Investigation of two technical toxaphene products by using isotope ratio mass spectrometry

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Introduction

Organochlorine compounds have been used in high quantities throughout the past 60 years [1]. Being long-lived in the environment and toxic to humans and wildlife, some of them were classified as persistent organic pollutants (POPs). One of the POPs of special concern is toxaphene which is produced by the chlorination of the natural product camphene (or α-pinene). The technical products consist of several hundred compounds, mainly of chlorobornanes with an average number of eight chlorine substituents [2,3]. Toxaphene has been produced in high quantities in different parts of the world [2,3]. Even though the use has been discontinued during the last two decades, there are still several ecosystems which are heavily contaminated with this chloropesticide. Due to the huge variety of the technical products accompanied with a severe change of composition in the environment, analytical tracing back of toxaphene residues to a specific product has not yet been achieved. One of the potential analytical tools for distinguishing substances that differ only in their way of production is the determination of ratios of stable isotopes ($^{13}C/^{12}C$; $^{2}H/^{1}H$; $^{15}N/^{14}N$) [4,5]. Since the synthesis of toxaphene is starting from natural compounds obtained from different continents, the technical products could have different ratios of stable isotopes. In this study, we investigated the $^{13}C/^{12}C$ ratio of two former major toxaphene products (**Figure 1**).

Figure 1: Photos of the technical products analyzed. Left: US-American product Toxaphene (Hercules Inc.), center: Former East-German product Melipax (powder, 10% content of active pesticide, right: Melipax waxy oil extracted from powder.

Material and Methods

Samples. Two 500g-units of the East-German technical product Melipax (Fahlberg-List, Germany) in original packing (**Figure 1b**) were found in a garden shed in Jena (Germany). The powder contained 10% of the active pesticide. The product was manufactured in 1979 (charge number “0008.10.79”.). Ironically, it was claimed on the packing that the product will be active for 30 months only. Upon extraction with *n*-hexane, the known amber waxy product was obtained (**Figure 1c**). 5 g of amber waxy Hercules Toxaphene (**Figure 1a**) manufactured in 1978 was obtained from L. Alder (BgVV, Berlin, Germany). The lot number printed on the label was “x-16189-9”, with the additional comment “Spectrum Typical, Chlorine 68.9%”. 50 mg of each of the products was accurately weighed and used for analysis. A sample of (+)-camphene (technical grade, purity 80%, cat. No. C30-1) was from Aldrich (Taufkirchen, Germany).

GC analysis of the samples. For GC/ECD analysis, samples were diluted to concentrations of about 10 ng/µL. Analysis was performed with a dual-column-GC/ECD system described elsewhere [6].

Carbon isotope ratio analysis of the samples. At MPI Jena, about 0.5 mg of sample material was combusted in an EA 1110 Elemental Analyzer (ThermoQuest) using tin capsules (oxidation furnace setting 1020 °C; reduction furnace setting 650 °C). CO_2 was separated by gas chromatography and analyzed for ^{13}C in a Delta^{Plus}XL isotope ratio mass spectrometer (ThermoFinnigan). δ^{13}C values were expressed in per mil (‰) relative to the international standard Vienna PeeDee belemnite (VPDB) [7]. Additional measurements were performed at the University of Hohenheim with a Delta^{Plus}XP IRMS (ThermoFinnigan). Parameters were identical with those reported above except that the δ^{13}C values were expressed in per mil (‰) relative to lab-intern standard provided by the supplier of the instrument. For these reason the absolute values varied due to the obvious non-uniformal calibration to the international VPDB scale.

Results and Discussion

Figure 2 shows the GC/ECD chromatograms of the two technical products manufactured at the end of the 1970s, respectively.

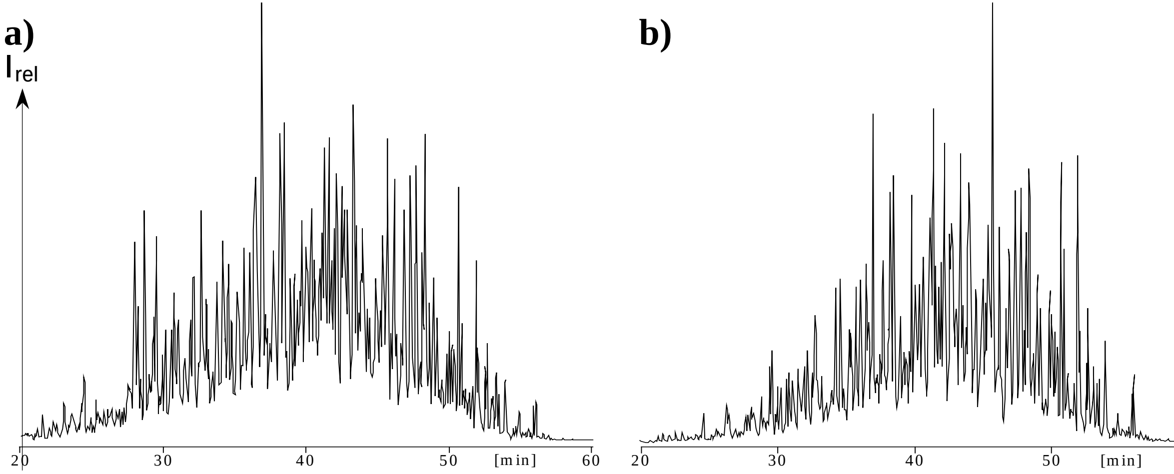

Figure 2: GC/ECD chromatograms of (a) Melipax® and (b) Toxaphene®

Both chromatograms exhibit the known complexity although the peak profiles were slightly different. The former East-German product Melipax was found in a garden shed where it was most likely stored for ~20 years. Since we cannot exclude that the sample was exposed to direct sunbeam and varied temperatures, it is possible that some CTT degradation occurred during this period. According to present knowledge, loss of carbon or change in the ratio of stable carbon isotopes is unlikely in this process. Therefore, there is no evidence that carbon isotope ratios have changed during the period of storage.

Since quality control in isotope ratio mass spectrometry in combination with a gas chromatograph requires an interference-free elution of the studied compounds (compound-specific isotope analysis), we could not apply this technique, and both products were analyzed as a whole without GC separation. For this reason, same amounts of both pure waxy Toxaphene and Melipax were combusted to CO_2. **Table 1** lists results of triple IRMS determinations of the two technical products.

Table 1: δ^{13}C values* of the East-German product Melipax and the US product Toxaphene

SAMPLE	**#1**	**#2**	**#3**	**Mean**	**STD**
Melipax	-27.70	-27.45	-27.59	**-27.58**	0.13
Toxaphene	-28.23	-28.43	-28.41	**-28.36**	0.11
ES1 (-29.81)**				**-29.81*****	0.08
ES2 (-40.14)**				**-40.13******	0.13

* $\delta^{13}C\ [‰] = ([^{13}C/^{12}C]_{sample} / [^{13}C/^{12}C]_{V\text{-}PDB} - 1) \times 1000$. *V-PDB is used as standard according to international conventions*

** *ES1 and ES2 are external standards (acetanilide and caffeine) measured in parallel for method validation. The reference values are given in parentheses.*

*** *n = 6;*

**** *n = 2*

Two external standards (ES1 and ES2 in **Table 1**) were used for calibration of the IRMS system. As can be seen from the results, the mean of the external standards matched the real value with high accuracy. Furthermore, the precision of the replicates was 0.32 % and 0.27%, respectively. The accuracy and precision was adequate for the investigation of the toxaphene standards, which only showed a slightly higher analytical error (0.39 and 0.47%, respectively). This confirms the good reproducibility and accuracy of the measurements. However, the δ^{13}C-values (for definition see footnote in **Table 1**) of Melipax and Toxaphene were different, and toxaphene was significantly depleted in its ^{13}C content relative to Melipax (variation: 2.8%).

Based three replicates, respectively, and 4 degrees of freedom the significance of the difference between Melipax and Toxaphene can be estimated to be $0.001 < p < 0.01$. The accuracy of the measurements demonstrated above clearly proves that the two products can be distinguished by their δ^{13}C-values.

The same samples were also analyzed on a second instrument at different location (see Experimental section). Both products showed the same variation in their δ^{13}C-values ($\Delta_{(\delta 13C,Toxaphene-\delta 13C,Melipax)}$ = 0.78 vs. 0.79) and similar standard deviation during repetitive measurements (details not shown). The δ^{13}C-values were in the typical range of C3-plants. In addition, a sample of technical camphene (not identical with the material used in the toxaphene syntheses) was 2.47 ‰ lighter in ^{13}C than toxaphene, and was thus also significantly different to the two toxaphene products. It is known that the starting material of the toxaphene synthesis, camphene, shows regionally different $\delta^{13}C$ values. Here it is important to note that toxaphene is the only important class of organohalogen compounds whose synthesis starts from a natural product. Unfortunately, the samples investigated were the only available batches of the products which have been produced for several decades. However, authentication controls in food by IRMS are accepted official methods in food monitoring. Such measurements are only possible if the $\delta^{13}C$ values are stable over a longer period. It is therefore realistic to assume that the carbon isotope ratios of individual products do not vary in the same content as the products from different manufacturers do.

We also attempted to determine the $\delta^{2}H$ values of the compounds. However, no sharp signal was detected which may be explained by the formation of HCl instead of H_2 upon pyrolysis.

Recently, Jarman et al.[8] analyzed PCB products from different manufacturers and found similar $\delta^{13}C$ values and variations as in this study for products from different sources. Furthermore, the authors observed increased ^{13}C depletion with increasing degree of chlorination of PCB congeners [8]. Although PCBs and toxaphene differ significantly (aromatic hydrocarbon backbone of an industrial chemical from oil production versus alicyclic hydrocarbon backbone of a natural product), we cannot exclude that such homolog-specific (minor) variation will also occur with toxaphene. However, the environmentally most relevant toxaphene compounds vary only little in their degree of chlorination. E.g. the most relevant CTTs in sediment and soils are hexa- and heptachlorinated compounds while the dominating congeners in higher organisms are octa- and nonachlorobornanes, respectively. Therefore, it is likely that calibration of the IRMS can be performed with the help of a few individual CTTs isolated from the two technical products.

The $\delta^{13}C$ results in this study indicate that it may be possible to distinguish different commercial toxaphene products by use of IRMS. For investigation of individual compounds, compound-specific isotope analysis has to be performed with the help of by GC/IRMS. This requires undisturbed peaks with a concentration of ~50 ng, which is a realistic frame for environmental samples with high toxaphene burden.

References

1. Bernes, C. (1998) Persistent Organic Pollutants. A Swedish view of an international problem. Swedish Environmental Agency, Monitor 16
2. Saleh, M. A. (1991) Rev. Environ. Contam. Toxicol. 118, 1-85
3. Vetter, W. and Oehme, M. (2000) Toxaphene. Analysis and Environmental Fate of Congeners. In: The Handbook of Environmental Chemistry, Vol. 3, Part K, New Types of Persistent Halogenated Compounds; J. Paasivirta, ed., Springer, pp. 237-287
4. de Groot, P. A., ed. (2002) The Handbook of Stable Isotope Analytical Techniques. Elsevier.
5. Schmidt, H.-L. and Gleixner, G. (1998) Isotopic patterns in natural compounds origin and importance in authenticity analysis. In: Natural product analysis, Schreier, P., Herderich, M., Humpf, H.-U. and Schwab, W. eds., Vieweg, Brauschweig, Germany, pp. 271-280
6. Vetter, W., Klobes, U. and Luckas, B. (2001) Chemosphere 43, 611-621
7. Werner, R. A. and Brand, W. A. (2001) Rapid Comm. Mass Spectrom. 15, 501-519
8. Jarman, W. A., Hilkert, A., Bacon, C., Collister, J. W., Ballschmiter, K. and Riseborough, R. W. (1998) Environ. Sci. Technol. 32, 833-836