COMPARATIVE ANALYSIS OF TOXAPHENE COMPOUNDS USING SPLIT/SPLITLESS AND ON-COLUMN INJECTION IN CAPILLARY GAS CHROMATOGRAPHY

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Introduction

Technical toxaphene is a complex pesticide mixture comprised of several hundred closely related compounds, including chlorobornanes and chlorocamphenes. Toxaphene analysis is difficult and challenging. Generally, capillary gas chromatography (GC) is used in combination with electron-capture, negative ionization mass spectrometry (ECNI-MS) or the electron-capture detector (ECD). In trace-level GC analyses, split/splitless injection (SSL), cold on-column injection (OC), or a hybrid technique, such as programmed temperature vaporization injection, are used [1-3]. In SSL, sample aliquots are injected into a heated vaporizer (inlet) and then transferred by the carrier gas into the analytical column. Because the temperature required to achieve efficient vaporization of high-boiling analytes in the injector is high, these conditions are detrimental to thermally unstable compounds. SSL also results in incomplete transfer of high-boiling compounds, which leads to discrimination between low- and high-boiling components [1, 4, 5]. In OC, sample aliquots are injected directly into the analytical column (or retention gap) at low temperature, and the analytes are subjected to less thermal stress. It is generally accepted that OC is more suitable for the analysis of thermally unstable compounds [1, 4].

In this study, we analyzed a technical toxaphene mixture and a reference mixture using both SSL and OC, under otherwise identical ECNI-MS conditions. Our SSL conditions were as described in a widely used analytical protocol by Swackhamer *et al.* [6], and our OC conditions were based on refs 7 and 8. In this study, we compare each technique. The two main disadvantages of SSL were thermal degradation and discrimination of some toxaphene components. OC, on the other hand, results in far less degradation and discrimination of these toxaphene components. We also discuss the common structural features of the congeners most affected by thermal degradation during SSL.

Materials and Methods

A Finnigan Voyager quadrupole mass spectrometer was used for analyte detection. The ion source was operated in the ECNI mode (70 eV; 120°C) using methane as the buffer gas. Analyses were carried out using the selected-ion-monitoring (SIM) mode, monitoring the $(M-Cl)^-$ anions of the chlorobornanes and related compounds at m/z 307, 309, 343, 345, 377, 379, 413, 415, 445, and 447. We also monitored m/z 342 (M^-) for the hexachloro compounds, m/z 406 $(M + 2)^-$ for $^{13}C_{12}$-PCB 180, and m/z 35 for chloride (Cl^-). Analyses were carried out on a Carlo Erba 8000 Top GC, equipped with SSL and OC injectors, and a 30-m DB5-MS capillary (0.25 mm i.d.) column. The column was temperature programmed under conditions as previously reported [7].

In SSL, the injector temperature was set at 285°C and the splitless time was set at 1.9 min, as suggested by Swackhamer *et al.* [6]. In OC, injections were made into the column at 80°C. Injection volumes were 1-2 μL in both SSL and OC.

Technical toxaphene (Hercules Corporation, Wilmington, DE) and a 22-component Reference Mixture (Ehrenstorfer, Augsburg, GFR) containing hepta- through decachlorobornanes, and hexa- through octachlorocamphenes (see Ref 3) were analyzed. Two test solutions were prepared in toluene (200 μL): the first contained 500 ng of technical toxaphene, and the second contained 5 ng of each component from the reference mixture; both solutions also contained 10 ng of $^{13}C_{12}$-PCB 180 as an internal standard (Cambridge Isotope Laboratories, Cambridge, MA) .

Results and Discussion

Comparative analyses of the Reference Mixture using SSL and OC revealed significant discrimination of several compounds as a result of incomplete transfer and/or thermal degradation in SSL. For example, the decachlorobornane P69 had a much lower signal intensity when using SSL than when using OC. The same phenomenon was observed for the nonachloro congeners, where P56, P58 and P59 exhibited the largest reduction in peak areas when using SSL. SSL also resulted in a lower peak area for the octachlorobornane P42 (toxicant A). This difference between SSL and OC is best evident when comparing the relative response ratios R_i of each component ($R_i = r_{i,\ SSL} / r_{i,\ OC}$ where $r_{i,\ SSL}$ and $r_{i,\ OC}$ are the relative response to the internal standard when using SSL and OC, respectively).

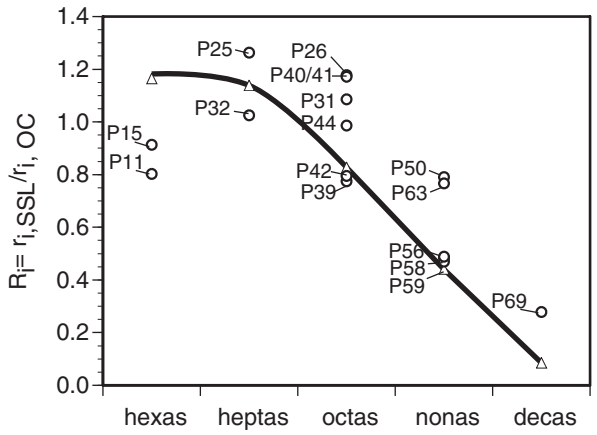

Figure 1: Relative response ratios of hexa- through decachloro compounds when using SSL and OC injection. O: chlorocamphenes and chlorobornanes from the Reference Mixture, Δ: homolog groups from technical toxaphene.

Figure 1 shows a plot of the R_i values for some compounds from the Reference Mixture, and exhibits a clear trend of lower R_i values for the highly chlorinated compounds. The lowest R_i

value (0.27, about one quarter detected when using SSL compared to OC) was observed for P69; the R_i values for the other compounds ranged from 0.42 (P59) to 1.27 (P25). R_i values >1 are likely the result of a less efficient transfer of the internal standard ($^{13}C_{12}$-PCB 180) than of the analyte in question when using SSL. The chlorobornanes with the highest values within the octa- and nonachloro homolog groups were P26 and P40/P41 (octas), and P50 and P63 (nonas). These compounds all have non-*gem* chloro substitution at the ring carbons (C-2, C-3, C-5 and/or C-6) and seem more resistant to thermal degradation than other chlorobornanes.

P32 is the heptachlorobornane with the lowest R_i value (1.03); P39 and P42 are the octachlorobornanes with the lowest R_i values (0.78, 0.80, respectively); and P56, P58 and P59 are the nonachlorobornanes with the lowest R_i values (0.48, 0.47, 0.49, respectively). These congeners all have a 2,2'- and/or 5,5'-*gem* dichloro substitution on the 6-member carbon-ring; they have the lowest R_i values within their respective homolog group most likely as a result of degradation in the SSL injector. This conclusion is supported by the extensive formation during SSL of Compound X1, likely a hexachlorobornene [8], that is a thermal degradation product. P32 (toxicant B) is likely a precursor of Compound X1 (Figure 2), which may be formed *via* loss of HCl (see below), although this has not been verified.

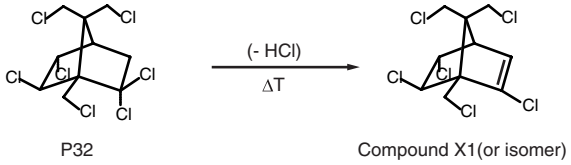

Figure 2: Thermal formation of Compound X1 from the P32 *via* HCl elimination.

Analysis of technical toxaphene yielded complex chromatograms, with dozens of peaks in the retention time windows of the hexa- through decachlorobornanes, when using both SSL and OC (Figure 3). As with the Reference Mixture, the nona- and decachloro compounds exhibited smaller peaks when using SSL compared to OC. For example, P58, P59 and P69 were clearly discriminated when using SSL, indicating incomplete transfer and/or decomposition. The discrimination of the octachloro compounds was less pronounced, except for P42. Like with the Reference Mixture, analysis of technical toxaphene using SSL resulted in the formation of Compound X1. Technical toxaphene contains HpSed, an environmentally persistent heptachlorobornane [9]. SSL resulted in the formation of additional HpSed, a phenomenon not observed when analyzing the Reference Mixture. Therefore, it is likely that the precursor of HpSed is not one of the Reference Mixture components.

SSL results in a clear trend of lower R_i values for the highly chlorinated compounds, indicating an incomplete transfer from the vaporizer to the analytical column and/or decomposition of these compounds in the vaporizer, and much lower response for nona- and decachloro congeners than when using OC. Figure 1 also shows a plot of the R_i values for each homolog group, calculated using the total area for the respective peak envelopes, versus the degree of chlorination. This same trend is observed when comparing analyses of the Reference Mixture using SSL and OC.

Several toxaphene components, especially the chlorobornanes with *gem* dichloro substitution on the six-member carbon ring, undergo thermal degradation when using SSL. Some of these congeners are major components of technical toxaphene, but generally are not present except at low concentrations in environmental and biological samples. Therefore, technical toxaphene may be discriminated and/or degraded differently than toxaphene compounds in environmental samples when using SSL. This results in significant bias of the quantitative data when using the technical material as a reference.

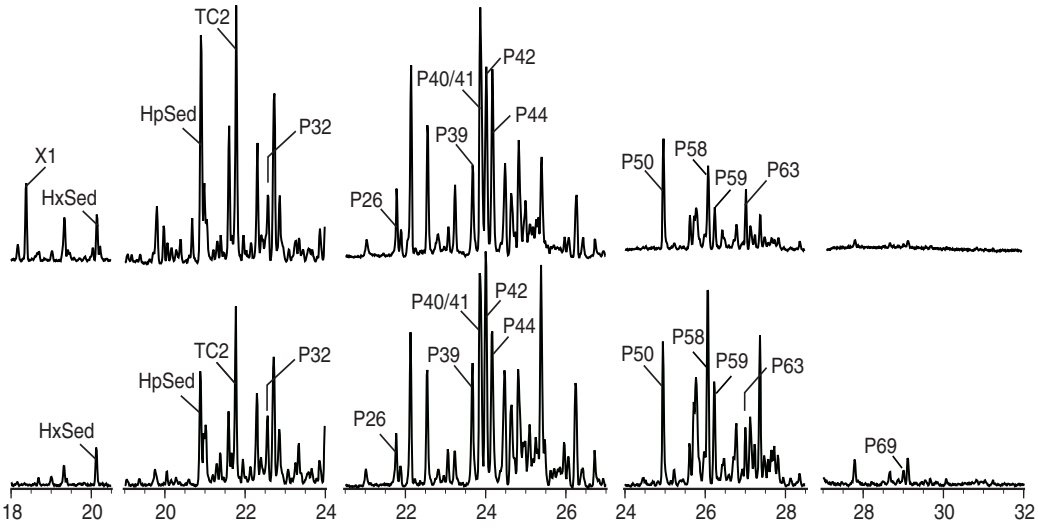

Figure 3: Partial ECNI SIM chromatograms (from left to right, m/z 307, 343, 377, 413 and 445) showing elution of hexa- through decachloro compounds in technical toxaphene when using SSL (upper panels) and OC (lower panels).

Clearly, well defined and characterized individual reference compounds are necessary to obtain reliable quantitative data [10-12], and particularly when using SSL. Such compounds have only recently become available for the analysis of chlorobornanes and related compounds [3]. In addition, toxaphene analysis should be carried out using a non-discriminatory injection technique such as OC.

Acknowledgment

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