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UTILIZATION OF GAS–LIQUID CHROMATOGRAPHY COUPLED WITH CHEMICAL IONIZATION AND ELECTRON IMPACT MASS SPECTROMETRY FOR THE INVESTIGATION OF POTENTIALLY HAZARDOUS ENVIRONMENTAL AGENTS AND THEIR METABOLITES

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1. INTRODUCTION

In order to have a thorough understanding of the potential health hazards to man produced by a particular environmental agent, one must first have a thorough knowledge of the chemistry of the particular compound. The chemistry of a specific environmental agent encompasses: (1) the chemical structure; (2) the physical properties of the material; (3) the analytical techniques required for the specific analysis of the entity in its environment; (4) the sources and/or uses of this environmental agent; (5) the chemical reactivity associated with the particular functionality of this compound; (6) the metabolism of the environmental agent in biological systems; (7) the biological interaction and/or biological activity of the agent and its metabolites; and (8) the toxicity of the environmental agent and components derived from this chemical species. Once this definitive information is available concerning the basic chemical and biochemical properties of the environmental agent, one must then evaluate the possible mode(s) of action by investigating: (a) the type of pathological changes produced by the agent; (b) the severity and longevity of the pathology; (c) the immediacy of the undesirable effects on man and/or his progeny; and (d) the minimization and/or reversibility of the events leading to the pathological end result. Finally, after one has considered the many complex interactive data concerning a specific environmental agent, one must then scientifically correlate all of this information so that man may be able to understand and control the impact that this particular environmental agent has on his existence.

The environment, the survival of its inhabitants and all biological systems may be affected by the presence of this chemical component. More important, the chemical form and the longevity of this form dictate the potential hazards that may be prevalent. Any environmental agent which would interrupt normal biological processes of the enzymatic, genetic and/or metabolic nature would be undesirable. The potentially hazardous environmental agents may be either natural products or "man-made" contaminants of the environment.

This review describes information that has been obtained by members of the Chemistry Group at this Institute over the last 6 years to assist in answering a small portion of the complex problems associated with the potential health hazards of noxious environmental agents. The major emphasis of this review is placed on the application of gas–liquid chromatography and mass spectrometry to environmental problems. The significance of the various types of information obtained by mass spectrometry will be applied towards answering questions concerning: (a) chemical purity, structure and functionality; (b) analytical methodology requirements; (c) metabolism; (d) biological interaction and biological activity; and (e) possible mode(s)

of action for the production of the pathology and undesirable effects. The general discussion on the utilization of gas–liquid chromatography coupled with mass spectrometry (GLC–MS) for the investigation of specific environmental agents will be oriented both to readers with an academic interest and to individuals with research involvement in these specific areas. This review also discusses the advantages and disadvantages of the various analytical systems with each specific type of environmental agent. A brief discussion is given on the uses of MS for the quantitation of environmental agents.

Probably the most versatile analytical technique available to the chemist is GLC[1–8]. Many types of environmental agents, including pesticides, non-polar natural products and synthetic chemicals, may be analyzed directly by gas chromatography. Still other compounds, because of increased polarity and low vapor pressure, must first be converted into a more volatile derivative and then be analyzed. Care must be exercised in choosing the type of derivative for a specific environmental agent and its metabolites in order to minimize the chemical and/or thermal lability of the reaction product which will be analyzed by gas chromatography. By the use of GLC alone, one cannot chemically verify the structure of a particular compound. By the use of many varied analytical conditions of GLC, one can determine the homogeneity of the particular sample. One can directly compare the retention time of the unknown with the known compound. In addition, one can quantitate the particular unknown with respect to the known compound. On the contrary, without further integration of the data from gas chromatography with data from other sources such as infrared (IR) and nuclear magnetic resonance (NMR) spectroscopy, one is not able to determine unequivocally the chemical structure of the unknown environmental agent. Furthermore, if the sample size of the unknown is limited to a few micrograms or, more commonly, to nanogram amounts, even IR and NMR spectroscopic analyses are not possible.

By the use of a GLC system which is directly interfaced with a mass spectrometer, one is able to examine thoroughly the chemical structure of microgram and even low nanogram amounts of numerous environmental agents and their metabolites. As described by Rosenstock and Krauss[9], mass spectra present a "chemical appearance of compounds". In addition to the analytical value of the information obtained by MS, these data reflect the chemistry, the reactivity and the stereochemical orientation of many functional groups of the molecule. The unimolecular ion reactions in the mass spectrometer are in many ways similar to the reactions that take place under thermal and photolytic conditions. The utilization of the mass spectrometer as a model to measure the decomposition and rearrangement propensities of certain environmental agents and their metabolites is very beneficial.

In particular cases, by use of a GLC system combined with a mass spectrometer which is controlled by a computer, one is able to detect and identify even picogram amounts of specific environmental agents. In many instances, because of the chemical nature of a compound, direct bombardment of the agent with electrons in the mass spectrometer will not produce the molecular ion in significant abundance for detection. Even by use of very low energy electron bombardment of 20 eV or less, the mass spectrum may not contain all of the descriptive mass fragments and the molecular ion.

Chemical ionization (CI)-MS is a type of high-pressure MS in which the compound of interest interacts with the reactant or carrier gas ions to form the positively

charged ions. The reactant ions are formed by a combination of electron impact (EI) and ion–molecule recombinations. The primary, secondary and tertiary reactant ions then combine with the desired sample and in this process transfer massive entities including protons (H^+), hydride ions (H^-) and alkyl carbonium ions (RCH_2^+) to the desired sample molecules. The amount of energy involved in the chemical ionization reactions is relatively low, depending on the reactant gas used. For a more detailed discussion on chemical ionization mass spectrometry, the reader is referred to articles by Munson and Field[10], Munson[11] and Fales *et al.*[12].

By the characterization of the crudely purified environmental agent by GLC coupled with CI- and EI-MS, one can unequivocally identify the chemical structure of many polyfunctional metabolites and reaction products of numerous potentially hazardous environmental agents. As will be seen in later discussions on specific compounds, ions present in the EI spectra of some compounds are descriptive of a specific portion of the molecule, whereas ions derived by the CI processes are descriptive of a different portion of the molecule to that described by the ions from the EI process. In some instances, without the CI data one would not be able to describe the complete chemical structure; on the contrary, very stable and rigid molecules may be studied very satisfactorily by EI-MS even at high electron voltages. In most instances, the limitations of a combined GLC–MS system are mainly those concerned with the chromatographic properties and chemical lability of the components being chromatographed. In many instances in which multi-component systems cannot be separated chromatographically, by careful computer analysis of the data, one can identify and differentiate the components present.

By further refinement of the application of MS to quantitation, one can quantitate very specifically many environmental agents which may or may not be chromatographically separable by monitoring specific characteristic ions by the technique of mass fragmentography. In this technique, the mass spectrometer becomes a specific ion detector. Molecules which yield the desired ions which are being monitored will be detected; on the contrary, numerous components and contaminants which do not yield the specific ions will not be detected. Quantitation of environmental agents by mass fragmentography in nanogram or even low picogram amounts routinely are very applicable. CI and EI mass fragmentography exhibit many desirable characteristics with specific classes of environmental agents.

2. GENERAL PROTOCOL

At least three major types of chemical investigations will be discussed with specific classes of environmental agents. The purpose of these types of investigations is to assist in answering the questions; "is this particular environmental agent harmful to man through his everyday exposure to low levels of this agent for periods up to a lifetime?" and "if this environmental agent is harmful, by what means may these harmful effects be minimized or eliminated?". As described earlier, in order to answer his portion of this problem, the chemist must be able to: (a) analyze very specifically for the well defined chemical component; (b) understand the chemical and biochemical reactivity of this agent; and (c) define the sequence of metabolic events that take place once this specific environmental agent enters the defined biological system.

Once a specific environmental agent has been deemed necessary for investiga-

tion as a potential health hazard, either because of preliminary toxicity information or because of ubiquitous distribution in the environment, the investigator must next evaluate the techniques available for the analysis of this specific agent without obtaining erroneous results from normal contaminants in the environment. The concern of this review will not be to consider the monitoring of samples routinely in order to determine the presence of a particular agent, but the emphasis is concerned mainly with determining what happens to the specified agent in defined chemical reactions and biological test systems. If the existing techniques do not suffice for the analysis of this environmental agent and its metabolites, then very specific methodology must be developed. In the discussion section, specific reference is made to the analytical requirements of individual environmental agents. The investigator who is more chemically oriented will study (a) methods for the synthesis of this environmental agent and (b) the types of reactivity which this molecule possesses. Informative investigation would include: (a) the chemical kinetics of reactions; (b) favored types of reactions including hydrolysis, reduction, oxidation and the tendency to eliminate chlorine atoms or a particular functional group; and (c) the synergistic effect of other contaminants on the chemical reactivity of this component. The more biologically oriented chemist would be concerned with: (a) translation of the pure chemical information into meaningful biological and metabolic activity; (b) the level of entrance of this environmental agent into the biological system; (c) the distribution and degree of modification of the agent in the biological system; and (d) the interaction of the environmental agent and its metabolites with normal components of the biological system.

TABLE I

GENERAL METABOLIC INVESTIGATIONS

No.	*Investigation*
1	Exposure of environmental agent to biological system
2	Distribution and rate of elimination of agent
3	Removal and purification of agent and metabolites
4	Characterization of metabolites
	(A) Chromatography
	(B) UV spectroscopy
	(C) IR spectroscopy
	(D) NMR spectroscopy
	(E) Mass spectrometry
5	Verification of structure of metabolites by organic syntheses
6	Determination of harmful effects caused by environmental agent and metabolites as illustrated by the pathological end-product

The normal sequence of events in these biological investigations may be summarized as in Table I. The investigation of the biochemistry of a particular environmental agent proceeds by administration of a single small dose of the chemically pure compound to the biological test system followed by the determination of the rate of absorption, the degree of storage and the routes and degree of elimination of the chemical component and its metabolites. After the biological data concerning the distribution of this environmental agent have been obtained, then one must determine in what chemical form this agent is present at a particular time in specific biological

tissues and fluids. Removal of the desired component from the biological medium must be accomplished before one can qualitatively or quantitatively characterize the chemical form of the metabolite. Numerous inorganic components require removal of protein and other organic materials by mild oxidation followed by atomic absorption, neutron activation or spark-source MS analyses. In other instances, inorganic components may first be complexed to form organic compounds, which then may be removed by selective organic extraction followed by analyses. Most organic environmental agents may be removed by pH-dependent organic or aqueous solvent extractions. Once the crude component has been removed from the biological environment, various analytical procedures may be used to characterize the composition of the complex mixture. In particular instances, because of the degree and nature of the polarity of the contaminants, biological extracts require some means of purification prior to initiation of structural characterization. This purification may be accomplished by solvent–solvent partition or by solvent–solid partition as exemplified by thin-layer[13], column[14,15] or high-pressure liquid chromatography[16].

The most important and the final remaining portion of the characterization is to determine the chemical form and the structural identity of the potentially hazardous environmental agent. Before final structural identification is possible, one must be able to verify that the component to be identified is chemically pure at the time of analysis.

Analysis of the chemical component by ultraviolet (UV) or visible absorption spectroscopy would require that the sample first be purified and then separately analyzed for its absorption spectrum in order to yield information concerning bond structure and the presence of chromophore groups. Measurement of the fluorescent and phosphorescent properties of the purified environmental agent is also desirable. Most spectroscopic methods require only microgram or nanogram amounts of material. This information alone would not verify a particular chemical structure.

IR spectroscopy generates more conclusive information concerning the presence of specific covalent bonds and functional groups. The environmental agent must first be isolated and purified, and then 100–200 μg of material are characterized by IR spectroscopy. On the contrary, by use of GLC integrated with a computerized IR system, one may analyze the various components by IR spectroscopy as they are eluted from the chromatograph. Most of the present integrated GLC and computerized IR systems require 10–100 μg of material[17–22].

Further characterization by conventional proton NMR spectroscopy of the pure unknown would require milligram amounts of the compound; however, by use of a computerized Fourier NMR system[23], one can obtain representative spectra on a few hundred micrograms.

Because of sample requirements, very few analytical techniques can produce structural information for the unequivocal identification of low microgram and, more commonly, nanogram amounts of a particular environmental agent. In the near future, we probably will find that the integrated GLC–IR computerized system[18,22] will be used more on low microgram amounts as the technology of instrument design advances. At the present time, for the characterization of low microgram, nanogram and, in some cases, picogram amounts of organic compounds, GLC–MS is the most informative analytical technique available to the chemist for obtaining structural data.

One of the major functions of this laboratory was to investigate the charac-

teristics and specific advantages of EI- and CI-MS coupled with GLC for the identification and characterization of numerous types of potentially hazardous environmental agents and their derivatives. In addition to studying singly the effects of various analytical parameters, numerous chemical and structural parameters were investigated in order to assist in the validation of some of the generalized information.

Routinely, with most of the specific environmental agents, the sample was characterized by EI-MS at 70, 20 and occasionally 10 eV and by CI-MS using methane, isobutane and occasionally helium as the reagent carrier gases. Analysis of the sample by GLC routinely was obtained prior to structural characterization by GLC–MS.

3. RESULTS AND DISCUSSION

A. Factors affecting gas chromatography (GC)

Although one speaks simply of "interfacing a mass spectrometer with a gas chromatograph", commercial GC–MS instruments generally involve a coupled system, each of the components of which has been designed for optimal performance in combination with the others. Thus a standard analytical gas chromatograph is not usually suitable, without extensive plumbing modifications, for coupling directly to the "interface" system of the molecular separator, transfer lines, vacuum baffle and other necessary components of the mass spectrometer. Nonetheless, it is advantageous and a common practice for investigators to use an auxiliary gas chromatograph to establish the conditions necessary for separating a sample mixture, prior to the actual GC–MS analysis. It then becomes necessary to transfer those previously established conditions to the gas chromatograph actually connected to the mass spectrometer. This translation of information requires an awareness of some of the limitations imposed on the chromatography by the nature of the interface and conditions operative in the mass spectrometer. Some of the major limitations are discussed below.

(a) Temperature

Assuming that sample decomposition in the injection port is not a problem, septum bleed will be the major factor limiting the injection port temperature. A constant level of septum bleed may be compensated for in the analytical gas chromatograph, but will necessitate careful background subtraction in the GC–MS system. Thus, the injection port temperature is limited to 225–250° as an upper limit in GC–MS, while the need for well-cured septums is nearly as important as the need for well-cured columns.

Similarly, the risk of generating mass spectra that represent mixtures of sample and column bleed generally limits the column temperature to values below those considered acceptable with the same liquid phases in analytical GC. As few GC–MS instruments permit dual column background compensation, temperature programming is understandably less popular in GC–MS. Temperature programming can be used to a great extent with the more stable silicone and Dexsil phases.

The desirability of keeping the interface region, whether separator, transfer lines or manifold, at a temperature above that of the column is analogous to the similar desirability in the case of the detector block in analytical GC, except that the interface connector region, once contaminated, may be considerably more difficult to

clean than an analytical GC detector. A special problem applies in the case of CI instruments. The ionizer region in chemical ionization must be maintained at a relatively low temperature in order to minimize sample fragmentation caused by "thermal cracking". Thus every possible means must be employed in GC–CI-MS to reduce the absolute amount of column bleed.

TABLE 2

METHANE-SUPPORTED CHEMICAL IONIZATION MASS SPECTRA OF COLUMN "BLEED"

Abbreviations: DC-200 = methyl silicone fluid; QF-1 = fluorosilicone; Ap.L = Apiezon L; EGS = ethylene glycol succinate; DEGS = diethylene glycol succinate; BDS = butanediol succinate; MPE-20 = polyphenyl ether; Carbowax 20M = polyethylene glycol.

m/e	*Relative abundance (%)*							
	DC-200	*QF-1*	*Ap.L*	*EGS*	*DEGS*	*BDS*	*MPE-20*	*Carbowax 20M*
61	28			3				12
64	18							
65		70	24					
71	27			3	6	9		22
73	83			7	27	22		100
77		8						
87				2	5			95
89				3	49			85
99				15				
100		100	100	8	33	8	100	
101				100	100	100		15
103								25
104	17							
107					37			
113								10
117								45
131								16
133								28
137		25						
145				20	48			
147	67							11
149	58							
159								5
161								5
175								8
177								8
189				9	13			
201								3
207	29							
219								5
221	100							
281	25							
295	31							
296	20							
355	31							
369	20							
370	15							
429	25							
735								

Mass fragments to be expected in methane-supported chemical ionization mass spectra of some common liquid phases deliberately bled off of the support at elevated temperatures are listed in Table 2. It can be seen that polyesters, Carbowax and the less stable silicones may produce considerable difficulties in GC–MS and probably should not be used extensively. On the other hand, the more stable silicones, polyethers and possibly the Apiezon greases seem to produce very minimal spectral interference in the mass range *m/e* 60–750.

(b) Carrier gas

Nitrogen and argon, popular carrier gases in analytical GC, are not ordinarily used in GC–MS because of their inefficient removal in molecular separators and by vacuum systems. Thus helium is the gas routinely used in GC–EI-MS, and should be used while setting up operating conditions. Methane and isobutane are commonly used as reagent gases in CI-MS and, for simplicity and reproducibility purposes, are generally used as carrier gases also. The Finnigan instrument permits changing carrier gases "at the flip of a switch", which is very convenient but has the following risk. It is essential to ensure that all traces of the first carrier gas are purged from the column by the second gas before samples are injected; otherwise, not only will the CI spectra be "mixed", but the chromatographic separation will be completely unreproducible.

The plumbing arrangement in the Finnigan CI system has a very fortuitous side-effect in that the pressure at the column outlet (before entering the mass spectrometer interface) is approximately 1 atm, just as it is in an analytical GC. This means that *j*-values used to correct for compressibility of the carrier gas, once determined for a given column in an analytical chromatograph, will also apply to the same column

TABLE 3

EFFECT OF CARRIER GAS ON RETENTION PARAMETERS

Abbreviations: Hex. = *n*-hexadecane; Oct. = *n*-octadecane; MPal. = methyl palmitate; T_R = retention time; T'_R = retention time corrected for dead space; V'_R = retention volume corrected for dead space; $V_n = jV'_R$ = retention volume corrected for dead space and carrier gas compressibility; V_g = specific retention = $V_n/W_L \cdot 273/T$ where W_L = weight of liquid phase, T = column temperature (°K); R.R.T.′ = corrected relative retention time; Ret. index = retention index (Kováts); K = partition coefficient; NTP = number of theoretical plates.

Operating conditions: injection port, 250°; column, 200°. Inlet pressures and volumetric flow-rates at column outlet: helium, 38–39 p.s.i.g., 33–34 ml/min; methane, 10 p.s.i.g., 10 ml/min; isobutane, 0.3 p.s.i.g., 2.24 ml/min. Column, 10 ft. × 1/8 in. O.D. stainless steel, 10% OV-17 on 100–120 mesh Supelcoport. Ion source pressure for all carrier gases, 1200 μ.

Parameter	*Helium, GC only*			*Helium, GC–MS*			*Methane, GC–MS*			*Isobutane, GC–MS*		
	Hex.	*Oct.*	*MPal.*	*Hex.*	*Oct.*	*MPal.*	*Hex.*	*Oct.*	*MPal.*	*Hex.*	*Oct.*	*MPal.*
T_R (min)	2.40	4.50	9.40	2.24	4.27	9.05	3.25	6.06	12.81	4.82	9.11	19.36
T'_R (min)	1.80	3.90	8.60	1.59	3.62	8.40	2.50	5.31	12.06	3.82	8.11	18.36
V'_R (ml)	59.4	128.7	284	54.0	122.9	285	25.0	53.1	120.6	8.55	18.2	41.1
V_n (ml)	23.0	49.8	110	21.2	48.2	112	18.2	38.7	87.7	7.95	16.9	38.2
V_g	26.5	57.5	127	24.4	55.4	129	21.0	44.7	101	9.17	19.5	44.1
R.R.T.′	0.47	1.00	2.30	0.44	1.00	2.31	0.47	1.00	2.27	0.47	1.00	2.26
Ret. index	1600	1800	2036	1600	1800	2037	1600	1800	2041	1600	1800	2036
K	46	100	220	42	96	224	36	77	175	16	34	76
NTP	2785	3120	3055	1362	2189	2695	1468	2345	2518	1490	2355	2542

in the GC–MS instrument. Therefore, specific retention parameters are not significantly altered by the fact that one is chromatographing "into a vacuum" in the GC–MS system.

As one would not ordinarily be able to use methane or isobutane in an analytical gas chromatograph because of incompatibility with the detector, it is necessary to have some idea of the effects on separation and retention parameters of changing from helium to these carrier gases. Table 3 lists some retention parameters for a three-component mixture analyzed on the same column in a Varian Model 1200 gas chromatograph (hydrogen flame ionization detector) and in the CI–GC–MS instrument. Helium was used with both instruments, methane and isobutane only with the latter. The ion source pressure for all three carrier gases was maintained at 1200 μ in the CI-MS system.

As methane and especially isobutane are removed more slowly than helium from the manifold and ionization chamber, it is necessary to use much lower flow-rates with the hydrocarbon gases. Fortunately, the partition coefficients for many compounds are significantly decreased when the hydrocarbon gases are used in comparison with helium, so that the retention times are not greatly increased. The particular mixture of compounds used in preparing Table 3 had sufficiently high partition coefficients on OV-17 that changing carrier gases, even to the moderately polar isobutane, which is unsymmetrical, had almost no effect on the relative retention times or retention index of methyl palmitate. Using SE-30, a relatively non-polar liquid phase, changing carrier gases did have a slight effect on relative retention times and retention indices, as the partition coefficients were much lower on SE-30 than on OV-17 (Table 4). In addition, the separation of methyl pentadecyl ketone from methyl palmitate was improved on changing from helium to the hydrocarbon gases.

(c) Peak shape

It has been reported[24] that the optimum peak shape for quantitative GC analyses is a Gaussian distribution in which the peak width is approximately half of the peak height. The area of such a peak can be measured with maximum accuracy.

TABLE 4

EFFECT OF CARRIER GAS ON RETENTION PARAMETERS WITH SE-30 AS LIQUID PHASE

Abbreviations as in Table 3; MPK = methyl pentadecyl ketone.
Chromatographic conditions: injection port, 250°; column, 200°. Inlet pressures and volumetric flow-rates at column outlet: helium, 19 p.s.i.g., 30 ml/min; methane, 0.8 p.s.i.g., 9.69 ml/min; isobutane, 0.02 p.s.i.g., 3.53 ml/min. Column, 5 ft. × 1/8 in. O.D. stainless steel, 5% SE-30 on 80–100 mesh Gas-Chrom Q. Ion source pressure for all carrier gases, 1200 μ.

Parameter	*Helium*			*Methane*			*Isobutane*		
	Oct.	*MPal.*	*MPK*	*Oct.*	*MPal.*	*MPK*	*Oct.*	*MPal.*	*MPK*
V_R' (ml)	67.0	102	100	33.4	48.2	44.8	17.8	26.5	24.1
V_R (ml)	38.5	58.8	57.7	23.4	33.9	31.5	17.1	25.4	23.1
V_g	11.2	17.0	16.6	6.72	9.80	9.11	4.84	7.32	6.69
K	19.3	29.4	28.8	11.7	17.0	15.8	8.55	12.7	11.6
R.R.T.	1.00	1.52	1.49	1.00	1.45	1.34	1.00	1.48	1.35
Ret. index	1800	1920	1904	1800	1902	1880	1800	1911	1889

In GC–MS, maximum sensitivity requires a sharp peak while maximum spectral reproducibility requires a fairly broad peak such that the concentration of sample molecules in the ionization chamber remains relatively constant during the mass scan (a few seconds). We generally prefer, as an acceptable compromise, to control the chromatographic conditions so that the peak width (15–25 sec) will permit 4–6 mass spectral scans. Thus in GC–MS the minimum acceptable peak width will depend upon the scanning rate and the mass range scanned.

B. Variables affecting mass spectra

(a) Temperature

The many variables described above for the chromatographic system also affect the appearance of the mass spectra of components eluted from the system. In addition, the analytical parameters of the mass spectrometer interact with those of the GC system. For any given set of operational conditions, one can obtain representative mass spectra of a particular compound. On the contrary, it has been assumed erroneously that mass spectra obtained from similar conventional mass spectrometers will appear to be identical[25].

Considering that all conditions are satisfactory for chromatographic separations, probably the most important variable of the integrated GC–MS system is temperature. The temperature requirements of the chromatographic separation dictate the operational temperature of the interface and manifold of the mass spectrometer. Excessive temperature requirements may cause decomposition of the components which are being chromatographed. In addition, excessive temperature requirements cause "thermal cracking" of ions in the mass spectrometer to yield a lower abundance of higher molecular weight ions. Temperature effects are very critical in CI-MS analyses. Table 5 illustrates the effect of temperature on CI spectra. It can be seen from these results that an increased ion source temperature reduced the abundance of the higher mass ions, even to the extent that one may obtain spectra on a CI system which in appearance mimic EI spectra because of excessive ion fragmentation caused by thermal effects. In addition to the effects of temperature on the abundance of ions, chemical decomposition in the system caused by thermal lability of the component to be analyzed is a major source of artifacts in the utilization of GC–MS systems. Only by thoroughly investigating the thermal lability of the specific compound and its derivatives to be analyzed by GC–MS will one be able to clarify the problems produced by the use of excessive temperature requirements.

(b) Efficiency of ionization

Ionization of a molecule can be accomplished by transfer of energy from an energy-rich species, such as a positively charged ion or an electron, as in the case of EI-MS, to a lower energy level component to produce another charged species. If the energy transfer or energy of repulsion is sufficient to remove one electron, the charged molecule is formed. For most molecules, the appearance potential is on the order of 7–16 V in order to produce the ionized or charged molecule. In cases where the electron energies used are greater (50–100 V) than the energy level required for ionization of the molecule, the molecular ion decomposes further to generate other ion fragments of the spectrum. In EI-MS, the efficiency of ionization is controlled directly by

TABLE 5

EFFECT OF TEMPERATURE ON METHANE CI MASS SPECTRA OF PERFLUOROTRIBUTYLAMINE (FC 43)

m/e	*Abundance (%)*		
	*Low temperature**	*Routine CI analytical conditions***	*Routine EI analytical conditions****
652	100	27.7	0
626	3.9	2.9	0
614	36.7	17.8	0.08
576	11.9	7.1	0.05
502	8.5	4.9	0.68
464	2.0	1.4	0.24
414	86.5	100	0.68
264	3.1	4.5	2.5
219	45.9	78.9	14.7
131	15.5	29.9	21.4
100	9.1	12.2	9.6
69	74.5	79.5	100

* Manifold heater off and ionizer heater off; ion source pressure 1000 μ; electron energy 70 eV; reagent gas methane; FC-43 administered to Finnigan Model 1015C CI mass spectrometer by use of a Granville Phillips Series 230 variable leak valve; overall pressure 10^{-5} torr.

** Manifold temperature 175°; ionizer heater off; temperature of source by heat transfer, 50–75°; all other conditions as in first footnote.

*** 70 eV. E.I. spectrum using Finnigan Model 1015C CI mass spectrometer in EI mode; no methane; repeller voltage 10 V; ion source pressure 80 μ with an overall pressure of 10^{-6} torr; all other conditions as in second footnote.

the amplitude of the electron energy. Furthermore, in the EI-MS system, the molecule becomes ionized by the interaction of an electron with ultimate ejection of another electron to yield the positively charged molecule which then generates lower mass fragments of varying degrees dependent upon the total electron energy. At the operating pressure of the EI ion source, only unimolecular reactions are appreciable; reaction between ions or ions and molecules are rarely important[26].

On the contrary, in CI-MS, the efficiency of ionization is controlled by the relative energy level of the charged species derived from the reagent gas, which in turn reacts with the sample molecule by charge transfer and by accepting or releasing of a proton (H^+), a hydride ion (H^-) or an alkyl carbonium ion (RCH_2^+) to generate the quasi-molecular ion and/or its recombination fragments. Under the operating pressure of the CI ion source, reactions between ions or between ions and molecules are very common. In relation to the three most commonly used reagent gases in CI-MS, the degree of energy transfer in the CI processes is greatest for helium, with methane being less and isobutane the least. Because of the nature of the secondary reactions between ions and molecules in the processes of chemical ionization mass spectrometry, the levels of energy transfer in CI dependent upon the reagent gases is much less than that in the EI processes, even at low electron energies (20 eV or less).

Tables 6A and 6B illustrate the effect of various levels of ionization energy on the EI and CI mass spectra of methyl palmitate. As represented in Table 6A, by reducing the electron energy there is an increased abundance of high-molecular-

TABLE 6A

PARAMETERS AFFECTING ELECTRON IMPACT MASS SPECTRA FOR METHYL PALMITATE

Chromatographic conditions as in Table 4. Fig. 1 represents the reconstructed gas chromatogram of the 70-eV EI system. Three micrograms of methyl palmitate were injected for each electron energy condition. Values in parentheses are background values.

m/e	*Abundance (%)*						
	70 eV, spectrum 21	*70 eV, spectrum 22*	*70 eV, spectrum 24*	*70 eV, spectrum 32*	*40 eV, spectrum 24*	*20 eV, spectrum 24*	*10 eV, spectrum 24*
271	—	—	0.07	(0.58)	0.03	0.09	—
270	1.52	0.74	0.60	(0.58)	0.40	0.45	—
239	—	0.46	0.21	(0.58)	0.40	0.36	—
227	1.81	1.30	0.81	(0.58)	0.91	1.08	—
199	1.21	1.11	0.74	1.17	0.73	1.08	—
185	1.82	1.57	0.99	(0.58)	1.21	1.72	—
171	1.82	1.85	1.06	1.17	1.79	1.90	—
157	0.91	0.83	0.81	(0.58)	0.73	1.17	—
143	4.87	4.64	5.25	4.70	4.03	7.80	—
129	2.43	2.60	1.70	2.35	3.44	2.72	—
115	1.21	1.48	1.13	—	1.54	2.17	—
111	0.60	0.74	0.63	1.17	0.73	0.90	—
101	2.43	2.23	3.48	3.52	2.42	4.53	—
97	2.43	3.71	2.13	2.35	3.66	2.17	—
87	51.82	44.05	35.50	37.64	48.93	35.75	33.3
83	4.87	5.01	5.61	5.88	4.47	5.62	—
74	100	100	100	100	100	100	100
71	5.48	4.83	3.55	3.52	5.28	2.35	—
69	12.19	10.40	11.50	12.94	10.40	6.17	—
60	—	0.18	0.28	1.17	0.29	0.18	—

weight ions of *m/e* 100–227. Upon reduction of the electron energy below 20 eV, there is a great increase in sample size requirements. Maintaining a constant sample size of 3 μg for the experiments in Table 6A, one will find that the total ion current for the 70 and 40 eV conditions are comparable; on the contrary, the sample requirements for 20 eV are at least twice those of 70 eV while the total ion current for the 10-eV analyses is only about 2% of that for the 70 or 40 eV conditions. As can be concluded from Table 6A, one may increase the abundance of ions from *m/e* 100 to the molecular range *m/e* 270 by reduction of the electron energy with the production of an increased sample requirement.

Another means of decreasing the effective ionization energy would be to analyze the sample by CI-MS. As indicative of Table 6B, the helium CI spectrum of methyl palmitate is very similar to the EI spectra in Table 6A. In addition, the molecular ion (M^+) of *m/e* 270 in the helium spectrum is much more abundant than even in the 20-eV EI spectrum. In cases where the investigator may have only a CI system, by use of the high-pressure system with helium as reagent gas, one can mimic EI spectra very satisfactorily. When the effective level of energy transfer is lowered further by use of methane or isobutane, an additional increase in the abundance of ions in the molecular region occurs. In the case of the methyl ester (Table

TABLE 6B

EFFECT OF REAGENT GASES ON CHEMICAL IONIZATION SPECTRA FOR METHYL PALMITATE

Analytical conditions as in Table 4.

m/e	*Abundance (%)*		
	Helium	*Methane*	*Isobutane*
311	—	4.11	0.49
299	—	13.14	—
285	—	1.38	—
271	1.72	100	100
270	8.23	17.14	3.49
269	0.57	69.44	4.81
239	2.10	10.14	0.66
227	2.10	2.02	0.22
213	0.38	1.54	—
199	0.76	1.44	0.14
185	1.34	1.01	0.09
173	—	1.01	—
171	1.53	1.01	0.07
159	—	1.17	—
157	—	0.90	0.14
145	—	1.17	—
143	6.13	2.67	0.63
131	—	1.06	—
129	4.21	1.81	0.19
117	—	1.06	—
115	1.91	1.92	0.24
111	2.29	0.64	—
103	—	1.17	
101	4.59	1.38	0.29
97	7.66	0.85	0.29
87	57.85	10.14	2.30
83	16.09	0.96	—
74	100	20.61	2.00
60	0.38	0.10	—

6B), the quasi-molecular $(M + 1)^+$ ion of *m/e* 271 is the base peak (100% abundance) with a much less abundant *m/e* 74 ion. In addition, the recombination ion $(M + CH_3)^+$ of *m/e* 285, $(M + C_2H_5)^+$ of *m/e* 299 and the $(M + C_3H_5)^+$ ion of *m/e* 311 are very abundant in the methane spectrum. These types of recombination fragments greatly assist in location of the molecular ion in CI spectra. By reducing the effective level of ionization by use of isobutane as reagent gas in the CI system, one may further increase the abundance of ions in the molecular region. In numerous cases, because of the temperature requirement for separation and elution of various components on the GC–MS system, one will note that because of thermal effects, the quasi-molecular ions and/or recombination fragments for a very non-volatile material may not be highly detectable even in the methane spectra. By use of the isobutane system with lower effective ionizing energy, one may be able to detect and clarify the presence of ions in the molecular region even with the high GC and manifold temperature requirements.

(c) Ion source pressure

As described earlier, CI-MS is a type of high-pressure MS in which ion and ion–molecule reactions take place to produce charged species. On the contrary, the electron impact process operates at a very low pressure to produce unimolecular reactions. In the EI system, increased ion source pressure caused either by an air leak, by admission of helium carrier gas through the separator or by excess sample concentration will result in reduction in overall sensitivity caused by the decreased ionization efficiency and by the inherent propagation of multi-molecular reactions. Most EI systems operate optimally in the 10^{-6}–10^{-4} torr range with lower sensitivity occurring at high-pressure conditions. Routinely, with the CI system the pressure of the closed ion source is operated optimally at about 1000 μ with an overall pressure of about 10^{-5} torr. Table 7 illustrates the effect of ion source pressure on CI methane spectra. As the ion source pressure was increased from 100 to 2000 μ, there was an increase in the ionization efficiency with maximum efficiency being in the region of 1000 μ. There were drastic changes in the characteristics of the mass spectra within this pressure range. At the lower ion source pressures (100–300 μ), one obtains very

TABLE 7

EFFECT OF ION SOURCE PRESSURE ON METHANE CI SPECTRA FOR METHYL PALMITATE

Analytical conditions: high-voltage power supply, 3000 Volts; manifold temperature, 100°; ion source heater, off (50° by heat transfer) from manifold; ion source pressure as specified in table; emission current, 500 μA; electron energy, 70 eV; lens voltage, 10 V; repeller voltage, 0 V; ion energy, 2 V; pre-amp sensitivity, 10^{-6} A/V. Scanning range: 60–150; 151–350. Solid probe temperature off. Scan time 4:8. Sample: 3.0 μg of methyl palmitate by solid probe.

m/e	*Abundance (%)*			
	I.S.P. 300 μ*	*I.S.P. 600 μ*	*I.S.P. 1000 μ*	*I.S.P. 1800 μ*
311	—	4.54	5.00	6.71
299	—	13.63	18.33	13.42
285	—	—	—	2.68
271	3.70	93.18	100	100
270	7.40	27.27	20.00	11.40
269	—	47.72	48.30	46.97
255	—	—	5.00	4.02
239	3.70	11.36	6.66	8.05
227	—	4.54	1.66	2.01
213	—	2.27	—	0.67
199	3.70	—	1.66	1.34
185	—	2.27	3.33	2.68
171	—	6.81	—	1.34
159	—	—	3.33	2.01
143	14.81	9.09	3.33	2.68
115	—	13.63	3.33	4.02
87	62.96	63.63	10.00	10.73
83	7.40	9.09	3.33	1.34
74	100	100	26.60	21.47
60	3.70	4.54	—	1.34

* I.S.P. = ion source pressure.

weak spectra with EI characteristics and a base peak of *m/e* 74 with the molecular ion of *m/e* 270, produced by the unimolecular reactions. As one increases the ion source pressure from 600 to 1200 μ, the very abundant quasi-molecular ion and base peak of *m/e* 271 with the appropriate recombination fragments and the less abundant *m/e* 74 ion are present. With increased ion source pressure from 1800 to above 2000 μ, the ionizing efficiency became less with the accompanying overall loss in sensitivity. As indicated by the results of Table 7, one may affect the spectral characteristics of the unimolecular reaction at low pressure which are similar to the EI spectra to coincide with the ion–molecule reaction CI spectra at high pressure.

(d) Other factors

Manual and computerized operation of GC–MS systems require daily calibration and resolution checks. Regardless of the superiority of spectra collected during an analysis, the final results are limited by the calibration conditions. An improperly numbered mass fragment is useless. Incorrect numbering of ions may occur much more readily in the manual mode than with computerized systems. Our normal operating procedure consists in calibration at least once every operating day for the appropriate EI or EI–CI system with day-to-day monitoring for characteristic changes in spectra that may be caused by electronic problems or by less than optimal operational conditions. FC-43 (perfluorotributylamine) is used for computer calibration of our system. Over-resolution of a system in order to increase the abundance of ions of high mass (350–750) will result in loss in sensitivity due to poor ion transmission. Routine utilization of a GC–MS system with or without a computer system requires a dedicated operator in order to minimize operational and electronic problems.

Considering that all operational parameters for the GC and MS systems are satisfactory for production of representative mass spectra, one must then consider the sample size for analysis. With most of the more recently designed mass spectrometers, one may obtain very satisfactory mass spectra with submicrogram amounts. Sample requirements for a GC–MS system are very dependent upon: (a) the chemical characteristics of the compound to be analyzed; (b) the operational parameters of the GC–MS system; and (c) the means of data collection for obtaining the mass spectra. In this laboratory with the dual Finnigan EI–CI computerized system and a very chemically stable compound, very representative mass spectra may be obtained routinely with 10–100 ng. As will be discussed later for mass fragmentography, detection in the picogram range is routinely possible. As has been noted in other reports[27], the sensitivity of a mass spectrometer is very dependent upon the degree of dedicated care that the system receives. Over-loading of samples to produce less than optimal electronic conditions will greatly increase the sample requirements.

After one has completed the GC–MS analyses of an appropriate aliquot of sample in which the compound to be identified resides in a single component GC–MS peak, one must then choose the most representative spectrum under these operating conditions for this compound. The most representative spectrum during the scanning mode of a GC–MS system would be obtained at the time when the concentration of samples is greatest and at which time the sample concentration in the ion source is relatively constant. Fig. 1 and Table 6A illustrate the effect of concentration of the compound on the appearance of mass spectra of methyl palmitate. During these analyses, the scan range of 60–350 was accomplished in about 2.4 sec. From Table 6A,

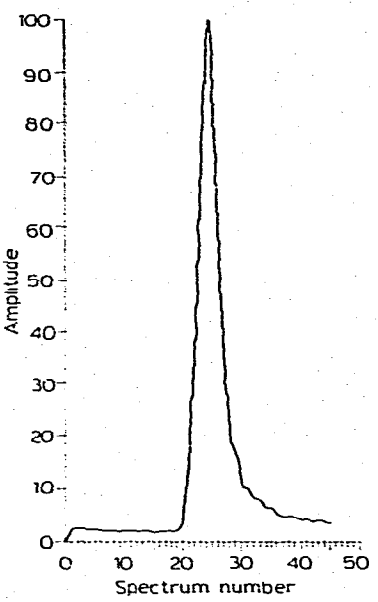


Fig. 1. Reconstructed chromatogram of the GC–EI-MS system (70 eV) for methyl palmitate (SE-30, 200°).

one can see that spectra on the leading side of the GC peak (spectra 21–23) have a much greater abundance of high-mass fragments than the spectra on the trailing portion of the peak (spectra 26–35), which favor the low-mass ions. This effect is more pronounced if the scan time is relatively long with respect to the GC peak width.

In addition to choosing the most representative spectra for the compound, one must choose the appropriate background spectrum very carefully. In the utilization of a GC–MS system, it becomes necessary to differentiate between ions which were generated from column bleed and pump oil and those which were produced by the compound to be identified.

C. Data prior to gas chromatography–mass spectrometry

As described earlier in section 2 on General protocol, it is always advisable to have additional information concerning (a) the UV–visible spectra; (b) the IR spectrum; and (c) the NMR spectrum of the unknown component in order to make the identification of this compound more conclusive. Often, because of sample requirements, one will be unable to obtain NMR and IR spectra.

Chromatographic data concerning the multiplicity of components in the sample, the GC retention characteristics and the chemical reactivity and thermal lability of the component are essential prior to GC–MS analyses. Even though initially more sample may be expended for the GC analyses prior to MS characterization, the information gained concerning column conditions and lability will be useful in the final completion of the structural identification.

Analysis of the suspected known compound with the unknown by all possible analytical means prior to and during the GC–MS analyses eliminates many false assumptions that may have been postulated concerning the structure of the environ-

mental agent or its metabolites. Many variations of computer mass spectral library[28–34] search routines exist for the comparison of an unknown spectrum with known spectra of a particular file. Some of these systems even project the degree of similarity of the known and unknown spectra by use of a similarity index[28–31,34]. It is very advantageous to use a mass spectral library for the purpose of obtaining the possible structure or type of compound that has been analyzed by the GC–MS system. On the contrary, the final conclusive identification should always be made by a direct comparison of the unknown spectrum with the spectrum of the standard known synthetic compound under identical analytical conditions.

D. Purpose and areas of inclusion

The main purpose of this discussion is to describe the usefulness and limitations of GLC and MS in the chemical evaluation of potentially hazardous environmental agents. The advantages of cross-referenced utilization of GC–EI-MS and GC–CI-MS for a wide variety of specific ubiquitous environmental agents and their metabolites are discussed, but there is no attempt to give a complete survey of the literature and this section will not serve the reader as a catalogued source of mass spectra. On the contrary, we discuss the significance of the mass spectral data for the investigation of the chemical, biological and metabolic processes of potentially hazardous environmental agents. For a general discussion of GC–MS, the reader may refer to a recent book by McFadden[35].

E. Characterization by gas chromatography and gas chromatography–mass spectrometry

(a) Man-made environmental agents

(i) Chlorinated hydrocarbons

DDT, its metabolites and related systems. For convenience of discussion, the DDT family of compounds (Fig. 2) is divided into three sub-groups, *viz.*, the saturated types, including DDT, DDD, DDMS and DDM; the unsaturated types, including DDE, DDMU and DDNU; and the oxidative types, including DBH, DBP, DDOH, DDOH acetate, and DDA ethyl esters. The importance of being able to determine the extent of contamination of the environment by these systems is derived from the extensive use and therefore widespread occurrence of DDT. DDT is one of the oldest, if not the oldest, chlorinated hydrocarbon pesticide and is still in use in some parts of the world today, especially in the control of malaria. Its dehydrochlorination product, DDE, is the most ubiquitous and abundant chlorinated hydrocarbon in the environment today, although the polychlorinated biphenyls are approaching the same level and may surpass DDE in the next few years as decreased usage of DDT and slow degradation of DDE render it less abundant. Only the salient features of their EI and CI spectra will be reported here, as a detailed comparison of their spectra has been previously reported[36]. With regard to the chromatographic separation of these systems, Fig. 3 illustrates the separation of this family of compounds on a mixed[37] GC phase (11% OV-17 + QF-1). This is an excellent example of the separation properties of this mixed phase for a complex family of related compounds by GC interfaced with EI and CI-MS systems.

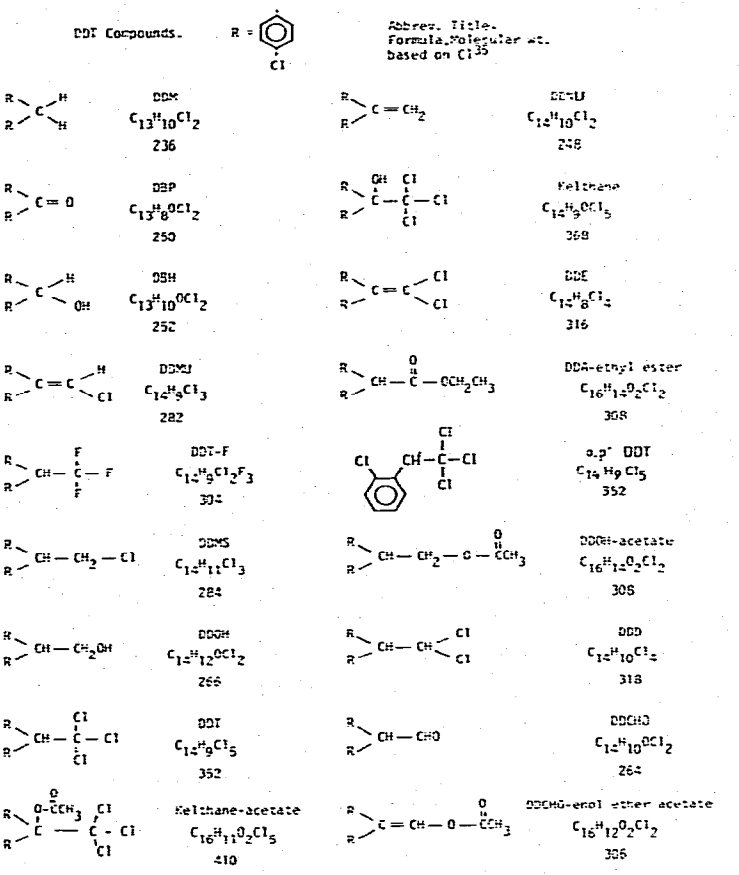


Fig. 2. Chemical structure of DDT compounds.

Saturated systems. The EI (70 eV) mass spectra of the saturated compounds have several fragments in common corresponding to the loss of HCl and Cl, singly or in combination, as well as the facile cleavage of the C–C bond of the ethane portion of the molecule. The latter process affords a fragment $(C_{13}H_9Cl_2)^+$ of *m/e* 235, which is the base peak for all of the saturated systems studied[36] except for DDM, for which *m/e* 236 is the parent peak and *m/e* 201 (M−Cl) is the base peak. In contrast to the EI spectra of these systems, the most characteristic fragment found in their CI spectra is that resulting from cleavage of a *p*-chlorophenyl group, which accounts for the base peaks of DDD, DDMS, DDM and, in some spectra, for DDT as well.

This apparently facile fragmentation process may reflect an electron-rich carbon–phenyl bond that is susceptible to attack by an alkyl carbonium ion. A similar enzymatic attack of an electrophilic oxygen species on these systems would be expected to lead subsequently to *p*-chlorobenzoic acid. There is at least one report[35] that *p*-chlorobenzoic acid is formed as a microbial metabolite of DDT. It is not unlikely that microorganisms are the chief agents of DDT degradation in nature.

The parent ions for DDT and DDD in EI-MS are very weak (<1%) with some improvement (*ca.* 4%) in DDMS. The CI spectra showed parent ions for these compounds varying in relative intensity from 3 to 8%.

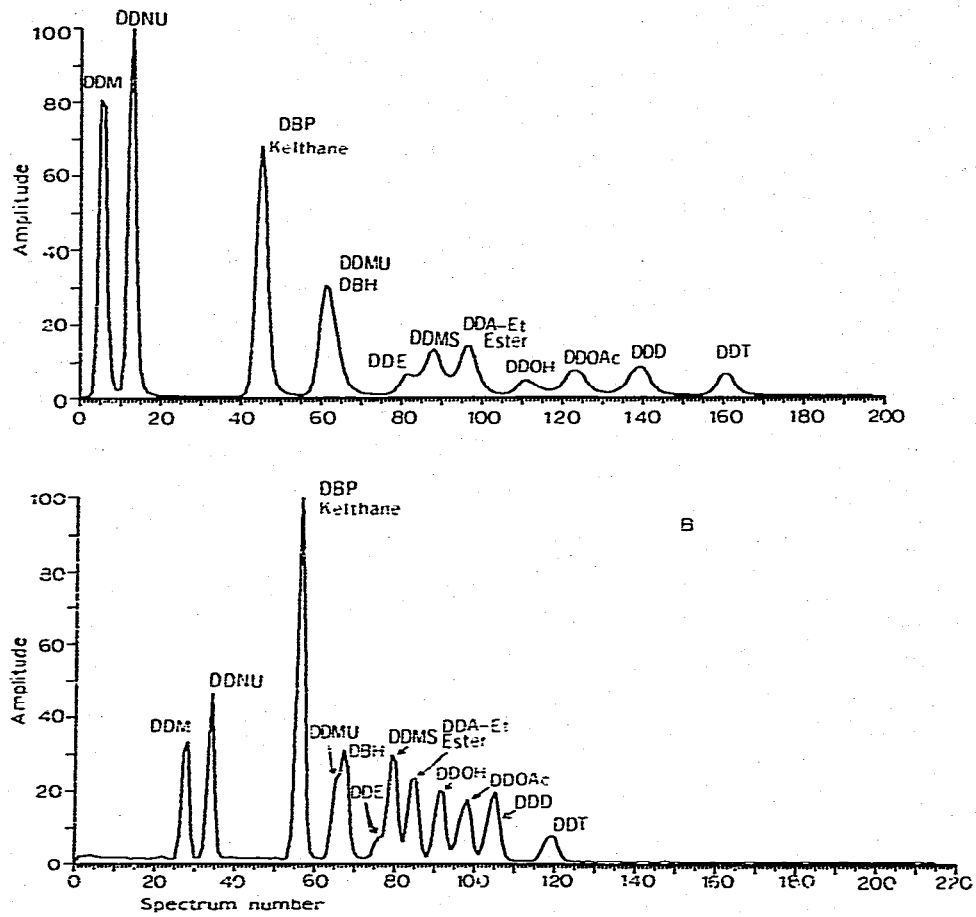


Fig. 3. Reconstructed chromatograms of related DDT compounds (OV-17—QF-1, 160–225° at 10°/min). A, GC–EI-MS, 70 eV. B, GC–CI-MS, methane.

Unsaturated systems. DDE, DDMU, and DDNU have in common the loss of two Cl moieties in one form or another to produce fragments $(M-2Cl)^+$ at *m/e* 246, 212, and 178 found as their base peaks. These systems also afforded a charasteristic and common fragment at *m/e* 233 reminiscent of the *m/e* 235 ion found for the saturated systems. One can reasonably propose dichlorofluorene-type structures for these fragments, as there is evidence[39] for their formation from DDE under photolytic conditions, and they are resonance stabilized. Again, in contrast to the EI spectra, the CI spectra of these unsaturated compounds contain very little, if any, of the *m/e* 233 fragment but reveal the facile cleavage of the *p*-chlorophenyl group to produce the fragment $[M-(p\text{-ClPh})]^+$ occurring as the base peak in DDMU and DDNU and of

moderate intensity (16%) in DDE as well. EI conditions were generally better for detecting parent ions (DDE, 57%; DDMU, 38%; DDNU, 83%) for the unsaturated systems studied. As a result, small amounts of these compounds can be readily detected as components of complex mixtures.

Oxidative systems. The oxidative metabolite systems afford somewhat more unique and charasteristic mass spectra as a result of the specific functionality they contain. Generally, those systems bearing oxygen at their benzhydryl carbon fragment in EI to form the *p*-chlorophenylcarbonyl (p-Cl·Ph·C≡O)$^+$ ion at *m/e* 139, which is usually the base peak. To a lesser extent, the charge is carried by the *p*-chlorophenyl fragment (*m/e* 111). However, under CI conditions, the ion of *m/e* 111 is found as the base peak for DBP, DBH and DDA ethyl ester and is also present in 50% and 4% of the relative intensities for DDOH and its acetate, respectively. Again, there is evidence for the facile EI C_α–C_β bond cleavage in the systems (base peak for DDOH and DDA ethyl ester, 75% for DDOH acetate). GC–EI-MS generally gave less abundant parent ions for the oxidative systems studied, but the CI quasi-molecular ion region of DDOH and its acetate were complicated by the appearance of recombination–elimination fragments. Nevertheless, such processes appear to be characteristic and diagnostic of those molecules in which an oxygen containing leaving group is available that can apparently be substituted at either the C_α or C_β atom of the ethane portion of the molecule, as kelthane acetate also afforded similarly derived fragments.

Both the EI and CI spectra obtained either by GC or direct probe for kelthane (DDT hydroxylated at the benzhydryl carbon) were essentially identical with those obtained for DBP, a known[40] thermal decomposition product of kelthane. In contrast, kelthane acetate (acetoxy-DDT) gave EI spectra very similar to that found for DDE, a known[41] thermal or photolytic decomposition product of kelthane acetate. On the other hand, CI spectra of kelthane acetate showed the expected fragments for the structure as indicated (Fig. 4). Although the parent ion was not present, a recombination–elimination fragment at *m/e* 378 confirms the molecular weight. Another unstable DDT system examined was the suspected aldehyde metabolic precursor of DDA, DDCHO. This aldehyde undergoes slow decomposition with or without solvent to form DBP. Although the EI spectra of DDCHO are descriptive, CI-MS appears to be more desirable for low-level detection of the aldehyde, as its CI spectra show $(M+1)^+$ as the base peak. In addition, the CI spectra of the stable enol ether acetate derivative can be used for confirmation [$(M-1)^+$, 40%].

DDT isomers and analogs. MS has been used with some success for distinguishing between stereoisomers and closely related analogues of various compounds[42]. The spectra of *p,p'*-DDT, its *o,p'*-isomer and its trifluoromethyl analog have been examined. The EI spectra of the *o,p'*-isomer closely resemble that of the *p,p'*-isomer, at least qualitatively, as one would expect. The CF_3 analog, although yielding some fragments in common with *p,p'*- and *o,p'*-DDT is clearly distinguishable from them, largely as a result of the effects of the lesser steric requirements of the CF_3 group

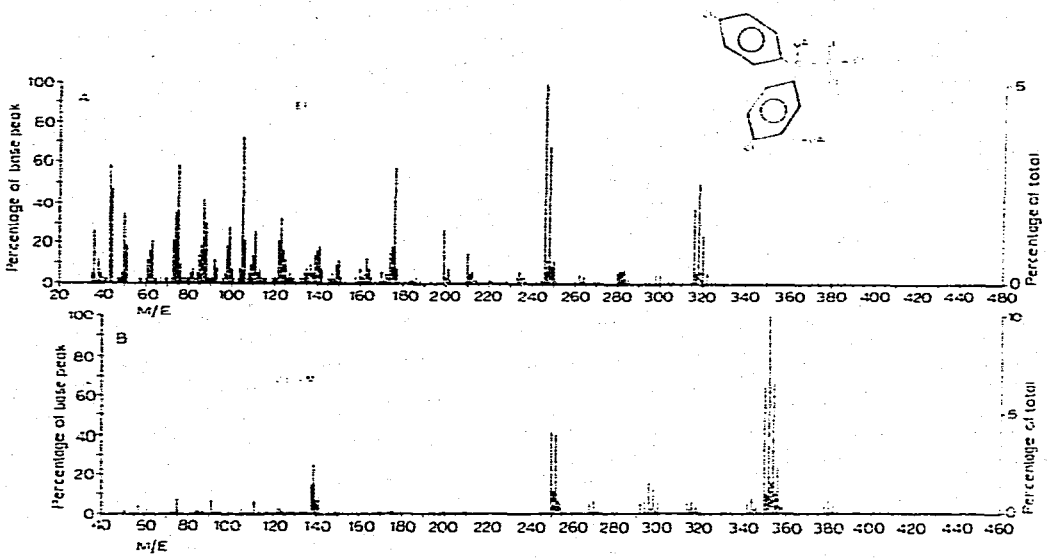


Fig. 4. Mass spectra of kelthane acetate (OV-1, 160–225 at 10 min). A, EI, 70 eV. B, CI, methane.

coupled with the increased stability of the CF bonds. Table 8 shows some characteristics and common fragments, some of which reflect the different steric requirements and bond strengths. The drastically different polarizing effects of the CF_3 group *versus* the CCl_3 group are also evident in the chromatographic behavior of these two compounds (Fig. 5). The reader is referred to a recent review by Fishbein[13] for a detailed discussion of the chromatographic and biological properties of DDT and its metabolites.

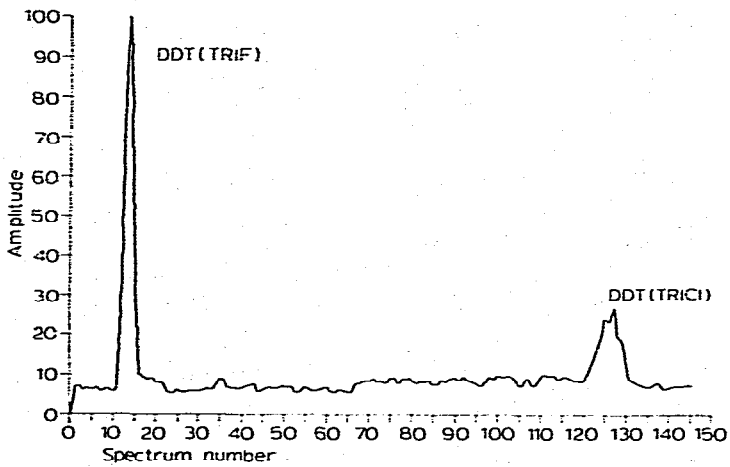


Fig. 5. Reconstructed chromatogram of trifluorinated DDT and trichlorinated DDT. GC–CI-MS, methane. (OV-17 + QF-1, 160–225 at 10 /min.)

TABLE 8

SELECTED MASS FRAGMENTS (EI, 70 eV) FOR *p,p'*- AND *o,p'*-DDT AND THE CF_3 ANALOG OF DDT

Compound	*Base peak m/e*	*Relative abundance (%)*			
		Parent ion	*199**	*176***	*165****
C*p'*-DDT	235	< 1	28	61	70
p,p'-DDT	235	< 1	33	16	67
o-F_3 analog	235	44	24	1	62

* $C_{12}H_5Cl_4 (M - CHX_3)^+$.
** $C_{11}H_5X_3 (M - HX_3)^+$.
*** $C_{12}H_5X_3 (M - CX_3)^+$.

Chlorinated polycyclodiene pesticides. The chlorinated polycyclodiene pesticides have also received extensive usage. This family of environmental agents are among the more persistant chlorinated hydrocarbons that contaminate the environment. Although many variations of the polycyclodiene are possible, the more commonly used are the so called "drin" insecticides: aldrin and dieldrin and their stereoisomers, isodrin and endrin, respectively. As a result of the structural rigidity of these systems, together with their specific group functionality, they possess unique stereochemistry and hence undergo descriptive fragmentation processes in the mass spectrometer.

The discussion of the mass spectral properties of the polycyclodienes will be limited to these "drin" insecticides, which will be divided into two groups, *viz.*, aldrin, dieldrin and their transformation products, and isodrin, endrin and their transformation products. The transformation products are derived from many sources, including synthesis, authentic metabolites, photolysis and thermolysis. This discussion will be limited to the more distinguishable and characteristic fragmentation processes for these compounds. The reader is referred to previous reports providing more detailed information[36,41–46] on the nature of the extensive fragmentation which these systems can undergo.

Aldrin, dieldrin and transformation products. The salient features of both the EI and CI mass spectra of these polycyclodiene systems include ions corresponding to a set of retro-Diels-Alder processes, ions resulting from successive losses of Cl, HCl, or both, ions produced by combinations of a retro-Diels-Alder process with a preliminary or subsequent loss of Cl or HCl, and ions more specifically involving the various groups substituted at the C_4 and C_5 positions (See Figs. 6A and 6B for structures and numbering systems). Abundant quasi-molecular ions, both $(M - 1)^+$ and, to a lesser extent, $(M + 1)^+$ have been noted in the CI spectra. The mass spectra of these compounds, which contain as many as six chlorine atoms, are complicated by the chlorine isotope distribution patterns.

Aldrin was the simplest polycyclodiene system examined. Its base peak under EI conditions was found to be at *m/e* 66, indicative of a preference for the retro-Diels-Alder process, releasing cyclopentadiene (*m/e* 66) and hexachloronorbornadiene (*m/e* 296), which is found in relatively low abundance (< 0.5%). The CI spectrum of aldrin, on the other hand, contains *m/e* 326 as its base peak, corresponding to the loss of HCl together with a quasi-molecular ion.

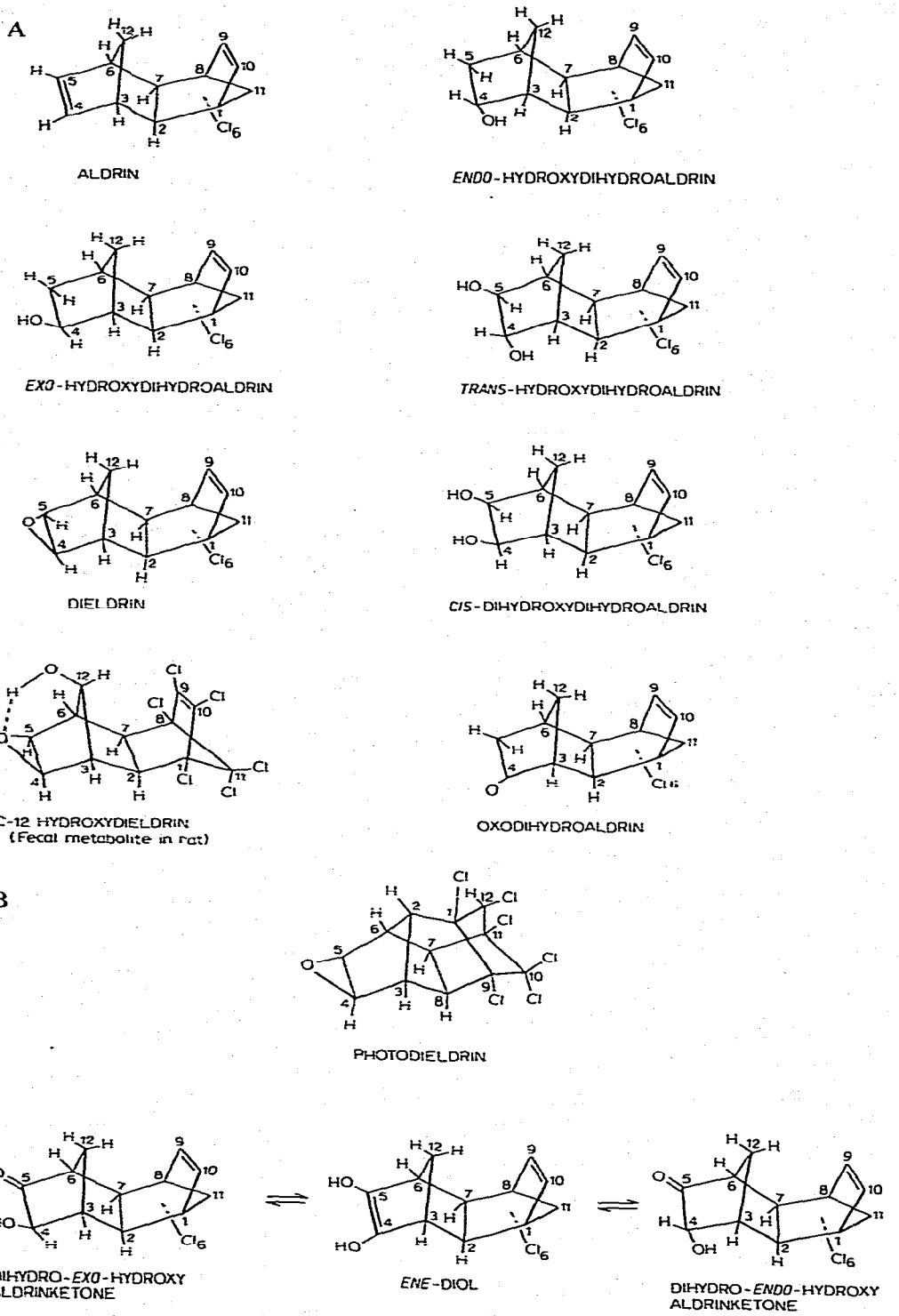


Fig. 6. Chemical structure of (A) aldrin and (B) dieldrin transformation products.

In comparison, the epoxide derivative of aldrin, namely dieldrin, affords an ion at *m/e* 79 as its base peak in both its EI and CI spectra. This ion occurs as the base peak in several other systems related to dieldrin and, apparently, indicates a favored retro-Diels-Alder process involving the formation of norbornadiene oxide with a subsequent loss of CHO to produce a cyclohexadiene system, $(C_6H_7)^+$. If one compares the CI spectrum of desmethylene dieldrin (no C_3–C_6 bridging methylene group), the base peak shifts to *m/e* 67, which most likely corresponds to protonated cyclopentadiene, $(C_5H_7)^+$. In addition, the fragment at *m/e* 79 is found together with an abundant fragment at *m/e* 95, corresponding to a protonated cyclohexadienone.

A ketone isomer (oxodihydroaldrin) of dieldrin, which is obtained via aldrin hydroboration and subsequent conversion to the alcohol followed by oxidation[47], yields EI spectra which contain an *m/e* 79 base peak, but in the CI spectra, the base peak is contained in the quasi-molecular region.

A second dieldrin isomer, which appears to be more toxic[48] than dieldrin, is obtained on photolysis[49] of dieldrin. This rearrangement product, known as photodieldrin, affords a base peak of *m/e* 81 in its EI spectra which can be assigned a protonated cyclopentadienone structure. The CI spectrum, on the other hand, contained its base peak in the $(M-Cl)^+$ pattern and was accompanied by much less relatively abundant (5%) quasi-molecular ion. The CI spectrum also contained a characteristic ion at *m/e* 163, the exact nature of which is not completely understood. Nevertheless, one would expect a somewhat reduced propensity for photodieldrin to undergo retro-Diels-Alder processes, as it has an additional bridging bond to be broken. It is interesting that the CI spectra of dieldrin also contain an ion at *m/e* 81, suggesting that some "photodieldrin-like" ions may occur during its fragmentation in the mass spectrometer.

Under the carbonium-ion-rich "solvolysis-like" conditions of the CI analysis, it is observed that many abundant ions are derived from chlorine losses. It has recently been shown in our laboratory[50] that, under conditions which also appear to be carbonium-ion-rich in nature, these chlorinated polycyclodiene "drin" insecticides can be stereoselectively and regiospecifically dechlorinated at the dichloromethylene bridge (chlorine *syn* to double bond). Photolyses[51] of aldrin and dieldrin have yielded deschloro-products, but they are primarily those derived from the loss of dichloroethylene chlorine atoms. As reductive dechlorination processes may be important in the degradation of these compounds in the environment, mass spectral elaboration of the resultant products is being undertaken.

The hydroxylic metabolites of aldrin and dieldrin include the *exo* and *endo* dihydroaldrin alcohols[52], the *cis*- and *trans*-dihydroaldrindiols[53] and C-12 *syn*-hydroxydieldrin[54]. In addition, two oxidation products of the *cis*-dihydroaldrindiol were analyzed. These hydroxylic compounds were generally analyzed as their trimethylsilyl ether (TMS) derivatives, as better chromatographic properties resulted, but in some instances they were analyzed directly or in the form of their acetate derivatives.

The normalized EI mass spectra of the TMS derivatives of these hydroxylic metabolites were generally not very informative, as the base peak at *m/e* 73 corresponding to the TMS group accounted for appreciable amounts of the total ion yield.

However, with the limited mass search capabilities of the computer and/or amplitude expansion, one can discern the usual fragment ions resulting from loss of Cl and HCl and retro-Diels-Alder processes. The CI spectra also generally contain *m/e* 73 as the base peak, but are much more abundant in ions at or near the quasi-molecular ion region. The quasi-molecular ion was discernible in addition to ions more specifically involving the TMS group, *viz.*, $(M-CH_3)$ and $[M-(TMSOH + HCl)]^+$.

An actual biological metabolite sample obtained from dieldrin metabolism in the rat, *viz.*, the major fecal metabolite (C-12 *syn*-hydroxydieldrin, now synthesized by other workers[55]) was examined directly with the EI- and CI-MS systems. The EI spectra are essentially lacking a parent ion, but the CI spectra clearly indicate a quasi-molecular ion, $(M+1)^+$, indicative of the hydroxylated dieldrin molecule, in addition to an abundant ion at *m/e* 145 and a shift in base peak from *m/e* 95 (EI) to *m/e* 79 (CI). The base peak shift, in addition to other changes, has been previously reported[51] as a problem associated with the direct probe sequential scan EI-MS analysis of this compound.

Other hydroxylic systems examined directly with the CI-MS system included the two oxidation products[53] of the *cis*-dihydroaldrindiol. The suspected hydroxyketone epimerase oxidation products are susceptive to tautomeric interconversion via an ene-diol intermediate. It was of interest to see which of these forms could be isolated, as at least one of them appeared to be a likely intermediate for the biological epimerization[53] of the *cis*-diol to the *trans*-diol catalyzed by microsomal enzymes found in rat liver.

As indicated earlier two systems were isolated and analyzed by GC–MS under CI conditions. The major component afforded a mass spectrum with a quasi-molecular ion $(M-1)^+$ at *m/e* 393 (22%) and a base peak of *m/e* 95 (C_6H_7O). In addition, other ions were present corresponding to the loss of CHO, CHOH and HCl, and CHO–CHOH–Cl. On the basis of the MS and IR data of this isomer, it was assigned the hydroxyketone structure (presumably the *exo*-isomer). The other component, with a longer retention time on the OV-1 column, showed a quasi-molecular ion, $(M+1)^+$, at *m/e* 395 and a base peak of *m/e* 79 (C_6H_7). Other fragment ions corresponded to losses of CHO and 2Cl, and $C_2H_2O_2$ and 2Cl. The spectrum was both qualitatively and quantitatively different from that of the other component. This isomer was assigned the ene-diol structure. This example illustrates the ability of CI-MS to distinguish between structurally similar tautomers.

Although MS is normally not desirable for the assignment of stereochemistry, it has some value[36] when applied to rigid polycyclic systems such as these and differences are expressed in terms of total ion yields. Chemical reaction studies involving opening of the oxirane ring of dieldrin have afforded a product mixture including the stereoisomeric *cis*- and *trans*-dihydroaldrin diacetates. As authentic samples of these diacetates were available (synthesized in our laboratory[56]) for comparison, it was possible to assign conveniently two of the peaks in the reconstructed gas chromatogram (RGC) to these diacetates as shown. However, without the aid of authenic samples, it is possible to obtain stereochemical information from the mass spectra of

these components. For example, the total ion RGC of the mixture shows that one diacetate (*m/e* 480 with the longest retention time) is present in a larger concentration than the other; however, the limited mass search RGC for the quasi-molecular ion region (*m/e* 480–486) indicates that about 2½ times the amount of *m/e* 480–486 ions are found for the diacetate with the shortest retention time. As the *cis*-oriented groups are more sterically compressed and, therefore, constitute a more unstable arrangement, one would expect to see a less abundant parent ion for the *cis*-diacetate with a corresponding higher abundance of the $(M-HOAc)^+$ fragment. This is indeed what is observed.

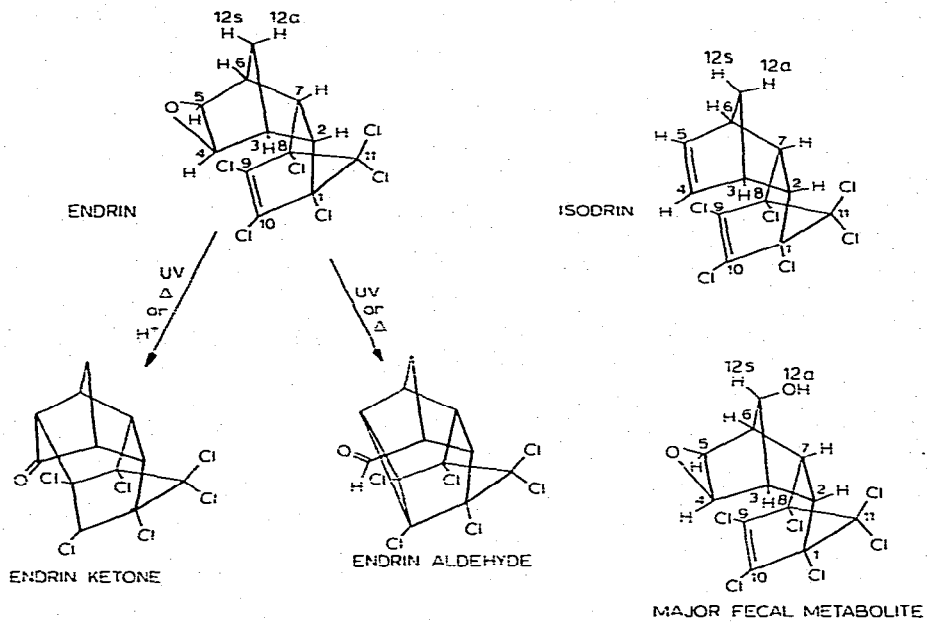


Fig. 7. Chemical structures of isodrin, endrin and transformation products.

Isodrin, endrin and transformation products (see Fig. 7 for structure). If one compares the mass spectral properties of isodrin and endrin *(endo-endo)*, skeletal isomers of aldrin and dieldrin *(endo-exo)*, the notable differences are primarily associated with the facile stereochemical rearrangements which occur between the two double bonds or the double bond and the oxirane ring. The more salient differences in the EI spectra of aldrin and isodrin which possibly reflect the different rearrangement propensities are the intensities of ions at *m/e* 193, 147, 180 and 101. Few, if any, of the first three ions (*m/e* 193, 147 and 180) are found in aldrin spectra with few ions of *m/e* 101 being found in isodrin spectra.

These differences in rearrangement propensities are perhaps more readily understandable from a comparison of endrin with dieldrin. For example, endrin gave by far the most complex EI spectra of the compounds investigated. Quantitatively, very different spectra could be obtained, depending on the mode of introduction (GC or direct probe) and the ionizing energy (20 or 70 eV) used. The endrin system rearranges readily[57] under a variety of conditions to form at least two other products, 1-ketoendrin (DKE) and a endrin aldehyde. Fig. 8 shows an RGC of the CI-MS system for a mixture of endrin and its two isomeric rearrangement products.

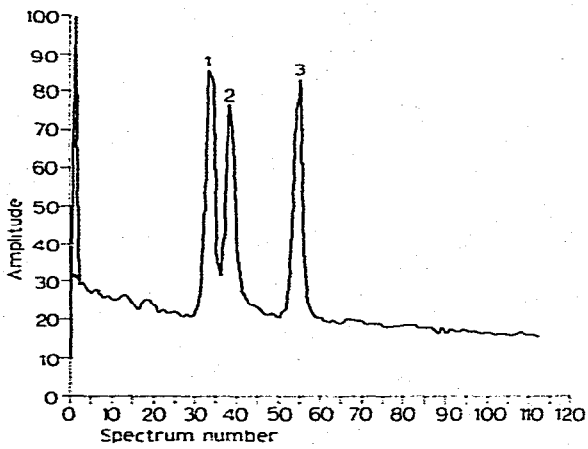


Fig. 8. Reconstructed chromatogram of endrin mixture, GC–CI-MS, methane (OV-1, 200–240° at 10°/min). 1 = Endrin; 2 = aldehyde; 3 = 1-ketoendrin.

On examination of the EI spectra of the aldehyde and ketone and re-examination of the endrin spectrum, it appears that the spectrum does contain fragments indicative of at least three structures of which 1-ketoendrin seems to be a major contributor. If one uses the computer to subtract the DKE spectra from endrin spectra, a fragment at *m/e* 261 ($C_7H_2Cl_5$) remains and appears to be a characteristic fragment of the true endrin structure. In a similar way, certain fragments can be shown to be characteristic of DKE, such as a fragment at *m/e* 315 (loss of CO, Cl). The base peak of both the aldehyde and the ketone was *m/e* 67. Again, utilizing the computer and the *m/e* 315 fragment, one can determine the percentage of total ions representing DKE in an endrin spectrum, and, therefore, under a given set of conditions measure the rearrangement propensities of endrin to DKE. The CI spectra of endrin, on the other hand, are generally much less complex and indicate less extensive rearrangement. At the same time, the CI spectra are rich in the quasi-molecular ions $(M+1)^+$. (See Fig. 9 for a comparison of an isobutane CI spectrum with a 70-eV EI spectrum.)

Less is known about the oxidative metabolism of endrin than that of dieldrin. The corresponding "diolic" metabolites have not been found, but hydroxylation at the C-12 methylene bridge appears to be a major route of metabolism. Although other portions of the molecule are apparently also attacked, the conversion is minor and the resulting products, which have not been fully identified, are unstable.

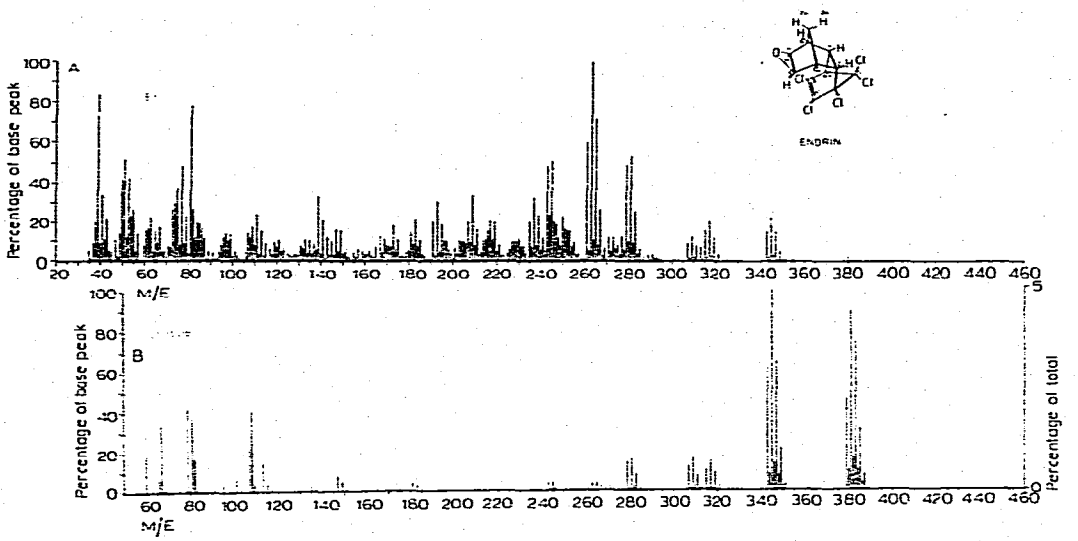
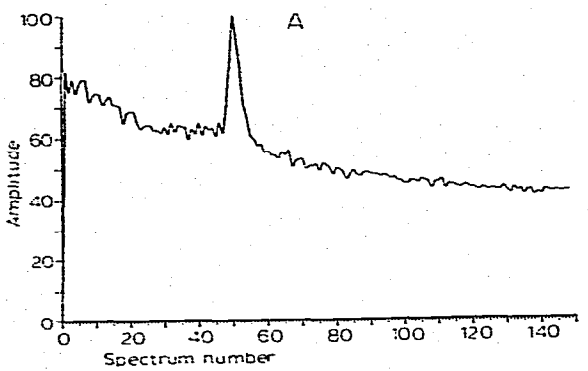


Fig. 9. Endrin mass spectra. A, GC–EI-MS, 70 eV. B, GC–CI-MS, isobutane.



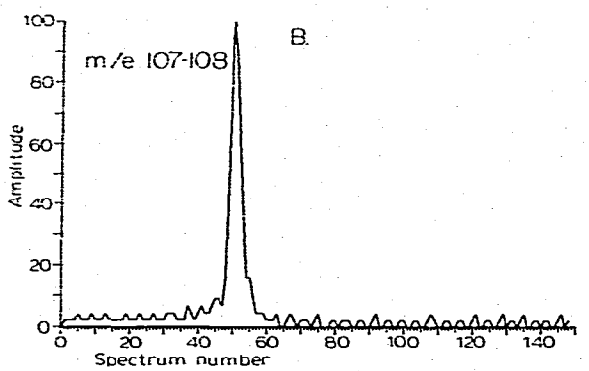


Fig. 10. Reconstructed chromatogram of endrin fecal metabolite, GC–CI-MS, methane (OV-1, 200–240° at 10°/min). A, RGC. B, Limited mass search, *m/e* 107–108.

Nevertheless, CI-MS has been used effectively[58] in the identification of the major fecal metabolite of endrin in the pine mouse and partial identification of other hydroxylic metabolites. It was shown[58] that a key fragment in detecting the presence of an uncaged hydroxylic metabolite of endrin (hydroxylated in the non-chlorinated portion) such as the major fecal metabolite is $(C_7H_7O)^+$ at *m/e* 107, resulting from a retro-Diels-Alder process (Fig. 10). On the basis of their spectral and chemical data[58], this metabolite was assigned the C-12 *anti*-hydroxyendrin structure. This was shown to be a correct assignment by recent workers[59] who synthesized both the *syn*- and *anti*-alcohol, which compelled them to reverse their previous assignment of *syn* stereochemistry to this metabolite isolated in their previous work[60] with rats. The *anti* structure is in contrast to the *syn* structure shown[55] to be the correct structure for the dieldrin rat fecal metabolite.

EI-MS analyses of the pine mouse metabolites[58] were of limited value as a result of the small amounts available and their increased lability to direct electron bombardment. However, it was observed that the base peak of the major endrin metabolite under EI conditions is *m/e* 95, as is found in spectra of the major dieldrin metabolite. CI-MS is particularly advantageous for aiding identification of such labile metabolite systems which are available in small amounts. (See Fig. 11 for methane and isobutane CI spectra of the major fecal metabolite.)

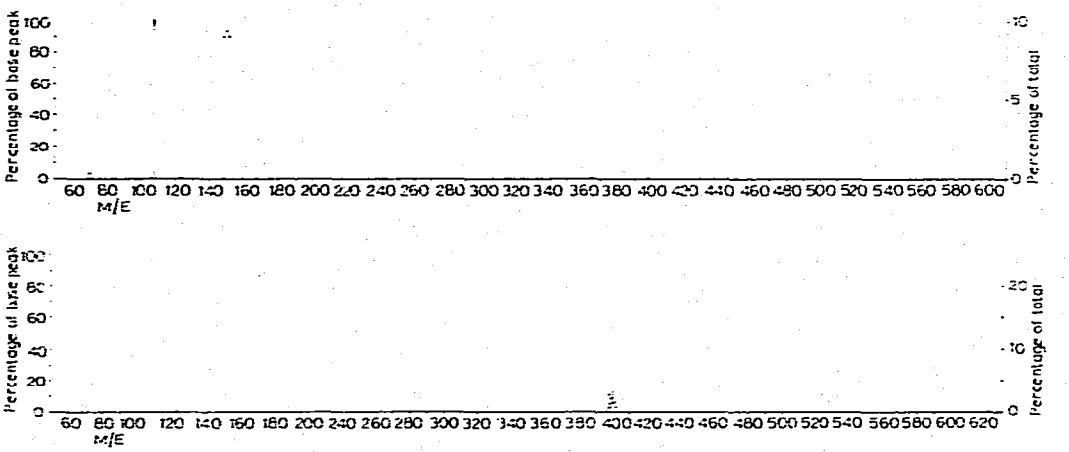


Fig. 11. Mass spectra of major endrin fecal metabolite (OV-1, 200–240° at 10°/min). A, GC–CI-MS, methane. B, GC–CI-MS, isobutane.

Polychlorinated biphenyls. Polychlorinated biphenyls (PCBs), together with DDE [1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethane], are reported[61] to be the most abundant chlorinated aromatic pollutants in the environment. PCBs have been found in extracts of pike, salmon, sea eagles[62], components of the food chain[63], and in human adipose tissue and human milk[61]. The commercial uses[61] of PCBs include coolant fluids for transformers and capacitors, plasticizers for electrical wire, high-pressure hydraulic fluids, machine cutting oils, heat transfer agents, and uses in formulations of epoxy paints, chlorinated rubber, printers' ink, textile dyes and carbonless reproducing paper.

Because of the ubiquitous nature of PCBs in the environment, together with

numerous ill-defined problems produced by complex mixtures of these compounds in respect to human health, greater clarification is needed in order to fully understand the potential health hazards associated with specific isomeric PCBs. The reader is referred to excellent review articles[61,65-67] on this subject in order to further understand the health problems associated with this family of compounds. The toxicology of PCBs is very poorly defined at present. Most of the results concerning toxicity and effects on biological systems have been obtained with complex commercial mixtures[61,66,67].

In this section, the discussion is limited to include problems of chemists concerned with the (a) analysis, (b) synthesis and (c) metabolism of PCBs.

Analysis. Suitable methods for the quantitative analyses of PCBs in biological systems are at present non-existent. Early investigations of PCBs further complicated the biological and analytical problems by use of complex isomeric PCB mixtures. Within the strict limitations of being able to define all contaminants of a mixture for the family profile of commercial PCBs for a specific type of sample, one may approximate, not quantitate, the PCB content by a combination of IR and GC. In the case of non-biological samples such as reported for immersion oils[68], with the contaminants being mainly hydrocarbons which could not be separated from the PCBs, prior screening of samples by IR spectroscopy followed by GC analysis of positive samples using an electron capture detector is useful. It must be emphasized that this type of approximation analysis for PCBs may be used only in very restricted instances in which one can define the contaminant and also can match, peak for peak, the components of the unknown chromatogram with all components of the chromatogram of a specific commercial PCB preparation.

Many attempts have been made to quantitate PCBs by GC[61-67] from biological samples derived by exposure to complex isomeric mixtures of PCBs. The validity of this type of GC quantitation of PCBs is highly questionable. Attempted approximations of the PCB content of biological samples by GC, regardless of the type of detection, are ineffective, not only because of the inability to separate completely these complex isomeric mixtures in its biological surroundings, but also because of the inability to: (a) define and/or eliminate non-polar lipid-soluble contaminants including other chlorinated hydrocarbon pesticides; (b) define the degree of response for each isomeric PCB in the mixture as monitored by specific GC detectors; and (c) quantitate all known PCBs of a complex standard in comparison with some of the similar components in biological media.

Verification of the presences of PCBs by GC–MS in biological systems has assisted the qualitative aspects of this enormous analytical problem. As shown by numerous types of biological experiments with commercial PCBs, the various isomeric PCBs are absorbed, concentrated and/or metabolized to varying degrees depending upon the degree of chlorination in the molecule, the solubility of the compound and its potential for metabolic conversion. Fig. 12 illustrates the order of complexity of one of the commercial PCB preparations. In the commercial process[61], theoretically over 200 isomers could be formed. As shown by the GC–MS analysis[69], Aroclor 1260 is a very complex mixture composed mainly of hexachloro- and heptachlorobiphenyls with lower concentrations of tetra-, penta- and octa-substitutents. Many of the isomeric components (hexachlorobiphenyls) similar to those of Aroclor 1260 have been found concentrated in human adipose tissue[61], human milk[70], sea eagles, pike and

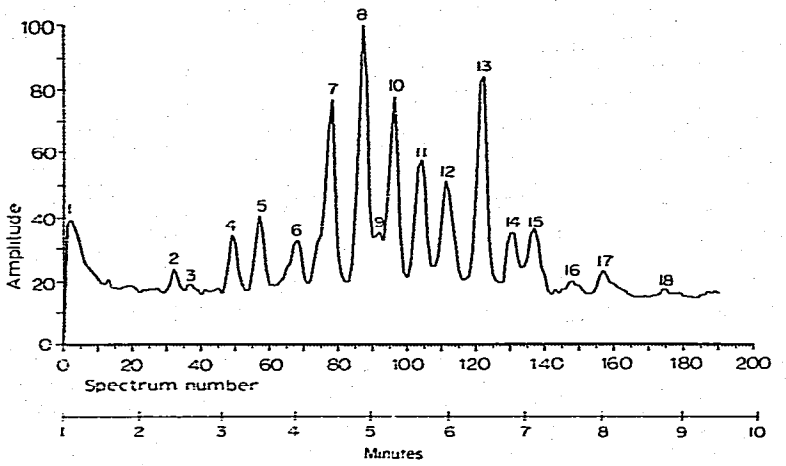


Fig. 12. Reconstructed chromatogram for Aroclor 1260, GC–EI-MS, 70 eV (OV-1, 200° for 1 min, 200–250° at 10°/min). Peak 4, pentachlorobiphenyls; peaks 6 and 7, hexachlorobiphenyls; peaks 9, 10 and 13, heptachlorobiphenyls; peak 14, octachlorobiphenyls.

salmon[62], sea water and phytoplankton[71], numerous land and sea animals and biological components of the food chain[61,66,67].

Other attempts to quantitate PCBs involved chemical modification followed by GC analysis. This laboratory has investigated in depth the problems associated with dechlorination[72] and perchlorination[73] of samples to yield (hopefully) a single component which could be correlated with an original PCB concentration. With respect to dechlorination, even under very vigorous conditions, the rate of conversion to biphenyl is not quantitative and is highly dependent upon the number of chlorine atoms originally present in the molecule.

Depending upon the complexity of the PCBs and the type of biological sample, dechlorination followed by quantitation of biphenyl is, in general, not desirable for the analysis of PCBs. Perchlorination has been applied to the analysis of many chlorinated hydrocarbons[73]. With respect to complex PCB mixtures, GC analysis of decachlorobiphenyl formed by perchlorination has been reported[74]. This type of approximation of the presence of PCBs is feasible within certain limitations. Excluding problems associated with normal contaminants and the elaborate clean-up procedures which produce very low recovery, it is very difficult to express meaningfully the PCB concentration in terms other than moles of decachlorobiphenyl present. Other problems associated with the quantitation of PCBs by perchlorination and GC analysis include (a) reduction in desirable analytical end-product affected by the presence of aliphatics; (b) production of chlorinated aromatics from aromatic contaminants; and (c) excessive quantitative values caused by biphenyl and by brominated and fluorinated biphenyls which undergo exchange reactions. In a few restricted instances, we have used the perchlorination procedure[74] with mirex as internal standard for the verification of the presence of PCBs in various types of biological samples.

The choice of the type of GC detector for various attempted quantitations of PCBs greatly affects the results. Bearing in mind the limitations and problems asso-

ciated with GC quantitation of PCBs, prior verification of the number of chlorine atoms in the molecule by GC–MS followed by GC quantitation of the chlorine in the molecule by use of a Coulson detector is of marginal use. Analysis of commercial PCB mixtures such as Aroclor 1254 by GC using a flame ionization detector affords one a characteristic pattern which may be recognized as 1254. From batch to batch of the commercial preparation, there is a significant variation in the abundance of minor components. By use of the electron capture detector, we have found that one cannot rely on the use of this detector even for comparison of various batches of commercial PCBs such as the Aroclor 1254, in which minor components are highly variable. The response factor for the electron capture detector with PCBs, including dichlorobiphenyl to hexachlorobiphenyl, varies as much as 10,000-fold.

Additional investigations using high-pressure liquid chromatography with commercial Aroclors in combination with other pesticides in the environment and natural contaminants of biological systems indicate that there is too great a degree of complexity for the generation of meaningful results as one considers the commercial PCB preparation. One would like to remove PCBs as a class from other contaminants and then concentrate on a more feasible type of analysis.

With respect to the use of chromatographic separations such as GC or high-pressure liquid chromatography, as the separation factors increase for the family of PCBs with respect to other contaminants such as lipid-soluble hydrocarbons or DDE, one begins to differentiate within the family of PCBs according to the individual isomers with the production of a general overlap in the chromatogram with many common contaminants of the environment.

Synthesis. As indicated earlier, the commercial preparation of PCBs from biphenyl and anhydrous chlorine in the presence of either iron(III) chloride or iron[61], produces numerous isomeric products. Other types of reactions used for the synthesis of specific isomeric PCBs include: (a) chlorination of benzidine derivatives[75] with ultimate removal and/or replacement of the nitrogen functions; (b) coupling of iodochlorobenzenes with copper dust by the Ullman reaction[76–79]; and (c) coupling of aromatic halides by a modified Grignard reaction[80]. For an in-depth discussion of reactions applied to PCB syntheses, the reader is referred to recent reviews[61,66,67,79].

One of the main functions of this laboratory is to prepare isomeric PCBs in the pure form, which may be applied to both biological and analytical investigations in order to clarify some of the potential health problems discussed above which were initially studied with complex commercial PCB preparations.

In general, chlorination processes may be directed towards the formation of a major product. In most instances there are trace amounts (up to 20%) of more or less chlorinated species formed during this type of reaction. As described earlier[69], reactions involving the chlorination of benzidine derivatives produce a mixture of PCBs. Fig. 13 illustrates the complexity of the Ullman reaction products for the preparation of 2,2-dichloro-[^{14}C]biphenyl. We have discussed in detail the possible sources of contamination produced by the Ullman reaction[69]. By use of a modified Grignard reaction, we have prepared pure unsymmetrical tetra-[69] and hexachlorobiphenyls[69,80].

For the worker concerned with the chemical synthesis and quantitation of PCBs, one must continue to strive to develop and evaluate synthetic reactions and methods of quantitation of PCBs in order to obtain a more conclusive understanding of the problems produced by PCBs on man and the environment.

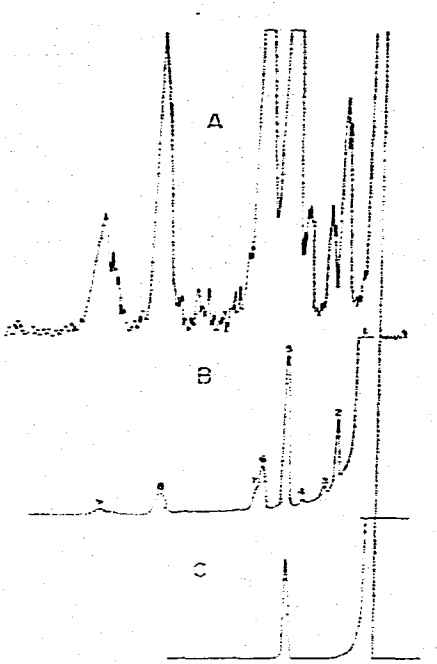


Fig. 13. Gas chromatograms for the Ullman reaction mixture for preparation of 2,2-dichloro-[^{14}C]-biphenyl (OV-1, 140). A. Distribution of ^{14}C label as monitored by GC proportional counter. B. Total mass as monitored by flame ionization detector (FID). C. Purified 2,2-dichlorobiphenyl (FID).

Metabolites. The syntheses of various individual PCB isomers, congeners, etc., have been reported[69,75,79,80] and are continuing to appear as the various suitable methods are being explored. However, reports of the synthesis of the various hydroxylated metabolites[81–83] occur much less frequently, as the synthetic methods involved are more difficult. The two approaches to their synthesis most readily envisioned are (a) the direct oxidation of the PCB in question by various chemical or electrochemical procedures and (b) the various coupling reactions involving the appropriately substituted chlorophenols and chloroanilines. The first approach is more desirable from the standpoint of illuminating the bio-mechanism of hydroxylation, as the product mixtures obtained chemically via such electrophilic oxidations are often the same.

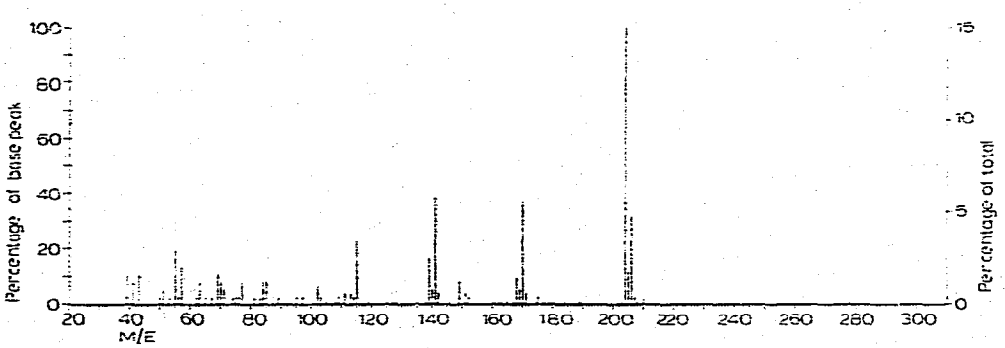


Fig. 14. Mass spectrum (EI, 70 eV) of 4-hydroxy-4′-chlorobiphenyl (direct probe).

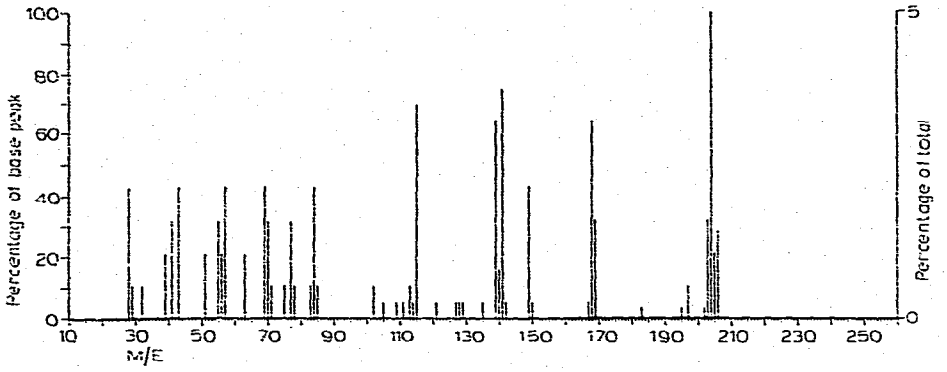


Fig. 15. Mass spectrum (EI, 70 eV) of 2-hydroxy-5-chlorobiphenyl (direct probe).

at least qualitatively as the metabolic pattern. The second synthetic approach is more speculative from the standpoint of obtaining authentic samples of metabolites, but is generally much more efficient in terms of the yield of hydroxylated products. In fact, efficient methods for the direct oxidation of aromatic rings are still limited in application at present.

Nevertheless, the direct peracid chemical oxidation of PCBs containing chlorine in only one ring has been accomplished in this laboratory[54], and other workers[55] have successfully applied electrochemical procedures to PCBs with chlorine atoms on both rings. For example, 4-chlorobiphenyl was oxidized with peroxytrifluoroacetic acid[56] to yield a mixture of products from which the 4′- and 2′-hydroxy- derivatives could be isolated as major monohydroxylated products. The 4′-hydroxy- compound has been found as a metabolite of 4-chlorobiphenyl in the rabbit[57]. The purified hydroxy- derivatives were analyzed by MS. Figs. 14–16 show the EI mass spectra (70 eV) on analysis via the direct probe. The EI spectra of the hydroxybiphenyls obtained in our laboratory generally contain intense parent ions which were usually the base peak as well. A 2-hydroxychlorobiphenyl containing the hydroxyl and chlorine groups in the same aromatic ring was synthesized by extension of a previously reported method[58] in which the hydroxychloroaniline was coupled via the N-acetyl-N′-nitroso derivative with benzene. The EI mass spectrum of this synthetic hydroxy-PCB is shown in Fig. 15. Although background spiking is evident in this

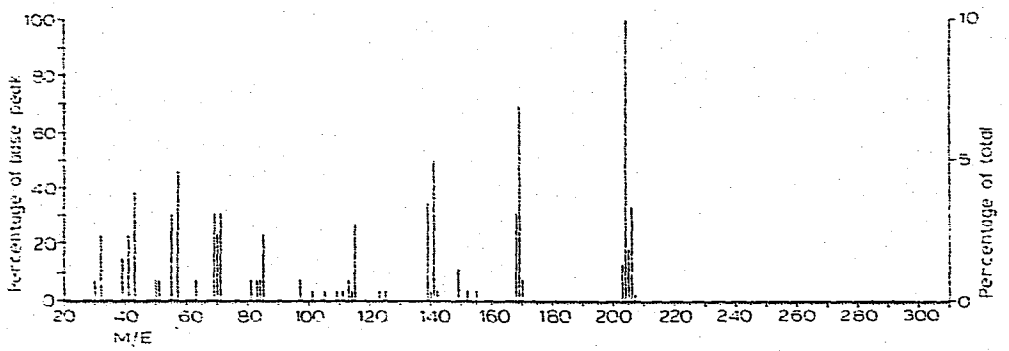


Fig. 16. Mass spectrum (EI, 70 eV) of 2-hydroxy-4′-chlorobiphenyl (direct probe).

spectrum, it shows the parent ion (*m/e* 204) as the base peak, as is seen for other hydroxymonochlorobiphenyls. However, there are characteristic differences in the spectra of these compounds associated with the loss of chlorine. The 2-hydroxy-5-chlorobiphenyl isomer appears to lose HCl preferentially to afford a fragment at *m/e* 168, whereas the 2-hydroxy-4′-chlorobiphenyl appears to lose only chlorine, and the 4-hydroxy-4′-chlorobiphenyl isomer (Fig. 16) shows an abundant ion at *m/e* 170, indicating that the chlorine loss is accompanied by a hydrogen entity transfer, possibly associated with a *p*-quinodal type of stabilization.

The CI spectra, on the other hand, were much less informative and less descriptive for these hydroxymonochlorobiphenyls. However, as the chlorine content is increased, the desirability of CI-MS improves as the parent ions generally constitute more unstable arrangements in the EI processes as a result of steric and/or stereoelectronic interplay. For example, CI-MS has been used in connection with GLC for the identification of a metabolite of 2,5,2′,4′,5′-pentachloro-[^{14}C]biphenyl excreted by rats[59]. This PCB is a major component of certain commercial Aroclor mixtures. The RGC and limited mass search RGC (*m/e* 340–345) are shown in Fig. 17. The CI

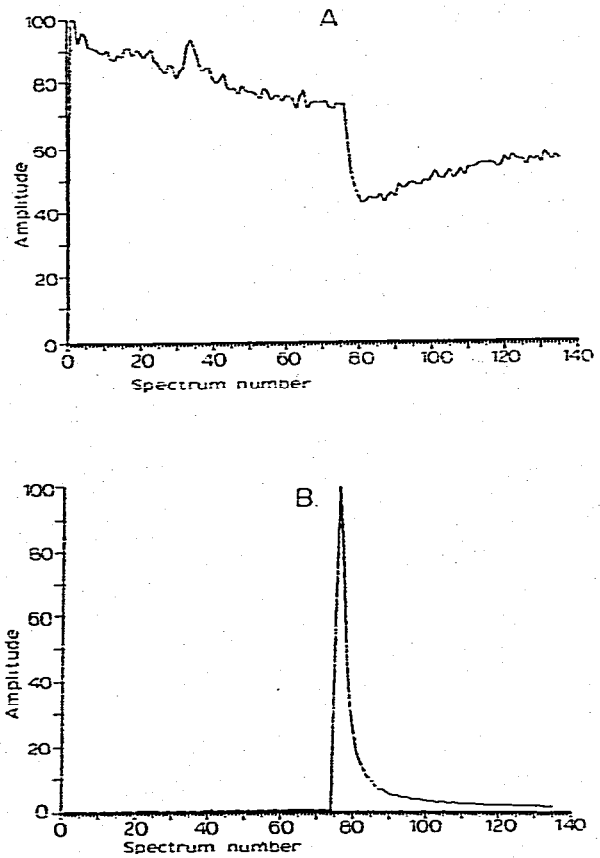


Fig. 17. Reconstructed chromatograms of the metabolite of [^{14}C]-2,5,2′,4′,5′-PCB (Dexsil 300, 160–240° at 10°/min). A. RGC. B. Limited mass search, *m/e* 340–345.

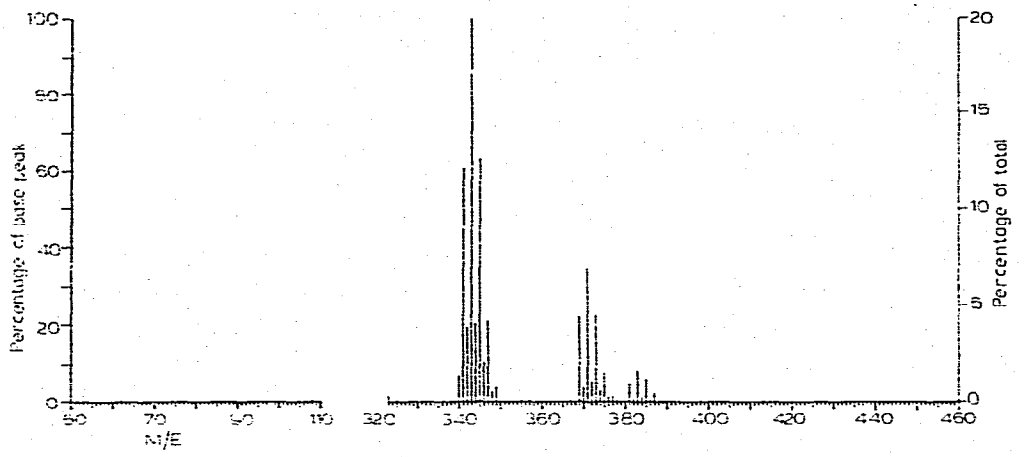


Fig. 18. Mass spectrum of [^{14}C]-2,5,2',4',5'-PCB metabolite (GC–CI-MS, methane).

methane spectrum (Fig. 18) is the simplest possible, with the only fragments seen in the normalized spectrum corresponding to the parent and quasi-molecular ions (*m/e* 340) and the expected recombination fragments $(M + 29)^+$ and $(M + 41)^+$. The isotope pattern is clearly that expected of five chlorine atoms. This metabolite[89] was shown by Fourier transform NMR analysis to be the 2,5-dichloro-3-hydroxy- derivative or the *meta*-substitution product of the dichloro-ring.

In concluding this section on PCBs, care must be exercised in choosing the types of organic reaction used for the preparation of PCBs that will be used for biological testing. Furthermore, one must evaluate very thoroughly the limitation of analyses used and analyze very scrupulously even the pure isomeric PCBs for trace impurities ($< 5\%$) before proceeding to introduce the compound into a biological system. Only after many types of chemical and biological investigations have been carried out with pure, well defined isomeric PCBs will one be able to assess fully the biological activity of the individual components and the potential health problems produced by these widely distributed compounds of the environment.

Mirex, BHCs and HCB. Mirex, dodecachlorooctahydro-1,3,4-metheno-2-cyclobuto[*c,d*]pentalene ($C_{10}Cl_{12}$), is an important pesticide used, among other things, in control of the fire ant in the south-east U.S.A. Because of its extreme chemical stability, convenient gas chromatographic elution characteristics and high electron capture detectability (20 pg) with a ^{63}Ni electron capture detector, we have used mirex as an internal standard for the analysis of PCBs. Mirex survives the reaction conditions under which PCBs are converted into decachlorobiphenyl with antimony pentachloride at elevated temperatures[71] and is eluted from OV-1 at 210° with a corrected retention time of 0.375 relative to decachlorobiphenyl. Furthermore, mirex is very stable to standard chemical oxidation conditions; on the contrary, under reductive dechlorination conditions[41], mirex is very reactive with the production of products similar to those described for DDT[41] and kelthane derivatives but absent for dieldrin. Hexachlorobenzene (HCB, C_6Cl_6) is an important herbicide which has been implicated in a mass poisoning of humans in Turkey due to the consumption of HCB-treated seeds[90]. Benzene hexachloride (BHC, $C_6H_6Cl_6$), better named as hexachloro-

cyclohexane, is a family of isomers which includes the well known pesticide lindane (γ-BHC). BHC is also a precursor in the commercial syntheses of HCB. We have been interested in the intestinal absorption of HCB and BHC isomers[91] and have accordingly studied "technical" BHC, which has the composition indicated in Table 9.

TABLE 9

PARTIAL MASS SPECTRA (EI, 70 eV) OF HEXACHLOROCYCLOHEXANE ISOMERS

Isomer	*Percentage of technical-grade BHC*	*Base peak m/e*	*Relative abundance (%)*	
			m/e 219[*]	*m/e 254*[**]
α	70.4	181	85	3
β	7.5	109	28	3
γ	15.7	181	75	11
δ	5.3	51	55	3

[*] $[(M - H) - 2HCl]^+$.
[**] $(M - HCl)^+$.

GC–MS procedures were employed to confirm the identities of the BHC isomer (and HCB) recovered from the feces, bile, etc., of treated rats. Mirex, BHC and HCB are discussed in the same section here because of the similarity of the principles apparently governing their mass spectral fragmentation patterns.

Hexachlorocyclohexane (BHC) isomers. The sample molecule may give rise to a molecular or quasi-molecular ion in one of three ways: (a) net elimination of an electron, as generally occurs in EI-MS; (b) elimination of a hydride ion, as occurs with normal alkanes in CI-MS; or (c) net addition of a proton, as occurs with most oxygenated or unsaturated compounds in CI-MS. The BHC isomers show neither tendency to any significant extent, and their EI, methane CI and isobutane CI spectra essentially lack a molecular or quasi-molecular ion. In general, the isotope patterns due to chlorine permit "identification" of the fragment "clusters" and provide information about the fragmentation mechanisms (at least on a net basis). GLC fractionation of the various isomers has been reported previously[92].

Of the three techniques used in this laboratory, methane CI provided the simplest and least useful spectra of the BHC isomers. The α-, β-, γ- and δ-isomers gave essentially identical methane CI spectra, with $C_6H_5Cl_4^+$ as the main fragment $[(M + H) - 2H Cl]^+$. As there was no quasi-molecular ion, either HCl or Cl^- was eliminated or proton capture was accompanied by expulsion of two molecules of HCl. A cluster corresponding to the fragment $(C_6H_6Cl_5)^+$ was present, which again could involve expulsion of Cl^- or expulsion of HCl with addition of a proton.

In isobutane CI and 70-eV EI spectra, the fragment $(C_6H_5Cl_5)^+$ predominated over $(C_6H_6Cl_5)^+$. Under these circumstances, expulsion of HCl (net) occurred without a net addition of a proton, but the expelled species could have been either (HCl + e) or (Cl^- + H^+). The isobutane CI spectra required larger samples than did the EI or methane CI spectra, and were of little use in distinguishing between the BHC isomers.

Some distinguishing features of the 70-eV EI spectra are summarized in Table 9. The base peaks alone permit the α-, β- and δ-isomers to be distinguished.

While the α-isomer has an m/e 146 $>$ m/e 147, the reverse is true for the γ-isomer. Thus these four BHC isomers can be distinguished by their EI mass spectra.

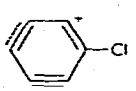
A peak cluster around m/e 145–151 probably involves fragments of three species, $C_6H_3Cl_2^+$ (m/e 145, 147, 149), $C_3H_4Cl_3^+$ (m/e 145, 147, 149, 151), and $C_6H_4Cl_2^+$ (m/e 146, 148, 150). Only in the case of α-BHC was m/e 146 $>$ m/e 147, suggesting that the α-isomer has the greatest tendency to eliminate Cl^- rather than HCl. The δ-isomer had m/e 146 $=$ m/e 147, while the β- and γ-isomers had the m/e 146 ion fragment less than m/e 147. For the latter two, either ring cleavage predominated or elimination of Cl^- was less favorable than elimination of HCl, or both reactions occurred. The relatively high abundance of the m/e 254 fragment from the γ-isomer suggests that this isomer has the greatest tendency to eliminate HCl (it is also the most toxic isomer). The conspicuously low abundance of the ion of m/e 219 for the β-isomer, together with its base peak at m/e 109, suggest that it is relatively easier for this stereoisomer to undergo ring cleavage than HCl elimination. The ion of m/e 109 would correspond to the fragment $Cl-\overset{+}{C}H-CH=CHCl$. Again, the β-isomer has extremely low toxicity compared with the γ-isomer.

Hexachlorobenzene (HCB). Again, methane CI gave less fragmentation than did either isobutane CI or EI at 70 eV. The methane CI spectrum shows only M^+, $(M + H)^+$ and $(M + C_2H_5)^+$ ions. While the EI spectrum shows only M^+ in the region of m/e 285 (*i.e.*, the relative abundance of the odd mass fragments in this region is constant with the ^{13}C abundance), the isobutane and methane CI spectra show a mixture of M^+ and $(M - H)^+$ ions. The chlorine isotope sets m/e 282, 284, 286, 288, 290 (M^+) were in ratios of 10:1, 1:2.5 and 5:1 with the m/e 283, 285, 287, 289, 291 sets $(M - H)^+$ for EI, methane CI and isobutane, respectively. Thus, the tendency to lose an electron, relative to the tendency to accept a proton, was greatest under electron impact, intermediate with isobutane, and very low with methane CI.

There was little or no tendency to expel Cl^- in either methane or isobutane CI. The few fragment ions that were produced in the isobutane CI spectrum (m/e 69, 71, 81, 85) are at present unassigned but were not particularly enlightening mechanistically.

The 70-eV EI spectrum of HCB is unusual in that the base peak is the molecular ion, emphasizing the stability of this structure. Fragments corresponding to $(M - Cl_n)^+$, where $n = 1$–3, occur to the exclusion of other contributing forms, having m/e $>$ 160. Thus the molecule either expels one Cl^- or $(n - 1)$ Cl^0 radicals or ($nCl^0 - e^-$). In any case, the unlikelihood of the persistence of di- and tri-radicals indicates that the fragments corresponding to $C_6Cl_3^+$ (14%) and $C_6Cl_4^+$ (16%) exist as benzyne structures.

Ring fragments appear having the formulae $C \equiv Cl^+$ (m/e 47, 49; 14%), $C_2Cl_2^+$ (m/e 94, 96, 98; 4%) and $C_3Cl_3^+$ (m/e 141, 143, 145, 147; 33%). The last ion, $(C_3Cl_3)^+$, adds to the peak "clump" associated with a fragment corresponding to $(C_6Cl_2)^+$, (m/e 142, 144, 146; 87%). In addition, a set of fragments at m/e 107, 109 apparently indicates an ion (56%) having the formula $(C_6Cl)^+$ or



which ordinarily would be expected to be violently reactive and a short-lived species.

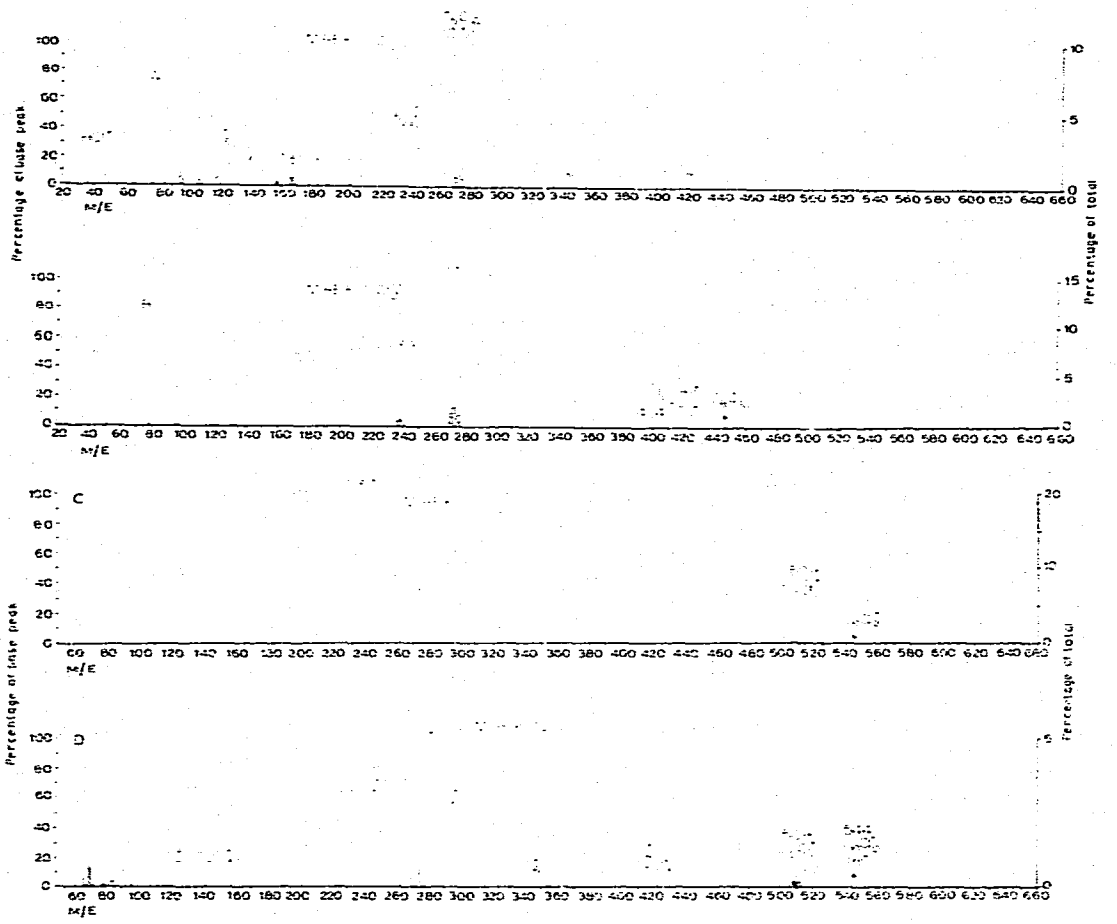


Fig. 19. Mass spectra of mirex (OV-17, 220–260 at 10 min). A, GC–EI-MS, 70 eV; B, GC–EI-MS, 10 eV; C, GC–CI-MS, methane; D, GC–CI-MS, isobutane.

Yet it is of very high relative abundance, suggesting that in fact if any ionization of HCB could be considered "probable", the other fragments observed here would be relatively negligible. One is tempted to conclude that the toxicity of HCB will probably be found to involve either the intact molecule or the product of a metabolic process involving nucleophilic substitution or addition. With regard to the latter, the presence in the methane CI mass spectrum of a 40% abundant $(M + C_2H_5)^+$ ion points out the relative ease with which addition can occur, in contrast to the apparent low probability of elimination reactions.

Mirex. Whereas HCB can form a quasi-molecular ion by accepting a proton, and BHC has hydrogen that theoretically could be expelled as hydride ions, mirex lacks hydrogen, yet is saturated and hence would not be expected to form any quasi-molecular ion. EI (70 and 10 eV) and methane CI mass spectra of mirex lack molecular or quasi-molecular ions. Isobutane CI gives only a molecular ion, $(M-e^-)^+$, of 2% relative abundance. These spectra are shown in Figs. 19A and 19B.

Electron impact spectra

These spectra are illustrated in Figs. 19A and B. The abundance of the low-molecular ions of m/e <220 are qualitatively similar, showing fragments that correspond in mass and chlorine isotope patterns to $(CHCl)^+$, $(CCl_3)^+$, $(C_2Cl_3)^+$, $(C_3Cl_3)^+$, $(C_4Cl_3)^+$ and $(C_5Cl_3)^+$. These fragments are increased in relative abundance in the 70-eV compared with the 10-eV spectrum.

The peak cluster beginning at m/e 235 corresponds to the ion $(C_5Cl_5)^+$, representing half of the mirex molecule less one chlorine atom. The base peak (cluster) starting at m/e 270 does represent half of the mirex molecule $(C_5Cl_6)^{\cdot+}$. Although the "270 cluster" appears to fit $(C_5Cl_6)^+$, the pattern is that expected for five rather than six chlorine atoms at 70 eV. At 10 eV, the isotope pattern for the "270 cluster" is not characteristic for any specific number of chlorine atoms. The isotopic pattern of ions in the m/e 235 region also changes on going from 70 to 10 eV. In this example, one cannot rely on the isotope pattern to characterize the fragments. Whether there is an isotopic effect influencing the ^{35}Cl–^{37}Cl distribution between two fragments of equal mass but different configuration and relative abundance, or an instrumental artifact, is uncertain.

Fragments apparently retaining all ten carbon atoms are represented only by $(C_{10}Cl_6)^{\cdot+}$ and $(C_{10}Cl_5)^+$ spectral clusters starting at m/e 330 and 400. Thus, mirex tends to eliminate chlorine atoms in pairs, either an $(Cl_2 + e^-)$ or $(Cl^- + Cl^{\cdot})$. Loss of single Cl^- ions is not seen, possibly because the remaining molecule is unable to stabilize carbonium ions in the absence of π-bonds. This further suggests that rapid exchange of chlorine atoms within the molecule (which would delocalize a carbonium ion charge) is unfavorable, a suggestion with possible useful implications concerning the degree or lack of chemical reactivity for mirex.

Chemical ionization spectra (Figs. 19C and D)

The methane and isobutane CI mass spectra of mirex are rather different, showing more fragments with isobutane than with methane in spite of the higher energy of the latter. In these spectra, unlike the EI spectra discussed above, the $(C_{10}Cl_{11})^-$ fragment $(M-Cl)^-$ is conspicuous. There are no peaks reflecting $(M + C_2H_5)^-$ or $[(M + C_2H_5)-Cl]^-$, suggesting that substitution into mirex does not account for the ability to expel Cl^- under CI conditions. We tentatively suggest that the expulsion of Cl^- to similar extents in isobutane and methane CI but not under EI conditions reflects a "pull from without" rather than a "push from within". Thus mirex may, under certain conditions, act as a nucleophilic reagent.

The base cluster in the methane CI spectra reflects the $(C_5Cl_5)^-$ fragment rather than the $(C_5Cl_6)^-$ fragment predominant under EI and isobutane CI conditions. Whether or not this involves derivation of the $(C_5Cl_5)^-$ from the $(C_5Cl_6)^-$ fragment could not be confidently determined, as metastable ions are not detected with our system.

In general, then, we found no particular advantage of CI over EI spectra in the identification of these types of polychlorinated hydrocarbons, and in some cases (the BHC isomer), EI spectra were more useful. However, the availability of both types of spectra was very useful in deducing the probable chemical properties (stabilities and reactivities) of the compounds, and it is not unlikely that an empirical correlation could be made between such biological properties as toxicity and the types of fragmentation reaction occurring in the mass spectrometer.

Chlorinated dibenzodioxins and dibenzofurans. Various types of contaminants of synthetic processes have been discussed throughout this review. Probably the most toxic trace contaminants are the chlorinated dioxins and dibenzofurans. Vos *et al.*[93] reported the identification of highly toxic tetra- and pentachlorinated dibenzofurans in commercial preparations of PCBs. During the distillation of crude PCBs in the presence of sodium hydroxide, it is possible that the PCBs reacted with sodium hydroxide at elevated temperatures[93] to yield phenolic compounds which, in the presence of base and elevated temperature, condensed to produce the dibenzofuran derivatives. Using the chick embryo[93] assay, a maximum dose per egg of 0.2 μg of the pentachlorodibenzofuran resulted in 100% mortality. The chlorinated dioxin and dibenzofurans are highly toxic trace contaminants found in certain pesticides and their synthetic precursors. A trace impurity formed during the manufacture of 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) from tetrachlorobenzene at high temperatures in the presence of sodium hydroxide is the 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD). Other dioxins and dibenzofurans may be formed by condensation of the chlorinated phenols[94,95] in the presence of alkali and heat.

This brief discussion on dioxin and dibenzofurans will be limited to (a) considering levels of analytical detection needed and (b) the use of highly radiolabeled TCDD in biological systems. For a detailed discussion of the toxicity of chlorinated dioxins and dibenzofurans, sources of contamination, implications of these compounds as chick edema factor, and general properties of these highly toxic compounds, the reader is referred to the report of a Symposium on this subject[96].

To the analytical chemist, an important intermediate in the formation of the chlorinated dioxins from alkali salts of phenols is the hydroxydiphenyl ether derivative or predioxin[97]. Analysis of chlorinated phenol preparations by GC without prior treatment of the mixture to remove the reactive predioxin will result in erroneously high values for the dioxin concentration. The hydroxydiphenyl ethers[97] will react within the GC to produce large amounts of the dioxins.

As described earlier, the chlorinated dioxins and dibenzofurans are condensation products of phenols produced in the presence of alkali and elevated temperature. Considering TCDD as representative of this class of compounds, dependent upon the species[98] the LD_{50} varies from 0.6 to about 100 μg/kg. As the lethal dose of this dioxin is in the low μg/kg or parts per billion range, very sensitive analytical procedures are required. Many of the procedures developed for the analysis of dioxins employ GC or GC–MS. Detection limits for the GC analysis of dioxins using the flame ionization detector[99] are in the low (0.5–2) ppm range, depending upon the nature of the sample, whereas detection limits of TCDD with the electron capture detector[99] are in the 10–30 ppb range. Crummett and Stehl[99] reported the detection limit of TCDD by GC–MS in the multiple ion detection (MID) mode (EI) to be about 6 pg, monitoring $(M)^+$, $(M + 2)^+$ and $(M + 4)^+$ ions. Other types of analyses that have been applied to the determination of dioxins and dibenzofurans include electron spin resonance spectroscopy[100], liquid–liquid chromatography[99] and high-resolution MS[101] in the MID mode with computer signal averaging. Baughman and Meselson[101] indicated that for a 1-g sample, the analytical methods for analysis of TCDD must have a sensitivity of about 1 pg. Detection limits for this reported method[101] for the detection of TCDD in animal tissues are indicated to be in the order of 10–30 ppb with varying degrees of recovery of TCDD from 27 ± 5 up to 60–80%.

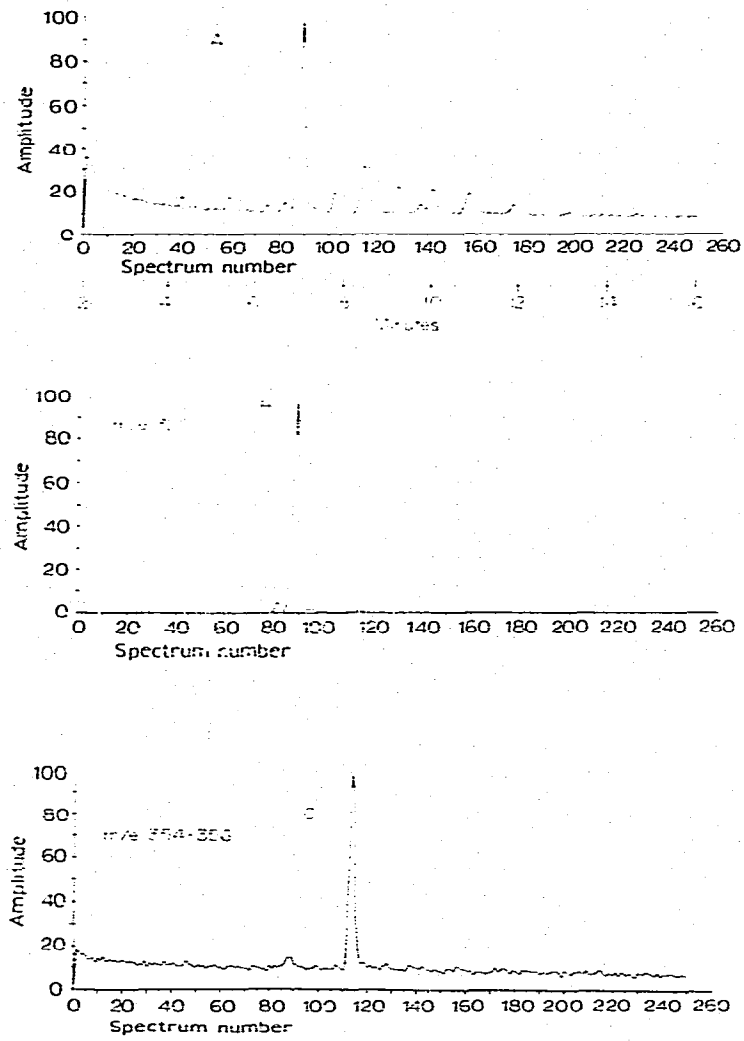


Fig. 20. Reconstructed chromatograms of crude synthetic preparation of 2,3,7,8-TCDD. GC–EI-MS, 70 eV (OV-1, 160–250 at 10 min). A, RGC. B, Limited mass search, *m/e* 320. C, Limited mass search, *m/e* 354–356.

To the synthetic chemist involved with chlorinated dioxins or dibenzofurans, similar problem exist for these chlorinated compounds to those described for the PCBs. Condensation of the dichlorocatechol[102] with 1,2,4,5-tetrachlorobenzene produces a mixture composed mainly of 2,3,7,8-TCDD (Fig. 20). As shown in Fig. 20C, the major contaminant of this synthesis is a pentachlorodibenzodioxin (*m/e* 354) which affords the appropriate mass spectrum (Fig. 21). Upon evaluation of the sources of impurities for the production of the pentachlorodioxin, it was found that the dichlorocatechol contained amounts of the trichlorocatechol. During the condensation[102] with 1,2,4,5-tetrachlorobenzene, the trichlorocatechol reaction produced the pentachlorodioxin (Fig. 21). The dichlorocatechol was prepared by chlorination[103]

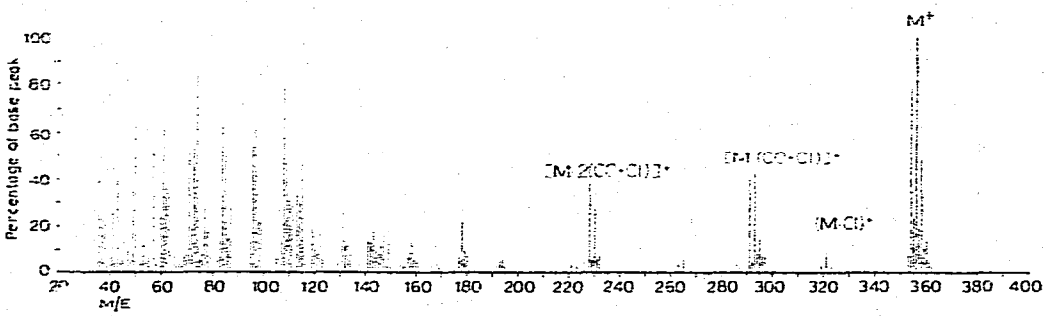


Fig. 21. Mass spectrum of major contaminant of 2,3,7,8-TCDD preparations. GC–EI-MS, 70 eV.

of the 4-chlorocatechol. It is very difficult, if not impossible, to control the chlorination reaction[103] to produce only the dichlorocatechol. Only after repetitive purification by recrystallization with an overall loss of the dichlorocatechol with the contaminant (the trichlorocatechol) is one able to obtain in very low yield a pure dichloro-compound.

Because of the high toxicity of TCDD, in order to study the general metabolism of this compound one must use a very small amount (few micrograms) of highly labeled compound. A ^{14}C-labeled 2,3,7,8-TCDD has been prepared by Kende and Wade[102] from highly radiolabeled 1,2,4,5-tetrachloro-[^{14}C]benzene with a specific activity of *ca.* 150 mCi/mmole. The final [^{14}C]TCDD had a specific activity of *ca.* 150 mCi/mmole. For the more biologically oriented worker, who may or may not be aware of the change in physical and/or chemical properties of radiolabeled compounds with very high percentage of radiolabel, some of the properties associated with the [^{14}C]TCDD are discussed below.

With respect to the [^{14}C]-2,3,7,8-TCDD with a specific activity of 150 mCi/mmole, one cannot distinguish by thin-layer chromatography using numerous solvent systems any separation difference between unlabeled and ^{14}C-labeled TCDD. On the contrary, as one evaluates the GC retention characteristics of the highly labeled compounds with respect to unlabeled TCDD, one will note very significant differences, as shown in Table 10. As one monitors the radioactivity by a GC proportional counter and total mass by a GC flame ionization detector either simultaneously or separately,

TABLE 10

RETENTION CHARACTERISTICS OF ^{14}C-LABELED AND UNLABELED TCDD

Specific activity of [^{14}C]TCDD = 150 mCi/mmole.

GC column	*Temperature (°C)*	*Component*	*Retention time* relative to aldrin*
OV-1	200	2,3,7,8-TCDD	4.60
		[^{14}C]TCDD	3.90
OV-17	210	2,3,7,8-TCDD	4.50
		1,2,3,4-TCDD	4.23
		[^{14}C]TCDD	4.05

* Independent and/or simultaneous detectors using flame ionization detector and GC proportional counter (Packard Model 894).

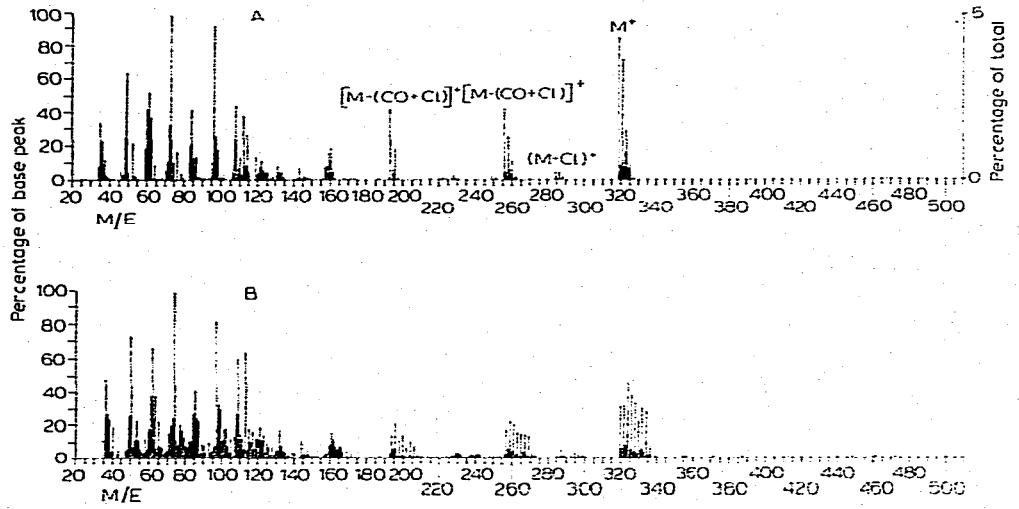


Fig. 22. Mass spectra of 2,3,7,8-TCDD, GC–EI-MS, 70 eV (OV-1, 180–240° at 10°/min). A, Unlabeled 2,3,7,8-TCDD. B, [^{14}C]TCDD, specific activity 150 mCi/mmole.

one notes that the highly ^{14}C-labeled TCDD is eluted earlier than the unlabeled TCDD. As one diluted the [^{14}C]TCDD with unlabeled TCDD to a specific activity below 50 mCi/mmole, the retention characteristics approached those of unlabeled TCDD. Analysis of the [^{14}C]TCDD by GC–MS indicated that this preparation had the expected mass spectrometric characteristics with a very high concentration of ^{14}C isotope overlapping with isotopic ^{35}Cl and ^{37}Cl patterns. As shown in Fig. 22, the chlorine–^{14}C cluster for the parent ion extends from *m/e* 320 in two mass intervals to *m/e* 336. As one diluted the highly labeled [^{14}C]TCDD (specific activity 150 mCi/mmole) with unlabeled TCDD, both the GC and MS characteristics approached those of the unlabeled compound. Additional investigations using UV spectroscopy indicated that the ^{14}C-labeled TCDD and the unlabeled TCDD have equivalent UV spectra with maxima at 305–306 nm with identical extinction coefficients.

Fig. 23 is a representative EI mass spectrum of 2,3,7,8-chlorinated dibenzofuran (TCDF). The use of GC–CI-MS for the analysis of chlorinated dioxins and dibenzo-

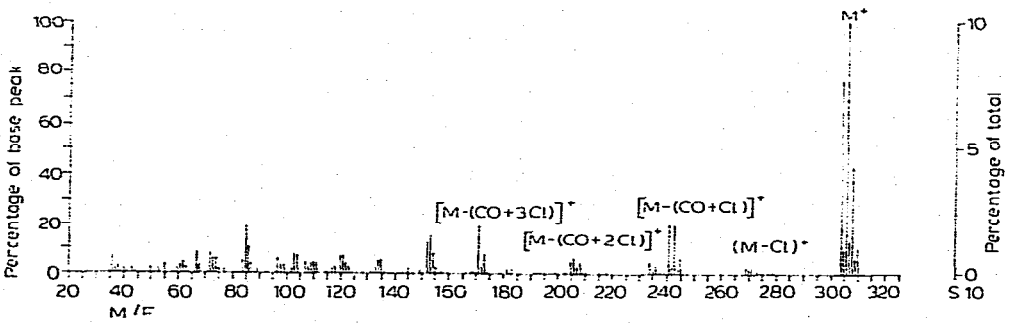


Fig. 23. Mass spectrum of 2,3,7,8-tetrachlorodibenzofuran (TCDF), GC–EI-MS, 70 eV (OV-1, 180–240° at 10°/min).

furans is not as informative as the EI mass spectra. The methane CI spectrum of 2,3,7,8-TCDF and other dioxins and dibenzofurans consists very simply of the ions in the quasi-molecular region $(M + 1)^+$ and the appropriate methane recombination fragments $(M + 29)^+$ and $(M + 41)^+$. Therefore, analysis of the chlorinated dioxins or dibenzofurans by GC–MS either in the qualitative scanning mode or in the quantitative multiple ion detection mode would be much more sensitive and useful under EI conditions.

Investigations in this laboratory using the highly labeled [^{14}C]TCDD (specific activity 150 mCi/mmole) indicated that one can very easily monitor the degree of absorption, distribution and excretion of the labeled TCDD molecule. Upon absorption, a large amount of the label is localized in the liver with chromatographic properties identical with those of the administered [^{14}C]TCDD. Upon single exposure of [^{14}C]TCDD with increasing time, the [^{14}C]TCDD seems to be removed from the liver and/or fat and excreted in the feces. Examination of the fecal material after 60 h by chromatographic means indicated that the radiolabeled components in feces are much more polar than the administered [^{14}C]TCDD. Whether the highly labeled [^{14}C]TCDD acts in biological systems as unlabeled TCDD cannot be answered definitively. Because of the amounts of the compounds involved in labeled and unlabeled biological and metabolic investigations with TCDD coupled with the high toxicity of these compounds, there is a great need for the development of more sensitive methods for both quantitation and unequivocal identification of subnanogram amounts of these environmental contaminants.

(ii) Plasticizers

EI-MS of plasticizers has been discussed several times recently[101–107]. Our experience has been that a combination of electron impact and chemical ionization mass spectrometry gives much more information about the structures of ester plasticizers and their metabolites than does either technique alone. As most of our experience has involved phthalate esters, the present discussion will be limited to these compounds.

The uses, biological aspects and chromatography of phthalate esters have been reviewed recently[108] and will not be discussed here in detail. Our interests have centered around confirming the identities of synthetic phthalate esters and determining the structures of their urinary metabolites[109–116]. These studies have so far been concerned primarily with dimethyl, di-*n*-butyl, di-*n*-octyl and di-(2-ethylhexyl) phthalates. EI mass spectra of which were given by Safe and Hutzinger[111]. It should be noted that di-*n*-octyl phthalate and di-(2-ethylhexyl) phthalate give such similar EI mass spectra that it would be impossible to distinguish them solely on that basis. CI mass spectra of the isomeric dioctyl phthalates also fail to provide unequivocal means of distinguishing between them. However, EI spectra can confirm the fact that a phthalate is present by showing a base peak[101] of *m/e* 149, or, for methyl phthalate[109,111], of *m/e* 163. Methane CI spectra can then, by the presence of a base peak at *m/e* 391, $(M - H)^+$, with smaller peaks[106] at *m/e* 279, 261 and 113, reveal that dioctyl esters are present. The particular octyl isomer(s) can then be confirmed from the GLC relative retention times (see Table 11).

EI mass spectra of phthalate esters generally give an adequate representation of the aromatic ring structure. The occurrence of a base peak at *m/e* 149 (usually attributed to a protonated phthalic anhydride) not only indicates the probable absence

TABLE 11

RELATIVE RETENTION TIMES FOR DIOCTYL PHTHALATES WITH HELIUM CARRIER GAS AT 240°

Octyl isomer (phthalate)	*Relative retention time*	
	OV-1	*OV-225*
Di-(2-ethylhexyl)	1.00	1.00
2-Ethylhexyl-6-methylheptyl	1.12	1.21
Di-(2-methylheptyl)	1.25	1.32
Di-(6-methylheptyl)	1.30	1.50
2-Ethylhexyl-*n*-octyl	1.40	1.66
Di-*n*-octyl	1.68	1.90

of additional ring substitution (–OH, $-OCH_3$, etc.), but confirms the *ortho* di-sibstitution of carboxyl moieties. For phthalates bearing at least one methyl ester moiety, the base peak at *m/e* 163 serves the same diagnostic function as *m/e* 149 in other phthalates. In addition, methyl phthalates generally give spectra containing significant fragments at *m/e* 181 and, for methyl phthalates other than dimethyl phthalate, *m/e* 149. These fragments may arise as suggested in Fig. 24. In any event, fragmentation of an unsymmetrical methyl phthalate appears inevitably to involve loss of the longer alcohol moiety in preference to methanol. But EI mass spectra generally give very little, if any, information about the nature of the non-methyl moiety.

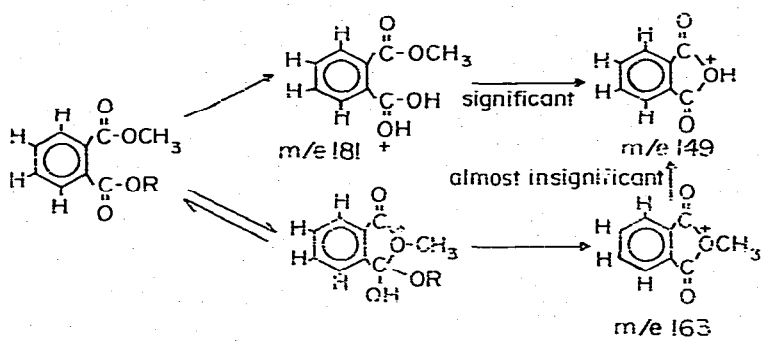
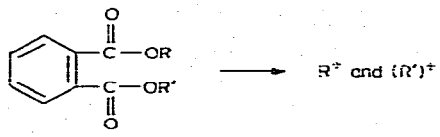


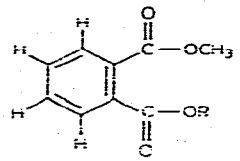
Fig. 24. Fragmentation of a methyl phthalate.

CI mass spectra utilizing methane as reagent gas provide considerable information about the moiety esterified to phthalic acid. Not only is the molecular weight of the intact diester clearly shown by the series of ions $(M - H)^+$, $(M - C_2H_5)^+$ and $(M - C_3H_5)^+$, but there is also a major fragment or fragments derived from cleavage of the side-chain(s) at oxygen atoms:



Thus, CI-MS will distinguish a heptyl nonyl phthalate (mol. wt. 390) from a dioctyl phthalate (mol. wt. 390). The former will have a major fragment at *m/e* 99 and at *m/e* 127, the latter at *m/e* 113.

Urinary metabolites of di-(2-ethylhexyl) phthalate (DEHP) from rats were isolated by a series of thin-layer chromatographic operations, as they were not easily resolved by GLC[109]. Although a variety of procedures were used in identification (IR, UV and NMR spectroscopy and GLC of various derivatives), GC–MS alone gave fairly complete characterization of the structures. The metabolites as excreted were found to be phthalate half-esters (monoesters), so most of the further characterization involved the diazomethane-produced methyl esters:



The analytical problem involved identifying R in the above structure.

The masses of the various R groups could be determined, as discussed above, directly from the methane-supported CI mass spectra. In two cases, R was terminated in a $-CH_2-COOCH_3$ moiety, giving rise to a prominent fragment of *m/e* 74 in the EI mass spectra but which was present in the CI mass spectra in very low abundance. This assignment was supported by the presence of the ion $(M-31)^+$ in the CI spectra, although traces of these fragments are seen with all methyl phthalates. In general, combining data for the mass of the R group with the presence of the *m/e* 74 fragment derived from R permitted identification of R (confirmed by NMR analysis).

Two of the other metabolites had interconvertable R groups, via reduction with sodium borohydride and oxidation with chromic oxide in pyridine. The metabolite corresponding to the more reduced state gave conspicuous CI fragments at *m/e* = $(M-17)^+$ and $(R-17)^+$, with a relatively low abundance fragment attributable to R^+. The more oxidized metabolite had a molecular weight 2 mass units less than that of the reduced species and did not have an $(M-17)^+$ fragment. The trimethylsilyl ether of the reduced metabolite formed readily and gave a prominent EI ion fragment

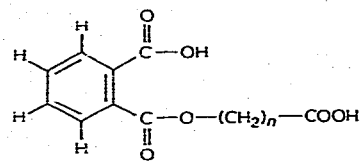
$$CH_3$$

at *m/e* 117, attributed to the fragment $(CH_3)_3-Si-O-CH$, indicating an $\omega-1$ (subterminal) secondary alcohol structure. The oxidized form was thus presumed to be a methyl ketone; and this was confirmed by NMR spectroscopy[109].

A minor metabolite of DEHP was later isolated and found to have an R group terminating in a primary alcohol structure. The TMS ether did not give a prominent fragment of *m/e* 117 but rather a prominent ion of *m/e* = 90 and 91 (trimethylsilanol)$^+$.

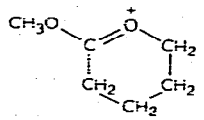
Dimethyl phthalate gave only phthalic acid and monomethyl phthalate as urinary metabolites; these metabolites could be identified by GC–MS of butyl esters in direct comparison with authenic standards. Dibutyl phthalate gave a combination of urinary metabolites analogous to those discussed above, and which were identified in the same manner. Di-*n*-octyl phthalate, in addition to metabolites having methyl ketone and secondary and primary alcohol functions, provided a homologous series

of urinary metabolites[110] having the structure



where n varied from 1 to 7. After esterification with diazomethane, GC–MS methane CI spectra revealed an interesting pattern of stabilities relative to the value of n in the structure shown above. This pattern is summarized in Table 12.

R^+ = *m/e* 115 was most conspicuous, with R = *m/e* 101 and 129 also very prominent. These fragments may be stabilized by ring formations, in which case rings with n = 3, 4 and 5 are thought to be stable[112], *e.g.*:

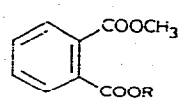


m/e = 115

The tendency to eliminate the methoxy group is greatest for n = 1 and n = 7, dipping smoothly towards a zero tendency for n = 3 and n = 4. The $(M-31)^+$ ion is commonly the base peak for methyl esters of aromatic acids[113], but ordinarily one expects a decreasing tendency to an $(M-31)^+$ fragment to correlate with an increasing tendency to produce an ion in the molecular region. As shown in Table 12, both $(M+1)^+$ and $(M-31)^+$ ions decrease in relative abundance with a minimum at n = 3 and 4. If the ester carboxyl groups normally accept a proton in forming the quasi-molecular $(M+1)^+$ ion, the above considerations suggest a vapor-phase interaction between methoxyl and carboxyl groups that is maximal at n = 3 or 4. Such an interaction, if it also occurs in solution, would influence molecular conformations and might be expected to be revealed in effects on rates of catabolism, enzyme specificities, etc., although there has been no investigation along these lines as yet.

TABLE 12

RELATIVE ABUNDANCE OF DIAGNOSTIC FRAGMENTS IN METHANE-SUPPORTED CI MASS SPECTRA OF SOME PHTHALATE DERIVATIVES

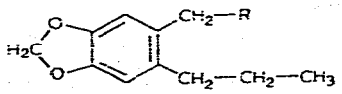
All phthalates had the structure 

R	R^+ *m/e*	*Relative abundance (% of base peak)*		
		R^+	$(M-31)^+$	$(M+1)^+$
CH_2COOCH_3	73	1	55	7
$(CH_2)_2COOCH_3$	87	15	4	4
$(CH_2)_3COOCH_3$	101	98	0	2.5
$(CH_2)_4COOCH_3$	115	100	0	2.8
$(CH_2)_5COOCH_3$	129	86.5	1	6
$(CH_2)_6COOCH_3$	143	67.5	11	15
$(CH_2)_7COOCH_3$	157	79	15	12

(iii) Synergists

In previous studies in this laboratory, we employed GC–MS together with IR and NMR spectroscopy and thin-layer chromatography (TLC) to identify the major impurities in commercial preparations of piperonyl butoxide (2-propyl-4,5-methylenedioxybenzyl-*n*-butyldiethylene glycol ether)[111]. Some of the impurities were later synthesized and found to have mixed function oxidase inhibitory abilities similar to that of piperonyl butoxide itself. For a more detailed discussion of the chemical and biological activities of piperonyl butoxide and other methylenedioxy synergists, the reader is referred to the literature by Fishbein and co-workers[115–120]. At the time that the investigations concerning the impurities present in piperonyl butoxide were completed (1971), we did not have the cross-referenced EI–CI mass spectrometer facilities available, so all of the spectra discussed were 70-eV EI spectra.

Most of the impurities had the partial structure



and gave rise to a molecular ion $(M)^+$ with a base peak at either *m/e* 176 or 149. The spectra will not be discussed in detail, as the salient features have been presented previously[111].

Even repetitive TLC procedures did not permit the isolation of pure samples of each of the impurities of interest, and the application of GLC was essential in order to obtain usable mass spectra. However, owing to the relatively high temperatures needed to elute some of the higher-molecular-weight components (two of which were dimeric in structure), it was necessary, because of unavailability of thermally stable polar columns, to use a liquid phase (Dexsil 300) that could not itself resolve all of the compounds. GC–MS studies, in this case, had to be applied to the individual

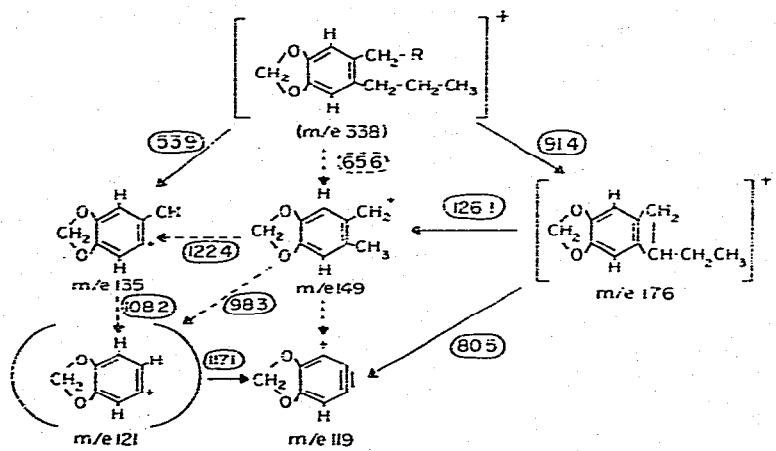


Fig. 25. Fragmentation patterns for dihydrosafrole derivatives, illustrated for piperonyl butoxide. The fragments indicated were observed in spectra from five substituted dihydrosafrole derivatives. The molecular ion illustrated as having *m/e* 338 refers to piperonyl butoxide, and the confirmatory metastable ions (*m/e* values circled) also refer to this compound. Solid vectors indicate fragmentations yielding major abundances (>5%) while dotted vectors indicate less favored fragmentation routes.

fractions previously obtained by preparative TLC. We have generally found that preliminary TLC, separating groups of compounds on the basis of difference in polarity, almost always permits subsequent GLC resolution on the basis of volatility or molecular weight. This allows one to use non-polar, highly stable GLC liquid phases for almost all types of GC–MS investigations.

The major fragmentation of the most concentrated impurities in commercial piperonyl butoxide are summarized in Fig. 25. The fragmentation sequence shown was confirmed by the metastable ions indicated in the figure, observable in this case because the spectra were obtained using a sector instrument without computerized data handling. Basically, the same fragmentation pattern was observed for compounds in which the methylenedioxy ring was replaced by two methoxyl groups, except that the corresponding ion fragments were shifted 16 mass units higher in the spectra.

The group designated as R in Fig. 25 could often be deduced from its molecular weight ($M^+ - 177$), but the deduction required independent confirmation by NMR or IR spectroscopy (or synthesis and GC–MS). The mass spectra did, however, provide information permitting the elimination of various possible structures for R. For example, the absence of a peak at *m/e* 57 indicated the absence of a butylcarbityl side-chain (the side-chain of piperonyl butoxide itself), while the absence of a peak at *m/e* 176 was associated with R = H. The findings reported in the section on *Plasticizers* (see above) suggest that confident identification of the R groups would have been greatly facilitated had CI been available in this study.

(iv) Medicinals and food additives

In this short section, no attempt will be made to discuss the broad and varied areas of drugs and specific food additives. On the contrary, the discussion will be limited to very specific examples in these areas in order to illustrate the limitations and the need for the use of MS prior to and during biological investigations. For the reader who may be concerned with specific health hazards associated with the repetitive exposure to common drugs, pharmaceuticals and medicants of animal feeds, one is referred to recent reviews by Fishbein and Flamm[121–123]. Applications of GC and GC–MS to the investigation of drug metabolism are too numerous to discuss within the scope of this presentation. A recent representative source of the utilization of GC–MS in clinical and forensic chemistry, in identification of drugs in body fluids and in toxicological problems may be found in the book by McFadden[35].

To the chemist who is concerned with the sequence of events that takes place once a compound has entered a biological test system, the most important consideration prior to initiation of the experiment is the chemical purity of the compound to be studied. As has been discussed earlier for other environmental agents which may contain contaminants, the utilization of an impure compound for metabolic, toxicological or other types of biological testing does not assist in obtaining the clarification needed on the specific compound. The use of contaminated compounds further complicates the interpretation of the biological data. Some examples of various types of contaminants include: (a) isomeric PCBs; (b) the dioxins in chlorinated phenols; (c) phthalates; and (d) drugs such as Stalinon which contain the active components diethyltin diiodide and linoleic acid. Stalinon contained as impurities the monoethyltin and the very toxic triethyltin iodides, and in an investigation of a series of poisonings[124], it was concluded that the triethyltin was the toxic contaminant which

resulted in the death of about 100 persons and in other affected patients resulted in the production of neurological symptoms.

A very difficult class of compounds for the analytical and synthetic chemist to evaluate is a family of drugs used to treat schistosomiasis (snail fever)[125]. The structures of some of the compounds are described in Fig. 26. Schistosomiasis[126] is endemic in 72 countries or islands with a total population of about 1.3 billion. Moreover, schistosomiasis imposes a severe economic burden due to the cost of medical care, time lost from gainful occupation and compensation for illness. In Egypt and Africa alone, the economic loss exceeds one billion U.S. dollars annually because of this parasitic disease.

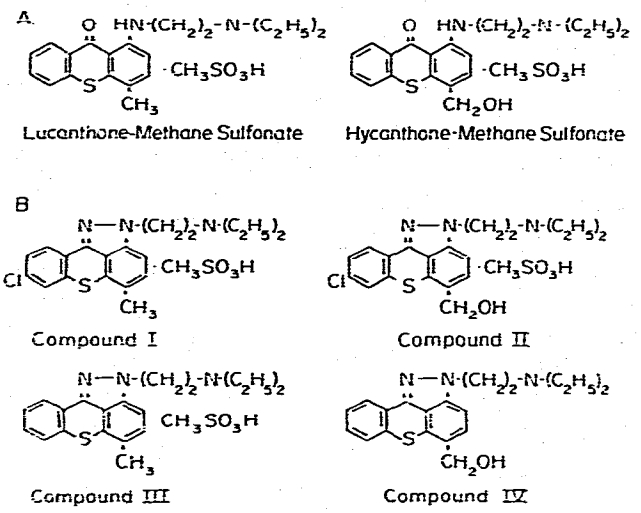


Fig. 26. Chemical structures of schistosomal drugs. A, Lucanthone, hycanthone. B, Indazole analogs.

It has been reported[127–130] that Miracil D (lucanthone) and hycanthone have been found to be mutagenic in Drosophila and in mammalian cells and induce lesions and chromatid breaks in human leukocytes. Since the first report by Hartman *et al.*[131] of the mutagenecity of hycanthone in Salmonella, additional evidence has been accumulated concerning the toxic side-effects of this drug, its hepatotoxicity[131,132], and its carcinogenicity[133] in *Schistosoma mansoni*-infected mice.

To the biologist, the problem is the potential health hazard produced by exposure of a large segment of human population to a mass-produced singly injectable drug which has some of the above undesirable side-effects. The medicinal chemist then becomes involved with this problem area in order to produce structure modifications of the hycanthone molecule in an attempt to dissociate its schistosomicidal and its host-toxicity properties[125]. The task of the analytical chemist in this area is concerned with the development of methods for analysis of purity and the detection of possible metabolites in biological systems.

It is most important to analyze the compound in the same chemical form as it enters the biological system. With the hycanthone analogs (Fig. 26), which hopefully would have less undesirable side-effects than the hycanthone, it is very difficult

to analyze these compounds in their salt form. As will be seen later, one may analyze these compounds by reversed-phase liquid–liquid chromatography. On the contrary, in order to analyze these analogs by GC and GC–MS, one must first convert the salt into the free base, which would be organic extractable. The free base was then analyzed by GC and GC–MS. These highly polyfunctional compounds are very polar and very non-volatile. One could derivatize the polar groups by silylation to convert the alcohol into the ether, but this additional process would remove the analysis still another step from the actual state in which the compound enters the biological system. Trace contaminants which may or may not react during the derivatization would become further obscured, even to the stage of non-detectability.

Therefore, with this set of compounds (Fig. 26), the organic extract which contained the free base was analyzed by TLC, GC and GC–MS. Because of the high degree of polarity and low volatility, the operational temperature of the GC system is near its maximum limit (about 300°). With this class of compound, if one operates the GC initially at too high a temperature (270–300°), one observes maximum decomposition with very little differentiation in components. By programming the temperature from below 200° to 280°, one obtains minimal thermal decomposition. The only indazole analogs that could be analyzed by GC or GC–MS were compounds I and III; all other analogs were too non-volatile to be eluted, even after maintaining the GC column temperature at 300° for long periods.

Specifically with compound III, a very trace contaminant was detected. The same chromatographable component with comparable retention time and spectra was also present in compound I. As can be seen from Fig. 27, even at the temperature limit of the GC system, these compounds do not produce a satisfactory GC chromatogram. The GC peak width under these specific conditions for compound III was almost 4 min in comparison with the GC peak width of compound I (over 8 min). In this instance, the contaminant with a peak width of about 30 sec can be much more

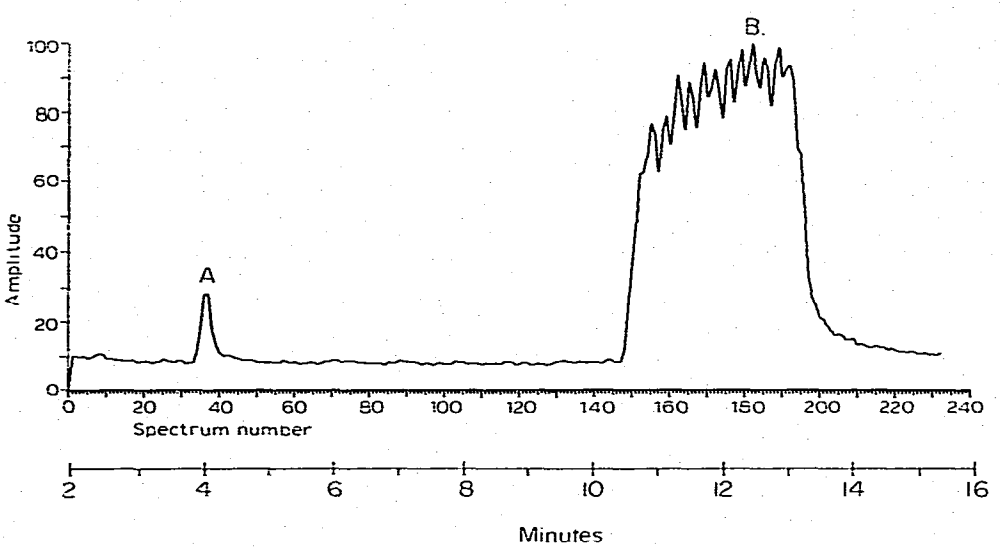


Fig. 27. Reconstructed chromatogram of the hycanthone indazole analog III (OV-1, 180° for 2 min, 180–280° at 10°/min).

satisfactorily analyzed than the major component by GC–MS. Similar contaminants which were described for compounds I and III with a retention time of about 4 min (Fig. 27) were also detectable in compounds II and IV. Because of the undesirable chromatographic behavior of these compounds on GC–MS, the material was analyzed by direct probe. Partial spectra of the hycanthone analog and this contaminant are given in Table 13. Even using the direct probe CI system, one may detect and differentiate the presences of the contaminant of compound III, as illustrated in Fig. 28. Careful control of the probe temperature enables one to remove the contaminant from the major component.

A common characteristic of all of the spectra of the hycanthone analogs (Fig. 26) is the base peak *m/e* of 86, derived by cleavage of the methylenediethylamino side-chain. In the EI mass spectra of these highly polyfunctional compounds, the ion in the molecular region was very weak (< 1%) or, in some instances, not detectable. As shown in Table 13, the CI spectra have very abundant (20–100%) quasi-molecular ions and the appropriate recombination fragments. The contaminant which was characterized by GC–MS and earlier detected by high-pressure liquid chromatography has a molecular ion at *m/e* 253 with a base peak of *m/e* 91. This trace contaminant does not have the ethylenediethylamino side-chain common to the ana-

TABLE 13

PARTIAL MASS SPECTRA OF HYCANTHONE INDAZOLE ANALOGS

Compound III, M *m/e* 337; compound I, M *m/e* 371 (Cl 35).

Compound III, GC–EI-MS, 70 eV		*Compound III, GC–CI-MS, methane*		*Contaminant III, GC CI-MS, methane*		*Contaminant III, GC–EI-MS, 70 eV*		*Contaminant I, GC–EI-MS, 70 eV*		*Compound III, direct probe CI, methane*		*Compound III, direct probe CI, isobutane*		*Compound I, direct probe CI, isobutane*	
m/e	%	*m/e*	%	*m/e*	%	*m/e*	%	*m/e*	%	*m/e*	%	*m/e*	%	*m/e*	%
337	0.53	367	2.5	282	6.9					367	1.1	397	0.43	429	0.45
264	0.29	339	8.3	255	10.3			254	1.6	339	4.3	395	4.1	428	2.2
251	1.5	338	23.3	254	100	253	6.7	253	8.0	338	9.5	394	2.2	417	0.34
238	1.6	337	18.3	210	10.3	224	2.5	224	2.7	337	11.2	382	1.5	416	1.6
222	0.23	336	3.3	186	6.9	210	28.3	210	37.2	336	3.0	381	3.2	415	1.3
208	0.71			172	93.1	184	1.7	184	2.1			380	3.7	414	5.1
163	0.53			162	13.8	172	4.2	172	6.4			379	8.86	413	0.11
100	0.59					155	48.3	155	48.4	100	4.3	340	6.1	377	0.11
98	0.71					139	3.3	139	3.2			339	21.2	376	1.6
87	5.2					98	43.0	98	41.5	87	5.2	338	100	375	8.4
86	100	86	100							86	100	337	20.3	374	30.8
85	6.0					91	100	91	100	85	3.4	254*	2.2	373	25.5
84	1.8			83	48.3							100	3.5	372	100
77	0.71					65	26.7	65	31.9			87	2.6	371	25.2
70	0.83					56	11.7	56	12.8			86	55.4	370	2.3
58	5.8					55	15.0	55	11.8			85	1.7	285	1.4
														100	7.7
														87	4.5
														86	98.1
														85	6.8

* As the probe temperature was increased from 50 to 150 , the contaminant volatilized during collection of spectra 9–13 (50–75), with spectrum 12 having the most abundant *m/e* 254 (see Fig. 28).

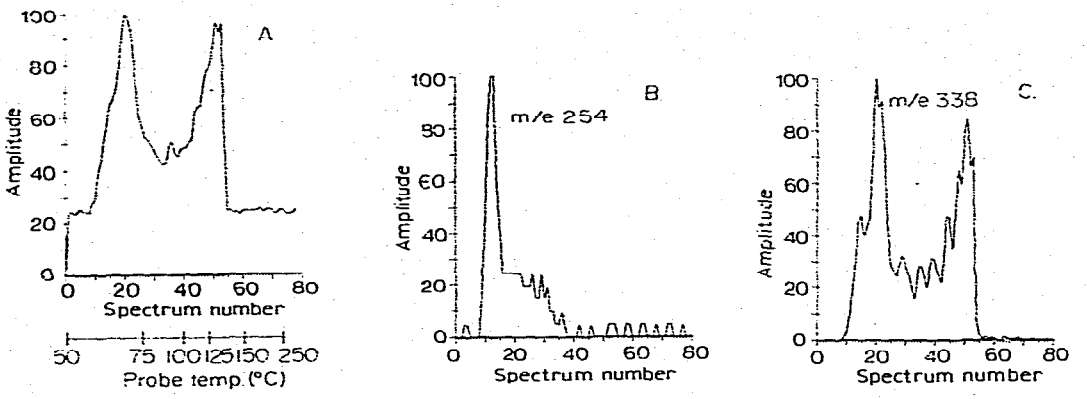


Fig. 28. Reconstructed chromatogram of total ion monitor for hycanthone indazole analog III, direct probe CI, isobutane. A, Total ion monitor response. B, Limited mass search, *m/e* 254. C, Limited mass search, *m/e* 338.

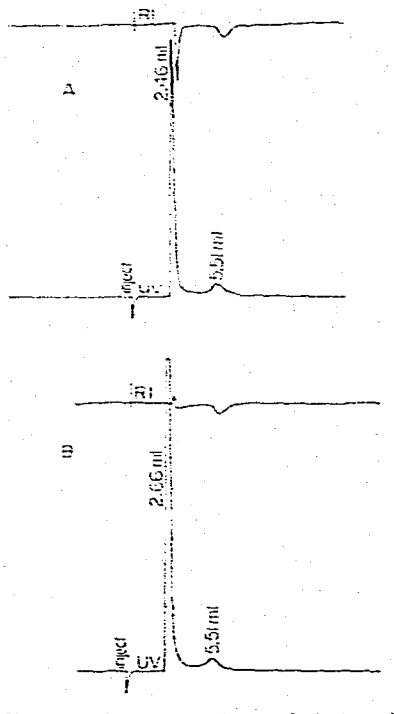


Fig. 29. Chromatograms of the analyses of hycanthone analogs by high-pressure reversed-phase liquid–liquid chromatography using Bondapak C_{18}/Corasil as the stationary phase and acetonitrile–ethanol (4:1) as the mobile phase. Instrument: Waters Ass. ALC 200/401 dual detector liquid chromatograph equipped with an M/6000 solvent delivery system. Flow-rate, 0.5 ml/min; column, 4 ft. × 2 mm; sample load, 0.4–0.5 mg. A, Compound III. B, Hycanthone. Detectors: UV = ultraviolet, 254 nm; RI = differential refractometer.

logs. Analysis of the intact salt form of these hycanthone indazole analogs indicated that all of these compounds contained a trace impurity, as described in Fig. 29. Because of the complexity of this family of compounds, the analytical chemist must rely not only on GC and GC–MS, but also on many other techniques in order to evaluate the chemical purity of these materials in the chemical forms in which they may be used. Analyses of these derivatives in the salt form by reversed-phase liquid–liquid chromatography, later followed by analysis of the free base by GC and GC–MS techniques, were necessary for determining the presence and the chemical identity of the impurity.

To the more biologically oriented chemist, another very important need for structural verification is concerned with the identification of a radioactive component which has been isolated from the biological system during metabolic investigation. Chromatographic techniques (TLC, liquid chromatography and GLC) assist in the initial determination of chemical characteristics. Many times, as described for the ^{14}C-labeled TCDD, the labeled compound has slightly different chromatographic properties dependent upon: (a) the degree of radiolabel present in the molecule; (b) the presence of natural contaminants; or (c) the effects caused by "overloading". Chromatographic data concerning the characteristics of the labeled component isolated from the biological systems in comparison with the labeled compound that was administered to the system are indicative, but not conclusive, of the chemical identity of the compound or its metabolite. Being able to differentiate the real metabolite from the artefact is not always as simple as one might expect.

For another specific example, consider the metabolism of [^{14}C]saccharin. Results of administration of a representative dose of [^{14}C]saccharin to the rat indicated that the labeled compound was rapidly removed from the gastrointestinal tract, from the blood and from the tissues and was excreted primarily in the urine[131]. Analysis of the urine for the radiolabeled compound by a chromatographic technique (TLC) and radiography indicated the labeled compound excreted in the urine did not have exactly the same chromatographic properties as those of the [^{14}C]saccharin which was administered. Several artefacts were present in the thin-layer chromatogram of the urine because of the interaction of normal contaminants with the unchanged [^{14}C]saccharin. These normal urinary constituents affected the mobility of the saccharin in the chromatographic system. Only after comparative analysis of the labeled compound which was administered to the rat with the labeled urinary component which was executed by the use of GC–MS could one conclude that the [^{14}C]saccharin remained unchanged in the biological test system upon excretion in the urine. In mixtures of more polar compounds, such as those containing amino- or hydroxyl-groups or combinations of them, the tendency for interference with chromatographic properties by normal substituents, especially in the urine and feces, are very great. The more rigidly one characterizes a component isolated from a biological system, the more reliably will one be able to differentiate the biological artefacts from the true metabolites. It is just as important to be able to recognize the normal contaminants from urine, feces or tissues as it is to determine the chemical structure of the metabolic component formed in the test system.

(v) Organometallics (methylmercury)

In the course of analyzing tissues for methylmercury, we observed that the

material eluting from a gas chromatograph during registration of the somewhat tailing peak assigned to methylmercury chloride[135] could be trapped in a chilled glass capillary for further examination. Upon re-injection into the gas chromatograph, this component gave a peak at the same position as standard methylmercury chloride, but no longer exhibited conspicuous tailing. Unfortunately, attempted GC–MS using GLC columns[135] traditional for methylmercury analysis gave spectra predominantly of column bleed. The material trapped from preparative GLC was too volatile for the sample handling necessary with the direct probe inlet.

At about that time, Baughman *et al.*[136] published observations on the GC–MS of organomercury compounds. Although they identified the material eluting from the gas chromatograph when various methylmercury halides were injected, and could account for the peak "tail" as involving the elution of methylmercury halides other than the one injected (exchange products), it was still not obvious why re-injection of the trapped component from preparative GLC did not result in another tailing peak. This phenomenon remains unexplained. As concluded by other reports[136,137], ionic methylmercury compounds, and especially phenylmercury compounds, decompose and undergo exchange reactions in the gas chromatograph to the extent that GLC is not a very suitable method for the analysis of these compounds, even after pre-treatment of the column. Other chromatographic and biological aspects of organic and inorganic mercury compounds have been discussed in depth in recent reviews[138–140].

(b) Natural product environmental agents

(i) Lipids

Hydrocarbons. Aliphatic hydrocarbons have been an environmental concern for many years, because of their association with follicular lipidosis[141,142], lipoid pneumonia[143,144] and possible artherosclerosis[145,146]. Some aliphatic hydrocarbons have shown co-carcinogenic properties with fused-ring aromatics[147,148] and, most recently, the saturated isoprenoid hydrocarbon pristane has been found to have as yet unexplained adverse effects on the immunogenic responses of the rat[149]. Paraffinic and isoprenoid hydrocarbons are both natural constituents of the diet and augmented by mineral oil "food additives", and their accumulation in animal tissues is facilitated by exposure to common pesticidal synergists such as piperonyl butoxide[150]. Although the metabolism of straight-chain hydrocarbons is becoming understood[151], little or nothing is known about the mammalian metabolism of cyclic or branched hydrocarbons.

Refsum's disease is one of the genetic lipidoses associated with inability to α-oxidize phytanic acid (3,7,11,15-tetramethylhexadecanoic acid) derived from dietary phytol[152,153], resulting in accumulation of isoprenoid fatty acids in various tissues including the brain[154]. If the hydrocarbon analogue of phytanic acid, phytane, were metabolically oxidized to phytanic acid (by analogy with the metabolic fate of normal paraffins), the further breakdown of the molecule could be accounted for by known pathways[155–157].

Our attempts to study the metabolism of isoprenoid hydrocarbons in rats have involved GC–MS in the identification of various metabolites and in the search for various potential metabolites. The GC properties of isoprenoid fatty acids have been discussed by Ackman[158,159] and by Ackman and Hansen[160]. As the phytane used in

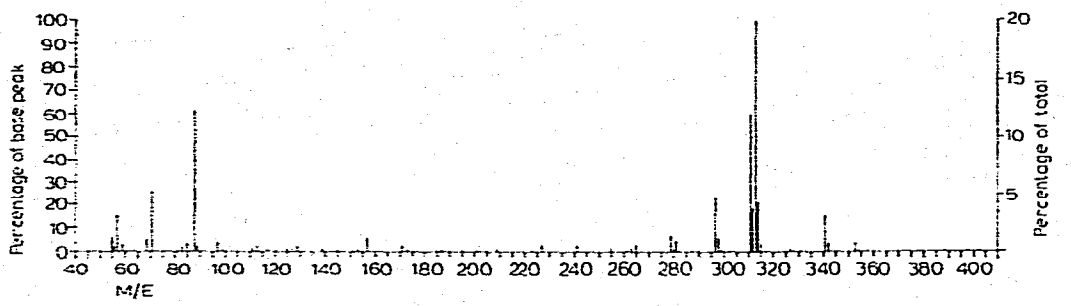


Fig. 30. GC–CI-MS methane spectrum of methyl pristanate.

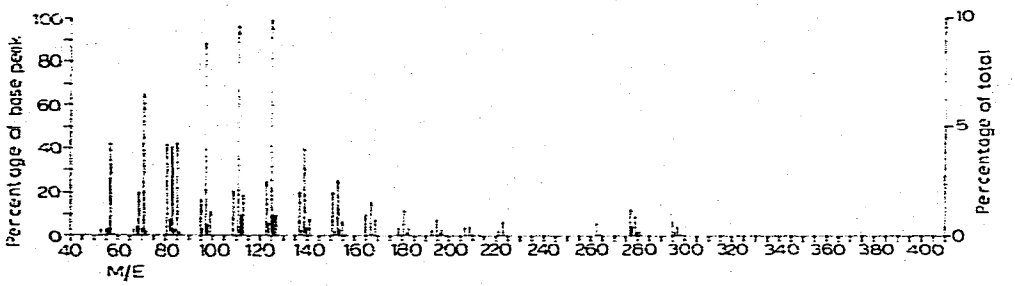


Fig. 31. GC–CI-MS methane spectrum of phytol.

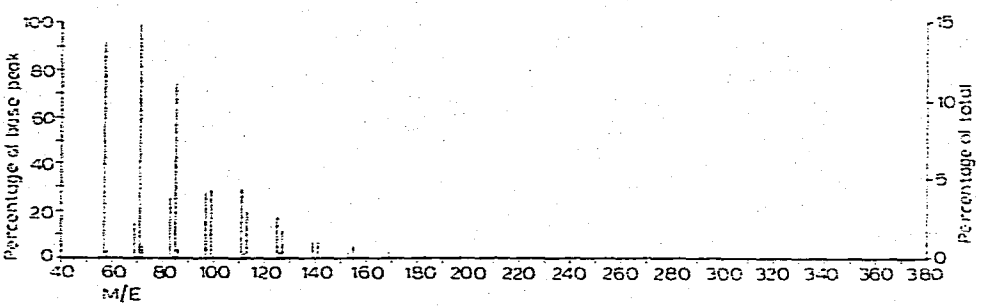


Fig. 32. GC–CI-MS methane spectrum of dihydrophytol.

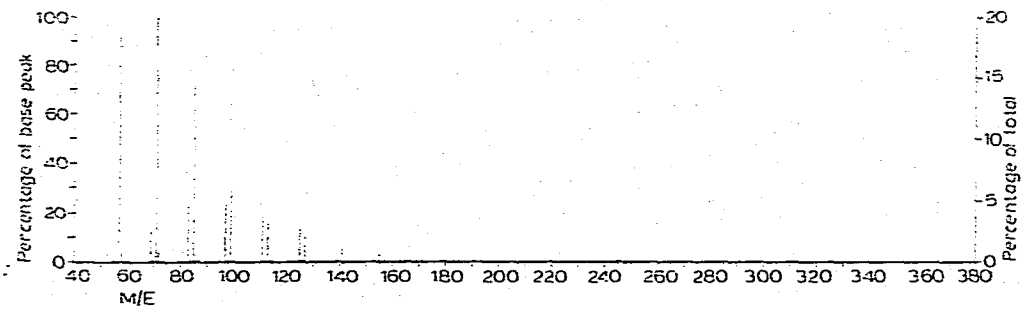


Fig. 33. GC–CI-MS methane spectrum of pristan-1-ol.

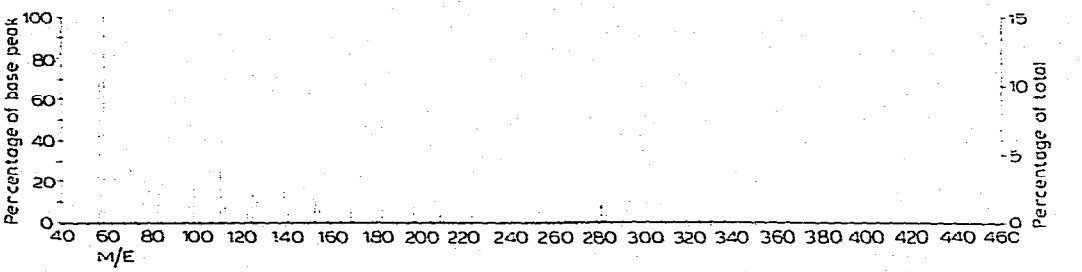


Fig. 34. GC–CI-MS methane spectrum of free phytane metabolite.

our studies was derived synthetically from [U-^{14}C]phytol, it would have had the potential of being metabolized to a mixture of diastereoisomers of phytanic and pristanic acids, and the isomers are also separable by GLC[160].

Among the published mass spectra possibly relevant to this problem are the electron impact mass spectra of pristane[161,162] and phytane[162], methyl pristanate[163], and a range of other multi-branched fatty acid methyl esters and their hydroxylated metabolites[153,156]. Although CI-MS has been applied to the study of hydrocarbons[164], no published applications to the study of isoprenoid structures have appeared as yet.

Although it was apparent on GLC that phytanic acid did not accumulate in rat liver or brain lipids after phytane exposure, the absence of pristanic acid was not certain. The methane CI spectrum of methyl pristanate is shown in Fig. 30. Limited mass searches of GC–CI-MS analyses of rat liver and brain fatty acids as methyl esters were made, but no component having the predominant fragments *m/e* of 313 and 88 were present. Examination of the spectra collected at the elution time of methyl pristanate failed to show the ion of *m/e* 313. This indicated that pristanic acid also did not accumulate when rats were fed phytane.

The only lipid material that could be shown to be a metabolite of phytane in rats (other than lipids derived from [^{14}C]acetate labeled from [^{14}C]phytane) was found by chemical means to be an alcohol.

Reduction of the mesylate with lithium aluminum hydride gave phytane, indicating that the C_{20} skeleton was intact (this was later confirmed by double $^{3}H/^{14}C$ labeling experiments). The problem was to locate the hydroxyl group on the chain. Methane CI spectra of phytol, dihydrophytol and pristan-1-ol are shown in Figs. 31, 32 and 33, respectively, while the corresponding spectrum of the phytane metabolite appears in Fig. 34. Unlike the standards, the unknown shows a base peak at *m/e* 59 and a fairly abundant peak at *m/e* 281 $(M-17)^+$. These spectra suggest that the hydroxyl group is more easily eliminated in the unknown than in dihydrophytol, yet is associated with an easily cleaved C_3 unit.

The 20-eV EI spectra for the trimethylsilyl ethers of phytan-1-ol (dihydrophytol), phytan-2-ol, phytan-3-ol, phytol and pristan-1-ol are shown in Figs. 35, 36, 37, 38 and 39, respectively, and for the trimethylsilyl ether of the phytane metabolite in Fig. 40. The spectra all show major fragmentation at *m/e* 73 and 75, derived from the trimethylsilyl moiety. The location of the hydroxyl groups can be readily deduced from the base peak in the spectra, as the favored cleavage appears to be as indicated in Fig. 41.

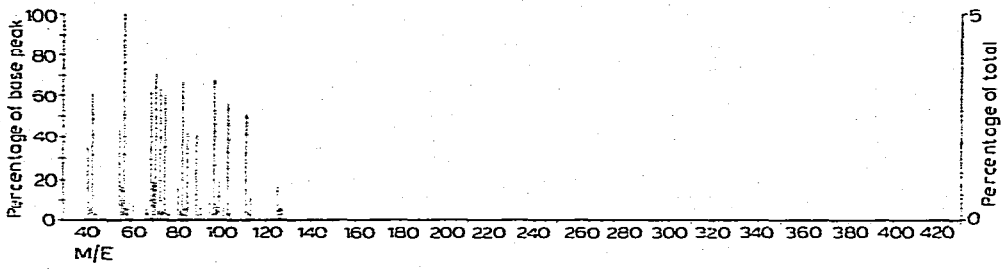


Fig. 35. GC–EI-MS 20 eV spectrum of dihydrophytol-TMS.

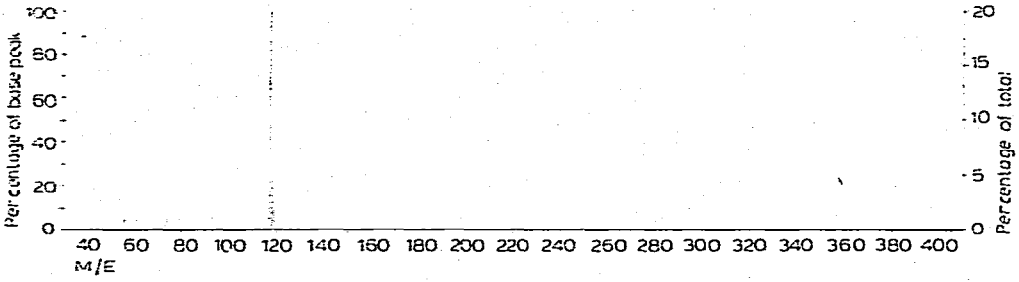


Fig. 36. GC–EI-MS 20 eV spectrum of phytan-2-ol-TMS.

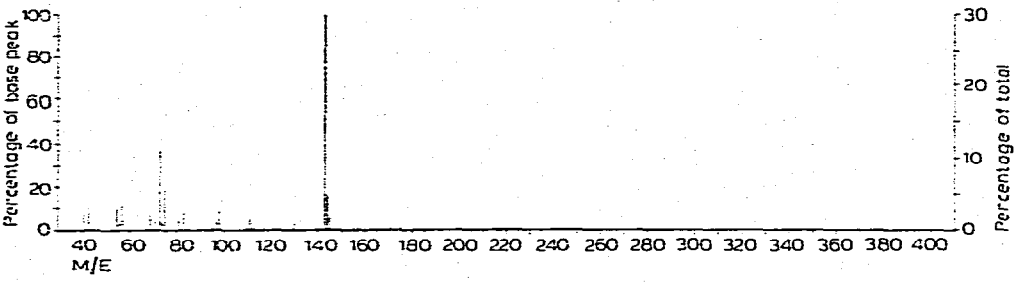


Fig. 37. GC–EI-MS 20 eV spectrum of phytan-3-ol-TMS.

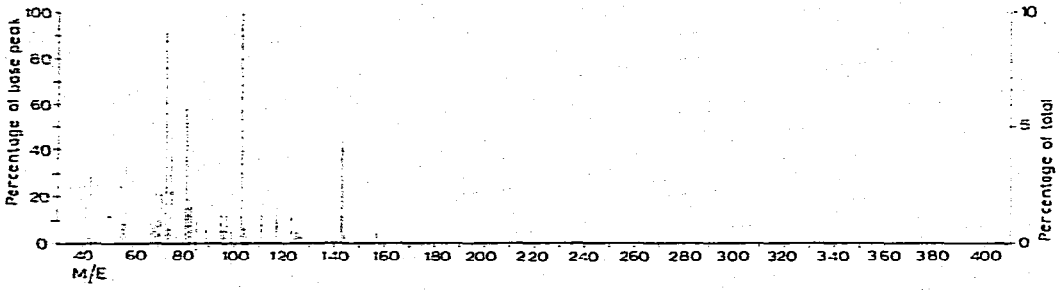


Fig. 38. GC–CI-MS 20 eV spectrum of phytol-TMS.

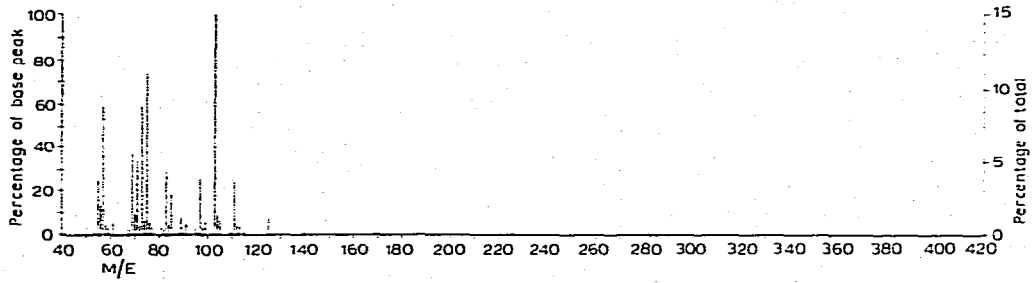


Fig. 39. GC–EI-MS 70 eV spectrum of pristan-1-ol-TMS.

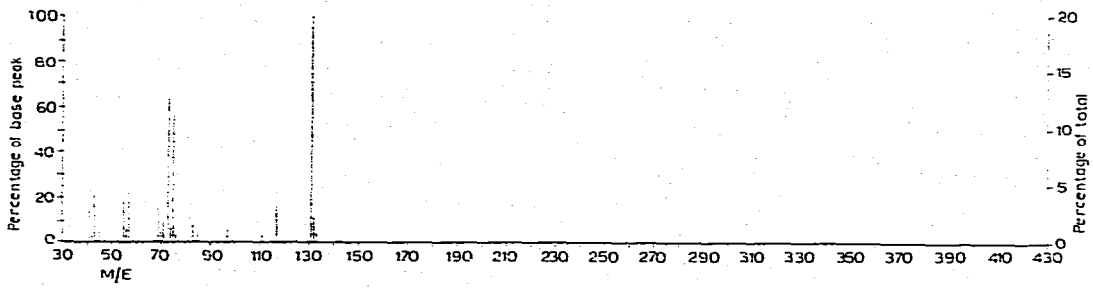


Fig. 40. GC–EI-MS 20 eV spectrum of phytane metabolite-TMS.

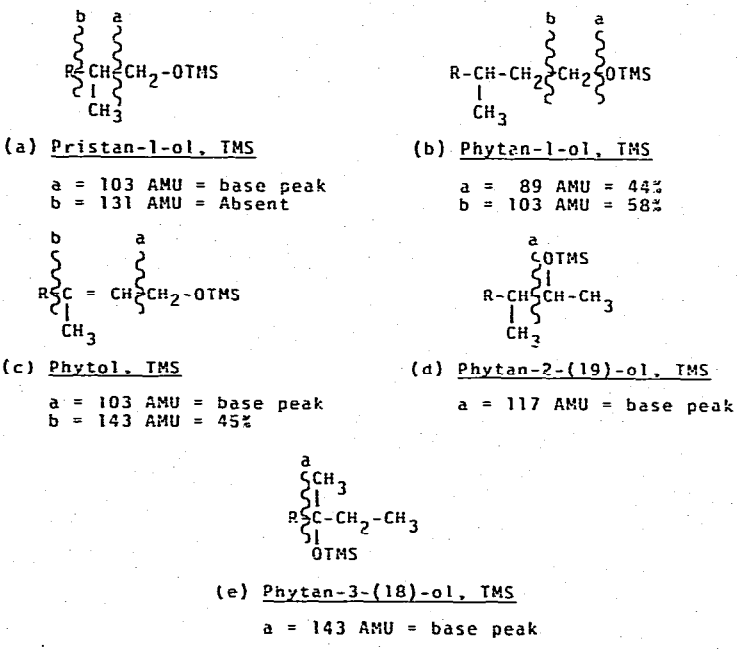


Fig. 41. Favored cleavage of trimethylsilyl (TMS) derivatives of isoprenoid alcohols at 20 eV.

The base peak at *m/e* 131 in the spectrum of the trimethylsilyl derivative of the phytane metabolite (Fig. 40) eliminates all three of the "phytan-*x*-ol" derivatives tested (Figs. 35–39) and suggests that the oxidation did not originally occur at the *anteiso*-end of the phytane molecule. However, if the hydroxyl group was located on one of the former methyl groups of the *iso*-end of the phytane molecule, the base peak should have been at *m/e* 103, because the cleavage "b" (Fig. 41) apparently does not occur in this type of structure.

The structure most consistant with the above observations is $R-\overset{\overset{CH_3}{|}}{\underset{\underset{CH_3}{|}}{C}}-OH$, which should split out H_2O very easily, eliminate an *m/e* 59 fragment fairly easily, and provide a TMS ether yielding a base peak at *m/e* 131. This postulated structure of the metabolite remains to be confirmed by organic synthesis. In passing, both CI and EI spectra of acetates and methyl ethers were obtained for the unknown and standard alcohols. No useful information was obtained from these spectra, as the substituent group was simply eliminated, yielding alkene spectra.

Another type of hydrocarbon ubiquitous in the environment is the group of polycyclic aromatics. Polycyclic aromatic hydrocarbons are probably the most abundant compounds found during analysis for air pollutants. Because of the complexity of the number and types of these hydrocarbons in air samples, gas chromatography alone as an analytical technique is very limited in its degree of usefulness in this area. Because of the carcinogenic and co-carcinogenic properties of some of the polycyclic

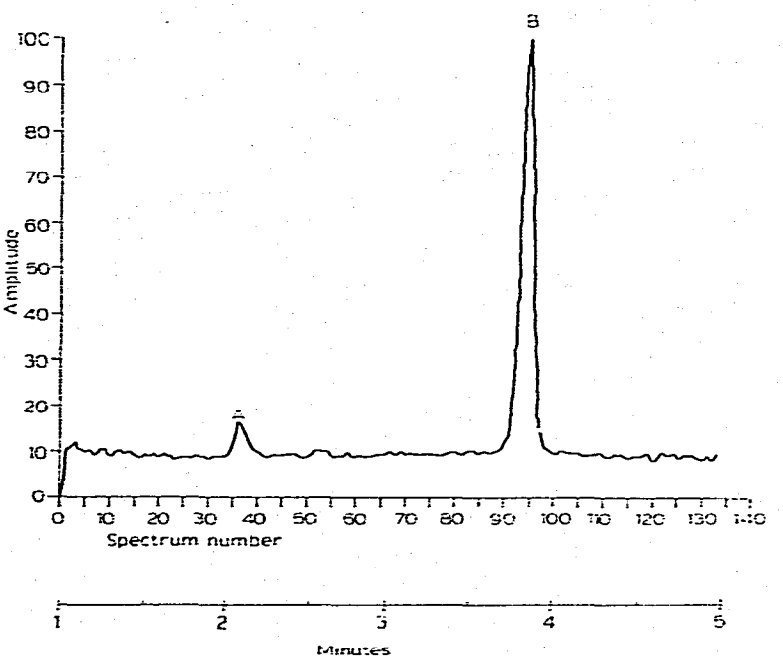


Fig. 42. Reconstructed chromatogram (GC–EI-MS 70 eV) of synthetic 1,4,5,9-tetramethylanthracene (OV-1, 200 for 1 min, 200–240° at 10°/min).

aromatic hydrocarbons which are pyrolysis products of fuels such as coal and petroleum, monitoring for these compounds as air-borne pollutants becomes very necessary in order to minimize human health problems associated with them.

A large volume of information is available concerning particulate polycyclic hydrocarbons[165] and the carcinogenic properties of these air pollutants. Within the scope of this review, one may refer to recent reports concerning the use of GC–MS in the analyses of polycyclic aromatic hydrocarbons in air samples[166–168].

Correlation of chemical structure and physical properties with biological activity is very advantageous to the chemist, especially in the case of the polynuclear aromatics and their carcinogenic properties. In this respect, we carried out the analysis of some very interesting methyl substituted anthracenes[169] in order to clarify the chemical structures and explore the possible presence of products of side-reactions during the synthesis. Fig. 42 represents the GC–MS analysis of synthetic 1,4,5,9-tetramethylanthracene. The major component (Fig. 42) with a retention time of $3\frac{1}{2}$–4 min is 1,4,5,9-tetramethylanthracene with a molecular ion at *m/e* 234 and a base peak at *m/e* 219 with less abundant *m/e* 203, 189, 117 and 102 ions (Fig. 43). In contrast to paraffins and unsaturated hydrocarbons, these anthracene derivatives in GC–methane CI-MS form very abundant $(M + 1)^+$ quasi-molecular ions with detectable $(M + CH_3)^+$ and $(M + C_2H_5)^+$ recombination fragments. As reported earlier[161], *n*-paraffins and isoparaffins in methane CI-MS produce very intense $(M-1)^+$ ions

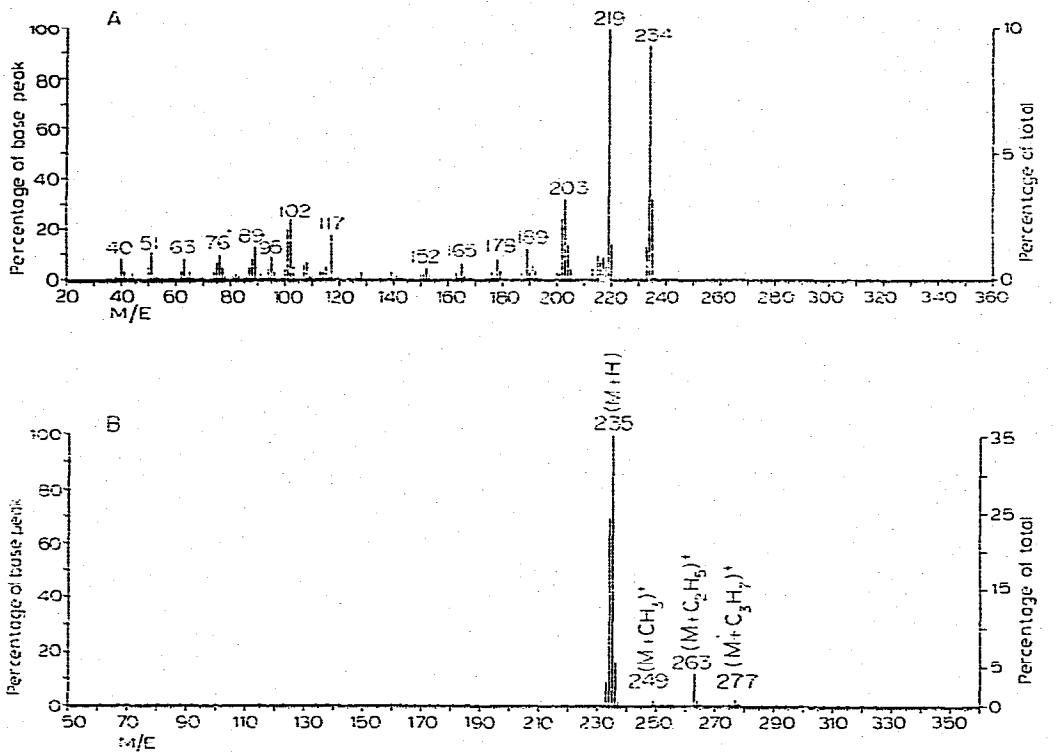


Fig. 43. Mass spectra of 1,4,5,9-tetramethylanthracene. A, GC–EI-MS, 70 eV. B, GC–CI-MS, methane.

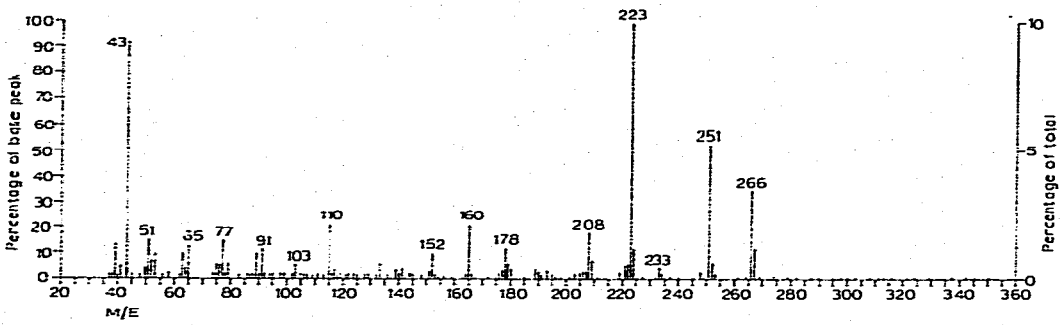


Fig. 44. Mass spectrum of synthetic contaminant of 1,4,5,9-tetramethylanthracene (GC–EI-MS, 70 eV).

without the production of the recombination fragments [$(M + C_2H_5)^+$, etc.]. As can be seen from the CI spectrum, this methyl-substituted anthracene readily undergoes adduct ion–molecule reaction with ions from methane to produce the recombination fragments similar to those described for esters[10] and more polar oxygenated compounds.

The earlier eluting contaminant of 1,4,5,9-tetramethylanthracene (Fig. 42) with a retention time of 2–2½ min has a molecular ion at *m/e* 266 with a base peak of *m/e* 223 (Fig. 44). The pentamethylanthracene[169] also contained a similar impurity which eluted before the parent hydrocarbon. This impurity of the tetra- and pentamethylanthracenes is an oxygenated compound which contains no reactive hydroxyl groups. This trace component (Fig. 42) produced during the reaction and/or storage is probably the endoperoxide of 1,4,5,9-tetramethylanthracene, which is similar to photooxidation products reported for other methylanthracenes[170,171]. The elution sequence of the endoperoxide before the parent hydrocarbon may be explained by the low degree of interaction of the oxygenated species with the GC phase in comparison with that of the hydrocarbon.

In the case of the substituted methylanthracene, the EI spectra are much more informative about the chemical structure than are the few fragments of the CI spectra. On the contrary, the CI spectra give much needed insight into the chemical reactivity and into the ability of these polycyclic aromatic hydrocarbons to undergo electrophic addition reactions.

Prostaglandins. The investigation of prostaglandins as possible hazardous environmental agents stems from at least two considerations. Firstly, because of the extreme potency and diverse effects[172] produced by minute amounts of various prostaglandins on biological systems, the evaluation as to whether exogenous prostaglandins either relative to ingestion or to topical and medicinal exposure should be considered environmental factors must be clarified. Secondly, one must determine whether endogenous prostaglandins play a significant role in the response of man and biological test systems to common environmental pollutants.

Prostaglandins[172–176] of the E type have been reported to be potent bronchial dilators, while those of the F type are potent constrictors. Both classes of prostaglandins have been implicated in inflammatory responses.

This laboratory, during the past 5 years, has investigated prostaglandins from the standpoint of: (a) development of analytical techniques for analysis of prostaglandins in biological systems, with emphasis on GC and GC–MS; (b) evaluation of common consumable food sources for the presence of prostaglandins and "prostaglandin-like" compounds which may enter the human system by way of the food chain; and (c) identification of metabolites from the biosynthesis and degradation of endogenous prostaglandins as affected by normal air pollutants.

Research into prostaglandins has increased rapidly since the early 1960s, as shown by the increasing size of the Upjohn bibliography[177] and the initiation of a journal in 1972 entitled, and dedicated solely to, *Prostaglandins*[178].

The use of GC and GC–MS for the analysis and elucidation of the structure of prostaglandins is almost as numerous as the bibliography[177] in this area. In most instances, the specific derivative of the prostaglandin analogue has been analyzed by GC–EI-MS. Some of these derivatives exhibit a very low or non-existent abundance of ions in the molecular region[176–179], even at reduced electron voltages (10–20 eV). Earlier reports from this laboratory described the GC characteristics[180] of various prostaglandin derivatives and the use of GC–CI-MS[181] for the structural identification of the intact prostaglandin molecule.

Because of the high degree of polyfunctionality of the various prostaglandins and their metabolites, care must be exercised in choosing the specific type of derivative for structural identification purposes in order to minimize the chemical and thermal lability of these biologically active lipids. As reported earlier for the trifluoroacetate derivative of $PGF_{2\alpha}$, the major chromatographable product is the methyl ester "di"-trifluoroacetate[181] with a molecular weight of *m/e* 542. In the presence of trace amounts of acid, $PGF_{2\alpha}$ tends to form elimination products. Table 14 illustrates the

TABLE 14

METHANE CHEMICAL IONIZATION MASS SPECTRA OF THE METHYL ESTER TRIMETHYLSILYL ETHER DERIVATIVES OF PROSTAGLANDIN $PGF_{1\alpha}$ (*m/e* 586) AND $PGF_{2\alpha}$ (*m/e* 584)

$PGF_{1\alpha}$		*$PGF_{2\alpha}$*	
Fragment m/e	*Abundance (%)*	*Fragment m/e*	*Abundance (%)*
615*	4.3	625***	0.7
585**	6.9	613*	10.3
571	52.6	583**	7.2
515	15.5	569	82.2
497	83.6	513	11.9
481	11.2	495	48.9
407	100.0	479	25.8
381	87.9	405	100.0
317	86.2	379	20.1
310	12.1	315	58.7
291	13.8	289	10.1
173	13.8	173	13.9
73	27.6	73	31.4

* Recombination fragments $(M + C_2H_5)^+$.
** M−1.
*** Recombination fragment $(M + C_3H_5)$.

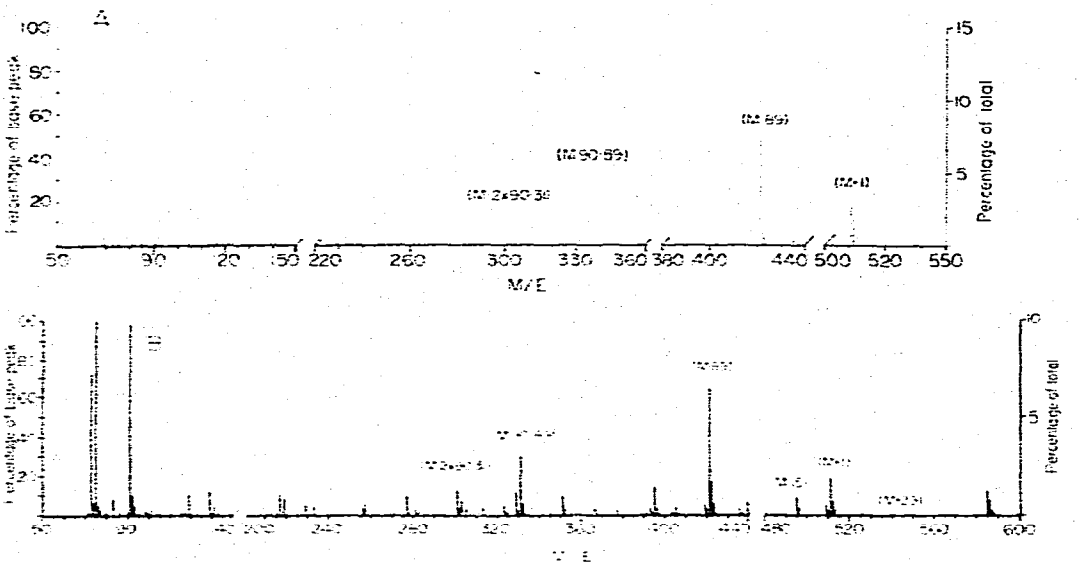


Fig. 45. Mass spectra of microsomal metabolite. A, GC–CI-MS, isobutane. B, GC–CI-MS, methane.

desirable characteristics of methane CI-MS for the analysis of the methyl ester trimethylsilyl ether derivatives of $PGF_{1\alpha}$ and $PGF_{2\alpha}$. In comparison with electron impact spectra even at low electron energies[181], in GC–CI-MS for these prostaglandins the sample requirements are less than that at 10–20 eV and the CI spectra have a greater abundance of ions in the molecular region and including the recombination fragment. The high-mass ions in the CI spectra are much more easily detected than in EI. Fig. 45 represents the CI mass spectra of a product of the microsomal biosynthesis of prostaglandins[182]. These biological investigations[182,183] are concerned with the effects of common atmospheric contaminants on the biological mechanisms of the lung as mediated by prostaglandins.

As will be discussed later for other compounds, with reference to the use of GC–CI-MS for the quantitative analysis of prostaglandins, CI mass fragmentography (refs. 184 and 185) has been used very advantageously to increase the detection limits of the methyl ester trimethylsilyl ether derivative of $PGF_{2\alpha}$ below 200 pg. The limits of detection and identification of prostaglandin derivatives by GC–CI-MS in both the specific ion monitoring and scanning modes are controlled mainly by the chemical and thermal lability of these lipids in the chromatographic system.

As discussed earlier, another reason for the evaluation of prostaglandins as possible environmental problems is concerned with the possible consumption of "prostaglandin-like" compounds through the food chain. During the discussion of this phase of investigations, the reader will note the limitation of chromatographic data when used alone.

GC–MS studies were involved in the identification of a compound isolated from wheat bran which initially was suspected on the basis of chromatographic properties to be di-nor-$PGF_{2\alpha}$. The compound co-chromatographed with $PGF_{2\alpha}$ on TLC plates in two solvent systems, and its methyl ester co-chromatographed during TLC with methyl-$PGF_{2\alpha}$. The methyl ester trimethylsilyl ether co-chromatographed with

TABLE 15

70-eV MASS SPECTRUM OF ACETYLATED METHYL ESTER OF THE FATTY ACID ISOLATED FROM WHEAT BRAN

m/e	*Relative abundance* (%)	*m/e*	*Relative abundance* (%)
29	29.2	211	3.8
30	1.5	213	5.4
31	4.2	226	36.9
32	13.8	237	8.5
57	100.0	238	2.3
58	7.8	251	2.2
59	10.8	252	1.5
60	17.2	253	1.7
83	46.6	256	3.1
84	18.6	267	1.5
85	40.0	268	3.1
86	6.2	269	1.2
87	12.6	277	5.4
88	—	285	2.0
140	52.3	290	1.7
149	57.0	308	6.5
151	12.3	309	2.9
155	13.1	314	1.5
156	2.9	322	1.5
166	8.3	323	1.1
171	4.5	324	0.6
173	7.5	325	0.9
182	14.0	326	0.9
194	23.1	350	0.6
195	6.0	351	0.8
197	3.7	368	1.4
199	3.2	411	0.6
208	10.2	439	0.6

the corresponding derivative of di-nor-$PGF_{2\alpha}$ during GLC on cyclohexane dimethanol succinate[180,186].

Identification of the bran acid eventually required the employment of IR, NMR and UV spectroscopy, MS, and a variety of chemical degradations[187]. The acetylated methyl ester of the bran acid (5,8,12-trihydroxy-*trans*-9-octadecenoic acid[187]) gave a 70-eV EI mass spectrum clearly incompatible with a prostaglandin structure[188]. This was the first indication that we were not dealing with di-nor-$PGF_{2\alpha}$.

The mass spectrum of the acetylated methyl ester of the wheat bran acid is summarized in Table 15. A scheme outlining the possible origin of the major even-*m/e* ion fragments[187] is given in Fig. 46. Other diagnostically useful fragments were those at *m/e* 439 (M − 31), 411 (M − 59), 351 (M − [59 + 60]), 277 (M − [73 + 2 × 60]), 350 (M − 2 × 60) and 290 (M − 3 × 60), confirming the presence of the three acetoxy groups and the methyl ester group. In this case, *m/e* = 74 was nearly absent, possibly because of the substitution on C_1 interfering with transfer of the γ-hydrogen[189]. Metastable ions, indicated by *( ), assisted in the development of the fragmentation scheme, as shown in Fig. 46.

Other lipids. An important area which has been only briefly mentioned but

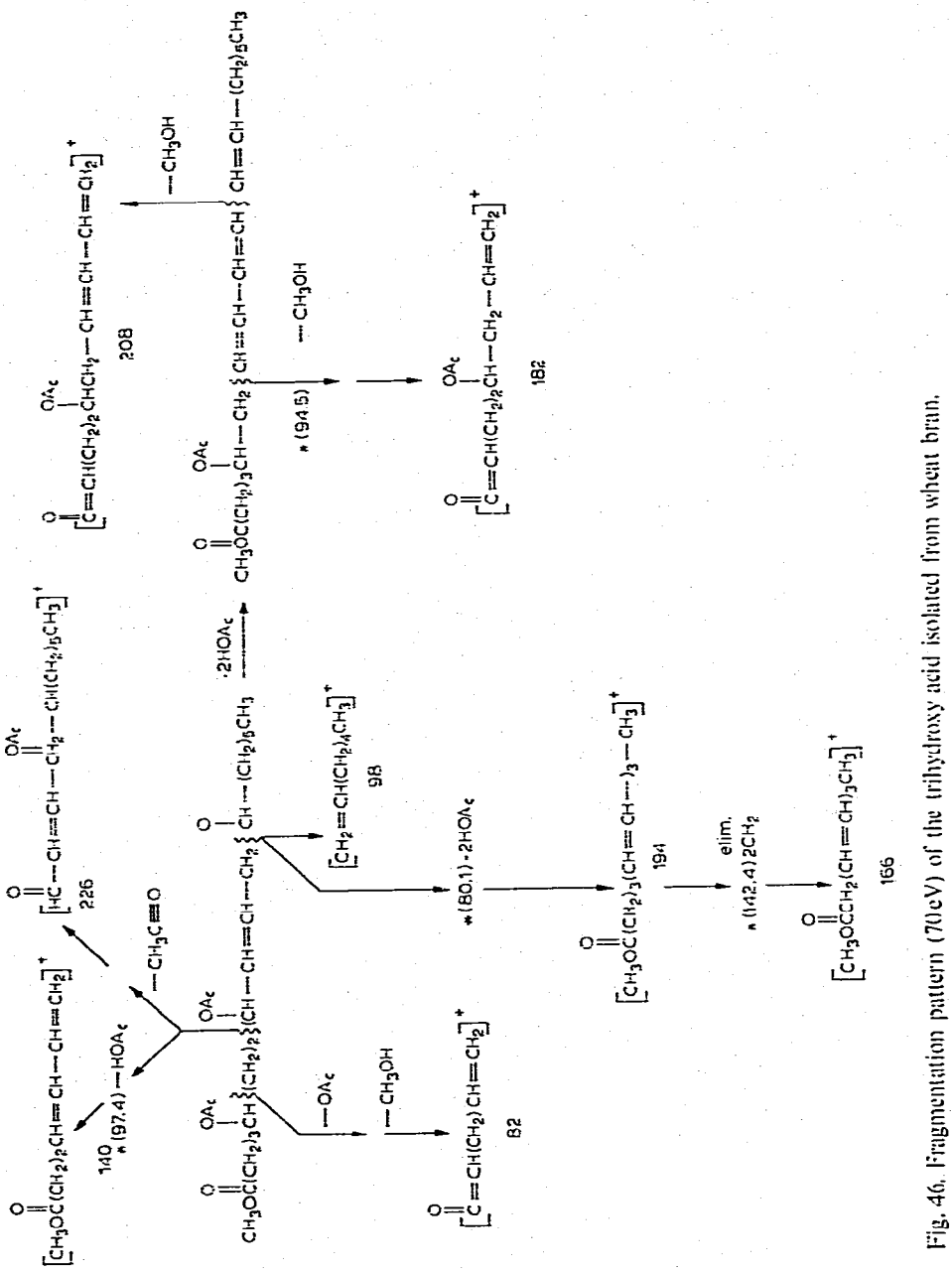


Fig. 46. Fragmentation pattern (70 eV) of the trihydroxy acid isolated from wheat bran.

which is very important during the isolation, characterization and identification of lipid soluble environmental agents, is concerned with one's ability to be able to differentiate in this case the normal lipids of the cell from the lipid-soluble agent under investigation. In the case of lipid-soluble environmental agents such as chlorinated pesticides which may be localized in the fat depot of the body, the lipids, including hydrocarbons, fatty acid esters, steroid derivatives and even the very polar complex lipids, would be normal contaminants from the biological system. In this review we do not discuss the use of GC and GC–MS in the broad field of lipids: the reader may refer to numerous other articles available on this topic[190–193]. In order to minimize and/or eliminate these interfering lipids, exhaustive means, including chemical, chromatographic and instrumental methods, must be used to assist in differentiation of the normal lipids from the lipid-soluble environmental agent and its metabolites. In instances where the investigator limits himself to one method and relies solely on chromatographic techniques to differentiate the natural contaminant from the lipid soluble compound being investigated, usually many erroneous results may arise, leading the investigator to make invalid conclusions concerning the environmental agent.

(ii) Essential oil components

Substituted allyl- and propenylbenzenes are a family of naturally occurring environmental agents with which man has many daily encounters. These essential oil components are present in foods, food additives and flavoring agent. These two classes of lipid-soluble compounds have been identified as natural substituents in nutmeg[194], bananas[195], processed tobacco[196], carrots[197], black pepper[198], parsley[194], parsnips[199], cloves[200], smoked meat products[201] and many other natural oils and flavoring materials[116]. Ingestion of considerable amounts of some of these allyl- and propenylbenzene derivatives produces physiological changes[202–204] dependent upon the drug —varying from (a) nausea, (b) cyanosis and (c) drop in blood pressure, to (d) insomnia, (e) heightening of ego, (f) inability to carry out intellectual processes and/or even (g) death. The mechanism(s) for the production of these responses are unknown. Furthermore, as reported for safrole[205–207] and more recently for other allylbenzenes[208–211], these allylbenzenes produce tumors[205–211], general fibrosis, mass adhesion, liver degeneration and very abrupt pathological changes in experimental animals.

MYRISTICIN

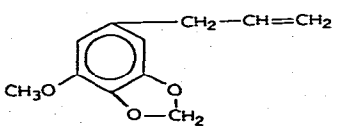


Fig. 47. Generally undesirable properties of myristicin and other allylbenzenes: 1. isolated from edible parts of parsnips, parsley, nutmeg, and processed tobacco; 2. naturally occurring insecticide; 3. possesses relatively strong synergistic activity; 4. possible psychoactive agent in nutmeg; 5. possible tumor producing agent.

Investigations in this laboratory have been concerned with the psychoactive and tumor-initiating properties of the allyl- and propenylbenzene derivatives. This research includes: (a) isolation of pure allyl- and propenylbenzenes from natural

sources; (b) synthesis of unlabeled and radiolabeled (^{14}C) components of essential oils; (c) development of analytical techniques required for chemical and biological investigations in this area; (d) general metabolism of the allyl- and propenylbenzenes and (e) correlation of structure–activity relationships at the enzyme level of the metabolites with the mechanism of psychoactive responses. Fig. 47 summarizes some of the generally undesirable properties of myristicin and other allylbenzenes. Fig. 48 represents the chemical structure of some of the essential oil components.

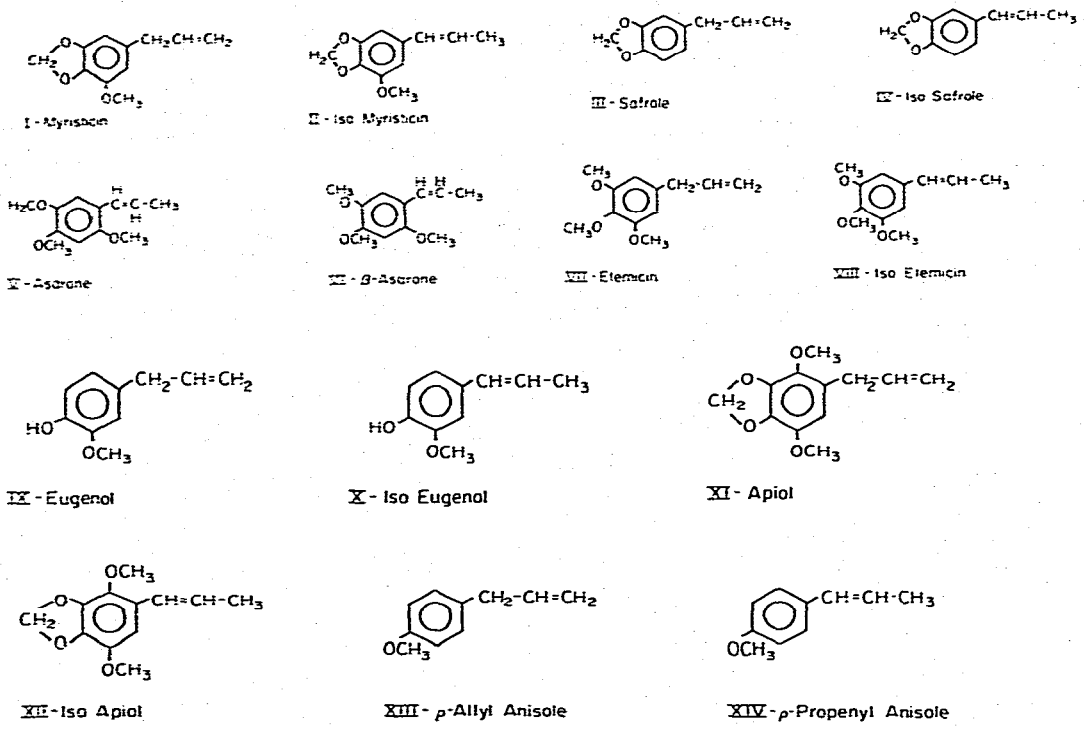


Fig. 48. Chemical structures of components of essential oils.

In order to account for the psychoactive responses produced by myristicin and other allyl- and propenylbenzenes, it was suggested that the substituted benzene derivatives may be converted biologically into amphetamines[191]. Beginning with the hypothesis that safrole or myristicin would be converted into the amphetamines which then would be responsible for the psychoactive responses, we investigated the production of urinary metabolites upon administration of a pure allyl- or propenylbenzene derivative. Using the "chemical label" of the methylenedioxy ring in the case of safrole and myristicin, together with the ninhydrin-positive characteristics of amines and amphetamines, theseinvestigations indicated that any allyl- or propenylbenzene which contained a double bond in the side-chain was converted into the ninhydrin-positive basic urinary metabolites[212] which were not present in control urine.

The urinary metabolites of safrole[212] co-chromatographed in the TLC system with the 3,4-methylenedioxyamphetamine. As the chemical haracterizaticon progressed further, it was found that these urinary metabolites reacted with the carbonyl

reagent (2,4-dinitrophenylhydrazine) and that these basic urinary metabolites decomposed very easily to yield ninhydrin-negative carbonyl-containing compounds[212]. Detailed characterization of the chemical structure of these urinary metabolites of safrole[213] using chromatography, UV, IR and NMR spectroscopy and MS in direct comparison with the synthetic standard indicated that the urinary metabolites were not amphetamines but were the more reactive *tert.*-aminopropiophenones[213]. Since the early identification of the urinary metabolites of safrole[213], in addition we have found that myristicin[209], eugenol[209,214] and elemicin[215] are also converted biologically into the appropriately ring-substituted *tert.*-aminopropiophenones.

As described earlier[212], the basic ninhydrin-positive urinary metabolites of the allylbenzenes[209,213–215] break down chemically very easily to produce ninhydrin-negative (non-nitrogen-containing) carbonyl compounds. Analyses of these metabolites[209,213–215] by GC–MS without prior chemical reduction of the carbonyl group with sodium borohydride results in almost complete conversion of the *tert.*-aminopropiophenone into the appropriately substituted allyl or vinyl ketone[213]. As has

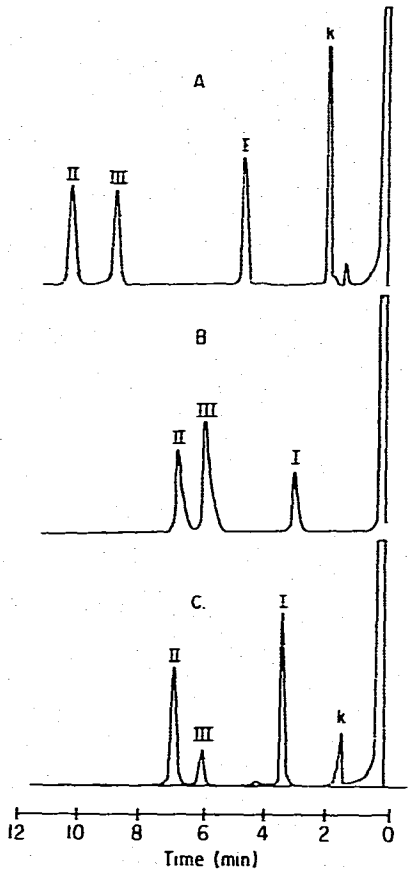


Fig. 49. Gas-chromatograms of reduced urinary basic metabolites of safrole, myristicin and elemicin (OV-1, FID). A, Safrole metabolites, 150–180° at 5°/min. B, Myristicin metabolites, 180–210° at 5°/min. C, Elemicin metabolites, 180–210° at 5°/min. k = Allylic ketone; I = N,N-dimethyl derivative; II = piperidine derivative; III = pyrrolidine derivitive.

been shown for the elemicin metabolites by GC–CI-MS analysis of the unreduced metabolites[215], very little of the nitrogen metabolite remains chemically intact. With this particular class of nitrogen-containing metabolite without prior reduction, the compound applied to the chromatograph is by no means the same compound which is eluted from the system. With thermally labile compounds of this nature, care must be taken to understand thoroughly their chemical and thermal reactivity before one attempts to chromatograph the compound by GLC.

Fig. 49 shows the gas chromatograms of the reduced metabolites of safrole, myristicin and elemicin[215]. In order to identify structurally these biologically active metabolites of the allylbenzenes, GC and GC–MS are obligatory. As one considers the ring substitution of these metabolites, it is noted that the chemical lability progresses with the eugenol metabolites being the most labile of the metabolites analyzed by GC and GC–MS[209,213–215]. This chemical lability of the eugenol metabolites is not

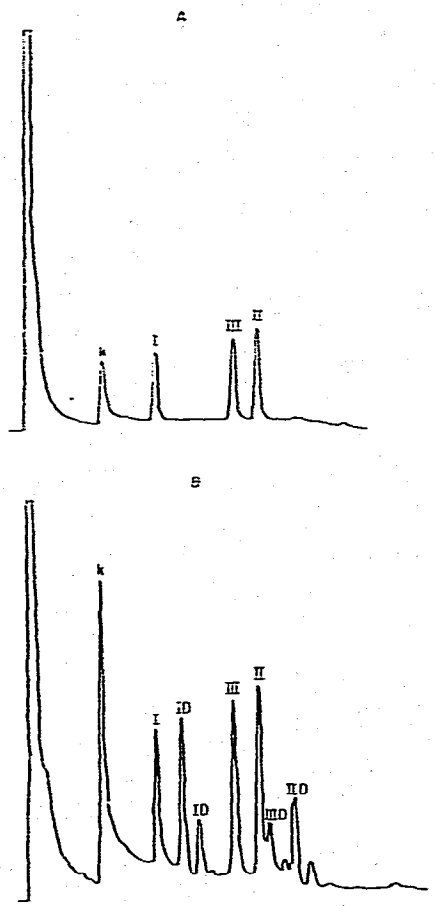


Fig. 50. Gas-chromatogram of the trimethylsilyl derivatives of the reduced eugenil metabolites (OV-1, 150° for 1 min, 150–180° at 5°/min). A, Freshly prepared, reduced and silylated metabolites of eugenol. B, Partially hydrolyzed derivatives of the silylated and reduced metabolites of eugenol. k = Allylic ketone; I = N,N-dimethyl derivative; II = piperidine derivative; III = pyrrolidine derivative; ID, IID and IIID = decomposition products of I, II and III, respectively.

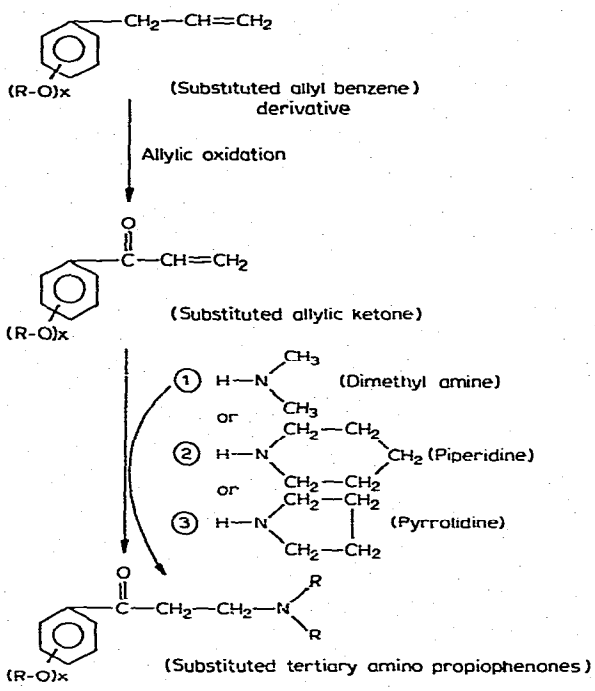


Fig. 51. Proposed pathway for the formation of *tert.*-aminopropiophenones from non-nitrogen-containing components of essential oils.

only associated with the carbonyl and nitrogen function but is also very dependent upon the phenol group on the aromatic ring.

Fig. 50 illustrates the chemical lability of the eugenol metabolites[214]. The silyl ether derivatives of the eugenol metabolites hydrolyze very easily to generate multiple components for each metabolite[214].

With respect to the metabolic sequences leading to the excretion of these urinary metabolites[209,213–215], Fig. 51 describes the proposed pathway[209]. To the more chemically oriented individual, these metabolites issue a challenge to understand the chemical mechanism. The most reactive intermediate in this sequence (Fig. 51) is the vinyl ketone. Upon oxidation of the benzyl carbon of the allylbenzene derivative to yield the vinyl ketone[216], the ketone will react with water, amines, sulfhydryl compounds and many other biologically important compounds to form the appropriate alkylated product. In the case of amines, the Mannich bases are formed[215]. Additional confirmatory data have also been reported for the *in vivo* formation of the vinyl ketones of safrole[210].

During the evaluation of reaction conditions for the synthesis of allylbenzenes, using procedures similar to those reported earlier[217] for the synthesis of [^{14}C]methylenedioxy ring-labeled myristicin from [^{14}C]iodoform, we found that two compounds with the same molecular weight as myristicin of *m/e* 192 were present in the reaction mixture. Analysis of this isomeric mixture by GC or GC–MS did not differentiate these positional isomers. Both myristicin isomers had comparable mass spectra with the molecular ion of *m/e* 192 with less abundant *m/e* 165, 147, 133, 119, 91, 77 and

57 fragments. One may very easily separate these two isomers of myristicin by silicic acid column chromatography with elution of the isomeric contaminant by carbon tetrachloride and the natural myristicin being eluted by benzene after a preceding solvent fraction of carbon tetrachloride–benzene (1:1). The isomeric contaminant of the synthetic myristicin has a R_F value (TLC on silica gel G, solvent system benzene) of 1.12 relative to the natural myristicin. Only after comparisons of the IR and NMR spectra was one able to conclude that the contaminant of this synthetic reaction was an isomer with a different positional substitution pattern on the aromatic ring. Considering natural myristicin to be the 3-methoxy-4,5-methylenedioxyallylbenzene, the isomeric reaction contaminant formed by a "double allylic rearrangement" is either the 2-methoxy-3,4-methylenedioxy- or the 4-methoxy-5,6-methylenedioxyallylbenzene. In this case, GC and GC–MS data did not assist in clarification of the chemical structure. As seen in many instances in this discussion, no one type of analytical data alone is conclusive for structural verification.

In summarizing this section, the nitrogen-containing metabolites of the essential oils chemically are very labile. Many different analytical techniques including GC, GC–EI-MS and GC–CI-MS are necessary for conclusive identification of these environmental agents. In the case of some of the CI spectra of these *tert.*-aminopropiophenones because of the low number of fragments in the spectra, it is very desirable to cross-reference this CI–EI information for identification of the urinary metabolites. Without prior reduction of these nitrogen-containing metabolites with sodium borohydride, the *tert.*-aminopropiophenones chemically decompose on the gas chromatograph to yield almost quantitatively the non-nitrogen-containing vinyl ketone which elutes from the chromatographic system.

(iii) Amines and amino acid derivatives

In this section, a brief discussion is given of: (a) biological properties and analyses for nitrosamines; (b) analysis of piperidine and pyrrolidine with emphasis on biosynthesis–precursor relationships; and (c) the derivatization and GC analysis of polyfunctional amino acid derivatives in urine and tissues.

N-Nitroso compounds. Nitrosamines and nitrosamides are very potent environmental hazards[218–221] to man because of their well documented carcinogenic, mutagenic, embryopathic and teratogenic properties. Studies by Magee and co-workers[222, 223] have shown that dimethylnitrosamine methylates both DNA and RNA *in vivo* to produce the abnormal 7-methylguanine. A proposed mechanism describing the *in vivo* metabolism and reactivity of dialkylnitrosamines has been presented by Druckrey *et al.*[224]. The occurrence of nitrosamines in foods[225–227] such as flour, milk, cheese, smoked meat and fish, and other food products has been reported. It has been demonstrated that amines and nitrite in foods can produce *in vitro* nitrosamines under conditions found in the stomach[228,229]. Within the scope of this discussion, nitrosamines have been analyzed by a wide variety of techniques including spectrophotometry[230], TLC[231,232], polarography[233], GLC[231–240], GLC–MS[241,242], and NMR and IR spectroscopy[243,244]. For a detailed discussion of nitrosamine and other alkylating agents, the reader is referred to reviews by Fishbein and co-workers[221,245]. Because of the carcinogenic properties of various nitroso compounds combined with a general lack of conclusive data on the interaction of oxides of nitrogen with naturally occurring amines present in foods to produce an "active carcinogen", the potential health

problem associated with very low-level exposure of nitrosamines to man are factual in nature but are very difficult to assess.

Piperidine and pyrrolidine. The next area of this discussion is concerned with biological origin and chemical reactivity of piperidine and pyrrolidine as associated with the earlier described metabolites of the essential oils[209,212–216]. Piperidine and pyrrolidine are natural substitutents of human and other mammalian brain tissues[246–248], skin[249], human urine[250–252], cerebrospinal fluid[253] and plant alkaloids[254]. These alicyclic amines possess biological activity[250,255–260] similar to that of nicotine. No conclusive information is at present available concerning the biosynthesis of piperidine or pyrrolidine in mammals. The suggestion that piperidine and pyrrolidine were produced in rats and other mammalian tissues by the decarboxylation of pipecolic acid and proline, respectively, is based on very scanty evidence[257–259]. In the case of pyrrolidine, Yamanishi *et al.*[258] indicated that the "TLC spot" indicative of pyrrolidine produced from brain slices to which proline had been added was "apparently larger and more dense" than that spot from slices without proline. It was concluded[258] on this evidence that pyrrolidine was produced from proline by a decarboxylase in brain tissue. To the analytical chemist involved in this area, a number of problems exist. The routine procedures[257–259] for the analysis of piperidine and pyrrolidine in *in vitro* biological systems use steam distillation prior to analysis of the DNP-amine by TLC. A similar conclusion to that described for pyrrolidine was made for piperidine[257] on the evidence of the production of a larger and dense TLC spot. In this laboratory, using numerous biological conditions including those reported earlier[257–259] for tissue slices and tissue homogenates, with carboxyl-labeled [^{14}C]pipecolic acid and proline, no decarboxylation occurred repeatly in brain or other tissues to form the respective piperidine or pyrrolidine. Analytical investigations using GC and GC–MS indicated that one can convert pipecolic acid by steam distillation into piperidine. These experiments involved steam distillation of pipecolic acid without tissue present under alkaline conditions to yield an aqueous distillate which was then converted into the DNP derivative. The DNP derivative was characterized by TLC, GC and GC–MS. The conversion of piperidine from pipecolic acid by steam distillation was very low and highly varied, with only a few micrograms of piperidine being formed per 100 mg of pipecolic acid. On the contrary, when excess of substrate was used[257,258] in comparison to the natural content of brain, this small conversion would account for the characteristic change in the size and density of the TLC spot[257,258]. The presence of the piperidine upon steam distillation of pipecolic acid was verified by GC–MS of the DNP derivative on OV-1 using EI- and CI-MS. The methane CI spectrum of DNP-piperidine has a base peak *m/e* 252 with the expected recombination fragments *m/e* 280 and 292 with less abundant *m/e* 234, 218, 206, 164, 85, 71 and 69 fragments. In the specific case of pipecolic acid, the artefact produced during steam distillation is piperidine.

During the investigation of the DNP-amine derivative, we found that DNP-pyrrolidine undergoes a chemical reaction or rearrangement upon exposure to sunlight. The entirety of this sequence is at present not completely understood. Analysis by GC and GC–MS of the DNP-pyrrolidine which had been irradiated with sunlight indicated no unusual change in the mass spectra or retention characteristics. On the contrary, the UV absorption maxima at 350 nm for the DNP-pyrrolidine upon irradiation with sunlight shifted to 335 nm. The DNP-piperidine derivative does not seem to undergo this reaction in sunlight.

In concluding the discussion on piperidine and pyrrolidine, concerning their analysis and reactivity, one must evaluate thoroughly the types of manipulations used for the isolation of these alicyclic amine from biological systems. Steam distillation of pipecolic acid results in non-biological formation of piperidine, which may lead to seemingly erroneous conclusions. Only after definitive data become available concerning the biological sequence of the biosynthesis of piperidine and pyrrolidine will one be able to coordinate the interaction of these alicyclic amines with the metabolic and psychoactive response of the *tert.*-aminopropiophenone formed from the essential oil components[212-216].

Derivatization of polyfunctional compounds. The final topic in this section of the discussion concerns the GC analysis of polyfunctional amino acid derivatives. Regardless of the research area, at some time one has a specific need for the analysis of a complex mixture of polyfunctional compounds. This laboratory has been concerned with the investigation of the metabolites of numerous environmental agents. Evaluation of the specific types of derivatives and derivatization agents become very necessary. Considering a common urine sample to contain a complex mixture of compounds, including urea, nicotinic acid, amino acids, amines, phenolic amines, and many possible combinations of functional groups (hydroxyl, amino, phenolic and carboxyl), derivatization of this complex group of compounds must be complete in order to produce single chromatographable products. Numerous silylation mixtures[261] were studied for the derivatization of acidic, neutral and basic metabolites of tyrosine and tryptophan. These derivatization conditions[261] enable one to analyze a complex body fluid such as urine simultaneously by GLC for carboxy, amino, phenolic, and many other polyfunctional metabolites. These conditions were also utilized in the investigation of the urinary metabolite of eugenol[214].

For additional information concerning the analysis of biogenic amines and amino acids by GC and GC–MS, including EI and CI, the reader is referred to articles by Fales and co-workers[262-264], Creveling *et al.*[261] and Steipta-Klauco[260].

(iv) Other natural product environmental agents

We have explored only a very small fraction of the potentially hazardous environmental agents that are naturally occurring. Other classes of compounds which will not be discussed but which are biologically active, toxic and/or carcinogenic to man by his exposure through the food chain either directly or indirectly may include: mycotoxins[265-271], mold and fungi metabolites, formed during food spoilage; plant constituents such as cycasin[272], pyrrolizidine alkaloids[273-275] and the furanoterpenoids[276] of damaged sweet potatoes, all of which are potent hepatotoxins; and trace elements[277], including lead, arsenic, selenium and cadmium. The reader is referred to more detailed articles[265-277] on these topics. Because of the very large areas concerned with the potential environmental health problems of man, it is impossible within this review even to mention one representative of each type of compound involved in man's daily survival within his environment.

(c) Mass fragmentography

Up to this point in the discussion, we have mentioned only the use of GC–MS for the qualitative identification of various environmental agents. Instead of scanning

an entire mass range with the mass spectrometer, one may concentrate only on one or more ions of a specified mass (*m/e*). In the use of mass fragmentography, the GC system serves as the means of chromatographic separation of the complex mixture, with the mass spectrometer functioning as a specific ion detector. By being able to concentrate on a small number of ions instead of all ions in a mass range, one has increased the sensitivity of the analytical mode greatly in this manner simply by being able to increase the time per ion that one monitors the system.

Mass fragmentography may be subdivided into single ion detection (SID) and multiple ion detection (MID). With a commercially available quadrupole mass spectrometer[278], one may monitor simultaneously up to eight ions. With conventional sector instruments[279,280], the mass spectrometer may be focused on one to four mass fragments within 10–30% of the mass range. SID may be used to analyze a complex mixture of compounds with different GC retention characteristics but which ionize to produce a common ion fragment. High-resolution[281] ion monitoring may be considered a further refinement of SID. By use of the high-resolution system one may analyze multiple components which produce similar ion fragments but were differentiated in mass only by a few parts per million. This technique[281] was applied to the detection of *p*-tyramine in the concentration range 10^{-12}–10^{-5} g with the ability to differentiate *p*-tyramine of *m/e* 108.0575 from a naturally occurring lipid hydrocarbon contaminant[281] of *m/e* 108.0939. MID may be used for the analysis of a complex mixture which may or may not be chromatographically separable. The components of MID analysis may all ionize to produce a common ion fragment but may be differentiated by one or more additional specific ions. By the use of multiple ion detection, one may very conclusively identify and quantitate simultaneously a specific component in a complex mixture without loss of specificity. As one reduces the number of ions being monitored by mass fragmentography, one also decreases the compound specificity of the analysis.

Mass fragmentography has been used more widely in the analyses of natural products and drugs than for the analysis of chlorinated hydrocarbons and other man-made environmental agents. The reader is referred to a review by Gordon and Frigerio[282] for a discussion of mass fragmentography up to 1972. As can be seen from their report[282], the detection limits for various psychoactive compounds and steroids vary from the low nanogram to the low picogram range, depending upon the type of compound and the mode of analysis.

More recent applications of mass fragmentography[283] have been oriented towards the areas of: prostaglandins, in the EI mode[284,285] with samples from 100 ng to 400 pg and in the CI mode[14] for $PGF_{2\alpha}$ TMS with a sensitivity limit below 200 pg[184]; steroids, catecholamines and amino acid derivatives[286] in the EI mode with analysis in the nanogram range; dansylated amines[260,287]; and guanido-containing drugs[288] with analysis at the low nanogram level.

Specific uses of mass fragmentography with man-made environmental agents include: pesticides of the DDT type in presence of PCBs[289] with detection limits below 10 ng; DDT by SID using a Varian-Mat[290] with a detection limit of about 10 pg; aldrin[291] monitoring *m/e* 263 with a sensitivity limit of about 500 pg; mercury halides in fish[292] with usable limits below 5 ng (1 μg of mercury per kilogram of fish); tetraethyllead[293] with detection limits in the low picogram range; and polychlorinated biphenyls[294] using specific ion sub-sets with sufficient sensitivity at least in the low nanogram range.

Most of the above discussion concerning mass fragmentography has been derived from EI analyses. A further refinement of mass fragmentography includes the use of this technique in the CI mode. As has been shown for many types of compounds in this discussion, the abundance of ion fragments of a polyfunctional compound in the molecular region is much greater in CI spectra than in EI spectra. Therefore, analysis of these specific compounds by CI mass fragmentography would further increase the sensitivity of analyses. As described earlier[184] for prostaglandins, propoxyphene and propoxypheneamide, the utilization of CI mass fragmentography is very advantageous. At least a 10-fold increase in sensitivity with reduction in interference from naturally occurring contaminants was exhibited for the analysis of propoxyphene[184] by CI mass fragmentography over the EI mode.

This powerful quantitative technique of mass fragmentography has been used only to a very small degree with specific environmental agents. A further exploration and expansion of applications of this mode of analysis in the area of both man-made and natural product environmental agents is needed. Additional consideration should be given to specific advantages of CI- and EI-MS characteristics for the quantitation of many potentially hazardous environmental agents and their metabolites. A greater utilization of CI mass fragmentography in the multiple ion detection mode will validate and generate a much more sensitive and specific analysis of many polyfunctional compounds in biological systems.

(d) Miscellaneous

Many very recent reports on the use of GC–MS which have not been included in this discussion may be of interest. This subject matter includes: computer analysis of MS data[295,296]; double detector GC–MS interface[297]; GC–MS in clinical biochemistry[298]; and the GC–EI-MS analyses of normal urine constitutents[299], barbiturates[300], amphetamines[301], hydroxy-steroids[302,303], catechols[304], arylglucuronic acid derivatives[305] and triglycerides[306]. The reader is also referred to reports concerning the use of GC–CI-MS in the analysis of marihuana smoke condensates[307] and biogenic amines[308].

As illustrated in this review, CI spectra may contain too few fragments to identify the compound conclusively. In numerous instances, only after cross-referencing of the CI and EI data is the structural determination conclusive. On the contrary, we have found that even though the structural CI data alone may not be sufficient for identification purposes, a large amount of useful information may be obtained from CI mass spectra concerning the chemical reactivity of the molecule. An excellent example of the use of CI-MS for investigation of chemical and functional groups reactivity has been reported by Hunt and co-workers[309,310]. By using various reagent gases (methane, isobutane, helium, deuterium oxide, argon–water or ammonia) one is able to study the types of chemical reactions that the compounds may undergo.

No mention has been given to the techniques of field ionization and field desorption MS in this review. Field desorption MS has an enormous advantage in that it is not necessary to volatilize the sample for the analysis[263]. The reader is referred to reports by Beckey[311], Fales[263], Schulten[312] and Damico and Barron[313] for further insight into the area of field ionization and field desorption MS.

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5. SUMMARY AND CONCLUSIONS

We have attempted to describe the utilization of gas chromatography and gas chromatography–mass spectrometry (GC–MS) as analytical techniques for the chemist concerned with the environmental health problems of man. Some of the more important potentially hazardous environmental agents and their metabolites were viewed through the eyes of chemists with respect to the areas of analysis, synthesis, chemical reactivity and metabolism in biological systems. Specific examples using various types of environmental agents were chosen to illustrate the advantages and/or limitations of data from GC and GC–MS. A detailed discussion was given of the complementary utilization of electron impact and chemical ionization mass spectrometry for the identification and quantitation of various types of compounds of environmental significance.

In concluding this review, we have presented the views of chemists using the valuable analytical technique of GC–MS to assist in solving problems associated with man's health as affected by the environment. Being able to obtain structural and quantitative data on submicrogram amounts of potentially hazardous compounds is the first prerequisite to fully understanding the problems produced by a compound. Only after the chemist has integrated the GC–MS data with those from other analytical techniques and with other types of biological investigations, will one be able to understand and control fully the undesirable health problems produced by the specific agent.

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