References and Notes

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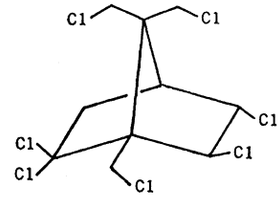
Toxaphene Insecticide: A Complex Biodegradable Mixture

Abstract. *Adsorption and gas-liquid chromatography separate toxaphene into at least 175 polychlorinated 10-carbon compounds including Cl_6, Cl_7, Cl_8, Cl_9, and Cl_{10} derivatives. One toxic component is 2,2,5-*endo*,6-*exo*,8,9,10-heptachlorobornane. Rats metabolically dechlorinate toxaphene, removing about half of the chlorine from the technical insecticide and from each of seven subfractions of varying composition and toxicity.*

Within the past 25 years 1 billion (10^9) pounds of toxaphene have been applied to crops and livestock for pest insect control. Its use continues at the rate of about 40 million pounds per year, in large part combined with methyl parathion for treatment of cotton. The toxaphene–methyl parathion combination has replaced the toxaphene–DDT combination employed until recently. Despite the fact that toxaphene is used in larger amounts in the United States than any other chlorinated hydrocarbon insecticide, there is insufficient information on several aspects of its chemistry, persistence, and environmental fate (*1*).

Toxaphene is produced by chlorination of camphene to about 67 to 69 percent chlorine by weight, yielding a reproducible but very complex mixture of compounds with an overall average elemental composition of $C_{10}H_{10}Cl_8$ (*2*). No individual component, toxic or otherwise, has previously been isolated in pure form. Despite the difficulties in evaluating toxaphene persistence, it is known from observations on its duration of effectiveness in insect control and from analyses of its residues by a variety of methods that many of the toxaphene constituents degrade under several different environmental conditions more rapidly than certain other chlorinated hydrocarbon insecticides, including DDT (*1*). Increased restrictions on the use of chlorinated insecticide chemicals and improved methodology for working with complex mixtures make it necessary and possible to define the nature of the toxaphene components and their metabolic fate in mammals. We have taken steps in this direction.

Analysis of chlorinated insecticides and other chlorine-containing environmental pollutants normally involves the use of gas-liquid chromatography (GLC) with an electron-capture detector. Technical toxaphene appears to contain 25 to 30 components when analyzed on appropriate GLC columns (*3*); however, many of the peaks detected are due to multiple components that do not separate on GLC. Fractionation of toxaphene on silica gel by thin-layer chromatography (TLC) with pentane as the developer or by column adsorption chromatography with hexane for elution resolves the toxaphene components on a different basis than GLC; the components elute from the GLC column in the general order of increasing degree of chlorination whereas this is not the case for elution from the adsorption column. Examination of the toxaphene fractions from silica gel column chromatography by combination gas-liquid chromatography–mass spectroscopy (MS) techniques (*4*) reveals a complex mixture of at least 175 C_{10} polychloro derivatives made up of $C_{10}H_8Cl_{10}$, $C_{10}H_{18-n}Cl_n$, and $C_{10}H_{16-n}Cl_n$ derivatives where the chlorine number (n) is 6, 7, 8, or 9. It appears likely that the majority of $C_{10}H_{18-n}Cl_n$ compounds are polychlorobornanes since one heptachlorobornane (**1**)



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has been identified, as described below, and 2-*exo*-10-dichlorobornane is a major intermediate (*5*) in the chlorination of camphene. The $C_{10}H_{16-n}Cl_n$ derivatives are likely to be polychlorobornenes or polychlorotricyclenes or both.

A procedure was devised for isolating individual toxaphene components in pure, crystalline form. It involves separation on a partition column with β-methoxypropionitrile and heptane and then on the silica gel–hexane adsorption column, followed by a repetition of these two steps in sequence, and then preparative GLC and further purification by either sublimation or crystallization (*6*). With suitable monitoring, this sequence of chromatographic steps should permit isolation of any individual component provided it is stable under the chromatographic conditions employed.

By using intraperitoneal acute toxicity in the mouse as the monitoring criterion, two toxic crystalline compounds were isolated, one a $C_{10}H_{11}Cl_7$ and the other a $C_{10}H_{10}Cl_8$ component. These crystalline materials are, respectively, 6 times and 14 times more toxic to mice than technical toxaphene and 2 times and 4 times more toxic to houseflies treated topically. The $C_{10}H_{11}Cl_7$ component has been characterized by x-ray crystallography and by MS and nuclear magnetic resonance studies as 2,2,5-*endo*,6-*exo*,8,9,10-heptachlorobornane (**1**). Crystallization of the racemate from a mixture of hexane and acetone (5 : 1) appears to lead to the separation of the two enantiomers; however, these enantiomers could not be differentiated by the x-ray study. The $C_{10}H_{10}Cl_8$ component has not yet been obtained in crystalline form appropriate for x-ray structure determination. The $C_{10}H_{11}Cl_7$ and $C_{10}H_{10}Cl_8$ toxic components each constitute 2 to 6 percent of technical toxaphene, based on a combination of TLC and preparative GLC analyses, but they are in relatively large amounts compared with many other components. While it is already evident from our study that other toxic materials are present in toxaphene, the two components isolated to date appear to contribute significantly to the mammalian toxicity of commercial toxaphene.

The availability of preparations labeled with ^{36}Cl and ^{14}C (*7*) made it possible to carry out initial studies on the metabolic fate of toxaphene in mammals. Toxaphene labeled with ^{36}Cl was administered orally to rats at about

14 mg/kg to determine the extent of dechlorination and rate of elimination of chlorinated compounds from the body over a 14-day period. For analysis, the ^{36}Cl ion was converted to phenylmercuric [^{36}Cl]chloride (*8*), which was recrystallized to a constant specific activity. Comparable studies with [^{14}C]-toxaphene yielded no metabolites labeled with ^{14}C in the recrystallized phenylmercuric chloride fraction, so metabolites of toxaphene other than chloride ion do not interfere in this method of isotope dilution analysis. Most of the [^{36}Cl]toxaphene is metabolized before excretion, less than 0.7 percent of the ^{36}Cl appearing in urine and less than 3 percent in feces as unmetabolized toxaphene. About 5 percent of the administered ^{36}Cl appears in urine and 21 to 24 percent in feces as partially dechlorinated metabolites of toxaphene. The remainder of the excreted ^{36}Cl, accounting for 44 to 57 percent of the administered [^{36}Cl]toxaphene dose, is chloride ion in the urine. For comparison, 90 percent of the ^{36}Cl from labeled NaCl administered to rats is excreted as chloride ion in the urine.

To get an idea of the variation in rate and the degree of metabolism of toxaphene components, we chromatographed [^{36}Cl]toxaphene on the silica gel–hexane column and combined the fractions in the order of elution so that seven subfractions were obtained, each containing one-seventh of the total ^{36}Cl content of [^{36}Cl]toxaphene. These subfractions do not differ greatly in their selectivity for poisoning mice and houseflies, but the toxicity of the subfractions to these organisms varies from one-sixth to three times that of technical toxaphene. The seven subfractions and toxaphene itself are dechlorinated to similar extents and eliminated at similar rates by rats despite a 16-fold toxicity difference for mice and a 10-fold difference for houseflies between the least toxic and the most toxic subfractions.

The results reported here suggest that many of the toxaphene components contain the same or similar biodegradable groupings that undergo in vivo dechlorination in mammals, with about half of the C-Cl bonds on an average being metabolically labile. This does not mean that half of the C-Cl bonds in each component are cleaved since the studies to date involve mixtures of many components, some of which may undergo a small degree of dechlorination while others are more extensively or even completely dechlorinated and fragmented. Two sets of structural features are therefore of interest relative to the individual toxaphene components. An appropriate steric relationship between certain chlorines present in only a few constituents may determine the neurotoxic potency. Biodegradable sites such as chloromethyl and other groupings are probably present in most if not all of the toxaphene components. The composition of technical toxaphene and the structure, metabolic fate, and environmental persistence of the toxaphene components should be defined more completely by the procedures described above.

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Phosphate Absorption Capacity and Acclimation Potential in Plants along a Latitudinal Gradient

Abstract. *The capacity for phosphate absorption by marsh plants is negatively correlated with the soil temperature of the habitat of origin. Species and races from thermally fluctuating environments achieve greater compensatory changes in the phosphate absorption rate through temperature acclimation than their counterparts from more stable environments.*

Related organisms living at very different temperatures may exhibit similar metabolic rates, as a result of both evolutionary modifications and acclimation (a compensatory change in the rate in response to a change in growth temperature). These forms of temperature compensation have been well-documented in animals (*1*) but have only recently received attention by botanists. Evolutionary temperature compensation of plant processes usually (*2, 3*) but not always (*4, 5*) occurs. Acclimation of photosynthesis and respiration generally compensates for seasonal temperature changes, but the extent of this compensation is not consistently correlated with the extent of temperature fluctuation in the habitats from which the species derive (*5, 6*), although this correlation was found in one comprehensive study (*3*). The results of the study presented here indicate that the rate of phosphate absorption is finely attuned to the local thermal regime and that both evolutionary and acclimatory forms of temperature compensation consistently occur in this process.

Species and races of marsh plants were selected for study from habitats along latitudinal gradients of temperature and thermal stability, ranging from the cold, thermally stable soils of the arctic tundra (Barrow, Alaska; latitude, 71°18′N) to the warm, thermally fluctuating soils of a desert oasis (Thousand Palms, California; latitude, 33° 49′N). The soil thermal regimes in this report are characterized by July mean soil temperatures; details of the thermal regimes and temperature measurements