Highly Chlorinated Dimethanofluorenes in Technical Chlordane and in Human Adipose Tissue

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Several new components of technical chlordane were discovered using electron capture, negative ionization gas chromatographic mass spectrometry. These compounds have 10–12 chlorines and are produced by the condensation of three cyclopentadiene molecules. The most abundant compound has a molecular weight of 606 and has the elemental composition $C_{15}H_6Cl_{12}$. This compound and a series of related compounds were also identified as contaminants in human adipose tissue samples. These compounds are approximately 0.01–0.03% of the technical chlordane mixture, and they have average concentrations in human adipose tissue of 0.4–0.7 ng per gram of fat. They are more highly retained in human adipose tissue than chlordane-like compounds containing eight or fewer chlorine atoms. (*J Am Soc Mass Spectrom* 1990, 1, 99–103)

Technical chlordane, a mixture of more than 50 compounds [1, 2], is a common pesticide. Because of its toxicity, its carcinogenic potential, and its persistence in the environment, chlordane was banned from general use in 1981, and it was voluntarily removed from the market by Velsicol in 1988. Chlordane's resistance to degradation and its widespread use have contributed to its ubiquity in the environment. For example, it is present in arctic air, fish, and mammals [3–5] and in people [6, 7].

Because of technical chlordane's commercial and environmental importance, there have been many efforts aimed at identifying (and quantitating) its components. Compounds with 10 carbons and up to 10 chlorines have been previously reported or identified [8–11]. We report here the presence of compounds with 15 carbons and 10–12 chlorines in technical chlordane and in human fat.

Experimental

Reagents

Hexachlorocyclopentadiene and chlordene (4,5,6,7,8,8-hexachloro-2,3,3a,4,7,7a-hexahydro-4,7-methano-1H-indene) were obtained from the Environmental Protection Agency repository and were used without

further purification. Various technical chlordane lots were also obtained from the Environmental Protection Agency repository. A 70% mixture of technical chlordane in an emulsifiable oil substrate was obtained from a local commercial pesticide applicator. All solvents were the highest purity available.

Synthesis

Hexachlorocyclopentadiene and chlordene were mixed in equimolar amounts (about 30 mg of each) in a small sealed vial and then heated to 190 °C for 30 min [12]. The mixture turned from a pale yellow to a medium orange-brown. The mixture solidified upon cooling. The product was isolated from the precursors by repeated washings with hexane, in which the product is only slightly soluble. (Caution: Hexachlorocyclopentadiene is toxic and reactive. At even minimal exposure, it will cause irritation to the mucous membranes of the throat and nasal passages.) The product was recrystallized from trichloroethylene.

Human Adipose Tissues

Human breast tissues were obtained from the pathologist at Bloomington Hospital, Bloomington, Indiana. The samples were excised tissues from radical mastectomy procedures. The tissues had been preserved in a 10% formalin mixture and stored in plastic containers prior to analysis. The fatty tissues were further excised from the gross tissue sample, and connective tis-

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sue, muscle, and cutaneous material were eliminated. The fatty tissue was homogenized into a fine paste, and 10–20 g of the homogenate was mixed with about 200 g of preextracted anhydrous Na_2SO_4 . This porous mixture was Soxhlet extracted for 24 h with a 1:1 (v/v) mixture of acetone and hexane. After Soxhlet extraction, the percent fat of the extract was determined by weighing 100 μL of the extract, allowing it to evaporate for 24 h, and reweighing the residual.

Two to three milliliters of the extract was used for gel permeation chromatographic separation. The column was 5×50 cm packed with Biobeads SX-2; the solvent mixture used to elute the column was 3:2 (v/v) cyclohexane and methylene chloride. The eluent was pumped at 4 mL/min through the column and through a UV detector set at 254 nm. These methods were described elsewhere [13]. The solvent was removed from the correct fraction by rotary evaporation, and a final cleanup was performed on 1% water-deactivated silica. The final volume was reduced by using a dry N_2 stream.

Analyses

Gas chromatographic analyses were performed on an HP 5890 instrument equipped with a $30 \text{ m} \times 0.25 \text{ mm}$ i.d. J & W DB-5 fused silica capillary column and an electron capture detector. Typical parameters were a helium flow rate of 1 mL/min and split/splitless injection (0.9 min splitless) with the injection port held at 285°C , oven programmed from a 1-min hold at 60°C , then $20^\circ\text{C}/\text{min}$ to 180°C , then $2^\circ\text{C}/\text{min}$ to 230°C , then $10^\circ\text{C}/\text{min}$ to 280°C , and held for 15 min.

Electron capture, negative ionization gas chromatographic mass spectrometry was performed on an HP 5985B mass spectrometer. The ion source temperature was set at 100°C ; the ion source methane pressure was monitored with an MKS Baratron capacitance manometer and adjusted to 0.43 torr. The chromatographic conditions were the same as given above. The gas chromatography combined with mass spectrometry (GC/MS) interface was maintained at 285°C . Other modifications and operating parameters were given elsewhere [13]. For trace analyses, selected ion monitoring of the most abundant ions in the mass spectra of the component of interest was used. Ions at the following nominal masses were monitored for the quantitation of the major chlordane-like components in both human fat and technical chlordane: m/z 237, 266, 300, 338, 374, 388, 410, 424, and 444. The exact masses used were adjusted for the expected negative mass defect due to the multiple chlorines. Full-scan mass spectra were obtained by scanning from 34 to 650 u in 1.5 s.

X-ray structures were determined on an instrument constructed locally [14]. Data were collected by using the standard moving crystal-moving detector technique with a scan speed of $4^\circ/\text{min}$. A total of 3077 reflections were collected. The structure was solved by direct methods and refined by full matrix least squares.

Figure 1. Mass spectrum of a C_{15} chlordane-like molecule found in human fat. ^{13}C isotope peaks omitted for clarity of plotting.

Results and Discussion

As part of a project on the bioaccumulation of pesticides, our laboratory analyzed human tissues for chlordane components with selected ion monitoring GC/MS in the electron capture, negative ion (ECNI) mode. We monitored selected ions from the ECNI mass spectra of the major components of technical chlordane. Responses were observed for the previously identified Cl_7 – Cl_9 components of technical chlordane; in addition, responses were observed for components with much higher GC retention times. Scrutiny of these GC/MS data revealed several components with unusual mass spectra. The mass spectrum of the most abundant of these unknown compounds (1) is shown in Figure 1. The other compounds gave very similar mass spectra, but the ions were shifted to lower masses by 34 u (except for the m/z 235–241 cluster, C_5Cl_5^-). It seems likely that these compounds were of similar structure, differing only in the total number of chlorines.

The mass spectrum shown in Figure 1 indicates that compound 1 has a molecular weight of 606 and 12 chlorines; the other compounds have molecular weights of 572 (with 11 chlorines) and 538 (with 10 chlorines). Mass chromatograms of these m/z values (integrated over the isotopic clusters) are shown in Figure 2. The first peak in the mass chromatogram of the m/z 606–616 cluster (Figure 2, top) is for a compound with a molecular weight of 608 (with 12 chlorines); its mass spectrum suggests that it is a dihydro analogue of compound 1, which is the second peak. Clearly, there is only one isomer of compound 1. The mass chromatogram of the m/z 576–582 cluster (Figure 2, middle) shows several peaks, but two (those marked with an X) are due to fragment ions from the molecular weight 608 compound and from compound 1. The other three peaks represent compounds differing from compound 1 by the loss of one chlorine. The peak for the most abundant of these compounds is labeled 2. The mass chromatograms of the 538–548 u cluster (Figure 2, bottom) show at least four compounds differing from compound 1 by the loss of two chlorines. The peak for the most abundant of these compounds is labeled 3.

Figure 2. Mass chromatograms from the selected ion monitoring analysis of a composite human fat extract. The peaks marked by an X in the middle and lower chromatograms are produced by fragment ions of the more chlorinated compounds.

We noted the same responses (with the exception of the molecular weight 608 compound) during the GC/MS analysis of technical chlordane. The same set of mass chromatograms is shown in Figure 3. The peaks for compounds 1, 2, and 3 are identified. The mass spectra of these peaks are the same as those noted during the analysis of the human fat samples. In all cases, the molecular ion isotope clusters agree exactly with those predicted from the specified number of chlorines.

These compounds can have at most 15 carbon atoms. This suggests the elemental compositions C₁₅H₆Cl₁₂, C₁₅H₇Cl₁₁, and C₁₅H₈Cl₁₀ for compounds 1, 2, and 3, respectively. From these elemental compositions we calculated that these molecules have seven

Scheme I

rings and/or double bonds. (An alternative composition of C₁₄H₁₈Cl₁₂ would be fully saturated; this seemed unlikely and was not considered further.) These compounds all show fragment ions corresponding to the sequential loss of 34 u (see Figure 1). This corresponds to the loss of a chlorine atom and the addition of a hydrogen; this is a fragmentation process often observed in the electron capture mass spectra of highly chlorinated compounds [15, 16].

At this point in the interpretation, we thought it wise to consider the industrial synthesis of chlordane and technical chlordane. Technical chlordane was produced by the chlorination of chlordene, a C₁₀H₆Cl₆ compound formed by the condensation of hexachlorocyclopentadiene and cyclopentadiene (see Scheme I). As in any industrial process, the precursor purities were only the minimum necessary or economically feasible. Therefore, technical grade hexachlorocyclopentadiene was typically a mixture of tetra- through hexachlorocyclopentadienes and cyclobutadienes [10]. The Diels-Alder reaction of this mixture with cyclopentadiene resulted in the various isomers of chlordene and other compounds that had been previously identified in technical chlordane. The further chlorination of this chlordene mixture produced chlordane (see Scheme I, lower right).

We believe that the Cl₁₀-Cl₁₂ compounds we observed were synthesized during the formation of chlordene. If an additional molecule of hexachlorocyclopentadiene were to add to the double bond of chlordene at C-1 and C-2, it would give a Cl₁₂ compound (see Scheme I, lower left). The less chlorinated compounds of this series are probably the products of the addition of pentachloro- and tetrachlorocyclopentadiene, minor components of the technical hexachlorocyclopentadiene mixture. The resulting compounds would be Cl₁₀ and Cl₁₁ analogues of the Cl₁₂ compound.

As discussed above (see Figure 3), there are several

Figure 3. Mass chromatograms from the selected ion monitoring analysis of technical chlordane. The peaks marked by an X in the middle and lower chromatograms are produced by fragment ions of the more chlorinated compounds.

geometric isomers of the Cl_{10} and Cl_{11} compounds, resulting from different positions of the chlorine atoms on the rings. For example, the $\text{C}_{15}\text{H}_7\text{Cl}_{11}$ compound can have up to six isomers due to the loss of one chlorine at each of the six possible sites. Similarly, there can be 66 isomers of the $\text{C}_{15}\text{H}_8\text{Cl}_{10}$ compound. In both cases, these are numbers larger than the total number of peaks detected in the mass chromatograms shown in Figures 2 or 3. Thus, a larger number of isomers can be produced than the few that were detected. More highly chlorinated analogues of these compounds are also possible, but we have not identified them in technical chlordane. Thus, compounds containing 13-18 chlorine atoms are probably not favored owing to steric constraints. Octa- and nonachloro compounds are also possible products of this synthetic scheme, and we detected many compounds that produce ions in the proper m/z region. Their abundances, however, were

Table 1. Average abundance of some components of technical chlordane and of human adipose tissue

Compound ^a	No. of Cl	Abundance in tech. chlordane (wt%)	Conc. in human fat (ng/g fat)	Ratio of columns 4 to 3
Heptachlor	7	6.0	—	0
Heptachlor epoxide	7	—	41	—
Oxychlordane	8	—	200	—
γ -Chlordane	8	10	0.3	0.03
α -Chlordane	8	9.2	0.8	0.09
Nonachlor-III	9	0.4	13	30
<i>trans</i> -Nonachlor	9	6.0	140	20
<i>cis</i> -Nonachlor	9	1.7	18	10
$\text{C}_{15}\text{H}_6\text{Cl}_{12}$ (1)	12	0.03	0.7	20
$\text{C}_{15}\text{H}_7\text{Cl}_{11}$ (2)	11	0.02	0.4	20
$\text{C}_{15}\text{H}_8\text{Cl}_{10}$ (3)	10	0.01	0.6	60

^a Nomenclature for the components is taken from ref 2.

too low to give usable mass spectra, and we have not pursued their identification further.

Of course, the structure labeled $\text{C}_{15}\text{H}_6\text{Cl}_{12}$ in Scheme 1 (1,2,3,4,5,6,7,8,10,10,11,11-dodecachloro-4,4a,4b,5,8,8a,9,9a-octahydro-1,4:5,8-dimethano-1H-fluorene) is only a hypothesis. To confirm this structure, we synthesized this compound (see Experimental section for details). The synthetic compound had the same mass spectrum and gas chromatographic retention index (2958) as that found in human fat and in the technical chlordane mixture. The structure of the synthetic compound was confirmed by x-ray crystallography. In fact, the conformation given in Scheme 1 was the result of this x-ray study.

To quantitate these compounds in human adipose tissue, the major ions (m/z 538-548, 572-582, and 606-616) in the mass spectra of these compounds were monitored during GC/MS analysis of a composite human adipose tissue extract. The same ions were also monitored during the analysis of a technical chlordane standard. It is apparent from a comparison of these two analyses that these compounds are well retained in human tissues.

Table 1 gives the abundances of the various components of technical chlordane and their metabolites in the commercial mixture and in human adipose tissue; the ratio of these two values is also given. These data suggest that the less chlorinated compounds (≤ 8 chlorines) are not retained in their original form but are metabolized. Heptachlor and γ - and α -chlordane are metabolized to epoxides (heptachlor epoxide and oxychlordane) and excreted. Compounds that have higher levels of chlorination, the nonachlors and the C_{15} molecules, are not as easily metabolized and are well retained in fatty tissues. The initial metabolic step appears to hinge on the availability of adjacent unchlorinated carbons for epoxidation. From the space-filling drawing of the $\text{C}_{15}\text{H}_6\text{Cl}_{12}$ compound (see Figure 4,

Figure 4. Space-filling drawing of the C₁₅H₆Cl₁₂ molecule (left) and chlordene (right) as determined by x-ray crystallography. Chlorine atoms are represented by the large hatched circles.

left), it is apparent that the carbons available on the periphery of the molecule are all blocked by chlorines. In contrast, a molecule such as chlordene (see Figure 4, right) has adjacent carbons available for epoxidation, and thus it is readily metabolized and excreted.

Although the concentrations in human fat are not high, it is clear that these compounds bioaccumulate in humans. They are not well metabolized and are highly lipophilic. This, coupled with their very low volatility, makes these compounds excellent tracers of human exposure to chlordane. Having such a tracer is important given that chlordane is a carcinogenic pesticide [17, 18] that was on the market until 1988.

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