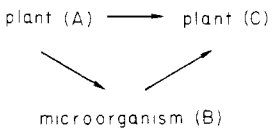
but may involve the mechanism



Interactions A–C and A–B were studied but a modification of the microorganism (B) can modulate the growth of other plants (C); thus, the edaphic environment must be considered in studying allelopathy in the real world.

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Registry No. Dhurrin, 499-20-7; taxiphyllin, 21401-21-8; *p*-hydroxybenzaldehyde, 123-08-0.

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Capillary Gas Chromatography–Electron Impact and Chemical Ionization Mass Spectrometry of Toxaphene

Mahmoud Abbas Saleh

Analysis of toxaphene by fused silica capillary column gas chromatography coupled with electron impact, positive and negative chemical ionization mass spectrometry reveals a complex mixture of at least 202 compounds. Among the toxaphene components, 76% are polychlorobornane isomers, 18% are polychlorobornenes, 2% are polychlorobornadienes, 1% are other chlorinated hydrocarbons, and 3% are nonchlorinated compounds. Single ion monitoring in the electron impact mode at m/e 159 provides a highly selective and sensitive technique for analysis of toxaphene and toxaphene-like materials in the presence of other chlorinated hydrocarbon insecticides.

Toxaphene is an extremely complex mixture of polychlorinated compounds with an average elemental composition of $C_{10}H_{10}Cl_8$ (Holmstead et al., 1974; Casida and Saleh, 1978). It is produced by intensive chlorination of technical-grade camphene to an overall chlorine content of 67–69% (Buntin, 1951). Only 10 toxaphene components have been isolated and identified, including the most toxic ingredients. However, these account for less than 25% of the mixture (Saleh and Casida, 1979).

Toxaphene is carcinogenic in rodents (National Institutes of Health, 1979) and is mutagenic in the Ames *Salmonella* test (Hopper et al., 1979). It is highly persistent in soils and lake sediments with a half-life estimated at 11–20 years (Nash and Woolson, 1967; Hermanson et al., 1971; Nash et al., 1973) and also accumulates in fish (Sanborn et al., 1976; Ribick et al., 1982).

More than 5×10^5 tons of toxaphene have been used in the United States since 1947. Toxaphene and toxaphene-like pesticides (Saleh and Casida, 1977) are still used extensively in many other countries (U.S. Trade Commission, 1977). Despite the wide use of toxaphene and its apparent health hazard, little is known about its distribution and residue in mammalian species or in the environment. This stems largely because of the difficulties in analyzing such a complex mixture and its degradation products (Pollock and Kilgore, 1978) and because of interference by other chlorinated hydrocarbons. This report presents the results of an investigation of capillary column gas chromatography–mass spectrometry (capillary GC–MS) analysis of toxaphene using electron impact (EI), positive chemical ionization (PICI), and negative chemical ionization (NICI) techniques. The use of the single ion monitoring (SIM) technique in the EI mode for residue analysis of toxaphene is also discussed.

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MATERIALS AND METHODS

Chemicals. The same sample of toxaphene (Lot X-18825-6 from Hercules, Inc., Wilmington, DE) was used throughout this investigation. The chlorinated hydrocarbon insecticide mixture containing hexachlorocyclohexane isomers, heptachlor, heptachlor epoxide, aldrin, dieldrin, chlordane, DDT isomers, DDE, DDD, endrin, mirex, kepone, and polychlorinated biphenyl isomers was obtained from Supelco-Chromatography Supplies (Bellefonte, PA).

Sample Preparation. A standard solution of toxaphene in hexane (1 mg/mL) was used for the GC/MS analysis. Samples of rat body fat (2g) were fortified either

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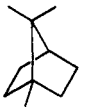
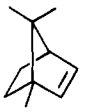
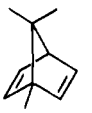
Table I. Composition and Abundance of Toxaphene Isomers

chlorine atoms per molecule			
2	4[a] (2.33 ± 0.10)[b]	2 (0.16 ± 0.02)	1 (0.02 ± 0.01)
3	2 (0.18 ± 0.03)		
4	3 (0.15 ± 0.01)	1 (0.02 ± 0.01)	3 (0.27 ± 0.03)
5	7 (1.19 ± 0.08)	11 (1.99 ± 0.09)	12 (0.50 ± 0.03)
6	11 (3.53 ± 0.11)	18 (8.88 ± 0.18)	3 (1.68 ± 0.04)
7	20 (23.22 ± 1.22)	7 (2.40 ± 0.11)	
8	23 (32.97 ± 2.31)	3 (4.68 ± 0.10)	
9	14 (7.82 ± 0.33)		
10	11 (2.47 ± 0.15)		
11	4 (0.59 ± 0.02)[c]		
other chlorinated hydrocarbons		19 (1.04 ± 0.02)	
nonchlorinated compounds		15 (3.41 ± 0.13)	

[a] Number of isomers present. [b] Percent present in toxaphene (mean ± standard deviation of three analyses). [c] Eight compounds of $C_{10}H_9Cl_{11}$ (0.14% of toxaphene) were also detected.

with toxaphene (5, 50, 500, and 1000 ppb) or with toxaphene in addition to the chlorinated hydrocarbon mixture (50 ppb of each chlorinated hydrocarbon). The fortified fat samples were extracted and cleaned up by using the fuming sulfuric acid–Celite column (Saleh and Casida, 1978) and analyzed by SIM GC–MS at m/e 159.

Gas Chromatography–Mass Spectrometry. A Finnigan 4530 EI, pulsed PI, NICI GC–MS data system equipped with a 30-m (0.25-mm i.d.) DB1 fused silica capillary column (J & W Scientific, Inc., Rancho Cordova, CA) was employed for the analyses. Helium was used as the carrier gas (40 cm/s) and methane as the makeup gas (20 mL/min) for CI runs. One microliter of each sample was injected (splitless), and data were acquired at the rate of 2 s/scan. Chromatographic conditions were as follows: injection port and interface temperatures, 250 °C; column temperature, 170 °C for 20 min and then 2 °C/min programmed to 290 °C. All spectra were acquired in the EI mode at 70 eV. The lowest mass ion of the ion cluster (due to ^{35}Cl), was used in all calculations of molecular formulas.

RESULTS AND DISCUSSION

The use of high-resolution fused silica capillary column gas chromatography resulted in a good separation of the toxaphene components (Figure 1), consistent with the results reported by Saleh and Casida (1977) using an electron capture detector. Thus, high-resolution GC eliminates the need for prefractionation of the toxaphene on a silica gel column as suggested by Holmstead et al. (1974).

Capillary GC–MS analysis of toxaphene using EI–PICI and NICI revealed the presence of at least 202 components. Among the toxaphene components, 76% are isomeric polychlorobornanes, 18% are polychlorobornenes, 2% are polychlorobornadienes, 1% are other chlorinated hydrocarbons, and 3% are nonchlorinated compounds [Table I and supplementary material (see paragraph at end of paper regarding supplementary material)]. The gas chromatographic separation revealed a close relationship between retention time and the number of chlorine atoms in the molecule. Components of low chlorine content were found to have shorter retention times.

The electron impact mass spectra (EIMS) of the various toxaphene components exhibit complex fragmentation patterns with molecular ions seldom visible except with compounds containing one or two double bonds. PICI using methane also gave very low intensity molecular ions; however, $[M - Cl - HCl]^+$ and $[M - Cl - 2HCl]^+$ ion

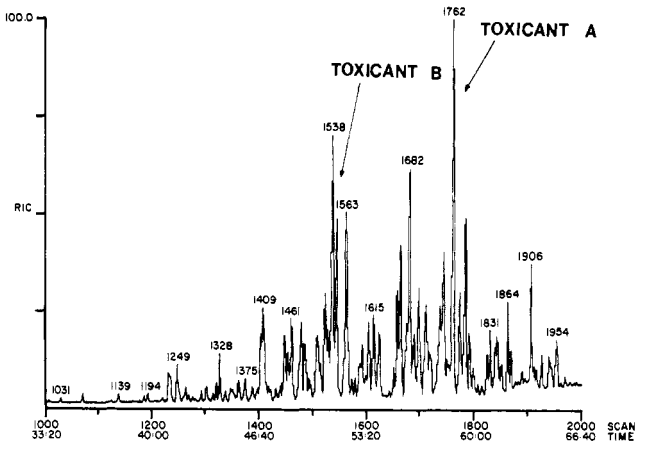


Figure 1. Fused silica capillary column gas chromatogram (RIC) of toxaphene.

clusters were observed at the major fragments with all of the toxaphene components. The large $[M - Cl]^+$ ion in the methane CI spectra is probably formed on rapid loss of Cl from the parent molecule as HCl resulting from protonation by the reagent gas ions $[CH_5]^+$; it then undergoes successive losses of HCl or Cl. Similar findings have been obtained with other chlorinated hydrocarbon insecticides such as lindane (Saleh, 1980), aldrin, and chlordane (Biros et al., 1972). NICI spectra of toxaphene generally showed a single $[M - Cl]^-$ ion cluster presumably formed by the loss of a chlorine atom from the parent compound. As toxaphene components having nine or more chlorine atoms per molecule gave very low intensity ions in the high-mass regions of EIMS, chemical ionization particularly in the negative mode proved to be very useful in identifying these components. The EI, PICI, and NICI fragmentation patterns of two of the most abundant and most toxic toxaphene components (Turner et al., 1975; Saleh et al., 1977) are shown in Figure 2.

Examination of the EI mass spectra of the toxaphene components revealed the presence of common fragment ions of m/e 83, 125, 159, and 197 for all of the compounds. Especially noteworthy, the ion at m/e 159 was highly abundant in all spectra and in many of the toxaphene components constituted the base peak. When the chromatography of toxaphene was determined by using SIM at m/e 159, an identical chromatogram to that of the total ions chromatogram (RIC) was obtained (Figure 1). However, it is significant that all of the chlorinated hydrocarbon

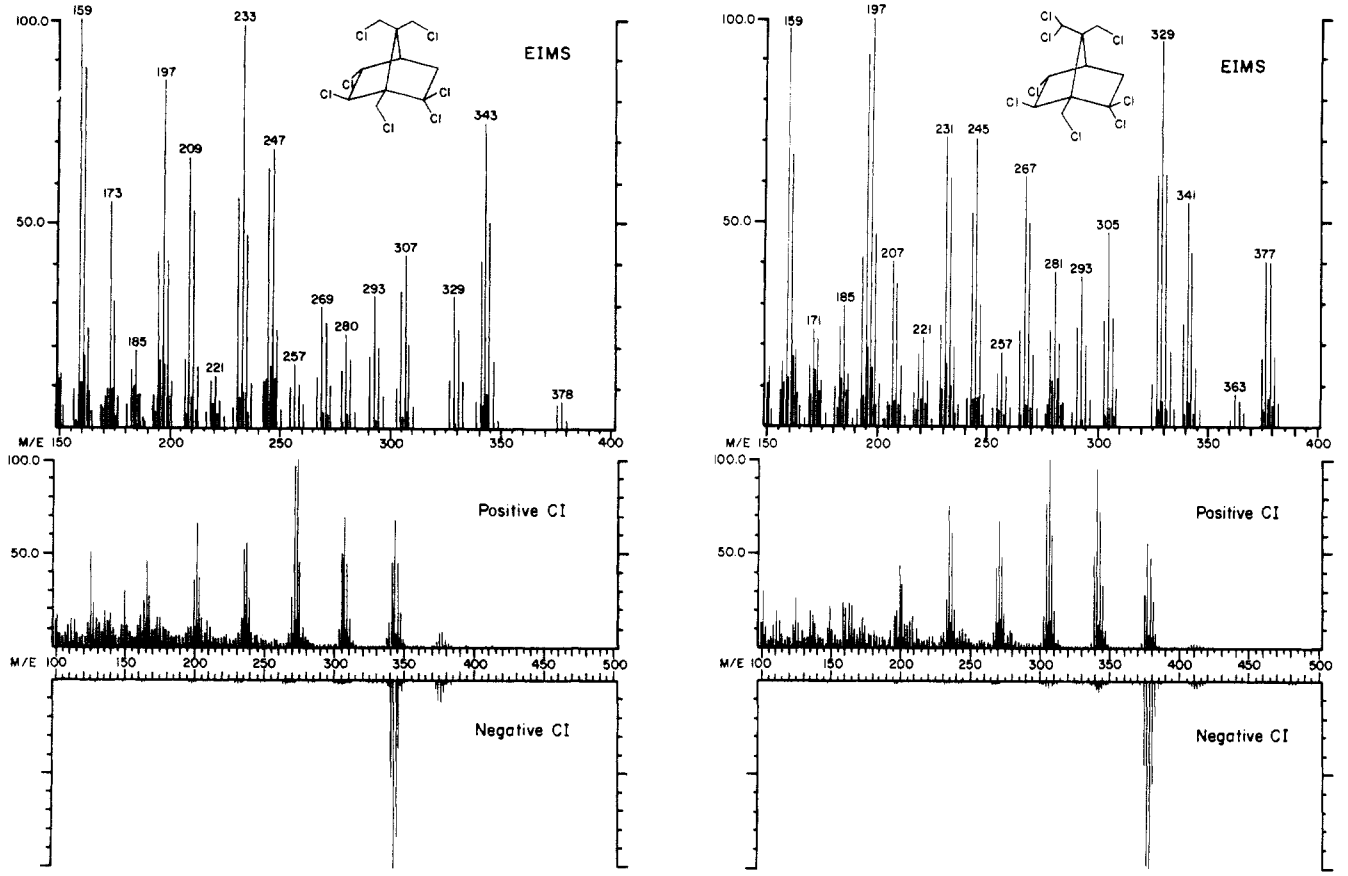


Figure 2. EI, PICI, and NICI mass spectra of toxicant A (right) and of toxicant B (left).

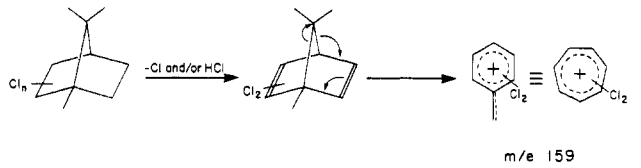


Figure 3. Suggested mechanism for formation of ion cluster m/e 159 in EIMS of toxaphene components.

insecticides and polychloroinated biphenyls which were examined under the same conditions did not give any of the m/e 159 ions. These compounds usually interfere with residue analysis of toxaphene when GC-ECD is used, but give no response with SIM 159 in the toxaphene chromatographic region. A possible mechanism for the formation of the ion cluster m/e 159 is shown in Figure 3.

The above observations suggested the possible use of the SIM m/e 159 for residue analysis of toxaphene. Thus, when a fortified fat sample containing a mixture of chlorinated hydrocarbon insecticides in addition to toxaphene was cleaned up as described by Saleh and Casida (1978) and analyzed by GC–SIM–EI at m/e 159, a chromatogram similar to the RIC of toxaphene was obtained (Figure 4). No interference from the other chlorinated hydrocarbon insecticide was observed. Therefore, the use of the SIM technique provides a highly selective and highly sensitive procedure for analyzing toxaphene in the presence of other chlorinated hydrocarbons. Toxaphene in fat samples (500 ppb) was easily detected (>98% recovery) by using this technique. A higher sensitivity may be achieved by using a short packed column operated at 200 °C where most of the toxaphene components are eluted at similar retention times. Also, it is of importance to notice that the toxaphene metabolites and chemical and photochemical decomposition products (Saleh et al., 1979; Saleh and Casida, 1978) may also be detected by the same technique.

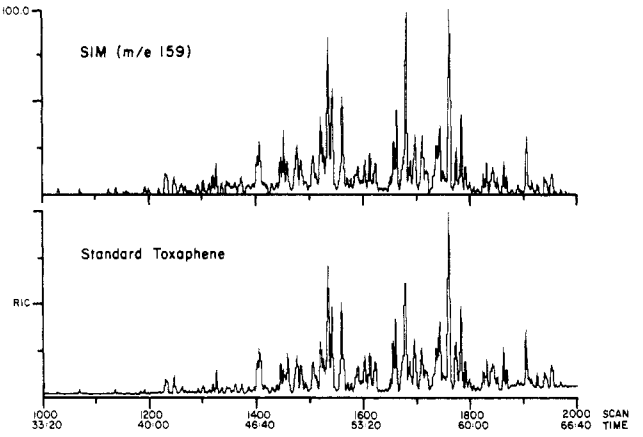


Figure 4. Comparison of the SIM (m/e 159) chromatogram of a mixture of chlorinated hydrocarbon insecticides and toxaphene and the total ion chromatogram of standard toxaphene.

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Registry No. Toxaphene, 8001-35-2.

Supplementary Material Available: Chromatographic and mass spectrometric properties of toxaphene components (9 pages). Ordering information is given on any current masthead page.

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Acifluorfen Increases the Leaf Content of Phytoalexins and Stress Metabolites in Several Crops

Tâmas Kömives[1] and John E. Casida*

Leaves treated with acifluorfen [sodium 5-[2-chloro-4-(trifluoromethyl)phenoxy]-2-nitrobenzoate] contain greatly increased levels of *N*-feruloyl-3-methoxytyramine (spinach) and of 10 phytoalexins, i.e., glyceollins I, II, and III and glyceofuran (soybean), phaseollin (bean and pinto bean), pisatin (pea), medicarpin and wyerone (broad bean), xanthotoxin (celery), and hemigossypol (cotton). Enhanced synthesis of these compounds is related to the acifluorfen concentration and exposure time to light. The phytotoxicity of acifluorfen and oxyfluorfen [2-chloro-1-(3-ethoxy-4-nitrophenoxy)-4-(trifluoromethyl)benzene] to spinach is counteracted by appropriate treatments with (aminooxy)acetic acid, L-2-(aminooxy)-3-phenylpropionic acid, or silver nitrate and by heat shock. Under certain conditions soybean injury is ameliorated by combining (aminooxy)acetic acid with acifluorfen and silver nitrate with oxyfluorfen. These relationships for diphenyl ether (DPE) herbicides and protective treatments resemble those for other stress factors with associated increases in lipid peroxidation, membrane permeability, ethylene production, and phenylalanine ammonia-lyase (PAL) activity. Although increased PAL activity is not a primary lesion, it may play an important role in DPE herbicide action.

Herbicide stress may lead to accumulation of secondary plant products: e.g., diphenyl ethers (DPEs) increase the *N*-feruloyl-3-methoxytyramine (FMT) content of spinach leaves (Kömives and Casida, 1982; Suzuki et al., 1981), 2,4-D and maleic hydrazide promote accumulation of scopolin and scopoletin in tobacco (Wender, 1970), and atrazine, bentazon, endothall, and oxadiazon increase the level of certain isoflavonoids in soybeans and navy beans (Rubin et al., 1979a,b). Some of the same secondary compounds accumulate in response to other abiotic elicitors (e.g., heavy metals, surfactants, and organic solvents) and to microorganisms (i.e., phytoalexins are produced as natural self-defense chemicals) (Bailey, 1982). Formation of these secondary products, stress metabolites, or phytoalexins is usually initiated by or associated with cell death caused by the chemical or microorganism (Bailey, 1982).

Acifluorfen [sodium 5-[2-chloro-4-(trifluoromethyl)-phenoxy]-2-nitrobenzoate], oxyfluorfen [2-chloro-1-(3-ethoxy-4-nitrophenoxy)-4-(trifluoromethyl)benzene], and other DPE herbicides are of particular interest since their phytotoxic action in many plants is accompanied by strong induction of a key enzyme in biosynthesis of several phytoalexins, i.e., phenylalanine ammonia-lyase (PAL, EC 4.3.1.5) (Kömives and Casida, 1982). Thus, preliminary studies indicate that acifluorfen treatment of beans and soybeans, as well as spinach, strongly incrases their levels of specific organosoluble phenolics and that these phenolics may include isoflavonoid phytoalexins (Kömives and Casida, 1982).

This study examines a variety of acifluorfen-treated plants for possible changes in their secondary products, with emphasis on crops with well-characterized but structurally divergent phytoalexins (Figure 1). It also

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