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SCREENING METHODS FOR ORGANOPHOSPHORUS PESTICIDES

USING TRIPLE QUADRUPOLE MASS SPECTROMETRY
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SCREENING METHODS FOR ORGANOPHOSPHORUS PESTICIDES USING TRIPLE QUADRUPOLE MASS SPECTROMETRY

By

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ABSTRACT

SCREENING METHODS FOR ORGANOPHOSPHORUS PESTICIDES
USING TRIPLE QUADRUPOLE MASS SPECTROMETRY

By
Mark Richard Bauer

The detection and identification of organophosphorus pesticides is an increasingly important problem. Detection methods normally require multiple extractions and long chromatographic separation times. The methods described here utilize the low energy CAD fragments of organophosphorus compounds to quickly screen for pesticides, their metabolites and decomposition products.

Methanol is used as the chemical ionization reagent gas in order to produce only $(M+H)^+$ ions in the source. The $(M+H)^+$ ions from each pesticide class follow characteristic decomposition pathways in the collision cell that may be monitored by neutral loss and parent ion scans. For a complete screening analysis, the triple quadrupole mass spectrometer automatically runs through a set of neutral loss and parent scans. The method can be used to detect many common pesticides and related compounds at the ng level in complex mixtures. The compound specific parent-daughter pairs exploited in this technique can also be used for targeted analysis using reaction monitoring. This limits the number of compounds that can be detected at one time, but it also lowers detection limits correspondingly. These methods illustrate how MS/MS can advantageously use the results of fragmentation studies for the direct analysis of entire classes of compounds.

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Background and Introduction

Pesticide Development

The use of chemicals to control insects and other pests has become commonplace in the world today. The fact is that chemicals have become necessary to preserve the way of life most people are now accustomed to. There are 4.7 billion people in the world today and the population is increasing at the fastest rate ever. At the same time, less land is devoted to agriculture each year. The only way that worldwide starvation is prevented is by increasing the productivity per acre through the use of chemical fertilizers and pesticides. If pesticides were not used, statistics show that only 37% of the potatoes grown would survive, 78% of the cabbage crop would be destroyed and only 10% of apples and 9% of the peaches grown would reach the market (1).

In a single season a house fly can, through seven generations, spawn 3.5×10^{12} individual flies. The woolly apple aphid can go through 20 generations in a season. Two aphids can mushroom into a phenomenal 7.6×10^{30} aphids (1). These insects along with others can completely wipe out a crop if left unchecked. They are so prolific that even with the use of pesticides, one third of all crops are lost to pests and disease. Insects, rodents, weeds, microorganisms and parasites represent a real threat of disease and destruction to plants, stored food and other dry goods, as well as threaten the

health of livestock and humans.

Fortunately science has developed chemical pesticides to control many of these problems. The use of pesticides has continued to increase yearly (1). The high economic efficiency achievable with the use of pesticides in agriculture and other branches of the economy has favored a rapid development of these compounds by the chemical industry. There has been continuous change and improvement in the assortment of pesticides available. In the middle of the nineteenth century the first pesticides used were powders such as sulfur for controlling mildew in vines, bordeaux mixture (copper sulphate solution plus lime) for controlling downy mildew in a variety of crops and paris green (a mixture of cupric oxide and arsenic oxides) against Colorado beetles. Further advances came at the beginning of this century with the use of nicotine solutions against aphids and tar distillate sprays to control aphids and lichens on dormant fruit trees. Ferrous sulfate and sodium chlorate were introduced as general herbicides and dinitrophenols and cresols were used to control weeds in cereal crops. These crude solutions and mixtures of multiple compounds have since become known as "first generation" pesticides (2).

In the 1940's a second generation of pesticides was developed. These new compounds included the organochlorines, organophosphoruses and carbonates. For many years the pesticides most commonly employed were organochlorines such as DDT, BHC, aldrin and dieldrin because they were so effective against a variety of problems and did not have to be reapplied very often. Recently however, this category of compounds has come under criticism and the organophosphoruses and carbonates have become the main pest control chemicals. The chemicals

in this second generation of pesticides have been developed to the point where they are optimized for specific applications. Different compounds are used for their particular effectiveness as insecticides, herbicides (weed killers), rodenticides, molluscocides (snail killers), acaricides (spider-mite killers), nematodacides (worm killers) or fungicides.

There is now a third generation of pesticides under development. These compounds are even more specific and are safer for the environment. New nontoxic chemicals such as pheromones and sex attractants are intended to disrupt the reproductive cycle of the insects rather than kill them outright (1,3,4,5). This new breed of compounds is especially useful in urban areas where toxic chemicals pose the greatest risk.

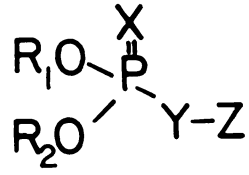
The major reason that organophosphorus pesticides have become so popular is that, unlike the organochlorine compounds, they are less persistent in the environment (1). DDT and dieldrin, the most common organochlorines used, were in use for over thirty five years will continue to be a part of our environment for years to come. Organochlorines are very persistent, having low volatility and water solubility. They are however, very soluble in fats and hence they collect in the fatty tissue of higher animals. The extent to which organochlorine residues have accumulated in soils and their side effects on the ecosystem have been well studied and documented. The result is that even though organochlorines are very effective in controlling insects, the shorter lived organophosphorus compounds are now used for most problem pests. Figure 1.1 shows several pesticides and their effective life times (6,7). The organophosphorus esters are

chemically unstable, susceptible to both acidic and alkaline hydrolysis by a variety of mechanisms which render the compounds harmless (1,8). The compounds are also more toxic than the organochlorines before they decompose. Therefore less of the chemical needs to be applied. This makes it cheaper for the farmer and better for his land.

In the same manner that the organophosphorus pesticides can be made specific for insects or weeds and not humans, they can also be made to be highly toxic to humans. By changing the molecular structure certain organophosphorus pesticides can also be used as chemical warfare agents. Before World War II, Dr. Gerhard Schrader made the first lethal organophosphorous pesticide that later became Toban, the first phosphorus chemical warfare agent. Much of the original research on the chemicals that became pesticides was done by the Germans during World War II in order to find a nicotine substitute (2). The toxins they discovered instead have found a place in peaceful non-mammalian uses.

Structure of Organophosphorus Pesticides

The organophosphorus compounds under discussion all have a similar structure. A generalized structure is shown in the following figure.



R_1 and R_2 are usually the same and can be either an ethyl or a methyl group. X and Y can be either sulfur or oxygen and Z is a fairly good

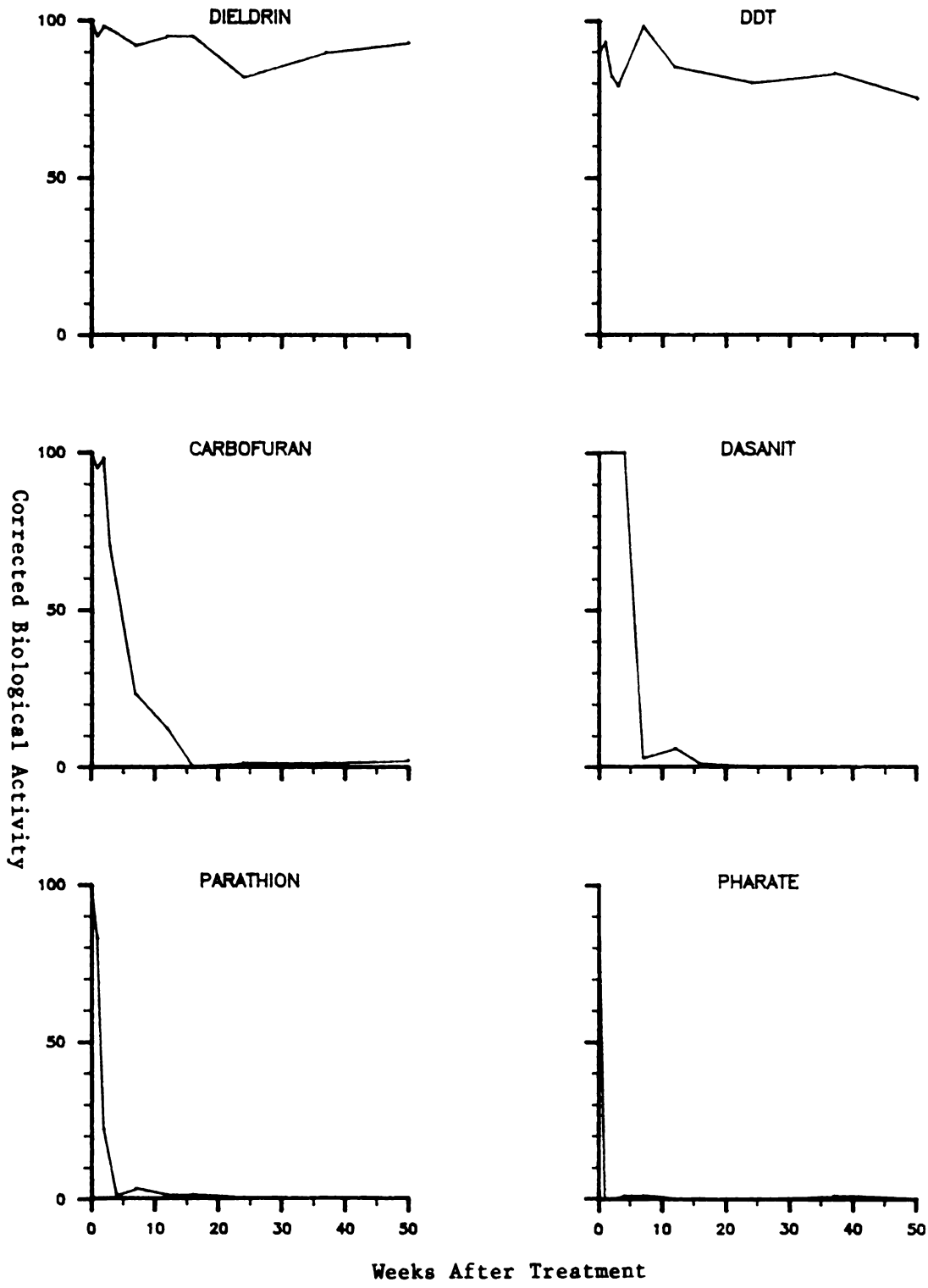
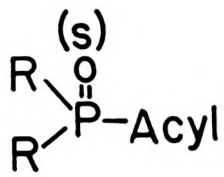


Figure 1.1

Persistance of some Pesticides

organic leaving group such as a substituted or heterocyclic ring, an aliphatic chain or an amine.

In 1963 Schrader came up with a formula dictating what functionilities are necessary to assure biological activity in an organophosphorus pesticide of the following general structure:



"It is likely that a biologically active phosphoric acid ester will be attained when the following prerequisites are satisfied: Either sulfur or oxygen must be directly bound to the pentavalent phosphorus, R_1 and R_2 may be alkoxy groups, alkyl groups or amines; while the "acyl" may be represented by the anions of organic or inorganic acids such as fluorine, cyanate and thiocyanate or one of the other acidic compounds."(8)

Schrader's definition of "acyl" was rather liberal and different from that which we use today, but he was the first to understand that the chemical mechanism of insecticidal action may depend on the phosphorylation of biological targets (8).

There are six main groups of organophosphorus pesticides made by the different possible combinations of sulfur and oxygen. The different classes are shown in Figure 1.2. Each group can also contain compounds with R groups which are either both methoxy or ethoxy. There are so many different types of organophosphorus pesticides because they can be manufactured to accomplish a specific mission. By changing the R groups or the type of acyl moiety used, specific insects, plants or other pests can be controlled. The compound's

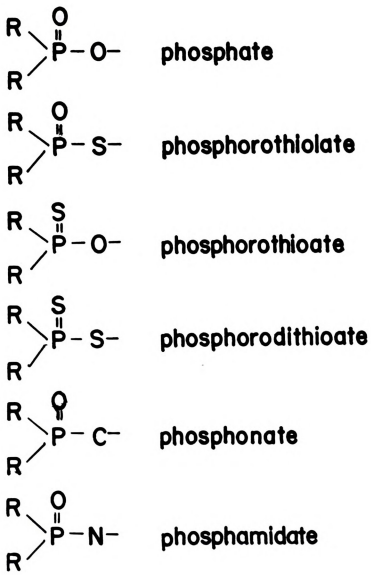


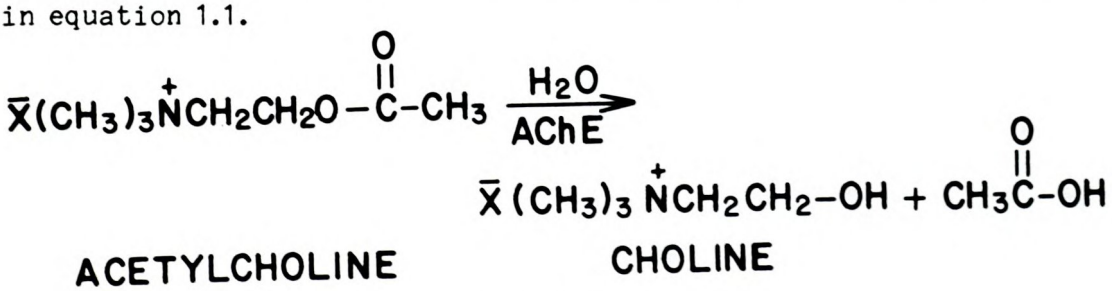
Figure 1.2

Common Organophosphorus Pesticide Structures

solubility, and hence persistence, may be tailored to last an entire growing season or be made such that produce can be sprayed one day and eaten safely the next. Certain structures are better if incorporated into the soil as herbicides; others are better if sprayed, others still are best if used as a systemic pesticide. Various forms of the molecule can be a deadly toxin to one species but perfectly harmless to another. This way the applied pesticide will kill the pest that tries to take a bite but will be harmless to birds or humans that may be hungry (4).

Toxicity

Organophosphorus pesticides are thought to work by the inhibition of enzymes involved in the functioning of nerves (1,2,4). In human cases the enzyme affected is acetylcholinesterase (AChE) which under normal circumstances catalyzes the hydrolysis of acetylcholine. During normal nerve activity when electrical impulses reach a nerve synapse, acetylcholine is released and diffuses across the synapse. Upon contacting the receptive site on the other side of the synaptic gap, a new impulse is triggered. acetylcholinesterase helps to hydrolyze the acetylcholine in order to free the receptive site and allow fresh impulses to be received. The hydrolysis reaction is shown in equation 1.1.



Equation 1.1

If the acetylcholinesterase is inhibited, the acetylcholine builds up at the synapse and the nerve becomes locked in the "on" position causing, for example, muscles to remain contracted. The actual mechanism for the hydrolysis of acetylcholine involves the formation of an initial enzyme substrate complex (equation 1.2) followed by the release of choline and the formation of esterified enzyme (equation 1.3).

$$\text{AChE} + \bar{X}(CH_3)_3\overset{+}{N}CH_2CH_2O-\overset{O}{\overset{\|}{C}}-CH_3 \rightleftharpoons \underset{\text{COMPLEX}}{\text{AChE}\cdots(CH_3)_3\overset{+}{N}CH_2CH_2O-\overset{O}{\overset{\|}{C}}-CH_3\bar{X}}$$

Equation 1.2

$$\text{COMPLEX} \longrightarrow \text{AChE}-\overset{O}{\overset{\|}{C}}-CH_3 + \underset{\text{CHOLINE}}{\bar{X}(CH_3)_3\overset{+}{N}CH_2CH_2-OH}$$

Equation 1.3

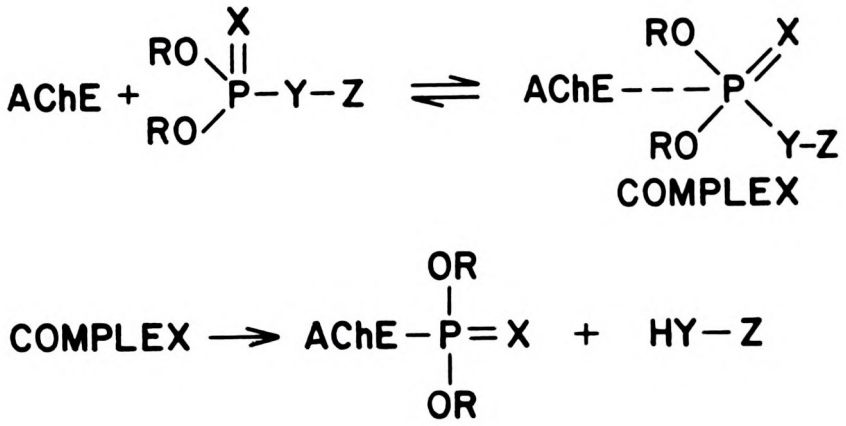
The esterified enzyme is then hydrolyzed back to free enzyme and acetic acid (equation 1.4).

$$\text{AChE}-\overset{O}{\overset{\|}{C}}-CH_3 \xrightarrow{H_2O} \text{AChE} + CH_3\overset{O}{\overset{\|}{C}}-OH$$

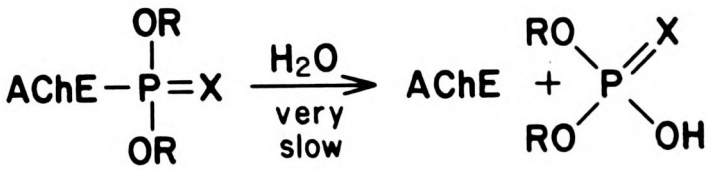
Equation 1.4

The organophosphorus compounds are effective inhibitors because they are phosphorylating agents due to the good leaving group Y. They are able to chemically combine with the enzyme forming a stable phosphorylated compound (equation 1.5). This new enzyme is unable to react with acetylcholine because it's active site is blocked and it's

hydrolysis is very slow (equation 1.6).



Equation 1.5



Equation 1.6

The factors affecting the inhibiting power of the organophosphorous pesticides include size, shape and polarity. These factors affect the relative stability of the phosphorylated enzyme. The nature of the leaving group also affects the reaction rate of the initial complex formation step (2).

The exact structure of AChE is not yet known, nor are the structures of the enzymes employed by the different insects. The nervous system of insects is thought to be somewhat similar to the human system but the differences allow some organophosphorus compounds to affect insects preferentially. The organophosphorus pesticide that is able to fit into the enzyme active site of the insect is unable to fit into a human's enzyme active site, thus making the chemical safer to humans(2).

The different physiologies of beings in the animal kingdom

cause another type of specificity. In some animals certain organophosphorous compounds are metabolized or hydrolyzed to harmless phosphorus acids before they reach the enzyme site (2,8). In many cases metabolism oxidizes the organophosphorus pesticides, creating a form which is more susceptible to hydrolysis than the reduced version. In other cases however, the oxidized form is even more deadly (1,2). This *in vivo* conversion is capable of converting an innocent compound that can be applied widely into a substance that is toxic to only certain targeted species.

The toxicity of organophosphorus pesticides is measured in terms of a factor called LD_{50} which is the average minimum dosage, in mg/kg of body weight, required to kill 50% of the population tested (2). LD_{50} values are determined using a variety of laboratory animals and are assumed to be similar for humans. The means of application also needs to be specified since injected doses, inhaled dosages and skin adsorption (the most common means) all have different LD_{50} values. The LD_{50} of malathion, a very common general purpose insecticide, is >4 g/kg dermally (9). Malathion, which was introduced in 1950, is a fast acting pesticide that is widely used on vegetables, fruits and cereals, both in the field and in storage. It is the compound used on humans to control lice and crabs and is also the toxin employed in animal flea collars. In 1982 it was the insecticide used in California against the Mediterranean fruit fly. It was mixed with molasses and yeast as bait and sprayed in residential areas by helicopter (4). It was used because it would take several hundred grams of malathion to affect an average size person and small amounts are quickly metabolized in man.

In contrast, the LD_{50} of the chemical warfare agents known as V-agents is about 0.03 mg/kg. It is estimated that 0.09 grams of a V-agent, a drop about the size of a pinhead, when placed on a man's skin will be lethal. It is not an easy death either. The first signs of organophosphorus pesticide poisoning are constricted pupils, blurred vision and pain behind the eyes. Subsequently, tightness of the chest with difficulty in breathing develops. This is followed by drooling, sweating, nausea, involuntary defecation or urination and eventually convulsions, coma and death by asphyxiation; all in less than ten minutes (2).

Most compounds fall in between the two extremes of totally safe and deadly. Researchers, manufacturers, distributers and farmers are urged to use appropriate safety measures when handling and applying these very helpful, but potentially dangerous chemicals. Caution must be used, not only to protect humans, but also to insure that no other creatures in the ecosystem are unintentionally harmed.

Detection Methods

For each different application of organophosphorus pesticides a detection method has been developed. Pesticide presence is monitored as they are manufactured, as they are applied, after they are applied and after they have claimed their victims. Analytical methodologies are also developed for new compounds or new applications during their experimental stage as an aid to the researchers so that they can analyze the pesticide's performance. Pesticide detection methods have also been developed to monitor their presence in different parts of the environment. When pesticides are applied most of it

ends up where desired, either in the soil or on a plant, depending on the method of application and intended use. However, through rain, wind, evaporation, diffusion and sometimes human carelessness, the pesticide is partitioned between the air, water, the soil and the rest of the ecosystem (10,11).

In the beginning of pesticide development, scientists were mainly concerned with the effectiveness of the compounds; how well they worked, how long they lasted and which application method worked best. During this phase, crude detection methods were often used. To see whether a certain variety of insect or plant was affected by the compound, they were simply exposed to the toxin and monitored to observe any affects. In his study of the persistence of certain pesticides in the soil Harris used cricket nymphs placed in a container with some soil. The number of nymphs that died was then plotted versus the weeks after application to obtain the results (6).

For humans however, this type of method cannot be used. So once LD_{50} levels were determined using laboratory animals, methods had to be developed to quantitatively detect the actual compounds in various species and in the environment. Finding the small residual amounts of pesticide present in food, plant and soil matrices is a classic analytical problem and classic analytical procedures were developed for their detection.

The first steps in soil and food analysis generally involve a lot of wet chemistry (12,13). The sample is first homogenized and then the pesticides are extracted out of the solids with appropriate solvents. The extract can then be filtered, buffered and subjected to several more extractions, dryings and evaporations. Column chromatography is often used as a last cleanup step before final detection.

Gas chromatography is the traditional detection method, but other detectors such as mass spectrometry and thin-layer chromatography are also used. A general flow chart of a typical multi compound analysis is shown in Figure 1.3. Depending on the target compound and the sample matrix, different procedures must be followed because of the differences in extractability, elution patterns in different absorbent solvent systems and the selectivity and sensitivity of the various filtration, cleanup and detection steps. Screening methods, used to qualitatively find which compounds may be present are usually different from quantitative analyses in that they can often be accomplished with less chemical work up.

The detection of pesticides in water samples involves many of the same steps outlined above. However, the concentrations of the target compounds in water are often very small and much smaller than the amounts found in many foods. Therefore large volumes of water must be sampled in order to get enough compound to detect. Processing water samples is also complicated by the fact that most pesticides are insoluble in water but are easily adsorbed by suspended organic matter in the sample. This necessitates detection techniques that address both adsorbed and dissolved pesticides (14). If the analyst is interested in the total pesticide contribution to lake or stream water, both states of the pesticide must be accounted for. If drinking water which is supposedly cleaned and filtered is under investigation, then only the dissolved pesticide need be considered. The water to be analysed is forced through a chemical trap or a physical filter, such as a macroreticular resin like XAD-2 or XAD-4 in order to preconcentrate the sample (15). The sample is eluted or extracted from the concentrator and then sent through the steps in the scheme shown in

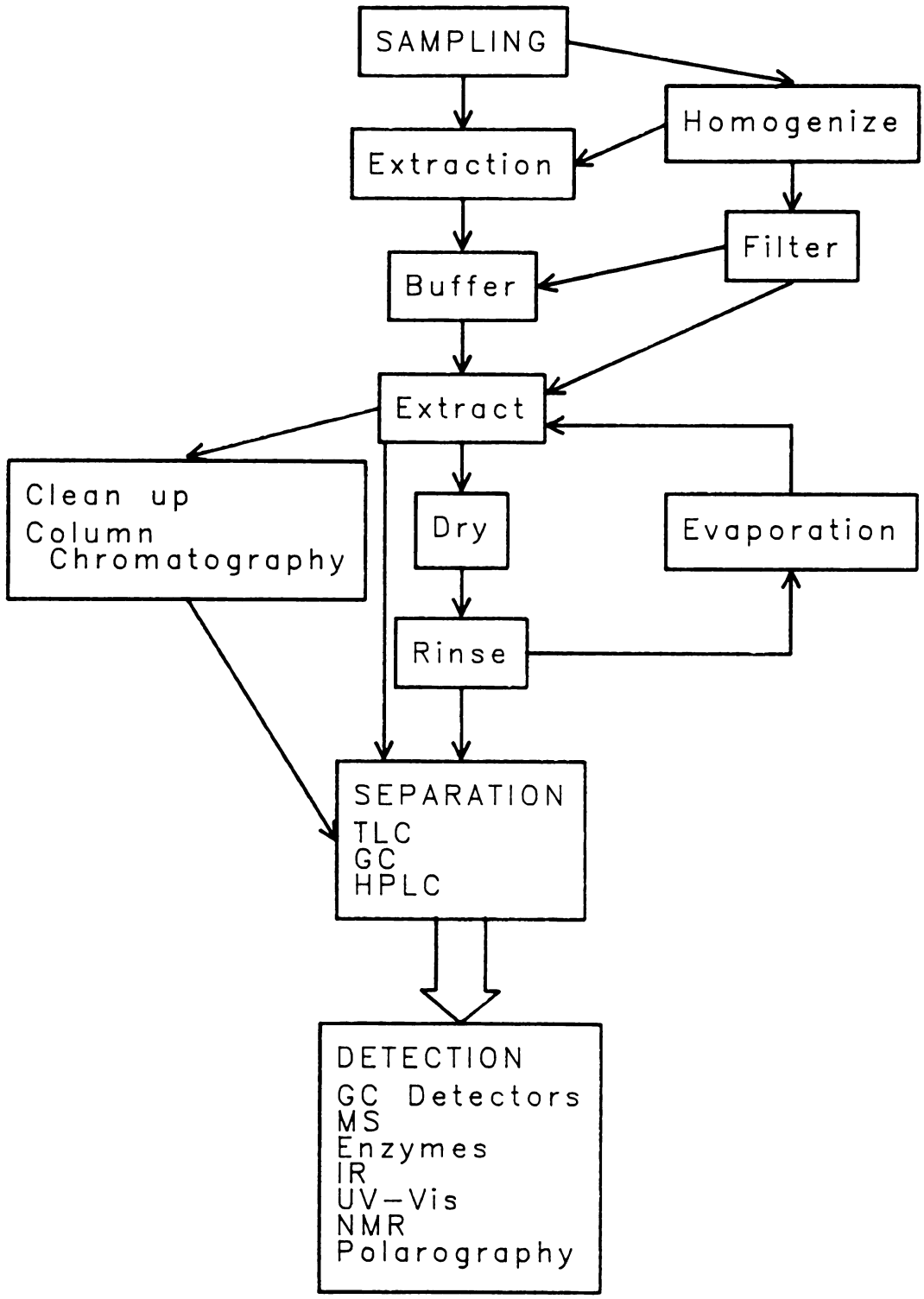


Figure 1.3

Flow Chart For Multi Compound Analysis

Figure 1.3.

Air samples pose similar problems. Particulate pesticides and pesticide vapors can be present in the air at the ng/m^3 level or lower. Air samples must also be preconcentrated by traps or filters. Pesticide vapors are usually chemically caught in resins, impingers or gas debubblers, while airborn particulate matter is trapped in filters and porous substances (16-19). The collection techniques employ large fans or pumps to sample 300 m^3 or more of air needed for analysis. The pesticides are then removed from the sampler with a soxhlet extractor, cleaned, concentrated and analyzed with a gas chromatograph.

Many of these methods are still used today but newer analytical techniques have been added to improve the methods. Lower detection limits for pesticides in air have been reached by using a combination of polyurethane foam and Tenex resin preconcentrators (20). Tenex is much better at trapping organic vapors and hence the formerly "hard-to-capture" high volatility pesticides are effectively collected. Organic interferences can be removed by a chromatographic cleanup and fractionation step which also aids in lowering detection limits below 1 ng/m^3 . Small portable air samplers have been developed that do not need large air flow rates and are ideally suited to detect vapors in the work place (21).

High Performance Liquid Chromatography (HPLC) has become a popular tool for water analysis (22-24). Samples as small as 1 ml can be analyzed on chemically bonded stationary phases without any preconcentration, and using solvent programming, part per billion detection limits are realized. Because of its ability to concentrate and separate, reversed phase liquid chromatography has also been used to detect organophosphorous pesticides in plasma and other body fluids. Recent

developments in HPLC detecters such as the LC/MS interface have given HPLC excellent sensitivity and selectivity for pesticide analysis (24).

Thin layer chromatography (TLC) can also be used without much cleanup for a variety of messy samples (13). It is often used as a quick screening method to find which longer quantitative methods should be run on a sample. A new very selective method for organophosphorus pesticides has been developed which utilizes cholinesterase inhibition during the development of the TLC plates (24).

Gas chromatography is still the most widely used detection device for organophosphorus pesticides (13,23-26). Most of the methods used for detection of organophosphorus pesticides on food (25,26) and in blood or urine (27), as well as the techniques mentioned above for air and water analysis involve gas chromatography. All the compounds can be detected with one or another form of gas chromatography. All the latest advances in GC technology have been incorporated into the standard detection schemes allowing better separations and sensitivity. GC/MS using selected ion monitoring has been shown to be an extremely sensitive technique with detection limits less than 1 ppb (28).

There are several inherent limitations in all of these detection methods, the most obvious of which is time. The time for one analysis can reach into days. With 24 hour collection times, 16 hour extractions, several hours of cleaning, evaporation and finally gas chromatography, rapid real time results are not possible. Furthermore, all the steps involved in the analysis cause errors due to incomplete extraction, adsorption on container walls and other solids and loss of sample during transfers. This thesis project is part of a

larger research effort to develop a method to detect organophosphorus pesticides in the air on a completly automated real time basis. None of the methods outlined above would be suitable to continuous monitoring or be easily automated. It will be shown in later chapters how triple quadrupole mass spectrometry can be used to detect organophosphorous pesticides .

Chapter 2

Mass Spectral Behavior of Organophosphorus Pesticides

Introduction

The mass spectrometer has become an important tool in the detection of organophosphorus pesticides. Unequivocal identification of pesticides and their metabolites in most cases requires the use of two analytical techniques (29). The retention time of a gas chromatographic peak does not provide enough information for absolute identification (30). Comparison of the retention times from two columns with different characteristics to pesticide standards or combined data from chromatography and an optical technique such as IR or UV-VIS can be used if the pesticide has been identified earlier and reference data are available for comparison. These techniques are not as useful, however, when looking for pesticide transformations during metabolism and decomposition studies because the altered compounds may not have reference data available for comparison.

A mass spectrum does contain enough definitive information to confirm and identify the presence of organophosphorus pesticide residues. Mass spectrometry fragmentation patterns resulting from bond fission and rearrangements are diagnostic and characteristic of the original compound. Therefore, by correlating the fragmentation patterns, pathways and mechanisms with the structure of known pure pesticidal compounds, the structure of unknown compounds can be de-

duced from their mass spectra. The theories and principles of the interpretation process in mass spectrometry has been discussed in several text books (31-33).

In order to use mass spectrometry to detect the many forms of organophosphorus pesticides, studies must first be made of the spectra of pesticides with known structures. Several researchers have studied the fragmentation of organophosphorus pesticides during their development of GC/MS or straight MS analysis methods (34-36). A general fragmentation scheme is shown in Figure 2.1 which compiles the possible ion-decomposition pathways deduced by the pioneers in organophosphorus mass spectrometry and given in several reviews on the subject (37,38).

There are four different types of organophosphorus compounds, made by different combinations of oxygen and sulphur in the X and Y positions. The structure of the phosphate, phosphorthioate, phosphorothiolate and phosphorodithioate are shown in Figure 2.2 . The relative intensities of the ions formed by each of the different decomposition pathways depicted in Figure 2.1 are dependent on the type of organophosphorus pesticide present, but are in general governed by five factors: (31,37)

1. Stability of the ion produced
2. Stability of the neutral lost
3. Bond lability
4. Steric factors
5. Inability of ions to refragment

The mass spectra of the four different types of organophosphorus pesticides do not have many features in common. Because the Z moiety is a good leaving group and often contains easily ionized

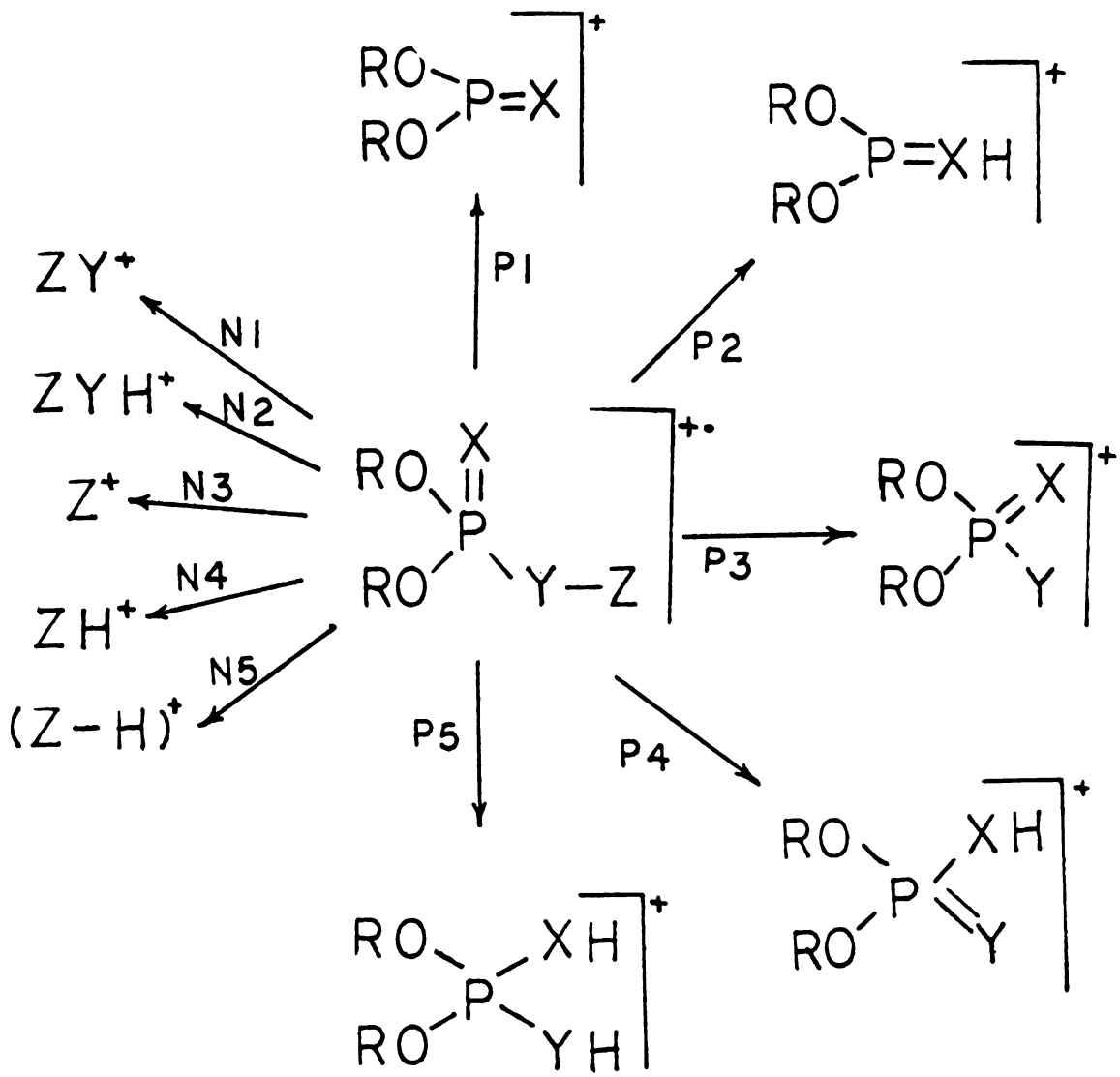


Figure 2.1

Initail Fragmentation Routes for a General Organophosphorous Pesticide

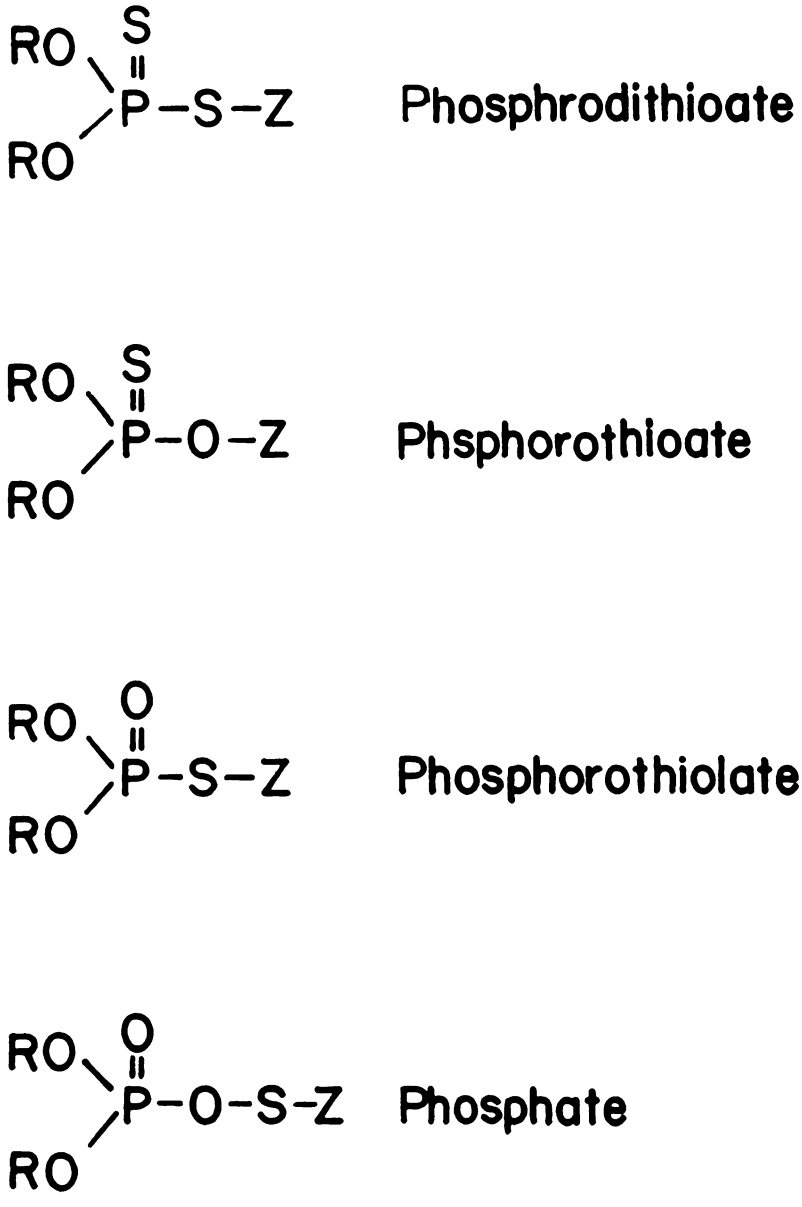


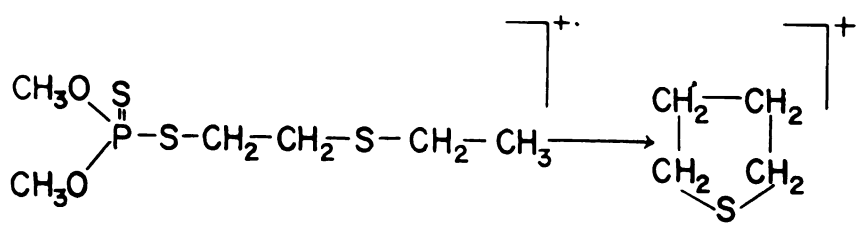
Figure 2.2

General Structures of Four Organophosphorous Pesticides

heteroatoms, the mass spectra of the pesticides is often dominated by peaks due to the Z group. Spectra of other compounds however are filled with peaks representative of ions containing phosphorus. Few generalizations about the spectral behavior of the organophosphorus pesticides exist to differentiate the different classes, and there are many exceptions to the few that have been deduced. Some of the generalizations that may be drawn and some of the notable exceptions are discussed in the following sections.

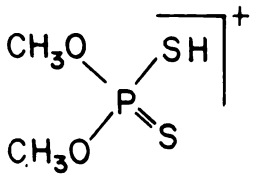
Phosphorodithioates

The predominant ions observed in the spectra of dithioates are due to the cleavage of the Y-Z bond with the charge on the Z group (paths N1 thru N5). The base peak of thiometon, phosmet, azinphos and others are due to N3 ions. Compounds that can undergo a hydrogen rearrangement usually do, giving an N5 or P4 type ion. The base peak of thiometon at m/z 88, is due to a hydrogen rearrangement with charge transfer, forming an N5 ion (34).

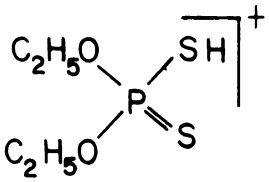


m/z 248 m/z 88

P4 type ions can be formed by a McLafferty type hydrogen rearrangement with charge retention. Examples of this are the ions at m/z 158 or 186 for corresponding dimethoxy and diethoxy ions, in the spectra of malathion and disulfoton (36).



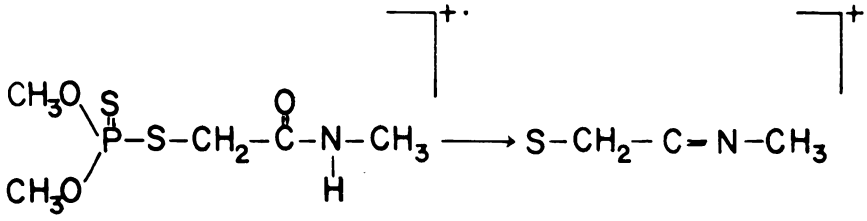
m/z 158



m/z 186

Azinphos, which does not have the gamma hydrogens needed for McLafferty rearrangements makes P3 ions at m/z 157 (36). Although a double hydrogen rearrangement is theoretically possible for some compounds, such as malathion, ions from this mechanism are not very abundant. Phosphorus containing ions of the P1 type are also found in most dithioates at m/z 125 for dimethoxy and 153 for diethoxy compounds, but at much lower abundance than the N type ions (34). The N ions can fragment further producing other ions indicative of the structure of the Z group. The mass spectrum of malathion and its fragmentation scheme are shown together in Figure 2.3.

The only unusual peak in the spectra of the dithioates is the base peak in the dimethoate spectrum at m/z 87. High resolution measurements gave a formula consistent with the structure shown below, but the mechanism of this fragmentation has not been explained (34).



m/z 229 m/z 87

Phosphates

The mass spectra of organophosphates can also be dominated by ion produced by the cleavage of the Y-Z bond, but unlike the

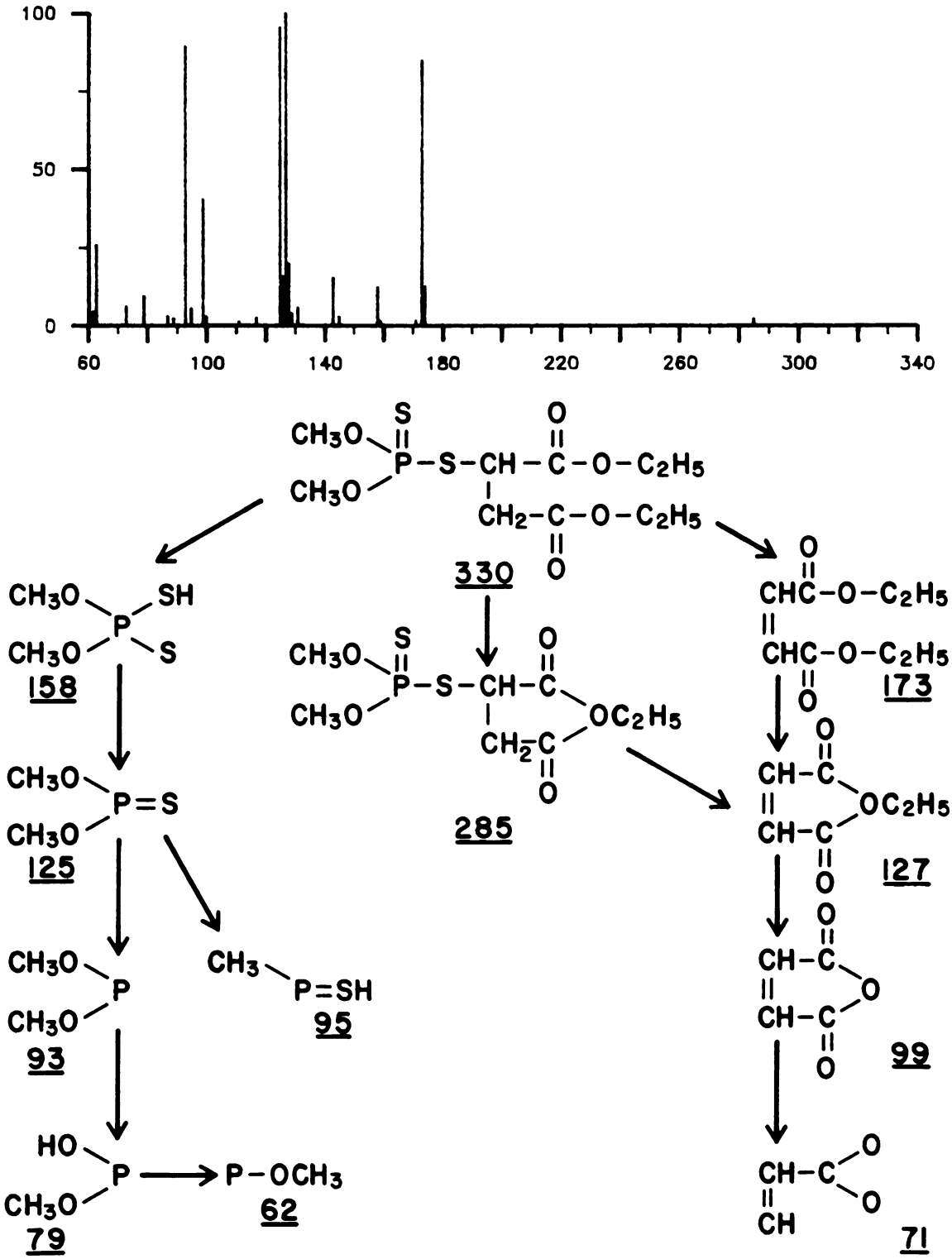
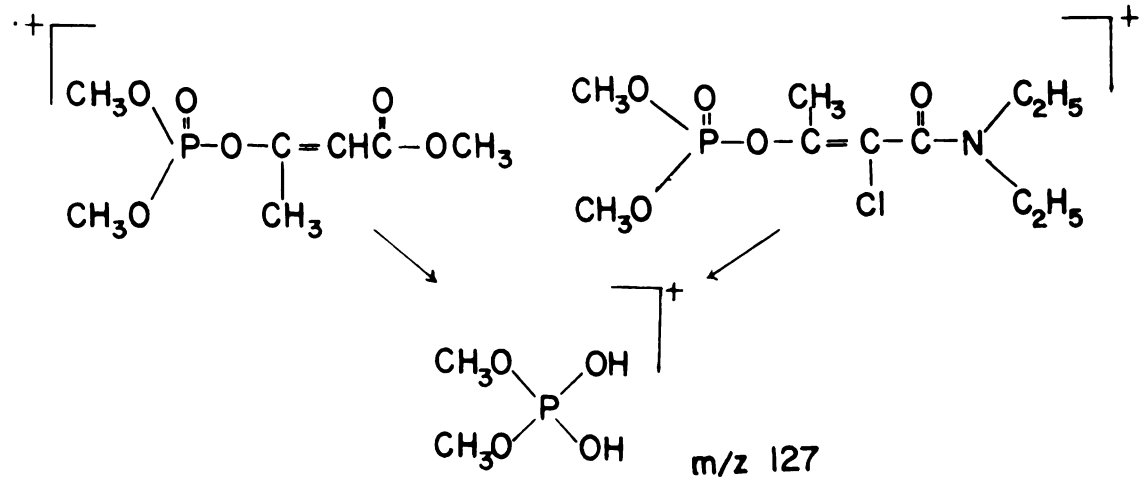


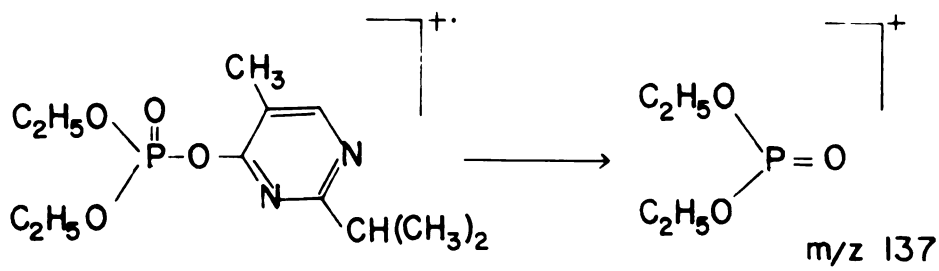
Figure 2.3

Mass spectrum and fragmentation routes of malathion

dithioates, the phosphorus fragment retains the charge. Double hydrogen rearrangements are often prevalent, producing P5 ions. This mechanism was proposed by Domico for the formation of the base peak at m/z 127 for phosdin and phosphamidon (34).



When hydrogens are not available for rearrangement, P1 ions are generally produced. The base peak in the spectrum of the oxygen analog of diazinon, at m/z 137, is due to a P1 type ion (37).



Single hydrogen rearrangements are not observed in the initial fragments of the Y-Z bond but the P1 and P5 ions fragment further with successive single hydrogen rearrangements, losing ethylene (29). Figure 2.4 shows the general scheme for dimethoxy and diethoxy phosphates (36). The ions in Figure 2.4 are responsible for the majority of the peaks in the mass spectra of the phosphates, but there are generally a

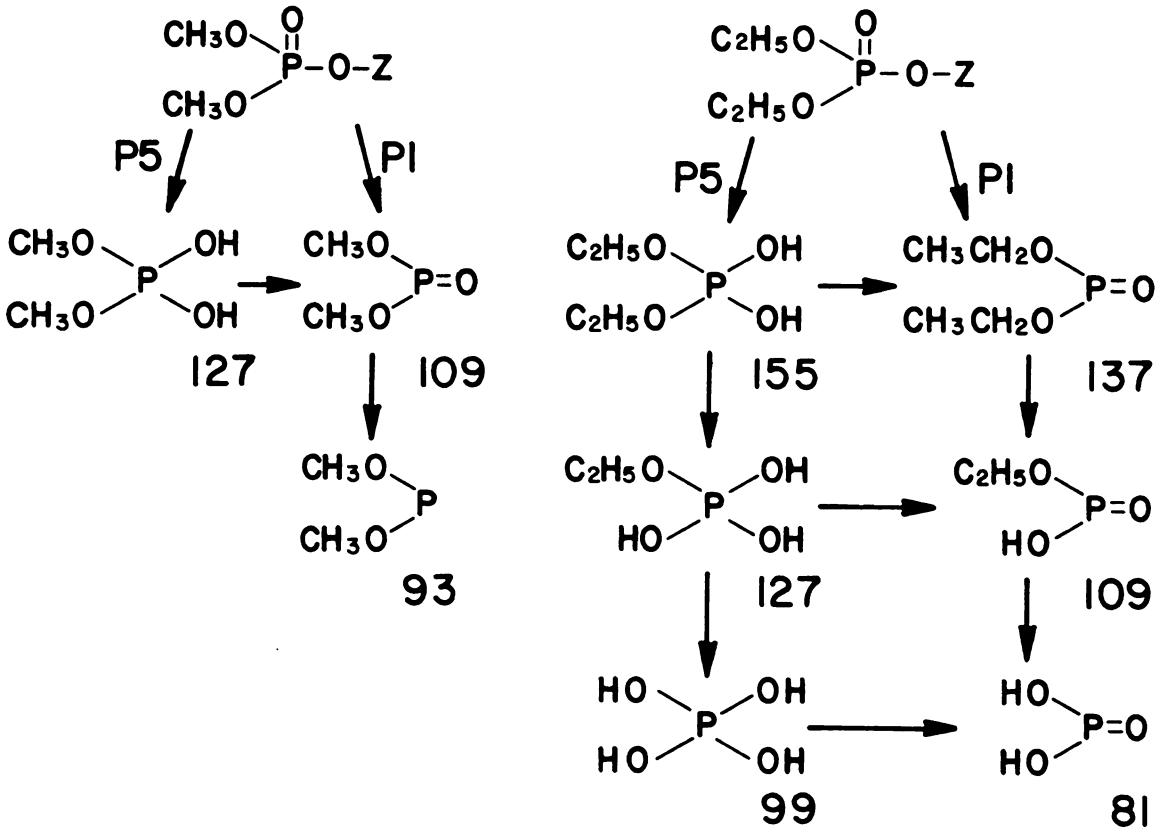


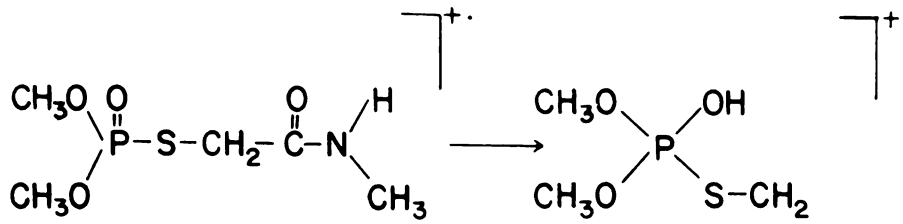
Figure 2.4

Fragmentation Routes for Dimethoxy and Diethoxy Phosphates

few small peaks due to the Z moiety. N2 type ions are typical and are useful in conjunction with the molecular ions and P1 ions in identifying the formula of phosphates (37).

Phosphorthiolates

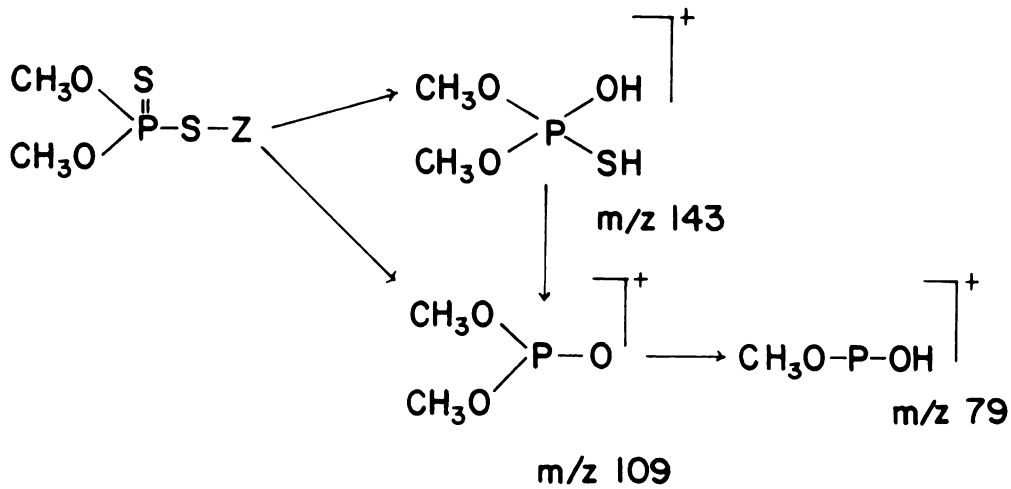
This group of compounds has the fewest general characteristics of any group. Each compound seems to undergo its own reactions. The N ions from the Z moiety dominate most of the spectra, but they are not formed by consistent mechanisms. Many of the ions formed have unexplained reactions. Exact mass measurements indicate the elemental composition of ions that use single or double hydrogen rearrangements, but where these hydrogens come from and how they migrate is not always obvious. An example of this is in the spectrum of the oxygen analog of dimethoate, in which the acidic amine hydrogen is postulated to be responsible for the m/z 156 ion (34,38).



m/z 213 m/z 156

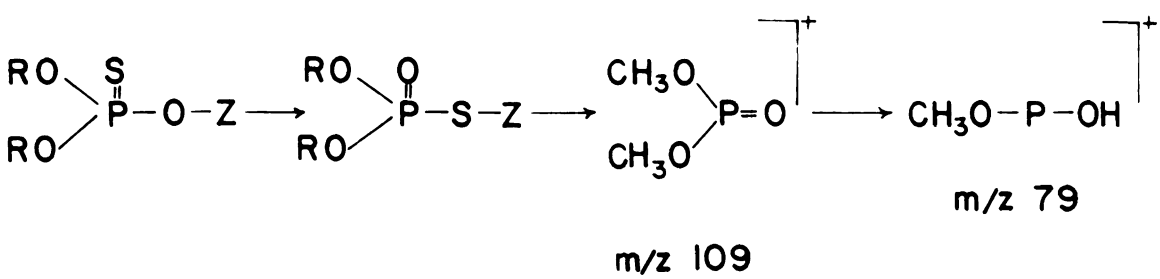
Fragmentation can occur on either side of the sulphur atom, producing P1, P3, N1 and N3 ions as well as the associated hydrogen rearrangement ions. The dimethoxy compounds generally produce at least some ion current due to the ions at m/z 109 and 79 (34,36). The 109 ion could be formed as a P1 ion or from the loss of H_2S from a P5 ion (36,38). The ion at m/z 79 is from the loss of CH_2O from the m/z 109 ion with hydrogen rearrangement (38). This reaction is preferred over

loss of $CH_3O\cdot$ because CH_2O is a more stable leaving group (37).



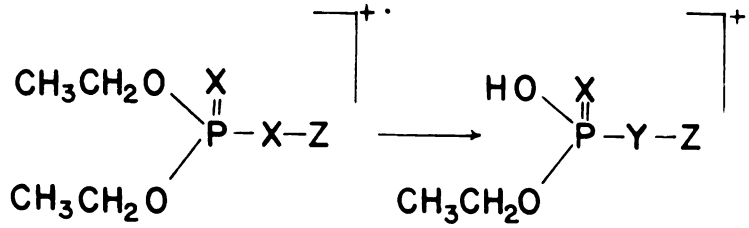
Phosphorothioates

The phosphorothioates are characterized by a rearrangement of the Z group from the oxygen to the sulphur (29,36,38-40). The migration is either initiated through thermal isomerization prior to ionization or by the electron impact process itself. Cleavage of the phosphorus-sulphur bond is preferred over cleavage of the phosphorus-oxygen bond because sulphur contributes less to the pi bonding (8). The ions at m/z 109 and 79 arise after the rearrangement(38).

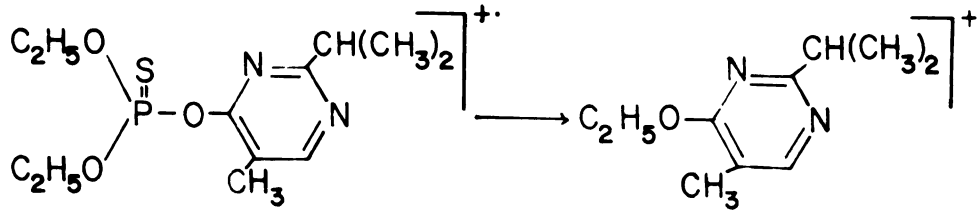


Other compounds do not rearrange as above but fragment with double hydrogen rearrangements making P5 ions (36). Many of the diethoxy compounds show a loss of one of the R groups as C_2H_4 (34,37). This rearrangement has been named after Quale who first observed the loss

of the stable ethylene neutral from trialkyl phosphates (41).



The ethyl group can also migrate to the Z moiety during fragmentation as is seen in the m/z 179 ion in diazinon (34).



m/z 179

CAD Spectra of Organophosphorus Pesticides

In the same way that correlations between fragment patterns and structures of compounds must be made before the spectra can be of use in analytical techniques involving a mass spectrometer, correlations between daughter spectra and parent ions must be made before they can be used in MS/MS techniques. This section reports the collisionally activated dissociation (CAD) spectra of the $(M+H)^+$ ion of 37 organophosphorus pesticides obtained on a triple quadrupole mass spectrometer. Table 2.1 lists the 37 pesticides by classes, with their structures and molecular weights, as well as the appendix page where the spectrum for each compound is presented.

Table 2.1 Pesticides Used

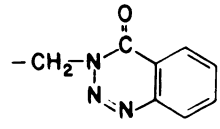
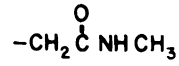
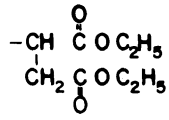
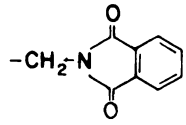
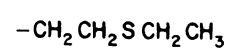
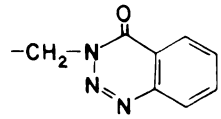
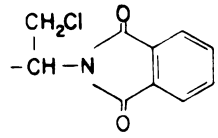
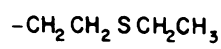
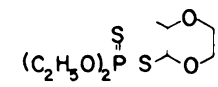
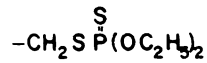
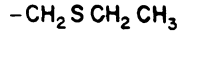
	MW	Z Group	Appendix Page
DIMETHOXYDITHIOATES			
azinphos	317		2
dimethoate	229		10
malathion	330		21
phosmet	317		29
thiometon	246		37
DIETHOXYDITHIOATES			
azinphos ethyl	345		3
dialifor	393.5		7
disulfoton	274		13
dioxathion	456		12
ethion	384.5		17
phorate	260		27

Table 2.1 (cont'd)

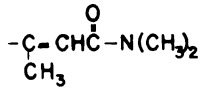
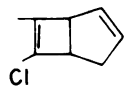
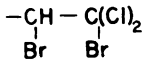
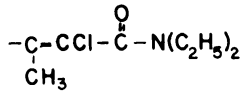
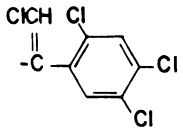
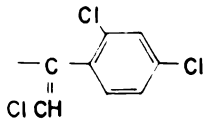
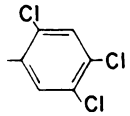
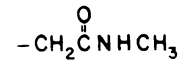
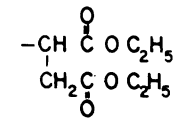
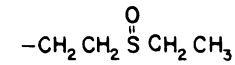
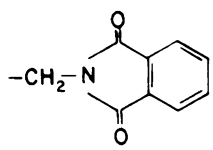
DIMETHOXYPHOSPHATES			
dicrotophos	237		9
heptenophos	251		20
naled	381		23
phosphamidon	299		31
tetrachlorvinphos	366		36
DIETHOXYPHOSPHATES			
chlorfenvinphos	359.5		4
oxygen analog of dursban	333		16
DIMETHOXYTHIOLATES			
oxygen analog of dimethoate	213		11
oxygen analog of malathion	314		22
oxydemeton	246		24
oxygen analog of phosmet	301		30

Table 2.1 (cont'd)

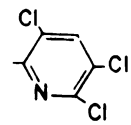
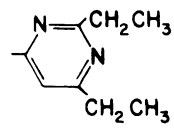
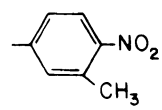
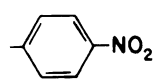
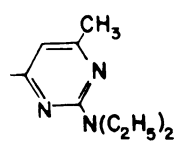
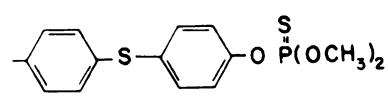
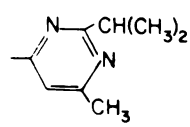
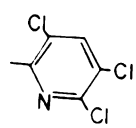
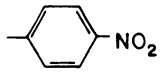
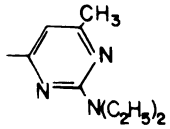
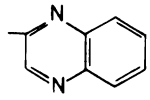
DIETHOXYTHIOLATES			
demeton-S	258	$-CH_2CH_2SCH_2CH_3$	6
oxygen analog of phorate	244	$-CH_2SCH_2CH_3$	28
DIMETHOXYTHIOATES			
methyl dursban	323		15
etrimphos	292		18
fentrothion	277		19
parathion-methyl	263		26
pirimiphos-methyl	305		33
temphos	466		35
DIETHOXYTHIOATES			
aspon	322	$-P(=S)(OC_2H_5)_2$	1
demeton-O	258	$-CH_2CH_2SCH_2CH_3$	5
diazinon	304		8
dursban	351		14

Table 2.1 (cont'd)

parathion	291		25
pirimiphos	333		32
quinalphos	298		34

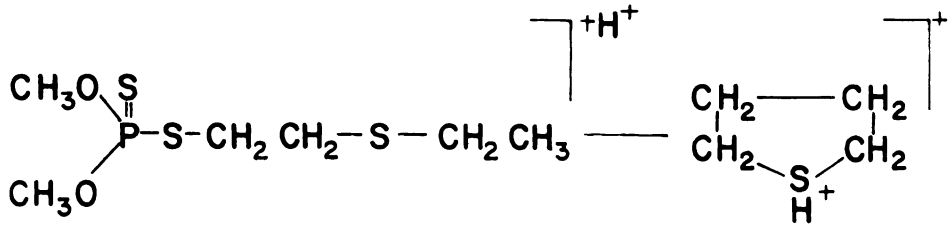
The spectra were not all taken at one time, and therefore were run under slightly different instrument conditions. Liquid samples were placed in a capillary tube. Solids were either put in a capillary tube also or dissolved in ether and then evaporated onto a quartz rod. All the samples were then introduced into the mass spectrometer by means of a solids probe. Methane, isobutane or methanol was used as the chemical ionization reagent gas. The argon collision gas pressure was adjusted until the molecular ion was no longer the base peak but was still present in the spectrum if possible. All spectra were taken with 20 volts of collision energy. Some of the spectral data were recorded with as oscillographic recorder and some were taken with an automated data system.

Instrumental conditions can affect the the ion intensities but do not change which ions are present in the spectrum. These are representative spectra of each compound but cannot be used for quantitative comparisons because identical instrument "tunes" could not be used during the acquisition of data.

In general the CAD spectra have fewer peaks than the corresponding EI or CI spectra. For many of the compounds, the peaks that are present in the CAD spectrum are the same as those found in the EI or CI spectrum. Other compounds however, have very different spectra. Ions that are formed by the CAD process may not be present in the normal spectrum and many of the ions from the EI spectrum, including abundant ones, are not produced by collision. This is due to the different energies involved in the collision and ionization processes.

Phosphorodithioates

Every one of the dimethoxy dithioate spectra contained a P1 ion at m/z 125 except phosmet. The only daughter ion, and the base peak, in the phosmet spectra is due to an N3 ion. The base peak of the azinphos spectrum is also an N3 ion at m/z 160. An N3 ion is also responsible for the base peak at m/z 89 for thiometon. This ion probably has a protonated tetrahydrothiophene structure.

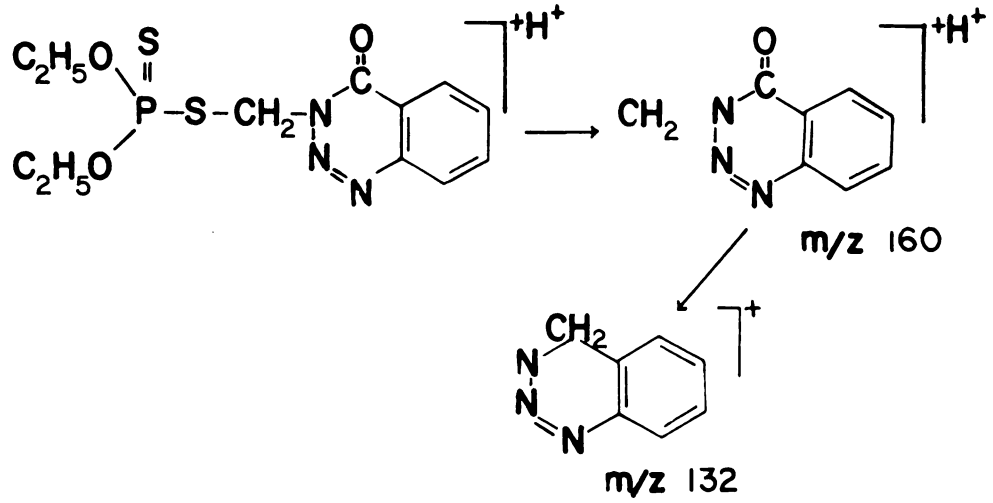


m/z 89

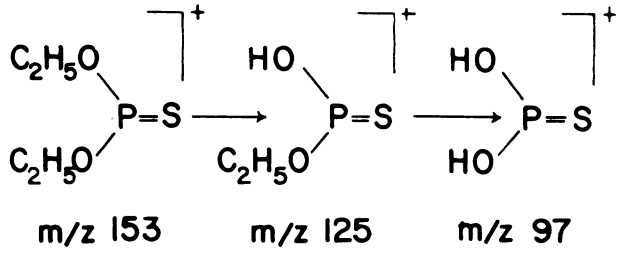
The dimethoate spectrum has a base peak due to loss of 31 mass units which is probably loss of a methoxy group. The ion at m/z 89 is probably the protonated version of the rearrangement proposed by Domico as discussed above (34).

The spectrum of malathion shows a base peak at m/z 127. This ion as well as the ion at m/z 99 are refragmentation products of an N4 ion following the same fragmentation path outlined above.

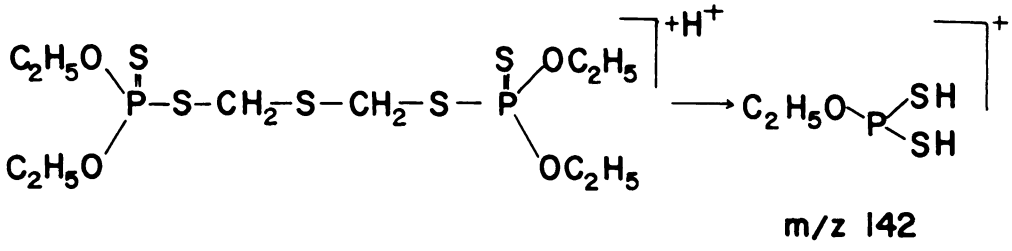
The mass spectrum of disulfoton, the diethoxy complement of thiometon, also contains only one peak. The N3 ion is the same as the one in thiometon. The mass spectrum of phorate also has only one peak and it too is an N3 ion. The base peak for azinphos ethyl at m/z 160 is the same, as expected, as the base peak in the azinphos spectra. This ion can then lose C=O to yield the ion at m/z 132 (34).



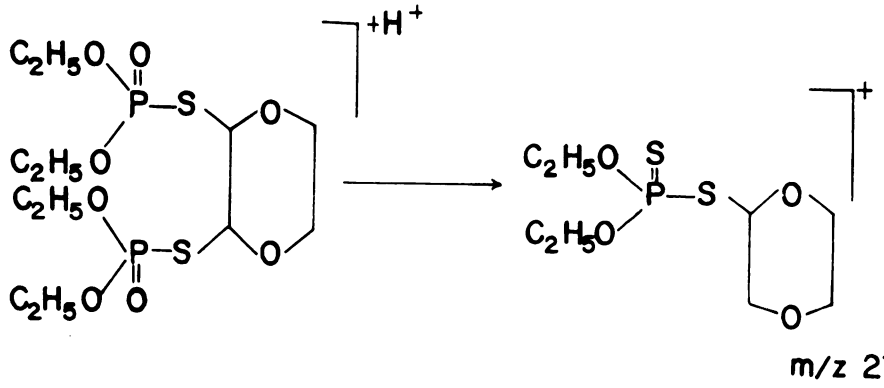
The spectrum for azinphos ethyl as well as those for ethion and dioxathion show P1 ions and daughters of P1 ions. The peaks in all three compounds at m/z 153, 125 and 97 have the structures



The base peak of the ethion spectrum is caused by a cleavage between the carbon and either sulphur and, because of the symmetry in the compound, it is both a P1 type Z ion and a phosphorus ion. The ion at m/z 142 is the result of a rearrangement and loss of an ethoxy group.



The peak at m/z 271 in the spectrum of dioxathion is also a Z ion and a phosphorus ion caused by the loss of one of the dithioate esters.

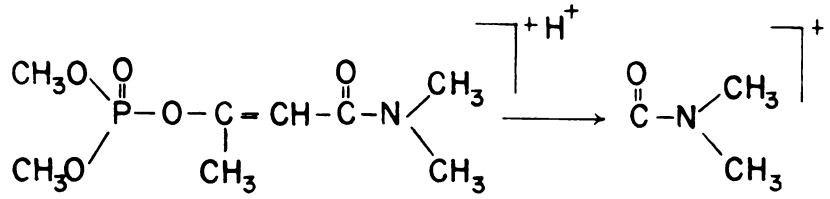


m/z 271

The only peaks that were identified in the CAD spectrum of dialifor are at m/z 186 and 208. They are the complementary P3 and N3 ions.

Phosphates

Few of the phosphate spectra contained ions due to the Z group. Because the Z moieties of most of the phosphates are halogenated aromatic groups, N type ions predominate. The exceptions are posphamidon and dicrotophos, both of which have P3 ions forming the base peak. Dicrotophos also has a peak at m/z 72 which is probably formed by alpha cleavage at the carbonyl group.



m/z 72

All the dimethoxy phosphates show an ion at m/z 127 which is the P5 ion. This peak is the only one present in the spectra of both naled and tetrachlor. 127 is the base peak in the spectrum of heptenophos which also shows a large peak at m/z 125 due to P3 ions.

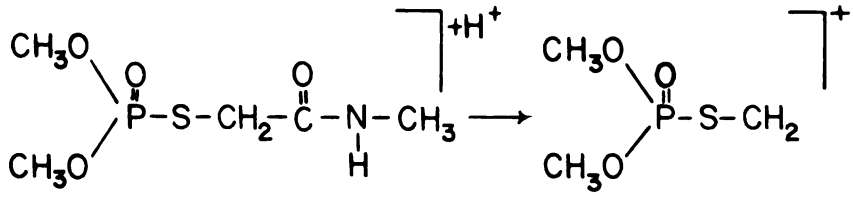
The spectrum of chlorfenvinphos also contains a peak at m/z 127 but it is the product of a Quale rearrangement of the P5 ion at m/z 155. There is also a small peak at m/z 205 which is due to some N3 ions.

The oxygen analog of dursban does not fragment by losing the Z group. It undergoes two Quale rearrangements making ions at m/z 306 and 278.

Phosphorothiolates

The spectra of the phosphorothiolates were very similar to their dithioate counterparts. N3 ions which are postulated to have the same structures that are described in the dithioate section, were the base peaks in demeton-S (oxidized disulfoton), the oxygen analog of phorate and the oxygen analog of phosmet. The oxygen analog of demeton forms an ion by alpha cleavage at the sulfoxide to yield m/z 169. Its other peak at m/z 105 is an N3 ion. The spectrum of the oxygen analog of malathion also has the same peaks as the malathion spectrum.

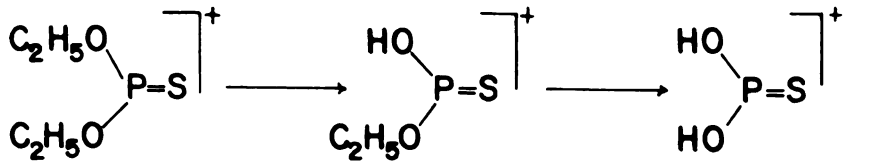
The oxygen analog of dimethoate, like dimethoate does not follow the same pattern as the other compounds in this class. Nor does it behave in the same manner as dimethoate. The m/z 88 ion, which is the base peak in dimethoate, is barely discernable. The spectrum of the oxygen analog has two P1 ions; one at m/z 125 is due to a rearrangement of the Z group on to the oxygen and then fragmentation, the other is due to the unrearranged species. The ions at m/z 155 are probable due to alpha cleavage at the carbonyl and m/z 180 ions are the molecular ion minus one methoxy group, as shown below.



m/z 155

Phosphorothioates

Many of the CAD spectra of the $(M+H)^+$ ion of the thioates contain peaks due to Quale rearrangements. These are the only peaks observed in the spectrum of parathion. The spectra of demeton-O has only one peak and it is from an N3 ion that has the same structure as demeton-S. Rearrangements of the Z moiety from the oxygen to the sulphur give rise to the two prominent peaks in the spectra of pirimiphos. The m/z ion at 197 is for $(P-Z)^+$, while the ion at m/z 181 is for $(O-Z)^+$. The spectra of diazinon, Quinalphos and Dursban all contain P1 or P5 ions and their fragments.



m/z 153 m/z 125 m/z 97

The base peak of dursban is created from N3 ions. However the spectrum of methyl dursban does not have any N3 ions. Instead it has a base peak at m/z 125, a P1 ion that is characteristic of the dimethoxy compounds. P1 ions are also the base peak in etrimphos, temphos, methyl parathion and in its oxygen form in fentrothion. Fentrothion and methyl parathion show both forms of the Z group rearrangement.

The base peak in the spectrum of temphos is due to P1 ions

which is not surprising since there are two thioate groups in the compound. The peak at m/z 244 is due to cleavage on either side of the central sulphur, and 341 is really an N3 ion.

The spectrum of methyl pirimiphos also contains the P1 ions at m/z 109 and 125 as well as a base peak due to N5 ions.

Chapter 3

Comparison of Ionization Techniques

Introduction

The choice of ionization method can be crucial to the success of mixture analysis with a mass spectrometer. The sensitivity and selectivity of the analysis can be greatly changed by the ionization technique employed. Many different types of ionization have been developed, each having advantages for the many different types of samples assayed with a mass spectrometer. Some ionization techniques ionize every compound in the sample, others ionize only the target molecules and leave the rest of the sample matrix alone. The traditional method, electron impact ionization (EI), is very energetic, causes many fragmentations and yields a mass spectrum with many peaks. The fraction of unfragmented molecular ions is often so low that the molecular weight of the compound cannot be determined.

Chemical ionization (CI), however, can be used to increase the relative amount of molecular ion present in a spectrum. $(M+H)^+$ protonated molecular ions are formed during an acid base reaction in the gas phase between an acid reagent ions and a basic reactant molecule.

$$BH^+ + M = B + MH^+$$

The exothermicity of the reaction depends on the relative proton affinities of the acid and the base. Any excess energy in the reaction is available as internal energy to the $(M+H)^+$ ion and promotes fragmentation. For maximum ionization efficiency one needs as exo

thermic a protonation as possible. But, to maximize the probability of $(M+H)^+$ ion formation, which provides molecular weight information, one needs the least exothermic reaction possible. On the other hand, the fragment ions, which provide information useful for structure elucidation, need excess energy for their formation. If the sample is a mixture, then fragmentation should be avoided because they may obscure $(M+H)^+$ ions of lower molecular wieght. Therefore, depending on the type of sample under study, different chemical ionization reagents can be tailored to give the right amount of excess energy to yield an adequate amount of $(M+H)^+$ ions and fragment ions.

Electron Impact Ionization

To date several different methods of ionization have been used to ionize organophosphorous pesticide samples. In 1966 Domico (34) used EI to study fragmentation and rearrangements in 23 organophosphorous pesticides because it was the predominant method available at the time. Domico was chiefly interested in the mechanisms and rearrangements involved in the fragmentation of organophosphorous esters. Basing his findings on work done by Quale (41), Domico published the first spectra of organophosphorous pesticides and explained many of their prominent peaks. The spectra were characteristic of EI spectra with little molecular ion and many lower mass fragment peaks, and have become the reference spectra for organophosphorous pesticides.

While Domico's work is very important in understanding the behavior of organophosphorous ions in a mass spectrometer, his study is not useful as an analytical technique. H.J.Stan (36) however, was

able to use EI mass spectroscopy in conjunction with gas chromotography to detect organophosphorous pesticide residues on food. Stan studied the four groups of pesticides and found five ions that were characteristic of the different classes. The ratio of the ions at m/z 93, 97, 109, 121 and 125 were found to be indicative of the type of organophosphorous pesticide present in a GC peak. These ions, along with the retention time, enable positive identification of the pesticides in food residues.

Chemical Ionization

Several people have also tried chemical ionization to analyze for organophosphorous pesticides. Holmstead and Cassida published the first CI spectra of organophosphorous pesticides in 1974 (42). They used methane as a reagent gas but found found the resulting spectra contained less $(M+H)^+$ ion than the M^+ in the EI spectra. The spectra contained some new ions, but lacked many of the EI ions, including some that formed base peaks. They suggested that a softer ionization gas, such as ammonia, be used to increase the amount of $(M+H)^+$ formed.

Stan also investigated the use of chemical ionization for organophosphorous pesticides (43). He compared the mass spectra obtained using methane, isobutane and methanol as reagent gases. Stan found that chemical ionization gave better results than electron impact. Unlike Holmstead and Cassida, Stan had more $(M+H)^+$ ion with methane than with EI, but he got even more with isobutane and still more with methanol. He suggested the use of isobutane because it gave, in almost every case, $(M+H)^+$ as the base peak, as well as some

useful fragment ions.

Busch etal. (44) also compared five ionization methods on several organophosphorous compounds. They compared EI, methane CI and three negative ion methods and found that none of the five techniques produced a satisfactory abundance of molecular or $(M+H)^+$ ions.

Negative chemical ionization of organophosphorus pesticides was also investigated in several studies. Daughtery and Wander (45) used dichloromethane as a reagent gas and formed characteristic $(M+Cl)^+$ ions. However, these ions were small and the spectra were dominated by $(M-Z)^+$ ions. Rankin (46) studied the negative ion spectra of pesticides including some organophosphorus compounds and he too found little or no molecular ions.

Stan and Kellner (47) used methane electron capture in the analysis of the negative ion spectra of 52 organophosphorus pesticides. The small amount of molecular ion formed was dependent on the structure of the compound and its ability to stabilize the charge. They found that fragmentation always led to a few intense ions characteristic of the structural group of organophosphorus pesticide. Selected ion monitoring of these ions in GC/MS were used to determine pesticides in food and biological and environmental materials.

Ionization for MS/MS

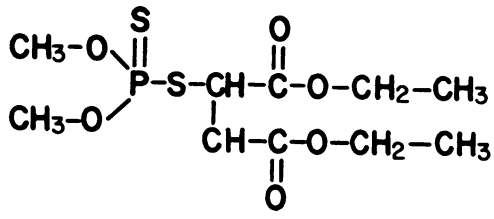
In an MS/MS experiment the ionization criteria depend on the purpose of the investigation. MS/MS is used primarily for either structure elucidation or for the analysis of mixtures. Structure elucidation experiments need fragments formed in the source to produce parent ions representing as many parts of the compound as possible.

Structure experiments are conducted with pure compounds, therefore there is no possibility of assigning a fragment to the wrong compound, or masking a lower weight molecular ion.

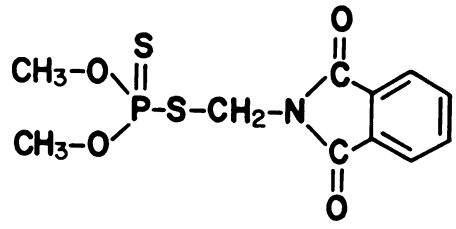
The most important consideration in TQMS mixture analysis is the amount of molecular ions formed. By keeping as much of the ion intensity concentrated in the molecular ion as possible, the sensitivity of the technique is optimized because the intensity of the daughter ion spectrum is maximized. Creating only molecular ions in the source also eliminates the possible confusion between fragments formed in the source and molecular ions of the same mass. The ability of the first quadrupole to select a single mass makes it easier to analyze mixtures by automatically subtracting the background peaks as well as all the peaks from other components in the mixture. Therefore, the ionization technique does not have to ionize only the species of interest but can ionize all compounds in the sample without any ill effects.

Experimental

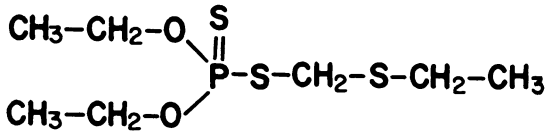
In this study six different ionization techniques were tested to find the technique that was best able to ionize organophosphorous pesticides for use in triple quadrupole mass spectrometry. The methods of ionization used were electron impact and positive chemical ionization using methane, isobutane, methanol, water and ammonia as reagent gases. Five representative organophosphorous compounds were chosen to test the different techniques. The set of compounds shown in Figure 3.1 contain dithioates and thioates, methyl and ethyl esters with aromatic and aliphatic Z groups containing a



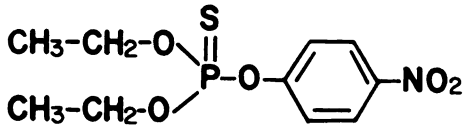
MALATHION MW 330



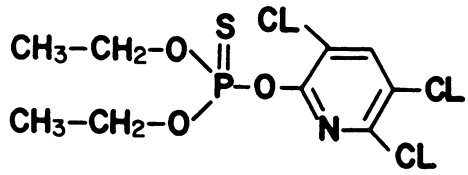
PHOSMET MW 317



PHORATE MW 260



PARATHION MW 291



DURSBAN MW 351

Figure 3.1

Compounds used for Ionization Experiments

variety of functional groups and hetero-atoms. The pesticides were obtained from Chem Service, Inc. and were used without further purification. Liquid and solid samples were placed in melting point capillaries which were then inserted into the mass spectrometer by means of the solids probe. Methane, isobutane and anhydrous ammonia specified as instrument grade or better were obtained from Matheson and introduced into the source using standard procedures. Absolute methyl alcohol was purchased from Mallinckrodt and the Milli Q water from Milli Pore. For use, approximately 3 ml of the liquids were injected with a syringe into an evacuated 250 ml expansion flask. Their vapors were then leaked into the source through a needle valve. Spectra were taken on an Extra Nuclear ELQ-400-3 triple quadrupole mass spectrometer which has an automated data system. The spectra were obtained by scanning the first quadrupole with the other two quadrupoles in the RF-only mode. There was no collision gas present in the second quadrupole. EI spectra were taken with 70 electron volt electrons, CI spectra were taken with 300 electron volt electrons. The CI gas pressures were varied for optimum ion intensities and were measured with an ion gauge attached to the ion source vacuum chamber.

Results and Discussion

The electron impict spectra were similar to those published by Domico (34) and also by Busch etal.(44) and contained many typical electron impact ionization features. There was little or no molecular ion present but many fragments were formed, especially at lower masses. The spectra can have many high intensity peaks, as in malathion (Figure 3.2), dursban (Figure 3.3) and parathion (Figure 3.4),

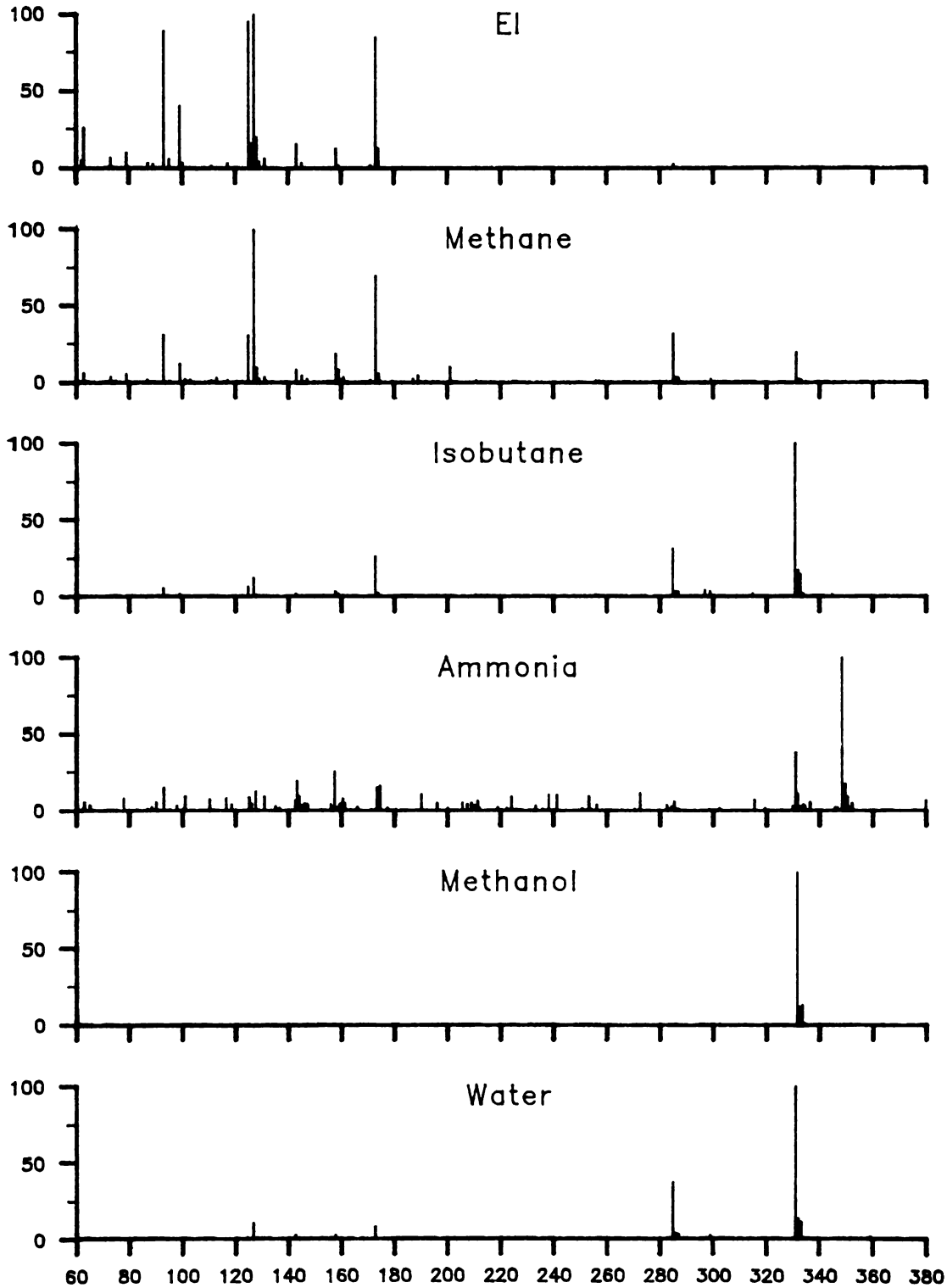


Figure 3.2
Mass Spectra of Malathion

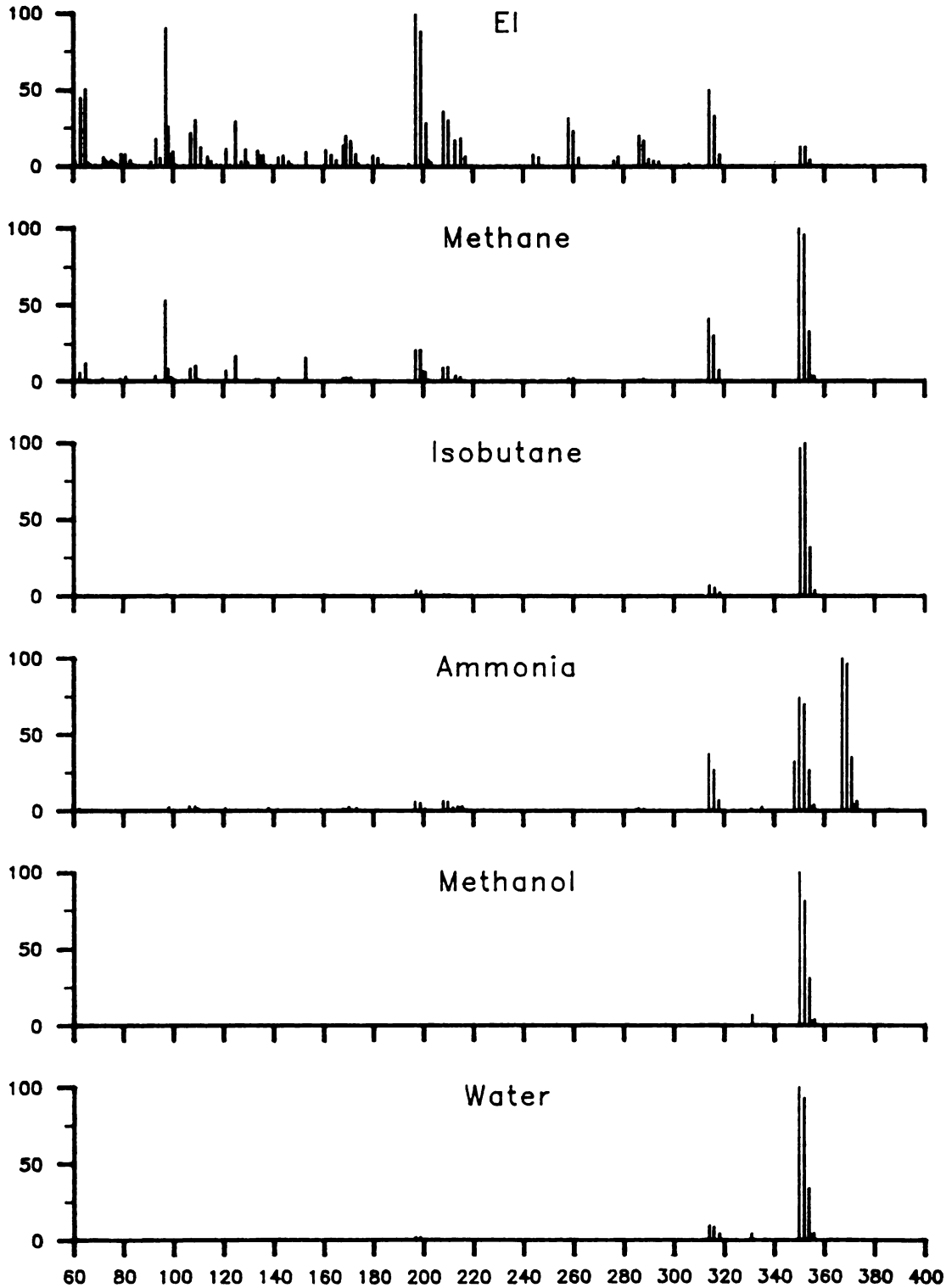


Figure 3.3
Mass Spectra of Dursban

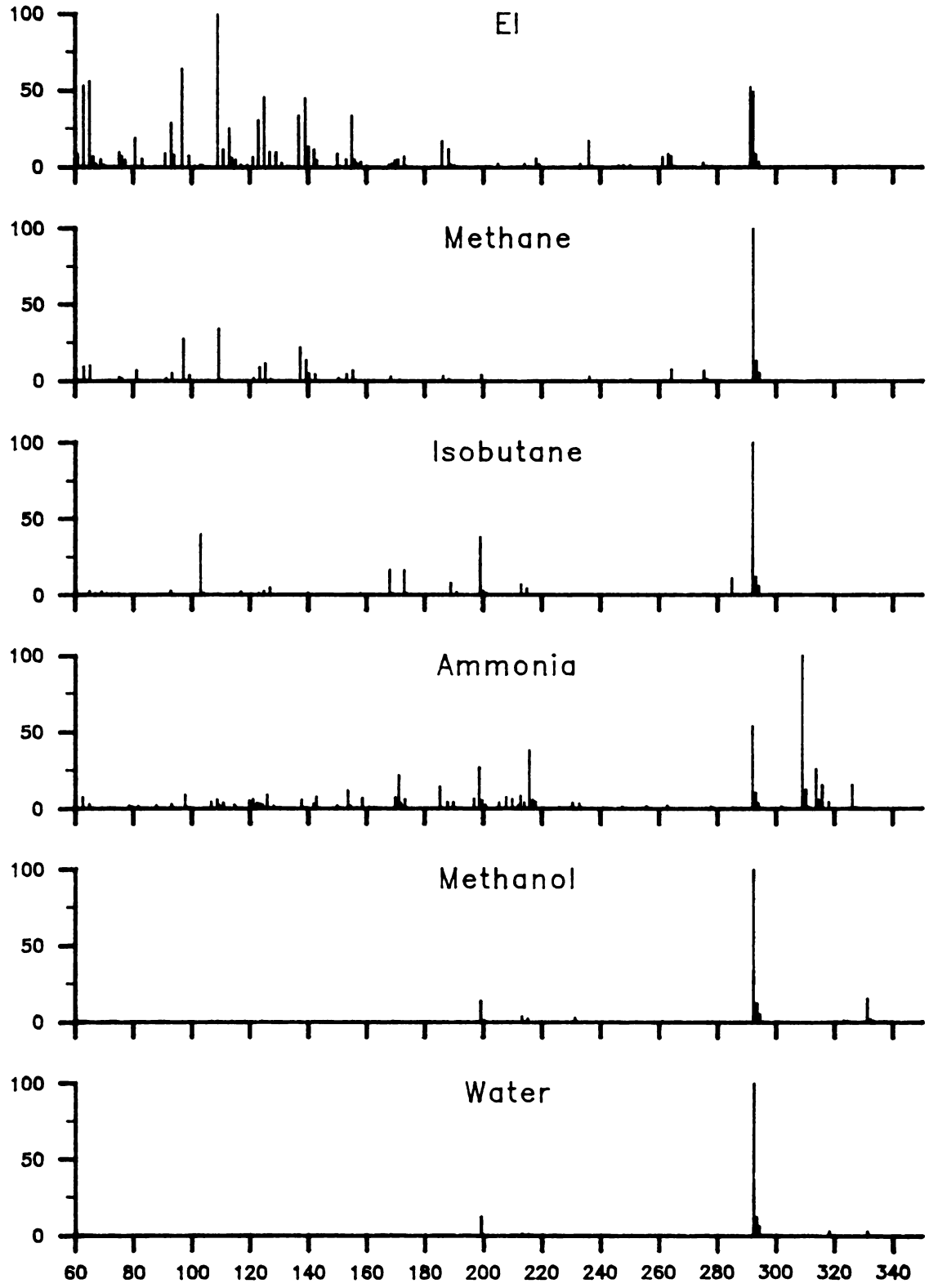


Figure 3.4
Mass Spectra of Parathion

or can be dominated by one peak from an especially stable ion like the m/z 160 ion peak in the phosmet (Figure 3.5) spectra or the m/z 75 ion from phorate (Figure 3.6). The spectra contain both fragments from the Z group, such as m/z 173, 127 and 99 in the malathion spectrum, and fragments from the phosphorous esters ie. m/z 158, 125 and 93 in the malathion spectrum. Because there are so many peaks and many of them are involved in multiple fragmentations and rearrangements, there are not many useful generalizations that can be drawn from these spectra.

The methane and isobutane spectra were similar in that there was more molecular ion present and fewer fragments than the EI spectra. The fragment ions still carried more of the ion current than was desired. Both gases gave $(M+H)^+$ ions with enough abundance to get good daughter spectra but were also energetic enough to cause fragmentation. Isobutane with a proton affinity of 196.0 kcal/mole is a much "softer" ionizing agent than methane which has a proton affinity of 130.0 kcal/mole. The isobutane spectra therefore, as expected, had a greater percent $(M+H)^+$ ion and fewer fragment ions. The base peaks in the isobutane spectra were always the $(M+H)^+$ ion but there were also a few other peaks present. The base peaks in the methane spectra were sometimes the $(M+H)^+$ ion but was often a peak due to a stable fragment ion. The other peaks in the isobutane spectra are usually caused by these same stable ions.

An even softer ionization gas, ammonia, with a proton affinity of 205.0 kcal/mole was tried in order to reduce fragmentation further. The ammonia spectra however, also contained many fragmentation peaks. The appearance of the spectra taken using ammonia as a reagent gas, like some other chemical ionization gases, was very

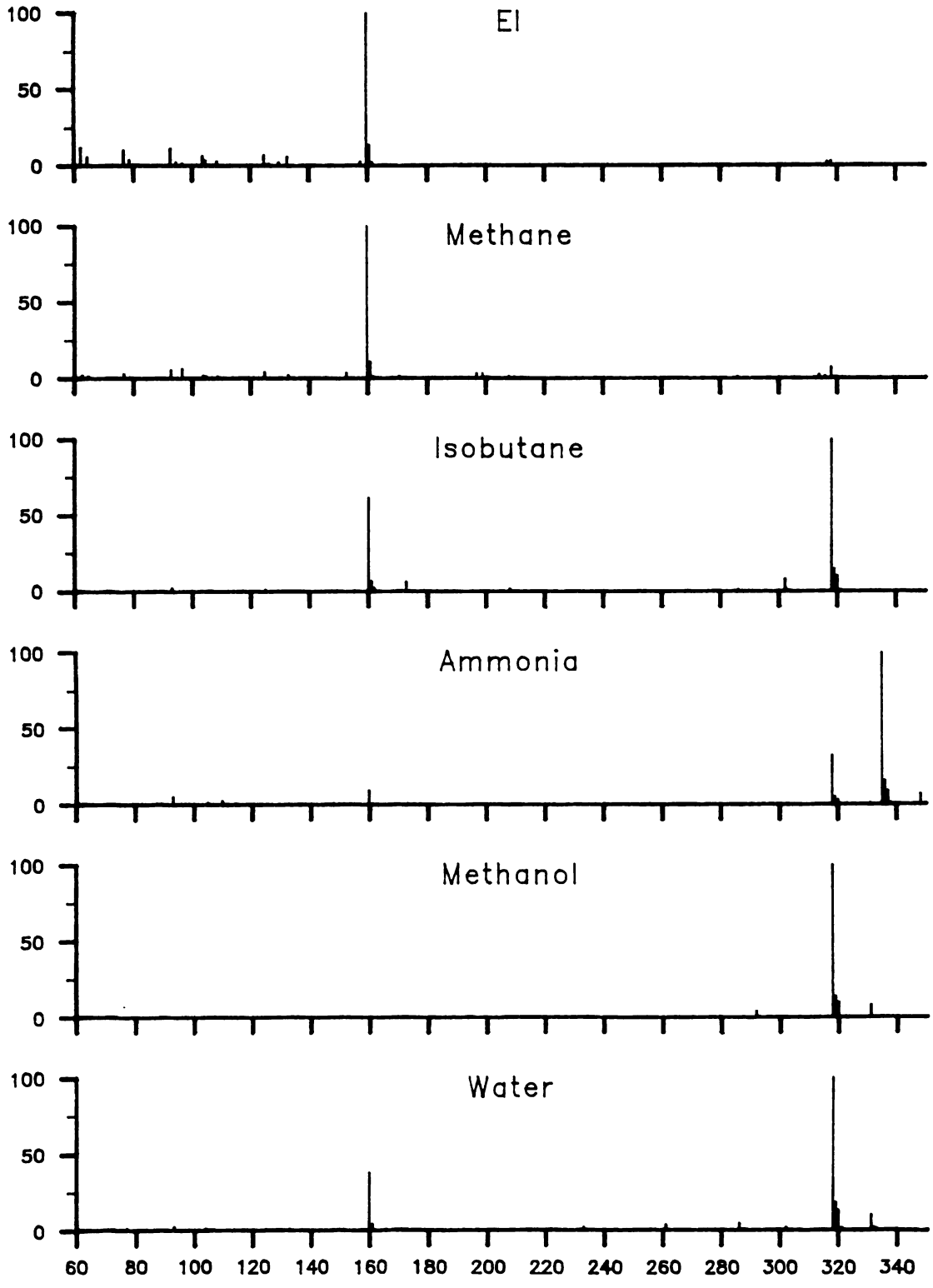


Figure 3.5
Mass Spectra of Phosmet

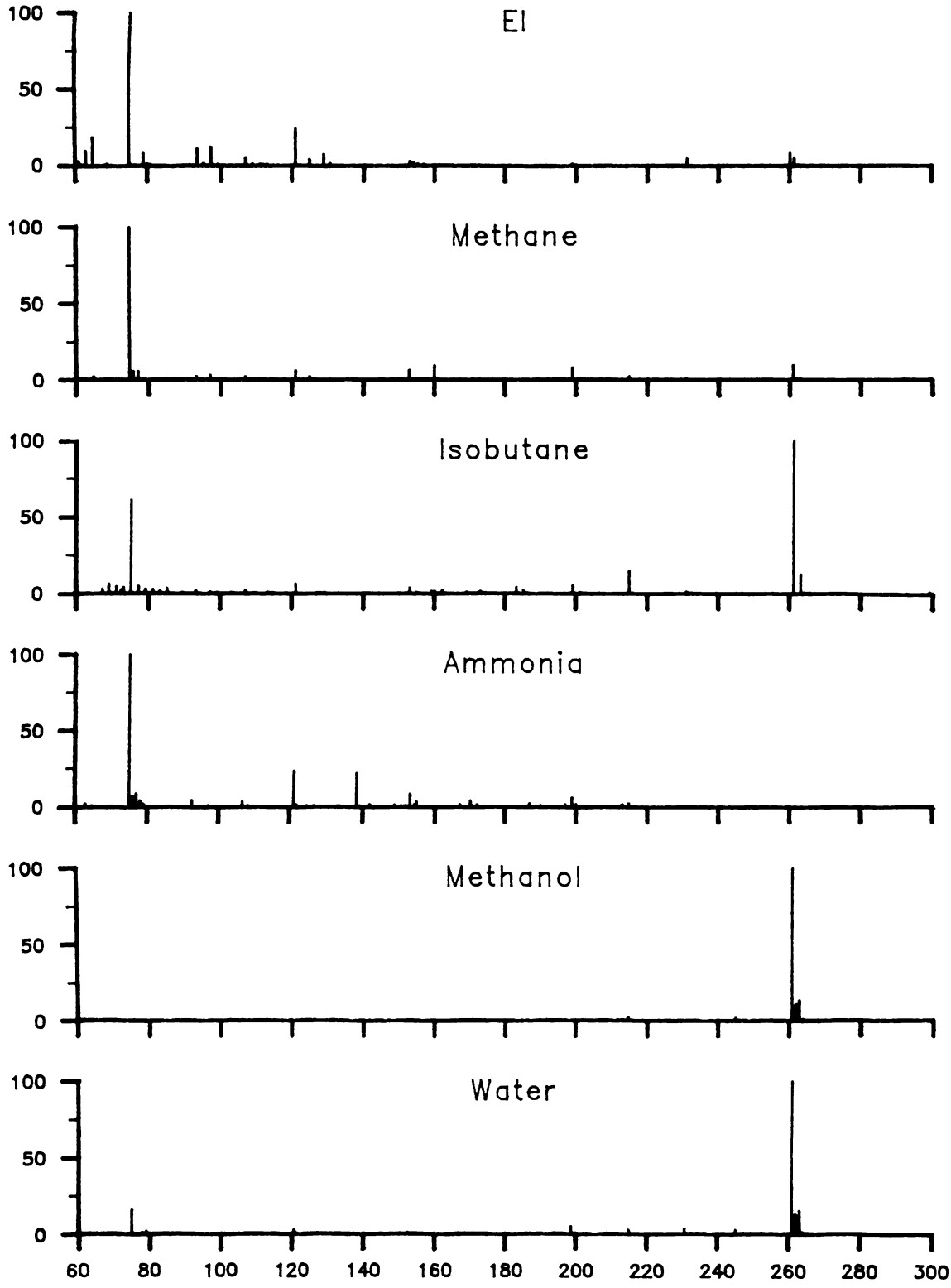


Figure 3.6
Mass Spectra of Phorate

dependent on the source pressure. The choice of reagent gas pressure in the source can determine whether the base peak is the $(M+H)^+$ ion or a fragment ion. At a source chamber pressure of 0.8×10^{-5} torr, the ammonia CI spectrum of malathion, shown in Figure 3.7, resembles its electron impact spectrum with fragment peaks at m/z 173, 158, 127, 125 and 93 predominating and little $(M+H)^+$ ion at either m/z 331 or 348. At this pressure there is not enough reagent gas to create a true chemical ionization environment. The spectrum observed is really an electron impact spectrum of a mixture of ammonia and malathion. At a pressure of 3.8×10^{-5} torr the spectrum begins to resemble a methane CI spectrum. Fragments are still prevalent but there is much more $(M+H)^+$ ion being formed. As the pressure in the source chamber is increased to 1.2×10^{-4} torr the fragment ions and the $(M+H)^+$ ions begin to decrease and a $(M+NH_3)^+$ ion begins to increase until it becomes the base peak in the normalized spectrum. The change in the spectrum is caused by a change in the proton affinity of ammonia which, like other polar reagent gases, is dependent on the pressure in the source. Polar reagents produce solvated protons, or clusters, of the type $(B)_nH^+$ with n as large as three or four. The 205.0 kcal/mole value for ammonia's proton affinity is for a monosolvated proton. The proton affinity for each cluster size is different and therefore the spectra are different. But, even though ammonia is known as one of the softest ionization gases, the protonation of the organophosphorous molecules by the ammonia clusters was still too exothermic and numerous fragment ions were formed.

Methanol was also tried as a chemical ionization reagent. H.J.Stan (43) reported that methanol produced only $(M+H)^+$ ions. He therefore made no further use of this reagent system because it did

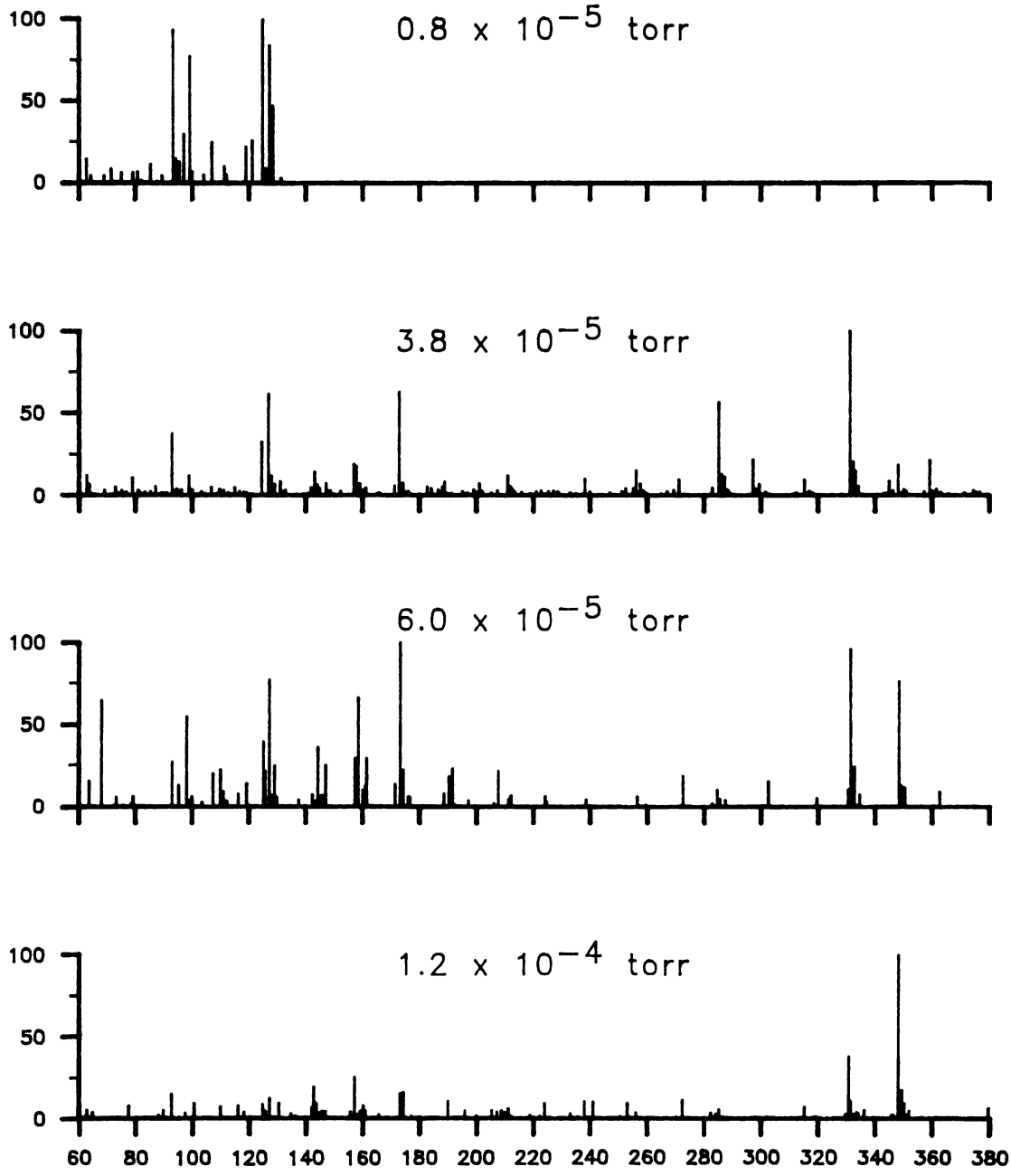


Figure 3.7
Ammonia chemical ionization spectra of Malathion

not give any fragments which he, like most other researchers, desired. Methanol therefore seemed to be the ideal gas for TQMS use and, in practice, this was born out. The spectra for almost all of the organophosphorous pesticides tested contain only one peak, that representative of the $(M+H)^+$ ions. That methanol should be such a tender ionization agent seemed at first unusual because the proton affinity of 184.9 kcal/mole is between that of methane and isobutane. Methanol however, being polar, can cluster and therefore can have a much different proton affinity. Figure 3.8 shows the spectrum of just methanol at chemical ionization pressure. The ions at m/z 33, 65, 97 represent $((MeOH)_{n+H})^+$ ions. Some researchers add other gases along with the methanol to prevent the clusters from forming, but for organophosphorous esters these protonated clusters are much better reagents than single methanol ions. The clusters seem to be able to give up a proton very gently.

Water, because it can also hydrogen bond and is known to cluster, was also tested as a chemical ionization reagent. The resulting spectra were, like the methanol spectra, very simple. The base peak was again from the $(M+H)^+$ ions, but this peak was not the only peak observed. Phosmet, parathion, phorate and malathion all had small peaks from fragment ions as well. These fragment ions are formed by simple cleavages or the loss of small stable neutrals and are very indicative of the sample compound. Water was not chosen as the optimum gas because it produced a small fragment ion current and it was not as easy to use as methanol. At room temperature, water's vapor pressure is not high enough to give a pressure that is easily regulated. The highest pressure reached was not optimum and fluctuated slightly giving pulsating peak intensities. Attempts to heat

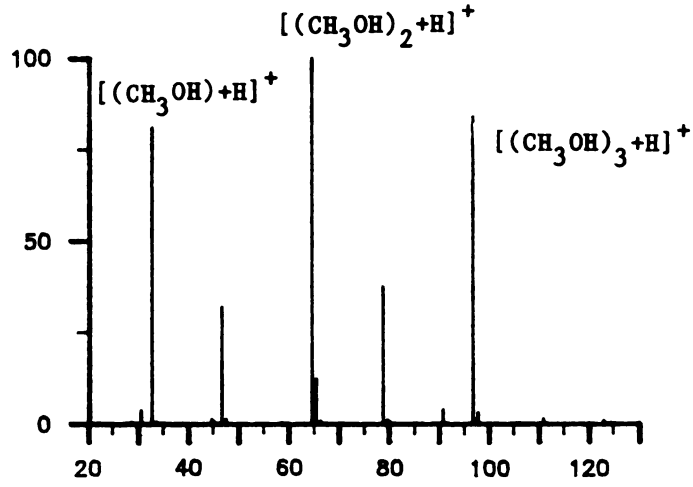


Figure 3.8

Background Spectrum of Methanol Reagent Gas

the water reservoir and inlet system in order to raise the pressure proved to be a lot of trouble and were eventually abandoned.

Methanol was chosen as the chemical ionization reagent. The resulting spectra have, in general, one peak representative of the $(M+H)^+$ ion. As is demonstrated in the next chapter, the $(M+H)^+$ ions formed can be fragmented in the collision cell to give good daughter spectra. Methanol is also readily available and has a vapor pressure at room temperature that is high enough to allow easy regulation.

Chapter 4

Detection of Organophosphorus Pesticides

Introduction

Looking at the daughter spectra of the pseudo-molecular ions of the organophosphorus pesticides revealed several ions and fragmentation mechanisms indicative of the parent compounds. The fragmentations, which were discussed in chapter two, can be employed by a triple quadrupole mass spectrometer to screen for organophosphorus pesticides. A small number of parent scans and neutral loss scans can be used to detect almost all the 38 compounds under study. The goal is to find the smallest number of scans that need to be used to detect all the possible organophosphorus pesticides. Table 4.1 lists twelve scans that could be used to detect all the pesticides listed. Other organophosphorus compounds, unknown pesticide metabolites and decomposition products could also be detected with these same scans. Several of the listed scans have been tested to determine how well they work.

Experimental I

The first detection study was done with seven phosphorothioates, four with methoxy R groups and three with ethoxy. Isobutane was used as the CI reagent, because the benefits of methanol had not yet been discovered. Six of the pesticides, dialifor, phosmet, azinphos ethyl, dimethoate, thiometon and disulfoton, were obtained from

Table 4.1 Scans Used to Detect Organophosphorus Pesticides

SCAN TYPE	MASS SELECTED	PESTICIDES FOUND		
parent	109	pirimiphos methyl	fentrothion	
parent	125	heptanophos	methyl parathion	quinalphos
		etrimphos	methyl dursban	diazinon
		oxygen analog of dimethoate	dimethoate	
parent	127	heptanophos	dicrotophos	naled
		phosphamidon	tetrachlorvinphos	malathion
		chlorfenvinphos	oxygen analog of malathion	
parent	153	diazinon	dioxathion	ethion
parent	155	chlorfenvinphos	oxygen analog of dimethoate	
parent	169	diazinon		
neutral loss	137	pirimiphos	quinalphos	
neutral loss	142	oxygen analog of demeton	oxygen analog of phosmet	pirimiphos methyl
		dimethoate		
neutral loss	152	dursban	pirimiphos	
neutral loss	158	phosmet	azinphos	thiometon
neutral	170	demeton-S	demeton-O	quinalphos
		oxygen analog of phorate		
neutral loss	186	disulfoton	dialifor	dioxathion
		azinphos ethyl	ethion	phorate

Chem Service, Inc. and were used without further purification. The malathion used was a commercial pesticide formula, ORTHO Malathion 50, obtained from a local garden store. Instrument grade isobutane and argon were obtained from Mathison. The solid and liquid pesticide samples were mixed together and diluted to a concentration of about 2 micrograms per microliter with ether and the ORTHO formula. One or two microliter samples were placed in a capillary melting tube and inserted into the mass spectrometer on a solids probe. Spectra were taken on an Extra-Nuclear ELQ-400-3 triple quadrupole mass spectrometer and data system. The spectra were obtained using neutral loss and parent scans using argon as the collision gas at a pressure of 1.8×10^{-4} torr.

Results and Discussion I

A normal mass spectrum of the sample mixture was obtained by scanning the first quadrupole and keeping the other two quadrupoles in the RF only mode. The base peak, at m/z 331, of the spectrum was due to the pseudo-molecular ion of malathion. Most of the abundant ions present were fragments of malathion. This was expected since there was 100 times more malathion in the mixture than the other pesticides. The pseudo-molecular ions of the other species in the mix were almost visible in the multitude of peaks with less than 1% abundance. Most of the other ions from the "inert ingredients", emulsifiers and xylene-range solvents present in the ORTHO sample do not show up in the spectrum because their masses are below 100. One can not scan below m/z 100 when using isobutane as a reagent gas because its ion at m/z 97 is much larger than any of the sample ion

intensities.

The other pesticides present in the mixture can be detected however, by using neutral loss scans. Neutral loss scans of 158 and 186 mass units were used to obtain the spectra shown in Figures 4.1 and 4.2 These two scans should be able to detect any dithioate.

Disulfoton, azinphos ethyl and dialifor were detected at m/z 275, 346 and 394-397 respectively using the neutral loss scan of 186 mass units. These three compounds were expected to show up in this scan because they are all ethoxydithioates whose daughter ion spectra indicated a tendency to lose a 186 mass neutral.

The peak at m/z 318 however was not expected. This peak is from phosmet, a methoxydithioate, which should lose 158, not 186 mass units. The 186 mass loss is from a double elimination fragmentation. The base peak in the daughter ion spectrum of phosmet is at m/z 160, from the breaking of the Y-Z bond and the charge staying with the Z group as discussed in Chapter 2. The daughter ion spectrum of the m/z 160 ion shows ions at mass 132, 104 and 76, indicating successive losses of 28 mass units. Therefore it can be assumed that the loss of 186 mass units from phosmet is the total from a loss of 158, yielding m/z 160, and then a loss of 28 to give m/z 132. This double neutral loss could either be caused by a too energetic collision or multiple collisions. The multiple collisions could possibly be corrected by using a lower collision gas pressure but if it is lowered too far, then not enough of the molecular ion is fragmented and sensitivity is lost. The 20 volt collision energy was determined during the acquisition of daughter spectra and was chosen because it gave the best abundance of the ion representing the desired neutral losses. The energy is therefore fixed as the best choice and for consistency

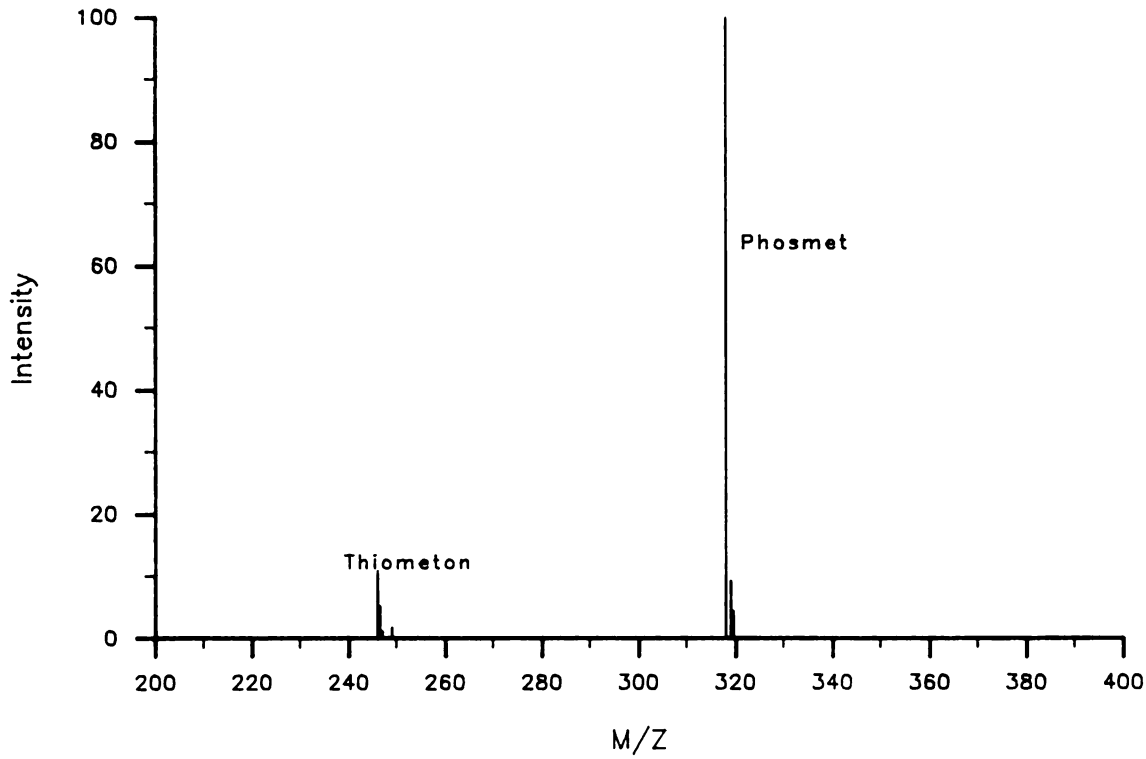


Figure 4.1
158 Neutral Loss

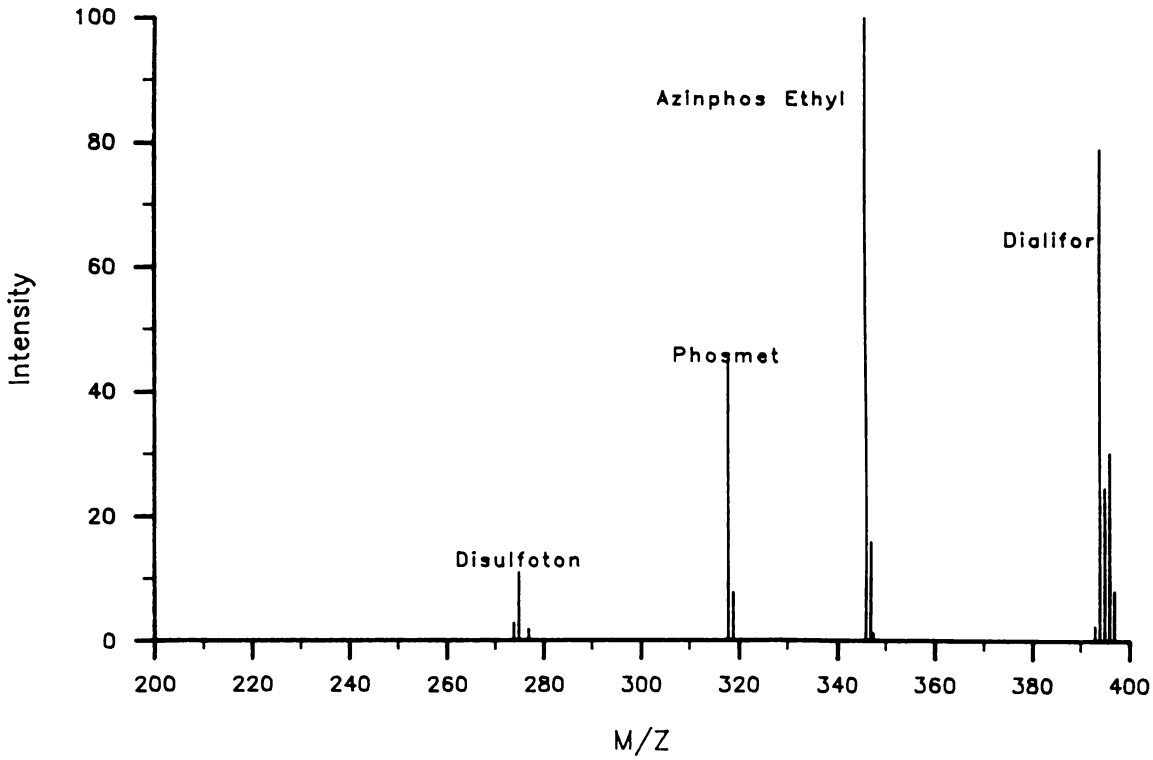


Figure 4.2
186 Neutral Loss

should not be adjusted for each compound.

The other compound in the mixture, dimethoate was not detected using neutral loss scans, as expected. Even though it is a methoxydithioate it does not fragment by breaking the Y-Z bond. It undergoes the unexplained rearrangement discussed in chapter two, and therefore does not lose 158 mass units.

A parent scan obtained by setting the third quadrupole to pass only ions with m/z 125 and scanning the first quadrupole is shown in Figure 4.3 . This scan should detect any of P1 ions from the dimethoxy dithioates and those diethoxy dithioate P1 ions that undergo a Quale rearrangement. This scan did detect dimethoate at m/z 230 as well as thiometon at m/z 246, phosmet at m/z 318, and two large malathion peaks at m/z 285 and m/z 331. There are also several other unexplained peaks, probably due to the fragmentation in the source of several of the compounds. Again this demonstrates the importance of creating only molecular ions in the source.

The success of these sample scans showed the feasibility of this screening method and prompted further experiments. After examining different CI reagent gasses and settling on methanol, a second detection experiment was carried out using a broader range of compounds.

Experimental II

Ten compounds were chosen that represent dithioates and thioates with both methoxy and ethoxy R groups. The ten compounds chosen are: fentrothion, dursban, quinalphos, parathion, etrimphos, malathion, dimethoate, phorate, phosmet and dialifor. In an attempt to eliminate the problems of fragmentation in the source, methanol was

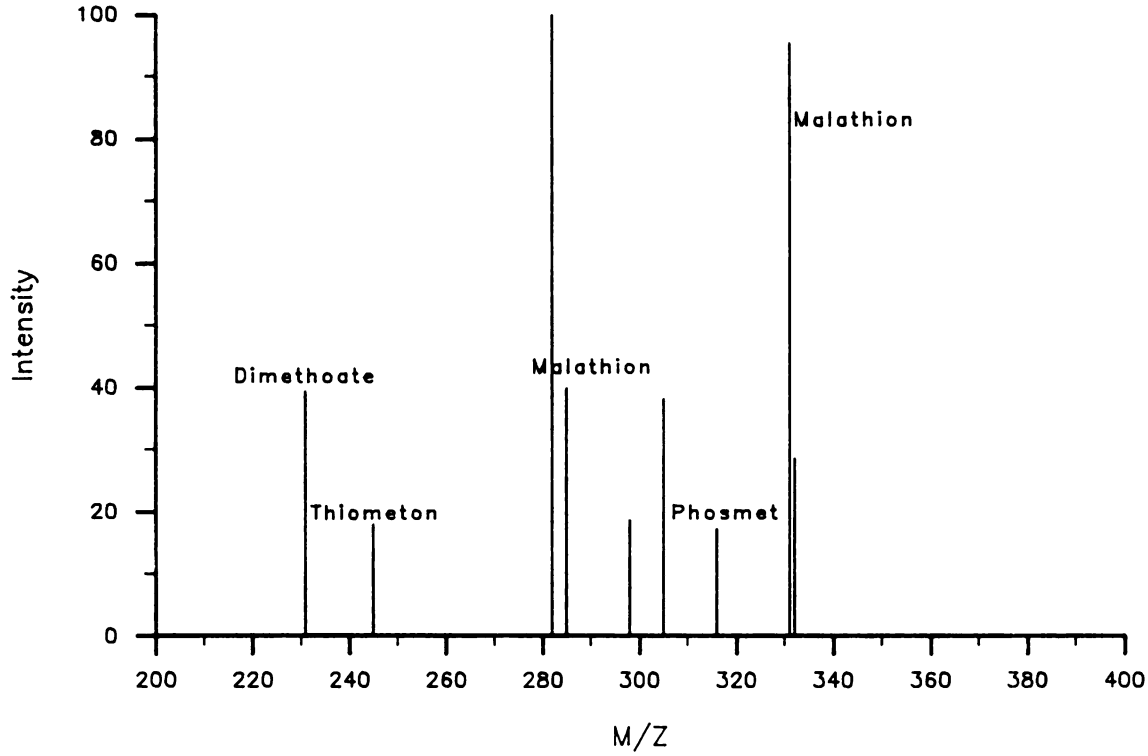


Figure 4.3
125 Parent Scan

used as the CI reagent gas. The methanol was injected with a syringe into an evacuated expansion bulb and its vapors were then leaked into the source through a needle valve. The pressure in the source was adjusted until the methanol cluster ions at m/z 65 and 97 were maximized and the ions at m/z 33 and 97 were approximately of equal intensity. Measured amounts of the ten pesticides were mixed in a xylene solvent and diluted to a concentration of about two micrograms per milliliter. The sample was introduced into the mass spectrometer with a solids probe into which was fitted a capillary tube containing one to two microliter samples of the mixture. The solids probe was slowly heated to 150 degrees centigrade, allowing the pesticides to volatilize.

Six scans were used to try to detect the pesticides. As in the first experiment, neutral losses of 158 and 186 mass units were used to detect the dithioates present. A 142 mass unit neutral loss was used to detect the methoxy thioates present and a 152 mass unit neutral loss was tried as a way to detect the diethoxy thioates. Two parent scans were included. Scans for the parents of ions with m/z 125 and 127 were used to detect fentrothion and malathion because their ions due to the above neutral losses were small.

Two different automated data collection software programs were used to take data. Software designed for chromatography has been implemented on the Extra Nuclear ELQ-400-3 mass spectrometer that continuously repeats a programed set of scans. In this case the instrument was instructed to do a scan for a neutral loss of 142, then successive scans for neutral losses of 152, 158 and 186, then do scans for all the parents of m/z 125 and 127. This sequence was repeated every two seconds for 600 seconds or until stopped by the operator.

The other program used utilizes multiple reaction monitoring to detect compounds as they elute off a gas chromatography column, or in this case, off the probe. Up to eight different specific parent-daughter ion pairs are entered into the program which, when run, continuously cycles through the pairs, setting quadrupoles one and three to the correct values and detecting any ions that get through in a specified amount of time. Selected ion monitoring experiments may also be done by setting the first quadrupole to pass only the ion of interest and putting quadrupole three into the RF only mode. Adduct ions, caused by the addition of mass during the collision process, may also be monitored by setting the daughter to a higher mass than the parent.

Results and Discussion II

Nine of the ten pesticides were detected using the first program. Because of differences in volatility, not all the pesticides were ever detected in one set of scans. Dimethoate could be detected as soon as the probe was inserted. Other compounds were detected as the probe slowly heated to 150 degrees where Dialifor could be detected. Figure 4.4 shows averaged composite spectra for the scans used.

The only compound not detected was Parathion. It has a small peak due to a loss of 152 mass units in its EI spectrum, but this ion is not formed by collision. The prominent ions in the Parathion collision spectrum are due to losses of 28 mass units. This neutral loss was not used to detect the pesticide because loss of 28 is a very common neutral loss and thus not indicative of just the pesticide. The other neutral losses used in this study are much more

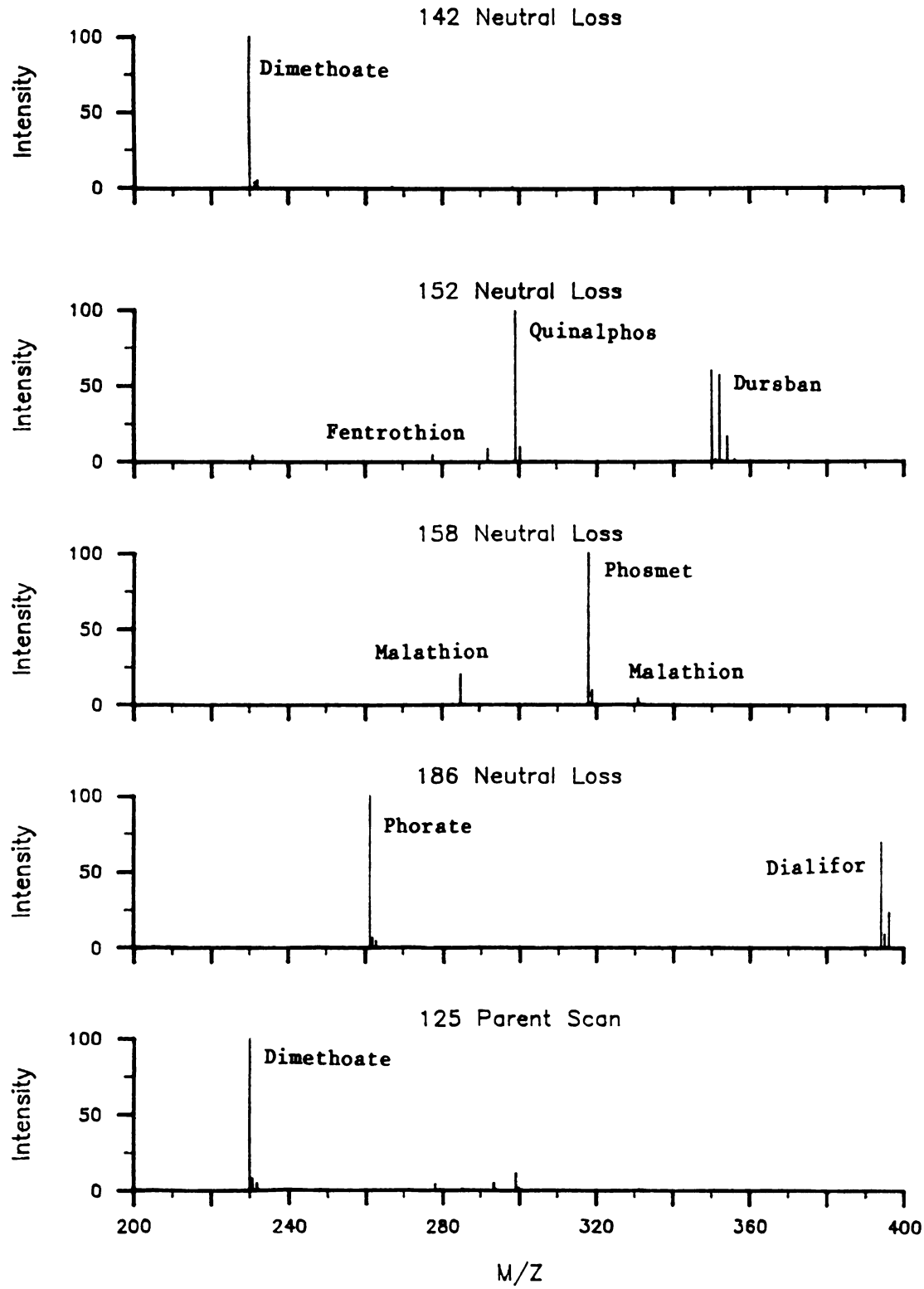


Figure 4.4

Spectra Obtained Using Scanning Techniques

uncommon and therefore should not suffer from interferences from sample matrix ions.

Dimethoate, which was not detected using the neutral loss scans in the first experiment, was detected in the 142 neutral loss scan. Dimethoate, a dimethoxy dithioate, should not be detected by a scan targeted for dimethoxy thioates but it is because the base peak at m/z 88 in its CAD spectrum (the unexplained rearrangement ion) just happens to be 142 mass units less than the m/z 230 pseudo-molecular ion.

This same sort of situation allows for the detection of malathion using an m/z 127 parent scan. Malathion can be detected with the expected 158 mass unit neutral loss, but because the m/z 173 peak that is needed for this scan to work is very small, malathion is not always picked up. Since the base peak in the CAD spectrum of the pseudo-molecular ion is at m/z 127, it can be detected much more easily, with potentially better sensitivity, using the m/z 127 parent scan. Using this scan does not increase the total number of scans used since the m/z 127 parent scan is also used to detect the dimethoxy phosphates.

Those scans that accidentally detected pesticides point out an unavoidable problem and an important consideration. Using a reagent gas that makes only pseudo-molecular ions does help eliminate ambiguity in compound identification, but does not get rid of all interferences. The two pieces of information in a neutral loss or parent scan, the m/z of the parent and the neutral or daughter mass can be used to identify a known compound but not an unknown compound. If for example, the 142 neutral loss scan yields a peak at an m/z that does not correspond to a known pesticide, the unknown compound does

not have to be a dimethoxy thioate, or even an organophosphorus compound. More information is needed to make an identification, such as a complete daughter scan of the detected ion.

By using unusual neutral loss and uncommon parent scans, chances of detecting only the desired compounds are better, but not assured. Any scanning technique which, by design looks for anything, can detect anything. This is good for metabolite or decomposition studies in which the metabolized species is not known, because a scanning technique will be able to detect the unknown compound. For targeted analysis however, in which only specific compounds need to be detected, a scanning technique that could potentially produce a peak at an unexpected mass could cause problems. Reaction monitoring of specific known ion pairs can eliminate this problem but then only the known compound can be detected. Therefore the goals of the experiment should be considered when choosing the type of scan used.

The ion current chronograms from the multiple reaction monitoring experiments are shown in Figure 4.5. All eight of the pesticides were detected. The peaks are broad because they represent heating profiles and not GC peaks. The temperature was initially allowed to rise to 100 degrees, but after approximately 280 seconds it was raised to 150 degrees in order to vaporize dialifor. The number in the upper left hand corner of each plot represents the ion current at the top of the peak in ADC units. The differences in the numbers is due in part to the different concentrations of the various compounds in the mixture, but also due to the relative abundances of the selected ions in the parent ion spectrum. The fragmentation efficiency and therefore the daughter ion abundance are dependent on the collision energy, collision gas species and pressure, and the mass of

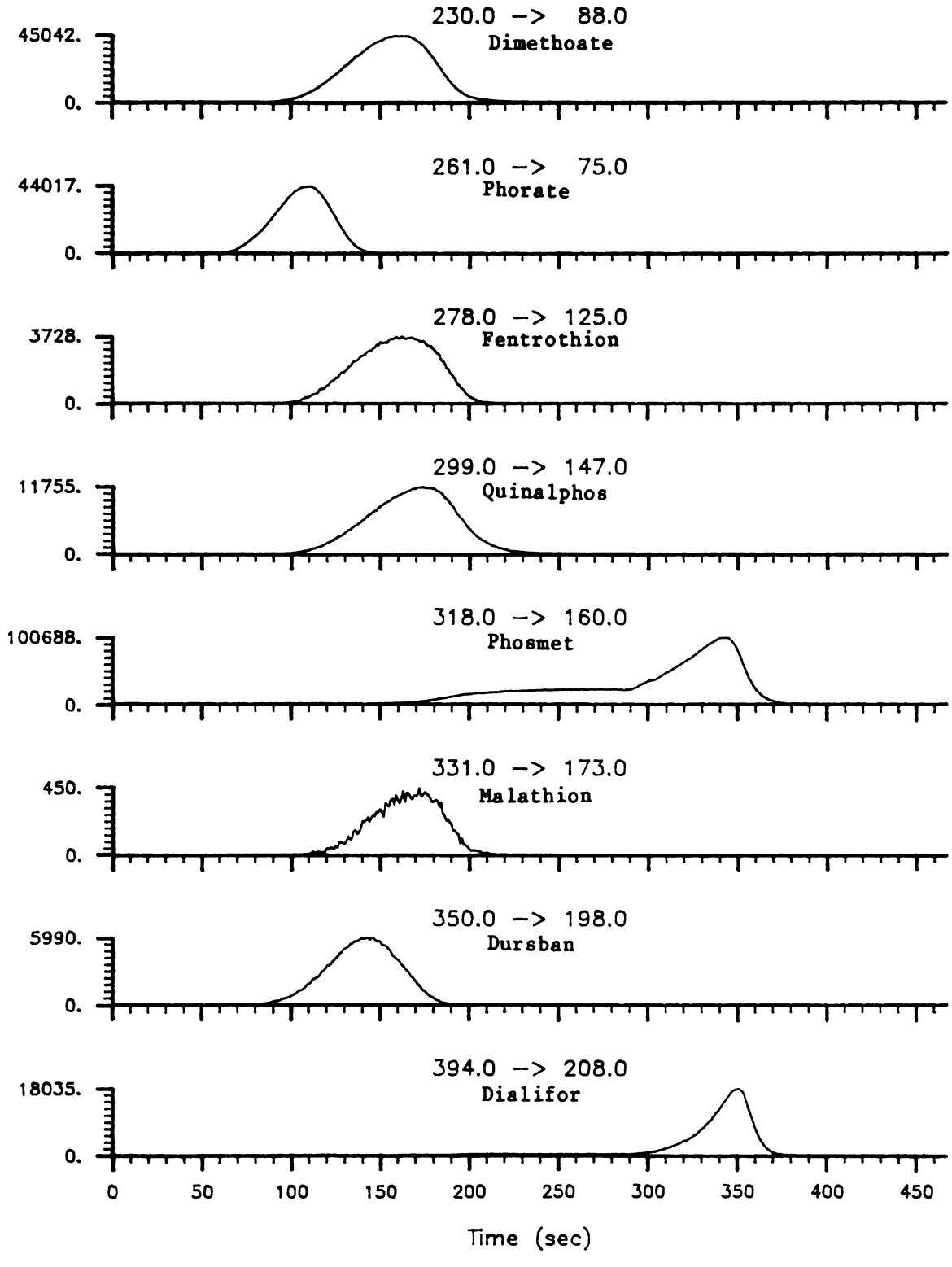


Figure 4.5

Multiple Reaction Monitoring Results

the parent and daughter. Each reaction has a different set of conditions for maximum efficiency and, when monitoring more than one reaction at a time, compromises must be made that adversely effect some of the reactions monitored. The malathion ion current is the smallest however, because the m/z 173 ion is not a very abundant ion in the CAD spectrum of the m/z 331 parent.

Reaction monitoring, because it does not have to scan the mass filters, is a much more sensitive technique than the method involving the neutral loss and parent scans. The ion currents detected using the multiple reaction monitoring programs were several times larger than those from the scanning programs which were near the detection limit of the instrument. A 100 fold dilution of the mixture was made and analyzed again using the same monitoring program. Approximately two nanograms of each compound was present in the sample analyzed. The ion current chronograms for the dilute mix are shown in Figure 4.6. This time only seven of the eight compounds were detected. The peaks in the dialifor chromatogram are just noise spikes with three ADC units of intensity. It is not known why the dialifor was not detected in the dilute solution. Malathion was still detectable, even though the signal to noise was not very good.

The sensitivity of this technique could be improved further by looking for only one compound and tuning the instrument to favor the reaction of interest. Detection limits in the picogram to femtogram range should be attainable.

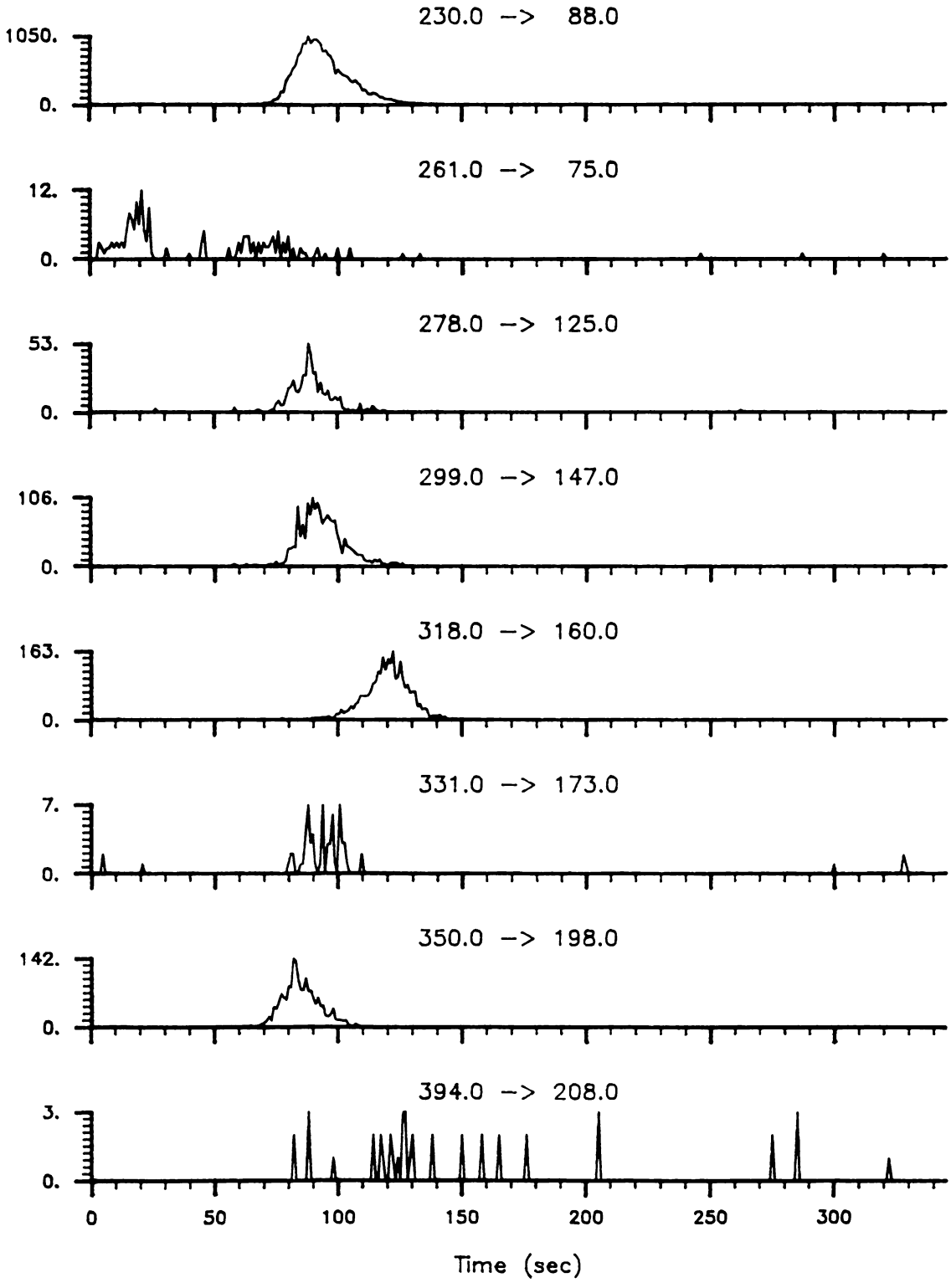


Figure 4.6

Multiple Reaction Monitoring Results (dilute sample)

Conclusion

By only looking for one specific collision reaction product the sensitivity and selectivity of the analysis are enhanced, but only the compound of interest can be detected. Knowing what to look for has the advantage of allowing the instrument to be tuned to favor the production and detection of the reaction components. By monitoring several reactions with one set of instrument conditions, sensitivity is lost. This could be corrected for by allowing the computer to adjust the variable instrument parameters so that the best settings for each compound are being used when data is being taken.

Unknown or unexpected compounds can only be detected using scanning techniques. Neutral loss and parent scans can identify known compounds, but are not as sensitive. Scanning techniques can detect an unlimited number of compounds at once with a common functionality, but the absolute identity of some may require confirmatory daughter scans.

Both methods are capable of routinely detecting many pesticides on a short time scale.

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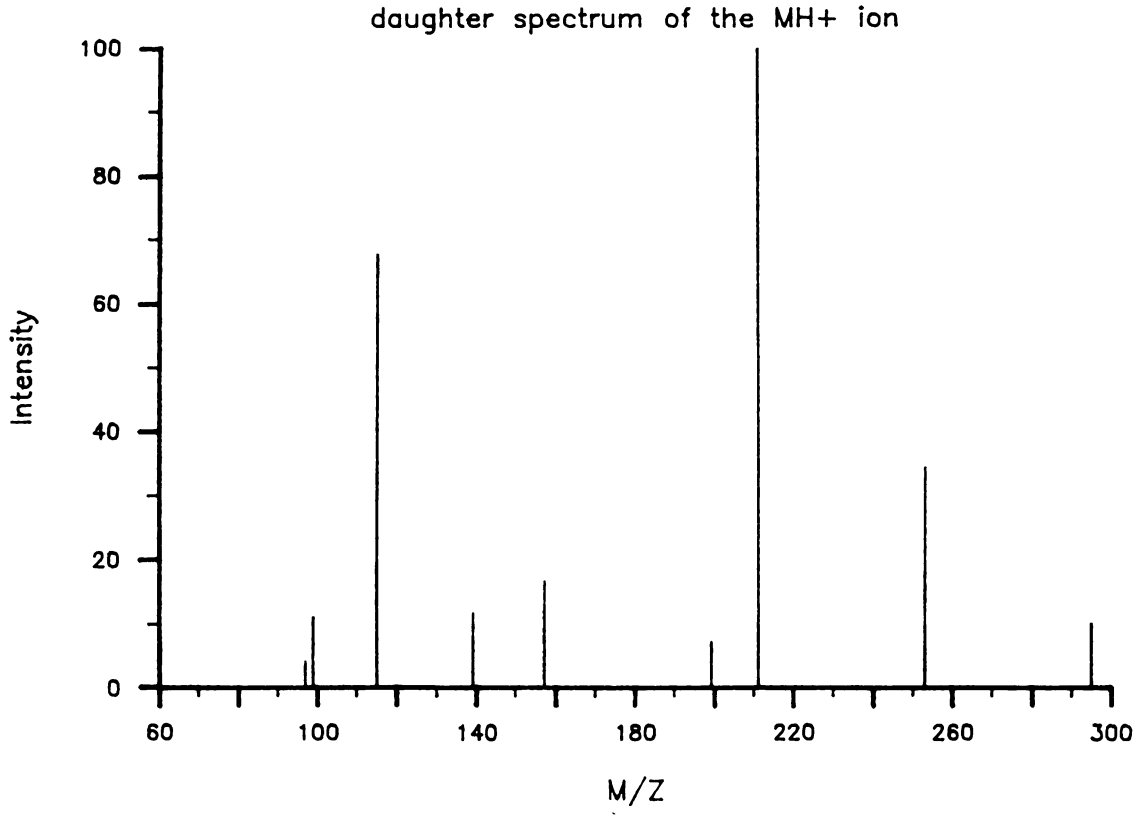
APPENDIX

Aspon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60
100
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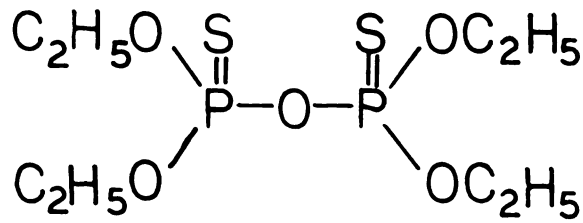
180
220
260
300
M/Z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OC2H5
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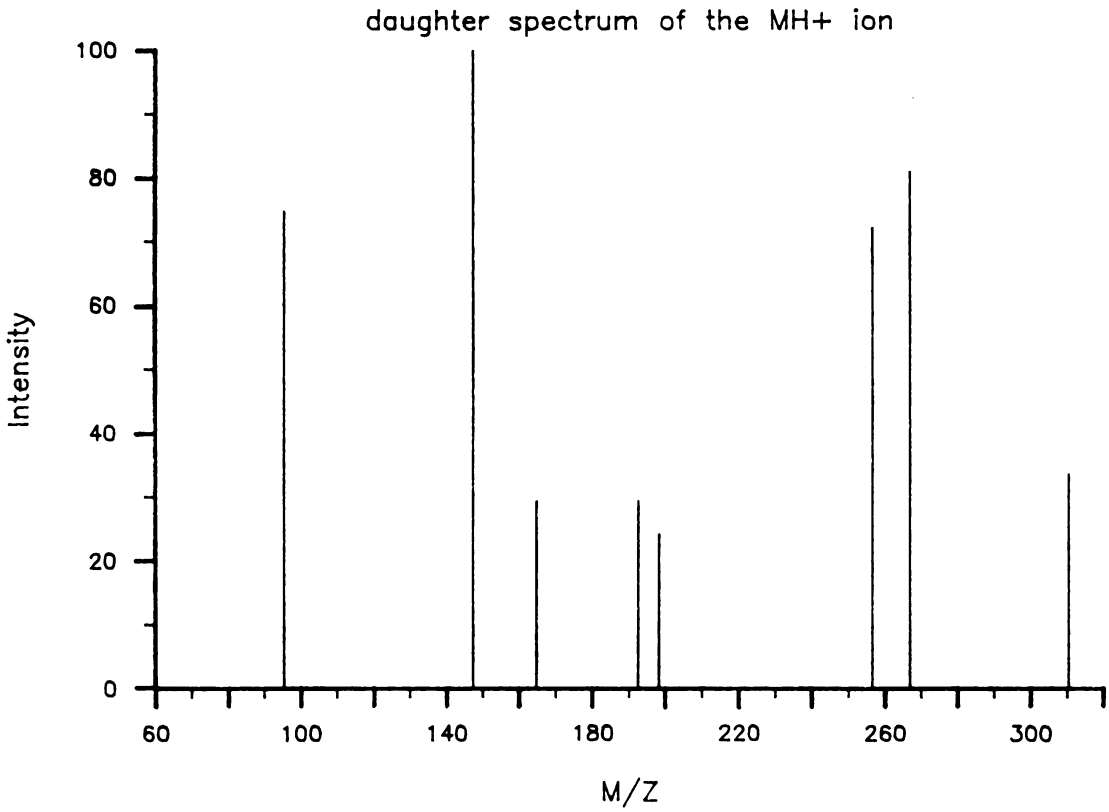
OC2H5

Azinphos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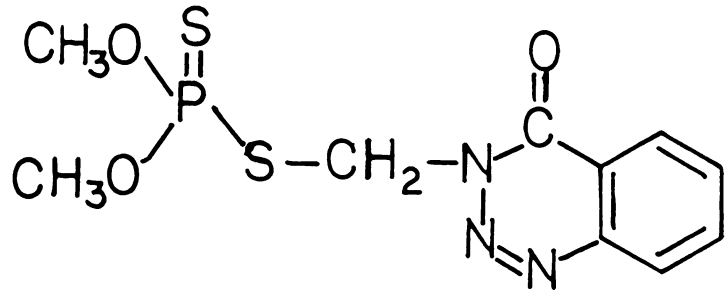
M/Z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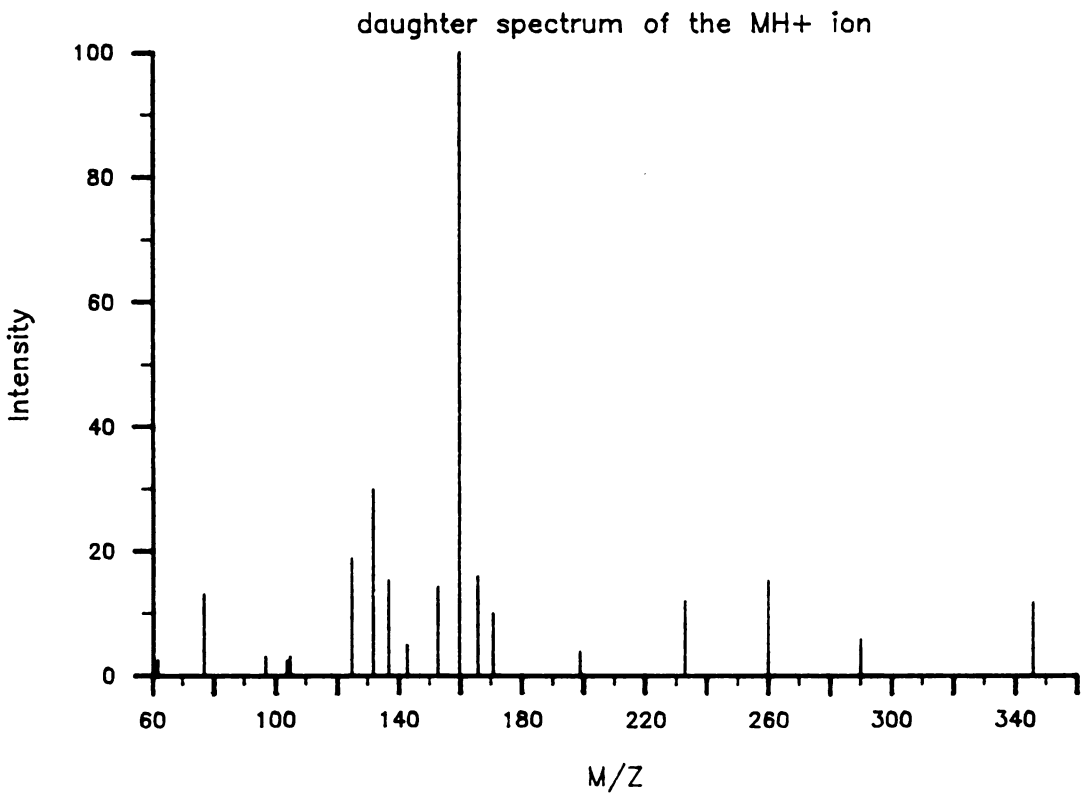
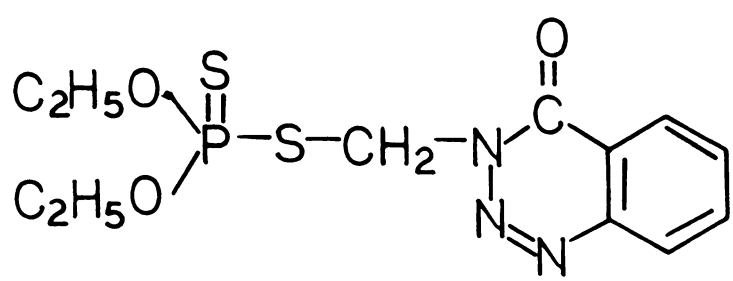
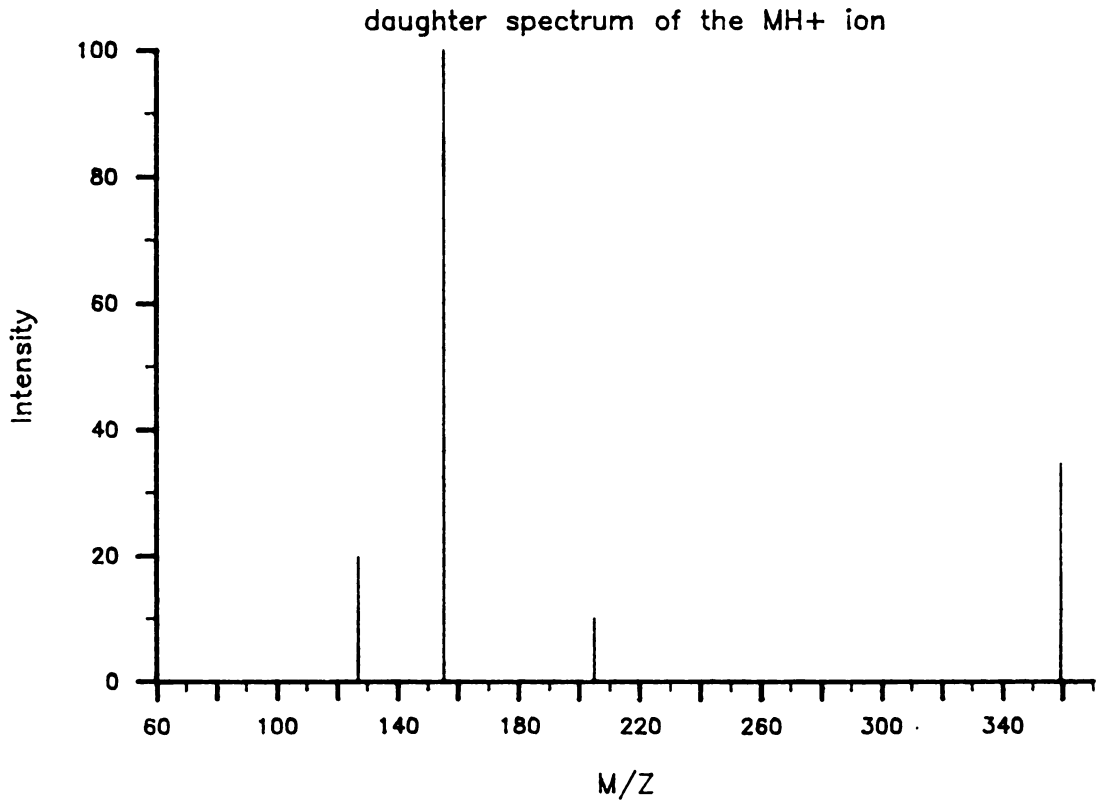
N

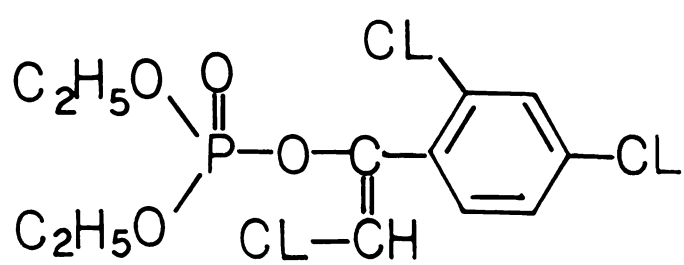
Azinphos Ethyl





Chlorfenvinphos





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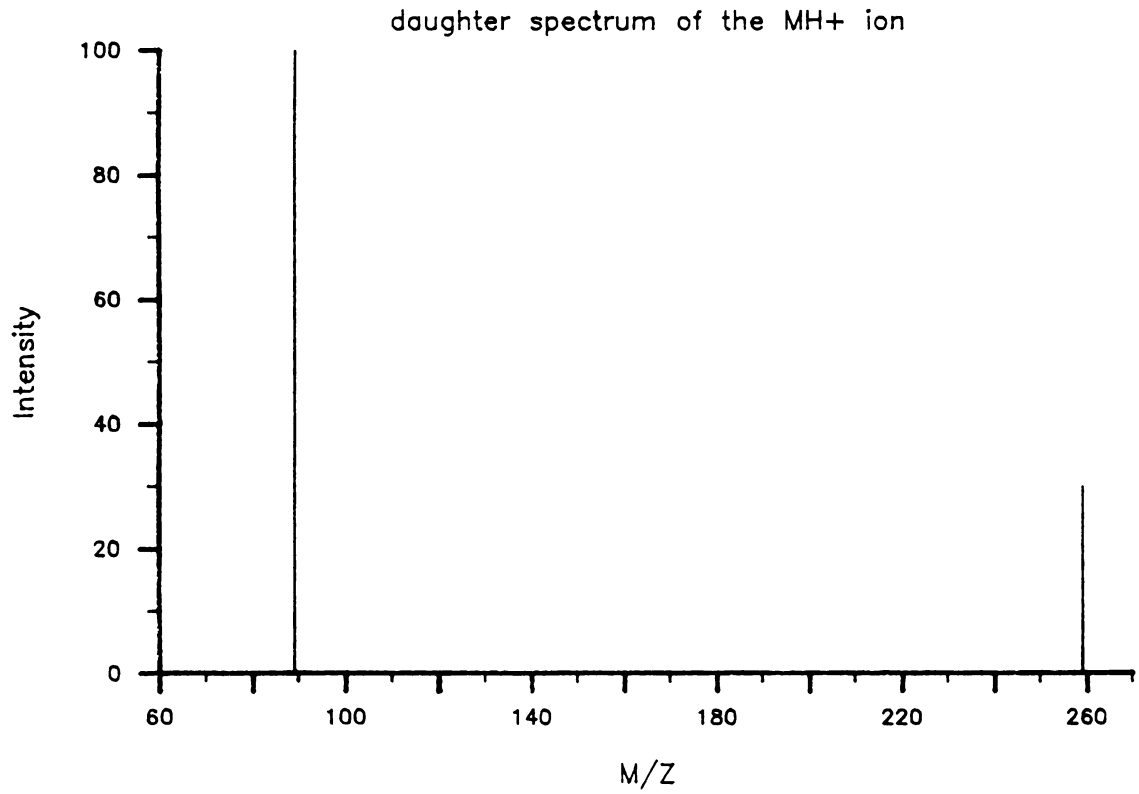
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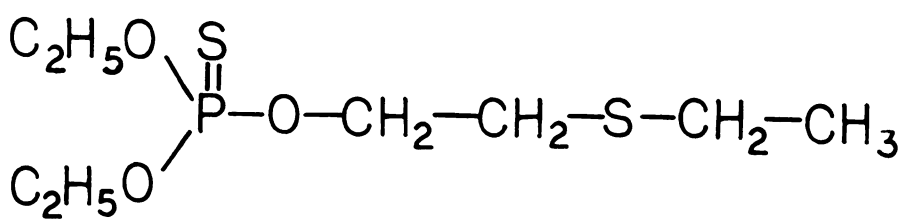
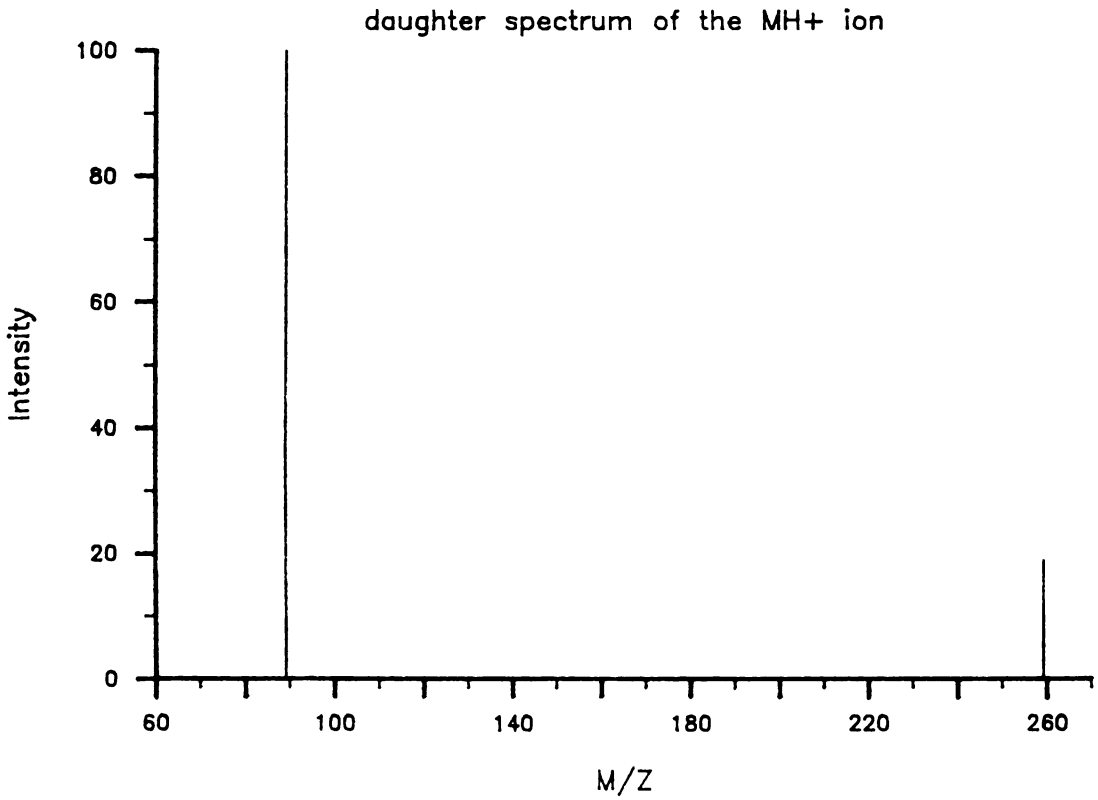
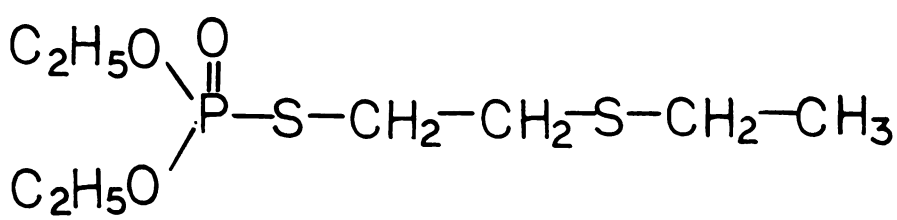
40
20
0
60
100
140
180
220
260
M/Z

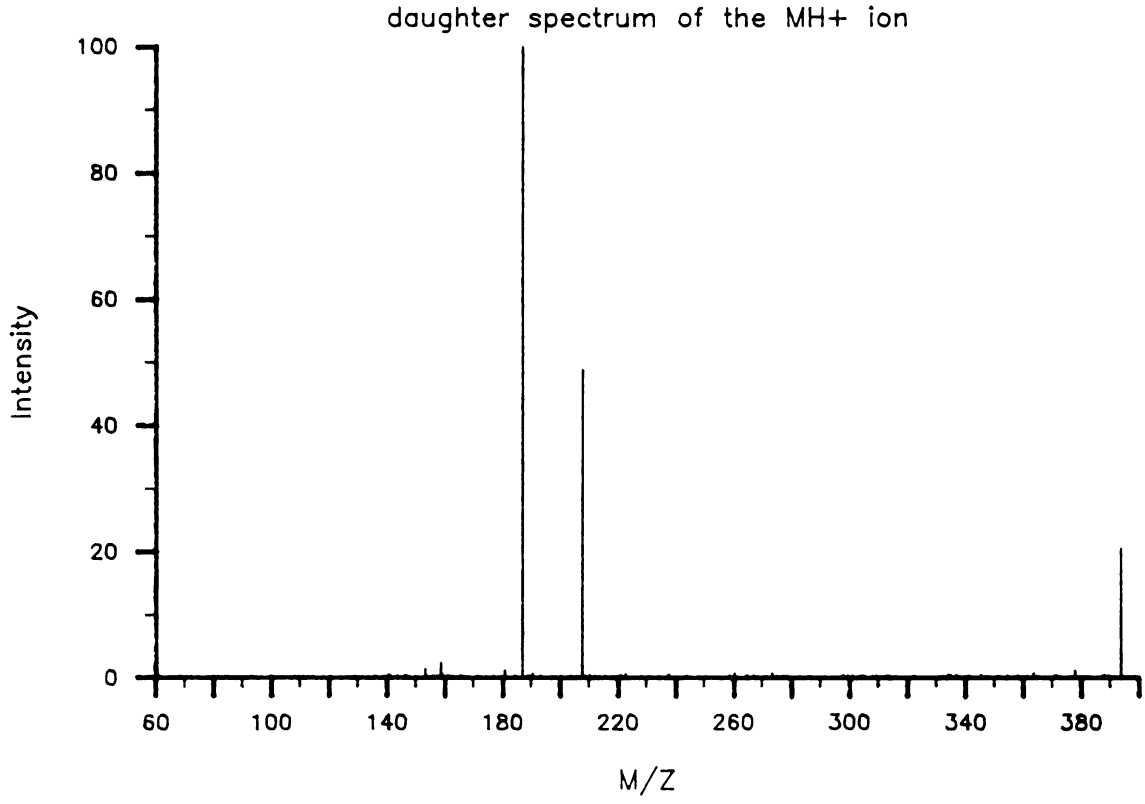

C2H5O
S
P–O–CH2–CH2–S–CH2–CH3
C2H5O

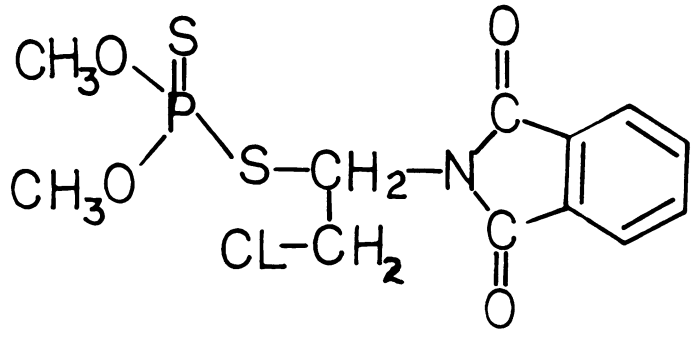
Demeton-S


daughter spectrum of the MH+ ion
Intensity
100
80
60
40
20
0
60
100
140
180
220
260
M/Z

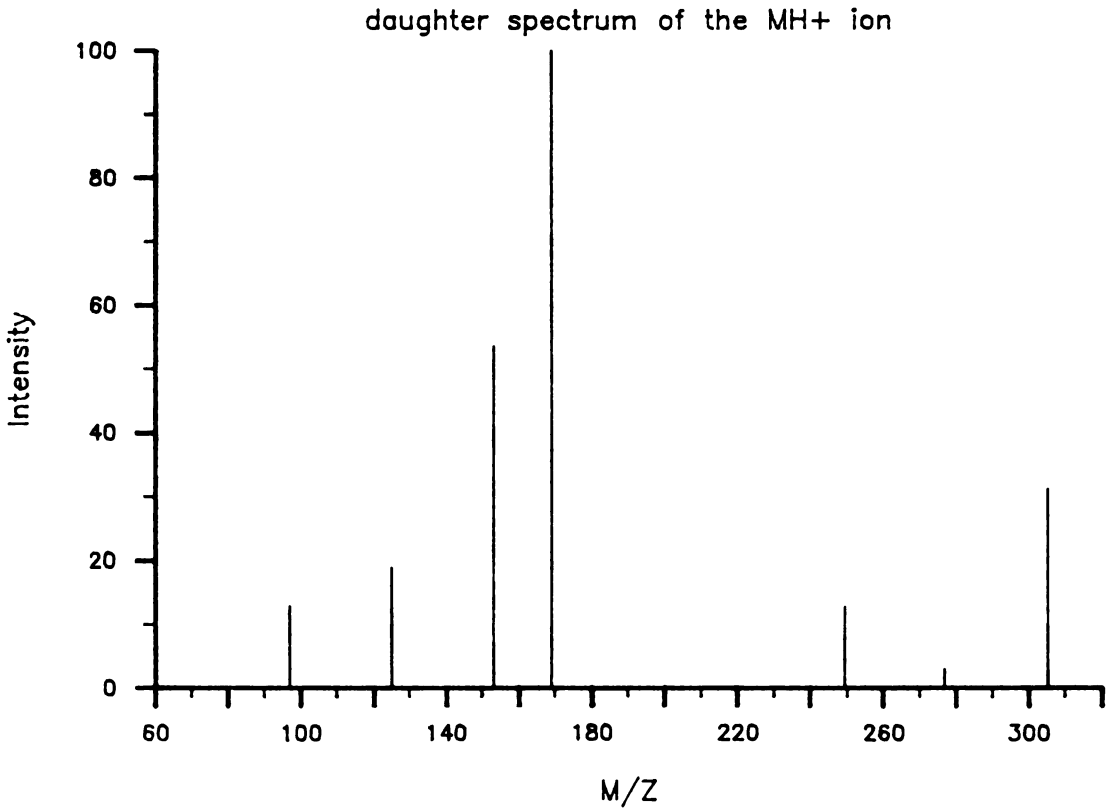

C2H5O
O
P
S
CH2
CH2
S
CH2
CH3
C2H5O

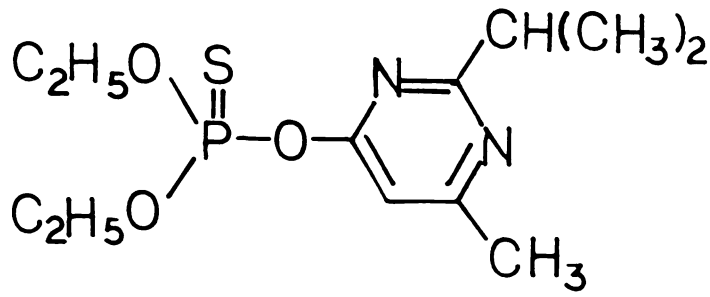
Dialifor


daughter spectrum of the MH+ ion
Intensity
100
80
60
40
20
0
60
100
140
180
220
260
300
340
380
M/Z

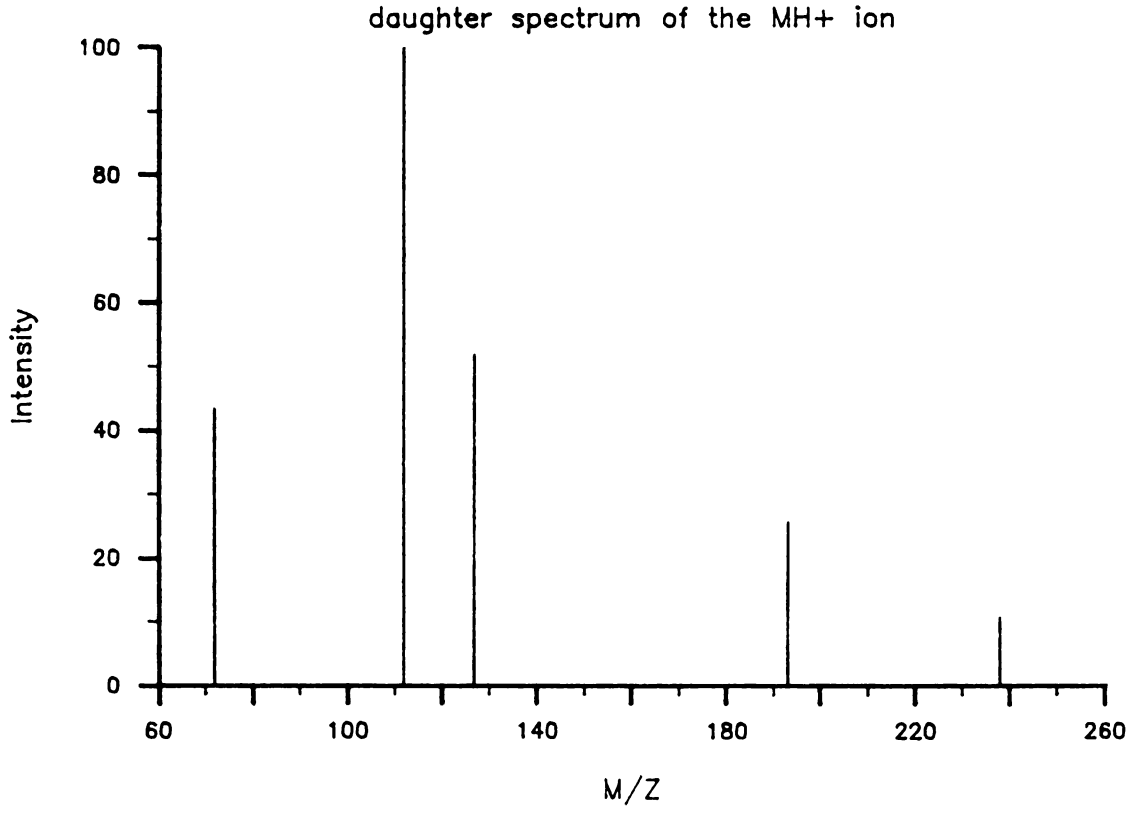

CH3O
S
P
CH3O
S–CH2–N
CL–CH2
O
C
C
O

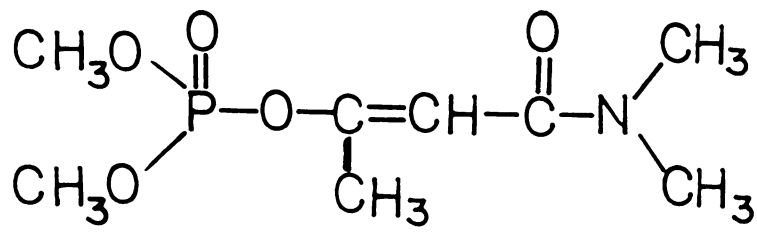
Diazinon


daughter spectrum of the MH+ ion
Intensity
100
80
60
40
20
0
60
100
140
180
220
260
300
M/Z

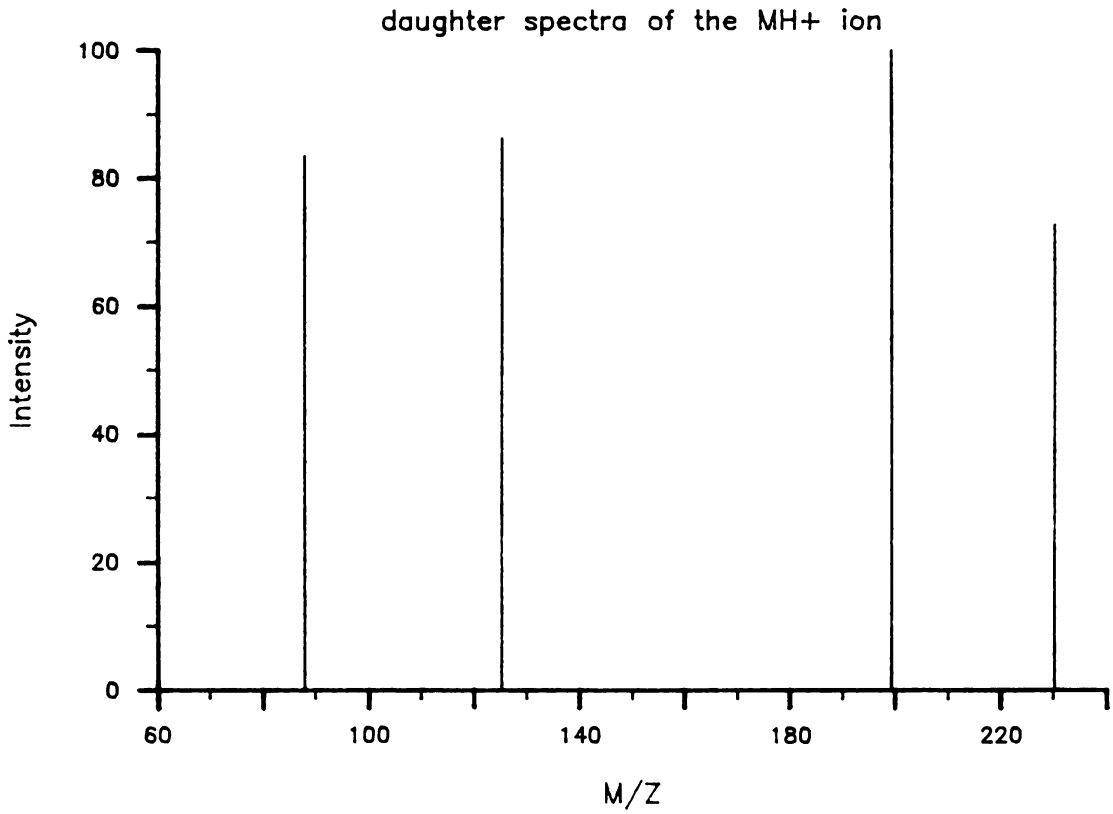

C2H5O
S
P
O
C2H5O
N
N
CH(CH3)2
CH3

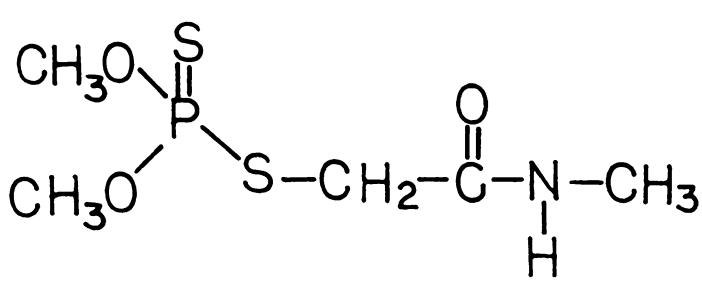
Dicrotophos



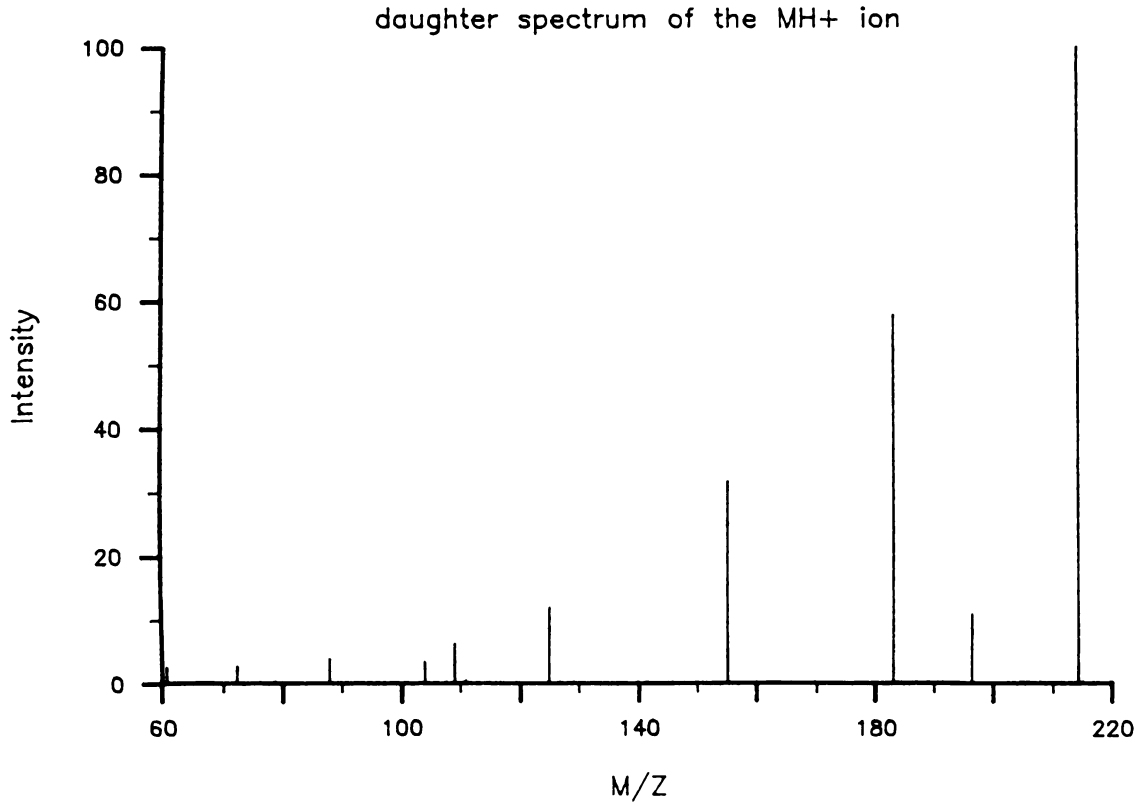


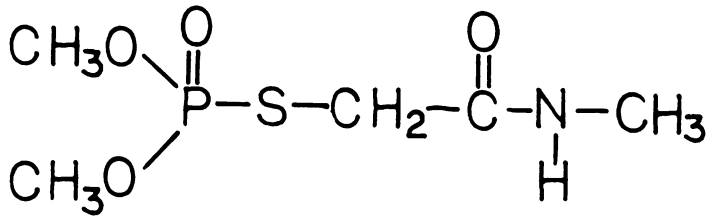
Dimethoate


daughter spectra of the MH+ ion
Intensity
100
80
60
40
20
0
60
100
140
180
220
M/Z

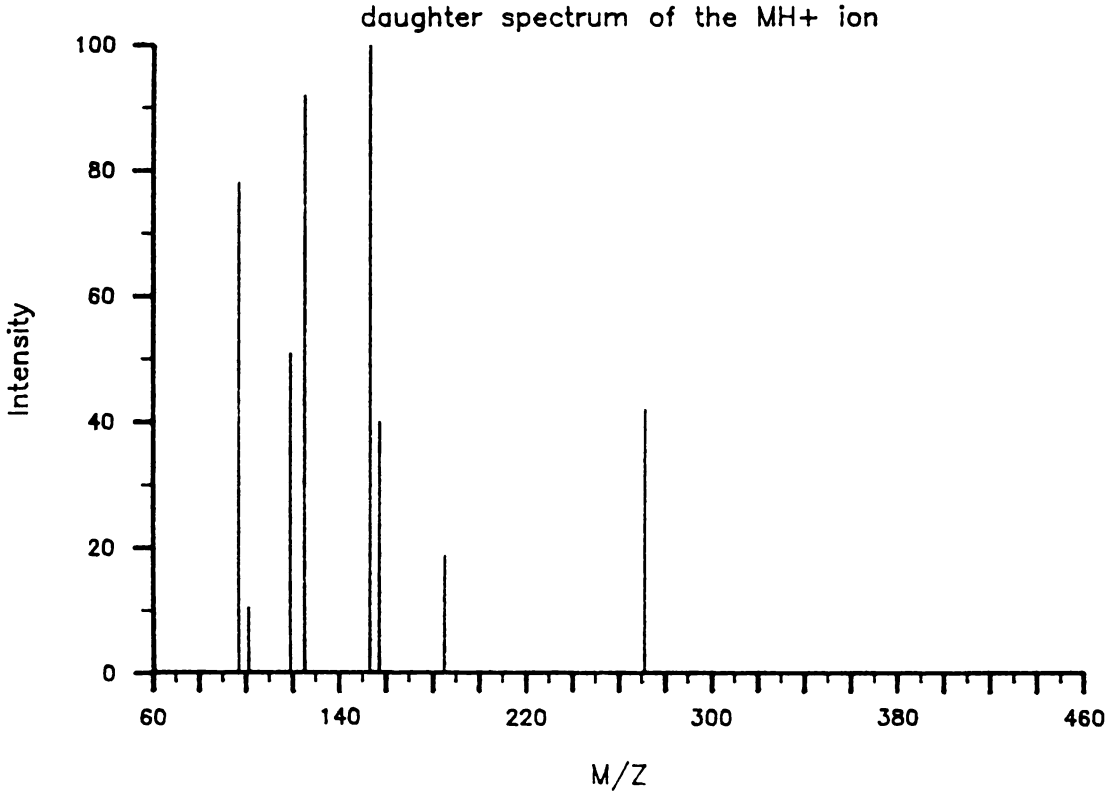

CH3O
S
P
CH3O
S
CH2
C
O
N
H
CH3

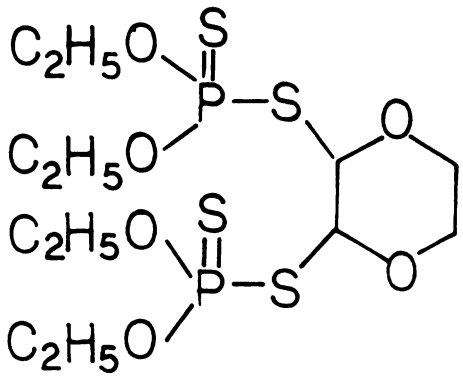
Oxygen Analog of Dimethoate



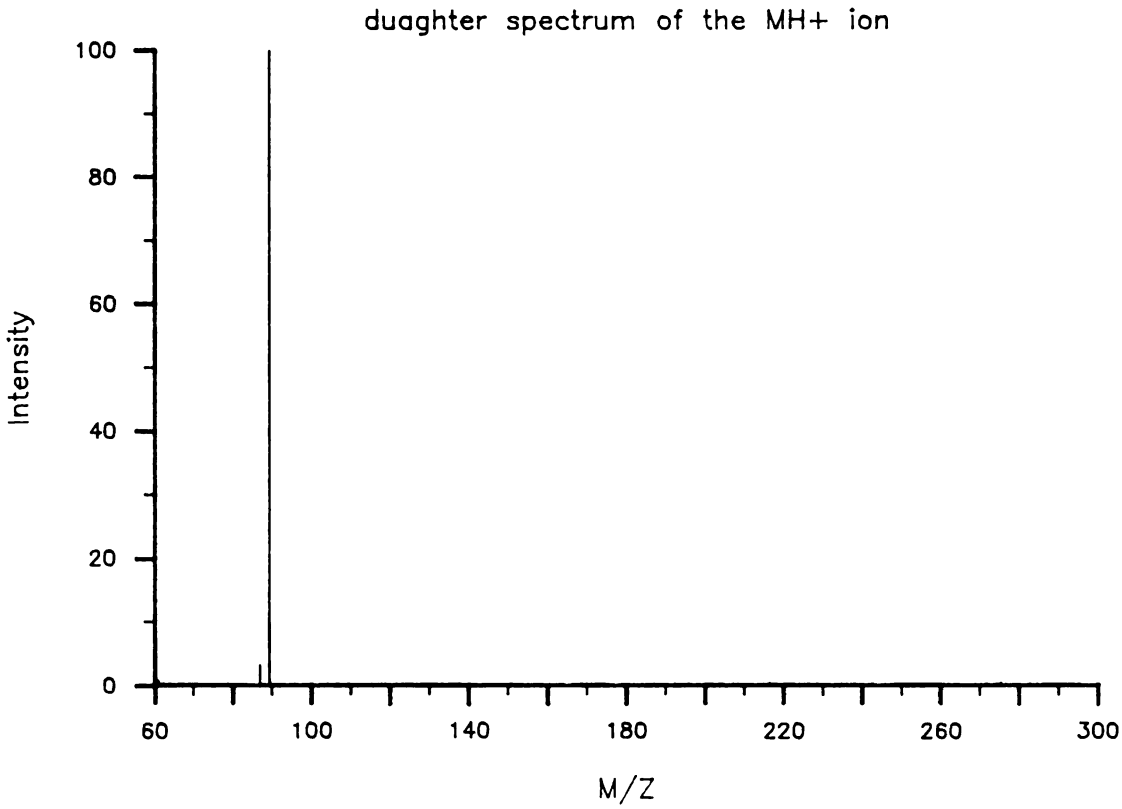


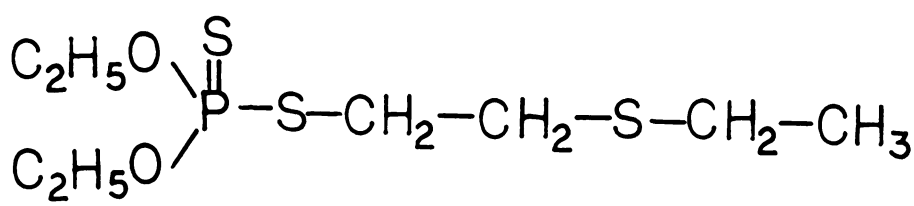
Dioxathion


daughter spectrum of the MH+ ion
Intensity
100
80
60
40
20
0
60
140
220
300
380
460
M/Z

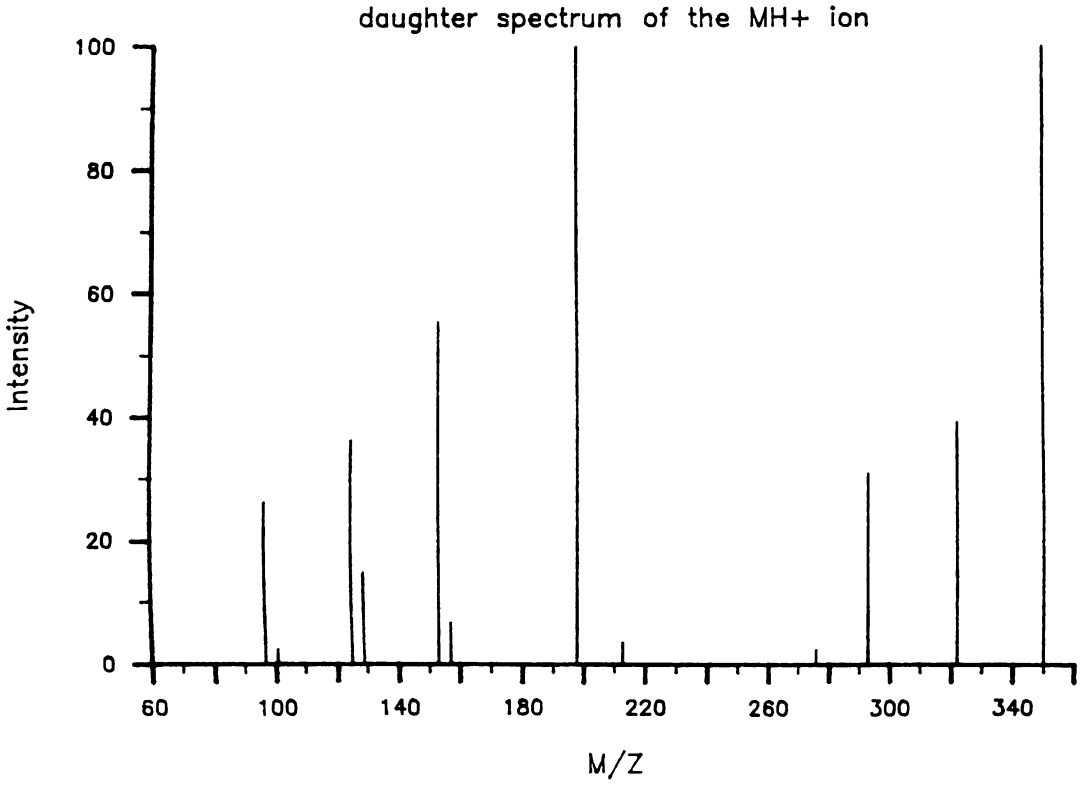

C2H5O
S
P
S
O
C2H5O
C2H5O
S
P
S
O
C2H5O

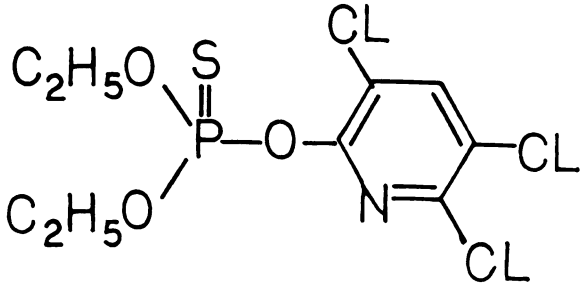
Disulfoton


duaghter spectrum of the MH+ ion
Intensity
100
80
60
40
20
0
60
100
140
180
220
260
300
M/Z

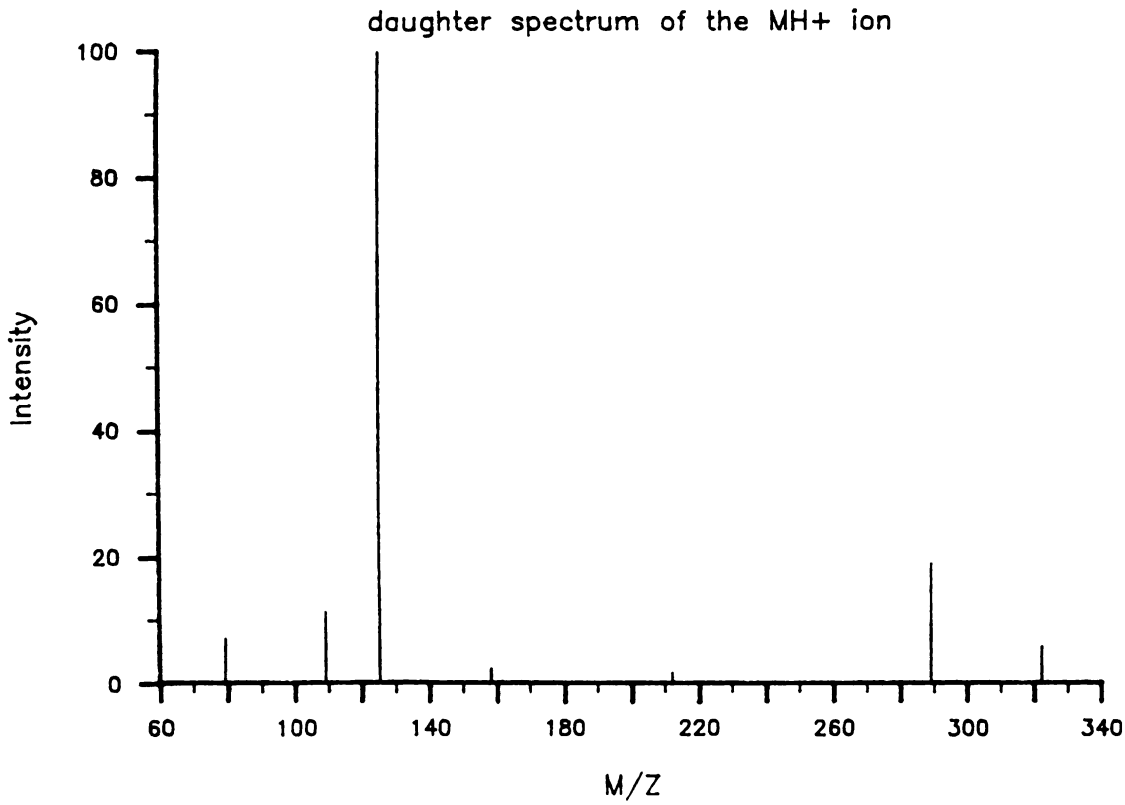

C2H5O
S
P
S
CH2
CH2
S
CH2
CH3
C2H5O

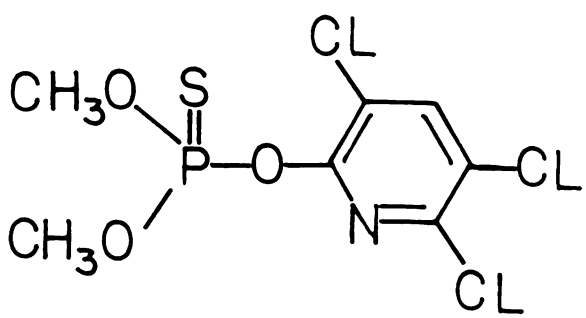
Dursban


daughter spectrum of the MH+ ion
Intensity
100
80
60
40
20
0
60
100
140
180
220
260
300
340
M/Z

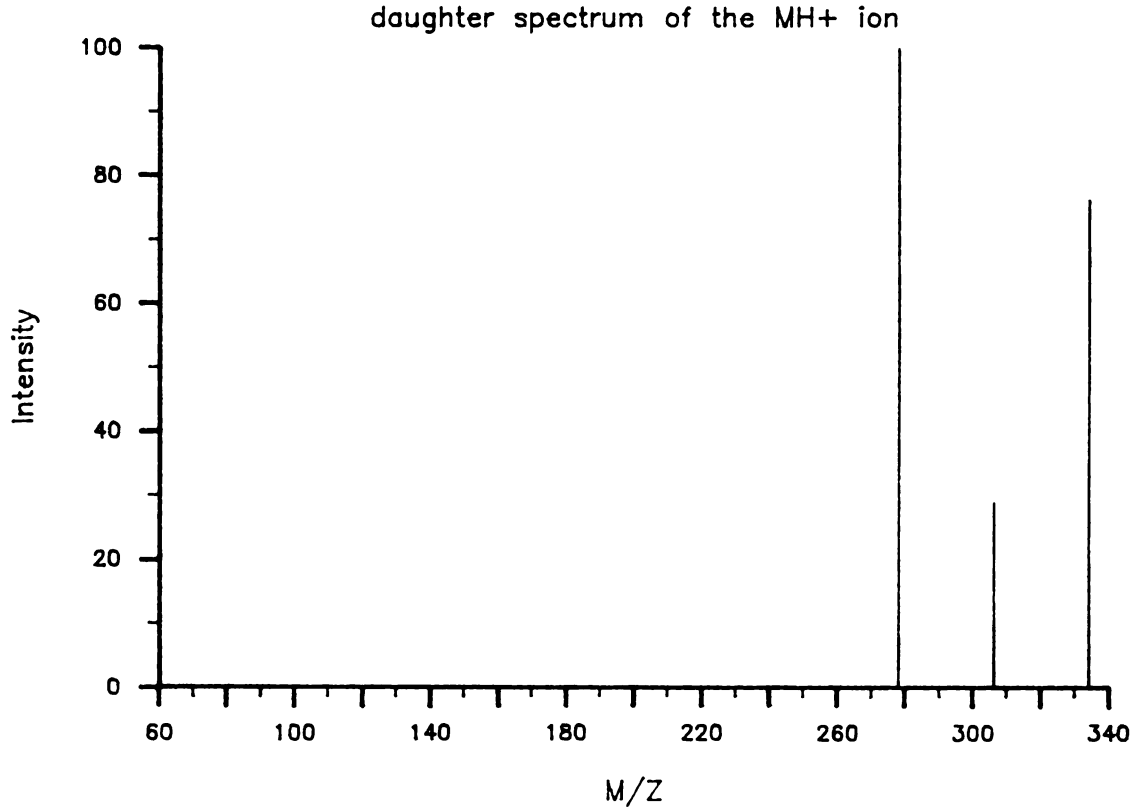

CL
C_2H_5O
S
P
O
CL
C_2H_5O
N
CL

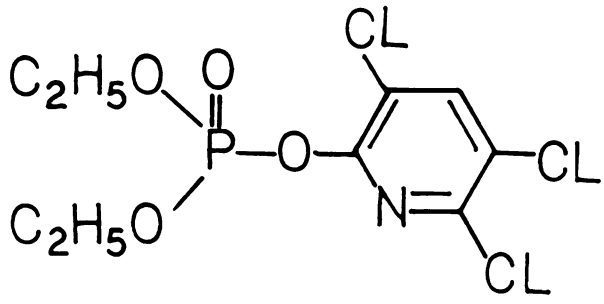
Methyl Dursban



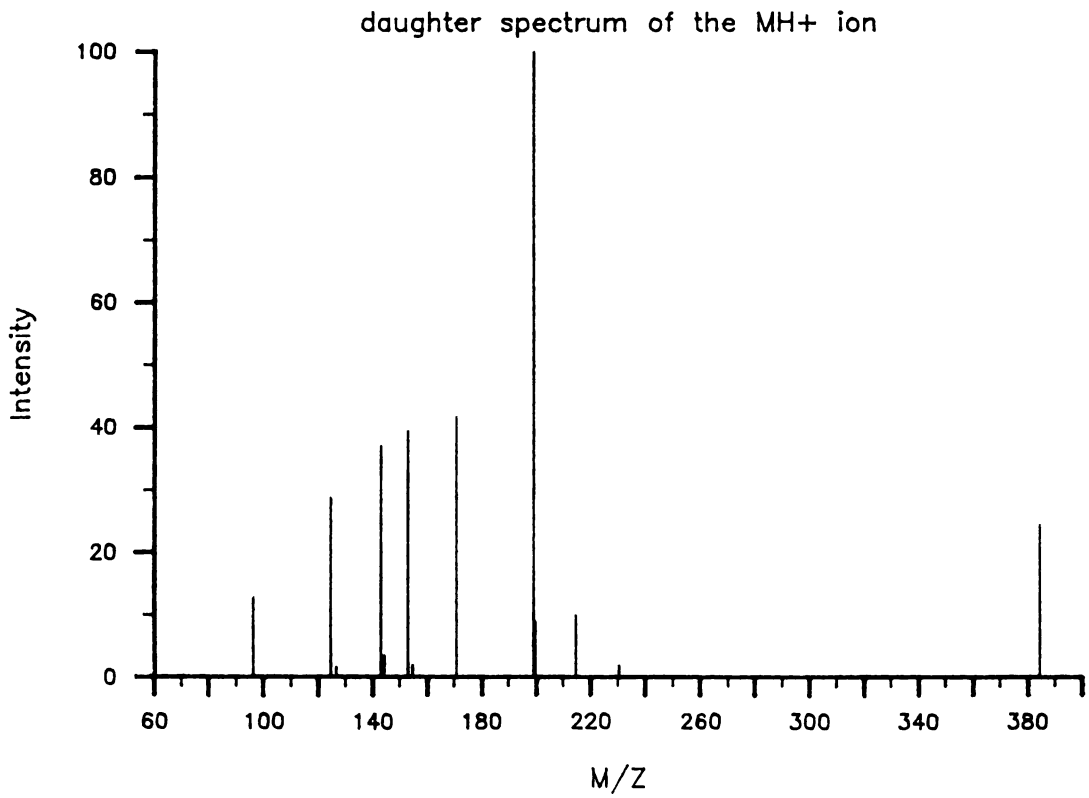


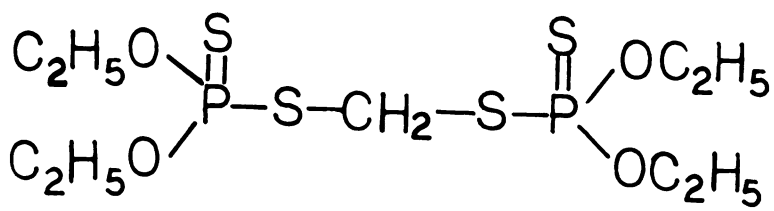
Oxygen Analog of Dursban



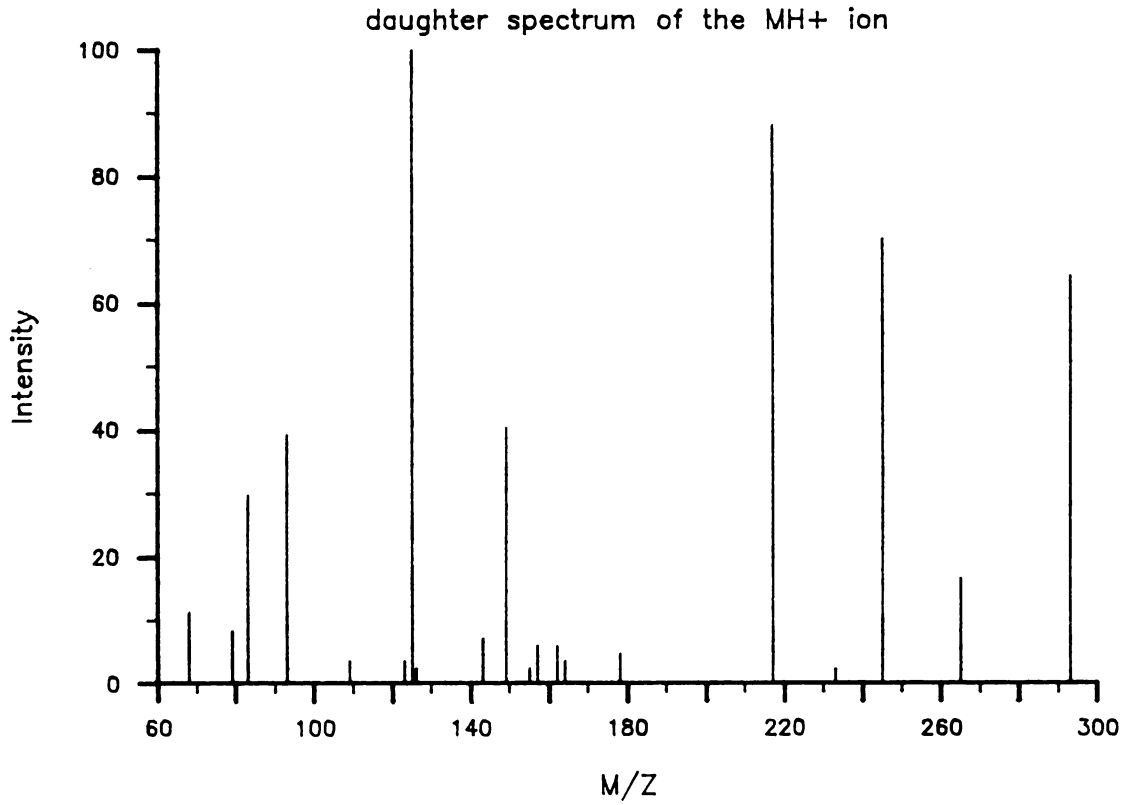


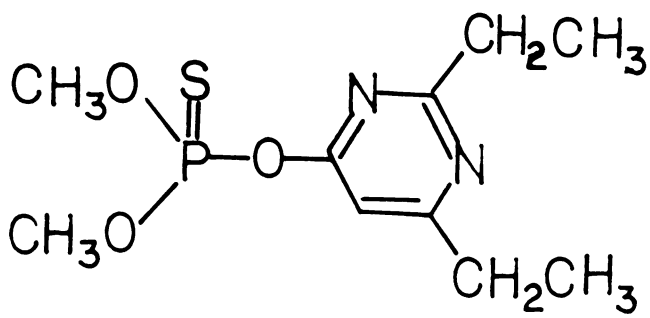
Ethion


daughter spectrum of the MH+ ion
Intensity
100
80
60
40
20
0
60
100
140
180
220
260
300
340
380
M/Z

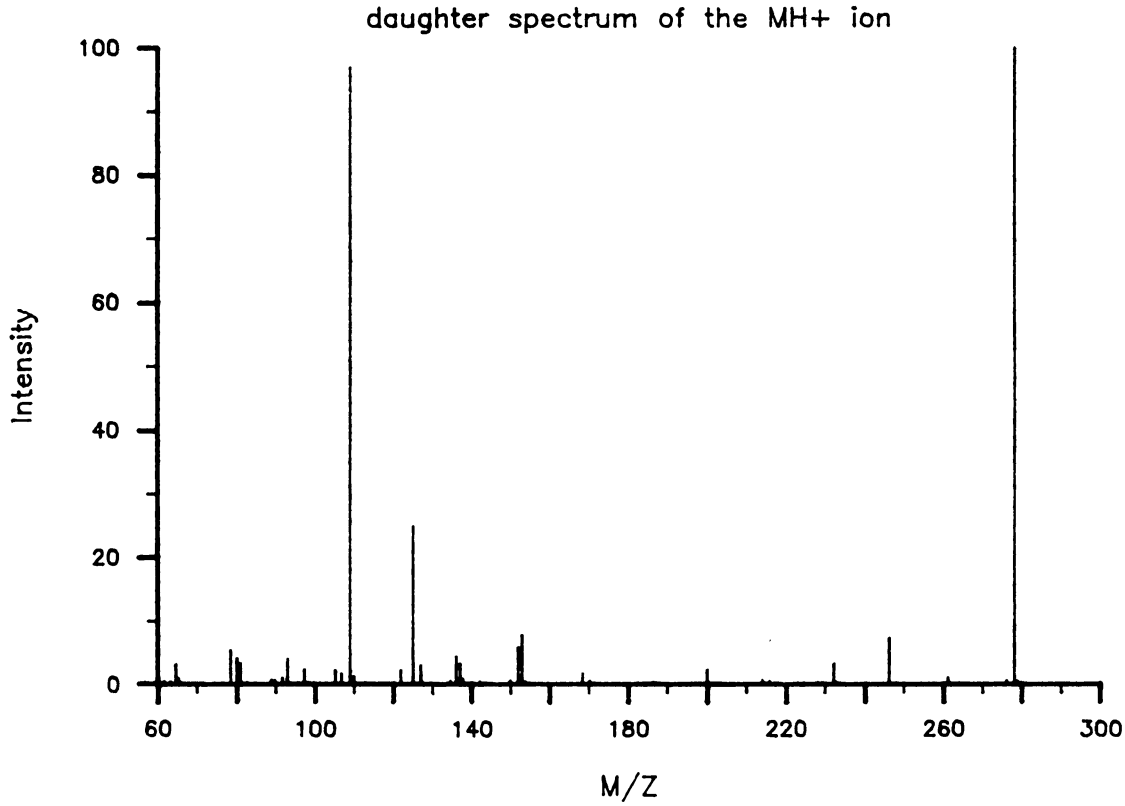

C2H5O
S
P
S
CH2
S
P
S
OC2H5
C2H5O
OC2H5

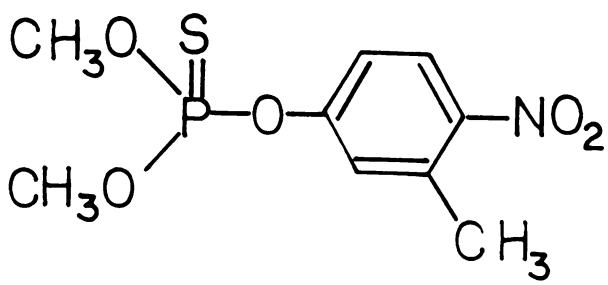
Etrimfos


daughter spectrum of the MH+ ion
Intensity
100
80
60
40
20
0
60
100
140
180
220
260
300
M/Z

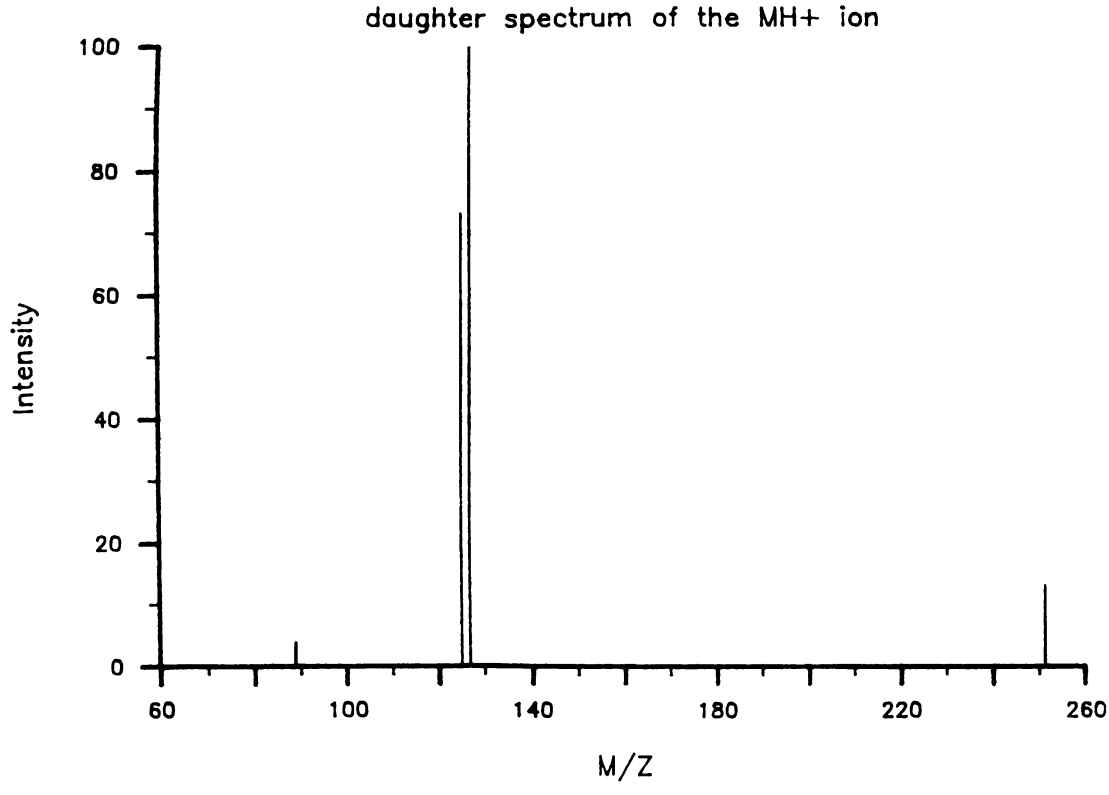

CH3O
S
P
O
N
N
CH2CH3
CH3O
CH2CH3

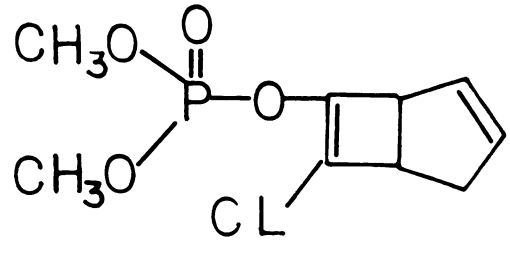
Fentrothion



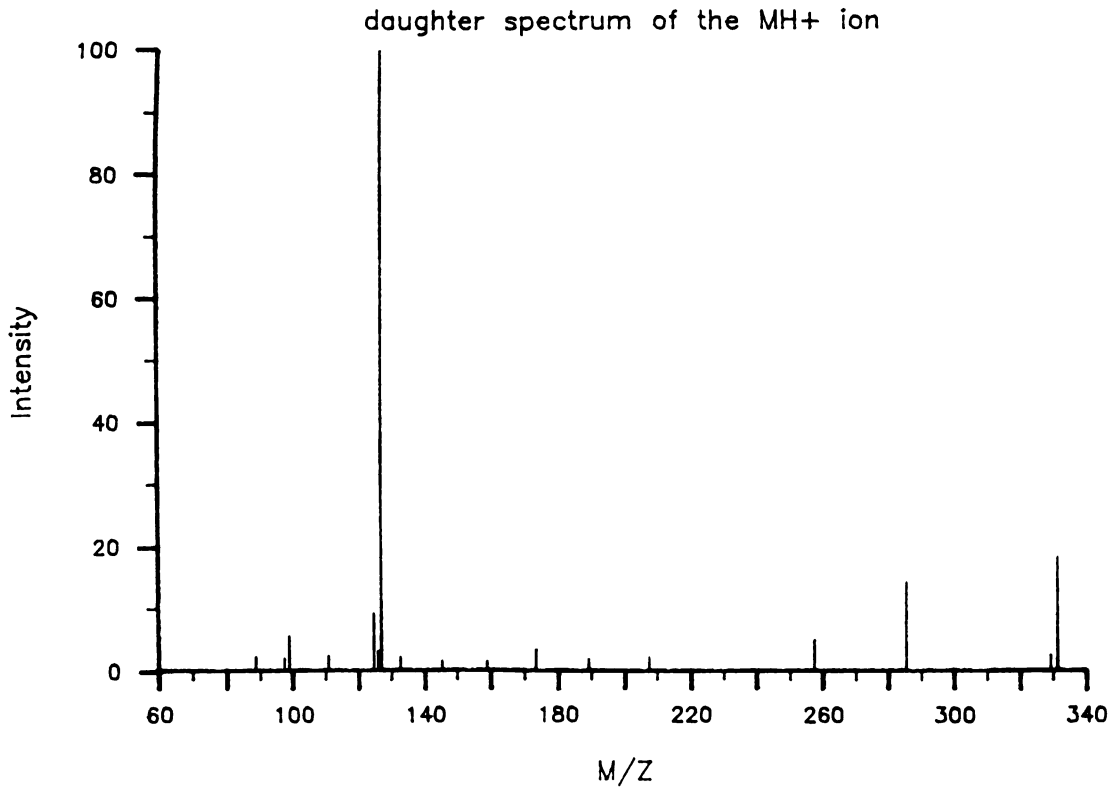


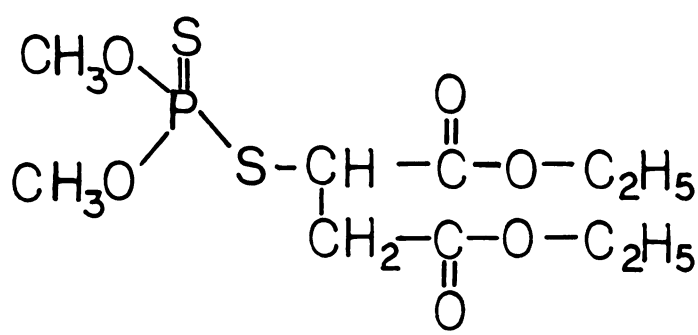
Heptenophos



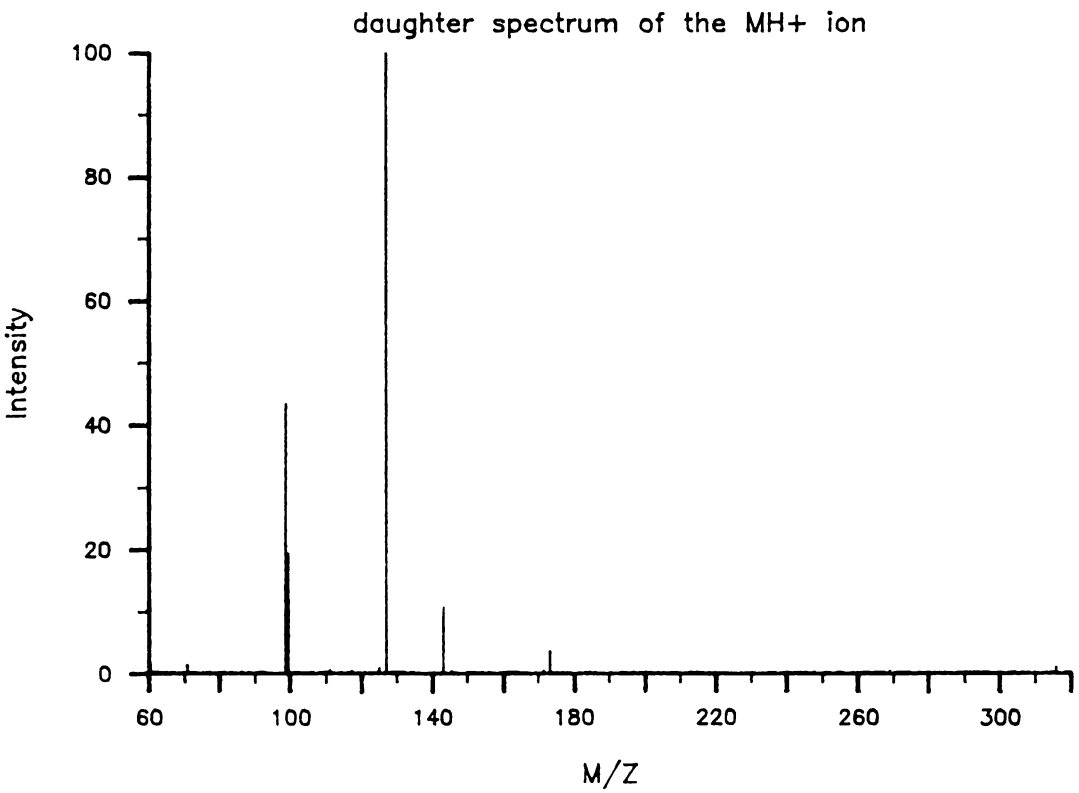


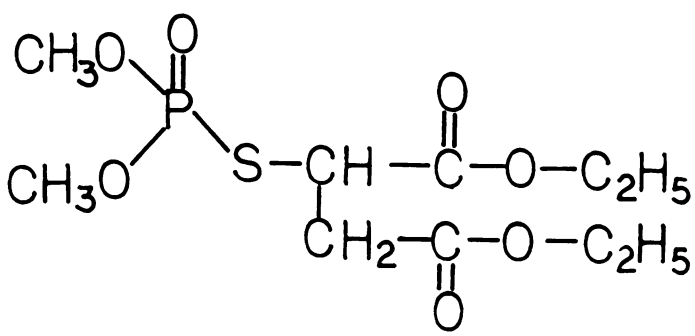
Malathion


daughter spectrum of the MH+ ion
Intensity
100
80
60
40
20
0
60
100
140
180
220
260
300
340
M/Z

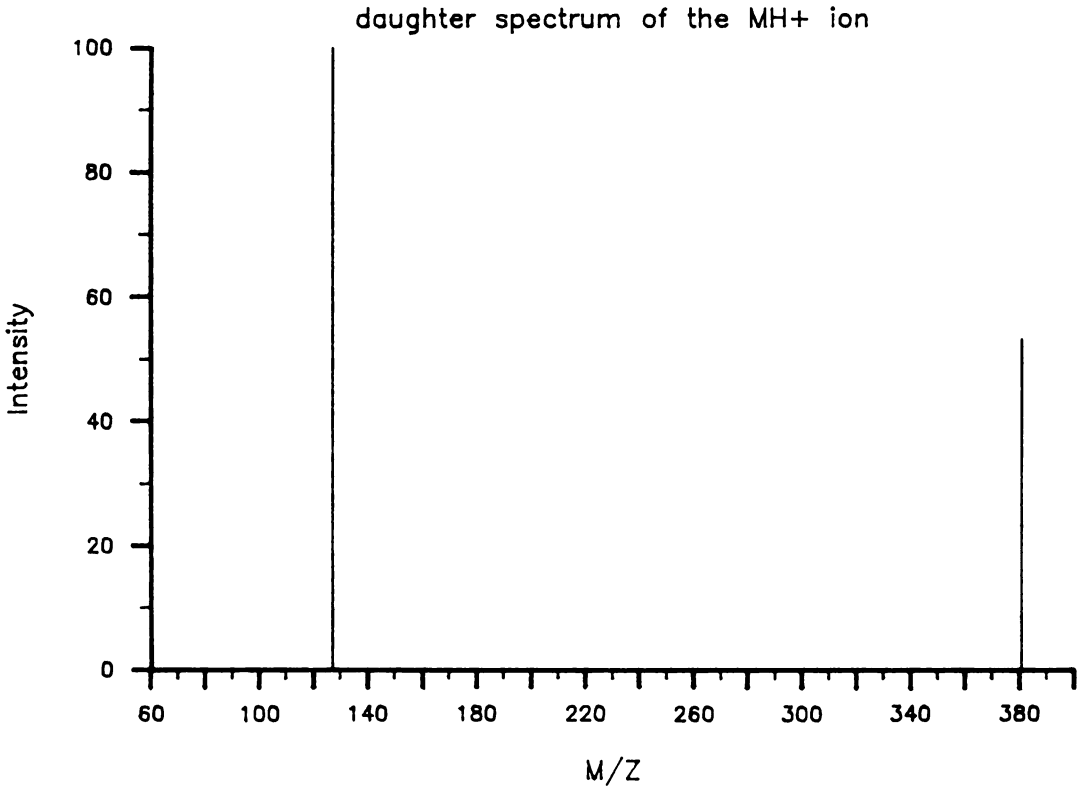

CH3O
S
P
CH3O
S-CH
C-O-C2H5
O
CH2-C-O-C2H5
O

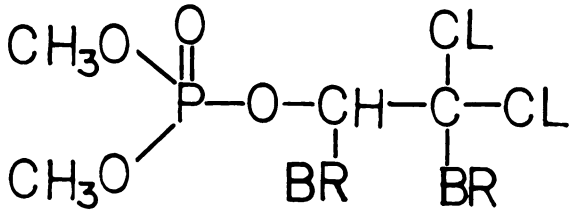
Oxygen Analog of Malathion



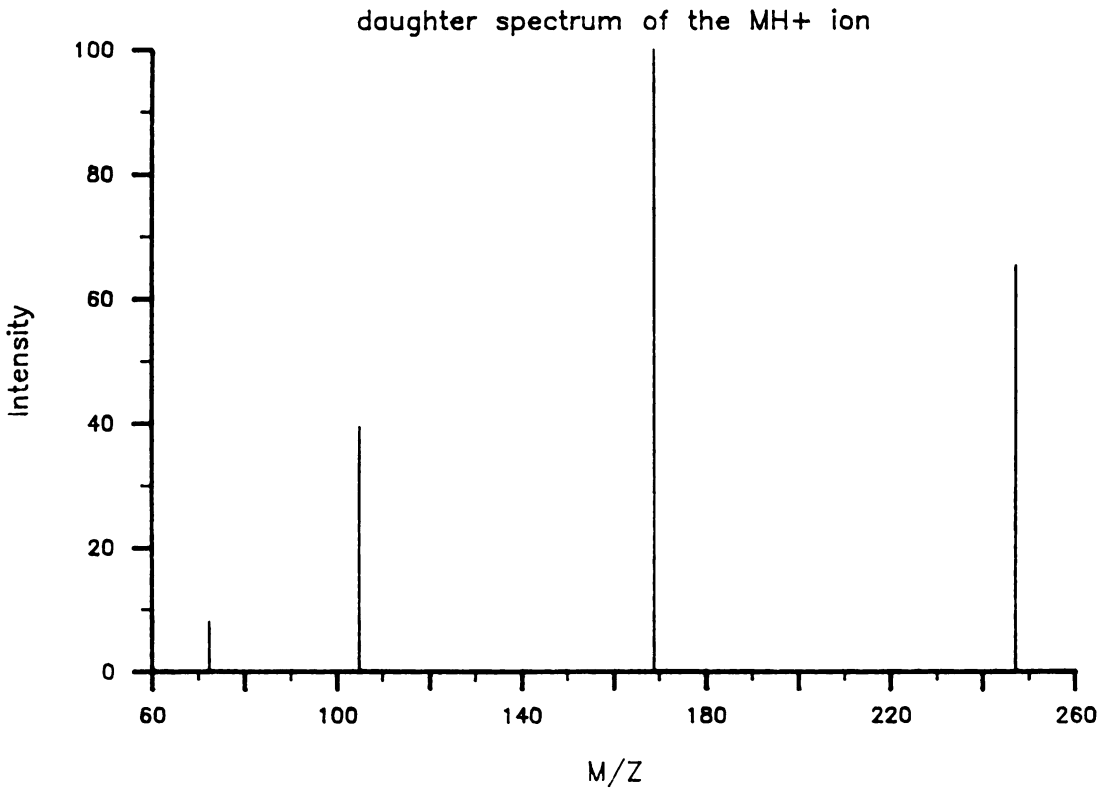


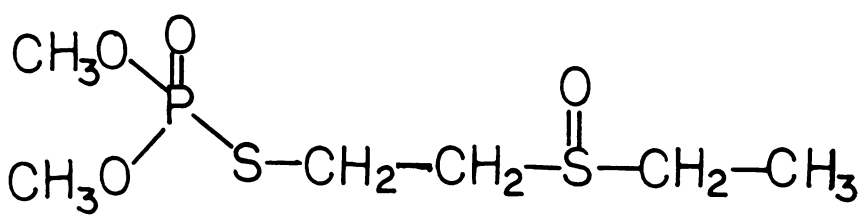
Naled


daughter spectrum of the MH+ ion
Intensity
100
80
60
40
20
0
60
100
140
180
220
260
300
340
380
M/Z

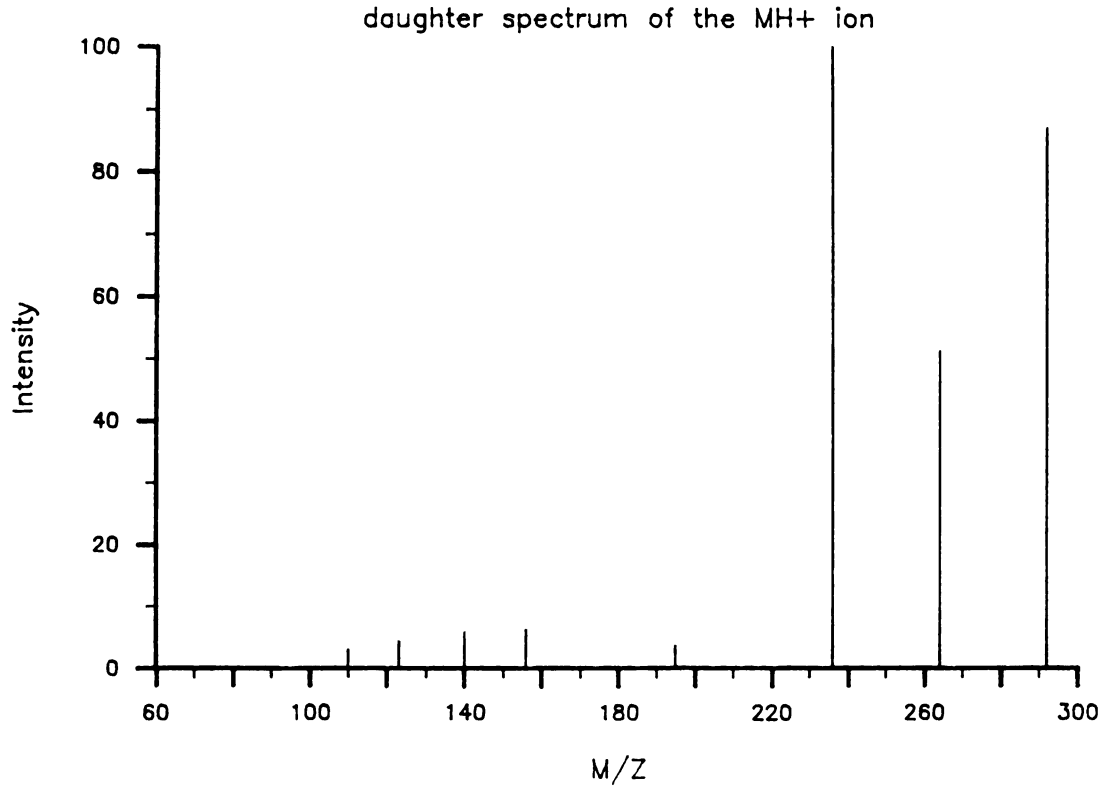

CH3O
O
P
O
CH
C
CL
CL
CH3O
BR
BR

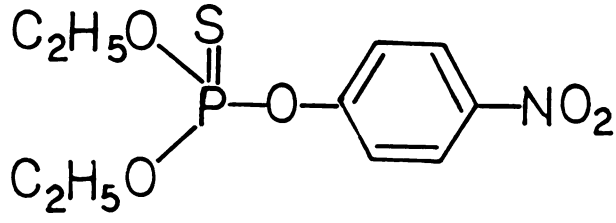
Oxydemeton Methyl



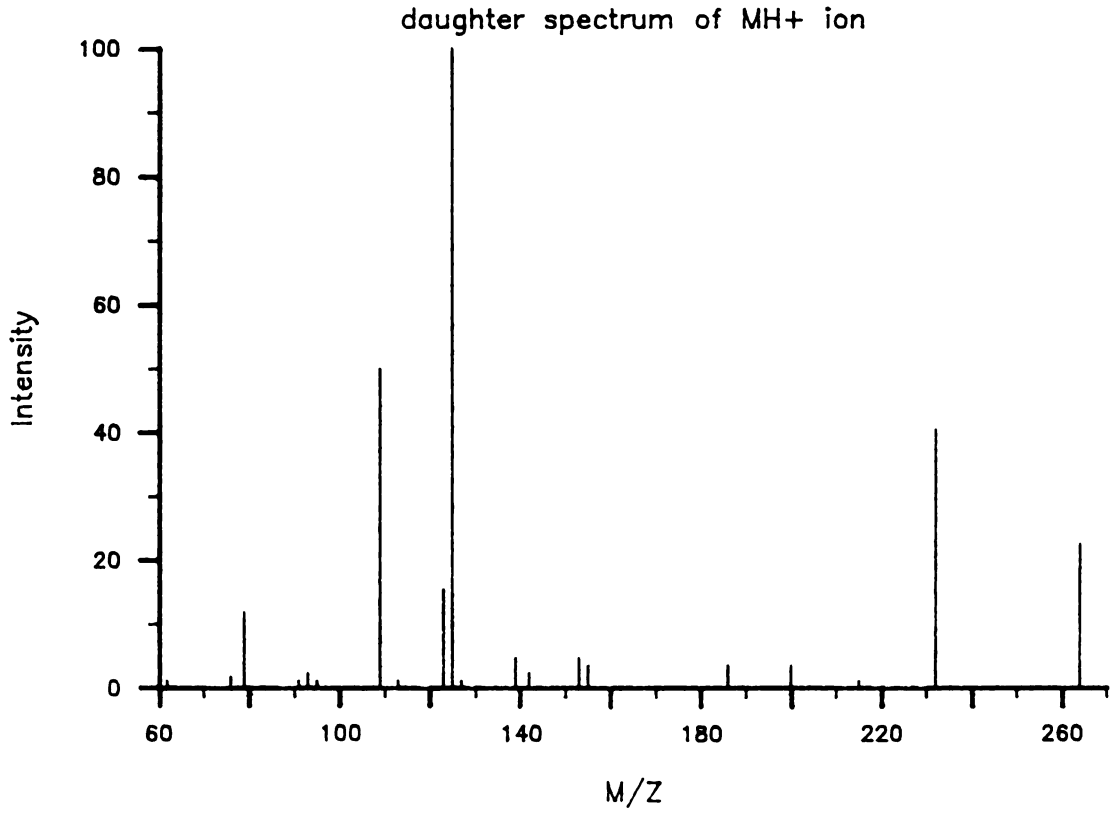


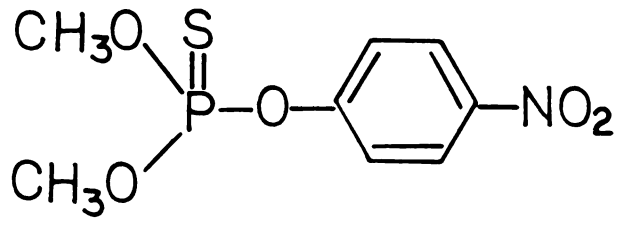
Parathion


daughter spectrum of the MH+ ion
Intensity
100
80
60
40
20
0
60
100
140
180
220
260
300
M/Z

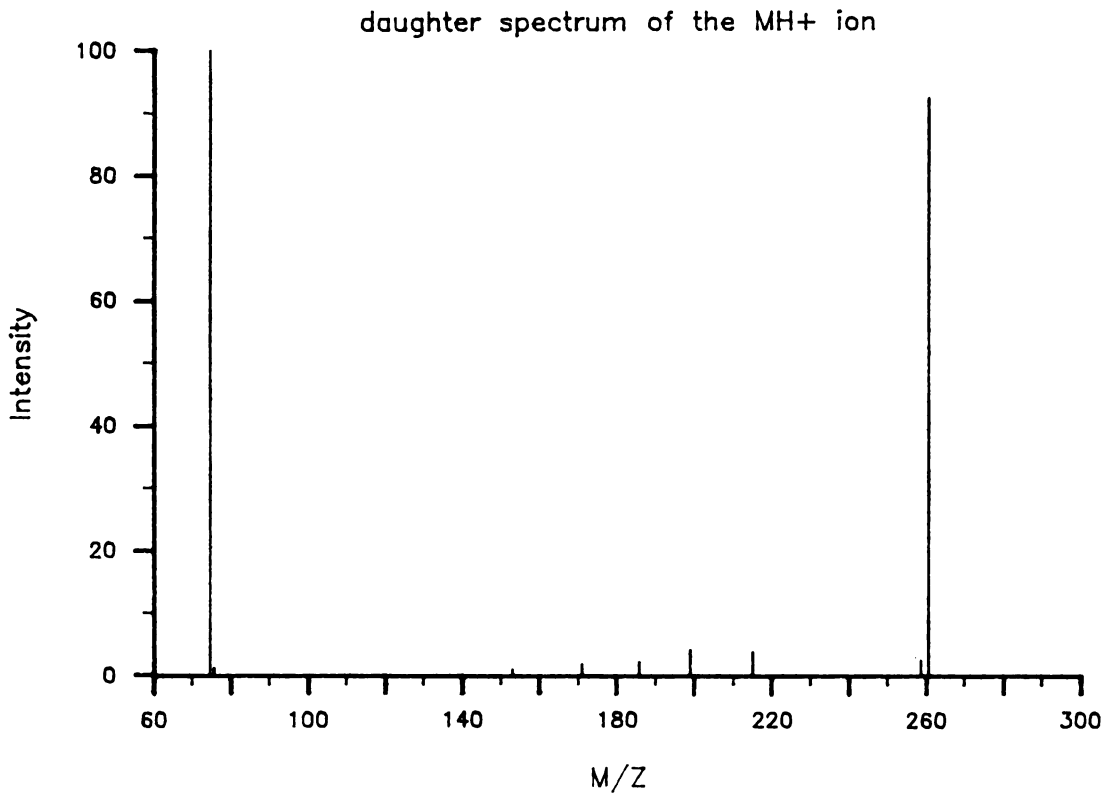

C2H5O
S
P
O
NO2
C2H5O

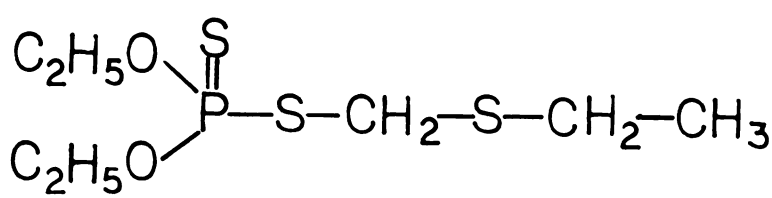
Methyl Parathion



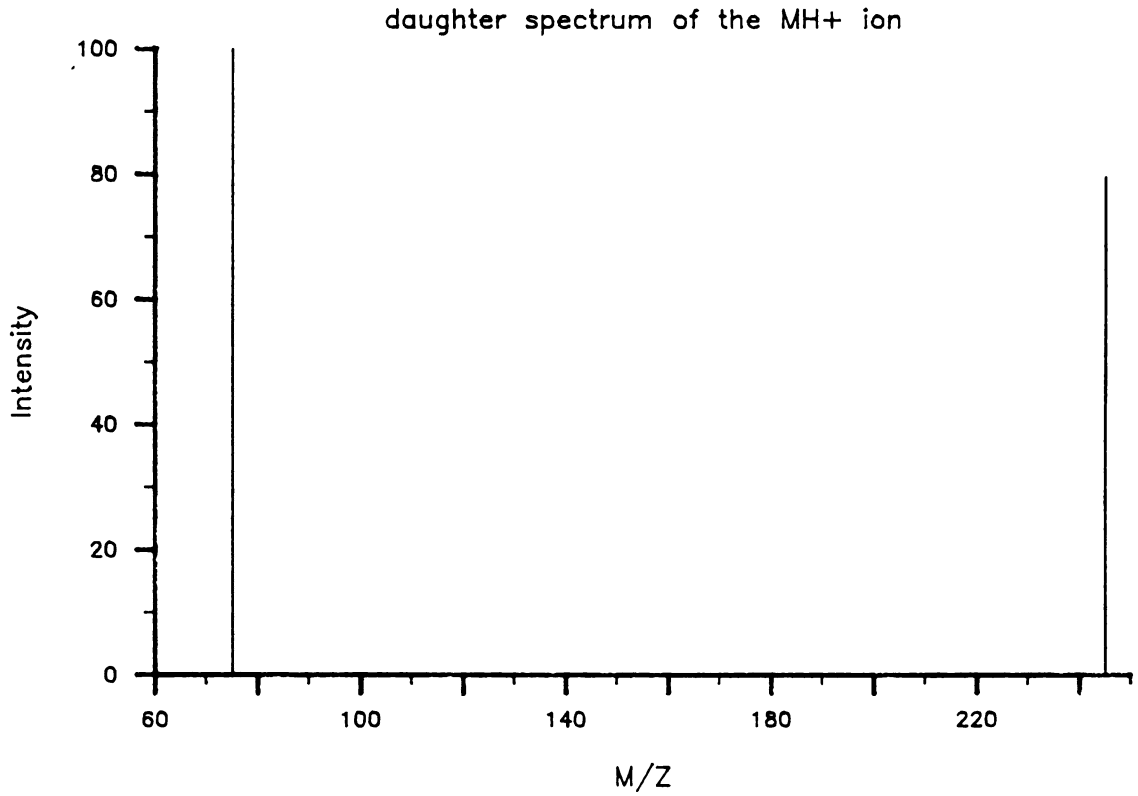


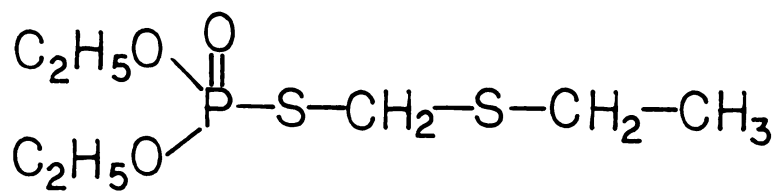
Phorate


daughter spectrum of the MH+ ion
Intensity
100
80
60
40
20
0
60
100
140
180
220
260
300
M/Z

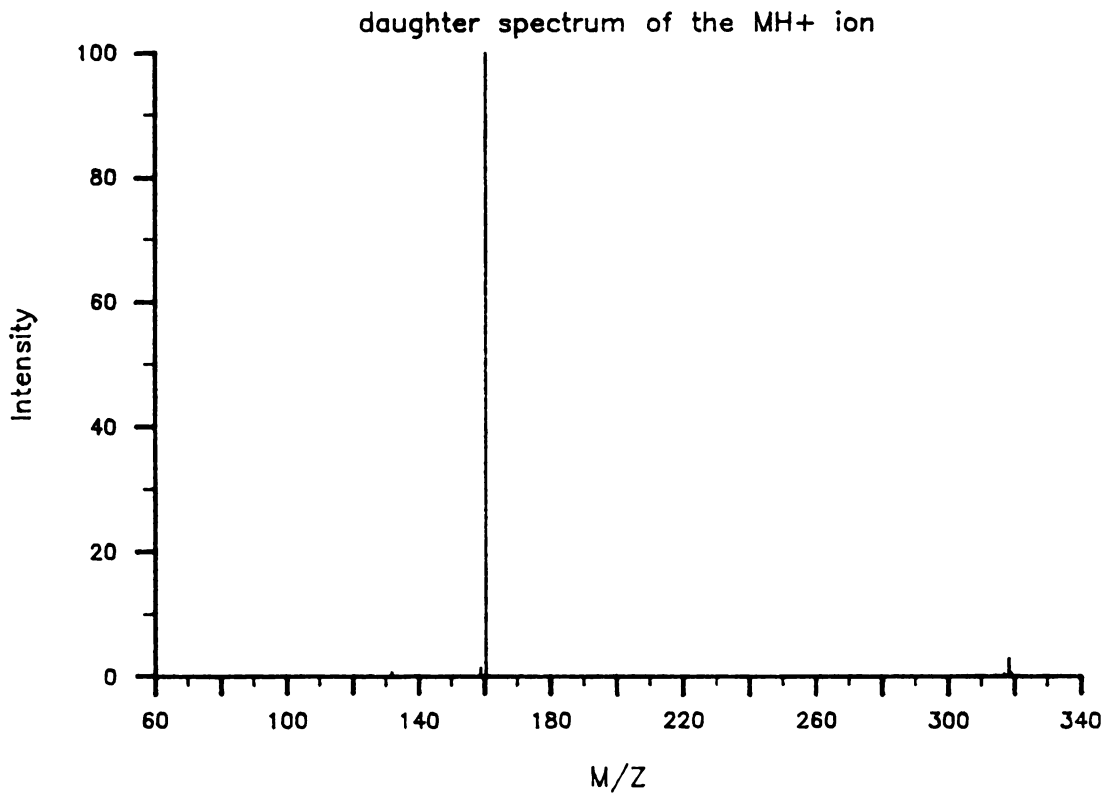

C2H5O
S
P
S
CH2
S
CH2
CH3
C2H5O

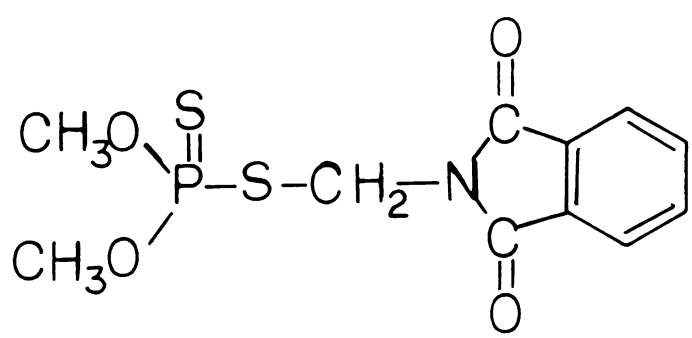
Oxygen Analog of Phorate



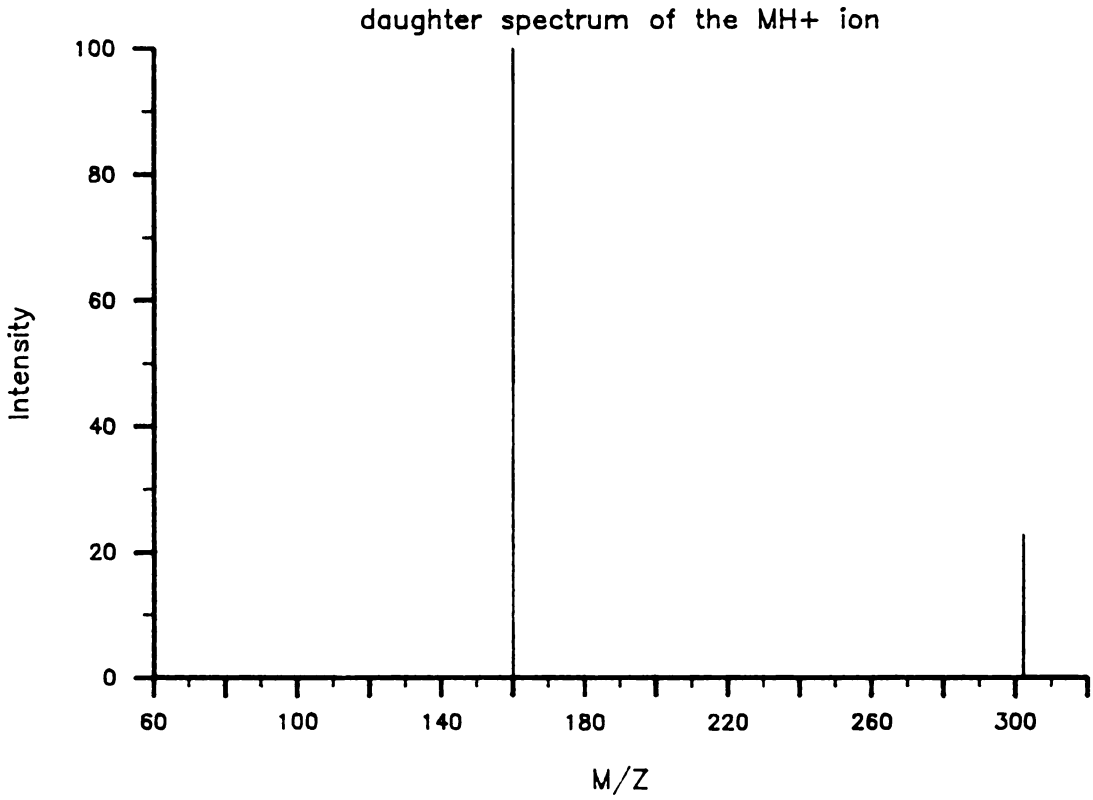


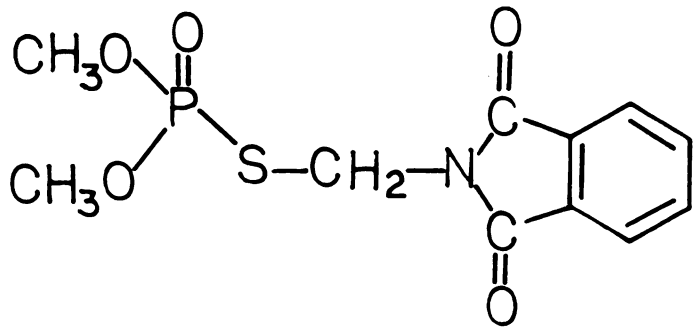
Phosmet


daughter spectrum of the MH+ ion
Intensity
100
80
60
40
20
0
60
100
140
180
220
260
300
340
M/Z

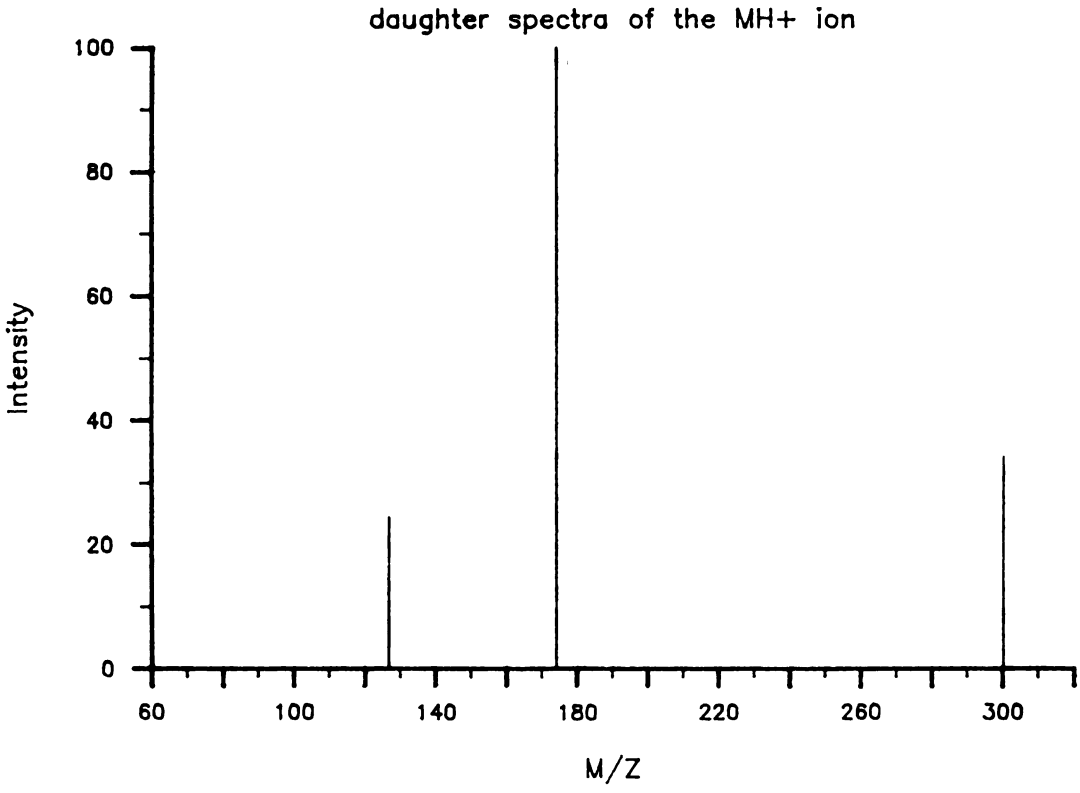

CH3O
S
P
S
CH2
N
CH3O
O
C
C
O

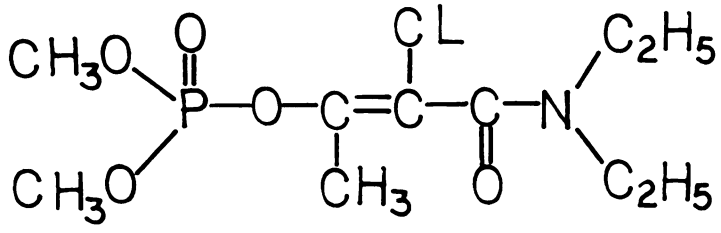
Oxygen Analog of Phosmet



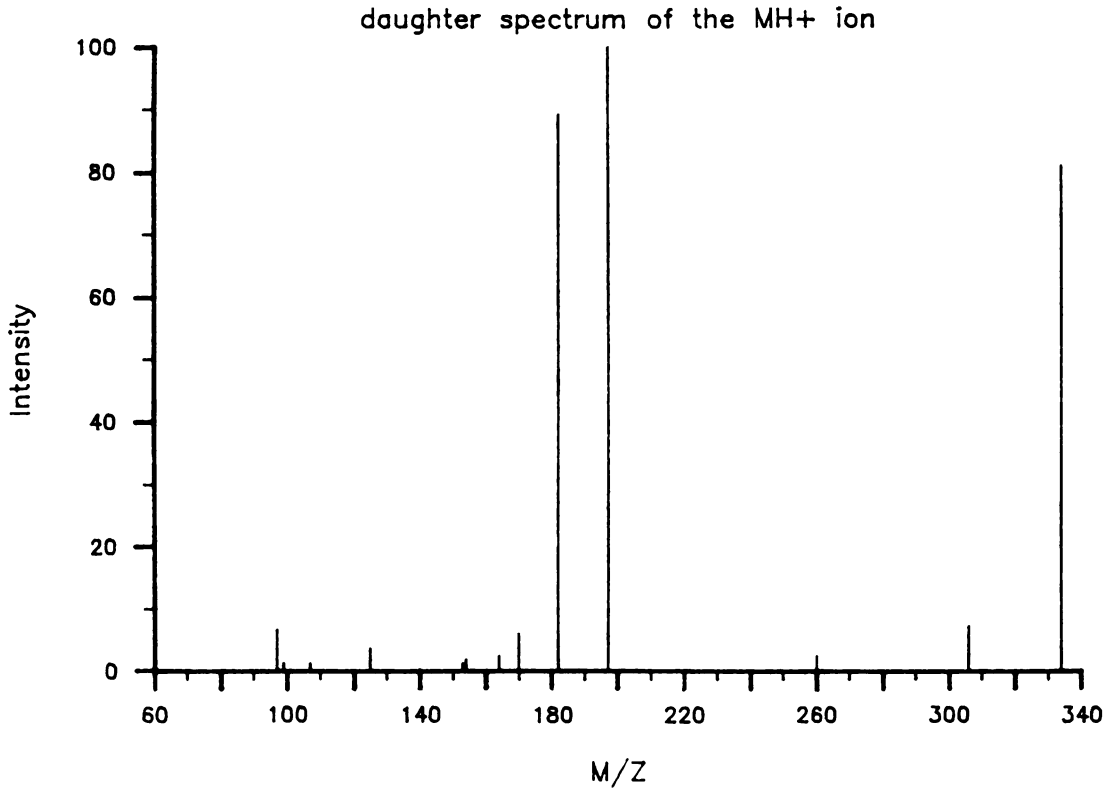


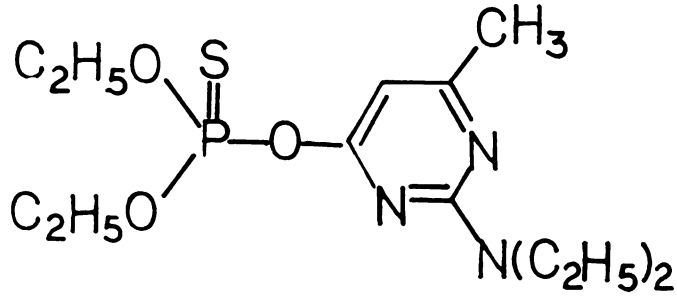
phosphamidon



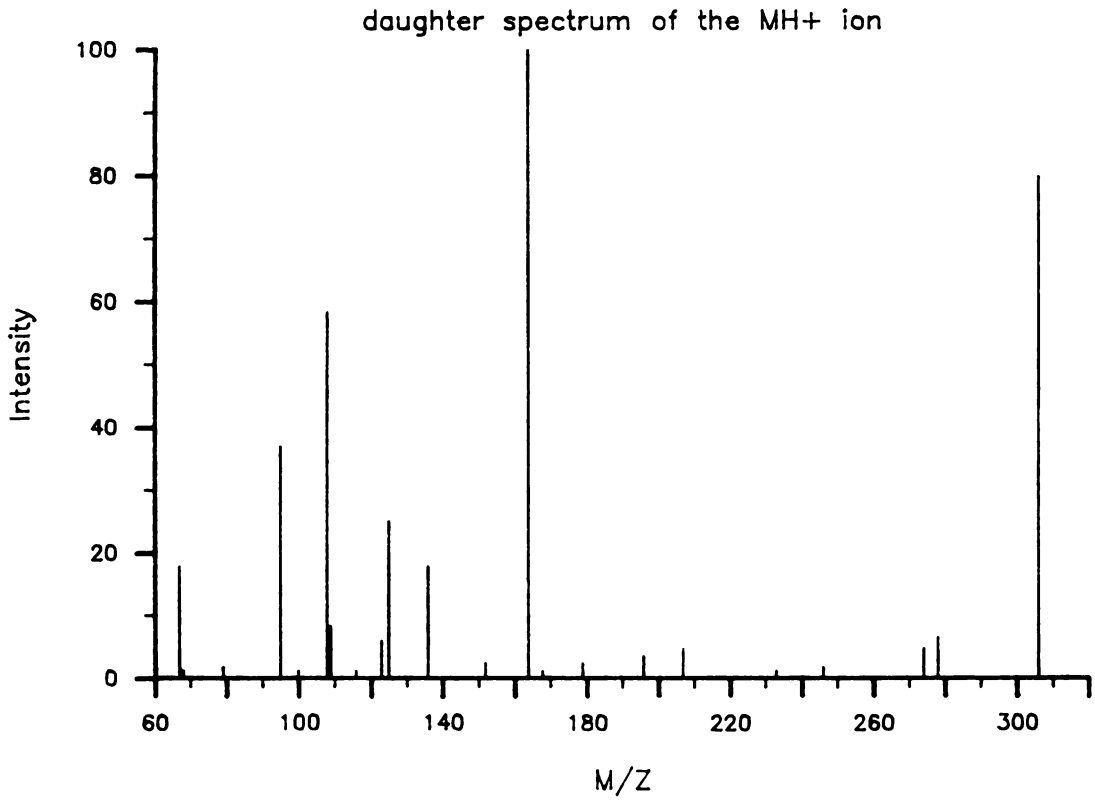


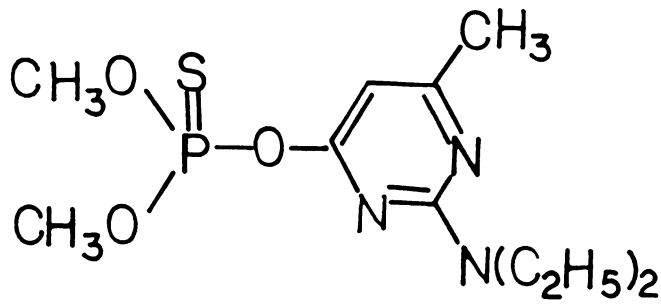
Pirimiphos


daughter spectrum of the MH+ ion
Intensity
100
80
60
40
20
0
60
100
140
180
220
260
300
340
M/Z

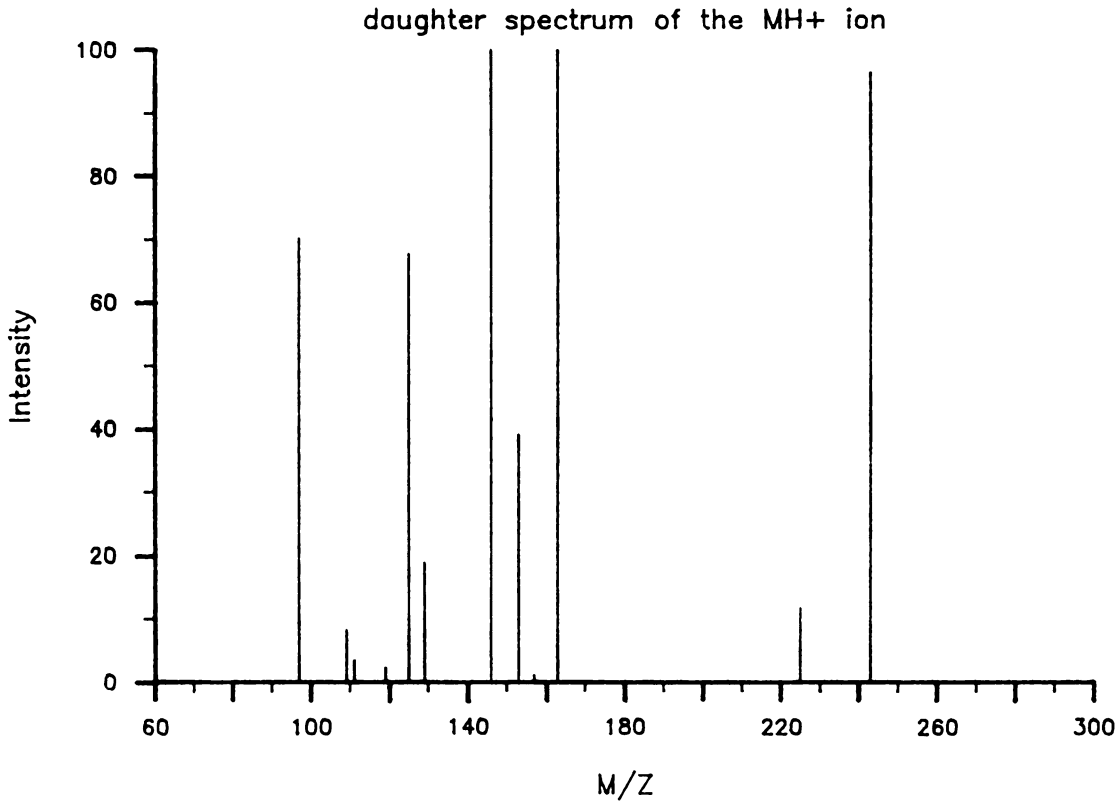

C_2H_5O
S
P
O
C_2H_5O
CH_3
N
N
$N(C_2H_5)_2$

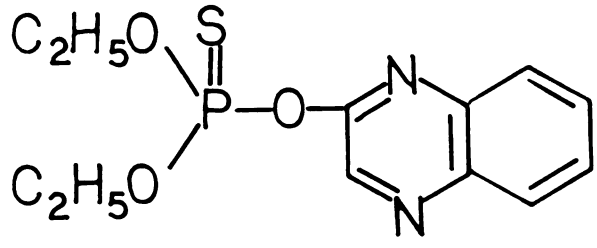
Pirimiphos Methyl



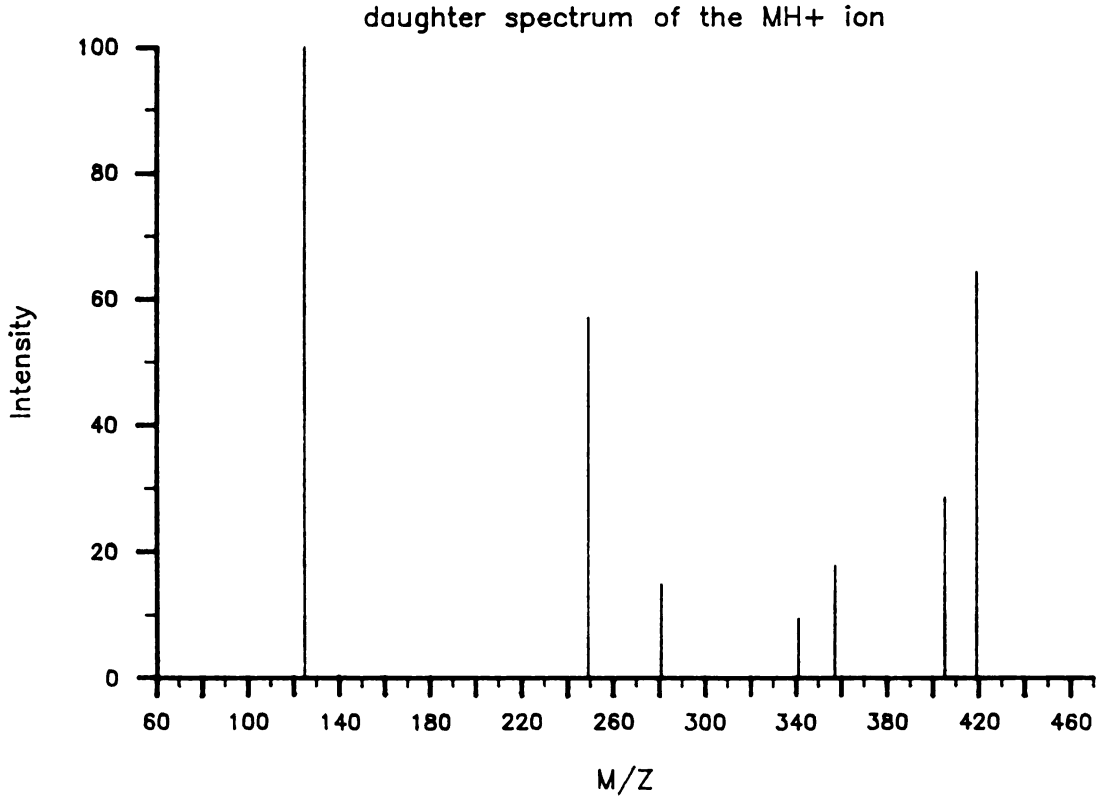


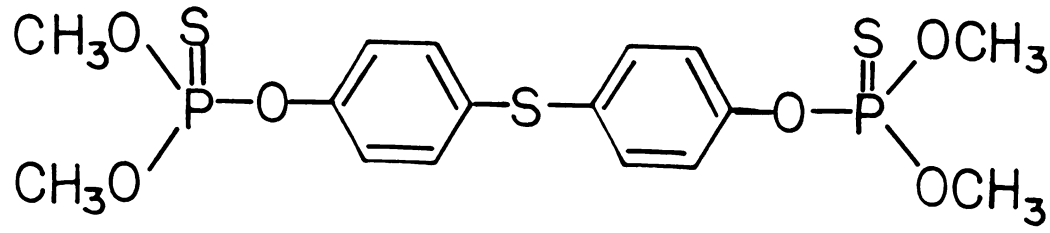
Quinalphos


daughter spectrum of the MH+ ion
Intensity
100
80
60
40
20
0
60
100
140
180
220
260
300
M/Z

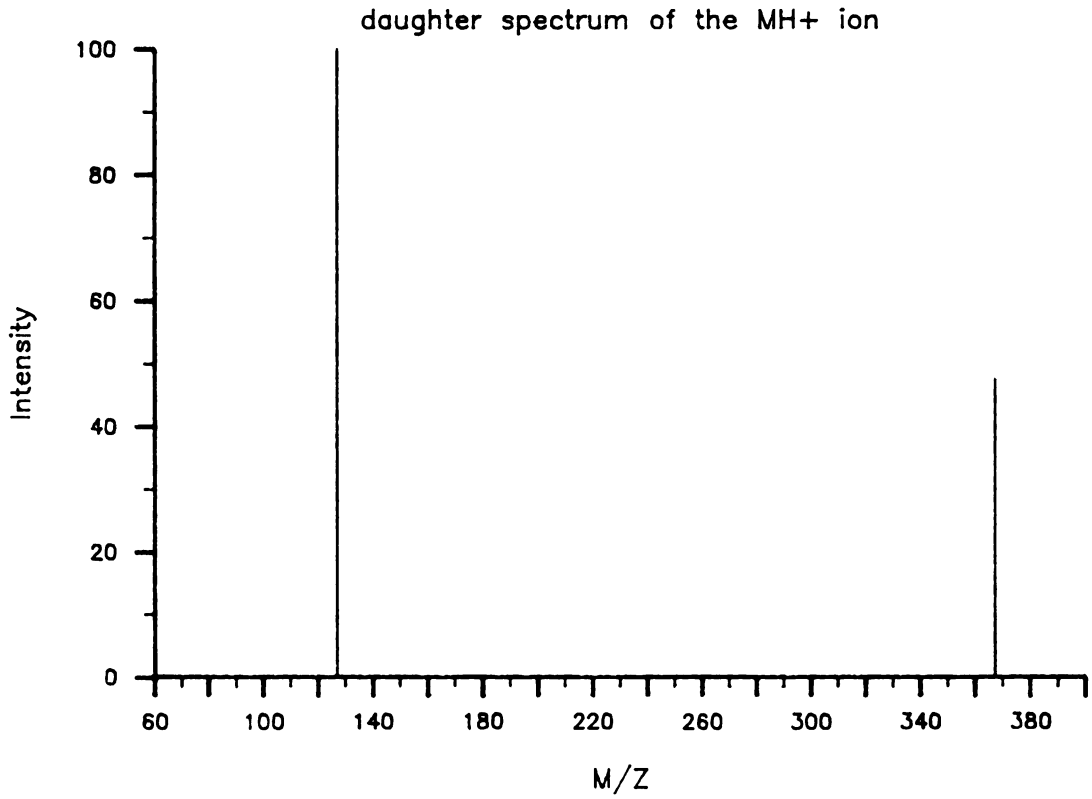

C2H5O
S
P
O
N
N
C2H5O

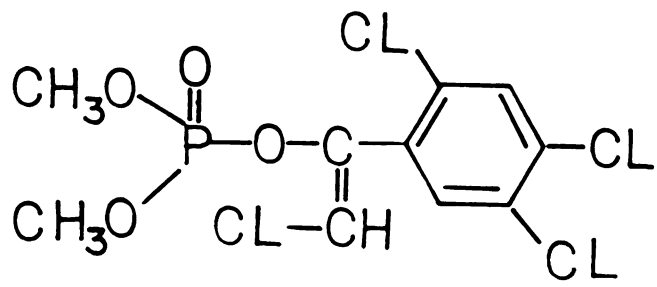
Temphos


daughter spectrum of the MH+ ion
Intensity
100
80
60
40
20
0
60
100
140
180
220
260
300
340
380
420
460
M/Z

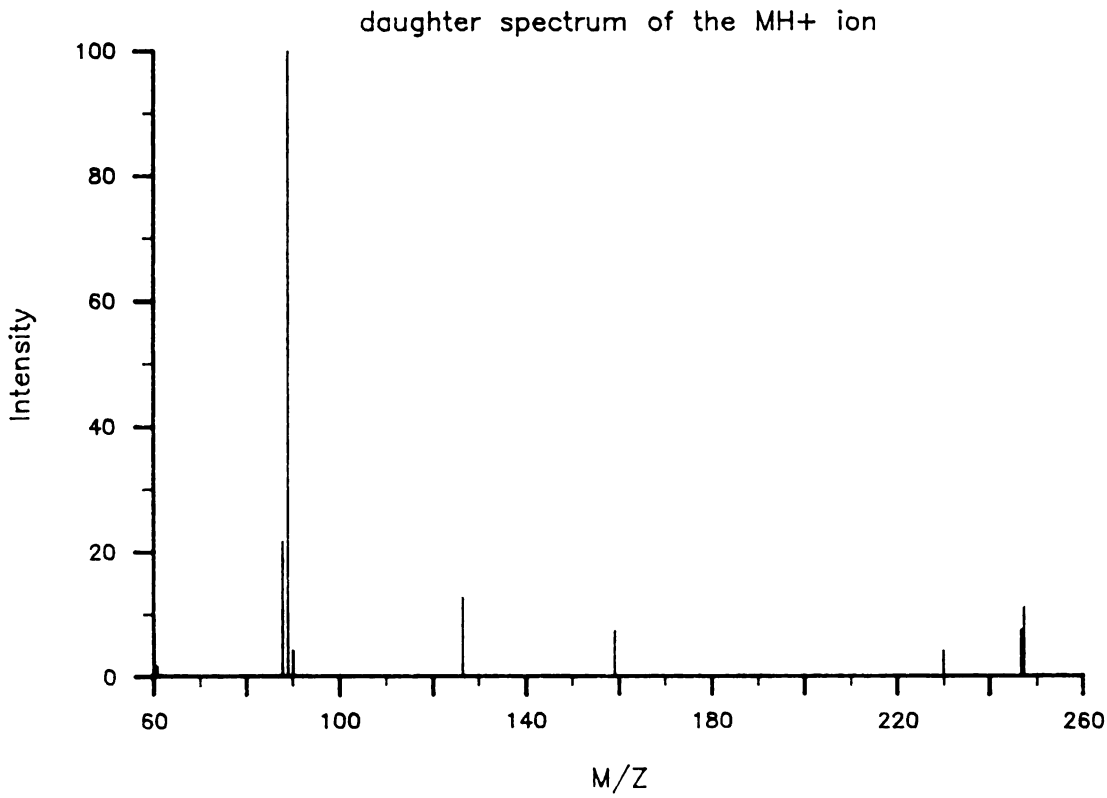

CH3O
S
P
O
S
O
P
S
OCH3
CH3O
OCH3

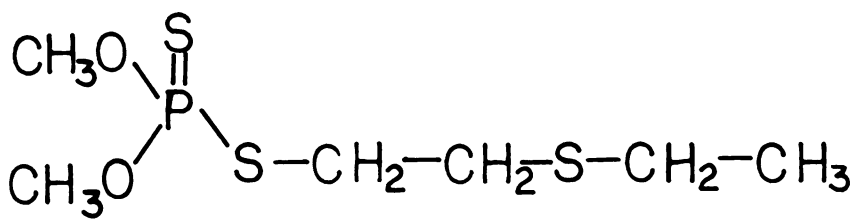
Tetrachlorvinphos





Thiometon


daughter spectrum of the MH+ ion
Intensity
100
80
60
40
20
0
60
100
140
180
220
260
M/Z


CH3O
S
P
CH3O
S–CH2–CH2–S–CH2–CH3