Toxaphene, a different environmental problem

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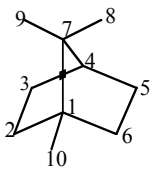
Production and toxic properities

This article give a condensed survey about the pesticide toxaphene concerning properties, environmental behaviour, toxicity, analysis and presence in the environment. It is partly based on a comprehensive review recently published in [1]. Therefore, only a limited number of literature citations is given here, and reference is made to ref. [1] in all other cases. Toxaphene was introduced by Hercules Powder Inc. in 1945. It is particularly efficient against pests in cotton, soybeans, peanuts and maize and not toxic for bees. Over the years it has become one of the most widely applied pesticides. Its estimated accumulated production of up to 1.3 10^6 t [2] exceeds those of e.g. DDT and hexachlorocyclohexanes. Due to its toxic properties described below, its use was banned in the U.S.A. in 1986 and during this decade in many other countries as well. There are indications of continuous usage in e.g. Mexico, the former Sovjet Union, Africa and South America [3]. Toxaphene (other trade names are Strobane T-90, Phenatox, Melipax etc.) consists of a complex mixtures of hundreds of mainly hexa- to nonachlorobornanes and is produced by chlorination of α-pinene or camphene including a Wagner-Meerwein rearrangement. The carbon skeleton structure of toxaphene is given below. However, most of the theoretically possible 16640 congeners (most are chiral in addition) are not formed during synthesis.

The large number of congeners present in the technical products and the lengthy naming according to IUPAC (e.g. 2-*endo*,3-*exo*,5-*endo*,6-*exo*,8,8,9,10,10-nonachlorobornane) has led to different approaches to use numeric or alphanumeric abbreviations. The existence of different assignment systems and incorrect numbering of the C-atoms of the carbon skeleton has caused considerable confusions in the past [1]. At present, it seems that the numbering according to Parlar (based on the elution order of about 70 isolated congeners on a rather non-polar stationary phase) and the complete structure related systematic numbering by Andrews and Vetter (AV-numbering) are most frequently used. The first one has the disadvantage that other congeners eluting in between cannot be labelled any longer. A detailed survey about nomenclature and its problems is given in [1]. It also contains a conversion table facilitating the identification of the structure assigned by different acronyms. Here, the Parlar and AV-numbering is used.

It was quite early detected that technical toxaphene is mutagenic [4]. The properties of some isolated congeners were investigated in the 1970s. It was found that some of them such as Toxicant A_c (P-50, B9-1679) were four times more toxic to insects than the technical product [5]. In 1995, a Nordic risk assessment of toxaphene exposure was established. It concluded that there was evidence for carcinogenic effects in animals at concentration levels around 100 ppm in the diet. The extrapolated doses assuming that toxaphene is a genotoxic carcinogen, were estimated to 0.05-1 µg kg^{-1} day^{-1} for a risk level of 10^{-5}. Supposing indirect mechanisms, a no observed adverse effect level of 0.2 mg kg^{-1} day^{-1} was established from a 13 weeks long dog study. Neurobehavioural and other effects observed in developmental studies of rats, however, indicated

a tolerable daily intake (TDI, assuming a safety factor of 100) could be around 1 μg/kg body weight. Toxaphene is listed as no. 32 on the CERCLA priority list in the U.S.A.



Carbon skeleton and atom numbering of bornane

The commercial availability of single congeners about 5 years ago and the generally increasing concern due to the ubiquitous presence of toxaphene in the environment, resulted in further toxicological studies. Steinberg et al. found mutagenic properties for the technical product with *Salmonella typhmurium* TA98 and TA100 but not for the tested single congeners [6]. Genotoxicity was tested with the Mutatox® test and activity was found for the technical pesticides in the direct assay [1]. Recently, synergistic embryotoxic effects on rat embryos were observed for the two most abundant congeners in human blood (P-26 and P-50) [7]. The estrogenic activity of toxaphene is still controversial [1]. Based on the knowledge available so far, the TDI for toxaphene was set to 0.2 μg kg^{-1} body weight day^{-1} by Health Canada [8].

Toxaphene has physical properties which makes it very mobile and suitable for a global dispersion via e.g. atmospheric long range transport (water solubility around 0.5 mg/L, vapour pressure 4 10^{-6} mm Hg, Henry's law constant 1.7 10^{-6} atm m^3 mol^{-1} [1]). The estimated log K_{OW} values of the higher chlorinated congeners are around 6-8 indicating a substantial bioaccumulation potential [1].

Analytical methodology

Despite the huge consumption, toxaphene can nearly be called the forgotten chlorinated pesticide mainly due insufficient and complex analytical techniques needed for detection. At the time where PCB and other chlorinated pesticides were already analysed routinely, toxaphene was only occasionally quantified mainly on the basis of the technical product though the congeners distribution is quite different in e.g. marine biota and sediments. Missing reference substances, a more complex gas chromatographic separation and a considerably lower thermal stability compared to chlorinated aromatic compounds are some further complications. The commercial availability of some of the most abundant congeners led to a revived interest in toxaphenes. This resulted in the situation that first violations of the German maximum residue limit for total toxaphene of 0.4 mg/kg lipid in fish introduced in 1971, were reported as late as in 1993 (at present: sum of P-26, 50, 62: ≤0.1 mg/g fresh weight). The main reason was not an increase of levels but the development of more suitable analytical techniques allowing the quantification of single congeners. Several intercalibrations have shown that such methods give the best performance [1].

Sample clean-up procedures for toxaphene are similar to those used for other chlorinated pesticides. Toxaphenes elute in the same fraction as DDT and chlordane. However, the extract

should be free for PCBs to avoid interferences by co-elutions. This can be best achieved by optimised column chromatography on silica [1, 9] (eventually preceded by nitration of the PCBs). For other methods using aluminium oxide or florisil always a few PCBs are still present in the toxaphene fraction or vice versa [1].

Compared to aromatic chlorinated compounds, toxaphenes dehydrochlorinate much more easily on hot and/or active surfaces. Therefore on-column injection or splitless transfer at temperatures in the range of 160 to <240 °C have been proposed as well as accelerated sample transfer by pressure-pulsed injection [1]. Degradation can also take place in retention gaps or on stationary phases. In general, more polar phases such as polyethylene glycols or polycyanopropyl phases show a strong degradation of mainly congeners with geminal chlorine positions at C2 of the six ring. So far, best separations were obtained on Carboran (HT-5) or methylbiphenylpolysiloxane (Optima δ3) phases. Multidimensional technique have also been applied to minimise the risk of co-elutions. This technique is particularly useful when separating toxaphenes simultaneously into isomers and enantiomers. This is important when studying biological degradation and the fate of these compounds in the environment. Unfortunately, the best enantioselective stationary phases available at present (heptakis(2,3,6-tri-O-t-butyl-dimethylsilyl)- and (2,3-di-O-methyl-6-O-t-butyl-dimethylsilyl)-β-cyclodextrin) show a high column-to-column variability. Another, simpler technique to minimise signal overlap is the use of tandem columns to disperse the obtained pairs of enantiomers more evenly over the total retention time range.

Detection is normally performed by electron capture detector or negative ion chemical ionisation (NICI) mass spectrometry (MS). The advantage of the first technique are much more uniform response factors for congeners with the same degree of chlorination compared to NICI where differences of up to one order of magnitude are observed. However, ECD requires a complete removal of PCBs. They also might disturb NICI-MS when a small leak is present resulting in the formation of $[M\text{-}Cl+O]^-$ PCB fragments which interfere with quantification masses of toxaphenes. High resolution MS in the NICI mode or registration of the EI fragment m/z 158.9769 have also been applied. The latter does not allow to determine the degree of chlorination. Recently, it could be shown that EI-MS/MS of the fragment ion m/z 301 in an ion trap give the same sensitivity as low resolution NICI-MS for some important congeners [10].

Since pure reference compounds are commercially available for many of the most important congeners and more will follow, quantification should now be carried out congener specific. Depending on the type of matrix, the most relevant congeners have to be selected. For example, in marine biota the eight toxaphenes P-26, P-40, P41, P-44, P-50, P-62 as well as B7-1453 and B8-1412 will account for 60 to >90 % of the total toxaphene amount. In sediments the hexa- and heptachloro congeners B6-923 and B7-1001 are most abundant while octa and nonachloro compounds are hardly detectable due to degradation. However, some regulations in North America require also the determination of total toxaphene based on a complete quantification of all signals present in a sample extract. A further problem is a suitable internal standard having the same properties as toxaphene. Isotope-labelled compounds are not yet available. 4,5-dichlorochlordene as well as the chlordane MC8 have been proposed as suitable compounds. Both are not found in the environment.

In connection with the revision of the German maximum allowable level of toxaphene in food, the toxaphene levels in a broad selection of fishes from the North Atlantic were determined. As can be seen from Table 1, the sum concentrations for P-26/P-50/P62 was in the range of 10-1000 μg/kg on lipid base [11]. In raw fish oils and fish liver high μg levels per kg lipid were

present [12,13]. Comparable concentration were also present in e.g. ringed seal blubber. The found toxaphene concentrations are comparable to those for PCBs.

Table 1: Selected toxaphene concentrations in marine biota from the North Atlantic

Species	Concentration range [µg/kg lipid]	Literature
Herring	131 (7.2-583, n=32)[a]	[11]
Greenland halibut	238 (124-500, n=15)[a]	[11]
Mackerel	46 (15-82, n=7)[a]	[11]
Redfish	343 (114-790, n=18)[a]	[11]
Farmed Salmon	85 (31-133, n=10)[a]	[11]
Raw fish oils	397 (38-1504)[b]	[13]
Ringed seal blubber (Arctic)	190-470[c]	[15]

[a] Sum of P-26, P-50 and P-62; [b] Sum of 6 congeners; [c] "Total toxaphene"

For the Inuit population at Baffin Island, the toxaphene intake via traditional food exceeded the TDI by nearly one order of magnitude [15]. In human milk total toxaphene levels were in the range of 0.1-0.5 mg/kg fat for some European countries. In milk from Northern Quebec, Canada, an average concentration of 0.3 mg/kg lipid was present. Much higher values were determined for Nicaraguan women (0.3-7.6 mg/kg). Only very few data are available for blood indicating a range of 2-200 pg/l whole blood.

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