DEVELOPMENTS IN ANALYSIS AND TOXICOLOGY OF TOXAPHENE COMPOUNDS

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Introduction

Over the last 50 years toxaphene has been produced and used as a pesticide extensively, mainly in cotton growing. Its production figures are comparable to those of polychlorinated biphenyls. The US Environmental Protection Agency banned it in 1982. However, in the early 1990s the presence of toxaphene in marine fish in Europe caused concern with regard to human health in relation with fish consumption (1). This short paper gives a brief overview of recent developments in the analytical and toxicological research on toxaphene.

Analysis

The complex mixture toxaphene is mostly determined as 'total toxaphene'. However, Carlin and Hoffman (2) showed that a large composition difference exists between the commercially available technical toxaphene standards. By using these standards concentrations between 19 and 131% of the true values were found. Furthermore, the detector response is, in general, not equal for all congeners. When the peak pattern of the sample under study does not resemble that of the standard, the true concentration may be over- or under-estimated. For example, marine mammals are known to metabolise toxaphene compounds to a great extent, whereas fish species change the toxaphene pattern less (3,4). The most relevant question may be, what does a total concentration imply when the composition is unknown? Actually, it is only useful when it is related to toxicological risk assessment. Therefore, the present trend is a congener-specific approach, which, despite their limitations (*cf.* below), offers more information. In Germany three indicator congeners were selected to monitor toxaphene residues in foodstuffs (5).

Nomenclature. Besides simple indications (e.g., Tox Ac, T12, Parlar 50), several authors have proposed systematic nomenclature systems for toxaphene compounds (6-10).The nomenclature by Wester et al. (9,10) is recommended since structural information can directly be deduced from it without the use of long lists or complex calculations. Every digit in the code represents the chlorine substitution of the carbon number. The first part of the code reflects the conformation of the six-membered ring (C2-C6) (0: none; 1: endo; 2: exo; 3: both), and the second part gives the number of chlorine atoms attached to C8, C9 and C10, respectively. Here, the Parlar no. will also be given when available.

Extraction and clean-up. In the literature little attention has been paid to the efficiency of extraction procedures. It is supposed that extraction procedures which are suitable for related compounds (PCBs, DDT, chlordanes) can be used for toxaphene compounds as well, because of their similar lipophilic and structural characteristics (11). Several stationary phases have been used for clean-up prior to analysis including Florisil, aluminium oxide and reversed phase C8 and C18 (12,13). In addition GPC can be used to remove lipids from the sample (5). Silica gel pre-separation of technical toxaphene can be used to obtain a separation over a wide range of compounds (14). Silica gel fractionation with 2.5 g (15) or 1.0 g (5) SiO_2 columns result in a separation between toxaphene and interfering compounds such as PCBs. However, compounds like B[12012]-(202) (Parlar #26) may elute partly in both fractions. Krock et al (16) improved this method by using 8.0 g activated silica. The first fraction (48 ml hexane) contains the PCBs and the toxaphene compounds are eluted with 50 ml hexane/toluene (65:35, v/v).

Gas chromatography. Alder et al. state that the injector temperature should not exceed 240°C because severe decomposition of compounds may take place (17). It should be mentioned here that care should be taken with active sites in the liner and the injector. Because of variable injector geometry it is recommended to verify the optimal temperature by a series of simple tests. Vetter et al. (18) reported the use of pressure pulse injection (PPI) at 225°C, which resulted in up to 4 times higher response factors compared with the widely used conventional splitless injection. One advantage of this technique is that the residence time of the compounds in the detector is short, and, therefore, the chance of degradation smaller.

In most cases the toxaphene compounds are separated on a relative non-polar DB-5 type stationary phase with lengths of 30 to 60 m and diameters of 0.25 and 0.32 mm I.D.. Krock et al. obtained a relatively good separation using a non-polar CP-Sil 2 stationary phase (polarity comparable to squalene) (19). On that column the same elution order as on the slightly more polar DB-5 column was found (20). The authors used the CP-Sil 2 successfully up to a temperature as high as 290°C, although the supplier advised a maximum temperature of 200°C. By comparing the retention times of B[12012]-(**20**2) with B[12012]-(**11**2) (Parlar #40) and B[30030]-(**02**2) (Parlar #38) with B[30030]-(**11**2) (Parlar #51) it was suggested that compounds with one chlorine on both C8 and C9 elute much earlier from this phase than compounds with two chlorines on one of these carbons. Furthermore, by comparing B[**12**0**12**]-(202) with B[**30**0**30**]-(202) and B[**12**0**12**]-(112) with B[**30**0**30**]-(112), it was found that compounds with an alternating endo-exo substitution elute earlier than compounds with two chlorines at both C2 and C5 (20). More polar phases such as DX-4 or FFAP can also be used (15). However, care should be taken because certain compounds may decompose on these phases. Baycan-Keller and Oehme (21) found degradation of B[32012]-(111), B[30012]-(211), B[30012]-(121), B[30012]-(212), B[30030]-(122), B[12012]-(212), B[32030]-(112) (Parlars #39, 42a, 42b, 56, 62, 50, 58) on the Rtx-2330 phase. 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 (17) which was not observed in the study of de Boer et al.

(15). This can partly be due to the fact that by the latter 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 only 220°C.

Detection. Negative chemical ionisation (NCI) MS shows a comparable profile as ECD. Xu et al. found identical results with these two techniques for the quantification of individual chlorobornanes in fish samples (22). Both techniques are less sensitive to low chlorinated congeners. The NCI technique is most widely used for MS detection of toxaphene. Often the M^- and $(M\text{-}Cl)^-$ ions are monitored (23) and good linearity over four orders of magnitude for five chlorinated bornane congeners has been obtained (24). Vetter et al. tentatively found that a 2,2,5,5-substitution of chlorobornane congeners had a negative effect on the NCI/MS response (20). The electron impact (EI)-mode is more sensitive to lower chlorinated congeners. Additionally 25 peaks from lower chlorinated compounds have been found with EI compared to NCI (14). Andrews et al. (25) used selected ion monitoring (SIM) at m/z 159 and 161 in the EI-mode to obtain a single 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 (26), but does not offer the possibility of structure elucidation.

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, but at a different time. This reduces loss of precursor ions and hence provides better sensitivity. The major ion produced by the m/z=159 ion is a fragment at m/z=125 amu. However, PCBs and some organochlorine compounds also produce this m/z in the MS/MS mode. Therefore, the ion at m/z=89 amu (totally dechlorinated toxaphene), which originates from the m/z=125 ion is more useful for quantification of toxaphene congeners (27). However, also with this method the response factor for individual congeners still varies considerable.

Alder and Vieth (5) determined the total toxaphene concentration in a standard reference sample (SRM 1588) on the basis of three indicator congeners, using ECD and NCI/MS, and concluded that the large difference in response factors of the NCI technique gives a considerable positive bias to the results. To obtain precise and comparable data, the use of indicator compounds on the basis of which the total concentration is calculated was recommended (5).

Enantiomers. A significant deviation from the racemic value is found, this suggests that one enantiomer is subject to a specific metabolic transformation (28). The comparison of the enantiomer ratios of different congeners in combination with their molecular structures can help to gain insight in the metabolism of these compounds. It has been shown that phases based on heptakis(2,3,6-O-tert.butyldimethylsilyl)-ß-cyclodextrins (TBDM-CD) are particularly suitable for the separation of polychlorinated bornane enantiomers (28-30). Unfortunately, this stationary phase is not very well defined and batch-to-batch differences have been observed (31). Baycan-Keller and Oehme (32) showed that a temperature ramp of 1°C resulted in much better separations compared to a 10°C ramp. This was also found by de Geus et al. (33) who used this phase in a multidimensional set-up to determine enantiomer ratios in wild-life samples. Vetter et al. (34) isolated the compound B[21020]-(022) from Melipax and found an enantiomer ratio of 1.26±0.03. This shows the risk of drawing conclusions on the assumption that the technical mixture is a racemate.

Interlaboratory study. Andrews found that in many laboratories only about 15-30% of the 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 (35). In a German round

robin recoveries of 77 to 100% were found for three compounds in a fatty matrix. The between laboratory agreement was good (repeatability 21±4%) (36). In a recent QUASIMEME laboratory performance study with 4 toxaphene congeners in standard solutions most of the 15 participants reported satisfactory results (37). An European research project called 'Investigation into the monitoring, analysis and toxicity of toxaphene' (MATT), started in 1997.

Toxicology

Although toxaphene has been shown to be extremely toxic to fish with mean acute toxicity values of 0.07 and 1.6 μg l^{-1} for marine and freshwater fish respectively, data on acute and chronic toxicity of toxaphene for aquatic organisms are not overwhelming (38). Chronic toxic effects of toxaphene are associated with inhibition of growth, reduced reproduction and backbone abnormalities. The effect of application of toxaphene as a piscicide was recently studied on a mesotrophic and eutrophic lake in central Alberta, Canada. The lake was subsequently restocked with a non-native fish species and the toxaphene effect was was measured on total chironomids, *Chaoborus* spp., and planktonic Cladocera (39). High concentrations of toxaphene resulted in a decrease in abundance of planktonic Cladocreans and dominance changes from small- to large-bodied types. The long-term changes in invertebrates of both lakes was most probably a result of the manipulation of fish communities rather then residual toxicity. Under natural living conditions species differ in elimination rates of toxaphene and elimination of two different chlorobornane components (B[12-12]-(202) and B[12012]-(212)) of toxaphene is different within a given species (40). Keller (41) observed that addition of sediment to the test chambers reduced the acute toxicity of toxaphene to freshwater mussels (*Anodonta imbecilic*) drastically. The application of toxaphene has also its effect on the reproductive success of aquatic species. Toxaphene exposure produces a dose-related decrease of the percentage of oviposition of female zebrafish (42). Hence, it was concluded that dietary exposure of zebrafish to toxaphene affects the reproductive process.

Legislation

Ideally, toxicity should play a major role in the selection of indicator compounds. Unfortunately, until now not much is known about acute and chronic toxicity of individual congeners to mammals. Occurrence determines in combination with toxicity the whether a compound is important or not, and is therefore another important selection criteria. Stereochemistry commonly plays an important role because the biological activity of enantiomers varies. In addition to these parameters the analytical convenience is important. The compounds should be detectable without interference of other compounds when common extraction, clean-up and separation/detection procedures are used. Next to that the compounds must be commercially available (5). In practice, the availability of standards and the analytical convenience dictate the choice of compounds, similar to the situation with PCBs. The concentrations of B[12012]-(202), B[12012]-(202) and B[30030]-(122) in fish are in the 0.05 - 0.08 mg kg^{-1} (fat basis) range and represent about 50% of the total toxaphene ECD response. Therefore, Alder and Vieth (5) suggested using them as indicator compounds. Xu et al. (24) proposed a second compound B[30032]-(122) (Parlar #69) for this purpose. However, this compound was found to degrade easily in the detector and is only present in minor amounts in technical formulations. The validated method for three indicator compounds (5,36) is applied in many German laboratories. In 1997 the German 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 (43), and for all other food of animal origin at 0.1 mg kg^{-1} on the basis of total toxaphene.

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