

MASS SPECTROMETRIC CHARACTERIZATION OF THE TWO MAIN TOXAPHENE CONGENERS IN BIOLOGICAL MATERIAL AND THEIR IDENTIFICATION IN THE TECHNICAL PRODUCT

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Toxaphene is a pesticide which has been used in large quantities (ca. $1.4 \cdot 10^6$ t totally until 1984). It consists of a complex mixture of several hundreds of polychlorinated norbornanes and related structures¹. Some congeners are highly accumulated in the food chain. Others are more easily excreted, or only their metabolites are enriched. This results in a significant difference between the congener pattern in higher organisms and of the technical product. One octa- and one nonachloronorbornane are especially bioconcentrated in marine mammals such as seals. At present no single congeners are commercially available as pure reference compounds. Toxaphene isomers are also very difficult to synthesize with a sufficient selectivity. Therefore, quantification of toxaphene has often been carried out by comparing the signal abundance of selected single compounds or isomer groups being present in both the technical standard and the sample extract. However, due to the strong metabolic transformation of the congener pattern, such approaches can only give semiquantitative results.

As a first approach to a real quantitative analysis method the two main toxaphene congeners in seal blubber were isolated in μg -quantities. Liquid extraction with hexane after matrix decomposition with different acids was used followed by several purification steps employing reversed phase HPLC². Mass spectrometry combined with high resolution gas chromatography (HRGC) was used as one of the methods for purity control and structure identification. Several strongly structure-related Retro-Diels-Alder-fragmentations were found which have not been reported before. They are of significant abundance in both mass spectra of the main octa- and nonachloro isomer in seal blubber. Only a limited number of congeners in the commercial product show these Retro-Diels-Alder fragments with a comparable abundance. Therefore, it was possible to identify both congeners as minor compounds in the technical mixture.

EXPERIMENTAL CONDITIONS

HRGC: 25m x 0.25 mm i.d., 0.1 μm RT_x5, 1 μl injected, splitless, 100 °C, 2 min, 100-180 °C, 20 °C/min, 180-280 °C, 4° C/min, 280 °C, 10 min.

Mass spectrometry: HP 5987, electron impact ionization: standard conditions, 70 eV; negative ion chemical ionization: CH₄, 0.5 torr source pressure; source temperature: 200° C.

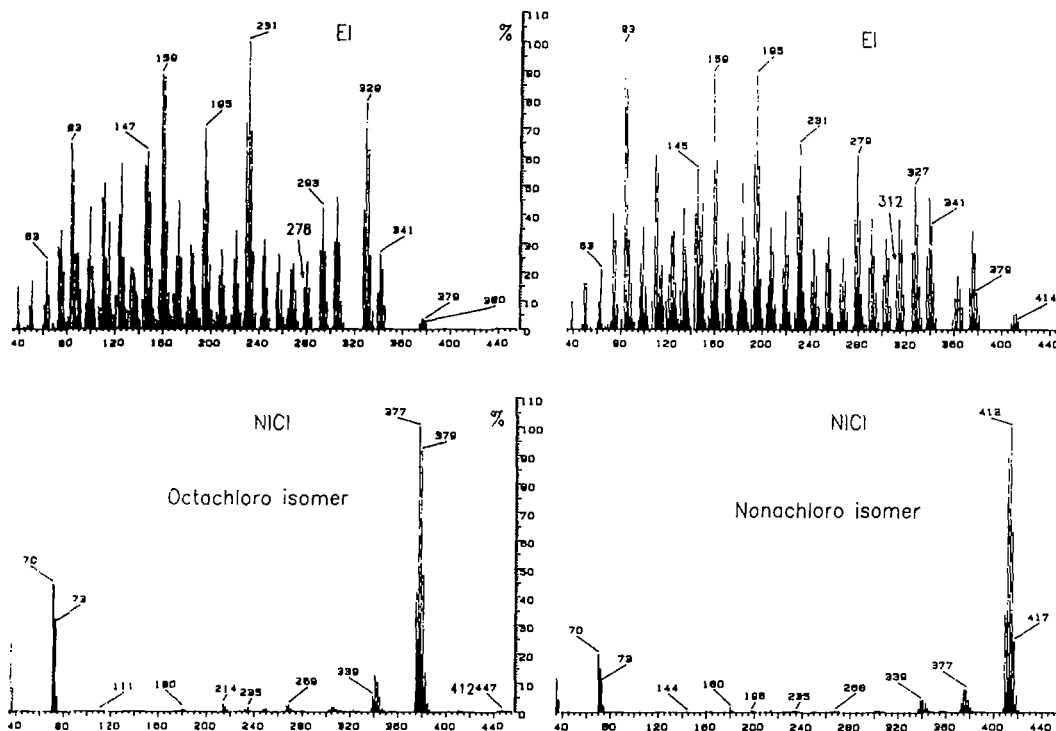


Figure 1: EI and NICI mass spectra of the two isolated toxaphene congeners

RESULTS AND DISCUSSION

Figure 1 shows both the EI and NICI mass spectra of the isolated octa- and nonachloro norbornane. As earlier discussed³, the EI-mass spectra of toxaphene congeners show an intensive fragmentation pattern caused by losses of Cl, Cl₂, HCl, CHCl₂ or CH₂Cl. Furthermore, the highly stable dichloro tropyliumcation (m/z 159) is formed as an abundant ion in the mass spectra of most congeners.

The NICI mass spectra show as expected (M-Cl)⁻ as base peak. They confirm the number of chlorine atoms which is in accordance with the earlier suggested structures. Furthermore, no interferences by co-eluting toxaphene congeners with different molecular weight or by other compounds (e.g. polychlorinated biphenyls) are observed.

An unusual even mass fragment pattern (corresponding to an odd electron species) which can only be formed by simultaneous cleavage of two bonds, was observed in the mass spectrum of the nonachloro congener. Figure 2 proposes as formation mechanism loss of HCl

(m/z 408) followed by a Retro-Diels-Alder reaction giving the substituted cyclopentadiene fragment $C_8H_6Cl_6^+$. The mass spectrum of the octachloro isomer shows an even mass Cl_5 -isotope cluster at m/z 278 but not the Cl_6 -pattern at m/z 312. It can be explained by a corresponding loss of HCl and a Retro-Diels-Alder reaction giving the cyclopentadiene fragment $C_8H_7Cl_5^+$ (see Figure 2). It is also present in the mass spectrum of the nonachloro isomer but disturbed by the ^{13}C -isotope cluster of $C_8H_6Cl_5^+$ (m/z 277). Both cyclopentadiene fragments allow to determine the number of Cl-atoms in the original norbornane structure as shown in Figure 2. Further work is on-going to combine this information with further substructure knowledges obtained by mass spectrometry and high resolution NMR.

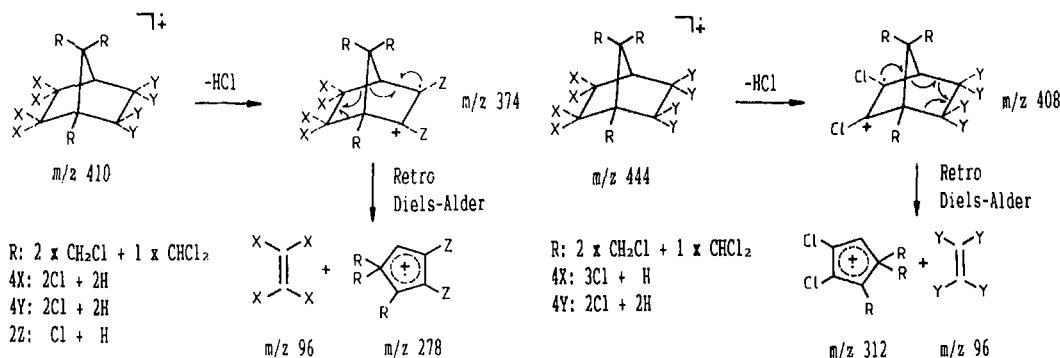


Figure 2: Retro-Diels-Alder reactions of the octa- and nonachloro congener.

As can be seen from Figure 3, only a limited number of octa- and nonachloro congeners show a rather abundant m/z 312 fragment pattern. The fragment cluster m/z 278 is somewhat more common. The fragmentation sequence m/z 408/312 was used to search for the nonachloro isomer in the technical mixture. It is present as a minor compound which is strongly overlapped by a slightly earlier eluting octachloro isomer with an abundant m/z 278 but no m/z 312 fragment in the mass spectrum (see mass fragmentograms in Figure 3). The interference corrected mass spectrum of the technical mixture was nearly identical with that of the isolated isomer. The strong interference with the very intensive octachloro isomer might lead to an erroneous quantitative result when the technical mixture is used as a reference. By using the fragment sequence m/z 374/278 the isolated octachloro isomer could also be identified as one of the less abundant congeners which partly co-elutes with another octachloro isomer.

CONCLUSIONS

The two main congeners in marine mammals have been identified as an octa- and nonachloro isomer, respectively.

Both congeners show even mass fragments in their mass spectra which are formed by Retro-Diels-Alder reactions. They are highly structure significant. First assignments of parts of their structure are given.

Both congeners were found in the technical mixture and are therefore no metabolites. However, they are minor constituents.

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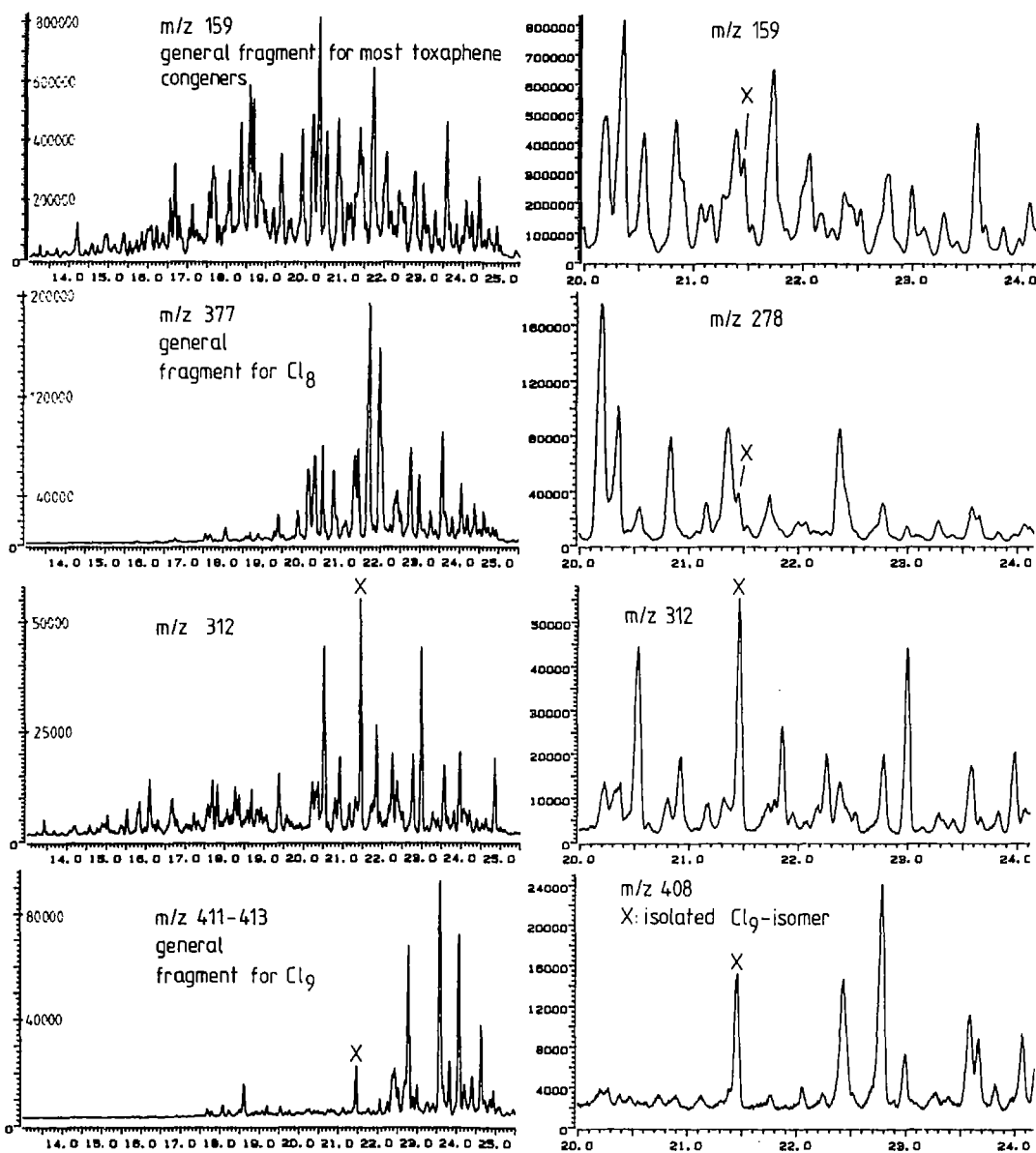


Figure 3: Presence of congeners with Retro-Diels-Alder fragments in the technical mixture and identification of the nonachloro isomer.

REFERENCES

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- 2 Vetter W, Oehme M, Luckas B., in preparation.
- 3 Saleh M A. Capillary gas chromatography-electron impact and chemical ionization mass spectrometry of Toxaphene. J. Agric. Food Chem. 1983;31:748-751.