Structure-Stability Relationship of Chlorinated Bornanes in the Environment

Gerda M. Fingerling, Mehmet Coelhan, Daniela Angerhöfer and Harun Parlar

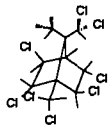
Department of Chemical Technical Analysis, Technical University of Munich, 85350 Freising-Weihenstephan

Abstract

In order to estimate the behavior of toxaphene components, a working knowledge of their conversion under simulated environmental conditions is necessary. In this work, representative toxaphene components were investigated under UV irradiation and in a flooded soil. The structure of the resulting products has been identified by X-ray spectroscopy as well as ^{1}H and ^{13}C-NMR, MS and FTIR spectroscopy. In combination with the results of kinetical experiments, the data suggests the dominance of abiotic degradation pathsways in atmosphere, water, and, perhaps, part of the aquatic biota whereas biotic pathways may dominate in soil.

Introduction

Toxaphene, an insecticidal mixture produced by the chlorination of camphene, 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. Today, more than 60 of these compounds have been identified in their structure (1-8). The peak area percentage of all components identified, measured by ECD, amounts to 50% of the total technical toxaphene (9). Of these compounds, only about 25 are regularly found in environmental samples. Most of the nona- and decachlorobornanes are normally absent, while many of the hexa- and heptachlorobornanes as well as some of the octa- and nonachlorobornanes are persistent and accumulate. Atmospheric transport of toxaphene to North Atlantic and Arctic has been demonstrated by residue analysis of air, water, and biotic samples (10-20). Sometimes, similarities in the gas chromatographic peak patterns are found, as for instance a shift to shorter retention times together with the dominance of partly the same peaks or peak groups. Tissues of some species, such as marine mammals, may contain only a few components in rather high amounts (19, 20). Nevertheless, there are some components which are present in nearly all samples containing toxaphene. The reason for this may be found by investigation of structure-stability relationships of the toxaphene components. Therefore, 14 pure chlorobornanes isolated from the technical mixture (Figure 1) were studied as to their stability under UV irradiation and in a flooded loamy silt in order to establish some rules for predicting the behavior of this substance class under environmental conditions.



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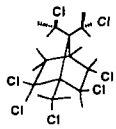

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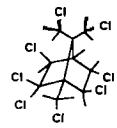

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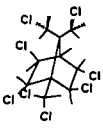

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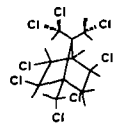

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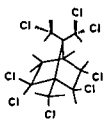

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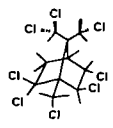

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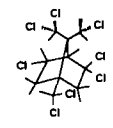

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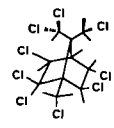

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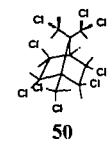

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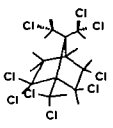

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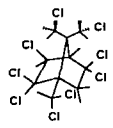

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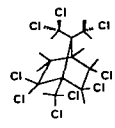

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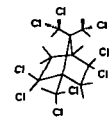

62
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Figure 1: Structure of chlorobornanes investigated in this study

Methods

Materials: Technical toxaphene standard (camphechlor) was obtained by Ehrenstorfer, Germany. Single toxaphene components were isolated as described previously (1, 21). All solvents were p. a. quality. The soil, a loamy silt (pH 6.7, 1.8% organic carbon) was collected from a field in the surroundings of Kassel. It had been chosen because of its lack of any detectable organochlorine contaminants. The soil was air-dried and passed through a 2-mm sieve prior to use.

Photolysis experiments: 1g of single substances was dissolved in 1.5L degassed n-hexane and irradiated for 12 h with a low pressure Hg lamp (Vycor 250mA/500V, Fa. Gräntzel, Karlsruhe, Germany, emission maximum at 254nm). 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: Incubation with soil 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 of 50mL of sterile, distilled water to each flask, these were shaken, and the dissolved oxygen 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. In the case of technical toxaphene, the total of 800µg (10µg/g of soil) was added to the soil. 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 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 nitrogen, 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. 1 mL. These extracts were used directly for HRGC analysis.

Gas chromatography: All routine analysis was carried out on a Varian 3400 gas chromatograph (injection splitless/split 230°C); column 30m DB-5, fused silica, i.d. 0.25mm; thickness 0.32µm; EC detector 280°C; carrier gas N_2, 1mL/min; temperature program (column): 120°C (0min) – with 20°C/min to 200°C (0min) – with 5°C/min to 230°C (1min) – with 1.5°C/min to 250°C (15min).

Isolation of major metabolites: The isolation of the major metabolites formed from all components was carried out by liquid chromatography. For that purpose, all samples were combined, dried over sodium sulfate, and concentrated to about 2 ml. The extracts were applied to a silica gel column (column 100 x 1.2cm, 50g silica gel 60, 70-230 mesh; mobile phase: petroleum ether 45-65°C; flow rate ca. 1.5mL; retention volume ca. 500mL). During slow evaporation of the solvent, one of them crystallized as colorless needles.

Mass spectrometry: The MS experiments were carried out using a Hewlett-Packard (HP) 5890/5988A GC/MS-system (column: 25 m HP-5, i.d. 0.2 mm; film thickness 0.33 µm; carrier gas He, 1 ml/min; temperature program: 140 °C (3 min) to 250 °C (20 min) with 4 °C/min; splitless (0.5 min)/split injection; injection block and transfer line 280 °C). The temperature of the ion source was 100 °C for ECNI/MS, with methane as moderating gas. The emission current was ca. 200 µA. EI was performed at 70 eV and 200 °C ion source temperature. Mass spectra were recorded within a mass range of m/z 40-500.

FTIR spectroscopy: An HP 5890/5965 GC/FTIR-system was used to record the IR spectra (column: HP-5, i.d. 0.32 mm, film thickness 0.52 µm; carrier gas: He, 1 ml/min); temperature program 140 °C (3 min) to 250 °C (20 min) with 4 °C/min; transfer lines: 250 °C; light pipe: 280 °C).

^{1}H-NMR spectroscopy: Proton NMR spectra were recorded with a Bruker AC 400 spectrometer (nominal frequency 400.13 MHz) at 303 K in $CDCl_3$ (δ = 7.25 ppm) using a 5 mm broadband inverse geometry probe (90° : 8.5 µs). DQF-COSY and NOE-diffrence spectra (mixing time: 1 s) were performed using Bruker standard software, employing 90-degree pulses (8.5 µs).

X-ray analysis: The X-ray measurements were taken with an Enraf-Nonius CAD4 V5.0 four-circle diffractometer with a MoKα radiation of λ = 71.073 pm (graphite monochromator). A total of 2639 reflections were collected in the 2θ range of 3-25°.

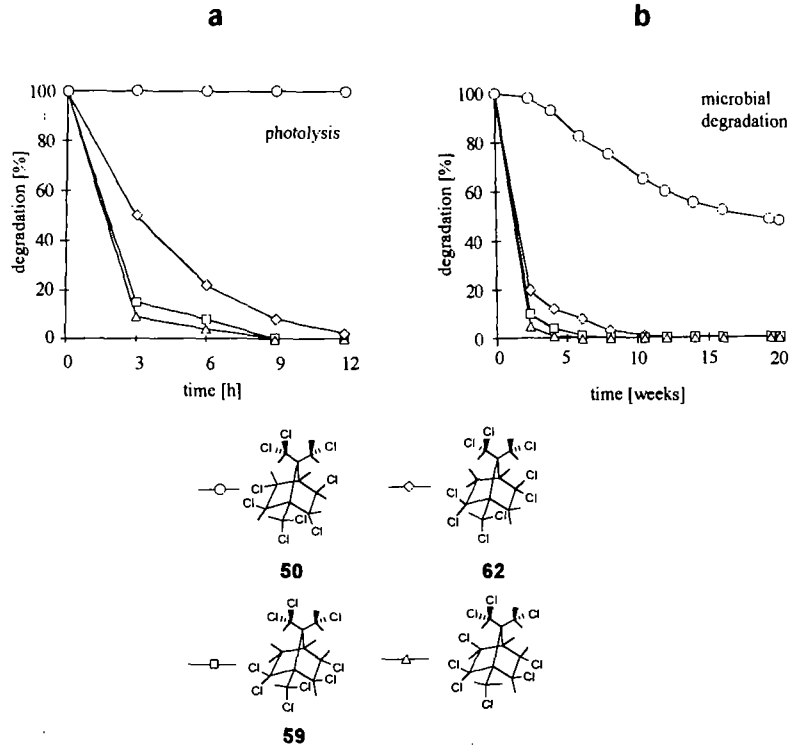

Fig. 2: Photolysis (a) and microbial degradation (b) of four single toxaphene components

Results

Irradiation of single toxaphene components results in dechlorination with reaction rates depending on the structure of the parent compound (Figure 2a). Generally, during irradiation in solvents, the bornane structure is preserved. Photolability seems to depend on the presence of a *geminal* dichloro group in C_2-position. The dechlorination rate is enhanced by an additional chlorine atom in C_3-position but not by a dichloro group in C_5-position. Components with only a single chlorine atom at each secondary ring atom in alternating orientation, such as Parlar numbers 26, 40, or 50, have been found to be extremely photostable (3).

The degradation of toxaphene in soil under aerobic conditions seems to proceed rather slowly, as residue analysis of aerated soil samples from a former toxaphene manufacturing plant exhibited chromatograms indistinguishable from those of technical toxaphene (3). Under anaerobic conditions, toxaphene is more easily degraded by microorganisms. HRGC-ECD analysis has shown that the highest chlorinated components (i. e. nona- and decachlorobornanes) as well as part of the octachlorobornanes are nearly completely degraded to lower chlorinated bornanes, while hexa- and heptachlorobornanes are accumulated. It is not clear whether the remaining hexa- and heptachloro compounds are produced by dechlorination of the higher chlorinated isomers or whether they are original constituents of the technical mixture. The stability pattern of single substances under these conditions resembles that under UV irradiation (Figure 2b). With technical toxaphene in a loamy soil under anaerobic conditions, a significant shift of the HRGC-ECD peak pattern of toxaphene towards lower retention times was observable after only one week. After three months, an additional decrease of detectable components could be seen, the main components being two hepta- and one hexachlorobornane. The peak

pattern became still simpler after 5 months. The main component then was 2-*exo*,3-*endo*,6-*exo*,8,9,10-hexachlorobornane, but hexachlorobornenes and a tetrachlorocamphene-2-one were also found.

Studies on the degradation of single toxaphene components in a loamy flooded soil have confirmed the dependence of microbial dechlorination rates on the chlorine substitution pattern of the six-membered ring. Components with only one chlorine atom at each C-atom in alternating orientation were highly persistent, while components with geminal dichloro groups on the ring were rather labile, especially when the dichloro group was localized at the C_2-atom (Figure 3). As in the case of photolysis, microbial degradation of all compounds investigated was enhanced by the presence of a second geminal dichloro group. The dead-end metabolite, 2-*exo*,3-*endo*,6-*exo*,8c,9b,10a-hexachlorobornane, has also been found in residue analysis of sediment and fish. Whether this is due to accumulation of this component or due to a high portion of components in technical toxaphene with a substitution pattern that leads to this product is unknown. Nevertheless, these results indicate that especially chlorobornanes with those substitution patterns shown in group 1 and 3 (Figure 3) are stable and can be expected in environmental samples whereas others with a substitution pattern such as group 2 will be absent after abiotic or biotic degradation.

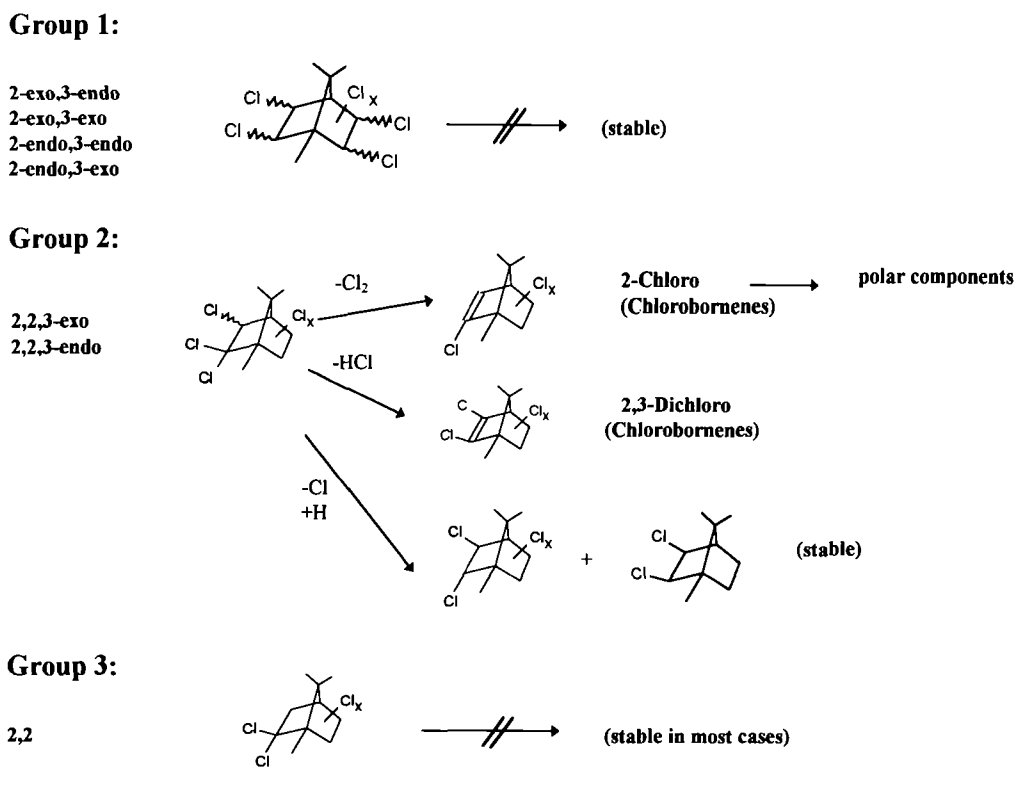

Fig. 3: Groups of chlorobornanes in technical toxaphene and their degradation pathways under varying environmental conditions

Literature

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