C (39min) to 180°C with 30°/min, 180°C to 250°C with 1°/min).

Photolysis: The single substances were dissolved in 1.5 l degassed n-hexane and irradiated for 12h with a low pressure Hg lamp (Vycor 250 mA/500 V, Fa. Gräntzel, Karlsruhe, Germany, emission maximum at 254 nm) at 254 nm. Samples were taken after 3, 6, and 9 hours and concentrated. The photoproducts were separated by column chromatography on silica gel 60 (70-230 mesh) with n-hexane and then analyzed.

Degradation in soil: The soil, a loamy silt (pH 6.7, 1.8% organic carbon) was collected from a field in the surroundings of Kassel, air-dried and passed through a 2-mm sieve prior to use. Incubation was done with portions of 80g of soil each placed in a 200mL Erlenmeyer flask and fortified with 1mL of acetone containing 400µg of the toxaphene component. After adding 150mL of sterile, distilled water to each flask, they were shaken, and the dissolved O_2 was removed with a stream of nitrogen for 30min. Then the flasks were tightly capped with Teflon-coated stoppers and kept in the dark at ca. 30°C. Two samples as well as two blanks were prepared in this way for each component. One series of the spiked soil samples was sterilized by autoclaving (121°C, 15psi) for two 1h periods at intervals of 24h before adding the components. Samples for analysis were taken weekly during the first two months and for the rest of the time – a total of 4 and 6 months, respectively – in intervals of 2 weeks. The flasks were shaken and then opened under a stream of N_2, and 10mL aliquots of the suspension were taken. Each sample was acidified with H_2SO_4 to pH ca. 1 and extracted with a mixture of 5mL of petroleum ether (45-65°C)/acetone (1:1) in an ultrasonic bath for 30min. The petroleum ether layer was separated, and the aqueous layer was reextracted twice with 2 x 5mL of petroleum ether (45-65°C). Finally, the organic phases were combined, dried over Na_2SO_4, and concentrated under N_2 to ca. 1mL. These extracts were directly used for GC analysis.

Results and Discussion

The similarity of the HRGC peak patterns of some kinds of environmental samples, especially fish samples, and UV irradiated toxaphene (Figure 1) indicates that similar degradation pathways lead to these residue patterns. Previously, it has been suggested that metabolism of chlorobornanes in fish should lead to the same products as photolysis of toxaphene [7-9]. However, enantiomer selective analysis of the toxaphene components Parlar #26, #44, #50, and #62 had shown that the enantiomer ratio in fish products was about 1. Therefore, it had then been assumed that no metabolism occurs in fish at all [12]. Now recent analysis of enantiomer ratios in cod liver and fish oil samples from different countries revealed significant deviations from 1 for the components Parlar #44 and #62, while #50 was found to be a racemate (Table 1). This can be interpreted as clear evidence for metabolism of at least some toxaphene components in cod fish and perhaps other fish species.

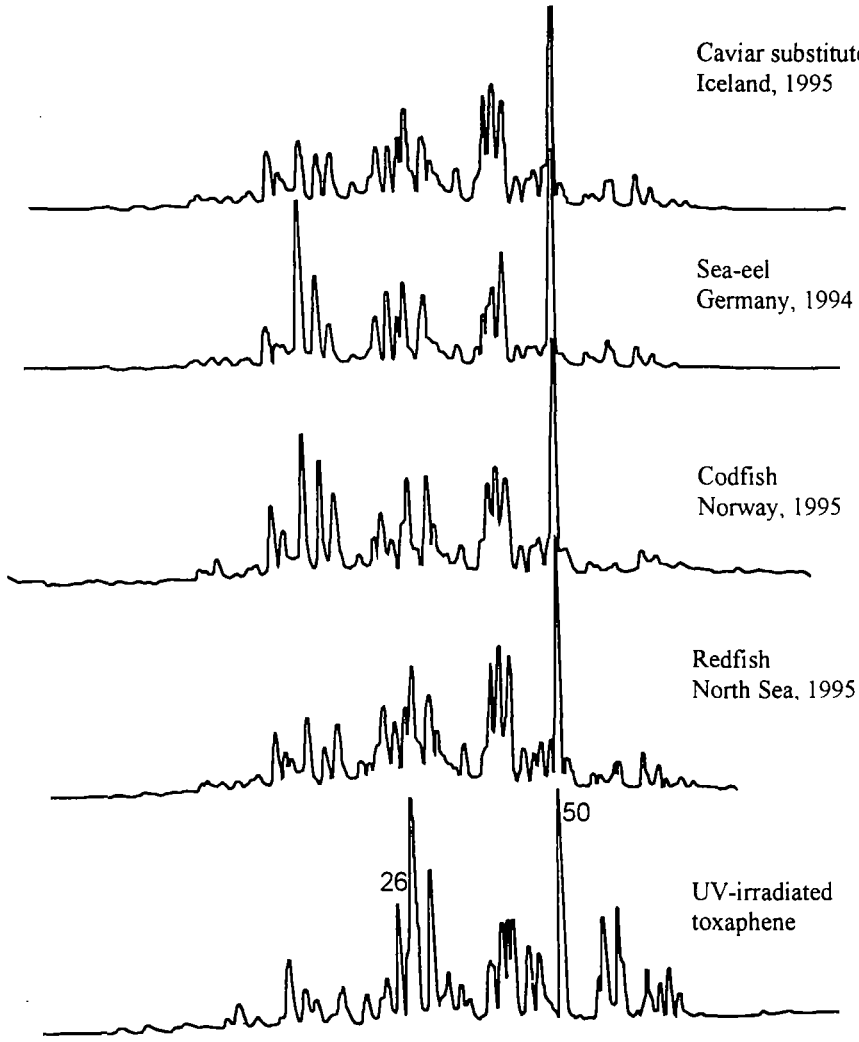

Figure 1. ECNI-MS-SIM chromatograms of different aquatic samples from the North Atlantic and North Sea compared to that of irradiated technical toxaphene

Table 2. Enantiomer ratios (+/–) of four toxaphene components in cod liver oils and fish oils

Substance	Cod Liver Oil	Fish Oil
toxaphene #26	1,00-1,41	1,00-1,02
toxaphene #44	1,10-1,28	1,20 1,28
toxaphene #50	1,00-1,11	1,01-1,02
toxaphene #62	1,01-1,09	1,75-1,89

Abiotic processes in the troposphere and microbial degradation in soil are the main known sinks for toxaphene; only few of the components are stable under these conditions. These are congeners with exactly one chlorine atom at the ring C-atoms in alternating orientation [10]. This can be seen with the component Parlar #50, which has been found to be a racemate. The same should be the case with Parlar #26, but here the enantiomer ratio in one sample of cod liver oil showed a deviation from 1. Whether this is due to accidental sample variations has yet to be checked. Toxaphene components with geminal dichloro groups are more reactive, especially when there are additional chlorines in α-position. The latter, which are easily degraded under different environmental conditions, amount to more than 70% of the technical toxaphene. The components Parlar #44 and #62 both have geminal dichloro groups, though without chlorines in α-position. The enantiomer ratio of #44 differed slightly from 1 in all samples investigated while that of #62 was 1 in cod liver oil, but significantly different from 1 in fish oils. Obviously, both components can at least be partly degraded, but whether in all fish species or only in some and to what extend remains to be examined.

References

1. Holmstead, R. L., Khalifa, S., and Casida, J. E., 1974, „Toxaphene composition analysed by combined gaschromatography-chemical ionisation mass spectrometry", *J. Agr. Food Chem. 22*, 939-944
2. Khalifa, S., Mon, T. R., Engel, J. L., and Casida, J. E., 1974, „Isolation of 2,2,3-endo,6-exo,8,9,10-heptachlorobornane and an octachloro toxicaqnt from technical toxaphene", *J. Agric. Food Chem., 22*, 653-657
3. Anagnostopoulos, M. L., Parlar, H., and Korte, F., 1974, „Ecological Chemistry LXXI. Isolation and toxicology of some toxaphene components", *Chemosphere, 3*, 65-70
4. Hainzl, D., 1994, „Isolierung und Identifizierung von C10-Chlorterpenen aus dem Insektizid Toxaphen", Thesis, University of Kassel, Germany
5. Hainzl, D., Burhenne, J., Barlas, H., and Parlar, H., 1995, „Spectroscopical characterization of environmentally relevant C_{10}-chloroterpenes from a photochemically modified toxaphene standard", *Fresenius J. Anal. Chem.*, 351, 271-285
6. Hainzl, D., Burhenne, J., and Parlar, H., 1993, „Isolation and characterization of environmental relevant single toxaphene components", *Chemosphere, 27*, 1857-1863
7. Lach, G., and Parlar, H., 1990, „Quantification of toxaphene residues in fish and fish products using a new analytical standard", *Chemosphere, 21*, 29-34
8. Lach, G., and Parlar, H., 1991, „Comparison of several detection methods for toxaphene residue analysis", *Toxicol. Environ. Chem., 31*, 209-219
9. Xu, L., Ramus, U., Pletsch, B., and Parlar, H., 1992, „Quantification of toxaphene residues in fish and fish products", *Fresenius Envir. Bull., 1*, 58-63
10. Fingerling, G., 1995, „Umwandlung von isolierten Toxaphenkomponenten unter abiotischen und biotischen Bedingungen"; Thesis, University of Kassel, Germany
11. Koske, G., Leupold, G., Parlar, H., 1997, „Gas chromatographic enantiomer separation of some selected polycyclic xenobiotics using modified cyclodextrins"; *Fresenius Envir. Bull. 6*, 489-493
12. Parlar, H., Fingerling, G., Angerhöfer, D., Christ, G., Coelhan, M., 1997, „Toxaphene Residue Composition as Indicator of Degradation Pathways"; in: Eganhouse, R. (ed.): Molecular Markers in Environmental Geochemistry: Source Indicators and Process Probes; ACS, Washington, 346-364