

**The analysis of pesticides & related compounds
using Mass Spectrometry**

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ABSTRACT

The determination of pesticides and related materials in food and environmental samples is important and presents an enduring challenge to analytical chemists. For practicality it is important that as many pesticides as possible are compared using a common technique. Mass spectrometry is the method of choice for multi-residue detection techniques, because of its sensitivity and specificity. This thesis comprises a detailed analysis and critical review of the mass spectrometric behaviour of over 600 commonly encountered pesticides and related compounds.

The work described in this thesis was undertaken in two tranches, one old and one new. The former experimental work was performed during the author's employment at the Ministry of Agriculture, Fisheries & Food (Harpenden, Hertfordshire, UK) and at Unilever Research (Colworth House, Sharnbrook, Bedfordshire, UK). The data helped underpin the analytical work of the UK national pesticide residues monitoring surveillance team and the pesticide formulations safety team. Qualitative and quantitative aspects were both important, e.g. for identification and characterisation of active ingredients, contaminants and degradation products in technical pesticide formulations, as well as unambiguous detection and/or confirmation of residue levels in UK fruit and vegetables. The latter experimental work was undertaken recently (2015) at the Cardiff School of Chemistry during the preparation of this thesis. The newly acquired data helped confirm the validity and robustness of the original data, and helped to better understand them.

Understanding the complex and sometimes unexpected behaviour of molecules during their extraction/analysis is essential, especially when performing trace analysis at the parts per billion level. Rationalisation of the mass spectrometric fragmentation pathways of these compounds was undertaken in order to better understand the fundamental processes taking place in the mass spectrometer. This improved understanding was essential in order to ensure the quality and validity of the data generated using these techniques. For comparison, some additional data are included, e.g. for chemical warfare agents, using literature data.

Mass spectrometry was chosen because of its power as an analytical technique. General approaches and specific precautions which should be taken when using mass spectrometry for

pesticide analysis are discussed and explained in this document and literature data were critically reviewed. It is hoped that these data and recommendations will find continued and future use as an adjunct to the plethora of literature data and MS instrument manufacturer databases.

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LIST OF ABBREVIATIONS

CW – chemical warfare

CWC - Chemical Weapons Convention (Organisation for the Prohibition of Chemical Weapons)

DDT – organochlorine insecticide

ECD – electron capture detector, a type of GC detector

EI – electron ionisation, a common gas phase ionisation technique used to create positive ions in mass spectrometry

ESI – electrospray ionisation

FAO – Food & Agriculture Organisation (United Nations)

FPD – flame photometric detector, a type of GC detector

GC – gas chromatography

LC – liquid chromatography

MS – mass spectrometry

MS/MS – tandem mass spectrometric analysis

MRM – multiple reaction monitoring, selected ion MS/MS monitoring

NPD – nitrogen/phosphorus detector, a type of GC detector

OP – organophosphorus

SIM – selected ion monitoring (same as SIM)

SIR – selected ion recording (same as SIR)

WHO – World health Organisation

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CHAPTER 1.

INTRODUCTION

1.1 Pesticides

The UN Food & Agriculture Organisation (FAO 2002), has defined a pesticide as “*any substance or mixture of substances intended for preventing, destroying, or controlling any pest, including vectors of human or animal disease, unwanted species of plants or animals, causing harm during or otherwise interfering with the production, processing, storage, transport, or marketing of food, agricultural commodities, wood and wood products or animal feedstuffs, or substances that may be administered to animals for the control of insects, arachnids, or other pests in or on their bodies. The term includes substances intended for use as a plant growth regulator, defoliant, desiccant, or agent for thinning fruit or preventing the premature fall of fruit. Also used as substances applied to crops either before or after harvest to protect the commodity from deterioration during storage and transport.*” Within this comprehensive definition, it is implicit that pesticides are toxic. They are intended to prevent, destroy or control specific plants or animals that threaten crops or other useful resources.

Pesticides have been used in agriculture for crop protection, and in public health programmes to help control disease vectors (of malaria, sleeping sickness, typhus etc.), as well as in the home, for thousands of years (Unsworth 2010). The first recorded use of insecticides was 4,500 years ago by the Sumerians, who used sulphur compounds to control insects and mites. About 3,200 years ago the Chinese were using mercury and arsenical compounds for controlling body lice (Banaszkiewicz 2010).

Total annual global usage of pesticides is now approximately 2.4 million tonnes, with an estimated market value of \$40-50 billion (EPA, 2007). By weight, herbicides represent 40% of total usage, insecticides 17%, and fungicides 10%.

Unintended toxic effects of pesticide use and application (“collateral damage”) are always possible. Beneficial insects or other crops which are inadvertently exposed to pesticides may be adversely affected or destroyed, and farm animals, wildlife or people may become ill or die after exposure to pesticides. *Integrated pest management* or (integrated pest control)

attempts to resolve the conflicting demands, by minimising the impact on beneficial species and the environment (FAO, 1966).

The main classes of pesticide, and their chemical classifications, are summarised in the table below (based on Wood 2015).

Table 1.1a. General types and chemical classes of pesticides

Insecticides & Acaricides	<p>Organophosphorus – acetylcholinesterase inhibition (nerve poison)</p> <p>Carbamate - acetylcholinesterase inhibition (nerve poison)</p> <p>Pyrethroid – sodium channel modulators</p> <p>Neonicotinoid – acetylcholine receptor agonist</p> <p>Organochlorine - sodium channel modulator etc.</p> <p>Inorganic, Botanical, Bacterial etc. - various</p>
Herbicides	<p>Amides & anilides</p> <p>Aromatic acid</p> <p>Arsenical</p> <p>Benzothiazole</p> <p>Carbamate</p> <p>Dicarboximide</p> <p>Dinitroaniline</p> <p>Dinitrophenol</p> <p>Inorganic</p> <p>Organophosphorus</p> <p>Oxadiazolone</p> <p>Oxazole</p> <p>Phenoxy & phenoxyacetic</p> <p>Pyridine</p> <p>Thiocarbamate</p> <p>Triazine & triazole</p> <p>Uracil</p> <p>Urea (phenylurea & sulphonylurea)</p>

Fungicides	<p>Aliphatic nitrogen</p> <p>Amide (acylamino acid, anilide, benzanilide, furamide)</p> <p>Antibiotic (strobilurin - QoI action)</p> <p>Aromatic</p> <p>Arsenical</p> <p>Aryl phenyl ketone</p> <p>Benzimidazole/precursor</p> <p>Benzothiazole</p> <p>Botanical</p> <p>Carbamate</p> <p>Conazole</p> <p>Dicarboximide</p> <p>Dinitrophenol</p> <p>Dithiocarbamate</p> <p>Inorganic (copper, mercury, sulphur)</p> <p>Organophosphorus</p> <p>Oxazole & Pyrazole</p> <p>Pyridine & Pyrimidine</p> <p>Quinoline, Quinone & Quinoxaline</p> <p>Thiazole & Triazole</p>
Rodenticides	<p>Botanical</p> <p>Coumarin - anticoagulant</p> <p>Inorganic (arsenic, phosphorus, thallium)</p> <p>Organofluorine</p> <p>Organophosphorus</p>

The WHO has produced a Classification Scheme based on degree of acute toxicity, and other factors (WHO IPCS 2009).

Table 1.1b. The WHO Recommended Classification of Pesticides by Hazard.

(WHO IPCS 2009)

WHO Class	Acute Toxicity Classification	Oral LD50 for rat (mg/kg)	Dermal LD50 for rat (mg/kg)
Ia	Extremely hazardous	<5	<50
Ib	Highly hazardous	5-50	50-200
II	Moderately hazardous	50-2,000	200-2,000
III	Slightly hazardous	>2,000	>2,000
U	Unlikely to present acute hazard	>5,000	>5,000

There are 28 active pesticide agents listed in Class Ia (“extremely hazardous”) in their unformulated, technical form. These include:

- Insecticides: aldicarb, chlorethoxyfos, chlormephos, disulfoton, EPN, ethoprophos, mevinphos, parathion, parathion-methyl, phorate, phosphamidon, sulfotep, tebupirimfos and terbufos.
- Rodenticides: brodifacoum, bromadiolone, bromethalin, chlorophacinone, difenacoum, difethialone, diphacinone, flocoumafen, sodium fluoroacetate.
- Fungicides: phenylmercury acetate, mercuric chloride (seed treatments)

It can be seen that many of the most toxic and hazardous pesticides are the organophosphorus insecticides.

Because of their toxicity, pesticides are subject to rigorous (and regularly updated) reviews and risk/benefit analyses (at least, in the EU and the US etc.) before they are approved for use. Monitoring of pesticide residues in foodstuffs forms an important part of the regulatory control of the risks of pesticide use. Modern pesticides have been widely and intensively used since the 1940s. Currently over 1,000 active ingredients are in use, formulated in many

thousands of different commercial forms. Modern pesticides encompass an enormous range of physico-chemical characteristics (MW, polarity, volatility and persistence), and their use has substantially benefited humanity. However there is always the potential for adverse effects to the environment and to public health. Once in the environment, most modern pesticides are relatively labile so their residues should not persist. But the use of pesticides is so widespread, it is difficult to avoid exposure (Barr 2010).

There are several comprehensive regulatory information resources available online. One is maintained by the European Union (EU Pesticides Database, 2015), which lists

- Active Substances
- Products
- Pesticide Residues (EU-MRLs - maximum residue levels)

Another is that of the US Environmental Protection Agency (EPA, 2015).

Unfortunately, the regulation of pesticides in developing countries is not so rigorously controlled. The vast majority (>99%) of deaths through occupational or accidental exposure occur in the developing world. Many deaths and cases of poisoning are caused by mishandling of pesticide waste and used pesticide containers; the common practice of re-using pesticide containers to store food and water is a prime example of this. Pesticides that are carelessly disposed of can contaminate the air, water and land, and poison people, livestock, fish and wildlife. The World Health Organization estimates that, worldwide, inadvertent exposure to pesticides causes an annual 20,000 deaths and at least 3 million cases of acute poisoning (Jeyaratnam 1990).

Unrestricted access to the most toxic pesticides also enables them to be used for suicide. An estimated 250,000-370,000 people die from deliberate ingestion of pesticide every year (Dawson 2010). In order to reduce this, the Food and Agriculture Organization of the United Nations recommends the withdrawal of the most toxic pesticides (WHO Class I pesticides) from agricultural use. This strategy has proven successful in Sri Lanka where a ban on Class I pesticides in 1995 and on the Class II pesticide endosulfan in 1998 has reduced pesticide deaths by 50% over the past 20 years without decreasing agricultural output (Dawson 2010).

1.2 Mass Spectrometry

What is mass spectrometry? John B. Fenn, the originator of electrospray ionization and 2002 Nobel Prize winner, penned this elegant and succinct description:

Mass spectrometry is the art of measuring atoms and molecules to determine their molecular weight. Such mass or weight information is sometimes sufficient, frequently necessary, and always useful in determining the identity of a species.

To practice this art one puts charge on the molecules of interest, i.e., the analyte, then measures how the trajectories of the resulting ions respond in vacuum to various combinations of electric and magnetic fields.

Clearly, the sine qua non of such a method is the conversion of neutral analyte molecules into ions. For small and simple species the ionization is readily carried by gas-phase encounters between the neutral molecules and electrons, photons, or other ions. In recent years, the efforts of many investigators have led to new techniques for producing ions of species too large and complex to be vaporized without substantial, even catastrophic, decomposition.

1.3 Historical perspective

James Lovelock's invention of the electron capture detector (ECD) in 1957 (Lovelock, 1958), coupled with the novel technique of gas chromatography (James, 1952), afforded unparalleled sensitivity for the trace detection of halogenated molecules. This was soon being exploited for detection of various environmental contaminants. The results triggered an explosion of interest in environmental analysis, especially on the organochlorine insecticides which had been developed during the early years of the twentieth century (DDT, aldrin, dieldrin etc.). This in turn prompted Rachel Carson's book "Silent Spring" (Carson, 1962), published in 1962, which explored the environmental impacts of the widespread and indiscriminate application of the organochlorine insecticides. The increased awareness of environmental issues led eventually to the banning of DDT and related persistent organic pollutants (POPs).

By the 1970s and 1980s, screening for many volatile (GC-amenable) contaminants (including pesticide residues) was being routinely performed using gas chromatographic separation and

a "conventional" (non-mass spectrometric) detection technique. Such detection techniques included improved electron capture detection (ECD), and several new innovations, such as nitrogen-phosphorus (NPD; Burgett 1977), flame photometric detection (FPD; Brody 1966) and atomic emission detection (AED) (Lee 1991).

However, the results obtained by these sensitive, specific techniques were potentially misleading. The precise nature of the analyte, other than its GC retention time and its response to the specific detector (e.g. that it captured electrons or contained sulphur) remained uncertain. To increase the confidence in positive findings obtained by such techniques, incontrovertible evidence was (and still is) obtained by the burgeoning and rapidly advancing technique of mass spectrometry.

Mass spectrometry was first demonstrated in 1912 by J. J. Thomson, when he separated neon into its two most abundant isotopes, ^{22}Ne , relative abundance 9%, and ^{20}Ne , 91% (Thomson 1912). It remained largely a gas analysis technique for some time. Following the introduction of GC and ECD, organic mass spectrometry was developed during the 1950s, originally by petrochemical analysts. The early GC-MS systems rapidly found application in many different areas (McLafferty, 1956), and underwent rapid development (Scripps Center for Metabolomics & Mass Spectrometry, 2015).

Processing the large amounts of data generated was a major hurdle with early MS systems. A widespread data capture approach was to record an oscillograph response on rolls of photosensitive paper. These were developed and the responses were measured and processed manually. Introduction of computerised systems massively increased data throughput and productivity, and permitted the routine use of libraries of mass spectrometric data (Chemical Heritage Foundation, 2015).

Mass spectrometry is now big business. The major application areas include pharmaceutical research, biotechnology, industrial chemistry, process and quality control, environmental testing, food and consumer product testing. The annual value of the global MS market has been estimated at \$4 billion and has been predicted to increase to \$6 billion by 2018 (Markets & Markets, 2014).

1.4 Analytical strategies and approaches

In many instances, low resolution (nominal mass) MS, in scanning or selected ion monitoring mode is sufficient to provide convincing data. In other cases, the use of more sophisticated MS techniques, such as increased MS resolution or MS/MS, is necessary. Generally, the less analyte present (or sought) and the more complex the substrate, the more difficult and expensive the analysis.

Mass spectrometric analysis relies upon the separation and detection of ionised fragments. Selection of appropriate ionisation and data acquisition regimes is crucial to the success of the analysis. Several techniques may have to be evaluated in order to obtain the desired sensitivity and specificity. It is essential when interpreting and evaluating the results of MS confirmation to consider all the available evidence, including that from the preliminary screening analyses.

In residues analysis, detection of a suspected pesticide is usually made in the presence of many other compounds (co-extractives and contaminants etc.) in the sample extract. Many of these may be present at much higher concentrations than the analyte and, because MS is a "universal" detector, these other compounds will produce responses which may cause interference. Removal of interfering compounds by means of an appropriate clean-up procedure will help to reduce this problem (though care must be taken to avoid the possibility of introducing further contaminants during such operations).

Although the results obtained by MS may be much less equivocal than those obtained by other analytical techniques, analytical quality control (AQC) data are just as important in evaluating their reliability. General guidelines for AQC are given elsewhere (e.g. SANCO 2009). Parameters important in confirming or disproving the presence of residues, and the precautions to be observed in interpretation of MS data, are outlined below. It is difficult to produce comprehensive rules for what is, and what is not, acceptable MS confirmation of a pesticide residue but acceptable AQC data provide essential support for the conclusions of the analyst.

The data presented in this document were generated using electron ionisation, acquiring positive ions (EI+). An advantage of this ionisation technique is that it is a "universal

detector”, with similar sensitivity for a wide range of volatile molecules. When analysing complex mixtures, this can produce a more representative picture of the sample composition.

Greater sensitivity (or, more accurately improved signal-to-noise) may be obtained for specific molecules using selective ionisation techniques such as chemical ionisation, in positive or negative ion acquisition modes.

1.5 Mass spectrometric introduction techniques

In most cases, the sensitivity and specificity of mass spectrometric detection is enhanced by means of a chromatographic sample introduction and separation procedure. Recognition of characteristic chromatographic behaviour adds considerably to the confidence in a result. This is especially so in the many cases where the pesticide sought is resolved into several chromatographic peaks, for instance chlorfenvinphos, dinocap and many synthetic pyrethroids.

The choice of ionisation technique is often determined by the method of sample introduction, which is in turn determined by the physical and chemical properties of the analyte.

Gas chromatography (GC) was the method of choice for sample extract introduction for most pesticide analysis. Its drawbacks include the requirement that analytes must be volatile and (usually) stable to heat. There were two main types, packed and capillary column GC. Packed column GC was cheaper, more rapid and more robust in operation, with direct (on-column) injection, but it could not give the chromatographic resolution of that of capillary systems and the degree of sample degradation on-column may be greater. Modern chemically-bonded capillary GC columns give excellent performance and less tailing, and GC injector design has helped overcome problems with transmission efficiency, reproducibility.

Previously, the vacuum systems of most mass spectrometers could not accommodate direct introduction of the effluent from packed column GC (carrier gas flow rates of 30-50ml/min). Jet separators were used to remove preferentially low molecular weight species (i.e. carrier gas and solvent) in order to reduce the flow into the mass spectrometer to an acceptable level. This process was sometimes called sample enrichment, though the overall transmission efficiency for analytes was reduced. The lower flow rates used with capillary GC (ca. 1

ml/min at atmospheric pressure) enable the effluent to be introduced directly into the mass spectrometer.

Generally, liquid chromatography (LC) is used for introduction of those pesticides that are not directly amenable to GC, i.e. those that are involatile and/or thermally labile. This method is being used increasingly for compounds that were previously analysed by GC.

Modern LC-MS instruments offer improved sensitivity and selectivity, occupy less laboratory space, are more robust and are easier to use and to maintain than their older counter-parts, whilst generally costing less (Hird 2014, Holčápek 2012).

However, new strategies are required for identification of unknown compounds using LC-MS, because searchable MS libraries are not available for the most frequently used LC-compatible MS ionisation techniques. An identification scheme using a combination of LC separation, followed by accurate mass (OA TOF) and MS/MS analysis (ion trap), followed by empirical formula and substructure database search, has been reported for identification of pesticide on tomato skins (Thurman 2005). This is an interesting approach, but in practice it would have been faster and simpler to compare the original accurate mass data with an index of pesticide molecular weights (see Appendix III). The positive electrospray (ESI+) ions for the unknown pesticides were observed at m/z 192.0771, 343.0530 and 306.1642. Subtracting 1.0078 (H) from these protonated pseudomolecular ions, $[M+H]^+$, yields accurate monoisotopic molecular weights of 191.0693, 342.0452 and 305.1564 Da, which are readily identified as carbendazim ($C_9H_9N_3O_2$, 191.0695 – potentially present as a degradation of thiophanate methyl), thiophanate methyl ($C_{12}H_{14}N_4O_4S_2$, 342.0457) and buprofezin ($C_{16}H_{23}N_3OS$, 305.1563).

Other separation techniques, such as supercritical fluid chromatography (SFC), capillary zone electrophoresis (CZE) and thin layer chromatography (TLC), have found application in MS analysis, but are not in widespread or routine use for pesticide residues confirmation.

1.6 Mass spectrometric ionisation techniques

The choice of mass spectrometric ionisation technique is largely dependent on the introduction technique. They all have their own advantages and disadvantages. The most widely used means of ionisation for pesticide residues analysis employed following GC separation, is that of electron ionisation (EI). It is a universal ionisation method and well understood.

The schematic below (Figure 1.6a) illustrates the general range of applicability of different MS ionisation techniques (Hernandez 2005). It reflects the suitability of GCMS (EI) for the analysis of compounds of low polarity and relatively low MW, such as the organochlorine insecticides (and other environmental contaminants, e.g. dioxins and polychlorinated biphenyls). It also indicates the usefulness of electrospray (ESI) for characterising biomolecules such as proteins, and even virus particles with MWs >2M Da (Tito 2001).

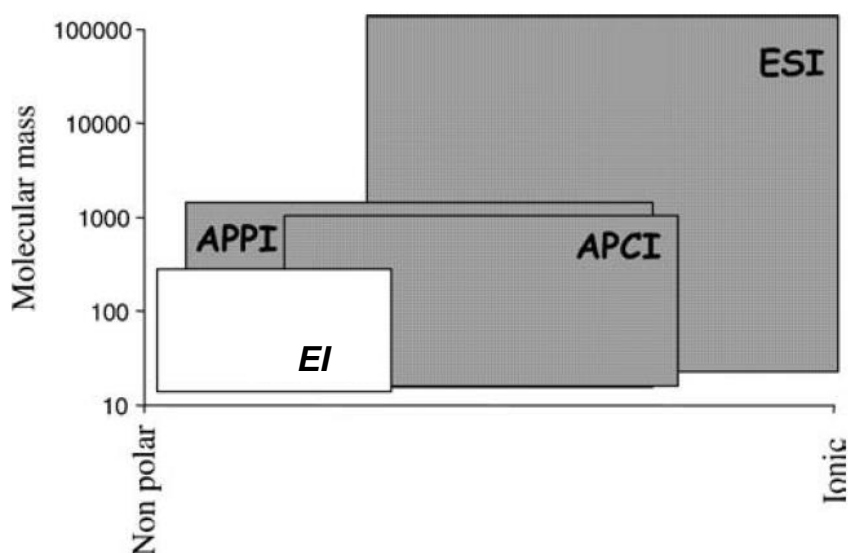


Figure 1.6a. Applicability of MS ionisation techniques according to analyte molecular weight and polarity (Hernandez 2005). Key: ESI electrospray ionisation; APPI atmospheric pressure photoionisation; APCI atmospheric pressure chemical ionisation.

Alternative techniques may also be needed when data obtained by EI are not sufficiently conclusive, e.g. if the EI spectrum of the analyte exhibits too few significant ions, there is too much interference from co-extractives, or when using a separation process where EI cannot be used (usually LC).

LC-MS and MS/MS are now much more widely used (Petrovic 2010). Typically, an atmospheric pressure ionisation method, such as electrospray, is employed. Direct LC introduction is invaluable for materials which are not amenable to GC introduction. When GC-MS was the only affordable technique available, complex derivatisation procedures were used, e.g. using silylation (trimethylsilyl/TMS or *tert*-butyldimethylsilyl/TBDMS) or trifluoroacetylation/TFA. Modern, direct LC-MS methods have made these largely obsolete.

1.6.1 Electron Ionisation (EI+)

Electron ionisation (EI+) generates positively charged ions. The introduced molecules are ionised by bombardment with energetic electrons (normally 70eV) in a region of low pressure (less than 10^{-5} torr). The heaviest charged fragment normally observed under EI conditions is the molecular ion, M^+ radical cation, produced by loss of one electron from the neutral molecule (smaller amounts of doubly or even triply charged ions may also be produced).



Figure 1.6.1a. Creation of a molecular ion M^+ (radical cation) by EI.

This species is unstable and fragments, to a greater or lesser extent. The fragmentation processes, and thus the mass and abundance of the fragments produced and detected, are dependent on the structure of the molecule. Simple fragmentation of the molecular ion, by loss of a radical or neutral molecule, is illustrated below (Downard 2004, Ch 2).



Figure 1.6.1b. Fragmentation of a molecular ion to produce either a charged fragment ion (F^+) and a neutral radical (R^\cdot), or a fragment radical cation ($F^{+\cdot}$) and a neutral molecule (N).

As well as simple bond cleavages, fragment ions are also produced following rearrangement, if the ion is sufficiently energetically excited to undergo bond cleavage and re-formation. For example, hydrogen atoms or protons may be transferred from a remote site to the ionic centre prior to cleavage of the molecular ion.

Systematic interpretation of mass spectra is assisted by recognition of certain, specific rearrangements; for example, a characteristic elimination of a neutral alkene from the molecular ion of a carbonyl compound with an adjacent gamma-hydrogen (see figure) to produce an enol radical cation. This is called the McLafferty rearrangement (McLafferty 1959).

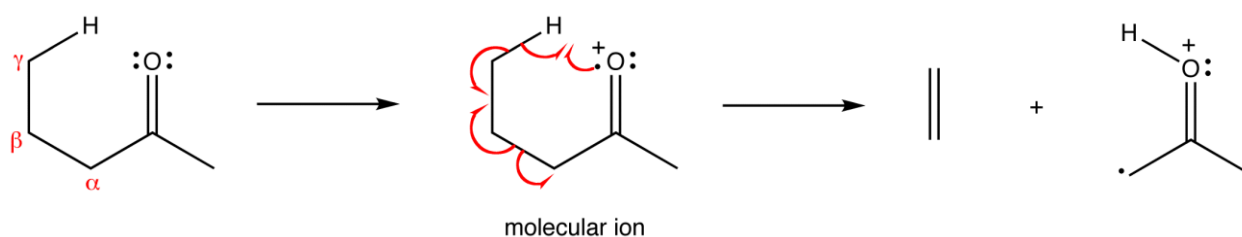


Figure 1.6.1c. The McLafferty rearrangement.

There are several useful guides on the interpretation and rationalisation of mass spectra, e.g. McLafferty & Tureček (1993), Downard (2004) and De Hoffmann & Stroobant (2007).

The mass spectra of some compounds exhibit intense molecular ions - some, such as quintozene (see accompanying data), with characteristic isotope patterns. Other spectra have weak or negligible molecular ions, but a few abundant ions which dominate the spectrum (e.g. DDT and dinocap). Some have many ions, none of which is particularly abundant (e.g. metalaxyl and endrin).

EI⁺ is widely used because of its simplicity, universality, high sensitivity and good reproducibility (which facilitates comparison of data) and also because EI spectra often contain useful structural information, which makes it useful for the identification of unknowns.

1.6.2 Chemical Ionisation

Chemical ionisation (CI) is a less energetic ionisation process, used for producing positive or negative ions. For CI, a reagent gas, such as methane or ammonia, is admitted to the ion source, at pressure of 0.1-1.0 torr. As with EI, a beam of energetic electrons is employed, but because the reagent gas molecules greatly outnumber the analyte molecules (by at least 100:1) it is the reagent gas molecules which are primarily ionised in greater numbers. At the

higher source pressure employed in CI these primary reagent ions are able to interact by collision with other gas molecules, to produce a plasma. It is the equilibrated, less energetic reagent ions that react with and ionise the sample molecules, often by proton transfer reactions in positive ion CI. The resulting analyte-related species are less energetic than the molecular ions produced by EI, and are therefore less prone to fragmentation. For this reason CI spectra are less complex and are usually dominated by an abundant pseudomolecular ion (often $[M+H]^+$ or $[M-H]^+$). In negative ion CI, the presence of the reagent gas may encourage electron-transfer reaction with the sample molecules, as well as ion transfer reactions, such as chloride ion transfer. Negative CI is far more effective at ionising molecules containing electronegative atoms which can stabilise the negative charge. This confers a high degree of selectivity, which may be exploited for the determination of, say, polychlorinated compounds.

CI is an alternative, complementary technique to EI. It is useful for producing molecular ion data. However, CI spectra can be less reproducible, being dependent on reagent gas pressure and purity. CI spectra are more dependent on source parameters such as temperature, design and cleanliness than are EI spectra. Selection of an appropriate reagent gas is critical.

It is also evident that CI spectra do not invariably provide unambiguous molecular ion data. For example, with ammonia CI, $[M-18]^+$ (due to $M-H_2O$), " M^+ " (due to $M+NH_4-H_2O$) and $[M+18]^+$ (due to $M+NH_4$) pseudomolecular ions are frequently observed, and in many cases ions of lower mass dominate the spectrum. CI is often particularly effective when employed using ion trap mass spectrometers, where the residence time before analysis is greater.

1.6.3 Other ionisation techniques

For gas chromatographic sample introduction into a mass spectrometer, which is still the most widely used combination applied to pesticide residue analysis, EI and CI are the only ionisation techniques in routine use.

Direct insertion (DI) sample introduction is of great importance for the validation of identity of pesticide standards and for ensuring that the spectrum obtained following chromatographic separation is identical to that from DI (to demonstrate that the compound is transmitted without degradation). For ionisation, following DI of sample extract/material into the mass

spectrometer, a large number of techniques may be used in addition to EI and CI, though few of these are in routine use for residues analysis (e.g. desorption CI, field desorption, fast atom bombardment (FAB), radioisotope and laser ionisation).

There are several increasingly important ionisation techniques which have been developed for use with LC sample introduction. Some interfaces have been developed which remove the LC solvent and transmit the sample, permitting production of conventional EI and CI spectra. Interfaces, such as the particle beam and moving belt, were effective for some compounds, but generally gave poor performance at trace levels. These are now rarely used. Direct liquid introduction interfaces, such as electrospray and APCI, produce CI-like spectra, with excellent sensitivity. They are designed to cope with solvent flow rates of 1-2ml/min. Coupled with MS/MS, to provide additional, structural information, these atmospheric pressure ionisation techniques are now commonplace.

1.7 Mass Analysis

Having generated the ions, they must be separated and detected in order to produce a mass spectrum. In most mass spectrometers used for residues analysis, the separation is achieved by means of a electric fields (quadrupoles and ion trap devices), by orthogonal acceleration time of flight (OA ToF) or by orbitrap. Magnetic sector instruments have been largely superseded, but for illustration of the spatial separation of the key processes, a figure is given below. For a concise introduction to the various types of mass spectrometers, see Downard (2004, Chapter 3) and a review by McLuckey (2001),

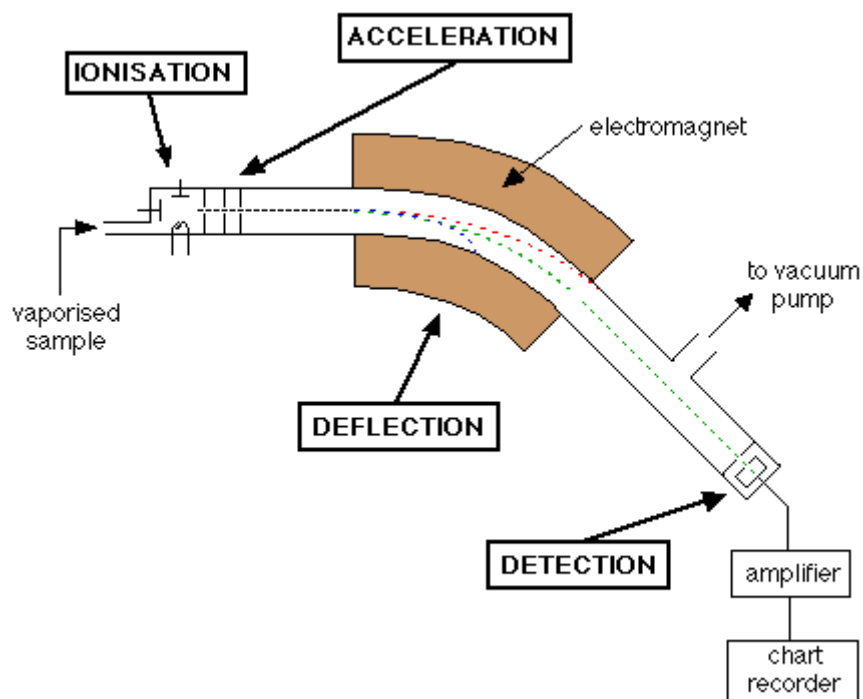


Figure 1.7. Schematic of a magnetic sector mass spectrometer
 (www.chemguide.co.uk/analysis/masspec/howitworks.htm)

1.7.1 Quadrupole and ion trap mass analysis

Mass spectrometers with quadrupole and ion trap analysers (which includes many bench-top instruments) are only capable of generating low (nominal mass) resolution mass spectral data. The concept of the quadrupole mass analyser was first reported in 1950s (Paul & Steinwedel 1953). Some bench-top instruments have limited mass ranges (e.g. up to 1,000 daltons), although this is sufficient for most pesticides. These instruments are usually compact and relatively simple to operate.

Ion trap devices differ from quadrupole mass spectrometers in that ion production, storage and analysis are effected in the same physical space. This leads to some operational differences: the main advantage is high sensitivity in full scan acquisition mode; the main disadvantages are that spectra may not be as reproducible as those obtained on other systems and overloading effects, particularly from co-eluting materials, may be more important. Some spectral differences may also be observed due to the longer period between generation and detection of the ions.

1.7.2 Orthogonal acceleration time of flight (OA-TOF) mass analysis

Orthogonal acceleration time-of-flight mass spectrometry has enjoyed a renaissance over recent years. It was one of the earliest types of mass analysers, having been proposed in 1946. Its engineering simplicity is certainly attractive, although ingenious refinements such as the reflectron, have greatly improved its performance (Guilhaus 2000). The basic principle is very simple. Ions from the analyte are accelerated by an applied voltage. The time they take to reach the detector indicates their mass, with lighter ions arriving more quickly. Coupling a TOF to a continuous ionisation source is achieved by applying the electric potential orthogonally – hence “orthogonal acceleration”.

1.7.3 Fourier transform ion cyclotron resonance (FT ICR) mass analysis

In FT ICR MS the mass to charge ratios of analyte ions are measured by detecting the image current produced by the ions whilst trapped in a cyclotron magnetic field (Marshall 1998). The ions are thus not destroyed by detection, as in most other types of MS. Benefits of FT ICR MS include very high resolution and ability to perform multiple MS/MS experiments.

1.7.4 Orbitrap mass analysis

In orbitrap MS systems, ions are electrostatically trapped in orbit around a central spindle shaped electrode (Hu 2005). As in FT ICR the ions are detected by their image current. Benefits of orbitrap MS include high mass accuracy, high sensitivity and good dynamic range.

Table 1.7a presents typical comparative performance characteristics for the types of modern mass spectrometer most frequently used for detection of pesticides and other chemical contaminants.

Table 1.7a. Performance characteristics of modern mass spectrometers (Hird 2014).

Mass Analyzer	Resolving power	Mass accuracy (ppm)	Mass range (m/z)	Acquisition rate (Hz)	Price
Q	3-5k	Low	2-3k	2-10	Low
Ion trap	4-20k	Low	4-6	2-10	Moderate
ToF	10-60k	1-5	10-20	10-100	Moderate
Orbitrap	100-240k	1-3	4	1-5	High

1.8 Modes of MS data acquisition

In most MS systems (except FT-ICR and orbitrap MS systems), following the separation of the ions according to their mass to charge ratios, the ions are detected by means of a device which multiplies the current associated with the ions, using a high voltage cascade process. Compiling these data into a histogram, with m/z value plotted versus relative abundance (%), results in a *mass spectrum*.

There are several modes in which mass spectral data may be acquired.

1.8.1 Scanning acquisition

The ultimate confirmation of the presence of a pesticide residue that can be achieved is the detection of a complete mass spectrum (in practice generally from m/z 50 to at least 20 daltons beyond the molecular ion region for EI, and at least 50 daltons for CI). Where the molecular ion is not observed in the EI spectrum, the generation of complementary CI data is recommended for improved validation. In general, ions lighter than 50 daltons are not routinely acquired as they are neither particularly informative nor diagnostic. Their detection may also reduce the rate of spectral acquisition. Spectra are usually acquired at least several times per second, commensurate with the time for elution of a capillary GC peak, which is usually of the order of a second.

OA TOF MS systems must be considered separately. Their mode of operation is quite different from quadrupole “mass filter” technology, in that full scan data are captured, at a high frequency (1,000+ Hz), and combined to produce full spectra. Selected ion recording is therefore not a practical option.

Modern bench-top GC-MS instruments can produce complete EI mass spectra, with acceptable signal to noise from 10-100pg of material eluting from a capillary GC, and perhaps from as little as 1pg for ion trap systems. In pesticide residues determination, this detection limit may not be so readily achieved. Many pesticides are polar and thermally labile, so they may be inefficiently transmitted and/or elute as broad GC peaks. Pesticides whose mass spectra exhibit many different ions of similar low overall abundance, such as metalaxyl or endrin, are also more difficult to detect than those whose spectra have fewer,

more abundant ions. Interference from co-extracted materials and contaminants usually reduces the effective sensitivity.

Positive ion CI sensitivity may be similar to that of EI, but is compound dependent. Negative ion CI may be 10-100x more sensitive, but is even more compound dependent. Electrospray LC-MS instruments can produce a full mass spectrum from as little as 10-100 pg of some compounds, such as ethylene thiourea (ETU), but this is also very compound dependent .

The plot of summed response of all ions detected per scan versus time is referred to as the total ion chromatogram (TIC). The TIC gives an overall indication of the amounts of analyte, co-extractives etc., detected. It is comparable to a GC chromatogram obtained using flame ionisation detection (FID).

Care must be exercised in the reporting of full mass spectral data, even though these are the least ambiguous of MS data. Modern MS datasystems make it very simple to "over-enhance" the spectrum by averaging and background subtraction - a great temptation when the spectrum is weak and interference is strong. For this reason, where enhanced mass spectra are reported, they should be accompanied by the "background" spectrum that was subtracted. This consideration is particularly relevant for data generated by ion trap systems, which may suffer from greater ion-abundance variability. For tabulation purposes, most MS datasystems can provide a numerical evaluation of spectral similarity (or "library fit factor"). In the absence of a generally agreed spectral similarity index, and in view of the great diversity of spectral "uniqueness", it must be left to the analyst to consider *all* the available information in deciding what is an acceptable degree of spectral similarity. For example, particular care should taken when determining low levels of 2,4,5-T *iso*-octyl ester, the EI spectrum of which is superficially similar to that of long chain *n*-alkanes (which are frequently encountered in sample extracts). In general, the *presence* of extraneous ions in the spectrum (due perhaps to a co-eluting compound) should cause less concern than the *absence* of expected ions (though this can be caused by over-zealous background subtraction): in all cases, checking the degree of correspondence of the reconstructed ion chromatograms (RICs) of the most significant ions is recommended.

In practice, acquisition of a complete spectrum is not often achieved because the amount of pesticide present is often too small or the interference from co-extractives is too great, but where residues substantially exceed a maximum residue level (MRL) requiring regulatory enforcement action, it is usually desirable and possible.

Where necessary, the use of a limited mass range acquisition (over say 5-10 daltons) may boost sensitivity, compared to full mass range acquisition. This is particularly useful for monitoring fragments which have characteristic isotope patterns distributed over several masses (because of the presence of polyisotopic elements such as chlorine or bromine). For example, to monitor the most abundant EI fragment produced by DDT and TDE, $(C_{13}H_9Cl)^+$, the expected response should be m/z 235 (relative intensity 100%), 236 (15%), 237 (65%), 238 (10%), 239 (10%).

1.8.2 Low resolution (nominal mass) SIM

For quadrupole instruments, the most sensitive MS detection technique is *selected ion monitoring* (SIM), also sometimes referred to as selected ion recording (SIR), in which only the characteristic ions of the analyte are monitored. The enhanced sensitivity is obtained at the cost of specificity, in that the technique provides a lower degree of confidence in the identification of the analyte. For ion trap (or OA TOF) systems the increase in sensitivity compared to full mass range scanning may be (is) negligible.

In general, results from monitoring ions at low mass (<100 daltons) are more likely to suffer from interference because most co-extractives and contaminants (especially aliphatic compounds such as alkanes and fatty acids) generate abundant ions in this region. However, monitoring certain low-mass ions may be worthwhile, particularly those with even-numbered mass, so they should not be ignored completely. As the mass spectra of some pesticides do not exhibit abundant ions at high mass (e.g. dinocap and dimethipin - as well as those whose molecular weight is less than 100 daltons, such as 2-aminobutane and aminotriazole), there may be no simple alternative for these compounds.

In order to obtain optimal sensitivity in SIM, the ions monitored should exhibit relative abundances greater than 20-30% of the base peak (the most intense ion in the spectrum). SIM with capillary GC introduction using positive ion EI or positive ion CI is capable of detecting

less than 10-100fg of analyte. With negative ion CI, SIM may detect sub-femtogramme levels of some polychlorinated compounds.

Potentially, the more ions monitored, the greater the confidence in the results. In practice there is no advantage to be gained from monitoring more than 6 ions per compound, as the enhancement in confidence is likely to be negligible and will result in reduced sensitivity and/or reduced sampling frequency. Monitoring a minimum of three significant ions is generally recommended. However, even where the spectrum of the analyte exhibits only one or two significant ions (e.g. the EI spectrum of dinocap), useful results may be generated by SIM, *where these are fully supported by appropriate analytical QC data*. Gilbert describes several successful pesticide residue determinations (carbaryl, dimethoate, and parathion) reported in the literature, which rely upon SIM of a single ion (Gilbert 1987). In such cases, monitoring the ions which are one and two daltons lighter than the analyte fragment may be more informative than attempting to monitor the weaker isotope ions at higher mass because it indicates whether any response detected is due to interference from the isotope ions of co-eluting compound. This is a form of limited scanning - providing supporting evidence by means of the *absence* of ions.

1.8.3 High resolution (accurate mass) SIM

Accurate mass SIM may eliminate unacceptable interference encountered with nominal mass techniques. Increasing the operating resolution of a magnetic mass spectrometer reduces its ion transmission efficiency and thus the absolute sensitivity obtainable. However, the removal of interference can result in a significant increase in observed signal to noise ratio (S/N). In many cases optimal performance is obtained at 3,000-5,000 resolution. Detection limits are very dependent upon introduction technique, analyte, substrate etc., but may be of the order of 10-100fg. Even when interference is not a problem, accurate mass SIM can be used to increase the confidence in a result obtained using nominal mass techniques.

1.8.4 Tandem mass spectrometry, MS/MS

Tandem mass spectrometry, or MS/MS, can be used to improve the reliability of SIM data. In this technique selected ions are encouraged to fragment by collision with neutral gas molecules, and the daughter ions are separated and detected. The highest sensitivity and specificity is obtained by monitoring a selected daughter ion of a selected primary or parent

ion. This technique is sometimes referred to as *multiple reaction monitoring* (MRM). An example is the thermospray LC-MS analysis of ETU, in which screening may be performed by SIM of the protonated pseudomolecular ion at m/z 103, and confirmation of positive findings may be performed by MRM of the m/z 44 daughter ion produced by fragmentation of the parent ion (Wilkins 1992). As with high resolution SIM, although absolute sensitivity is reduced versus low resolution SIM, signal to noise ratios may be significantly enhanced. Detection limits are very dependent upon introduction technique, analyte, substrate etc., but may be of the order of 10-100fg of analyte injected.

The performance of high-resolution MS versus low resolution MS/MS has been critically compared, for determination of a suite of veterinary drug residues at trace levels in several matrices (Kaufmann 2010), and for nerve agent (CW) metabolites in urine (Hamelin 2013). For the veterinary drugs, the high-resolution MS (single-stage Orbitrap operated at 50,000 resolution) and MS/MS (triple quadrupole) gave similar quantitative performance, but for confirmation of analytes present at low levels the MS/MS proved superior. For the nerve agent metabolites, the precision, accuracy and sensitivity of the two techniques were similar, but high resolution MS showed additional capabilities by confirming the presence of an unexpected metabolite.

Confirmation of identity and quantity by both primary ion (i.e. SIM) and daughter ion (MRM) data is generally regarded as being as convincing as limited scan data. As with other MS techniques, full supporting analytical QC data greatly increases the confidence in the results produced.

1.9 Quantification and Confirmation

Quantification is one of the key aspects of the determination of residues by MS. It is not uncommon to find that the identity of a residue detected using a less specific detector is confirmed by MS but that the concentration present has been over-estimated (due to interference) by the screening system.

If the analyte is present at high concentration, and interference is minimal (as evidenced by spectral purity), the TIC obtained in full scan acquisition mode may be used for quantification purposes. For most practical purposes in residues analysis these criteria are not

met and the reconstructed ion chromatograms (RICs) of characteristic ions (from full scan data) must be used in order to distinguish the response of the analyte from the background.

With magnetic and quadrupole instruments, the accuracy of quantitative data obtained by TIC or RIC response measurement is usually inferior to that obtained using SIM. Where no interference is observed, the relative SIM responses (peak heights or areas) of each of the ions monitored for the analyte should correspond to those obtained from a standard. In reality, SIM data for some ions often suffer from some degree of interference. When assessing such data it is most informative to overlay the SIM chromatograms obtained for the sample extract with those from the standard solution (preferably spiked at a similar level in an extract of the same substrate): this greatly facilitates recognition of any response due to the analyte in the sample extract, and allows the similarity of chromatographic peak characteristics to be assessed (i.e. peak shape and retention time). Using this technique, the presence of interference in any particular chromatogram should be easily discerned and it should be obvious if it is necessary to disregard data for these ions. If data for more than one ion are obtained by SIM, the inter-ion abundance ratios of the responses permit more thorough comparison. The ratios should be similar to those obtained from the corresponding standard (within 20%). If the response from one SIM channel is significantly greater than expected, it is probably indicative of interference from a co-eluting compound. Data from this SIM channel should not be included in the quantification (but should not be ignored as they may imply that the other ions monitored are not totally free from interference and that further confirmation is necessary).

When interpreting SIM data where more than one ion has been monitored without interference, satisfactory quantification may be based on the data obtained for the most abundant ion. The other SIM data then form supporting evidence. Where the ions monitored are of similar abundance, averaging the quantification data obtained for each ion is recommended.

Where the analyte is not detected, the validity of the reporting limit should be determined experimentally rather than estimated by extrapolation. The criteria for defining the limit of determination using MS are similar to those adopted for all other chromatographic analyses.

1.10 Future developments in Mass Spectrometry

The use of mass spectrometry in pesticide residue and environmental analysis is likely to continue to expand, particularly as the performance and ease of use of bench-top GC-MS and LC-MS instruments improves. These help to expand the range of compounds amenable to trace detection by MS. LC- atmospheric pressure ionisation MS (especially electrospray) has demonstrated its value, allowing routine, direct determination of compounds which were difficult or impossible to determine directly by GC. Improved chromatographic introduction techniques, miniaturisation and cost reduction are also extending the potential applications of MS (Wang 2013).

The potential for MS, and related technologies such as ion mobility spectrometry, to leave the laboratory and be used at the point of application – literally in the field for agrochemicals – for the detection of toxic environmental contaminants, including chemical warfare agents, is particularly exciting (Satoh 2015, Utabe 2014).

1.11 Aims and Objectives

The purpose of this thesis is to review several decades of practical application of mass spectrometry for the characterisation of pesticides and related substances. Salient scientific literature and other resources are described. Several case histories are provided, to illustrate the different types of challenge that may be met, and how they may be addressed. General recommendations are given and unexpected observations are described and explained.

The Appendices contain several compilations of mass spectrometric data accrued during this period:

- Appendix I contains key data for 600 pesticides, related compounds, CW agents and GC artefacts and contaminants. As well as molecular information, pesticide class, acute toxicity, amenability to GC etc., it summarises the eight most abundant ions in the EI+ mass spectrum, and gives tentative assignments for key fragments (based on rational review of the data, and in some cases by accurate mass data).
- Appendix II summarises the MS data from Appendix I in a searchable format, for the identification of unknown spectra by their by most abundant ions.
- Appendix III is a comprehensive database of pesticide molecular weights, intended to facilitate the identification of unidentified compounds. It contains data for (approximately 2,000 pesticides listed in ascending order by accurate monoisotopic MW, from “prussic acid” (hydrogen cyanide, M^+ m/z 27.0109) to streptomycin (M^+ m/z 1456.4337)).

Much of the effort in the compilation of Appendix I went into rationalising the fragmentation processes which gave rise to the mass spectra. Understanding these processes is enormously helpful when comparing and exploiting MS data; for example, when determining related classes of compound, technical contaminants or unidentified environmental contaminants. The multi-dimensionality of chromatographic separation coupled with mass spectrometric analysis can be enormously powerful analytical tool. Understanding the underlying principles is essential for successful exploitation.

It is hoped that this thesis will be of interest and utility to analysts working in this field.

CHAPTER 2.

EXPERIMENTAL

2.1 Equipment

2.1a Facility - Ministry of Agriculture, Fisheries & Food Laboratory (MAFF, 1979 -1993)

Three different gas chromatograph - mass spectrometer (GC-MS) systems were used for the bulk of this work:

- Varian 1400 GC – VG Micromass 12B MS,
- Dani 3800 GC - VG Analytical 7035 MS
- Hewlett-Packard 5790 GC - JEOL DX300 MS.

For those compounds that decomposed completely under gas chromatographic conditions, spectra were obtained by direct insertion of the pure compounds. All the spectra reported here were produced by EI with an ionisation energy of 30 or 70 eV, acquiring ions over the range m/z 20-620. In those situations where convincing relative molecular mass information was not provided by EI, chemical ionisation (CI) using 2-methylpropane or ammonia as the reagent gas, was employed. In addition, accurate mass measurement and/or metastable ion correlation was used to help identify apparently important fragment ions whose formation appeared to be due to unexpected or complicated rearrangements. GC was performed using packed columns at temperatures from 150 to 240°C, with OV-1701 or OV-17 as the stationary phase. Some relative retention times were measured on a 0.5 m X 2 mm column of 7% OV-1701 on 100-120-mesh Chromosorb W(HP), at a temperature of 220°C, with a helium carrier gas flow-rate of 30 ml min⁻¹. When better gas-chromatographic resolution was required, a 25 m X 0.2 mm CP-Sil 19CB capillary column (Chrompak Ltd.) was employed, with splitless injection, on the HP 5790.

2.1b Facility - Cardiff University School of Chemistry (2014 - 2015)

Further GC-MS investigations were undertaken at Cardiff University Department of Chemistry, using capillary GC/accurate mass OA-TOF MS (orthogonal acceleration time of flight mass spectrometry) using an Agilent 6890 Gas Chromatograph coupled to a Micromass/Waters GCT MS. Pesticide standards were diluted in Analar hexane.

- GC conditions: Manual injection of 1ul, with split ratio of 1:2, into GC injector at 230°C, onto Supelco Equity-5, 30m x 0.25mm capillary GC column coated with 5%

phenyl/95% methyl silicone stationary phase (thickness 0.5µm)

- GC oven temperature programme: held at 40°C for 5min, then raised at 5°C/min to 300°C, and held for 5min.
- MS conditions: Ion source temperature 200°C. EI+ ionisation mode, using 70eV energy.
- Full scan data acquisition (m/z 40-1,500).

2.2 Chemicals

The pesticide names used here are generally those quoted in *The Pesticide Manual* (Worthing 1990).

2.2.1 MAFF

Aphidan (S-ethylsulphinylmethyl O,O-diisopropyl phosphorodithioate, also known as IPSP) was obtained from Berk Ltd. (London). Carbophenothion and its metabolites were obtained from Stauffer Chemical Company (Westport, CT, USA). Demeton, demeton-S-methyl, disulfoton, fenamiphos, fensulfothion, fenthion, oxydeprofos, sulprofos and some of their metabolites were obtained from Bayer UK Ltd. (Bury St. Edmunds, Suffolk) and Bayer AG (Leverkusen, FRG). Phorate, temephos, terbufos and some of their metabolites were obtained from Cyanamid of Great Britain Ltd (Gosport, Hampshire). Chlorthiophos, ethion, sulfotep and TEPP were obtained from the Laboratory of the Government Chemist (London). Bensulide, famphur and methyl carbophenothion were obtained from Greyhound Chromatography Ltd. (Birkenhead, Cheshire, UK). Demephion and thiometon were isolated from Pyracide (BASF) and Ekatin (Sandoz) formulations, respectively. Vamidothion and its metabolites were obtained from May & Baker Ltd. (Brentwood, Essex).

Aphidan sulphide was found as a contaminant in the parent sulphoxide. The sulphides of fensulfothion and oxydeprofos were prepared by reduction of the respective parent sulphoxides with concentrated hydrochloric acid and solid potassium iodide, in glacial acetic acid solution, at room temperature for 2-5 min. After dilution with water, the sulphides were extracted with dichloromethane. The extract was dried by passing it through anhydrous sodium sulphate and, after addition of toluene (to assist removal of the acetic acid) and heptane (to assist removal of the iodine generated during the reaction), the solvent was removed using a rotary evaporator.

A similar method of extraction was employed after the oxidations given below, with the addition of toluene where acetic acid was used. The sulphoxides of chlorthiophos, demephion, demeton, sulprofos and thiometon were prepared by oxidation with 8M aq. hydrogen peroxide (“100 volume”) in glacial acetic acid, containing a trace amount of concentrated sulphuric acid (approx. 1% by volume), at room temperature for 10-15 min. The oxon sulphones of Aphidan, chlorthiophos, demephion, sulprofos and the oxon of famphur were prepared in a similar manner to the sulphoxides but with the reaction carried out at 40-80°C for 10-20 min. Aphidan oxon sulphide was observed as a contaminant in the oxon sulphone preparation, presumably arising from oxidation of the Aphidan sulphide. The sulphones of chlorthiophos, demephion, sulprofos and thiometon were prepared from their respective sulphides, and those of Aphidan, oxydeprofos and temephos were prepared from their respective sulphoxides, by oxidation with potassium permanganate, using a method similar to that employed for residue determination (except that the oxidant concentration was 10g/l). Although the majority of the sulphones were produced in 10-30 min at room temperature, those of chlorthiophos and temephos required 30-60 min at 80°C. In a few instances, further purification of the products was required, and this was achieved by column chromatography using silica gel (Merck Art. 7734, Kieselgel 60) eluted with mixtures of hexane/acetone appropriate to the polarity of the product required.

2.2.2 Cardiff School of Chemistry

The following 25 pesticides and related materials were analysed:

Azinphos methyl

Butocarboxim

Chlorpyrifos

Diazinon

Dichlorvos

Dimethoate

Disulfoton

Ethoprophos

Famphur

Fenamiphos

Isofenphos

Methiocarb sulphoxide

Parathion
Paratahion methyl
Pirimiphos methyl
Prothiofos
Pyraclostrobin
Pyrazophos
Pyridaphenthion
Quinalphos
Sulfotep
Thiofanox
Thionazin
Triazophos
Triethyl thiophosphate

These were either kindly provided by ex-colleagues at FERA (ex CSL/MAFF York, UK), or purchased from Sigma Aldrich:

- Pyridaphenthion PESTANAL[®] (Cat No 32538)
- EPA 8270 Organophosphorus Pesticide Mix (Cat No 5-07202, Lot LC02194, Exp Aug 2016), which contained dimethoate, disulfoton, famphur, methyl parathion, O,O,O-triethylphosphorothioate, parathion, phorate, sulfotep and thionazin.

The data for some compounds, particularly highly toxic and/or CWC (chemical weapons convention) restricted materials, were taken from the literature. These are referenced accordingly.

2.3 Software & interpretation

ChemBioDraw Ultra (Version 14.0.0.117, Cambridge Soft Corp., Perkin Elmer) was used to produce the molecular structures and generate some MS fragmentation data.

Two online resources were also used:

ChemCalc software (Patiny 2013) was used to interpret the accurate mass GCT data; **Exact Mass calculator, Single Isotope Version** (SIS, Scientific Instruments Services, Inc, 2015).

CHAPTER 3. RESULTS & DISCUSSION

3.1 Analytical strategies & Case Studies

Safety First – It is essential when considering working with a new compounds, particularly unknowns, that the safety of oneself and of one's colleagues must be respected and preserved. Gather the appropriate information, do a risk assessment and take appropriate protective measures.

3.1.1 Case Study 1: Identification of residue of Pirimicarb

My first example is the successful identification of a nitrogen compound. A nitrogen-containing GC peak was detected by GC-NPD during analysis of an extract of a lettuce sample during a routine pesticide surveillance programme. A GC peak with corresponding retention time was identified in the GCMS total ion current (TIC) chromatogram. The compound exhibited ions at m/z 238 (25%), m/z 166 (100%) and m/z 72 (80%). Comparison of this spectrum with the available MS libraries did not yield a convincing identification. Accurate mass study of the presumed molecular ion gave m/z 238.145, which indicated a likely empirical formula of $C_{11}H_{18}N_4O_2$ (theoretical 238.1431). This corresponds with **pirimicarb**. The tentative identification was confirmed by analysis of a reference standard. This identification was unexpected, because at the time, it was believed that carbamate compounds were not amenable to GC (using packed GC columns).

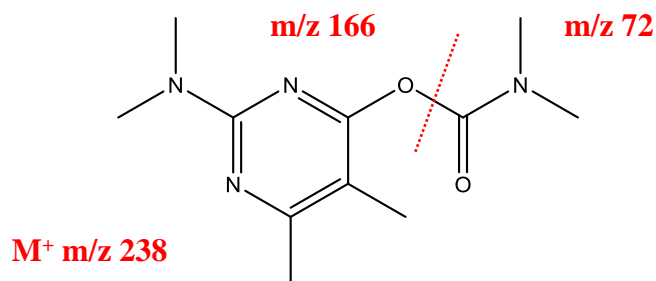


Figure 3.1. Pirimicarb (a carbamate insecticide). MW 238.

See (more recently compiled) NIST mass spectrum at

<http://webbook.nist.gov/cgi/cbook.cgi?ID=C23103982&Mask=200#Mass-Spec>

To facilitate the identification of other unidentified pesticides, I produced an index of pesticide molecular weights. See Appendix III.

However, not all MS investigations are as successful as this. Here are two examples where interpretation of the analytical findings was correct, but the actual problem or question remained unresolved.

3.1.2 Case Study 2: Ice cream factory closure due to potential chemical contamination

During employment at Unilever, the urgent investigation of a potential chemical contamination incident at a major European ice cream production unit arose. There had been a significant number of consumer complaints about recent batches of ice cream produced at the factory suffering from an unpleasant chemical taint/off-flavour. The Quality Control personnel at the factory identified a particularly badly affected batch and sent samples for analysis at Unilever Research Colworth.

I undertook a rapid SPME / GC-MS analysis of the samples, and detected significant levels of several organic solvents in the sample. These included dichloromethane, chloroform and ethyl acetate – materials that clearly should not have been present.

An urgent follow-up investigation was mounted, as it was feared that wilful adulteration may have been perpetrated, by, e.g., a disaffected employee. The QC personnel at the factory quickly investigated the issue. Fortunately, the explanation turned out to be very simple. The original tainted samples had been sent to a local university Chemistry Department for analysis. After cursory analysis, nothing significant had been found, so the samples had been consigned to a fridge. Unfortunately the fridge was used for storing various organic solvents (I was able to tell the QC personnel which solvents were present). After a week or so, the solvent contaminated samples had been shipped to Unilever Colworth.

Further investigation, using more representative, unadulterated samples, indicated that the taint was probably due to microbial degradation of vanillin to guaiacol (2-methoxyphenol).

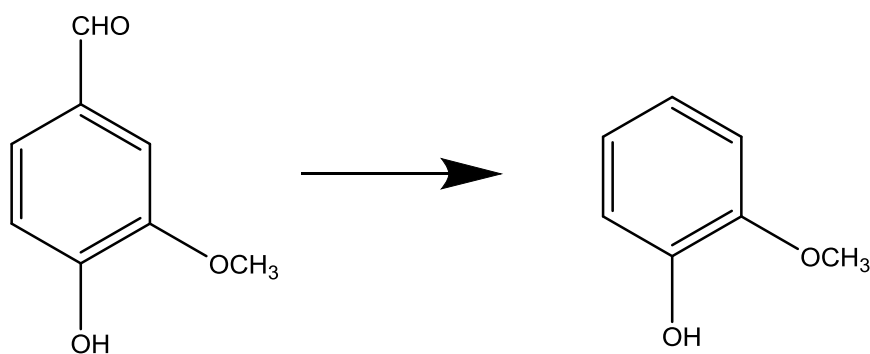


Figure 3.1.2(a). Microbial degradation of vanillin to guaiacol (2-methoxyphenol).

This can occur with poor storage and hygiene practices (Jensen 2008). Guaiacol imparts a potent smoky taint. It has an odour threshold of 20 ng/g (ppb) in pure water (Leffingwell 2015)

3.1.3 Case Study 3: Suspected poisoning of wildlife by pesticides

During my time at MAFF Harpenden, regular requests were received to use MS analysis to help identify or confirm cases of wildlife poisoning suspected to be due to agrochemicals, in support of the Wildlife Incident Investigation Scheme (WIIS, 2015). Often this would involve the GCMS identification of a compound detected using GC-NPD or GC-FPD. Poisonings were usually due to organophosphorus (e.g. famphur, parathion, fenitrothion, mevinphos) or carbamate (aldicarb, carbofuran) insecticides and rodenticides (difenacoum, *alpha*-chloralose, strychnine). Sometimes GCMS was used to confirm detection of compounds not amenable to GC, following specific derivatisation procedures. Examples include *alpha*-chloralose as its TMS (trimethylsilyl) derivative, and fluoroacetic acid as its methyl ester (following diazomethane treatment).

The pesticide-related poisonings arose either following approved agricultural practice (e.g. wild geese consuming cereal seed that had been treated with a toxic seed treatment, and honeybees collecting nectar from sprayed crops) or due to misuse (e.g. crop spraying when the crop was in flower) or intentional abuse, e.g. gamekeepers placing animal carcasses or eggs containing OP pesticides or strychnine to attract and kill raptors (buzzard, red kite, eagle).

On one occasion, the cause of death of a magpie was linked to a nitrogen containing compound observed on GC. Tissue extract was analysed by capillary GCMS and identified the nitrogen compound as pentobarbital, a barbiturate, by its prominent EI+ MS fragment ions at m/z 141 and 156 (the molecular ion, at m/z 226, was absent). (See NIST MS spectrum at <http://webbook.nist.gov/cgi/cbook.cgi?ID=C76744&Mask=200#Mass-Spec>)

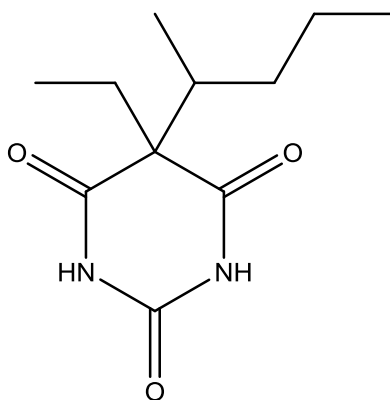


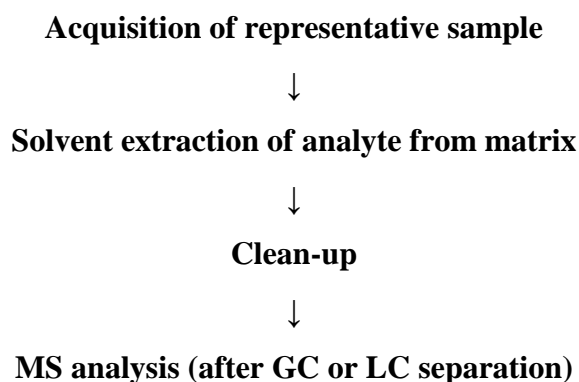
Figure 3.1.3(a). Pentobarbital, C₁₁H₁₈N₂O₃, mw 226. (NIST MS C76744)

This unexpected result was reported back to the submitter of the dead magpie, who was rather embarrassed. He explained that he was a vet, and having found the magpie in a distressed state by the side of a country road, he had euthanized it with an injection of barbiturate. Unfortunately he had omitted to inform the WIIS of this fact. So, although the actual cause of death of the unfortunate bird was indeed acute barbiturate poisoning, the reason for its distressed state remained unknown.

3.2 Specific analytical considerations for pesticide residue analysis

There is a rich literature on the recommended conduct of pesticide residue analysis. General guidelines have been produced by e.g. Codex Alimentarius (FAO/WHO 2003).

For pesticide residue MS analysis, the basic process can generally be summarised as:



It is necessary to confirm pesticide stability throughout each of these steps. Unexpected losses can occur, especially at the very low levels ($\mu\text{g-mg/kg}$) involved in trace analysis. Sample integrity must be maintained, and contamination and/or cross contamination, must be avoided.

When selecting the type of MS analysis, it is essential to identify the most appropriate means of sample introduction. One must consider volatility and stability. Can the molecule be delivered intact into the vapour phase / vacuum? This will predicate the use of GC or LC.

Many standardised analytical protocols for pesticide analysis are now available, e.g. from the EU and the US EPA, for the most frequently encountered pesticides and their toxicologically significant metabolites or degradation products.

The “QuEChERS” (quick, easy, cheap, effective, rugged & safe) sample extraction method (Lehotay 2007). Full method details and much other information is available at www.quechers.com

The EU Reference Laboratories (see <http://www.eurl-pesticides.eu/docs/public/home.asp?LabID=100&Lang=EN>) have not been

idle. See for example an impressive, recent report of a rapid, sensitive, accurate and reliable multiresidue method for the identification and quantification of 210 relevant pesticides in four representative fruit and vegetable commodities (tomato, potato, spring onion and orange). This was developed and validated using GC in tandem with triple quadrupole MS. The method has been fully validated and applied to 292 samples from different countries. Prior to analysis, an extraction procedure based on a sample extraction of multiclass analytes, using ethyl acetate was employed. Mass spectrometric conditions were individually optimized for each compound in MS/MS selected reaction monitoring (SRM) mode to achieve optimal sensitivity. The pesticides were separated and analysed in less than 25 min. GC retention time locking was used. System maintenance was reduced by using a purged capillary flow device that provided backflush capabilities by reversing column flow immediately after elution of the last compound of interest. Isotopically labelled internal standards were employed to improve the quality of the analytical results (Ucles 2014).

Another helpful report, from the US, accompanied with comprehensive GC and MS data, describes a method for detecting 119 pesticides in environmental samples (Hladik 2012).

Over the past two decades, LC-MS instrumentation has become much more widely used. The comparative effectiveness of GCMS and LCMS/MS detection for pesticide analysis has been studied (Alder 2006). The capabilities of mass spectrometry (MS) in combination with gas chromatography (GC) and liquid chromatography (LC) for the determination of a multitude of pesticides were evaluated. The selection of pesticides was based on the status of production, the existence of regulations on maximum residue levels in food, and the frequency of residue detection. GC-MS with electron impact (EI) ionization, and the combination of LC with tandem mass spectrometers (LC-MS/MS) using electrospray ionization (ESI), were identified as techniques most often applied currently in multi-residue methods for pesticides. The applicability and sensitivity obtained with GC-EI-MS and LC-ESI-MS/MS was individually compared for each of the selected pesticides. For one substance class only, the organochlorine pesticides, did GC-MS achieve better performance. For all other classes of pesticides, the assessment demonstrated a wider scope and better sensitivity when detection was based on LC-MS, although the difference was not great in many instances.

3.3 Artefacts & other confounding processes

Analytical studies can sometimes produce unexpected results, because of the complexity and variety of the physicochemical processes involved (Middleditch 1989). Below are described several examples of unexpected reactions or effects observed when using GC-MS systems:

3.3.1 Reduction of Parathion to Aminothion in GC injector

Some materials cannot survive the high temperatures experienced during GC injection. For most GC-MS analyses, the pesticide, typically in 1-5 μl of organic solvent, is introduced into the GC system via the injector at 200-250°C. Many pesticides (approx. 30%) are not sufficiently volatile, or are too thermally unstable, to survive this process. Most of these are amenable to LC. Some largely survive GC introduction, but a proportion undergoes specific chemical modification. This is the case with parathion and parathion-methyl.

By introducing the pesticide solution slowly, over several seconds, into a hot GC injector, at 250-300°C, it is possible to convert up to 5% of the parathion into its “aminothion” analogue, in which the nitro (NO_2) group is reduced by exposure to the hot metal surfaces in the injector, into the corresponding aniline (NH_2 , Figure 3.3.1a). It is perhaps surprising that the organophosphorus moiety is unaffected during this process, and remains unchanged.

This process may be exploited as a convenient means of generating amino-analogues of nitro-aromatic compounds.

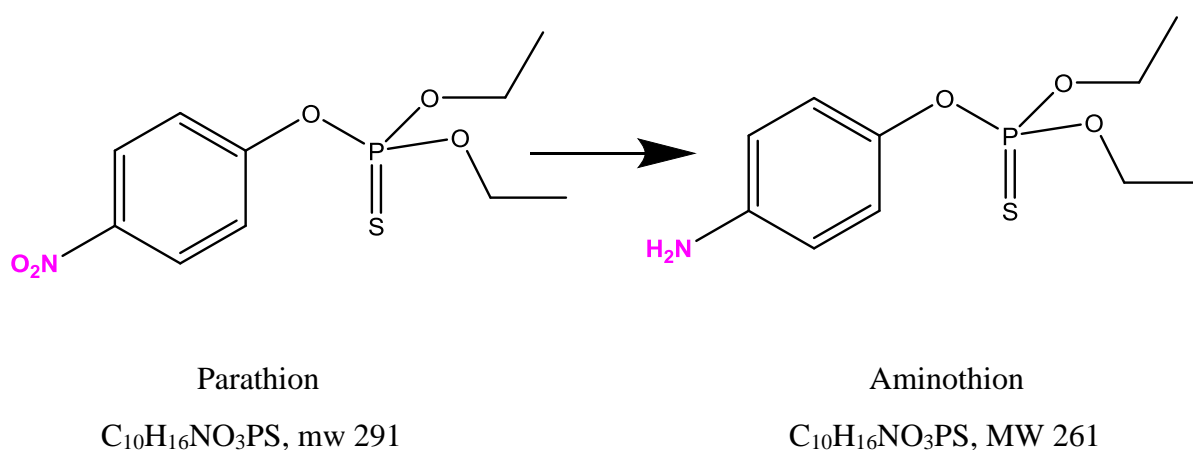


Figure 3.3.1(a). Reduction of parathion to aminothion, e.g. in hot GC injector.

3.3.2 Reduction of sulfoxides and azides in the GC and/or in MS ion source

Injection chemical modification effects were also observed with the aromatic sulfoxide, organophosphorus compound fensulfothion, where a proportion was converted into the sulphide form at elevated GC injector temperatures. The sulphide product eluted as a shorter retention time GC peak.

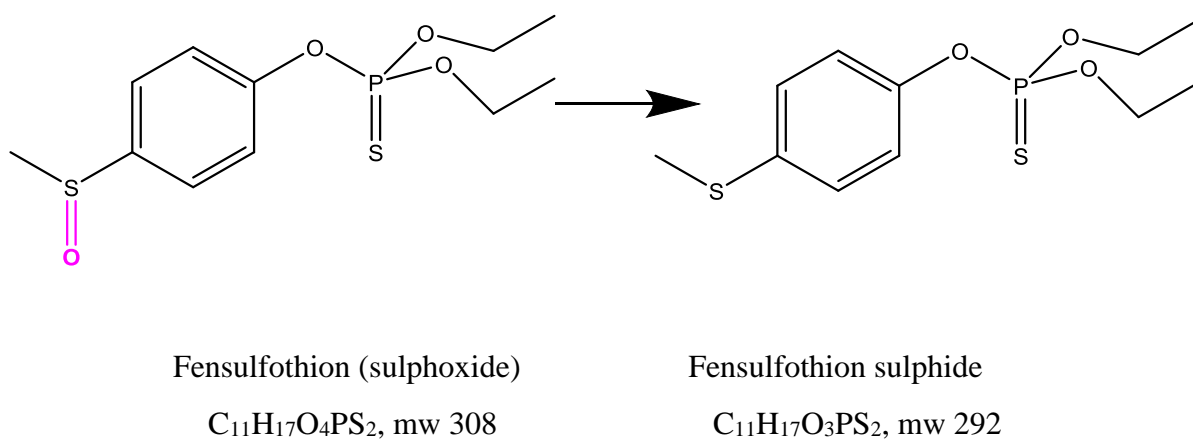
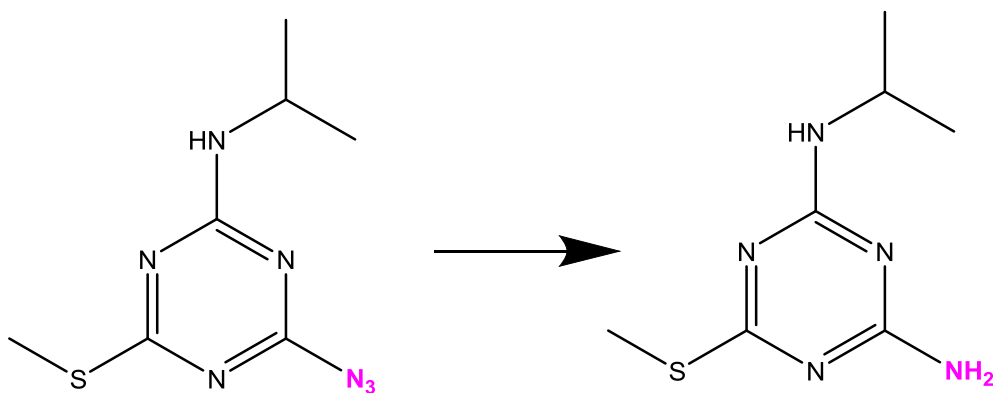


Figure 3.3.2(a). Reduction of fensulfothion to its sulphide.

Interestingly, the effect of the reactivity of the fensulfothion molecule was also observed during the MS ionisation process. At low ion source concentrations, a larger proportion of the sulfoxide molecule spectrum was observed to be due to the sulphide reduction product, giving rise to an apparently concentration dependent mass spectrum. Presumably, the sulfoxide molecules are so reactive that a proportion is reduced in the MS ion source on the hot metal surfaces (typically 200°C). At greater concentrations, the relative amount of reduction is less as a proportion of the whole, as the reductive process is swamped. It is interesting to note that pre-treatment of the ion source with polyethylene glycol 300 (PEG300) has been reported to help reduce this effect for fensulfothion (Sugitate 2012).

Similar ion source reduction effects were observed with other aromatic sulfoxide OP compounds (see e.g. carbophenothion sulfoxide, fenthion sulfoxide, temephos sulfoxide) and other classes of pesticide (e.g. fipronil and methiocarb).

Aziprotryne is also susceptible to reductive processes, either during GC or in the MS ion source. In this case reduction of its azide $-N_3$ substituent to $-NH_2$ results in a reduction in MW of 26 ($-42+14$) daltons, from m/z 225 to m/z 199:



Aziprotryne

$C_7H_{11}N_7S$, mw 225

Aziprotryne reduction product

*N*²-isopropyl-6-(methylthio)-1,3,5-triazine-2,4-diamine

$C_7H_{13}N_5S$, mw 199

Figure 3.3.2(b). Reduction of aziprotryne.

3.3.3 Dichlorophen – reaction with silicone GC stationary phase

Dichlorophen is too polar to be readily amenable to GC analysis. A tailing GC peak can be obtained if very large quantities (100ng- μ g) are injected. However, GC artefact peaks may sometimes be observed, which are due to reaction of the dichlorophen molecule with the GC dimethylsilicone stationary phases. These unexpected products are cyclic silicones, of which the dimethyl form is usually dominant. See figure below and data in Appendix 1.

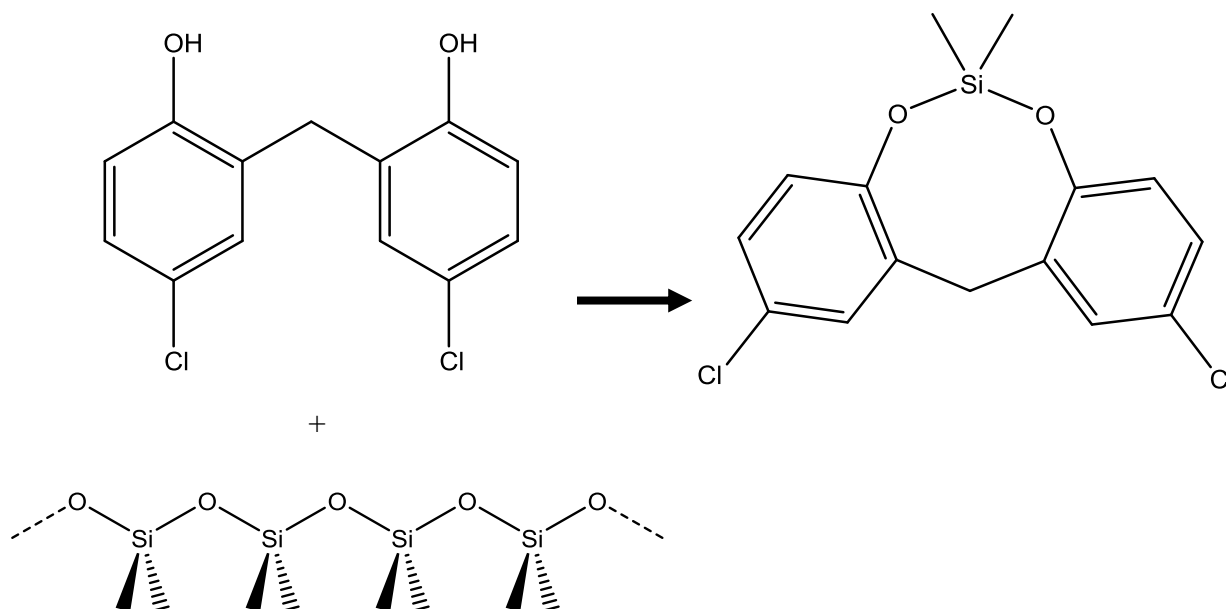


Figure 3.3.3(a). Reaction of dichlorophen (MW 268) during GC transmission with dimethylsilicone stationary phase to produce cyclic dimethylsilicone derivative (MW 324).

$$\text{Change in Mass } (\Delta M) = +56 \text{ daltons } (58 - 2)$$

The mechanism of this reaction presumably involves nucleophilic attack of one of the dichlorophen hydroxyl group oxygens onto a silicon in the dimethylsilicone polymer, causing scission of the silicone chain, followed by nucleophilic attack of the second dichlorophen hydroxyl group oxygen onto the same silicon, to form the cyclic dichlorophen silicone compound and a cleaved silicone polymer.

3.3.4 PFTBA/Heptacosia - anomalous m/z 197 ion in ion trap MS

Another unexpected mass spectrometric analysis related artefact was observed with a quadrupole ion trap MS system (Finnegan GCQ MS). When assessing the potential low mass/high mass spectrum balance of the ion trap MS system, as compared to a magnetic sector MS instrument, the EI mass spectra of perfluoro-tri-*n*-butylamine (PFTBA), the recommended MS calibrant, were compared on the two systems. For comparison, the NIST WebBook mass spectrum of PFTBA is summarised below (with my elemental formulae assignments):

Perfluoro-tri-*n*-butylamine

$C_{12}F_{27}N$

MW 671 (0%)

Theoretical molecular ion: m/z z 670.9510

Average MW: 671.0



Mass spectrometer calibrant. "Heptacosia" (heptacosafuorotributylamine)

671 (0) – M^+ $C_{12}F_{27}N^+$ absent m/z 670.95995

614 (1) – [M-57] loss of 3F (not usual aliphatic compound loss of C_4H_7) to $C_{12}F_{24}N^+$ m/z 613.96475

502 (5) – [M-169] loss of C_3F_7 to $C_9F_{20}N^+$ m/z 501.9711

414 (4) – [M-257] loss of C_4F_9 & F_2 to $C_8F_{16}N^+$ m/z 413.9775

264 (13) – [M-407] $C_4F_9NCF^+$ $C_5F_{10}N^+$ m/z 263.9871

219 (65) – [M-452] $C_4F_9^+$ m/z 218.9856

169 (3) – [M-502] $C_3F_7^+$ m/z 168.9888

131 (40) – [M-540] $C_3F_5^+$ m/z 130.9920

119 (9) – [M-552] $C_2F_5^+$ m/z 118.9920

114 (3) – [M-557] $C_2F_4N^+$ m/z 113.9967

113 (4) – [M-558] $C_3HF_4^+$ m/z 113.0014 – unexpected H from ion/molecule reaction(?)

100 (12) – [M-571] $C_2F_4^+$

69 (100) – [M-602] CF_3^+

Data from NIST mass spectrum: <http://webbook.nist.gov/cgi/cbook.cgi?ID=C311897&Units=SI&Mask=200#Mass-Spec>

Interestingly, the quadrupole ion trap mass spectrum of PFTBA, whilst exhibiting all the ions observed using the magnetic sector mass spectrometer, and with roughly similar relative abundances, also exhibited an abundant ion at m/z 197, with relative abundance of 20-100% of the base peak. The appearance of this unexpected ion was troubling, not least because it

proved impossible to reconcile its mass with the elemental composition of the precursor molecule.

MS/MS experiments were therefore undertaken, which indicated unambiguously that the m/z 197 ion was being generated from the ion at m/z 219, due to the perfluorobutyl ion $C_4F_9^+$. The transition of m/z 219 to m/z 197 is equivalent to a loss of 22 daltons. This was puzzling, as it does not correlate to any combination of C, F or N atoms. The most likely explanation for this transition was that the m/z 197 ion was being generated via an ion-molecule interaction between the highly reactive $C_4F_9^+$ ion and residual water vapour in the ion trap (Creaser & Wilkins, 2000). This reaction was facilitated by the extended residence time in the ion trap ion source as compared to the magnetic sector MS system.

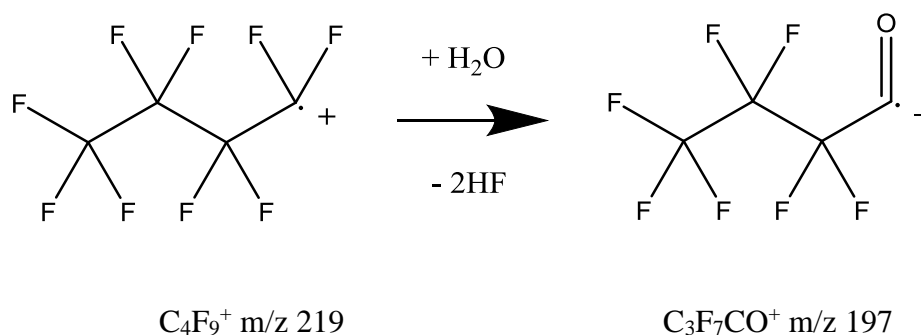
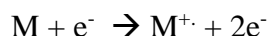


Figure 3.3.4(a). Reaction of PFTBA fragment ion m/z 219 ion with water in ion trap MS.

This is an example of a reactive ion being modified during storage and mass analysis in the MS ion trap. Although rare, the potential for this type of effect should be borne in mind when trying to interpret unexpected ions, especially during extended MS/MS studies.

3.3.5 Diphenylamine – doubly charged molecular ion m/z 84.5

The 30-70eV electron ionisation (EI⁺) spectra of most organic compounds exhibit only singly charged species, due to loss of one electron from the analyte molecule.



However some molecules, particularly those with delocalised electrons such as porphyrins and polynuclear aromatic hydrocarbons, can more readily lose two electrons and generate significant levels of doubly charged ions.

The mass spectrum of diphenylamine is the only pesticide where a doubly charged ion is detected or evident in this collection of spectra. The singly charged molecular ion of diphenylamine occurs at m/z 169. The doubly charged ion is at m/z 169/2, i.e. m/z 84.5. This explains the curious appearance of the NIST MS spectrum for diphenylamine (see <http://webbook.nist.gov/cgi/cbook.cgi?ID=C122394&Mask=200#Mass-Spec>), in which all the significant ions are accompanied by ^{13}C satellites, apart from m/z 84 (17% relative abundance). The m/z 84 ion is not due to a plausible, singly charged ion arising from diphenylamine (C_7^+ and $\text{C}_5\text{H}_{10}\text{N}^+$ are not likely).

Biphenyl may exhibit an M^{2+} at m/z 154/2 i.e. m/z 77, but this would be hidden beneath the $(\text{M}/2)^+$ ion due to C_6H_5^+ . The key diagnostic feature is the presence of ^{13}C isotope peaks, which would occur at 0.5 dalton separation. Unfortunately the data processing (smoothing and centroiding) algorithms of most MS data acquisition systems may typically remove these peaks.

3.3.6 Dimethylsilanediol – unusual SPME artefact GC peak

When performing solventless solid phase microextraction (SPME) experiments of aqueous samples, a short GC retention time peak was frequently observed. Its mass spectrum exhibited m/z 77 and little else. Surprisingly for such a small molecule, computer library searches produced no plausible fits. The ion at m/z 77 is often indicative of an aromatic compound, because it is the phenyl C_6H_5^+ ion - but generally a molecular ion would be observed, and no higher mass ions were evident in this case, other than the m/z 77 isotope peaks.

Closer inspection of the abundances of the isotope peaks was informative. The m/z 79 ion was approx. 4% of the base peak m/z 77, indicating the presence of sulphur or silicon. Initially the presence of silicon was discounted, because the low mass dimethylsilicone fragment ion most usually observed is $(\text{CH}_3)_2\text{Si}^+$ at m/z 73. However, TMS ethers exhibit an ion 2 daltons heavier at m/z 75 due to $(\text{CH}_3)_2\text{Si}(\text{OH})^+$. Logically, replacement of another CH_3

with OH would give $(\text{CH}_3)\text{Si}(\text{OH})_2^+$, with the required m/z 77 and associated silicon isotope peaks.

The most likely source of the material was hydrolysis of dimethylsilicone of the SPME fibre, so the missing fragment was most likely a methyl group, indicating that the mystery GC peak was due to dimethylsilanediol, $(\text{CH}_3)_2\text{Si}(\text{OH})_2$ ($\text{C}_2\text{H}_8\text{O}_2\text{Si}$ mw 92)

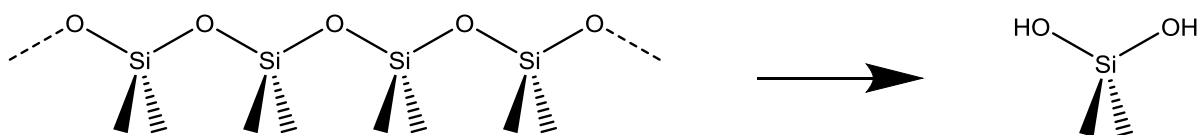


Figure 3.3.6(a). Hydrolysis of polydimethylsiloxane to dimethylsilanediol.

Ammonia chemical ionisation MS gave a pseudomolecular ion at m/z 110, which was ascribed to $[\text{M}+\text{NH}_4]^+$, confirming molecular weight of 92.

Accurate mass study confirmed that the elemental composition of the m/z 77 ion was consistent with the proposed ion formula of $(\text{CH}_3)\text{Si}(\text{OH})_2^+ \text{CH}_5\text{O}_2\text{Si}^+$ m/z 77.0059.

The synthesis of dimethylsilanediol, its chemistry, and analysis by GCMS and NMR has been reported (Varaprath 1997). This study confirmed the importance of this molecule in environmental fate studies of siloxanes, and describes its propensity to re-polymerise if not stored in dilute solution.

MS data and other details for dimethylsilanediol are included with the **GC Contaminants & Artefacts** section at the end of Appendix I.

The dimethylsilanediol spectrum and assignment were submitted and included in the NIST MS Database:

NIST MS Database (NIST 2015)

Name: Dihydroxydimethylsilane

Formula: C₂H₈O₂Si

MW: 92 Exact Mass: 92.029356 CAS#: 1066-42-8 NIST#: 282495 ID#: 220749 DB:
ar20110516

Other DBs: None

Comment: John P.G. Wilkins, Unilever Research, UK

I was pleased to discover that this NIST MS Database entry was instrumental in the identification of this dimethylsilicone hydrolysis product in the potable water supply of the NASA International Space Station (Rutz 2011).

3.4 Pesticides

3.4.1 Pesticide MS Data in Appendix I

Electron impact ionisation, positive ion (EI+) MS data for >500 pesticides and related compounds are summarised in Appendix 1. The choice of these compounds was based primarily on their importance for the UK national pesticide residue surveillance programme and for pesticide formulation quality studies, and the availability of reference materials. MS data for novel pesticides and for some chemical warfare agents have also been included, for completeness, and for comparison.

The data are annotated with rationally derived, fragment ion compositions for significant ions. Other observations regarding compound, toxicity, regulatory status etc. are also included.

3.4.2 Comparison with reference MS Data (e.g. NIST) & significant anomalies

When available, reported MS data were compared in order to validate these results. One of the most useful resources is the US National Institute of Standards & Technology (NIST) *Chemistry WebBook* (NIST 2014), which contains many pesticide mass spectra. The mass spectra were provided by the *NIST Mass Spectrometry Data Center* (supplementary data, such as the source of the spectrum, instrument type, instrument parameters, and the EPA MS number are displayed below the individual spectrum). Approximately half of the mass spectra described in this thesis are also represented in the NIST WebBook, and generally, the agreement was excellent. Some variation in relative ion abundances was observed, but this is to be expected when acquiring mass spectra on different systems. However, in some cases the NIST spectra were “weak”, i.e. they did not contain low abundance ions (such as ¹³C isotope peaks), and/or poorly resolved, so, e.g. polychlorinated species lacked weaker ions.

Occasionally, spectra which exhibited weak or absent molecular ions in this collection, in the NIST WebBook spectra were observed to exhibit protonated molecular (M+H)⁺ ions at “M+1”. This is most likely due to excessive material being introduced into the EI source, causing “auto-chemical ionisation”. (See e.g. carbophenothion oxon sulphoxide spectrum at <http://webbook.nist.gov/cgi/cbook.cgi?ID=C16662865&Units=SI&Mask=200#Mass-Spec> which exhibits m/z 343 (2%) rather than m/z 342.)

Major disparities between the spectra described in this document and with the NIST spectra were rare, but three exceptions were found:

Aldicarb sulphoxide - The NIST spectrum was different from that described here. It is very similar to that for aldicarb sulphone. This could be due to sample error or degradation.

Bensulide – The NIST spectrum of bensulide at <http://webbook.nist.gov/cgi/cbook.cgi?ID=C741582&Mask=200#Mass-Spec> exhibits abundant ions at m/z 77, 170 and 141. They appear to be due to N-butyl benzene sulphonamide, a known GC contaminant, rather than bensulide. See GC artefact compounds at end of Appendix I. (This has been reported to NIST.)

Butocarboxim – The EI mass spectral data reported here for butocarboxim are rather different from the NIST & Restek data, which both report a base peak at m/z 86 (100%) $C_6H_8NO^+$, and a weaker ion at m/z 108 (5%).

See <http://webbook.nist.gov/cgi/cbook.cgi?ID=C34681102&Units=CAL&Mask=3F92> and <http://www.restek.com/compound/view/34681-10-2/Butocarboxim>

The reason for the disparity of the butocarboxim spectra was not clear. This appears to be a very labile compound, perhaps sensitive to MS ion source conditions.

3.4.3 Similar MS fragmentations in EI+ data and ESI+ MS/MS data

The collected EI+ MS data contained in Appendix I present an interesting and useful resource, displaying the range of compounds used as pesticides and how they behave during GC and MS analysis. Although they are based on EI+ ionisation, the fragments and fragmentation processes observed often find parallels in the ions and fragmentation pathways found during soft ionisation (e.g. electrospray) MS/MS studies. For example, in the ESI+ MS/MS data reported by Greulich (2013) for 300 pesticides, the daughter ions of the protonated molecular ions are described:

Aldicarb m/z 209 to m/z 86 and 116 (m/z 115 in EI MS)

Acephate m/z 184 to 143 (m/z 142 in EI MS)

Azamethiphos m/z 325 to 183 and 139 (both present in EI MS)

Azinphos methyl m/z 318 to m/z 132 and 160 (both present in EI MS)

Azoxystrobin m/z 404 to 372 and 344 (both present in EI MS)

Demeton-S-methyl m/z 248 (M+18) to m/z 89, 61 (m/z 88 and 60 in EI MS)

Malathion m/z 331 to m/z 127, 99 (both present in EI MS)

Pyridaphenthion m/z 341 to m/z 189, 205 (m/z 188 and 204 in EI MS)

However, some compounds appear to exhibit different processes: e.g.

Buprofezin m/z 306 → m/z 201 and 186, but the most abundant EI ions are at m/z 105, 172, 57, 106, 104, 77, 41, 83. It can be seen that the first loss (306 to 201) is equivalent to the most abundant ion (m/z 105) in the EI MS.

In this small but representative selection of compounds, many of the ESI MS/MS ions observed are also present as abundant ions in the EI+ spectra (although some are 1 dalton (H) lighter), or the loss corresponds to EI ions.

For this reason, the proposed ion structures reported in this work should find application in ESI+ MS/MS studies.

3.5 Organophosphorus compounds

Organophosphorus (OP) compounds comprise a significant proportion of the data collection. These compounds are of particular interest and importance, not least because of their potentially extreme toxicity (Eto 1974). The following review of potential toxic effects of OP compounds is from HSE (2015). The Health and Safety Executive (UK) are charged with ensuring that damaging effects of pesticides used in the UK are properly understood and suitably risk assessed. It is included here because it addresses several specific areas of public concern regarding the use of OP pesticides.

There are many different organophosphorus esters and they differ in their properties. Many OPs inhibit an enzyme known as acetylcholinesterase. Some OPs react with other proteins such as neuropathy target esterase. Inhibitors of acetylcholinesterase affect certain nerve junctions in animals, as well as parasympathetic effector sites (the heart, lungs, stomach, intestines, urinary bladder, prostate, eyes and salivary glands). The transmission of impulses across nerve junctions involves the release of a transmitter chemical, which, in the case of many nerves, is acetylcholine [$(\text{CH}_3)_3\text{N}^+\text{CH}_2\text{CH}_2\text{OCOCH}_3$]. To stop the nerve continuing to transmit the message, the transmitter, acetylcholine, must be broken down immediately after

it has had its effect. This breakdown is brought about by an enzyme, acetylcholinesterase. By inhibiting the enzyme acetylcholinesterase, OPs prevent the nerve junction from functioning properly (Colovic 2013).

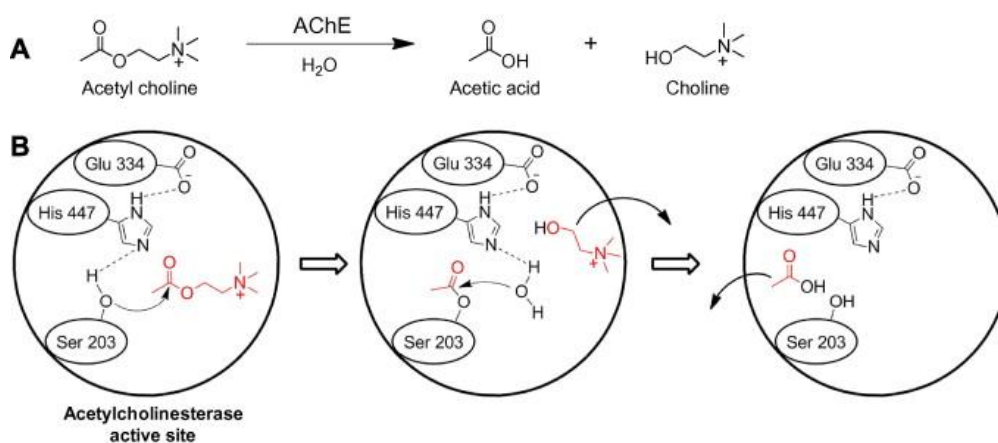


Figure 3.5(a). Acetylcholinesterase mode of action. OP compounds inhibit irreversibly this action by phosphorylating the active site (Ser 203).

(Image courtesy of Dr Amit Kessel website: amit1b.wordpress.com)

In the case of most OPs and all medicinal and pesticidal anticholinesterase OP products this effect is either reversible, the rate of re-activation of the enzyme being dependent on the chemical structure of the OP, or recoverable by synthesis of new enzyme.

OPs can be carefully selected, on the basis of their chemical structure, so that they are very effective agents against their target pest or insect and the risk to humans can be controlled by following the recommended precautions. The efficacy of OP products as pesticides and as human and veterinary medicines relates to the inhibition of acetylcholinesterase in the target pest species.

In humans, anticholinesterase OPs have broadly similar actions to those seen in other species. Acetylcholinesterase inhibition causes acute effects in humans and other mammals. The symptoms in humans, which generally occur when acetylcholinesterase activity has been reduced by about 50%, may include: headache, exhaustion and mental confusion together with blurred vision, sweating, salivation, chest tightness, muscle twitching and abdominal cramps. The severity of the effects depends on the degree of acetylcholinesterase inhibition. The more severe effects can include muscle paralysis leading to severe difficulty in breathing, so requiring respiratory support. Convulsions and unconsciousness can occur. Recovery

depends on elimination of the OP product from the body and return of acetylcholinesterase activity. However, as noted in paragraph 1, not all OPs are anticholinesterases, and compounds such as glyphosate exhibit quite different toxic effects. Furthermore some non-OPs are anticholinesterases and these compounds have similar toxicity to anticholinesterase OPs, an example of this being the carbamate insecticides. Some OPs may also work by another mechanism, that is, causing an OP-induced delayed effect on the peripheral nerves. This is known as OP induced delayed polyneuropathy (OPIDP). OPIDP is a delayed effect caused by die-back in the long nerves, thus affecting the limb extremities. OPIDP is associated with, but not necessarily caused by, inhibition of another enzyme known as neuropathy target esterase (NTE). The capacity of OPs to inhibit NTE and cause OPIDP does not correlate with their capacity to inhibit acetylcholinesterase. Any OP product which is shown by laboratory tests to be likely to produce OPIDP in humans, will not be authorised in the UK. A number of studies of OP products currently or previously used in UK sheep dip, have shown them to have no potential to produce delayed polyneuropathy in animal tests.

Another known toxicological effect of OPs in humans has been termed the intermediate syndrome. This can follow severe acute poisoning, sometimes as a result of a suicide attempt, and causes temporary paralysis of the proximal muscles (muscles nearest to the central line of the body e.g. respiratory, neck and upper part of limb muscles; the distal muscles of the limb are not affected so grip strength may be preserved). Since this includes the respiratory muscles, respiratory support is necessary to keep the patient alive. The precise reasons for the development of intermediate syndrome are not clear but explanations which have been advanced include myopathy (muscular damage), depolarisation blockade (blocking impulses at the neuromuscular junction and paralysing the muscles) and Guillain-Barre syndrome-like effects (muscle weakness, numbness and pins and needles in limbs).

There are postulated long-term effects of OPs following long-term low-level exposure. Some studies on low-level exposure have shown subtle effects (e.g. slower reaction times) in specialised tests for neurological function, whereas others have shown no change in different neuropsychological and neurophysiological tests. The alleged theories and mechanisms are sometimes not related to acetylcholinesterase activity.

More detailed descriptions of the underlying chemical processes are also available (e.g. Marrs 2004). One particularly significant issue is the potential impact of chemical transformations

of OP pesticides (e.g. oxidation or isomerisation):

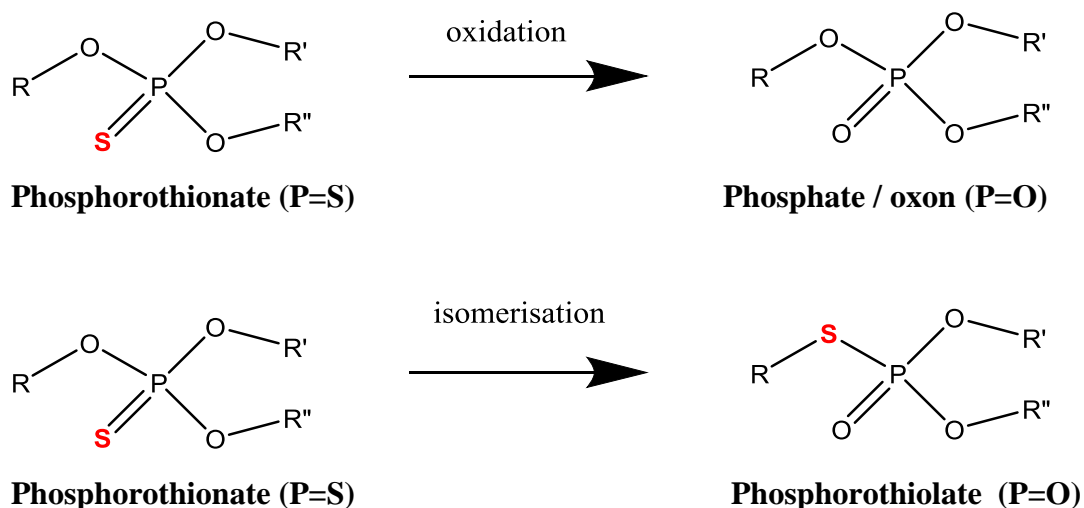


Figure 3.5(b). Potentiation of OP toxicity by oxidation and isomerisation.

Both of these processes can dramatically increase the toxicity of the OP compound. An example is malathion - malaoxon and isomalathion (see Appendix I) are both much more toxic than the parent (P=S) compound.

Table 3.5(a). Toxicity of malathion and related compounds.

	Acute oral LD50 for rat
Malathion	1,500 mg/kg
Malaoxon	100 mg/kg
Isomalathion	100 mg/kg

There are 193 OP compounds in the data collection in the Appendix:

107 pesticides

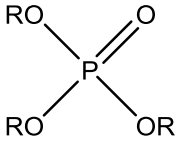
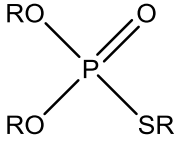
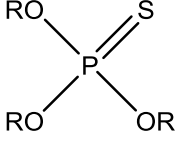
82 pesticide metabolites, technical contaminants and related compounds.

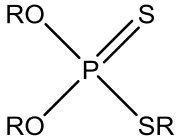
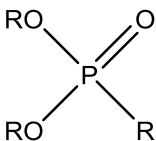
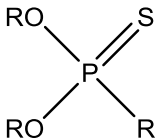
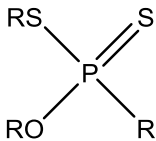
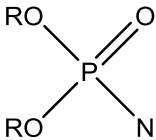
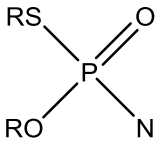
4 chemical warfare (CW) agents Cyclosarin, Sarin , Soman & Tabun

The chemical classes of the 107 OP pesticides and CW agents are described in Table 1.

An attempt to rationalise the MS fragmentation pathways of the organophosphorus esters is described below.

Table 3.5(b). Organophosphorus pesticides and chemical warfare agents included in Appendix I, grouped by OP ester type.

Organophosphorus ester class & number of compounds	Compound name	
A. Phosphates (9) 	Chlorfenvinphos Dichlorvos Heptenophos Mevinphos / Phosdrin Monocrotophos	Naled Phosphamidon Tetrachlorvinphos / Stirofos TEPP (tetraethyl pyrophosphate)
B. Phosphorothiolate (9) 	Azamethiphos Demephion-S Demeton-S Demeton-S-methyl Iprobenfos / Kitazin	Omethoate Oxydeprofos Profenofos Vamidothion
C. Phosphorothionate (40) 	Bromophos Bromophos-ethyl Chlorethoxyfos Chlorpyrifos Chlorpyrifos-methyl Chlorthiophos I Chlorthiophos II Chlorthiophos III Coumaphos Cyanophos Demephion-O Demeton-O Diazinon Dicapthon Dichlofenthion Dioxabenzofos / Salithion Etrimfos Famphur Fenchlorphos / Ronnel Fenitrothion	Fensulfothion Fenthion Iodofenphos Isazofos Methacrifos Parathion Parathion-methyl Phoxim Pirimiphos-ethyl Pirimiphos-methyl Pyrazophos Pyridaphenthion Pyrimitate Quinalphos Sulfotep Tebupirimfos Temephos / "Abate Thionazin Tolclofos-methyl Triazophos
D. Phosphorodithioate (27)	Anilofos Azinphos-ethyl Azinphos-methyl	Malathion Mecarbam Methidathion

	<p>Bensulide Cadusafos Carbophenothion / Trithion Chlormephos Dialifos / Dialifor Dimethoate Dioxathion Disulfoton Ethion Ethoprophos Formothion</p>	<p>Methyl-trithion / Methyl Carbophenothion Phenkapton Phenthoate Phorate Phosalone Phosmet Prothiofos / Tokuthion Sulprofos / Bolstar Terbufos Thiometon</p>
<p>E. Phosphonates (2)</p> 	<p>Trichlorfon / Metrifonate Ethephon (chloroethyl phosphonic acid)</p>	
<p>F. Phosphonothioates (5)</p> 	<p>EPN Leptophos Trichloronat Cyanofenphos Quintiofos</p>	
<p>G. Phosphonodithioate (1)</p> 	<p>Fonofos</p>	
<p>H. Phosphoramidates (5)</p> 	<p>Crufomate Fosthietan Mephosfolan Phosfolan Fenamiphos</p>	
<p>I. Phosphoramidothiolates (2)</p> 	<p>Acephate Methamidophos</p>	
<p>J. Phosphoramidothionates (4)</p>	<p>Butamifos Propetamphos</p>	

$\begin{array}{c} \text{RO} \quad \text{S} \\ \diagdown \quad \diagup \\ \text{P} \\ \diagup \quad \diagdown \\ \text{RO} \quad \text{N} \end{array}$	Ditalimfos Isofenphos
K. Phosphorodiamide (1) $\begin{array}{c} \text{RO} \quad \text{O} \\ \diagdown \quad \diagup \\ \text{P} \\ \diagup \quad \diagdown \\ \text{N} \quad \text{N} \end{array}$	Schradan (di-ester)
L. Phosphorotriamide (1) $\begin{array}{c} \text{N} \quad \text{O} \\ \diagdown \quad \diagup \\ \text{P} \\ \diagup \quad \diagdown \\ \text{N} \quad \text{N} \end{array}$	Triamiphos
M. Phosphonofluoridate (3) $\begin{array}{c} \text{RO} \quad \text{O} \\ \diagdown \quad \diagup \\ \text{P} \\ \diagup \quad \diagdown \\ \text{R} \quad \text{F} \end{array}$	Cyclosarin / GF Sarin / GB Soman / GD
N. Phosphoroamidocyanidate (1) $\begin{array}{c} \text{RO} \quad \text{O} \\ \diagdown \quad \diagup \\ \text{P} \\ \diagup \quad \diagdown \\ \text{N} \quad \text{CN} \end{array}$	Tabun

3.6 Review of MS data for characteristic OP ions

The main phosphorus containing EI+ MS fragment ions of the pesticides and CW agents are reviewed and rationalised below, in order to assess the feasibility of identifying characteristic, diagnostic ions which could be exploited in screening for a range of OP compounds:

A. Phosphate pesticides (RO)₃P=O

A.1 O,O-dimethyl phosphates

Dichlorvos

145,147 (10,3) – [M-75] (CH₃O)₂(HO)PCl⁺ C₂H₇ClO₃P⁺ m/z 144.9821 etc.
109 (100) – [M-111] (CH₃O)₂PO⁺ C₂H₆O₃P⁺ m/z 109.0055
79 (15) – [M-141] (CH₃O)(HO)P⁺ CH₄O₂P⁺ m/z 78.9949
47 (10) – [M-173] PO⁺ m/z 46.9687

Heptenophos

127 (20) – [M-123] (CH₃O)₂(HO)₂P⁺ m/z 127.0106
109 (10) – [M-141] (CH₃O)₂PO⁺ C₂H₆O₃P⁺ m/z 109.0055

Mevinphos

127 (100) – [M-97] (CH₃O)₂(HO)₂P⁺ C₂H₆O₄P⁺ m/z 127.0160
109 (20) – [M-115] (CH₃O)₂P=O⁺ C₂H₆O₃P⁺ m/z 109.0055
79 (5) – [M-145] (CH₃O)(HO)P⁺ CH₄O₂P⁺ m/z 78.9949

Monocrotophos

127 (100) – [M-96] (CH₃O)₂(HO)₂P⁺ C₂H₈O₄P⁺ m/z 127.0160
109 (15) – [M-114] (CH₃O)₂P=O⁺ C₂H₆O₂P⁺ m/z 109.0055

Naled

145,147 (40,10) – [M-233] (CH₃O)₂(HO)PCl⁺ C₂H₇ClO₃P⁺ m/z 144.9821 etc. [rearrangement]
109 (100) – [M-269] (CH₃O)₂P=O⁺ C₂H₆O₃P⁺ m/z 109.0055
79 (15) – [M-141] (CH₃O)(HO)P⁺ CH₄O₂P⁺ m/z 78.9949
47 (10) – [M-173] PO⁺ m/z 46.9687

Phosphamidon

127 (100) – [M-172] (CH₃O)₂(HO)₂P⁺ C₂H₈O₄P⁺ m/z 127.0160
109 (20) – [M-190] (CH₃O)₂PO⁺ C₂H₆O₃P⁺ m/z 109.0055

Tetrachlorvinphos

109 (100) – [M-255] (CH₃O)₂P=O⁺ C₂H₆O₃P⁺ m/z 109.0055
93 (5) – [M-271] (CH₃O)₂P⁺ C₂H₆O₂P⁺ m/z 93.0105
79 (15) – [M-285] (CH₃O)(HO)P⁺ CH₄O₂P⁺ m/z 78.9949

A.2 O,O-diethyl phosphates

Chlorfenvinphos –

109 (45) – [M-249] (CH₃CH₂O)(HO)PO⁺ C₂H₆O₃P⁺ m/z 109.0055

81 (60) – [M-277] (HO)₂PO⁺ H₂O₃P⁺ m/z 80.9742

TEPP (tetraethyl pyrophosphate)

99 (40) – [M-191] (HO)₄P⁺ H₄O₄P⁺ m/z 98.9847

81 (40) – [M-209] (HO)₂P=O⁺ H₂O₂P⁺ m/z 80.9742

Table A1. Phosphate pesticides phosphorus-containing ions.

Observed ion	Notional assigned structure	Empirical formula	Theoretical accurate mass
m/z 145	(CH ₃ O) ₂ (HO)PCl ⁺	C ₂ H ₇ ClO ₃ P ⁺	m/z 144.9821
m/z 127	(CH ₃ O) ₂ (HO) ₂ P ⁺	C ₂ H ₈ O ₄ P ⁺	m/z 127.0160
m/z 109	(CH ₃ O) ₂ P=O ⁺ or (CH ₃ CH ₂ O)(HO)PO ⁺	C ₂ H ₆ O ₃ P ⁺	m/z 109.0055
m/z 99	(HO) ₄ P ⁺	H ₄ O ₄ P ⁺	m/z 98.9847
m/z 81	(HO) ₂ PO ⁺	H ₂ O ₃ P ⁺	m/z 80.9742
m/z 79	(CH ₃ O)(HO)P ⁺	CH ₄ O ₂ P ⁺	m/z 78.9949
m/z 47	PO ⁺	PO ⁺	m/z 46.9687

Table A2. Relative abundances (%) of phosphorus ions of phosphate compounds.

Pesticide	m/z 145	m/z 127	m/z 109	m/z 99	m/z 81	m/z 79	m/z 47
<i>A1. O,O-dimethyl phosphates</i>							
Dichlorvos	10		100			15	10
Heptenophos		20	10				
Mevinphos		100	20			5	
Monocrotophos		100	15				
Naled	40		100			15	10
Phosphamidon		100	10				
Tetrachlorvinphos		100					15
<i>A2. O,O-diethyl phosphates</i>							
Chlorfenvinphos			45		60		
TEPP				40	40		
Total (out of 9)	2	5	7	1	2	3	3

The most commonly observed phosphorus containing ions for the phosphate compounds were:

m/z 109 – exhibited by 77% (7 out of 9 compounds)

and m/z 127 – exhibited by 56% (5 out of 9 compounds).

Other lower mass ions were also observed (m/z 99, 81, 79 and 47) and these could be used to provide additional supporting evidence of the identification of an unknown phosphate ester.

B. Phosphorothiolate pesticides (RO)₂P=O(SR)

B.1 O,O-dimethyl phosphorothiolates

Azamethiphos

125 (80) – [M-199] (CH₃O)₂P=O.S⁺ C₂H₆O₃PS⁺ m/z 124.9826
109 (100) – [M-183] (CH₃O)₂P=O⁺ C₂H₆O₃P⁺ m/z 109.0055
79 (20) – [M-213] (CH₃O)(HO)P=O⁺ CH₄O₂P⁺ m/z 78.9949

Demephion-S

142 (15) – [M-74] (CH₃O)₂(HO)PS⁺ C₂H₇O₃PS⁺ m/z 141.9854
112 (13) – [M-104] (CH₃O)(HO)(HS)P⁺ CH₅OPS⁺ m/z 111.9748
109 (100) – [M-107] (CH₃O)₂P=O⁺ C₂H₆O₃P⁺ m/z 109.0055
79 (7) – [M-137] (CH₃O)(HO)P⁺ CH₄O₂P⁺ m/z 78.9949

Demeton-S-methyl

142 (12,5) – [M-88] (CH₃O)₂(HO)P=S⁺ C₂H₇O₃PS⁺ m/z 141.9854
112 (8) – [M-118] (CH₃O)(HO)(HS)P⁺ CH₅O₂PS⁺ m/z 111.9748
109 (100) – [M-121] (CH₃O)₂P=O⁺ C₂H₆O₃P⁺ m/z 109.0055
79 (8) – [M-151] (CH₃O)(HO)P⁺ CH₄O₂P⁺ m/z 78.9949

Omethoate

156 (100) – [M-57] loss of CH₃NCO to CH₂SP(OH)(OCH₃)₂⁺ C₃H₉O₃PS⁺ m/z 156.0010
141 (10) – [M-72] (CH₃O)₂P=O.S⁺ C₂H₆O₃PS⁺ m/z 140.9775
126 (15) – [M-87] (CH₃O)₂(HS)P⁺ C₂H₇O₂PS⁺ m/z 125.9904
110 (100) – [M-103] (CH₃O)₂(HO)P⁺ C₂H₇O₃P⁺ m/z 110.0133
109 (25) – [M-104] (CH₃O)₂P=O⁺ C₂H₆O₃P⁺ m/z 109.0055
79 (30) – [M-134] (CH₃O)(HO)P⁺ CH₄O₂P⁺ m/z 78.9949

Oxydeprofos

143 (10) – [M-117] (CH₃O)₂(HO)(HS)P⁺ C₂H₈O₃PS⁺ m/z 142.9932
125 (35) – [M-135] (CH₃O)₂P=S⁺ C₂H₆O₂PS⁺ m/z 124.9826
109 (25) – [M-151] (CH₃O)₂P=O⁺ C₂H₆O₃P⁺ m/z 109.0055
79 (10) – [M-181] (CH₃O)(HO)P⁺ CH₄O₂P⁺ m/z 78.9949

Vamidothion

169 (5) – [M-118] (CH₃O)₂P=S.SCH₂CH₂⁺ C₂H₇O₃PS⁺ m/z 169.0088
142 (15) – [M-145] (CH₃O)₂(HO)PS⁺ C₂H₇O₃PS⁺ m/z 141.9854
109 (15) – [M-178] (CH₃O)₂P=O⁺ C₂H₆O₃P⁺ m/z 109.0055

B.2 O,O-diethyl phosphorothiolates

Demeton-S

170 (5) – [M-88] (CH₃CH₂O)₂(HO)P=S⁺ C₄H₁₁O₃PS⁺ m/z 170.0167
142 (5) – [M-116] (CH₃CH₂O)(HO)₂P=S⁺ C₂H₇O₃PS⁺ m/z 141.9854
114 (10) – [M-144] (HO)₃P=S⁺ H₃O₃PS⁺ m/z 113.9541

B.3 O,O-di-isopropyl phosphorothiolate

Iprobenfos

65 (10) – [M-223] (HO)₂P⁺ H₂O₂P⁺ m/z 64.9792

B.4 O-ethyl,S-propyl phosphorothiolate

Profenofos

139 (100) – [M-233] (C₃H₇S)(HO)P=O⁺ C₃H₈O₂PS⁺ m/z 138.9983

125 (45) – [M-247]] (CH₃CH₂O)(HS)P=O⁺ CH₆O₂PS⁺ m/z 124.9826 - not usual OP m/z 125

97 (85) – [M-275] (HO)₂PS⁺ H₂O₂PS⁺ m/z 96.9413

Table B1. Phosphorothiolate (RO)₂P=O.SR' pesticide phosphorus containing EI+ ions.

Observed ion	Notional assigned structure	Empirical formula	Theoretical accurate mass
m/z 170	(CH ₃ CH ₂ O) ₂ (HO)P=S ⁺	C ₄ H ₁₁ O ₃ PS ⁺	m/z 170.0167
m/z 169	(CH ₃ O) ₂ P=S.SCH ₂ CH ₂ ⁺	C ₂ H ₇ O ₃ PS ⁺	m/z 169.0088
m/z 156	CH ₂ SP(OH)(OCH ₃) ₂ ⁺	C ₃ H ₉ O ₃ PS ⁺	m/z 156.0010
m/z 143	(CH ₃ O) ₂ (HO)(HS)P ⁺	C ₂ H ₈ O ₃ PS ⁺	m/z 142.9932
m/z 142	(CH ₃ O) ₂ (HO)PS ⁺	C ₂ H ₇ O ₃ PS ⁺	m/z 141.9854
m/z 141	(CH ₃ O) ₂ P=O.S ⁺	C ₂ H ₆ O ₃ PS ⁺	m/z 140.9775
m/z 139	(CH ₃ CH ₂ CH ₂ S)(HO)P=O ⁺	C ₃ H ₈ O ₂ PS ⁺	m/z 138.9983
m/z 126	(CH ₃ O) ₂ (HS)P ⁺	C ₂ H ₇ O ₂ PS ⁺	m/z 125.9904
m/z 125	(CH ₃ O) ₂ P=O.S ⁺	C ₂ H ₆ O ₃ PS ⁺	m/z 124.9826
m/z 114	(HO) ₃ P=S ⁺	H ₃ O ₃ PS ⁺	m/z 113.9541
m/z 112	(CH ₃ O)(HO)(HS)P ⁺	CH ₅ OPS ⁺	m/z 111.9748
m/z 110	(CH ₃ O) ₂ (HO)P ⁺	C ₂ H ₇ O ₃ P ⁺	m/z 110.0133
m/z 109	(CH ₃ O) ₂ P=O ⁺	C ₂ H ₆ O ₃ P ⁺	m/z 109.0055
m/z 97	(HO) ₂ PS ⁺	H ₂ O ₂ PS ⁺	m/z 96.9413
m/z 79	(CH ₃ O)(HO)P=O ⁺	CH ₄ O ₂ P ⁺	m/z 78.9949
m/z 65	(HO) ₂ P ⁺	H ₂ O ₂ P ⁺	m/z 64.9792

Table B2. Relative abundances (%) of phosphorus ions of phosphorothiolate pesticides.

Pesticide	Phosphorus containing ions (m/z)															
	65	79	97	109	110	112	114	125	126	139	141	142	143	156	169	170
<i>B.1 O,O-dimethyl phosphorothiolates</i>																
Azamethiphos		20		100				80								
Demephion-S		7		100		13						15				
Demeton-S-methyl		8		100		8						12				
Omethoate		30		25	100				15		10			100		
Oxydeprofos		10		25				35					10			
Vamidothion				15							15					5
<i>B.2 O,O-diethyl phosphorothiolates</i>																
Demeton-S							10					5				5
<i>B.3 O,O-di-isopropyl phosphorothiolate</i>																
Iprobenfos	10															
<i>B.4 O-ethyl,S-propyl phosphorothiolate</i>																
Profenofos			85					45*		100						
Total number of times ion observed in 9 OP spectra	1	5	1	6	1	2	1	3	1	1	2	3	1	1	1	1

The most commonly observed phosphorus containing ions for the phosphorothiolate compounds were:

m/z 109 again – exhibited by 67% (6 out of 9 compounds)

and m/z 79 – exhibited by 56% (5 out of 9 compounds).

C. Phosphorothionate pesticides (RO)₃P=S

C.1 O,O-dimethyl phosphorothionates

Bromophos

125 (75) – [M-237] (CH₃O)₂P=S⁺ C₂H₆O₂PS⁺ m/z 124.9826

109 (30) – [M-255] (CH₃O)₂P=O⁺ C₂H₆O₃P⁺ m/z 109.0055

47 (30) – [M-317] PO⁺ m/z 46.9687

Chlorpyrifos-methyl

125 (100) – [M-196] (CH₃O)₂P=S⁺ C₂H₆O₂PS⁺ m/z 124.9826

109 (20) – [M-212] (CH₃O)₂P=O⁺ C₂H₆O₃P⁺ m/z 109.0055

93 (25) – [M-228] (CH₃O)₂P⁺ C₂H₆O₂P⁺ m/z 93.0105

79 (35) – [M-242] (CH₃O)(HO)P⁺ CH₄O₂P⁺ m/z 78.9949

63 (15) – [M-258] PS⁺ m/z 62.9458

47 (30) – [M-274] PO⁺ m/z 46.9687 and/or CH₃S⁺ m/z 46.99555

Cyanophos

125 (60) – [M-118] (CH₃O)₂P=S⁺ C₂H₆O₂PS⁺ m/z 124.9826
109 (100) – [M-134] (CH₃O)₂P=O⁺ C₂H₆O₃P⁺ m/z 109.0055 [O/S swap]
79 (30) – [M-164] (CH₃O)(HO)P⁺ CH₄O₂P⁺ m/z 78.9949
63 (15) – [M-180] PS⁺ m/z 68.9458
47 (30) – [M-196] PO⁺ m/z 46.9687

Demephion-O

143 (9) – [M-75] (CH₃O)₂(HS)(HO)P⁺ C₂H₈O₃PS⁺ m/z 142.9932
125 (5) – [M-91] (CH₃O)₂P=S⁺ C₂H₆O₂PS⁺ m/z 124.9826
109 (3) – [M-107] (CH₃O)₂P=O⁺ C₂H₆O₃P⁺ m/z 109.0055 [O/S swap]

Dicapthion

125 (65) – [M-172] (CH₃O)₂P=S⁺ C₂H₆O₂PS⁺ m/z 124.9826
109 (20) – [M-188] (CH₃O)₂P=O⁺ C₂H₆O₃P⁺ m/z 109.0055
79 (25) – [M-218] (CH₃O)(HO)P⁺ CH₄O₂P⁺ m/z 78.9949
63 (20) – [M-234] PS⁺ m/z 62.9458

Etrimfos

125 (55) – [M-167] (CH₃O)₂PS⁺ C₂H₆O₂PS⁺ m/z 124.9826
93 (20) – [M-199] (CH₃O)₂P⁺ C₂H₆O₂P⁺ m/z 93.0105
79 (20) – [213] (CH₃O)(HO)P⁺ CH₄O₂P⁺ m/z 78.9949

Famphur

125 (25) – [M-200] (CH₃O)₂PS⁺ C₂H₆O₂PS⁺ m/z 124.9826
109 (15) – [M-216] (CH₃O)₂PO⁺ C₂H₆O₃P⁺ m/z 190.0055
93 (30) – [M-232] (CH₃O)₂P⁺ C₂H₆O₂P⁺ m/z 93.0105

Fenclorphos

125 (60) – [M-195] (CH₃O)₂PS⁺ C₂H₆O₂PS⁺ m/z 124.9826
109 (25) – [M-211] (CH₃O)₂P=O⁺ C₂H₆O₃P⁺ m/z 109.0055 [O/S swap]
93 (25) – [M-227] (CH₃O)₂P⁺ C₂H₆O₂P⁺ m/z 93.0105
79 (25) – [M-241] (CH₃O)(HO)P⁺ CH₄O₂P⁺ m/z 78.9949
47 (20) – [M-273] PO⁺ m/z 46.9687

Fenitrothion

125 (100) – [M-152] (CH₃O)₂P=S⁺ C₂H₆O₂PS⁺ m/z 124.9826
109 (90) – [M-168] (CH₃O)₂P=O⁺ C₂H₆O₃P⁺ m/z 109.0055
93 (35) – [M-184] (CH₃O)₂P⁺ C₂H₆O₂P⁺ m/z 93.0105
79 (30) – [M-198] (CH₃O)(HO)P⁺ CH₄O₂P⁺ m/z 78.9949
63 (15) – [M-214] PS⁺ m/z 62.9458
47 (35) – [M-230] PO⁺ m/z 46.9687

Fenthion

125 (10) – [M-153] (CH₃O)₂P=S⁺ C₂H₆O₂PS⁺ m/z 124.9826
109 (10) – [M-169] (CH₃O)(HO)P=O⁺ C₂H₆O₃P⁺ m/z 109.0055
93 (10) – [M-185] (CH₃O)₂P⁺ C₂H₆O₂P⁺ m/z 93.0105
79 (10) – [M-199] (CH₃O)(HO)P⁺ CH₄O₂P⁺ m/z 78.9949

Iodofenphos

125 (35) – [M-287] (CH₃O)₂P=S⁺ C₂H₆O₂PS⁺ m/z 124.9826
109 (20) – [M-303] (CH₃O)₂P=O⁺ C₂H₆O₃P⁺ m/z 109.0055 [O/S swap]
93 (20) – [M-319] (CH₃O)₂P⁺ C₂H₆O₂P⁺ m/z 93.0105
79 (20) – [M-333] (CH₃O)(HO)P⁺ CH₄O₂P⁺ m/z 78.9949

47 (15) – [M-365] PO⁺ m/z 46.9687

Methacrifos

125 (100) – [M-115] (CH₃O)₂P=S⁺ C₂H₆O₂PS⁺ m/z 124.9826

110 (35) – [M-130] (CH₃O)₂P(OH)⁺ C₂H₇O₃P⁺ m/z 110.0133

93 (60) – [M-147] (CH₃O)₂P⁺ C₂H₆O₂P⁺ at m/z 93.0105

79 (30) – [M-161] (CH₃O)(HO)P⁺ CH₄O₂P⁺ m/z 78.9949

47 (20) – [M-193] PO⁺ m/z 46.9687

Parathion-methyl

125 (80) – [M-138] (CH₃O)₂P=S⁺ C₂H₆O₂PS⁺ m/z 124.9826

109 (100) – [M-154] (CH₃O)₂P=O⁺ C₂H₆O₃P⁺ m/z 109.0055 (O/S swap)

93 (20) – [M-170] (CH₃O)₂P⁺ C₂H₆O₂P⁺ m/z 93.0105

79 (30) – [M-184] (CH₃O)(HO)P⁺ CH₄O₂P⁺ m/z 78.9949

Pirimiphos-methyl

125 (50) – [M-180] (CH₃O)₂PS⁺ C₂H₆O₂PS⁺ m/z 124.9826

93 (30) – [M-212] (CH₃O)₂P⁺ C₂H₆O₂P⁺ m/z 93.0105

Temephos

125 (20) – [M-341] (CH₃O)₂PS⁺ C₂H₆O₂P⁺ m/z 124.9826

109 (5) – [M-357] (CH₃O)₂PO⁺ C₂H₆O₃P⁺ m/z 109.0055

93 (20) – [M-373] (CH₃O)₂P⁺ C₂H₆O₂P⁺ m/z 93.0105

Tolclofos-methyl

125 (30) – [M-175] (CH₃O)₂P=S⁺ C₂H₆O₂PS⁺ m/z 124.9826

79 (20) – [M-221] (CH₃O)(HO)P⁺ CH₄O₂P⁺ m/z 78.9949

63 (10) – [M-237] PS⁺ m/z 62.9458

C.2 O,O-diethyl phosphorothionates

Bromophos-ethyl

125 (25) – [M-67] (CH₃CH₂O)(HO)P=S⁺ C₂H₆O₂PS⁺ m/z 124.9826

109 (20) – [M-283] (CH₃CH₂O)(HO)P=O⁺ C₂H₆O₃P⁺ m/z 109.0055

97 (100) – [M-295] (HO)₂P=S⁺ H₂O₂PS⁺ m/z 96.9513

Chlorethoxyfos - O,O-diethyl (RS)-O-(1,2,2,2-tetrachloroethyl) phosphorothioate (P=S)

334,336,338 (5,8,4) – M⁺

299,301,303 (50,50,10) – [M-35] loss of Cl to C₆H₁₁Cl₃O₃PS⁺ m/z 298.9232 etc.

271,273,275 (10,10,3) – [M-63] loss of Cl & C₂H₄ to C₄H₇Cl₃O₃PS⁺ m/z 270.8919 etc.

263,265,267 (10,7,2) – [M-71] loss of HCl₂ to C₆H₁₀Cl₂O₃PS⁺ m/z 262.9465 etc.

243,245,247 (10,12,3) – [M-91] loss of Cl & 2C₂H₄ to C₂H₃Cl₃O₃PS⁺ m/z 242.8606 etc.

153 (100) – [M-181] (CH₃CH₂O)₂P=S⁺ C₄H₁₀O₂PS⁺ m/z 153.0139

125 (40) – [M-209] (CH₃CH₂O)(HO)P=S⁺ C₂H₆O₂PS⁺ m/z 124.9826

97 (85) – [M-207] (HO)₂P=S⁺ H₂O₂PS⁺ m/z 96.9513

65 (7) – [M-269] (HO)₂P⁺ H₂O₂P⁺ m/z 64.9792

Chlorpyrifos

153 (5) – [M-196] (CH₃CH₂O)₂P=S⁺ C₄H₁₀O₂PS⁺ m/z 153.0139

125 (40) – [M-224] (CH₃CH₂O)(HO)P=S⁺ C₂H₆O₂PS⁺ m/z 124.9826

97 (100) – [M-252] (HO)₂P=S⁺ H₂O₂PS⁺ m/z 96.9513

65 (15) – [M-258] (HO)₂P⁺ H₂O₂P⁺ m/z 64.9792

47 (30) – [M-274] PO⁺ m/z 46.9687 and/or CH₃S⁺ m/z 46.99555

Chlorthiophos I

125 (35) – [M-235] (CH₃CH₂O)(HO)P=S⁺ C₂H₆O₂PS⁺ m/z 124.9826
109 (42) – [M-251] (CH₃CH₂O)(HO)P=O⁺ C₂H₆O₃P⁺ m/z 109.0055
97 (99) – [M-263] (HO)₂P=S⁺ m/z 96.9513

Chlorthiophos II

97 (97) – [M-263] (HO)₂P=S⁺ m/z 96.9513

Chlorthiophos III

97 (99) – [M-263] (HO)₂P=S⁺ m/z 96.9513
65 (18) – [M-295] (HO)₂P⁺ m/z 64.9792

Coumaphos

125 (35) – [M-237] (CH₃CH₂O)(HO)P=S⁺ C₂H₆O₂PS⁺ m/z 124.9826
109 (100) – [M-253] (CH₃CH₂O)(HO)P=O⁺ C₂H₆O₃P⁺ m/z 109.0055
97 (90) – [M-265] (HO)₂P=S⁺ H₂O₂PS⁺ m/z 96.9513

Demeton-O

171 (10) – [M-87] (CH₃CH₂O)₂(HO)(HS)P⁺ C₄H₁₂O₃PS⁺ m/z 171.0245

Diazinon

125 (10) – [M-179] (CH₃CH₂O)(HO)P=S⁺ C₂H₆O₂PS⁺ m/z 124.9826
97 (35) – [M-207] (HO)₂PS⁺ H₂O₂PS⁺ m/z 96.9513
93 (45) – [M-211] (CH₃CH₂O)(HO)P⁺ C₂H₆O₂P⁺ m/z 93.0105

Dichlofenthion

125 (30) – [M-189] (CH₃CH₂O)(HO)P=S⁺ C₂H₆O₂PS⁺ m/z 124.9826
109 (45) – [M-205] (CH₃CH₂O)(HO)P=O⁺ C₂H₆O₃P⁺ m/z 109.0055
97 (80) – [M-217] (HO)₂P=S⁺ H₂O₂PS⁺ m/z 96.9513
63 (15) – [M-251] PS⁺ m/z 62.9458

Fensulfothion

153 (50) – [M-155] (CH₃CH₂O)₂P=S⁺ C₄H₁₀O₂P⁺ m/z 153.0139
125 (85) – [M-183] (CH₃CH₂O)(HO)P=S⁺ C₂H₆O₂PS⁺ m/z 124.9826
109 (50) – [M-199] (CH₃CH₂O)(HO)P=O⁺ C₂H₆O₃P⁺ m/z 109.0055
97 (95) – [M-211] (HO)₂PS⁺ H₂O₂PS⁺ m/z 96.9513

Isazofos

97 (50) – [M-216] (HO)₂P=S⁺ H₂O₂PS⁺ m/z 96.9513
65 (25) – [M-248] (HO)₂P⁺ H₂O₂P⁺ m/z 64.9792

Parathion

125 (45) – [M-166] (CH₃CH₂O)(HO)P=S⁺ C₂H₆O₂PS⁺ m/z 124.9826
109 (95) – [M-182] (CH₃CH₂O)(HO)P=O⁺ C₂H₆O₃P⁺ m/z 109.0055
97 (95) – [M-196] (HO)₂P=S⁺ H₂O₂PS⁺ m/z 96.9513

Phoxim

109 (50) – [M-189] (CH₃CH₂O)(HO)P=O⁺ C₂H₆O₃P⁺ m/z 109.0055

Pirimiphos-ethyl

125 (50) – [M-208] (CH₃CH₂O)(HO)PS⁺ C₂H₆O₂PS⁺ m/z 124.9826

109 (25) – [M-224] (CH₃CH₂O)(HO)PO⁺ C₂H₆O₃P⁺ m/z 109.0055

97 (20) – [M-236] (HO)₂P=S⁺ H₂O₂PS⁺ m/z 96.9513

93 (30) – [M-240] (CH₃CH₂O)(HO)P⁺ C₂H₆O₂P⁺ m/z 93.0105

Pyrazophos

97 (10) – [M-276] (HO)₂P=S⁺ H₂O₂PS⁺ m/z 96.9513

65 (5) – [M-308] (HO)₂P⁺ H₂O₂P⁺ m/z 64.9792

Pyridaphenthion

125 (50) – [M-215] (C₂H₅O)(HO)P=S⁺ C₂H₆O₂PS⁺ m/z 124.9826

109 (25) – [M-231] (C₂H₅O)(HO)P=O⁺ C₂H₆O₃P⁺ m/z 109.0055

97 (85) – [M-243] (HO)₂P=S⁺ H₂O₂PS⁺ m/z 96.9513

65 (20) – [M-275] (HO)₂P⁺ H₂O₂P⁺ m/z 64.9792

Pyrimitate

153 (100) may be partly due to (CH₃CH₂O)₂P=S⁺ C₄H₁₀O₂PS⁺ m/z 153.0139, but probably due mainly to C₇H₁₁N₃O⁺ m/z 153.0902 (needs accurate mass study).

Quinalphos

97 (30) – [M-201] (HO)₂PS⁺ H₂O₂PS⁺ m/z 96.9513

Sulfotep

125 (10) – [M-197] (C₂H₅O)(HO)P=S⁺ C₂H₆O₂PS⁺ m/z 124.9826

121 (55) – [M-201] (CH₃CH₂O)₂P⁺ C₄H₁₀O₂P⁺ m/z 121.0418

97 (40) – [M-225] (HO)₂P=S⁺ H₂O₂PS⁺ m/z 96.9513

93 (50) – [M-229] (CH₃CH₂O)(HO)P⁺ C₂H₆O₂P⁺ m/z 93.0105

65 (45) – [M-257] (HO)₂P⁺ H₂O₂P⁺ m/z 64.9792

Thionazin

97 (85) – [M-151] (HO)₂PS⁺ H₂O₂PS⁺ m/z 96.9513

65 (20) – [M-183] (HO)₂P⁺ H₂O₂P⁺ m/z 64.9792

Triazophos

125 (15) – [M-188] (CH₃CH₂O)(HO)P=S⁺ C₂H₆O₂PS⁺ m/z 124.9826

97 (25) – [M-216] (HO)₂PS⁺ H₂O₂PS⁺ m/z 96.9513

65 (20) – [M-248] (HO)₂P⁺ H₂O₂P⁺ m/z 64.9792

C.3. “Other” phosphorothionates

Tebupirimfos (*RS*)-[*O*-(2-*tert*-butylpyrimidin-5-yl) *O*-ethyl *O*-isopropyl phosphorothioate] P=S

318 (100) – M⁺ C₁₃H₂₃N₂O₃PS⁺

303 (20) – [M-15] loss of CH₃ to C₁₂H₂₀N₂O₃PS⁺ m/z 303.0932

276 (40) – [M-42] loss of C₃H₆ to C₁₀H₁₇N₂O₃PS⁺ m/z 276.0698

261 (60) – [M-57] loss of (CH₃)₃C to C₉H₁₄N₂O₃PS⁺ m/z 261.0463

234 (55) – [M-84] loss of (CH₃)₃C & HCN to C₈H₁₃NO₃PS⁺ m/z 234

152 (35) – [M-166] (CH₃)₃C.C₄H₂N₂.OH⁺ C₈H₁₂N₂O⁺ m/z 152.0950

137 (20) – [M-181] (CH₃)₂C.C₄H₂N₂.OH⁺ C₇H₉N₂O⁺ m/z 137.0715

110 (15) – [M-208] (CH₃)₂C.C₃HN.OH⁺ C₆H₈NO⁺ m/z 110.0606

Dioxabenzofos - Cyclic phosphorothionate

201 (25) – [M-15] loss of CH₃ to C₇H₆O₃PS⁺ m/z 201.9775

183 (55) – [M-33] loss of SH to C₈H₈O₃P⁺ m/z 183.0211

153 (30) – [M-63] loss of CH₃OS to C₇H₆O₂P⁺ m/z 153.0105

138 (20) – [M-78] loss of C₆H₆ to C₂H₃O₃PS⁺ m/z 137.9541 (interesting rearrangement)

63 (10) – [M-153] PS⁺ m/z 68.9458

47 (10) – [169] PO⁺ m/z 46.9687

Table C1. Phosphorothionate (RO)₃P=S pesticides, phosphorus-containing EI⁺ ions.

Observed ion	Proposed ion structure	Empirical formula	Theoretical accurate mass
m/z 171	(CH ₃ CH ₂ O) ₂ (HO)(HS)P ⁺	C ₄ H ₁₂ O ₃ PS ⁺	m/z 171.0245
m/z 153	(CH ₃ CH ₂ O) ₂ P=S ⁺	C ₄ H ₁₀ O ₂ P ⁺	m/z 153.0139
m/z 143	(CH ₃ O) ₂ (HS)(HO)P ⁺	C ₂ H ₈ O ₃ PS ⁺	m/z 142.9932
m/z 125	(CH ₃ O) ₂ P=S ⁺ or (CH ₃ CH ₂ O)(HO)P=S ⁺	C ₂ H ₆ O ₃ PS ⁺	m/z 124.9826
m/z 121	(CH ₃ CH ₂ O) ₂ P ⁺	C ₄ H ₁₀ O ₂ P ⁺	m/z 121.0418
m/z 110	(CH ₃ O) ₂ (HO)P ⁺	C ₂ H ₇ O ₃ P ⁺	m/z 110.0133
m/z 109	(CH ₃ O) ₂ P=O ⁺ or (CH ₃ CH ₂ O)(HO)P=O ⁺	C ₂ H ₆ O ₃ P ⁺	m/z 109.0055
m/z 97	(HO) ₂ PS ⁺	H ₂ O ₂ PS ⁺	m/z 96.9513
m/z 93	(CH ₃ O) ₂ P ⁺ or (CH ₃ CH ₂ O)(HO)P ⁺	C ₂ H ₆ O ₂ P ⁺	m/z 93.0105
m/z 79	(CH ₃ O)(HO)P=O ⁺	CH ₄ O ₂ P ⁺	m/z 78.9949
m/z 65	(HO) ₂ P ⁺	H ₂ O ₂ P ⁺	m/z 64.9792
m/z 63	PS ⁺	PS ⁺	m/z 68.9458
m/z 47	PO ⁺	PO ⁺	m/z 46.9687

Table C2. Relative abundances (%) of phosphorus ions of phosphorothionate pesticides.

	Phosphorus containing ions (m/z)												
	47	63	65	79	93	97	109	110	121	125	143	153	171
Pesticide Name	<i>C.1 O,O-dimethyl phosphorothionates</i>												
Bromophos	30						30			75			
Chlorpyrifos-Me	30	15		35	25		20			100			
Cyanophos	30	15		30			100			60			
Demephion-O							3			5	9		
Dicaphon		20		25			20			65			
Etrimfos				20	20					55			
Famphur				10	30		15			25			
Fenchlorphos	20			25	25		25			60			
Fenitrothion	35	15		30	35		90			100			
Fenthion				10	10		10			10			
Iodofenphos	15			20	20		20			35			
Methacrifos	20			30	60			35		100			
Parathion-methyl				30	20		100			80			
Pirimiphos-Me					30					50			
Temephos					20		5			20			
Tolclofos-methyl		10					20			30			
SUB-TOTAL out of 16	7	5	0	11	11	0	13	1	0	16	1	0	0
	<i>C.2 O,O-diethyl phosphorothionates</i>												
Bromophos-ethyl						100	20			25			
Chlorethoxyfos			7			85				40		100	
Chlorpyrifos	30		15			100				40		5	
Chlorthiophos I							42			35			
Chlorthiophos II						97							
Chlorthiophos III			18			99							
Coumaphos						90	100			35			
Demeton-O													10
Diazinon					45	35				10			
Dichlofenthion		15				80	45			30			
Fensulfothion						95	50			85		50	
Isazofos			25			50							
Parathion					30	20	25			45			

Phoxim							50						
Pirimiphos-ethyl				30	20	25			50				
Pyrazophos			5		10								
Pyridaphenthion			20		85	25			50				
Pyrimitate											0-100		
Quinalphos					30								
Sulfotep			45	50	40			55	10				
Thionazin			20		85								
Triazophos			20		25				15				
<i>SUB-TOTAL out of 22</i>	<i>1</i>	<i>1</i>	<i>8</i>	<i>0</i>	<i>4</i>	<i>17</i>	<i>9</i>	<i>0</i>	<i>1</i>	<i>12</i>	<i>0</i>	<i>4</i>	<i>1</i>
	<i>C.3 Other phosphorothionates</i>												
Tebupirimfos													
Dioxabenzofos	10	10											
<i>TOTAL out of 40</i>	<i>9</i>	<i>7</i>	<i>8</i>	<i>11</i>	<i>15</i>	<i>17</i>	<i>22</i>	<i>1</i>	<i>1</i>	<i>28</i>	<i>1</i>	<i>3</i>	<i>1</i>

The phosphorothionates were the largest class of OP compounds studied here. For greater discrimination they are divided into three groups: O,O-dimethyl, O,O-diethyl and “others”.

The spectra of all 16 of the O,O-dimethyl phosphorothionate compounds exhibited m/z 125 due to $(\text{CH}_3\text{O})_2\text{P}=\text{S}^+$. Many (13) also exhibited m/z 109 due to $(\text{CH}_3\text{O})_2\text{P}=\text{O}^+$ produced following phosphorothionate/phosphorothiolate O/S rearrangement. The other most common ions were m/z 93, 79, 63 and 47.

Twelve of the 22 O,O-diethyl compounds also exhibited the m/z 125 ion, but for these the notional structure was assigned to $(\text{CH}_3\text{CH}_2\text{O})(\text{HO})\text{P}=\text{S}^+$, generated by loss of ethene from m/z 153 $(\text{CH}_3\text{CH}_2\text{O})_2\text{P}=\text{S}^+$ (which itself was only observed in four of the O,O-diethyl compound spectra).

The most frequently observed ion in the O,O-diethyl phosphorothionate spectra was m/z 97, due to $(\text{HO})_2\text{PS}^+$, which was evident in 78% (17 out of 22) of the spectra. The next most commonly observed ion was m/z 65 due to $(\text{HO})_2\text{P}^+$.

The spectra of the “other” phosphorothionates exhibited fewer phosphorus-containing ions: The ions analogous to the O,O-diethyl fragments m/z 153/125/109 in the spectrum of

tebupirimiphos (an O-ethyl,O-isopropyl ester) would be expected at m/z 167 $(\text{CH}_3\text{CH}_2\text{O})((\text{CH}_3)_2\text{CHO})\text{P}=\text{S}^+$ and m/z 137 $(\text{HO})((\text{CH}_3)_2\text{CHO})\text{P}=\text{S}^+$. These were not observed, and neither was the was m/z 97, due to $(\text{HO})_2\text{PS}^+$. The most abundant ions were derived from the pyrimidine moiety.

The spectrum of dioxabenzofos (a cyclic OP ester) exhibited the lower mass OP fragments at m/z 63 and 47.

D. Phosphorodithioate pesticides, $(\text{RO})_2\text{P}=\text{S}(\text{SR})$ & $(\text{RO})\text{P}=\text{S}(\text{SR})_2$

D.1 O,O-dimethyl phosphorodithionates (P=S)

Anilofos

125 (75) – [M-242] $(\text{CH}_3\text{O})_2\text{PS}^+ \text{C}_2\text{H}_6\text{O}_2\text{PS}^+$ at m/z 124.9826

93 (35) – [M-274] $(\text{CH}_3\text{O})_2\text{P}^+ \text{C}_2\text{H}_6\text{O}_2\text{P}^+$ at m/z 93.0105

Azinphos-methyl

125 (20) – [M-192] $\text{S}=\text{P}(\text{OCH}_3)_2^+ \text{C}_2\text{H}_6\text{O}_2\text{PS}^+$ m/z 124.9826

93 (30) – [M-224] $(\text{CH}_3\text{O})_2\text{P}^+ \text{C}_2\text{H}_6\text{O}_2\text{P}^+$ m/z 93.0105

Dimethoate

157 (5) – [M-72] $(\text{CH}_3\text{O})_2\text{PS.S}^+ \text{C}_2\text{H}_6\text{O}_2\text{PS}_2^+$ m/z 156.9547

143 (5) – [M-86] $(\text{CH}_3\text{O})(\text{HO})\text{PS.S}^+ \text{CH}_4\text{O}_2\text{PS}_2^+$ m/z 142.9390

125 (30) – [M-104] $(\text{CH}_3\text{O})_2\text{PS}^+ \text{C}_2\text{H}_6\text{O}_2\text{PS}^+$ m/z 124.9826

93 (35) – [M-136] $(\text{CH}_3\text{O})_2\text{P}^+ \text{C}_2\text{H}_6\text{O}_2\text{P}^+$ m/z 93.0105

Formothion

126 (100) – [M-131] $(\text{CH}_3\text{O})_2\text{P}(\text{SH})^+ \text{C}_2\text{H}_7\text{O}_2\text{PS}^+$ m/z 125.9904

125 (50) – [M-132] $(\text{CH}_3\text{O})_2\text{P}=\text{S}^+ \text{C}_2\text{H}_6\text{O}_2\text{PS}^+$ m/z 124.9826

93 (70) – [M-164] $(\text{CH}_3\text{O})_2\text{P}^+ \text{C}_2\text{H}_6\text{O}_2\text{P}^+$ m/z 93.0105

Malathion

158 (45) – [M-172] $(\text{CH}_3\text{O})_2(\text{HS})\text{P}=\text{S}^+ \text{C}_2\text{H}_7\text{OPS}_2^+$ m/z 157.9625

125 (85) – [M-205] $(\text{CH}_3\text{O})_2\text{PS}^+ \text{C}_2\text{H}_6\text{O}_2\text{PS}^+$ m/z 124.9826

93 (85) – [M-237] $(\text{CH}_3\text{O})_2\text{P}^+ \text{C}_2\text{H}_6\text{O}_2\text{P}^+$ m/z 93.0105

79 (15) – [M-251] $(\text{CH}_3\text{O})(\text{HO})\text{P}^+ \text{CH}_4\text{O}_2\text{P}^+$ m/z 78.9949

63 (10) – [M-267] PS^+ m/z 62.9458

Methodathion

157 (5) – [M-145] $(\text{CH}_3\text{O})_2\text{PS}_2^+ \text{C}_2\text{H}_6\text{O}_2\text{PS}_2^+$ m/z 156.9547

125 (25) – [M-177] $(\text{CH}_3\text{O})_2\text{PS}^+ \text{C}_2\text{H}_6\text{O}_2\text{PS}^+$ m/z 124.9826

93 (15) – [M-209] $(\text{CH}_3\text{O})_2\text{P}^+ \text{C}_2\text{H}_6\text{O}_2\text{P}^+$ m/z 93.0105

63 (10) – [M-239] PS^+ m/z 62.9458

47 (10) – [M-255] PO^+ m/z 46.9687

Methyl-trithion

125 (45) – [M-189] $(\text{CH}_3\text{O})_2\text{PS}^+ \text{C}_2\text{H}_6\text{O}_2\text{PS}^+$ m/z 124.9826

93 (40) – [M-221] $(\text{CH}_3\text{O})_2\text{P}^+ \text{C}_2\text{H}_6\text{O}_2\text{P}^+$ m/z 93.0105

Phenthoate

125 (90) – [M-195] $(\text{CH}_3\text{O})_2\text{P}=\text{S}^+ \text{C}_2\text{H}_6\text{O}_2\text{PS}^+$ m/z 124.9826
93 (95) – [M-227] $(\text{CH}_3\text{O})_2\text{P}^+ \text{C}_2\text{H}_6\text{O}_2\text{P}^+$ m/z 93.1054
79 (25) – [M-241] $(\text{CH}_3\text{O})(\text{HO})\text{P}^+ \text{CH}_4\text{O}_2\text{P}^+$ m/z 78.9949

Phosmet

125 (5) – [M-192] $(\text{CH}_3\text{O})_2\text{PS}^+ \text{C}_2\text{H}_6\text{O}_2\text{PS}^+$ m/z 124.9826
93 (15) – [M-224] $(\text{CH}_3\text{O})_2\text{P}^+ \text{C}_2\text{H}_6\text{O}_2\text{P}^+$ m/z 93.0105
63 (5) – [M-254] PS^+ m/z 62.9458

Thiometon

158 (5) – [M-86] $(\text{CH}_3\text{O})_2(\text{HS})\text{P}=\text{S}^+ \text{C}_2\text{H}_7\text{O}_2\text{PS}_2^+$ m/z 158.9625
125 (10) – [M-121] $(\text{CH}_3\text{O})_2\text{P}=\text{S}^+ \text{C}_2\text{H}_6\text{O}_2\text{PS}^+$ m/z 124.9826
93 (5) – [M-153] $(\text{CH}_3\text{O})_2\text{P}^+ \text{C}_2\text{H}_6\text{O}_2\text{P}^+$ m/z 93.1054

D.2 O,O-diethyl phosphorodithionates (P=S)

Azinphos-ethyl

97 (20) – [M-248] $(\text{HO})_2\text{PS}^+ \text{H}_2\text{O}_2\text{PS}^+$ m/z 96.9513
65 (15) – [M-280] $(\text{HO})_2\text{P}^+ \text{H}_2\text{O}_2\text{P}^+$ m/z 64.9792

Carbophenothion

153 (20) – [M-189] $(\text{CH}_3\text{CH}_2\text{O})_2\text{PS}^+ \text{C}_4\text{H}_{10}\text{O}_2\text{PS}^+$ m/z 153.0139
121 (50) – [M-221] $(\text{CH}_3\text{CH}_2\text{O})_2\text{P}^+ \text{C}_4\text{H}_{10}\text{O}_2\text{P}^+$ m/z 121.0418

Chlormephos

154 (50) – [M-80] loss of SCHCl to $(\text{CH}_3\text{CH}_2\text{O})_2(\text{HS})\text{P}^+$ m/z $\text{C}_4\text{H}_{11}\text{O}_2\text{PS}^+$ m/z 154.0217
125 (10) – [M-109] $(\text{CH}_3\text{CH}_2\text{O})(\text{HO})\text{P}=\text{S}^+ \text{C}_2\text{H}_6\text{O}_2\text{PS}^+$ m/z 124.9826
121 (90) – [M-113] $(\text{CH}_3\text{CH}_2\text{O})_2\text{P}^+ \text{C}_4\text{H}_{10}\text{O}_2\text{P}^+$ m/z 121.0418
97 (100) – [M-137] $(\text{HO})_2\text{P}=\text{S}^+ \text{H}_2\text{O}_2\text{PS}^+$ m/z 96.9513
65 (50) – [M-169] $(\text{HO})_2\text{P}^+ \text{H}_2\text{O}_2\text{P}^+$ m/z 64.9792

Dialifos

130 (10) – [M-263] $(\text{HO})_2(\text{HS})\text{PS}^+ \text{H}_3\text{O}_2\text{PS}_2^+$ m/z 129.9312
129 (10) – [M-264] $(\text{HO})_2\text{PS}_2^+ \text{H}_2\text{O}_2\text{PS}_2^+$ m/z 128.9234
97 (10) – [M-296] $(\text{HO})_2\text{PS}^+ \text{H}_2\text{O}_2\text{PS}^+$ m/z 96.9513
65 (10) – [M-328] $(\text{HO})_2\text{P}^+ \text{H}_2\text{O}_2\text{P}^+$ m/z 64.9792

Dioxathion

185 (30) – [M-271] $(\text{CH}_3\text{CH}_2\text{O})_2\text{PS}_2^+ \text{C}_4\text{H}_{10}\text{O}_2\text{PS}_2^+$ m/z 184.9860
153 (70) – [M-303] $(\text{CH}_3\text{CH}_2\text{O})_2\text{PS}^+ \text{C}_4\text{H}_{10}\text{O}_2\text{PS}^+$ m/z 153.0139
125 (60) – [M-331] $(\text{CH}_3\text{CH}_2\text{O})(\text{HO})\text{PS}^+ \text{C}_2\text{H}_6\text{O}_2\text{PS}^+$ m/z 124.9826
97 (100) – [M-357] $(\text{HO})_2\text{PS}^+ \text{H}_2\text{O}_2\text{PS}^+$ m/z 96.9513

Disulfoton

153 (10) – [M-121] $(\text{CH}_3\text{CH}_2\text{O})_2\text{P}=\text{S}^+ \text{C}_4\text{H}_{10}\text{O}_2\text{PS}^+$ m/z 153.0139
142 (10) – [M-132] $(\text{HO})_2\text{PS}.\text{SCH}^+ \text{CH}_4\text{O}_2\text{PS}_2^+$ m/z 141.9312

Ethion

153 (65) – [M-231] $(\text{CH}_3\text{CH}_2\text{O})_2\text{PS}^+ \text{C}_4\text{H}_{10}\text{O}_2\text{PS}^+$ m/z 153.1039
125 (45) – [M-259] $(\text{CH}_3\text{CH}_2\text{O})(\text{HO})\text{P}=\text{S}^+ \text{C}_2\text{H}_6\text{O}_2\text{PS}^+$ m/z 124.9826
121 (45) – [M-263] $(\text{CH}_3\text{CH}_2\text{O})_2\text{P}^+ \text{C}_4\text{H}_{10}\text{O}_2\text{P}^+$ m/z 121.0418
97 (50) – [M-287] $(\text{HO})_2\text{P}=\text{S}^+ \text{H}_2\text{O}_2\text{PS}^+$ m/z 96.9513

Mecarbam

- 153 (30) – [M-174] (CH₃CH₂O)₂PS⁺ C₄H₁₀O₂PS⁺ m/z 153.1039
125 (45) – [M-204] (CH₃CH₂O)(HO)PS₂⁺ C₂H₆O₂PS⁺ m/z 124.9826
121 (35) – [M-208] (CH₃CH₂O)₂P⁺ C₄H₁₀O₂P⁺ m/z 121.0418
97 (60) – [M-232] (HO)₂PS⁺ H₂O₂PS⁺ m/z 96.9513
93 (30) – [M-236] (CH₃CH₂O)(HO)P⁺ C₂H₆O₂P⁺ m/z 93.0105
65 (30) – [M-] (HO)₂P⁺ H₂O₂P⁺ m/z 64.9792

Phenkapton

- 199 (40) – [M-177] (CH₃CH₂O)₂P=S.SCH₂⁺ C₅H₁₂O₂PS₂⁺ m/z 199.0016
153 (65) – [M-223] (CH₃CH₂O)₂P=S⁺ C₄H₁₀O₂PS⁺ m/z 153.0139
125 (50) – [M-251] (CH₃CH₂O)(HO)P=S⁺ C₂H₆O₂PS⁺ m/z 124.9826
121 (100) – [M-255] (CH₃CH₂O)₂P⁺ C₄H₁₀O₂P⁺ m/z 121.0418
97 (80) – [M-279] (HO)₂P=S⁺ H₂O₂PS⁺ m/z 96.9513
65 (45) – [M-311] (HO)₂P⁺ H₂O₂P⁺ m/z 64.9792

Phorate

- 121 (25) – [M-139] (CH₃CH₂O)₂P⁺ C₄H₁₀O₂P⁺ m/z 121.0418
97 (10) – [M-163] (HO)₂PS⁺ H₂O₂PS⁺ m/z 96.9513
93 (10) – [M-167] (CH₃CH₂O)(HO)P⁺ C₂H₆O₂P⁺ m/z 93.0105
65 (10) – [M-195] (HO)₂P⁺ H₂O₂P⁺ m/z 64.9792
47 (10) – [M-213] PO⁺ m/z 46.9687

Phosalone

- 200 (30) – [M-167] (CH₃CH₂O)₂(CH₂S)PSH⁺ C₅H₁₃O₂PS₂⁺ m/z 200.0095 - interesting rearrangement
154 (25) – [M-213] (CH₃CH₂O)₂(HS)P⁺ C₄H₁₁O₂PS⁺ m/z 154.0217
153 (20) – [M-214] (CH₃CH₂O)₂PS⁺ C₄H₁₀O₂PS⁺ m/z 153.0139
121 (50) – [M-246] (CH₃CH₂O)₂P⁺ C₄H₁₀O₂P⁺ m/z 121.0418
97 (40) – [M-270] (HO)₂PS⁺ H₂O₂PS⁺ m/z 96.9513
65 (25) – [M-300] (HO)₂P⁺ H₂O₂P⁺ m/z 64.9792

Terbufos

- 186 (10) – [M-102] (CH₃CH₂O)₂PS.SH⁺ C₄H₁₁O₂PS₂⁺ m/z 185.9938
153 (15) – [M-135] (CH₃CH₂O)₂PS⁺ C₄H₁₀O₂PS₂⁺ m/z 153.0139
142 (10) – [M-146] (HO)₂PS.SCH⁺ CH₄O₂PS₂⁺ m/z 141.9312
129 (10) – [M-159] (HO)₂PS₂⁺ H₂O₂PS₂⁺ m/z 128.9234
125 (10) – [M-163] (CH₃CH₂O)(HO)P=S⁺ C₂H₆O₂PS⁺ m/z 124.9826
121(10) – [M-167] (CH₃CH₂O)₂P⁺ C₄H₁₀O₂P⁺ m/z 121.0418
97 (10) – [M-191] (HO)₂PS⁺ H₂O₂PS⁺ m/z 96.9513
65 (15) – [M-223] (HO)₂P⁺ H₂O₂P⁺ m/z 64.9792

D.3 Other phosphorodithionates (P=S)

Bensulide – O,O-di-isopropyl phosphorodithioate

- 215 (100) – [M-182] (C₃H₇O)₂(HS)₂P⁺ C₆H₁₆O₂PS₂⁺ m/z 215.0329
214 (70) – [M-183] (C₃H₇O)₂PS(SH)⁺ C₆H₁₅O₂PS₂⁺ m/z 214.0251
172 (75) – [M-225] (C₃H₇O)(HO)P=S.SH⁺ C₃H₉O₂PS₂⁺ m/z 171.9782
131 (75) – [M-266] (HO)₂(HS)₂P⁺ H₄O₂PS₂⁺ m/z 130.9390

Prothiofos – O-ethyl, S-propyl phosphorodithioate (P=S)

- 183 (30) – [M-161] (CH₃CH₂O)(CH₃CH₂CH₂S)P=S⁺ C₅H₁₁OPS₂⁺ m/z 183.0067
155 (55) – [M-189] (HO)(CH₃CH₂CH₂S)P=S⁺ C₃H₈OPS₂⁺ m/z 154.9754
141 (30) – [M-203] (CH₃CH₂O)(HS)P=S⁺ C₂H₆OPS₂⁺ m/z 140.9598
113 (85) – [M-231] (HO)(HS)PS⁺ H₂OPS₂⁺ m/z 112.9285

63 (30) – [M-281] PS⁺ m/z 62.9458

Sulprofos – O-ethyl, S-propyl phosphorodithioate (P=S)

125 (20) – [M-197] (CH₃CH₂O)(SH)P=O⁺ C₂H₆O₂PS⁺ m/z 124.9826

113 (30) – [M-209] (HO)(HS)P=S⁺ H₂O₂PS₂⁺ m/z 112.9285

97 (15) – [M-225] (HO)₂PS⁺ H₂O₂PS⁺ m/z 96.9513

63 (10) – [M-259] PS⁺ m/z 62.9458

D.4 Other phosphorodithiolates (P=O)

Cadusafos – S,S-di-*sec*-butyl O-ethyl phosphorodithioate (P=O)

270 (15) – M⁺ C₁₀H₂₃O₂PS₂⁺

213 (20) – [M-57] loss of CH₃CH₂CH(CH₃) to C₆H₁₄O₂PS₂⁺ m/z 213.0173

159 (100) – [M-111] loss of C₄H₈ & C₄H₇ to (CH₃CH₂O)(HS)₂POH⁺ C₂H₈O₂PS₂⁺ m/z 158.97033

158 (80) – [M-112] loss of 2C₄H₈ to (CH₃CH₂O)(HS)₂PO⁺ C₂H₇O₂PS₂⁺ m/z 157.9698

97 (50) – [M-173] (HO)₂PS⁺ H₂O₂PS⁺ m/z 96.9513

88 (20) – [M-182] C₄H₈S⁺ m/z 88.0347

Ethoprophos – S,S-dipropyl,O-ethyl phosphorodithioate (P=O)

200 (25) – [M-42] loss of C₃H₆ to C₅H₁₃O₂PS₂⁺ m/z 200.0095

168 (15) – [M-74] loss of C₃H₆S to C₅H₁₃O₂PS⁺ m/z 167.0296

167 (5) – [M-75] loss of C₃H₇S to C₅H₁₂O₂PS⁺ m/z 167.0296

158 (100) – [M-84] loss of 2C₃H₆ to (HS)₂PO.OCH₂CH₃⁺ C₅H₁₃O₂PS₂⁺ m/z 157.9625

139 (50) – [M-103] C₃H₈OPS⁺ m/z 138.9983

126 (50) – [M-116] loss of C₃H₆ & C₃H₆S to C₂H₇O₂PS⁺ m/z 125.9904

97 (70) – [M-145] (HO)₂PS⁺ H₂O₂PS⁺ m/z 96.9513

93 (40) – [M-149] (CH₃CH₂O)(HO)P⁺ C₂H₆O₂P⁺ m/z 93.0105

Table D1. Phosphorodithioate $(RO)_2P=S(SR)$ & $(RO)P=S(SR)_2$ pesticides, phosphorus-containing EI⁺ ions.

Observed ion	no	Proposed ion structure	Empirical formula	Theoretical accurate mass
m/z 158	3	$(CH_3O)_2(HS)P=S^+$	$C_2H_7OPS_2^+$	m/z 157.9625
m/z 157	2	$(CH_3O)_2PS.S^+$	$C_2H_6O_2PS_2^+$	m/z 156.9547
m/z 153	8	$(CH_3CH_2O)_2P=S^+$	$C_4H_{10}O_2PS^+$	m/z 153.0139
m/z 142	2	$(HO)_2PS.SCH^+$	$CH_4O_2PS_2^+$	m/z 141.9312
m/z 129	2	$(HO)_2PS_2^+$	$H_2O_2PS_2^+$	m/z 128.9234
m/z 125	16	$(CH_3O)_2P=S^+$ or $(CH_3CH_2O)(HO)P=S^+$	$C_2H_6O_3PS^+$	m/z 124.9826
m/z 121	8	$(CH_3CH_2O)_2P^+$	$C_4H_{10}O_2P^+$	m/z 121.0418
m/z 97	12	$(HO)_2PS^+$	$H_2O_2PS^+$	m/z 96.9513
m/z 93	13	$(CH_3O)_2P^+$ or $(CH_3CH_2O)(HO)P^+$	$C_2H_6O_2P^+$	m/z 93.0105
m/z 79	2	$(CH_3O)(HO)P^+$	$CH_4O_2P^+$	m/z 78.9949
m/z 65	8	$(HO)_2P^+$	$H_2O_2P^+$	m/z 64.9792
m/z 63	5	PS^+	PS^+	m/z 62.9458
m/z 47	2	PO^+	PO^+	m/z 46.9687

Table D2. Relative abundances (%) of main phosphorus ions of 27 phosphorodithioate pesticides [25 phosphorodithionates (RO)₂P=S(SR) and 2 phosphorodithiolates (RO)P=O(SR)₂]

	Phosphorus containing ions (m/z)												
	47	63	65	79	93	97	121	125	129	142	153	157	158
Pesticide Name	<i>D.1 O,O-dimethyl phosphorodithionates</i>												
Anilofos					35			75					
Azinphos-methyl					30			20					
Dimethoate					35			30				5	
Formothion					70			50					
Malathion		10		15	85			85					45
Methidathion	10	10			15			25				5	
Methyl-trithion					40			45					
Phenthoate				25	95			90					
Phosmet		5			15			5					
Thiometon					5			10					5
<i>SUB-TOTAL out of 10</i>	<i>1</i>	<i>3</i>	<i>0</i>	<i>2</i>	<i>10</i>	<i>0</i>	<i>0</i>	<i>10</i>	<i>0</i>	<i>0</i>	<i>0</i>	<i>2</i>	<i>2</i>
	<i>D.2 O,O-diethyl phosphorodithionates</i>												
	47	63	65	79	93	97	121	125	129	142	153	157	158
Azinphos-ethyl			15			20							
Carbophenothion							50				20		
Chlormephos			50			100	90	10					
Dialifos			10			10			10				
Dioxathion						100		60			70		
Disulfoton										10	10		
Ethion						50	45	45			65		
Mecarbam			30		30	60	35	45			30		
Phenkapton			45			80	100	50			65		
Phorate	10		10		10	10	25						
Phosalone			25			40	50				20		
Terbufos			10			10	10	10	10	10	15		
<i>SUB-TOTAL out of 12</i>	<i>1</i>	<i>0</i>	<i>8</i>	<i>0</i>	<i>2</i>	<i>10</i>	<i>8</i>	<i>6</i>	<i>2</i>	<i>2</i>	<i>8</i>	<i>0</i>	<i>0</i>
	<i>D.3 Other phosphorodithionates</i>												
Bensulide													

Prothiofos		30											
Sulprofos		10				15		20					
<i>D4. Phosphorodithiolates (P=O compounds)</i>													
Cadusafos						50							80
Ethoprophos					40	70							100
TOTAL out of 27	1	2	8	2	13	13	8	17	2	2	8	2	4

The 27 phosphorodithioates were the second largest class of OP compounds studied here.

Again, for greater discrimination they are divided into several sub-groups:

O,O-dimethyl phosphorodithionates (10)

O,O-diethyl phosphorodithionates (12)

“other” phosphorodithionates (3)

and phosphorodithiolates (2).

The spectra of all ten of the O,O-dimethyl phosphorodithionate compounds exhibited ions at m/z 125 due to $(\text{CH}_3\text{O})_2\text{P}=\text{S}^+$ and m/z 93 due to $(\text{CH}_3\text{O})_2\text{P}^+$. The other most common ions were m/z 79 and 63.

The commonest ions in the spectra of the twelve O,O-diethyl phosphorodithionates were:

m/z 97, $(\text{HO})_2\text{PS}^+$, in 10 out of 12 compounds (85%)

m/z 153 $(\text{CH}_3\text{CH}_2\text{O})_2\text{P}=\text{S}^+$, in 8 out of 12 compounds (75%),

m/z 121 $(\text{CH}_3\text{CH}_2\text{O})_2\text{P}^+$, in 8 out of 12 compounds (75%)

m/z 65 $(\text{HO})_2\text{P}^+$, in 8 out of 12 compounds (75%)

m/z 125 $(\text{CH}_3\text{CH}_2\text{O})(\text{HO})\text{P}=\text{S}^+$, in 6 out of 12 compounds (50%).

Structural differences ensured that the spectra of the three “other” phosphorodithioates exhibited few common ions: bensulide – none, prothiofos only m/z 63, and sulprofos the most with m/z 63, 97 and 125.

Regarding the spectra of the two phosphorodithiolates, cadusafos exhibited m/z 97 and 158, and ethoprophos m/z 93, 97 and 158.

E. Phosphonates (RO)₂P=O(R)

E.1. O,O-dimethyl phosphonates

Trichlorfon O,O-dimethyl trichlorohydroxyethylphosphonate

256 (0) – M⁺ absent C₄H₈Cl₃O₄P⁺
221,223 (5,3) – [M-35] loss of Cl to give C₄H₈Cl₂O₄P⁺ m/z 220.9537 etc.
185,187 (6,2) – [M-71] loss of HCl to give C₄H₇ClO₄P⁺ m/z 184.9771
145,147 (35,10) – [M-111] loss of Cl₂CCOH to (CH₃O)₂(HO)PCl⁺ C₂H₇ClO₃P⁺ m/z 144.9821 – rearrangement
139 (30) – [M-117] loss of CCl₃ to give (CH₃O)₂PO.CHOH⁺ C₃H₈O₄P⁺ m/z 139.0160
110 (100) – [M-146] (CH₃O)₂P(OH)⁺ C₂H₇O₃P⁺ m/z 110.0133
109 (100) – [M-147] (CH₃O)₂P=O⁺ C₂H₆O₃P⁺ m/z 109.0055
79 (85) – [M-177] (CH₃O)(HO)P⁺ CH₄O₂P⁺ m/z 78.9949
47 (15) – [M-209] PO⁺ m/z 46.9687

Ethephon chloroethylphosphonic acid [i.e. (HO)₂P=O(R)]

144,146 (0) – M⁺ absent
109 (25) – [M-35] loss of Cl to (CH₂CH₂)(HO)₂P=O⁺ C₂H₆O₃P⁺ m/z 109.0055
91 (10) [M-53] loss of Cl & H₂O to (CH₂CH₂)PO₂⁺ C₂H₄O₂P⁺ m/z 90.9949
82 (100) – [M-62] (HO)₃P⁺ H₃O₃P⁺ m/z 81.9820
81 (100) – [M-62] (HO)₂PO⁺ H₂O₃P⁺ m/z 80.9742
65 (25) – [M-79] (HO)₂P⁺ H₂O₂P⁺ m/z 64.9792
47 (10) – [M-97] PO⁺ m/z 46.9687

Two common phosphorus-containing fragment ions were observed for the two phosphonates trichlorfon and ethephon:

m/z 109 C₂H₆O₃P⁺ due either to (CH₃O)₂P=O⁺ or (CH₂CH₂)(HO)₂P=O⁺
and m/z 47 PO⁺

F. Phosphonothionates (RO)₂P=S(R)

F.1 O-methyl

Leptophos O-methyl,O-bromodichlorophenyl phenylphosphonothionate (P=S)

410,412,414 (0,0,0) – M⁺ absent
375,377,379 (40,50,15) – [M-35] loss of Cl to C₁₃H₁₀BrClO₂PS⁺ m/z 374.9011 etc.
171 (100) – [M-239] (C₆H₅)(CH₃O)P=S⁺ C₇H₈OPS⁺ m/z 171.0034
155 (25) – [M-255] (C₆H₅)(CH₃O)P=O⁺ C₇H₈O₂P⁺ m/z 155.0262 [O/S swap]
124 (15) – [M-286] C₆H₅PO⁺ C₆H₅OP⁺ m/z 124.0078
109 (20) – [M-301] C₆H₅PH⁺ C₆H₆P⁺ m/z 109.0207
77 (35) – [M-333] C₆H₅⁺ m/z 77.0391
63 (20) – [M-347] PS⁺ m/z 62.9458 or PO₂⁺ m/z

F.2 O-ethyl

EPN O-ethyl,O-nitrophenyl phenylphosphonothionate (P=S)

323 (10) – M⁺
278 (5) – [M-45] loss of CH₃CH₂O to C₁₂H₉NO₃PS⁺ m/z 278.0041
248 (5) – [M-75] loss of CH₃CH₂O & NO to C₁₂H₉O₂PS⁺ m/z 248.0061
185 (40) – [M-138] C₆H₅.PS.OCH₂CH₃⁺ C₈H₁₀OPS⁺ m/z 185.0190
169 (60) – [M-127] C₆H₅.PO.OCH₂CH₃⁺ C₈H₁₀O₂P⁺ m/z 169.0418
157 (100) – [M-166] C₆H₅.PS.OH⁺ C₆H₆OPS⁺ m/z 156.9877
141 (40) – [M-182] C₆H₅.PO.OH⁺ C₆H₆O₂P⁺ m/z 141.0105

77 (20) – [M-246] C₆H₅⁺ m/z 77.0391
63 (25) – [M-260] PS⁺ m/z 62.9458

Trichloronat O-ethyl,O-trichlorophenyl ethylphosphonothionate (P=S)

332,334 (0,0) – M⁺ absent
297,299,301(40,20,5) – [M-35] loss of Cl to give C₁₀H₁₂Cl₂O₂PS⁺ m/z 296.9673 etc.
269,271,273 (30,20,5) – [M-63] loss of Cl & C₂H₄ to give C₈H₈Cl₂O₂PS⁺ m/z 268.9360 etc.
137 (15) – [M-195] (C₂H₅)(C₂H₅O)P=S⁺ C₄H₁₀OPS⁺ m/z 137.0190
109 (100) – [M-223] (C₂H₅)(HO)P=S⁺ C₂H₆OPS⁺ m/z 108.9877
93 (25) – [M-239] (C₂H₅O)(HO)P⁺ C₂H₆O₂P⁺ m/z 93.0105

Cyanofenphos O-ethyl,O-cyanophenyl phenylphosphonothionate (P=S)

303 (15) – M⁺
185 (35) – [M-118] loss of OC₆H₄CN to C₈H₁₀OPS⁺ m/z 185.0190
169 (55) – [M-134] loss of SC₆H₄CN to C₈H₁₀O₂P⁺ m/z 169.0418 [O/S swap]
157 (100) – [M-146] loss of OC₆H₄CN & C₂H₄ to C₆H₆OPS⁺ m/z 185.0190
141 (35) – [M-162] loss of SC₆H₄CN & C₂H₄ to C₆H₆O₂P⁺ m/z 141.0105
77 (25) – [M-226] C₆H₅⁺ m/z 77.0391

Quintiofos O-ethyl, O-quinoliny phenylphosphonothionate (P=S)

329 (20) – M⁺
252 (60) – [M-77] loss of C₅H₃N to give C₁₂H₁₃O₂PS₂⁺ m/z 252.0374
237 (100) – [M-92] loss of CH₃ & C₆H₅ to give C₁₀H₈NO₂PS⁺ m/z 237.0013 (and/or loss of CH₃ & C₅H₃N)
157 (80) – [M-172] ?
145 (70) – [M-184] C₉H₇NO⁺ m/z 145.058

Within the 5 phosphonothionates, which have rather diverse structures, common phosphorus-containing fragment ions were few. Those observed were m/z 63, 93 and 109 in EPN and trichloronat spectra only.

The phenylphosphonothionates cyanofenphos and EPN both exhibited m/z 141 due to C₆H₅.PO.OH⁺ C₆H₆O₂P⁺ (though leptophos and quintiofos did not).

G. Phosphonodithionate (RO)(RS)P=O(R)

Fonofos O-ethyl, S-phenyl ethylphosphonothioate
137 (60) – [M-109] (CH₃CH₂)(CH₃CH₂O)P=S⁺ C₄H₁₀OPS⁺ m/z 137.0190
109 (100) – [M-137] (CH₃CH₂)(HO)P=S⁺ C₂H₆OPS⁺ m/z 108.9877
81 (15) – [M-165] (HO)(HS)P⁺ H₂OPS⁺ m/z 80.9564
63 (10) – [M-183] PS⁺ m/z 62.9458

In the single phosphonodithionate spectrum (fonofos), three previously observed common phosphorus-containing fragment ions were observed: m/z 63, 81 and 109.

H. Phosphoramidates (RO)₂P=O(NR)

Crufomate O-methyl,O-chloro/t-butylphenyl,O-methylaminophosphate (P=O)
291,293 (20,7) – M⁺
276,278 (80,5) – [M-15] loss of CH₃ to C₁₁H₁₆ClNO₃P⁺ m/z 276.0556
256 (100) – [M-35] loss of Cl to C₁₂H₁₉NO₃P⁺ m/z 256.1103
182 (60) – [M-109] C₁₀H₁₁ClO⁺ m/z 182.0498 etc.
169,171 (65,25) – [M-122] C₉H₁₀ClO⁺ m/z 169.0420 etc.

108 (95) – [M-183] (CH₃O)(CH₃NH)P=O⁺ C₂H₇NO₂P⁺ m/z 108.0214

Fosthietan O,O-diethyl CH₂S₂C=N- phosphate (P=O)

241 (0) – M⁺ absent

196 (85) – [M-45] loss of CHS to C₅H₁₁NO₃PS⁺ m/z 196.0197

168 (45) – [M-73] loss of CHS & C₂H₄ to C₃H₇NO₃PS⁺ m/z 167.9884

140 (100) – [M-101] loss of CHS & 2C₂H₄ to (HO)₃P-NCS⁺ m/z CH₃O₃PNS⁺ m/z 139.9571

109 (50) – [M-132] (CH₃CH₂O)(HO)P=O⁺ C₂H₆O₃P⁺ m/z 109.0055

81 (55) – [M-160] (HO)₂PO⁺ H₂O₃P⁺ m/z 80.9742

46 (50) – [M-195] CH₂S⁺ m/z 45.9877

Mepfosfolan O,O-diethyl C₃H₆S₂C=N- phosphate (P=O)

269 (10) – M⁺

227 (45) – [M-42] loss of C₃H₆ to S₂CN-PO(OCH₂CH₃)₂⁺ C₅H₁₀O₃PNS₂⁺ m/z 226.9840

196 (100) – [M-73] loss of C₃H₅S to SCN-P(OH)(OCH₂CH₃)₂⁺ C₅H₁₁NO₃PS⁺ m/z 196.0197

168 (60) – [M-101] loss of C₃H₅S+C₂H₄ to SCN-P(OH)₂(OCH₂CH₃)⁺ C₃H₇NO₃PS⁺ m/z 167.9884

140 (95) – [M-129] loss of C₃H₅S+2C₂H₄ to SCN-P(OH)₃⁺ CH₃NO₃PS⁺ m/z 139.9571

106 (90) – [M-163] SCN-P(OH)⁺ m/z 105.9517

81 (35) – [M-188] (HO)₂PO⁺ H₂O₃P⁺ m/z 80.9742

74 (90) – [M-195] C₃H₆S⁺ m/z 74.0190

41 (70) – [M-228] C₃H₅⁺ m/z 41.0391

Phosfolan O,O-diethyl C₂H₄S₂C=N- phosphate (P=O)

255 (35) – M⁺ C₇H₁₄NO₃PS₂⁺ m/z

227 (25) – [M-28] loss of C₂H₄ to C₅H₁₀NO₃PS₂⁺ m/z 226.9840

196 (55) – [M-59] loss of CH₂CHS to (HSCN)(C₂H₅O)₂PO⁺ C₅H₁₁NO₃PS⁺ m/z 196.0197

168 (45) – [M-87] loss of CH₂CHS & C₂H₄ to (HSCN)(C₂H₅O)(HO)PO⁺ C₃H₇NO₃PS⁺ m/z 167.9884

140 (65) – [M-115] loss of CH₂CHS & 2C₂H₄ to (HSCN)(HO)₂PO⁺ CH₃NO₃PS⁺ m/z 139.9571

92 (100) – [M-163] ring scission to SCH₂CH₂S⁺ C₂H₄S₂⁺ m/z 91.9754

60 (55) – [M-195] CH₂CH₂S⁺ C₂H₄S⁺ m/z 60.0034

Fenamiphos O,ethyl,O-aryl,isopropylaminophosphate (P=O)

303 (100) – M⁺

288 (40) – [M-15] loss of CH₃ to C₁₂H₁₉NO₃PS⁺ m/z 288.0823

260 (30) – [M-43] loss of C₃H₇ to C₁₀H₁₅NO₃PS⁺ m/z 260.05103

217 (15) – [M-86] loss of CH₃ & C₃H₇ & C₂H₄ to C₇H₈NO₃PS⁺ m/z 216.9963

195 (25) – [M-108]

154 (30) – [M-149] phenol (CH₃S)(CH₃)C₆H₃.OH⁺ C₈H₁₀OS⁺ m/z 154.0452

80 (15) – [M-223] (HO)₂(NH)P⁺ H₃O₂NP⁺ m/z 79.9901

44 (20) – [M-259] C₂H₆N⁺ m/z 44.0500

Within the 5 phosphoramidates, which again have rather diverse structures, common phosphorus-containing fragment ions were few. Those observed in previous sections were m/z 81 and 109 in the fosthietan and mepfosfolan spectra.

I. Phosphoramidothiolates (RO)(RS)P=O(NR)

Acephate O-methyl,S-methyl, acetylaminophosphate

183 (5) – M⁺

142 (10) – [M-41]⁺ due to loss of ketene C₂H₂O to [H₂N.P=OH(SCH₃)(OCH₃)]⁺ C₂H₉NO₂PS⁺ m/z 142.0092

136 (100) – [M-47]⁺ loss of (CH₃S) to C₃H₇NO₃P⁺ m/z 136.0164

125 (15) – [M58] loss of CH₃CONH to (CH₃O)(CH₃S)P=O⁺ m/z 124.9826

94 (50) – [M-89] loss of CH₃CO & CH₂S to give H₂N.P=O.(OCH₃)⁺ CH₅NO₂P⁺ m/z 94.0058

42 (80) – [M-141] CH₂=C=O⁺ C₂H₂O⁺ m/z 42.0106

Methamidophos O-methyl,S-methyl, aminophosphate

141 (40) – M⁺
 126 (5) – [M-15] loss of CH₃ to CH₅NO₂PS⁺ m/z 125.9779
 110 (5) – [M-31] loss of CH₃O to CH₅NOPS⁺ m/z 109.9829
 95 (60) – [M-46] loss of CH₂S to NH₂P(OH)(OCH₃)⁺ CH₆NO₂P⁺ m/z 95.0136
 94 (100) – [M-47] loss of CH₃S to NH₂P=O(OCH₃)⁺ CH₅NO₂P⁺ m/z 94.0058
 79 (10) – [M-62] (CH₃O)(HO)P⁺ CH₄O₂P⁺ m/z 78.9949
 64 (20) – [M-76] SO₂⁺ m/z 63.9619
 47 (20) – [M-94] PO⁺ m/z 46.9687 and/or CH₃S⁺ 46.9955

Within the two phosphoramidothiolates, which again have rather diverse structures, common phosphorus-containing fragment ions were few. Those observed in previous sections were m/z 79 in methamidophos, and m/z 125 in acephate.

J. Phosphoramidothionate (4) (RO)(RO)P=S(NR)

Butamifos O-ethyl, O-(5-methyl-2-nitrophenyl),N-(1-methylpropyl)phosphoramidothionate

332 (0) – M⁺ absent
 286 (100) – [M-46] loss of NO₂ to C₁₃H₂₁NO₂PS⁺ m/z 286.1031
 260 (5) – [M-72] loss of NHCH(CH₃)CH₂CH₃ to C₉H₁₁NO₄PS⁺ m/z 260.0146
 258 (5) – [M-74] loss of NO₂+C₂H₄ to C₁₁H₁₇NO₂PS⁺ m/z 258.0718
 232 (50) – [M-100] loss of NHCH(CH₃)CH₂CH₃+C₂H₄ to C₇H₇NO₄PS⁺ m/z 231.9833
 202 (50) – [M-130] loss of NHCH(CH₃)CH₂CH₃+C₂H₄+NO to C₇H₇O₃PS⁺ m/z 201.9854
 200 (90) – [M-132] C₇H₇NO₄P⁺ m/z 200.0113
 152 (5) – [M-180] CH₃C₆H₃(NO₂)O⁺ C₇H₆NO₃⁺ m/z 152.0348
 96 (95) – [M-236] H₃NOPS⁺ m/z 95.9673
 72 (60) – [M-260] NHCH(CH₃)CH₂CH₃⁺, C₄H₁₀N⁺ m/z 72.08132

Propetamphos O-methyl,O-alkyl, N-ethyl,phosphoramidothionate

281 (0) – M⁺ absent
 236 (25) – [M-45] loss of CH₃CH₂NH₂ to C₈H₁₃O₄PS⁺ m/z 236.0272
 194 (35) – [M-187] loss of (CH₃)₂CHO.CO to C₆H₁₃NO₂PS⁺ m/z 194.0405
 156 (15) – [M-125] (CH₃O)(C₂H₅NH)P(SH)(OH)⁺ C₃H₁₁NO₂PS⁺ m/z 156.0248
 138 (100) – [M-143] (CH₃O)(C₂H₅NH)P=S⁺ C₃H₉NOPS⁺ m/z 138.0143
 122 (25) – [M-143] (CH₃O)(C₂H₅NH)P=O⁺ C₃H₉NO₂P⁺ m/z 122.0371
 110 (25) – [M-171] (CH₃O)(NH₂)P=S⁺ CH₅NOPS⁺ m/z 109.9830
 106 (10) – [M-175] (CH₃O)(C₂H₅NH)P⁺ C₃H₉NOP⁺ m/z 106.0422
 44 (60) – [M-237] CH₃CH₂NH⁺ C₂H₆N⁺ m/z 44.0770

Ditalimfos O,O-diethyl, N-phthalimido, phosphoramidothionate

299 (85) – M⁺
 271 (35) – [M-28] loss of C₂H₄ to C₁₀H₁₀NO₄PS⁺ m/z 271.0068
 243 (55) – [M-56] loss of 2C₂H₄ to C₈H₆NO₄PS⁺ m/z 242.9789
 209 (60) – [M-90] loss of 2(CH₃CH₂O) to C₈H₄NO₂PS⁺ m/z 208.9700
 194 (50) – [M-105] loss of C₆H₄CO+H to C₅H₉NO₃PS⁺ m/z 194.0041
 148 (75) – [M-151] C₆H₄(COH)₂N⁺ C₈H₆NO₂⁺ m/z 148.0399
 130 (100) – [M-169] C₆H₄NC₂O⁺ C₈H₄NO⁺ m/z 130.0293
 102 (25) – [M-197] C₆H₄NC⁺ C₇H₄N⁺ m/z 102.0343
 76 (20) – [M-223] C₆H₄⁺ m/z 76.0313

Isofenphos O-ethyl,O-aryl,N-isopropyl phosphoramidothionate

345 (0) – M⁺ absent (but present in NIST spectrum) C₁₅H₂₄NO₄PS
 286 (5) – [M-59] loss of (CH₃)₂CHO to C₁₂H₁₇NO₃PS⁺ m/z 286.0667
 255 (40) – [M-90]
 213 (60) – [M-132] loss of (CH₃)₂CHOCO & CH₃CH₂O to C₉H₁₂NOPS⁺ m/z 213.0377
 185 (40) – [M-160]
 138 (20) – [M-207] (C₃H₇NH)(HO)P=S⁺ C₃H₉NOPS⁺ m/z 138.0143
 121 (40) – [M-224] C₃H₇NH.P=S⁺ C₃H₈NPS⁺ m/z 121.0115
 96 (40) – [M-249] (HO)(NH₂)P=S⁺ H₃NOPS⁺ m/z 95.9673

58 (100) – [M-287] (CH₃)₂CHNH⁺

Within the 4 phosphoramidothionate spectra, which again have rather diverse structures, no common phosphorus-containing fragment ions were observed.

K. Phosphorodiamide (1) (RO)P=O(NR)₂ & L. Phosphorotriamide (1) (RN)₃P=O

Schradan (di-ester)

286 (20) – M⁺

243 (20) – [M-43] loss of CH₂NCH₃ to C₆H₁₉N₃O₃P₂⁺ m/z 243.0902

199 (40) – [M-87] loss of CH₂NCH₃ & (CH₃)₂N to C₄H₁₃N₂O₃P₂⁺ m/z 199.0401

153 (45) – [M-133] [(CH₃)₂N]₂(HO)₂P⁺ C₄H₁₄N₂O₂P⁺ m/z 153.0793

135 (65) – [M-153] [(CH₃)₂N]₂P=O⁺ C₄H₁₂N₂OP⁺ m/z 135.0687

92 (45) – [M-151] (CH₃)₂N(HO)P⁺ C₂H₇NOP⁺ m/z 92.0265

44 (100) – [M-242] (CH₃)₂N⁺ C₂H₆N⁺ m/z 44.0500

Triamphos N,N-di(dimethylamino),N-heterocycle,phosphorotriamide

294 (20) – M⁺

160 (100) – [M-134] C₆H₅.C₂N₃.NH₂/H⁺ C₈H₈N₄⁺ m/z 160.0749

135 (40) – [M-159] [(CH₃)₂N]₂P=O⁺ C₄H₁₂N₂OP⁺ m/z 135.0687

104 (10) – [M-190] C₆H₅.CNH⁺ C₇H₆N⁺ m/z 104.0500

92 (15) – [M-202] [(CH₃)₂N]P=OH⁺ C₂H₇NOP⁺ m/z 92.0265

44 (40) – [M-250] (CH₃)₂N⁺ C₂H₆N⁺ m/z 44.0500

Schradan and triamphos, because of the structural similarity of their OP ester groups, were found to share two phosphorus-containing ions: m/z 92 and 135.

M. Phosphonofluoridate (3) (RO)(R)P=O(F)

Cyclosarin / GF O-cyclohexyl,methylphosphonofluoridate

180 (0) – M⁺ absent

137 (2) – [M-43] loss of C₃H₇ to C₃H₄O(CH₃)FP=O⁺ C₄H₇FO₂P⁺ m/z 137.0168

125 (1) – [M-55] loss of C₄H₇ to C₂H₄O(CH₃)FP=O⁺ C₃H₇FO₂P⁺ m/z 125.0168

99 (100) – [M-81] loss of C₆H₉ to CH₃(HO)₂FP⁺ CH₃FO₂P⁺ m/z 99.0011

82 (10) – [M-98] cyclohexene C₆H₁₀⁺ m/z 82.0783 and/or CH₃(HO)FP⁺ CH₄FOP⁺ m/z 81.9984

81 (10) – [M-99] C₆H₁₀⁺ m/z 81.0704

67 (20) – [M-113] C₅H₇⁺ (as in cyclohexanol spectrum) m/z 67.0548 (or FPOH⁺ m/z 66.9749?)

54 (15) – [M-126] C₄H₆⁺ m/z 54.0470

Sarin / GB O-isopropyl,methylphosphonofluoridate

140 (0) – M⁺ absent

125 (35) – [M-15] loss of CH₃ to C₃H₇FO₂P⁺ m/z 125.0168 (NOT the typical OP ion!)

99 (100) – [M-41] loss of C₃H₅ to CH₃(HO)₂FP⁺ CH₃FO₂P⁺ m/z 99.0011

81 (10) – [M-59] loss of C₃H₅ & H₂O to give CH₃FOP⁺ m/z 80.9906

Soman / GD O-pinacolyl,methylphosphonofluoridate

182 (0) – M⁺ absent

126 (35) – [M-56] loss of C₄H₈ to C₃H₈FO₂P⁺ m/z 126.0246

99 (100) – [M-83] loss of C₆H₁₁ to CH₃(HO)₂FP⁺ CH₃FO₂P⁺ m/z 99.0011

82 (10) – [M-100] loss of C₆H₁₀ & H₂O to give to CH₃(HO)FP⁺ CH₄FOP⁺ m/z 81.9984

The three phosphonofluoridates appear to share one common phosphorus-containing ion:

m/z 99 - $\text{CH}_3(\text{HO})_2\text{FP}^+$

and the related m/z 81 $\text{CH}_3\text{FP}=\text{O}^+$ CH_4FOP^+ and/or m/z 82 $\text{CH}_3(\text{HO})\text{FP}^+$ CH_4FOP^+ ions.

Unfortunately the PF^+ ion (m/z 50) was not detected at significant intensities. This would have been a useful marker ion.

N. Phosphoramidocyanidate (1) $(\text{RO})(\text{RN})\text{P}=\text{O}(\text{CN})$

Tabun O-ethyl N,N-dimethyl phosphoramidocyanidate

162 (30) – M^+

147 (5) – [M-15] loss of CH_3 to $\text{C}_4\text{H}_8\text{N}_2\text{O}_2\text{P}^+$ m/z 147.0323

133 (45) – [M-29] loss of CH_3CH_2 to $\text{C}_3\text{H}_6\text{N}_2\text{O}_2\text{P}^+$ m/z 133.0167

117 (15) – [M-45] loss of $\text{CH}_3\text{CH}_2\text{O}$ to $\text{C}_3\text{H}_6\text{N}_2\text{OP}^+$ m/z 117.0218

106 (20) – [M-56] loss of CH_3CH_2 & HCN to $(\text{CH}_3)\text{CH}_2\text{N.PO}_2^+$ $\text{C}_2\text{H}_5\text{NO}_2\text{P}^+$ m/z 106.0058

70 (85) – [M-59] dimethyl cyanamide, $\text{N}\equiv\text{C}-\text{N}(\text{CH}_3)_2$ $\text{C}_3\text{H}_6\text{N}_2^+$ m/z 70.0531

43 (100) – [M-119] $(\text{CH}_3)\text{NCH}_2^+$ $\text{C}_2\text{H}_5\text{N}^+$ m/z 43.0422

Tabun's phosphoramidocyanidate structure did not lead to any phosphorus-containing ions similar to those observed in other OP compounds. The most promising ion was m/z 106, which was also observed in propetamphos and mephosfolan spectra. Unfortunately they all due to different ions:

- tabun $(\text{CH}_3)\text{CH}_2\text{N.PO}_2^+$ $\text{C}_2\text{H}_5\text{NO}_2\text{P}^+$ m/z 106.0058
- propetamphos $(\text{CH}_3\text{O})(\text{C}_2\text{H}_5\text{NH})\text{P}^+$ $\text{C}_3\text{H}_9\text{NOP}^+$ m/z 106.0422
- mephosfolan $\text{SCN-P}(\text{OH})^+$ m/z 105.9517

3.7 Overview of characteristic OP ions

In conclusion, the search for universal diagnostic EI+ MS fragment ions for OP compounds is challenging, because of the wide diversity of chemical structures of the OP pesticides and CW agents. However, for the commonest classes of OP, e.g.. the O,O-dimethyl or O,O-diethyl phosphates, phosphorothioates and phosphorodithioates, several characteristic ions were observed. These included :

- For phosphates: m/z 127 $(\text{CH}_3\text{O})_2(\text{HO})_2\text{P}^+$ and m/z 109 $(\text{CH}_3\text{O})_2\text{P}=\text{O}^+$ / $\text{CH}_3\text{CH}_2\text{O})(\text{HO})\text{PO}^+$
- For the phosphorothioates: m/z 109 $(\text{CH}_3\text{O})_2\text{P}=\text{O}^+$ / $\text{CH}_3\text{CH}_2\text{O})(\text{HO})\text{PO}^+$ and m /z 79 $(\text{CH}_3\text{O})(\text{HO})\text{P}=\text{O}^+$.
- For the phosphorothionates: m/z 125, 109, 97, 65.

- For the phosphorodithionates: m/z 125, 93, 97, 153 and 121.
- The other OP classes generally exhibited few common ions, but the two phosphonates represented (EPN and ethephon) both exhibited m/z 109.
- Some phenylphosphonothionates (cyanofenphos and EPN) exhibited m/z 141 due to $C_6H_5.PO.OH^+$.
- The sole phosphonodithionate spectrum (fonofos), also exhibited 109, as did one of the 5 phosphoramidates (fosthietan). Of the 6 phosphoroamidothioates, acephate exhibited m/z 125 and methamidophos m/z 79.
- The spectra of the two phosphorodiamides (schradan and triamiphos) both exhibited ions at m/z 92 and 135.

The three phosphonofluoridate CW agents (cyclosarin, sarin and soman) share m/z 99 due to $CH_3(HO)_2FP^+$, and the related m/z 81 $CH_3FP=O^+$ CH_4FOP^+ and/or m/z 82 $CH_3(HO)FP^+$ CH_4FOP^+ ions. The m/z 50 PF^+ ion was not observed.

And finally the spectrum of the single phosphoroamidocyanidate (tabun) did not exhibit any phosphorus-containing ions similar to those observed in other OP compounds. The most promising ion was m/z 106, which was also observed in propetamphos and mephosfolan spectra. Unfortunately they all due to different ions:

- tabun $(CH_3)CH_2N.PO_2^+$ $C_2H_5NO_2P^+$ m/z 106.0058
- propetamphos $(CH_3O)(C_2H_5NH)P^+$ $C_3H_9NOP^+$ m/z 106.0422
- mephosfolan $SCN-P(OH)^+$ m/z 105.9517

3.8 Proportion of phosphorus-containing ions in the EI+ mass spectrum

Another consideration which may help shed light on the possibility of developing a universal analytical method for detecting OP compounds using MS, is to understand the effects which determine the proportion of the total ion current (TIC) generated by ionisation of an OP molecule that is due to ions that contain phosphorus. Some initial attempts, based on calculating the proportion using the eight most abundant ion data in Appendix I produced some interesting results. For some compounds, e.g. parathion-methyl, all 8 EI+ ions contained a phosphorus atom. In other compounds, only 1 or 2 ions contained phosphorus, e.g. triazophos and demeton-S-methyl.

Table 3.8(a). OP EI+ mass spectra: Estimation of proportion of phosphorus containing ions in total ion current (TIC).

Pesticide	Eight most abundant ions (m/z & % rel. abundance)										% OP ions
	m/z	88	60	142	109	89	61	79	112		
DEMETON-S-METHYL	m/z	88	60	142	109	89	61	79	112		
OP	%			12	12			8	8	40	18.8%
non OP	%	100	54			10	9			173	
MALATHION	m/z	173	127	125	93	158	99	143	79	total	
OP ions	%			85	85	45			15	230	48.4%
non-OP ions	%	100	90				35	20		245	
OXYDEPROFOS	m/z	183	41	125	109	102	143	79	29		
OP	%	100		35	25		10	10		180	73.5%
non OP	%		40			20			5	65	
PARATHION	m/z	<u>291</u>	109	97	137	139	125	155	123		
OP	%	100	95	95	60		45	45		440	85.4%
non OP	%					55			20	75	
PARATHION-METHYL	m/z	109	125	<u>263</u>	79	93	47	63	200		
OP	%	100	80	65	30	20	20	10	5	330	100.0%
non OP	%									0	
PIRIMIPHOS-METHYL	m/z	290	276	<u>305</u>	125	233	180	262	93		
OP	%	100	90	85	50	40		30	30	425	93.4%
non OP	%						30			30	
TRIAZOPHOS	m/z	161	162	172	177	257	97	285	91		
OP	%					30	25	25		80	22.2%
non-OP	%	100	75	50	30				25	280	

This is clearly a crucial criterion when attempting to identify characteristic OP EI+ MS fragments. Molecular modelling may be helpful in investigating this with known OP compounds, and for predicting this effect for novel compounds or unknowns.

A recent detailed study of the EI MS fragmentation pathways of twenty seven O,S-dialkyl alkylphosphonothionate and O,O-dialkyl alkylphosphonothiolate isomers (CW agent homologues) with empirical formula C₆H₁₅O₂PS and molecular weight 182 (Kathikraj 2013), revealed that these OP esters underwent rather similar fragmentation pathways to those described here. Fragmentations included “*alpha*-cleavage, McLafferty rearrangement, McLafferty + 1 rearrangement, O/S-alkyl radical loss, and an alkene loss with a hydrogen shift”.

3.9 Accurate Mass Investigations (GCT MS at Cardiff)

The fragments in the mass spectra of several organophosphorus (and some other) compounds proved difficult to assign unambiguously (or their generation appeared to involve unexpected rearrangements), using nominal mass data. Therefore, accurate mass MS study, using capillary GC/accurate mass OA-TOF MS (orthogonal acceleration time of flight mass spectrometry), was undertaken at Cardiff School of Chemistry by Tom Williams. The data and main empirical formula assignments are presented and summarised in Appendix IV.

As can be seen, it was reassuring to confirm that the accurate mass data confirmed nearly all of the previous tentative identifications, based on nominal mass data. Several ambiguous fragmentation pathways e.g. involving loss of nominally isobaric fragments (such as N₂, CO and C₂H₄ which are all 28 Da) were clarified. Some fragments proved intractable (the spectrum of isofenphos was particularly bewildering).

3.9.1 Elucidation of an unexpected OP rearrangement

A particularly puzzling fragment ion, due to [M-141]⁺, was observed in the spectra of several O,O-diethyl phosphorothionate compounds (see Table 3.9.1a).

OP pesticide	MW	[M-141] ⁺ ion (m/z)	Rel abundance (%)
Bromophos-ethyl	364	223	4 (NIST)
Chlorethoxyfos	334	193	<1 (NIST)
Chlorpyrifos	349	208	23 (NIST)
Chlorthiophos isomers	360	219	<10
Coumaphos	362	221	10 (NIST)
Demeton-O	258	117	<1
Diazinon	304	163	14 (NIST)
Dichlofenthion	314	173	5 (NIST)
Fensulfothion	308	167	1
Isazofos	313	172	30
Parathion	291	150	7 (NIST)
Phoxim	298	157	2 (NIST)
Pirimiphos-ethyl	333	192	<5 (NIST)
Pyrazophos	373	232	40
Pyridaphenthion	340	199	80
Pyrimitate	305	164	8 (NIST)
Quinalphos	298	157	65
Sulfotep	322	181	<1 (NIST)
Thionazin	248	107	80
Triazophos	313	172	50

Table 3.9.1(a). Relative abundance of [M-141]⁺ ions in O,O-diethyl phosphorothioate pesticide EI mass spectra.

A review of the literature, and consultation with expert pesticide residue analysts at FERA (York), LGC and SASA (etc.) did not shed light on the nature of this fragmentation.

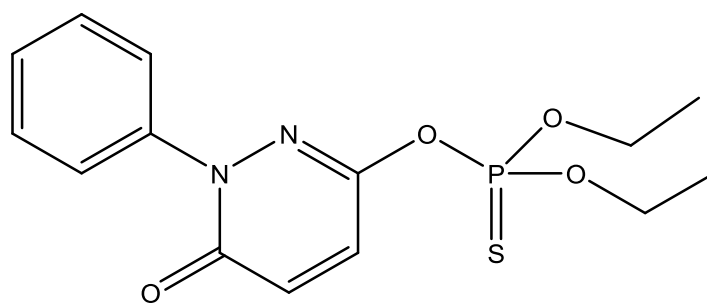


Figure 3.9.1(a). Pyridaphenthion, $C_{14}H_{17}N_2O_4PS$, MW 340.

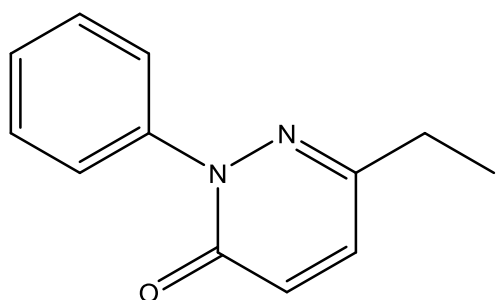


Figure 3.9.1(b). Nominal structure of pyridaphenthion $[M-141]^+$ m/z 199 ion $C_{12}H_{11}N_2O^+$

The GCT MS spectrum of pyridaphenthion (see Appendix IV) exhibited the $[M-141]^+$ ion at m/z 199.0871. Therefore, the loss from the molecular ion at m/z 340.0572 was 140.9747 Da. Possible empirical formulae for this loss are given in Table 3.4.2b below. The most likely candidate appears to be $C_2H_6O_3PS$, which is equivalent to “ $(C_2H_5O)(HO)POS$ ”.

This is equivalent to loss of the diethyl phosphorothioate ester moiety ($C_4H_{10}O_3PS$, mass 169), apart from an ethylene (C_2H_4 , mass 28). It appears to be due to a complex rearrangement which results in transfer of the ethyl group to the aromatic ring, followed by expulsion of the OP moiety. Rearrangement of the nominal structure given in Fig 3.4.2a(ii) seems likely, e.g. transfer of the C_2H_4 moiety to the adjacent nitrogen atom as $[>N-CH=CH_2]^+$

	Formula	Monoisotopic mass	PPM	mDa	unsaturation
1	C ₅ H ₂ O ₃ P	140.9742	3.863	-0.545	5.5
2	C ₄ HN ₂ O ₂ S	140.9759	8.32	1.173	5.5
3	C ₈ NP	140.9768	15.148	2.136	10
4	C ₂ H ₆ O ₃ PS	140.9775	20.048	2.826	0.5
5	C ₉ HS	140.9799	36.854	5.196	9.5
6	C ₅ H ₄ NPS	140.9802	39.058	5.506	5
7	C ₄ H ₂ N ₂ PS	140.9676	50.151	-7.07	5.5
8	CH ₄ NO ₃ PS	140.9650	69.165	-9.75	1
9	C ₅ HO ₃ S	140.9646	71.368	-10.06	5.5
10	C ₄ H ₂ N ₂ O ₂ P	140.9854	75.815	10.689	5.5

Table 3.9.1(b). Possible empirical formulae for fragment corresponding to loss of 140.9747 Da, constrained by pyridaphenthion formula, obtained using ChemCalc (Patiny 2013).

Colour by difference: ≤-0.0010 ≤-0.01 ≤-1.0

This extraordinary rearrangement [M-141]⁺ ion was also observed with significant relative abundance (>10%) in the spectra of chlorpyrifos, coumaphos, diazinon, isazofos, pyrazophos, pyridaphenthion, quinalphos, thionazin and triazophos. A common structural feature shared by these compounds (except for coumaphos) is the presence of a nitrogen-containing aromatic ring adjacent to the organophosphorus ester moiety. This shared feature indicates that the nitrogen is involved in the rearrangement reaction.

Analogous [M-141]⁺ ions were also observed in O,O-dimethyl phosphorothionate spectra. These are more easily rationalised as being due to loss of “(CH₃O)₂POS”, which requires scission of only one bond. Examples include:

- Azamethiphos m/z 183 (40%)
- Demephion-O m/z 75 (33%)
- Fenthion m/z 137 (10%)
- Fenthion sulphoxide m/z 153 (20%)

Cf. analogous $[M-157]^+$ ion at m/z 226 (100%) in the spectrum of anilofos, due to loss of the 16 dalton heavier fragment $(\text{CH}_3\text{O})_2\text{PS}_2 / \text{C}_2\text{H}_6\text{O}_2\text{PS}_2$, of 156.9547 Da.

The m/z 141 $\text{C}_2\text{H}_6\text{O}_3\text{PS}^+$ ion (not neutral fragment loss) is also observed in the spectra of omethoate (10%), sulprofos oxon sulphoxide (30%), and amiton (10%).

Interestingly, an alternative cyclic structure for the m/z 141 ion of amiton (CW agent) produced by electrospray ionisation has been reported (Ellis-Steinborner et al. 2006. The fragmentation pathways of protonated Amiton in the gas phase. *Rapid Comm. in Mass Spec.*, 20(12), 1939-1948). See figure below.

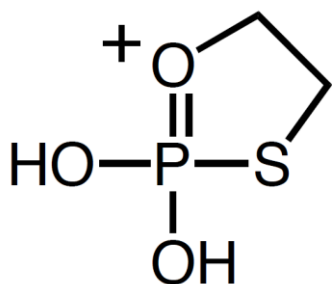


Figure 3.9.1(c). Proposed structure of m/z 141 ion from ESI+ spectrum of *Amiton* (Ellis-Steinborner, 2006).

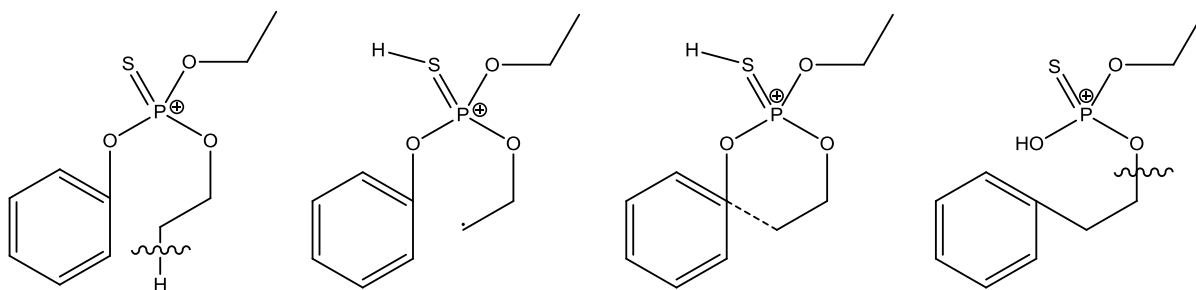


Figure 3.9.1(d). Putative mechanism for formation of $[M-141]^+$ ion from molecular ion of O-aryl-O,O-diethyl phosphorothionate (based on Zeller 1993).

Further studies of this unexpected rearrangement, to understand the mechanism and driving force for it, could be worthwhile, e.g. by MS/MS of the species involved by isotopic labelling of the OP ester ethoxy group (e.g. native form versus OCD_2CH_3), and by study of homologues (e.g. do the di-*n*-propyl or di-*i*-propyl esters undergo similar rearrangement).

3.10 Author's related published papers

Four published papers describing related areas of the author's research are included:

- Organophosphorus sulphides, sulphoxides and sulphones: Part 2. Characterisation by GCMS.

Wilkins, Hill & Lee (1985).

- Investigation of the transesterification products of Malathion.

Wilkins & Mason (1987).

- Rationalisation of the mass spectrometric and gas chromatographic behaviour of organophosphorus pesticides. Part 1. Substituted phenyl phosphorothioates.

Wilkins (1990).

– Reactions of perfluoro-tri-*n*-butylamine fragment ions in the quadrupole ion trap.

Creaser, West & Wilkins (2000).

Organophosphorus Sulphides, Sulphoxides and Sulphones Part 2.* Characterisation by Gas Chromatography - Mass Spectrometry

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The gas-chromatographic behaviour and electron-impact mass spectrometric characteristics are reported for nearly 90 organophosphorus sulphides, sulphoxides and sulphones, used as or derived from pesticides.

Keywords: *Organophosphorus pesticides; metabolites and contaminants; residues analysis; gas chromatography; electron-impact mass spectrometry*

Organophosphorus sulphides, sulphoxides and sulphones are widely used as agricultural pesticides, despite their generally high toxicity to man. Residual levels of these pesticides and, where appropriate, their oxidation or reduction products, must consequently be very low in harvested crops and derived foodstuffs to ensure consumer safety. Determination of these residues is usually performed by gas chromatography (GC), exploiting the high sensitivity and relatively specific response of currently available phosphorus detectors. However, as described in Part 1,¹ the lability of many of these compounds requires that gas-chromatographic results must be interpreted with caution and, where possible, be supported by mass spectrometric (MS) evidence.

In support of a programme for surveying pesticide residues in UK foodstuffs, and also as part of a PhD project for one of us (J.P.G.W.), we have studied the gas-chromatographic behaviour and electron-impact and chemical ionisation (EI and CI) magnetic-sector mass spectrometric characteristics of nearly 90 organophosphorus sulphides, sulphoxides and sulphones used as, or derived from, pesticides, and present here a summary of data useful in their identification. Gas-chromatographic and mass spectral data are also given for some phosphorus-containing contaminants observed during this work.

Mass spectral information on 45 members of this group of compounds has been published²⁻¹² but little of this has yet been incorporated into the libraries of mass spectrometer data systems. Mass spectra for many of the toxic metabolites of these compounds have not been published and it can be difficult for the analyst to acquire analytical standards of them. We have therefore included as many of these pesticides and their toxic metabolites as were available, or could readily be prepared. We have included some appropriate corrections/additions to published mass spectral data where the published material appeared to be incomplete, confusing or assigned to the wrong compound.

Experimental

Apparatus

Three different gas chromatograph - mass spectrometer combinations were used for this work: Varian 1400 - VG Micromass 12B; Dani 3800 - VG Analytical 7035; and Hewlett-Packard 5790 - JEOL DX300. For those compounds that decomposed completely under the gas-chromatographic conditions used, spectra were obtained by direct insertion of the pure compounds. All the spectra reported here were produced by EI with an ionisation energy of 30 or 70 eV

(mostly at 30 eV) and a source temperature of 150–200 °C, acquiring ions over the range m/z 20–620. In those situations where convincing relative molecular mass information was not provided by EI, CI, using 2-methylpropane or ammonia as the reagent gas, was employed. In addition, accurate mass measurement and/or metastable ion correlation was used to help identify apparently important fragment ions whose formation appeared to be due to complicated rearrangements. GC was performed as previously described,¹ using packed columns at temperatures from 150 to 240 °C, except that OV-1701 was used instead of OV-17 as the stationary phase. Relative retention times were measured on a 0.5 m × 2 mm column of 7% OV-1701 on 100–120-mesh Chromosorb W(HP), at a temperature of 220 °C, with a helium carrier gas flow-rate of 30 ml min⁻¹. When better gas-chromatographic resolution was required, a 25 m × 0.2 mm CP-Sil 19CB capillary column (Chrompak Ltd.) was employed, with splitless injection, on the HP 5790.

Chemicals

The pesticide names used here are those quoted in "The Pesticide Manual."¹³ Aphidan (*S*-ethylsulphinylmethyl *O*,*O*-diisopropyl phosphorodithioate, also known as IPSP) was obtained from Berk Ltd. (London). Carbophenothion and its metabolites were obtained from Stauffer Chemical Company (Westport, CT, USA). Demeton, demeton-*S*-methyl, disulfoton, fenamiphos, fensulfothion, fenthion, oxydeprofos, sulprofos and some of their metabolites were obtained from Bayer UK Ltd. (Bury St. Edmunds, Suffolk) and Bayer AG (Leverkusen, FRG). Phorate, temephos, terbufos and some of their metabolites were obtained from Cyanamid of Great Britain Ltd (Gosport, Hampshire). Chlorthiophos, ethion, sulfotep and TEPP were obtained from the Laboratory of the Government Chemist (London). Bensulide, famphur and methyl carbophenothion were obtained from Greyhound Chromatography Ltd. (Birkenhead, Cheshire, UK). Demephion and thiometon were isolated from Pyracide (BASF) and Ekatin (Sandoz) formulations, respectively. Vamidothion and its metabolites were obtained from May & Baker Ltd. (Brentwood, Essex).

Aphidan sulphide was found as a contaminant in the parent sulphoxide. The sulphides of fensulfothion and oxydeprofos were prepared by reduction of the respective parent sulphoxides with concentrated hydrochloric acid and solid potassium iodide, in glacial acetic acid solution, at room temperature for 2–5 min. After dilution with water, the sulphides were extracted with dichloromethane. The extract was dried by passing it through anhydrous sodium sulphate and, after addition of toluene (to assist removal of the acetic acid) and heptane (to assist removal of the iodine generated during the reaction), the solvent was removed using a rotary evaporator.

* For Part 1 of this series, see reference 1.
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A similar method of extraction was employed after the oxidations given below, with the addition of toluene where acetic acid was used. The sulphoxides of chlorthiophos, demephion, demeton, sulprofos and thiometon were prepared by oxidation with hydrogen peroxide (100 volume) in glacial acetic acid containing a trace amount of concentrated sulphuric acid, at room temperature for 10–15 min. The oxon sulphones of Aphidan, chlorthiophos, demephion, sulprofos and the oxon of famphur were prepared in a similar manner to the sulphoxides but with the reaction carried out at 40–80 °C for 10–20 min. Aphidan oxon sulphide was observed as a contaminant in the oxon sulphone preparation, presumably arising from oxidation of the Aphidan sulphide. The sulphones of chlorthiophos, demephion, sulprofos and thiometon were prepared from their respective sulphides, and those of Aphidan, oxydeprofos and temephos were prepared from their respective sulphoxides, by oxidation with potassium permanganate, using a method similar to that employed for residue determination¹ (except that the oxidant concentration was 1% *m/V*). Although the majority of the sulphones were produced in 10–30 min at room temperature, those of chlorthiophos and temephos required 30–60 min at 80 °C. In a few instances further purification of the products was required, and this was achieved by column chromatography using silica gel (Merck Art. 7734, Kieselgel 60) eluted with mixtures of hexane - acetone appropriate to the polarity of the product required.

Results and Discussion

Using similar experimental conditions (and an ionisation energy of either 30 or 70 eV) on our three mass spectrometers, the spectra produced from a given compound were almost identical.

There is a lack of uniformity in the presentation of mass spectral data in the literature and, where analogue spectra are given, the inaccuracies in printing can make the assignment of nominal mass difficult. Many of the spectra previously reported were generated using quadrupole mass spectrometers (as opposed to magnetic-sector instruments), which tended to discriminate against high mass ions. In spite of this, there is a good level of agreement between the spectra obtained and the majority of those which have already been published.^{2–12} In those instances where differences are present, these generally appear to be due to exaggerated relative intensities of the low mass ions in the previously published spectra. Another source of disparity is that some workers have reported spectral data below *m/z* 20. We did not monitor ions below this value because of the critical dependence of their relative intensities upon operational conditions and their generally high background levels, which make it difficult to generate reproducible, uncontaminated spectra. We have found that enhanced production of high mass ions, which are generally more useful in characterising compounds, can be effected by the use of a lower source temperature (which, however, may cause problems due to source contamination and loss of chromatographic efficiency) and a lower ionisation energy (which can result in reduced sensitivity). Mass spectral data, presented in the format used in the "Eight Peak Index of Mass Spectra"¹² (EPI), recorded for the compounds either eluting as gas chromatographic peaks or from the direct insertion of the pure compounds, are given in Table 1. Reference materials of the organophosphorus sulphides, sulphoxides and sulphones may contain organophosphorus contaminants that can mislead the analyst (especially if the contaminant exhibits a shorter retention time and/or a better chromatographic peak than the desired compound) and therefore we have included mass spectral data on some of these compounds at the end of the table.

The sulphides of Aphidan, fensulfothion and oxydeprofos were included because biological reduction^{14,15} of these

pesticides, which are sulphoxides, could result in the sulphides appearing in residues.

In contrast to Ripley and Braun,¹⁶ we were unable to obtain a GC peak corresponding to bensulide, but observed several related compounds apparently produced by pyrolysis. The spectrum that we recorded for bensulide (by direct insertion), is in marked contrast to that presented in EPI (Q3918), which exhibits a preponderance of low mass ions, possibly for the reasons outlined above. Our spectrum of carbophenothion is similar to those of Damico,³ Lovins,⁴ Mestres *et al.*⁵ and Stan *et al.*⁶ However, Mestres *et al.* reported a significant fragment ion at *m/z* 108 (which they claimed to be characteristic of both ethyl thiophosphates and ethyl dithiophosphates, a claim that we dispute), which we observed only as a minor component of the spectrum, and which is probably due to (C₆H₅S)⁺. Support for this suggestion comes from the appearance of this ion as a significant component of the spectra of bis(4-chlorophenyl) disulphide and bis(4-chlorophenylthio)methane (both found in technical grade carbophenothion). Stan *et al.*⁶ quoted a characteristic ion at *m/z* 172, which we consider should be at *m/z* 171. Of the three spectra represented in EPI, one (Q4128) is markedly different from that obtained by us, no ions outside the range *m/z* 127–173 being reported. The spectra represented in EPI for carbophenothion metabolites are in good agreement with those obtained by us.

Chlorthiophos I (using the isomer assignment given by Worthing and Walker¹³) is the only isomer represented in EPI but, again, the spectrum has a preponderance of low mass ions. The three isomers of chlorthiophos, and their metabolites, present an interesting complexity. In each group of spectra produced for the sulphides, sulphoxides and sulphones, respectively, two were very similar whereas the third was markedly different. Liquid chromatographic separation of the isomers of the sulphide and their subsequent conversion into the sulphoxides and sulphones, followed by GC - MS examination, indicated that the singular spectra were due to chlorthiophos II and its metabolites. A preliminary study by proton and carbon-13 nuclear magnetic resonance spectroscopy (NMR) did not assist in the elucidation of the structure of the isomers.

The spectrum we recorded for demephion is very different from that given by Stan *et al.*⁶ EPI contains eight spectra of demeton and its metabolites, which are in general agreement with our spectra, except for one (Q0295) of the three given for demeton-O, and that given for demeton-O sulphone (R0915). Our spectra of demeton-S-methyl, its sulphoxide and sulphone are similar to those of Stan *et al.*⁶ Skinner and Greenhalgh⁷ and EPI, except that the spectrum recorded by Stan *et al.*⁶ for the sulphoxide appears to represent a pyrolysis product (which is, in part, the subject of another report we currently have in preparation) and one (R6601) of the spectra of demeton-S-methyl sulphone presented in EPI is misleading in that no ions that we consider to be characteristic of this compound are given. The disulfoton spectra given by Damico³ and Hattori *et al.*⁸ agree with ours but those given by Stan *et al.*⁶ and Mestres *et al.*⁵ show some disparity, including an apparent lack of ions that we found in high abundance (*m/z* 60, 61, 88 and 89). Of the spectra of disulfoton and its metabolites presented in EPI, only one, of the sulphide (Q0301), shows a significant discrepancy from those that we present.

Our spectrum of ethion agrees with those of Damico,³ Stan *et al.*⁶ and Mestres *et al.*⁵ (except that in reference 5 the ion at *m/z* 231 appears to have been misprinted as *m/z* 281). One (Q2111) of the two spectra given for ethion in EPI is in close agreement with our spectrum. The spectra of famphur and its oxon represented in EPI are in good agreement with our spectra. Our spectra of fenamiphos and its sulphoxide agreed very closely with those of Skinner and Greenhalgh⁷ (although, owing to inadequate resolution in the printing of their spectra, some of the ions in these and other spectra appear to be one

Table 1. Mass spectral data for organophosphorus compounds

Compound name	Empirical formula	Molecular weight*	Mass to charge ratios		Relative intensities of most abundant ions										M ⁺ reference number	EPI†				
			43	47	75	107	140	140	140	140	140	140	140	140			140	140	140	140
Aphidan sulphide	C ₈ H ₂₁ O ₃ S ₃ P ₁	288	75	43	107	47	98	288	140	100	24	19	13	10	9	8	8	8	—	—
Aphidan	C ₉ H ₂₁ O ₃ S ₃ P ₁	304	139	43	41	97	27	78	45	100	68	31	26	18	17	17	17	17	—	—
Aphidan oxon sulphide	C ₉ H ₂₁ O ₄ S ₃ P ₁	320	143	139	43	159	41	97	47	100	94	58	32	25	24	23	22	5	—	—
Aphidan oxon sulphide	C ₉ H ₂₁ O ₄ S ₃ P ₁	272	75	74	43	41	128	115	47	100	82	48	28	22	22	17	10	—	—	—
Aphidan oxon sulphide	C ₉ H ₂₁ O ₄ S ₃ P ₁	304	169	127	43	123	211	59	41	100	74	52	50	30	29	21	21	0.1	—	—
Bensulide†	C ₈ H ₂₀ O ₄ N ₁ S ₃ P ₁	397	215	131	172	77	214	173	141	100	72	70	66	58	54	53	—	—	—	—
Carbophenothion	C ₁₁ H ₁₆ O ₂ Cl ₁ S ₃ P ₁	342	157	121	153	342	159	199	125	97	100	51	45	43	38	37	37	43	—	—
Carbophenothion sulphoxide	C ₁₁ H ₁₆ O ₃ Cl ₁ S ₃ P ₁	358	199	153	125	97	109	171	201	200	98	66	62	17	13	10	8	—	—	—
Carbophenothion sulphone	C ₁₁ H ₁₆ O ₄ Cl ₁ S ₃ P ₁	374	153	199	125	97	159	171	201	185	100	87	52	34	17	15	9	8	—	—
Carbophenothion oxon	C ₁₁ H ₁₆ O ₃ Cl ₁ S ₂ P ₁	326	183	109	326	139	157	111	155	75	100	95	55	53	44	44	42	40	—	—
Carbophenothion oxon sulphoxide	C ₁₁ H ₁₆ O ₄ Cl ₁ S ₂ P ₁	342	109	183	139	81	137	75	155	127	100	61	32	31	29	27	21	15	—	—
Chlorthiophos I	C ₁₁ H ₁₅ O ₃ Cl ₁ S ₂ P ₁	358	183	109	329	137	75	155	127	184	100	45	30	23	19	18	9	7	—	—
Chlorthiophos II	C ₁₁ H ₁₅ O ₃ Cl ₁ S ₂ P ₁	360	269	97	325	297	360	271	109	125	100	99	79	51	44	43	42	35	—	—
Chlorthiophos III	C ₁₁ H ₁₅ O ₃ Cl ₁ S ₂ P ₁	360	269	325	97	271	297	327	65	299	100	97	77	61	45	43	41	40	—	—
Chlorthiophos I sulphoxide	C ₁₁ H ₁₅ O ₄ Cl ₁ S ₂ P ₁	376	97	341	269	325	271	343	285	297	100	86	80	58	40	37	36	34	—	—
Chlorthiophos II sulphoxide	C ₁₁ H ₁₅ O ₄ Cl ₁ S ₂ P ₁	376	257	259	97	313	222	45	224	315	100	85	56	53	36	33	32	32	—	—
Chlorthiophos III sulphoxide	C ₁₁ H ₁₅ O ₄ Cl ₁ S ₂ P ₁	376	97	341	269	325	271	343	285	297	100	86	80	58	40	37	36	34	—	—
Chlorthiophos I sulphone	C ₁₁ H ₁₅ O ₃ Cl ₁ S ₂ P ₁	392	301	97	357	303	329	359	125	65	100	65	43	18	11	11	11	11	—	—
Chlorthiophos II sulphone	C ₁₁ H ₁₅ O ₃ Cl ₁ S ₂ P ₁	392	257	259	97	313	222	45	224	315	100	64	49	43	35	25	22	22	—	—
Chlorthiophos III sulphone	C ₁₁ H ₁₅ O ₃ Cl ₁ S ₂ P ₁	392	97	357	301	125	109	329	359	240	100	81	60	49	46	39	35	32	—	—
Demephion-O	C ₈ H ₁₉ O ₃ S ₂ P ₁	216	74	75	143	41	76	125	47	109	100	33	9	7	6	5	4	3	—	—
Demephion-O sulphone	C ₈ H ₁₉ O ₄ S ₂ P ₁	248	125	169	79	109	111	63	47	107	100	65	43	18	11	11	11	11	—	—
Demephion-O oxon sulphone	C ₈ H ₁₉ O ₅ S ₂ P ₁	232	153	109	127	79	96	95	154	63	100	82	64	15	10	9	7	7	—	—
Demephion-S	C ₈ H ₁₉ O ₃ S ₂ P ₁	216	74	142	112	75	109	41	76	79	100	15	13	12	10	9	8	7	—	—
Demephion-S sulphoxide†	C ₈ H ₁₉ O ₄ S ₂ P ₁	232	169	109	125	74	91	171	142	110	100	69	42	10	7	6	5	5	—	—
Demephion-S sulphone	C ₈ H ₁₉ O ₃ S ₂ P ₁	248	169	109	125	168	110	142	170	127	100	52	48	32	21	20	10	8	—	—
Demeton-O	C ₈ H ₁₉ O ₃ S ₂ P ₁	258	88	89	60	61	29	171	115	59	100	53	39	27	12	10	9	8	—	—
Demeton-O sulphone	C ₈ H ₁₉ O ₄ S ₂ P ₁	290	197	153	29	45	141	125	97	121	100	89	84	77	71	51	44	42	—	—
Demeton-O oxon sulphone	C ₈ H ₁₉ O ₅ S ₂ P ₁	274	125	99	181	121	127	153	155	29	100	85	73	45	44	43	34	2	—	—
Demeton-S	C ₈ H ₁₉ O ₃ S ₂ P ₁	258	88	60	29	89	61	114	115	93	100	42	14	13	13	10	9	9	—	—
Demeton-S sulphoxide†	C ₈ H ₁₉ O ₄ S ₂ P ₁	274	109	197	141	29	81	137	45	61	100	89	77	28	27	26	24	23	—	—
Demeton-S sulphone	C ₈ H ₁₉ O ₃ S ₂ P ₁	290	109	197	141	29	81	169	45	61	100	95	73	40	32	28	28	27	—	—
Demeton-S-methyl	C ₉ H ₁₅ O ₃ S ₂ P ₁	230	88	60	142	109	89	61	79	112	100	54	12	12	10	9	8	7	—	—
Demeton-S-methyl sulphoxide†	C ₉ H ₁₅ O ₄ S ₂ P ₁	246	169	109	125	29	79	105	60	142	100	92	48	13	11	9	9	7	—	—
Demeton-S-methyl sulphone	C ₉ H ₁₅ O ₃ S ₂ P ₁	262	169	109	125	110	168	142	29	170	100	51	33	15	13	9	8	7	—	—
Disulfoton	C ₈ H ₁₉ O ₃ S ₃ P ₁	274	88	89	60	61	153	186	142	274	100	34	20	13	12	12	12	12	—	—
Disulfoton sulphoxide†	C ₈ H ₁₉ O ₄ S ₃ P ₁	290	125	213	153	97	185	61	157	29	100	83	82	81	59	41	34	25	—	—
Disulfoton sulphone	C ₈ H ₁₉ O ₃ S ₃ P ₁	306	213	153	61	125	97	157	185	186	100	75	39	38	29	21	13	12	—	—
Disulfoton oxon, see Demeton-S																				
Famphur	C ₁₀ H ₁₆ O ₅ N ₁ S ₂ P ₁	325	218	93	125	217	44	109	219	220	100	29	24	21	14	11	11	6	—	—
Famphur oxon	C ₁₀ H ₁₆ O ₆ N ₁ S ₂ P ₁	309	202	201	44	265	109	186	93	309	100	67	39	21	18	18	12	9	—	—
Fenamiphos	C ₁₃ H ₂₂ O ₄ N ₁ S ₁ P ₁	303	303	288	260	154	195	44	217	304	100	37	32	31	26	18	15	15	—	—
Fenamiphos sulphoxide	C ₁₃ H ₂₂ O ₅ N ₁ S ₁ P ₁	319	304	122	303	319	154	196	80	44	100	84	35	29	25	24	21	29	—	—
Fenamiphos sulphone	C ₁₃ H ₂₂ O ₅ N ₁ S ₁ P ₁	335	320	292	292	58	44	321	122	293	100	66	22	17	15	12	9	8	—	—

Table 1. Continued

Compound name	Empirical formula	Molecular weight*	Mass to charge ratios	Relative intensities of most abundant ions	M ⁺ reference number	EPI†
Fensulfothion sulphide	C ₁₁ H ₁₇ O ₃ S ₂ P ₁	292	156 140 97 125 109 29	100 59 47 28 28 21	100	—
Fensulfothion	C ₁₁ H ₁₇ O ₄ S ₂ P ₁	308	308 141 97 125 153 109 292	100 55 49 47 47 33 33	19 55	P8887, P8888
Fensulfothion oxphone	C ₁₁ H ₁₇ O ₃ S ₂ P ₁	324	324 109 97 188 125 157 170	100 99 83 67 46 35 30	25 100	—
Fensulfothion oxon sulphide	C ₁₁ H ₁₇ O ₄ S ₂ P ₁	276	276 140 220 248 125 139 81 29	100 67 45 28 23 22 16	14 100	—
Fensulfothion oxon	C ₁₁ H ₁₇ O ₃ S ₂ P ₁	292	277 141 109 249 292 221 278 81	100 63 42 33 25 25 20	17 25	—
Fensulfothion oxon sulphone	C ₁₁ H ₁₇ O ₄ S ₂ P ₁	308	109 127 182 201 99 280 308 119	100 68 64 55 55 48 48	44 48	—
Fenthion	C ₁₀ H ₁₅ O ₃ S ₂ P ₁	278	278 109 279 169 280 125 245 137	100 19 14 14 12 11 9	100	P4678, P4679
Fenthion sulphoxide	C ₁₀ H ₁₅ O ₄ S ₂ P ₁	294	279 125 294 109 169 138 153 93	100 67 58 38 24 23 19	14 58	—
Fenthion sulphone	C ₁₀ H ₁₅ O ₃ S ₂ P ₁	310	125 310 109 93 136 105 79 137	100 94 62 37 36 32 24	17 94	—
Fenthion oxon	C ₁₀ H ₁₅ O ₄ S ₂ P ₁	262	262 247 263 264 135 217 109 215	100 31 20 10 10 9 8	6 100	R2356
Fenthion oxon sulphoxide	C ₁₀ H ₁₅ O ₃ S ₂ P ₁	278	263 109 278 262 127 79 45 77	100 70 27 25 19 13 12	11 27	R2357
Fenthion oxon sulphone	C ₁₀ H ₁₅ O ₄ S ₂ P ₁	294	294 215 104 109 231 230 295 279	100 60 28 23 19 19 18	17 100	R4948
Methyl carbophenothion	C ₈ H ₁₂ O ₂ Cl ₁ S ₃ P ₁	314	157 125 45 93 159 314 316 171	100 46 37 36 35 19 9	9 19	—
Oxydeprofos sulphide	C ₇ H ₁₇ O ₃ S ₂ P ₁	244	102 74 89 41 142 61 109 143	100 68 23 22 18 16 13	12 3	—
Oxydeprofos sulphone	C ₇ H ₁₇ O ₄ S ₂ P ₁	260	183 41 125 109 29 102 143 74	100 55 34 22 16 14 13	12 0.2	—
Phenkapton	C ₁₁ H ₁₅ O ₂ Cl ₁ S ₃ P ₁	376	121 153 199 97 125 191 376 193	100 79 58 52 51 45 39 34	39	—
Phorate	C ₇ H ₁₇ O ₂ S ₃ P ₁	260	75 121 97 93 47 260 65 29	100 25 12 11 10 9 9	6 9	O0288, O0290
Phorate sulphoxide	C ₇ H ₁₇ O ₃ S ₃ P ₁	276	97 125 153 199 29 65 171 75	100 84 80 73 20 15 13	12	—
Phorate sulphone	C ₇ H ₁₇ O ₄ S ₃ P ₁	292	153 97 199 125 171 65 29 93	100 84 79 79 17 16 15	14 6	Q8375
Phorate oxon	C ₇ H ₁₇ O ₃ S ₂ P ₁	244	74 75 171 111 138 109 47 244	100 72 43 28 17 16 15	13 13	—
Phorate oxon sulphoxide	C ₇ H ₁₇ O ₄ S ₂ P ₁	260	109 183 75 137 81 75 155 127	100 46 37 27 26 26 22 21	—	Q8377
Phorate oxon sulphone	C ₇ H ₁₇ O ₃ S ₂ P ₁	276	109 183 139 137 81 75 155 127	100 90 38 32 31 31 30	20	—
Sulprofos	C ₁₂ H ₁₉ O ₃ S ₂ P ₁	322	322 156 140 139 113 43 155 125	100 80 59 50 28 26 24	21 100	—
Sulprofos sulphoxide	C ₁₂ H ₁₉ O ₄ S ₂ P ₁	338	296 141 43 281 139 156 113 140	100 95 86 80 75 73 71	67 42	—
Sulprofos sulphone	C ₁₂ H ₁₉ O ₃ S ₂ P ₁	354	188 312 43 113 172 141 125 155	100 70 41 31 28 21 17 16	15 15	—
Sulprofos oxon	C ₁₂ H ₁₉ O ₄ S ₂ P ₁	306	140 306 139 43 125 156 307 97	100 90 35 21 18 16 14	14 90	—
Sulprofos oxon sulphoxide	C ₁₂ H ₁₉ O ₃ S ₂ P ₁	322	307 139 167 43 97 141 125 140	100 77 50 39 33 32 21	22 21	—
Sulprofos oxon sulphone	C ₁₂ H ₁₉ O ₄ S ₂ P ₁	338	172 196 43 139 296 188 97 157	100 61 56 51 37 36 36	28 15	—
Temephos‡	C ₁₆ H ₂₀ O ₆ S ₃ P ₂	466	466 467 93 468 203 125 357 341	100 20 16 16 14 11 6	4 100	Q9302, Q9303, Q9304
Temephos sulphoxide‡	C ₁₆ H ₂₀ O ₇ S ₃ P ₂	498	125 434 109 93 233 435 482 466	100 66 25 23 16 15 14	12 14	R6693
Temephos sulphone‡	C ₁₆ H ₂₀ O ₆ S ₃ P ₂	482	57 231 103 153 186 142 97 288	100 67 51 48 40 14 14	12 51	—
Terbufos	C ₉ H ₂₁ O ₃ S ₂ P ₁	288	57 97 153 29 125 97 264 200 172	100 83 57 49 47 38 36	29	—
Terbufos sulphoxide‡	C ₉ H ₂₁ O ₄ S ₂ P ₁	304	153 199 57 125 97 264 200 172	100 62 57 56 36 33 25	24 5	—
Terbufos sulphone	C ₉ H ₂₁ O ₃ S ₂ P ₁	320	171 57 215 170 272 143 115 126	100 63 60 56 31 29 19	15 31	—
Terbufos oxon	C ₉ H ₂₁ O ₄ S ₂ P ₁	272	170 232 109 183 57 139 41 137	100 47 35 27 20 17 17	16	—
Terbufos oxon sulphoxide‡	C ₉ H ₂₁ O ₃ S ₂ P ₁	288	156 183 184 109 140 57 75 155	100 86 85 85 84 80 47	37	—
Terbufos oxon sulphone	C ₉ H ₂₁ O ₄ S ₂ P ₁	304	88 89 60 125 61 246 90 158	100 21 19 11 8 5 4	5 4	—
Thiometon	C ₈ H ₁₅ O ₂ S ₂ P ₁	246	125 185 157 93 59 159 187 88	100 83 78 14 14 8 8	7	—
Thiometon sulphoxide‡	C ₈ H ₁₅ O ₃ S ₂ P ₁	262	125 185 157 93 59 159 187 88	100 83 78 14 14 8 8	7	—
Thiometon sulphone	C ₈ H ₁₅ O ₄ S ₂ P ₁	278	125 185 93 157 29 158 186 61	100 57 36 20 14 11 8	8 0.5	—
Thiometon oxon, see Demeton-S-methyl						
Vamidothion	C ₈ H ₁₈ O ₇ N ₁ S ₂ P ₁	287	87 145 146 142 109 88 58 60	100 46 18 16 15 14	12 9	1 Q7762
Vamidothion sulphoxide‡	C ₈ H ₁₈ O ₈ N ₁ S ₂ P ₁	303	169 109 125 58 87 143 142 86	100 48 28 22 20 14 13	10	—

Table 1. Continued

Compound name	Empirical formula	Molecular weight*	Mass to charge ratios								Relative intensities of most abundant ions								EPI* M ⁺ reference number
			319	87	169	109	125	58	110	86	142	100	34	19	18	8	7	7	
Vamidithion sulphone	C ₈ H ₁₈ O ₆ N ₁ S ₂ P ₁	319	87	169	109	125	58	110	86	142	100	34	19	18	8	7	7	6	—
<i>O,O</i> -Diethyl- <i>O</i> -phenyl phosphorothioate	C ₁₀ H ₁₅ O ₃ S ₁ P ₁	246	94	246	110	109	97	105	141	190	100	78	63	43	26	20	17	17	78
<i>O,O</i> -Diethyl- <i>O</i> -(2,4,5-trichlorophenyl) phosphorothioate	C ₁₀ H ₁₂ O ₃ Cl ₃ S ₁ P ₁	348	97	313	257	315	259	125	285	109	100	64	59	47	40	35	33	33	—
Ethion	C ₈ H ₁₂ O ₄ S ₄ P ₂	384	231	153	121	125	384	97	233	199	100	50	35	24	22	15	14	13	22
Oxydeprofos sulphone isomer	C ₇ H ₁₇ O ₅ S ₂ P ₁	276	183	41	125	109	142	143	29	79	100	49	37	18	17	14	11	9	0.1
Sulfotep	C ₈ H ₂₀ O ₅ S ₂ P ₂	322	322	202	121	93	65	174	238	266	100	56	52	51	41	40	37	36	100
TEPP	C ₈ H ₂₀ O ₇ P ₂	290	263	161	179	235	162	207	99	191	100	94	82	73	67	55	43	23	7
Tetraethyl pyrophosphorothioate	C ₈ H ₂₀ O ₄ S ₃ P ₂	338	97	121	65	93	125	29	338	153	100	90	76	65	52	46	41	33	41

* Molecular weights are given according to the "Eight Peak Index"² format, in which molecular weights are calculated from the integral values of atomic weights of the most abundant isotope of each element present, not according to the usual definition of average relative molecular mass.
 † Reference number of spectral data presented in the Eight Peak Index.²
 ‡ Spectra obtained by direct insertion of the pure compound.

mass unit different from the values that we have obtained), whereas those presented in EPI appear to have exaggerated abundances of low mass ions. The spectra of fensulfothion presented in EPI, and of fensulfothion and its oxon presented by Skinner and Greenhalgh,⁷ agree closely with our data. Our spectrum of fenthion is similar to that of Stan *et al.*⁶ and those of EPI but the oxon metabolites given in EPI show an exaggerated low-mass ion abundance. Our methyl carbophenothion spectrum is very similar to those given by Damico,³ Stan *et al.*⁶ and two of those presented in EPI, whereas the third EPI spectrum (Q4826) lacks the important molecular ion.

Our phenkapton spectrum compares well with two of the spectra in EPI whereas the third (Q7760) is in complete disagreement. Our spectrum of phorate agrees with those of Stan *et al.*,⁶ Skinner and Greenhalgh⁷ and Mestres *et al.*,⁵ except that the last of these did not report a peak at m/z 75. Our spectra of phorate oxon and phorate sulphone agree with those of Skinner and Greenhalgh⁷ but the spectrum that Singh and Cochrane⁹ assigned to phorate oxon sulphoxide is identical with that which we obtained from phorate sulphoxide.

The spectra presented in EPI for phorate, and four of its metabolites, have ions in common with our spectra but several of them exhibit enhanced low mass ion abundances and thus, again, do not record the more characteristic high mass ions. Our sulfotep spectrum agrees with those of Damico,³ Stan *et al.*⁶ and EPI. Our TEPP spectrum is similar to that of Tatematsu *et al.*¹⁰ and one of those (G0523) given in EPI, whereas the other two spectra given in the latter appear to represent triethyl phosphate, a commonly observed decomposition product of TEPP. The spectrum we obtained for temephos, by direct insertion, is not very different from that published by Biros and Ryan¹¹ and is similar to two (Q9302 and Q9304) of those given in EPI, but that given by Stan *et al.*⁶ (obtained by GC), unlike our spectrum, has ions of high abundance at m/z 263, 344, 360 and 435. Our spectrum of temephos sulphoxide has a greater abundance of characteristic high mass ions than that reported in EPI. The spectra we observed for terbufos and its metabolites agree very closely with those reported by Wei and Felsot,¹² except that their spectrum of the oxon sulphone lacks ions at m/z 140, 156 and 184, which we consider to be important components of the spectrum. The EPI spectra for terbufos, and for thiometon, are very similar to our spectra. The spectrum of vamidothion that we report is similar to that presented in EPI but appears to differ considerably from that given by Mestres *et al.*⁵

The spectral data that we have reported, for the compounds listed in Table 1, appear to be consistent with the structures that have been published for them.

Chemical ionisation would usually be the preferred ionisation technique to confirm the presence of low concentrations of most of these compounds in extracts of biological samples, especially where relatively high concentrations of co-extracted lipids are also present, but there seems little point in our reporting such spectra here because they convey little extra information, other than the relative molecular mass, and the spectra produced are dependent upon source design, source temperature and reagent gas employed.

However, we found that the compounds that produced little, or no, detectable molecular ion peak (intensity less than 0.1% of base peak) under EI often gave a relatively more abundant (though still weak) protonated molecular species, $(M + H)^+$. Detection of such relatively low abundance ions required a high sample pressure in the ion source (produced by direct insertion of microgram amounts of sample) but did allow us to obtain molecular weight information for these compounds, under EI conditions.

We regard relative retention times as an important part of the characterisation of these compounds because, in addition to the possibility of generating artifacts from them by pyrolysis

during GC, a few of them produced very similar mass spectra. Gas-chromatographic relative retention times are given in Tables 2 and 3.

Relative retention times may vary with temperature and the individual column used, but we found that the order of elution of these compounds was similar over a wide range of conditions, using packed or capillary columns.

We were unable to achieve gas-chromatographic elution of even a small proportion of the injected material of bensulide, temephos and its sulphoxide and sulphone, or of the sulphoxides of demephion-O, demephion-S, terbufos, terbufos oxon,

Table 2. Retention times of organophosphorus sulphides, sulphoxides and sulphones, relative to malathion (= 1.00)

	Sulphide	Sulphoxide	Sulphone
Aphidan	0.40	1.20	1.25
Aphidan oxon	0.30		1.00
Carbophenothion	3.40	8.50	7.90
Carbophenothion oxon	2.70	6.70	6.25
Chlorthiophos I	2.85	5.65	7.50
Chlorthiophos II	2.35	4.60	4.50
Chlorthiophos III	2.50	4.90	6.55
Demephion-O	0.20	*	0.70
Demephion-S	0.25	*	1.10
Demephion-O oxon			0.55
Demeton-O	0.30		1.00
Demeton-S	0.40	1.60*	1.65
Demeton-O oxon			0.85
Demeton-S-methyl	0.35	1.30*	1.35
Disulfoton	0.55	2.20*	2.45
Disulfoton oxon, see Demeton-S			
Famphur			4.40
Famphur oxon			3.75
Fenamiphos	2.05	7.10	7.30
Fensulfothion	1.15	3.50	4.00
Fensulfothion oxon	0.95	3.00	3.35
Fenthion	1.15	3.80	4.05
Fenthion oxon	1.00	3.30	3.45
Methyl carbophenothion	2.85		
Oxydeprofos	0.35	1.15*	1.35
Phenkapton	5.70		
Phorate	0.30	1.20	1.30
Phorate oxon	0.25	0.85	0.95
Sulprofos	3.40	10.7	11.7
Sulprofos oxon	2.70	8.00	9.00
Terbufos	0.30	*	1.50
Terbufos oxon	0.25	*	1.25
Thiometon	0.40	*	1.80
Thiometon oxon, see Demeton-S-methyl			
Vamidothion	2.50	*	5.35*

* Largely or wholly decomposed during injection and/or chromatography.

Table 3. Retention times of some organophosphorus contaminants observed in pesticide standards, relative to malathion (= 1.00)

Contaminant	Relative retention
<i>O,O</i> -Diethyl- <i>O</i> -phenyl phosphorothioate, found in fensulfothion	0.25
<i>O,O</i> -Diethyl- <i>O</i> -(2,4,5-trichlorophenyl) phosphorothioate, found in chlorthiophos	0.95
Ethion, found in terbufos sulphoxide	2.95
Oxydeprofos sulphone isomer, by-product of oxidation of oxydeprofos	1.15
Sulfotep, found in technical demeton	0.35
TEPP, found in oxidised technical demeton	0.25
<i>O,O,O,O</i> -Tetraethyl pyrophosphorotrithioate, found in phorate sulphone	0.85

thiometon and vamidothion. Coupled liquid chromatography - mass spectrometry would appear to offer possible advantages (because of the obvious instability of some of these compounds during gas chromatography) but we have had very little experience of this. For most of the labile sulphoxides, it is convenient to convert them into the sulphones,¹ which are more stable. Temephos and its metabolites may be detected in some biological extracts using a direct-insertion probe (at temperatures of up to 400 °C), exploiting the very low volatility and the relative abundance and high mass of their molecular ions. However, for the confirmation of trace levels of bensulide, vamidothion sulphoxide and its sulphone, liquid chromatography may be the only satisfactory means of introduction into the mass spectrometer.

Nonetheless, we find that the identity and concentration of the majority of these compounds, occurring as residues, can be readily confirmed using GC - MS.

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A Study of the Trans-esterification Products of Malathion by Capillary Gas Chromatography–Mass Spectrometry

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ABSTRACT

The organophosphorus insecticide malathion undergoes trans-esterification reactions with many simple alcohols under mild conditions. The products of a series of such reactions were prepared and characterised by capillary gas chromatography–mass spectrometry. For the trans-esterification reaction of malathion with a given alcohol, the mass spectra of the two initial products indicated that they were formed by exchange of one or other of the two non-equivalent ethoxy groups of the succinate moiety of the malathion molecule. The mass spectra were not sufficiently diagnostic to allow the site of trans-esterification for each product to be assigned. Synthesis and ¹³C-nuclear magnetic resonance spectroscopy were used to show that the major initial product is formed by trans-esterification of the β-carboxylate group of the succinate moiety. The two methoxy groups of the phosphorodithioate ester moiety exchange much less readily and, in the alcohols examined, such exchange was only apparent with ethanol. Gas chromatographic retention times and mass spectral data for the series of trans-esterification products examined are presented and discussed. The data and techniques presented provide a means of determining trace amounts of malathion α- and β-monoacids, which are important metabolites of malathion, after their conversion to a suitable ester (the monoacids themselves were not amenable to gas chromatographic analysis under the conditions described).

1 INTRODUCTION

Malathion, diethyl (dimethoxythiophosphorylthio)succinate (Fig. 1), is a widely used organophosphorus insecticide of low mammalian toxicity. Examination of commercial emulsifiable concentrate (e.c.) formulations of malathion has shown the presence of malathion-related contaminants which could have arisen from reaction of the malathion with alcohols present in the formulation. The results of this survey are reported separately.¹ Malathion analogues have also been observed in crop extracts,^{2,3} and in an e.c. formulation of malathion,² but the structures of these compounds were not elucidated. To facilitate the identification of the formulation contaminants mentioned above, the reaction of malathion with a variety of alcohols has been studied under physical conditions similar to those in which malathion formulations are stored. As the principal interest was in the products rather than the kinetics of the trans-esterification reaction the reaction conditions were not optimised, and no detailed kinetic data are presented. It was necessary to acidify the solutions to catalyse the trans-esterification reaction because neutral solutions reacted very slowly. The malathion formulations examined¹ were acidic. The alcohols used were methanol, ethanol, 1-propanol, 2-propanol, 1-butanol, 2-butanol, 2-methylpropan-1-ol (*iso* butyl alcohol), 2-methylpropan-2-ol (*tert* butyl alcohol), 2-methoxyethanol and 2-ethoxyethanol.

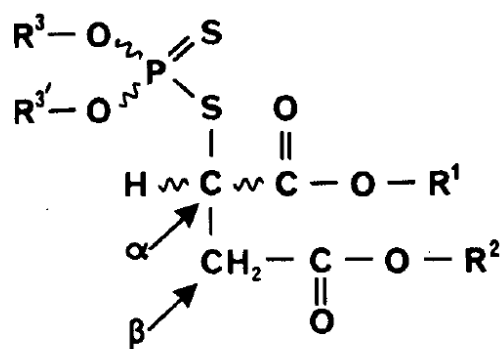


Fig. 1. The structure of malathion and its trans-esterification products.

	R ¹	R ²	R ³ and R ^{3'}
Malathion	ethyl	ethyl	methyl, methyl
Series A	ALK	ethyl	methyl, methyl
Series B	ethyl	ALK	methyl, methyl
Series C	ALK	ALK	methyl, methyl
Series D	ethyl	ALK	ETHYL, methyl
Series E	ethyl	ALK	ETHYL, ETHYL
Series F	ALK	ALK	ETHYL, methyl

R³ and R^{3'} are not stereochemically equivalent, and have not been distinguished.

Substituents differing from those in malathion are denoted by capital letters. ALK represents the alkyl or alkoxyalkyl group of the reaction alcohol.

Compounds are identified throughout this paper by a Roman numeral, to specify the reaction alcohol (ethanol **I**, methanol **II**, 2-propanol **III**, 1-propanol **IV**, 2-butanol **V**, 2-methylpropan-1-ol **VI**, 1-butanol **VII**, 2-methoxyethanol **VIII** and 2-ethoxyethanol **IX**) and a letter, to indicate the series to which it belongs. Malathion is a special case where **IA=IB=IC**.

These alcohols were used in order to produce a range of reaction products and because methanol, 2-methylpropan-1-ol, 2-methoxyethanol and 2-ethoxyethanol are used as formulating agents for malathion.¹

The structures of selected trans-esterification reaction products were determined by comparison of their g.c./m.s. characteristics with those of the products of synthesis and esterification of malathion α - and β -monoacids ($R^1=H$ or $R^2=H$ respectively in Fig. 1). The identity of the malathion β -monoacid was confirmed by ¹³C-nuclear magnetic resonance spectroscopy. The gas chromatographic retention times (relative to malathion) and mass spectral data for malathion and thirty-four of its trans-esterification products are presented, with the addition of those for malaoxon and isomalathion, which are also sometimes found in malathion formulations.

2 EXPERIMENTAL

2.1 Chemicals

All reagents were AR grade unless otherwise specified. The following chemicals were used as purchased: methanol HPLC grade from May & Baker Ltd; ethanol from James Burrough plc; 2-propanol, 2-methylpropan-1-ol LR grade, 2-methoxyethanol LR grade and 2-ethoxyethanol LR grade from BDH Chemicals Ltd; 1-propanol, 1-butanol, 2-butanol SLR grade and maleic anhydride 99.5% SLR grade from Fisons plc; 2-methylpropan-2-ol GPR grade, fumaric acid monoethyl ester 95%, and dimethyl dithiophosphate ammonium salt 95%, from Aldrich Chemical Co. Ltd. Technical malathion was premium grade supplied by Albright and Wilson Ltd (purity confirmed by g.c./m.s.).

2.2 Preparative methods

2.2.1 Trans-esterification products

Malathion (250 mg) was dissolved in each of the ten alcohols (25 ml) listed above, and the solutions were acidified with aqueous 6M sulphuric acid (0.1 ml). These solutions were allowed to stand at room temperature for six months. At intervals aliquots (1 ml) were taken, diluted with the same alcohol (99 ml) used originally and neutralised with solid sodium hydrogen carbonate.

The proportion of the series A compounds in the reaction products was increased by diluting an aliquot (1 ml) of aged trans-esterification reaction solution with ethanol (99 ml), and standing for 1–2 weeks at room temperature. The solution was neutralised with solid sodium hydrogen carbonate.

2.2.2 Synthesis of malathion monoacids

Malathion α - and β -monoacids were prepared using modifications of procedures described by Chen *et al.*⁴ and Wolfe *et al.*⁵

2.2.2.1 Malathion α -monoacid (Fig. 1, $R^1=H$): A mixture of *O,O*-dimethyl phosphorodithioic acid (0.55 g, 3.5 mmol) and maleic anhydride (0.34 g, 3.5 mmol) was heated in a stoppered tube, in a water bath at 85°C for 18 h to produce

TABLE 1
Mass Spectral Data in Eight Peak Index^a Format for Malathion, Isomalathion, Malaoxon and some Malathion Trans-esterification Products:

Compound	Substituents ^a			<i>m/z</i>	Molecular weight	Relative intensities of most abundant ions	<i>M⁺</i>	Empirical formula				
	<i>R'</i>	<i>R²</i>	<i>R', R²</i>					<i>C</i>	<i>H</i>	<i>O</i>	<i>P</i>	<i>S</i>
I ^b	Ethyl	Ethyl	Methyl, Methyl	173 127 125 93 158 99 143 29	330	100 87 80 67 45 28 24 18	2	10	19	6	1	2
ID	Ethyl	Ethyl	ETHYL, Methyl	173 127 111 79 29 172 107 99	344	100 90 90 68 65 61 50 40	1	11	21	6	1	2
IE	Ethyl	Ethyl	ETHYL, ETHYL	127 186 173 153 97 121 128 142	358	100 90 81 50 47 45 45 47	—	12	23	6	1	2
IIA	METHYL	Ethyl	Methyl, Methyl	159 125 93 113 158 127 55 79	316	100 88 79 69 57 39 14 13	—	9	17	6	1	2
IIIB	Ethyl	METHYL	Methyl, Methyl	159 125 93 113 158 127 99 143	316	100 90 81 56 54 35 17 16	1	9	17	6	1	2
IIIC	METHYL	METHYL	Methyl, Methyl	113 125 93 145 158 59 55 85	302	100 82 78 77 54 19 14 13	—	8	15	6	1	2
IID	Ethyl	METHYL	ETHYL, Methyl	111 159 79 172 113 107 139 127	330	100 93 74 66 59 54 35 33	1	10	19	6	1	2
IIIA'	1-PROPYL	Ethyl	Methyl, Methyl	143 127 125 158 187	344	100	—	11	21	6	1	2
IIIB'	Ethyl	2-PROPYL	Methyl, Methyl	187 125 145 127 93 158 143 99	344	100 75 71 71 65 56 47 33	1	11	21	6	1	2
IIIC'	2-PROPYL	2-PROPYL	Methyl, Methyl	143 125 159 93 43 201 41 158	358	100 41 38 34 31 27 27 23	0.5	12	23	6	1	2
IIID'	Ethyl	2-PROPYL	ETHYL, Methyl	187 145 127 172 111 79 99 157	358	100 80 77 70 54 46 45 35	—	12	23	6	1	2
IVA	2-PROPYL	Ethyl	Methyl, Methyl	127 187 125 143 99 93 141 145	344	100 92 76 53 49 47 47 46	—	11	21	6	1	2
IVB	Ethyl	1-PROPYL	Methyl, Methyl	187 125 93 127 99 158 145 141	344	100 70 63 57 50 47 31 30	1	11	21	6	1	2
IVC	1-PROPYL	1-PROPYL	Methyl, Methyl	201 143 125 141 99 93 158 43	358	100 78 74 72 68 51 50	1	12	23	6	1	2
IVD	Ethyl	1-PROPYL	ETHYL, Methyl	187 127 172 99 141 111 93 145	358	100 91 66 64 58 49 43 47	—	12	23	6	1	2
VA	2-BUTYL	Ethyl	Methyl, Methyl	143 127 125 93 145 158 56 201	358	100 48 42 35 25 23 21 14	—	12	23	6	1	2
VB	Ethyl	2-BUTYL	Methyl, Methyl	145 127 201 125 158 93 143 99	358	100 84 81 72 69 61 53 34	1	12	23	6	1	2
VC	2-BUTYL	2-BUTYL	Methyl, Methyl	143 125 57 173 93 257 117 158	386	100 30 25 24 23 22 22 21	—	14	27	6	1	2
VD	Ethyl	2-BUTYL	ETHYL, Methyl	145 127 172 201 111 157 79 99	372	100 88 86 85 54 48 46 38	—	13	25	6	1	2
VIA	2-METHYLPROPYL-1	Ethyl	Methyl, Methyl	143 125 127 93 57 158 145 201	358	100 47 46 42 36 29 27 25	—	12	23	6	1	2
VIB	Ethyl	2-METHYLPROPYL-1	Methyl, Methyl	125 145 93 57 127 158 201 143	358	100 97 87 87 83 76 61	0.5	12	23	6	1	2
VIC	2-METHYLPROPYL-1	2-METHYLPROPYL-1	Methyl, Methyl	143 57 125 93 158 173 117 229	386	100 79 35 30 27 19 16 15	0.5	14	27	6	1	2
VID	Ethyl	2-METHYLPROPYL-1	ETHYL, Methyl	172 57 127 111 99 201 145 79	372	100 71 64 56 54 52 50 41	—	13	25	6	1	2
VIIA	1-BUTYL	Ethyl	Methyl, Methyl	143 201 125 93 127 99 145 158	358	100 99 87 78 77 54 52 49	—	12	23	6	1	2
VIIIB	Ethyl	1-BUTYL	Methyl, Methyl	201 125 93 127 99 158 145 143	358	100 72 67 60 54 52 45 38	1	12	23	6	1	2
VIIIC	1-BUTYL	1-BUTYL	Methyl, Methyl	143 229 57 125 93 99 173 155	386	100 70 63 58 54 51 47 43	1	14	27	6	1	2
VIIID	Ethyl	1-BUTYL	ETHYL, Methyl	201 99 145 111 172 127 56 79	372	100 96 76 68 66 62 58 57	—	13	25	6	1	2
VIIIA	2-METHOXYETHYL	Ethyl	Methyl, Methyl	143 59 125 93 127 158 58 203	360	100 72 59 53 50 37 35 14	—	11	21	7	1	2
VIIIB	Ethyl	2-METHOXYETHYL	Methyl, Methyl	59 145 125 158 93 127 99 203	360	100 98 98 86 81 76 38 33	—	11	21	7	1	2
VIIIC	2-METHOXYETHYL	2-METHOXYETHYL	Methyl, Methyl	59 143 125 93 158 58 157 274	390	100 92 40 33 27 27 20 14	—	12	23	8	1	2
VIIID	Ethyl	2-METHOXYETHYL	ETHYL, Methyl	59 172 127 111 145 58 99 157	374	100 97 87 77 68 57 47 46	—	12	23	7	1	2
IXA	2-ETHOXYETHYL	Ethyl	Methyl, Methyl	143 125 93 127 158 73 171 217	374	100 44 39 37 35 34 19 7	—	12	23	7	1	2
IXB	Ethyl	2-ETHOXYETHYL	Methyl, Methyl	145 158 125 127 93 73 171 143	374	100 82 74 66 60 56 42 34	—	14	27	7	1	2
IXC	2-ETHOXYETHYL	2-ETHOXYETHYL	Methyl, Methyl	143 73 45 125 158 93 72 274	418	100 64 62 29 27 26 25 14	—	14	27	8	1	2
IXD	Ethyl	2-ETHOXYETHYL	ETHYL, Methyl	145 172 73 127 111 107 139 171	388	100 97 84 58 47 41 30 37	—	13	25	7	1	2
X		Isomalathion		127 99 55 128 173 47 126 283	330	100 57 31 24 20 20 19 12	—	10	19	6	1	2
XI		Malaoxon		127 99 55 126 128 268 173 195	314	100 52 29 16 15 8 8 8	1	10	19	7	1	1

^aCompound codes are defined in Fig. 1. Substituents differing from those in malathion are denoted by capital letters

^bCompound I (malathion) is a special case: I=IA=IB=IC.

^cThe spectrum of this compound was too weak to report in full.

the mercaptosuccinic anhydride *S*-ester of *O,O*-dimethyl phosphorodithioic acid (the identity of which was confirmed by direct insertion m.s.). Ethanol (0.08 g, 1.8 mmol) was added, to produce a mixture of malathion α - and β -monoacids. A sample of several milligrams of monoacids, enriched in the α -isomer, was obtained using the thin-layer chromatographic method of Welling *et al.*⁶ Acetone was used to extract the product from the silica gel.

2.2.2.2 Malathion β -monoacid (Fig. 1, $R^2=H$): A mixture of *O,O*-dimethyl phosphorodithioic acid (0.55 g, 3.5 mmol) and ethyl hydrogen fumarate (0.43 g, 3.5 mmol) was heated in a stoppered tube, in a water bath at 85°C for 18 h to produce malathion β -monoacid (yield *ca* 70%).

2.2.3 Derivatives of malathion monoacids

Compounds **IIA** and **IIB** (Table 1) were produced by methylation of the malathion α - and β -monoacids respectively, with diazomethane⁷ in acetone solution. Compounds **VIIA** and **VIIB** were produced by reacting the appropriate monoacid with an excess of butyl *p*-toluenesulphonate in 1-butanol, with a drop of aqueous 6M sulphuric acid as catalyst, for 30 min at room temperature, followed by neutralisation with solid sodium hydrogen carbonate.

2.3 Analytical methods

2.3.1 Capillary gas chromatography–mass spectrometry (g.c./m.s.)

The trans-esterification products and malathion monoacid derivatives were analysed by capillary g.c./m.s., using a Hewlett-Packard 5790A gas chromatograph coupled directly to a JEOL JMS-DX300 double-focusing mass spectrometer. A 25 m×0.22 mm i.d. Chrompack WCOT fused silica CP-Sil-19 CB (film thickness 0.22 μ m) capillary column was used, with a Hewlett-Packard capillary injector at 230°C operated in a splitless mode (0.7 min valve time). Helium carrier gas flow rate was *ca* 1 ml min⁻¹. The oven temperature was held at 40°C for 2 min after injection, programmed to 100°C at 20°C min⁻¹ and held for 1 min, then programmed to 270°C at 3°C min⁻¹, and held for 10 min (total run time 72.7 min). This temperature programme was used because it combined optimum g.c. resolution and good sensitivity for the longer retention time components with the shortest practicable run time. All of the compounds described in Table 1 eluted during the second temperature ramp, facilitating comparison of retention times.

The mass spectrometer was operated in electron impact ionisation mode, with an electron energy of 70 eV, a source temperature of 200–210°C, an accelerating voltage of 4 kV and a resolution of 1000. Ammonia chemical ionisation m.s. was used to confirm molecular weights.

2.3.2 Nuclear magnetic resonance spectroscopy (n.m.r.)

Samples were dissolved in deuteriochloroform with tetramethylsilane as internal standard. The proton n.m.r. spectra of the malathion α - and β -monoacids were recorded at 60 MHz on a Varian EM360 proton spectrometer. The proton-noise-decoupled ¹³C-n.m.r. spectrum of the β -monoacid was recorded at 25.15 MHz on a JEOL PFT-100 spectrometer, using a spectral width of 5000 Hz with 8192 data

points. Approximately 20 000 scans were used to obtain the averaged ^{13}C spectrum.

3 RESULTS AND DISCUSSION

3.1 Determination of identity and purity of the synthesised malathion monoacids

3.1.1 Nuclear magnetic resonance spectroscopy

Analysis by proton n.m.r. spectroscopy did not provide the unambiguous differentiation of the α - and β -monoacids that was required, but Welling *et al.*⁹ have shown that malathion α - and β -monoacids produce clearly distinguishable ^{13}C -n.m.r. spectra. The distinction arises from their observation that in compounds related to malathion, carbon nuclei separated from the phosphorus atom by four or more bonds do not exhibit a measurable long-range phosphorus-carbon coupling, while interactions involving up to three bonds are observable. Hence, the critical observations are the coupling of the phosphorus with the α -carbonyl carbon, and the absence of coupling with the β -carbonyl carbon. For the spectrum of malathion β -monoacid Welling *et al.* reported a doublet ($\delta=170.1$) with a coupling constant of 5.9 Hz for the α -carbonyl carbon, and a singlet ($\delta=176.2$) for the β -carbonyl carbon. The ^{13}C -n.m.r. spectrum of the synthetic malathion β -monoacid used here exhibited a doublet ($\delta=170.1$) with a coupling constant of 6.1 Hz and a singlet ($\delta=176.0$). This provides unequivocal evidence that the synthesised malathion β -monoacid used in this study is identical to that characterised by Welling *et al.*

3.1.2 Thin-layer chromatography (t.l.c.) and g.c./m.s. after methylation

T.l.c. analysis⁶ of the mixed preparation of malathion α - and β -monoacids (Section 2.2.2.1 above) indicated two major components with R_F values of 0.4 and 0.55. These are in good agreement with the reported values (0.39 and 0.57 for α - and β -monoacid respectively). G.c./m.s. analysis of this preparation, after methylation,⁷ indicated approximately equal proportions of the α - and β -monoacids. In the sample of monoacids enriched in the α -isomer the ratio of α to β was 7:3.

Similar t.l.c. analysis of the synthesised β -monoacid (Section 2.2.2.2) revealed one major component (R_F value 0.55), as expected. G.c./m.s. analysis of the unpurified synthetic material, after methylation, indicated a ratio of greater than 10:1 for β - to α -monoacid.

3.2 The trans-esterification reactions

For all the alcohols with which reaction occurred, the first two trans-esterification products to be observed were due to exchange of one or other of the two non-equivalent ethoxy groups (R^1O or R^2O) of the succinate moiety of the malathion molecule. The observed rates of reaction varied greatly for different alcohols. For example, with methanol, which reacted most rapidly, products were observable after only a few days. With 2-butanol, detectable conversion took months, and with 2-methylpropan-2-ol no reaction was apparent even after a year. Exchange of the second ethoxy group to produce the disubstituted (series C) compounds

occurred after longer reaction times, and with many alcohols the series **C** compound was the major reaction product after six months.

The derivatisation of the malathion monoacids to produce the methyl esters **IIA** and **IIB**, and the butyl esters **VIIA** and **VIIB** was performed using diazomethane and butyl *p*-toluenesulphonate respectively. Esterification of these monoacids by reaction with methanol or 1-butanol was not used because unwanted trans-esterification could occur under the conditions required for such reactions. Comparison of these derivatives with the products of the trans-esterification reactions enabled us to show that for methanol and 1-butanol, the major initial trans-esterification product of malathion is due to reaction at the β -carboxylate group (to give series **B** compounds) rather than at the α -position (to give series **A** compounds). The similarity of the results obtained from the analysis of the other trans-esterification reaction solutions indicates that this generalisation applies to all the alcohols studied. This result can be rationalised on simple steric grounds, in that the β -carboxylate group is more accessible to reaction with an alcohol than the α - because it is adjacent to a primary rather than a secondary carbon. It was also observed that the slower the rate of the trans-esterification reaction the greater was the predominance of the series **B** products over the **A** products, e.g. with methanol their ratio was *ca* 10:1, but with 2-butanol, it was *ca* 100:1. In other words, the more hindered alcohols reacted more slowly and more selectively. The concentrations of series **A** products were consequently often relatively low, and although their retention times could be determined, their mass spectra were often too weak to measure accurately. However, increased relative concentrations of series **A** compounds were produced by adding ethanol in excess to aged trans-esterification reaction solutions rich in series **C** product. The ethanol reacted relatively rapidly with the series **C** compound at the β -carboxylate group to produce preferentially the series **A** analogue, rather than the **B** analogue by reaction at the α -carboxylate group. Sufficient material was produced by this 'back-trans-esterification' method to determine both g.c. and m.s. characteristics of the series **A** compounds.

The exchange of an ethoxy group of the succinate moiety of malathion for that of an unlabelled ethanol molecule is undetectable. Slow reaction between malathion and ethanol was observed, however, due to exchange of one or both methoxy groups (R^3O or/and $R^{3'}O$) of the phosphorodithioate moiety, to give **ID** and **IE**, respectively. Trans-esterification of the phosphorodithioate moiety was not observed with any other alcohol (exchange with unlabelled methanol being undetectable). In most of the aged malathion trans-esterification reaction solutions series **D** and **F** compounds were observed, even when the reaction alcohol was not ethanol. The ethanol involved in this reaction was presumably that released from the initial succinate trans-esterification reactions.

3.3 Gas chromatography

The g.c. retention times (relative to malathion which eluted after 2710 s) of the trans-esterification products prepared in this study are presented diagrammatically in Fig. 2. This presentation of the data allows the overall trends to be easily observed: for example, the reversed g.c. elution order of **IIA** and **IIB**

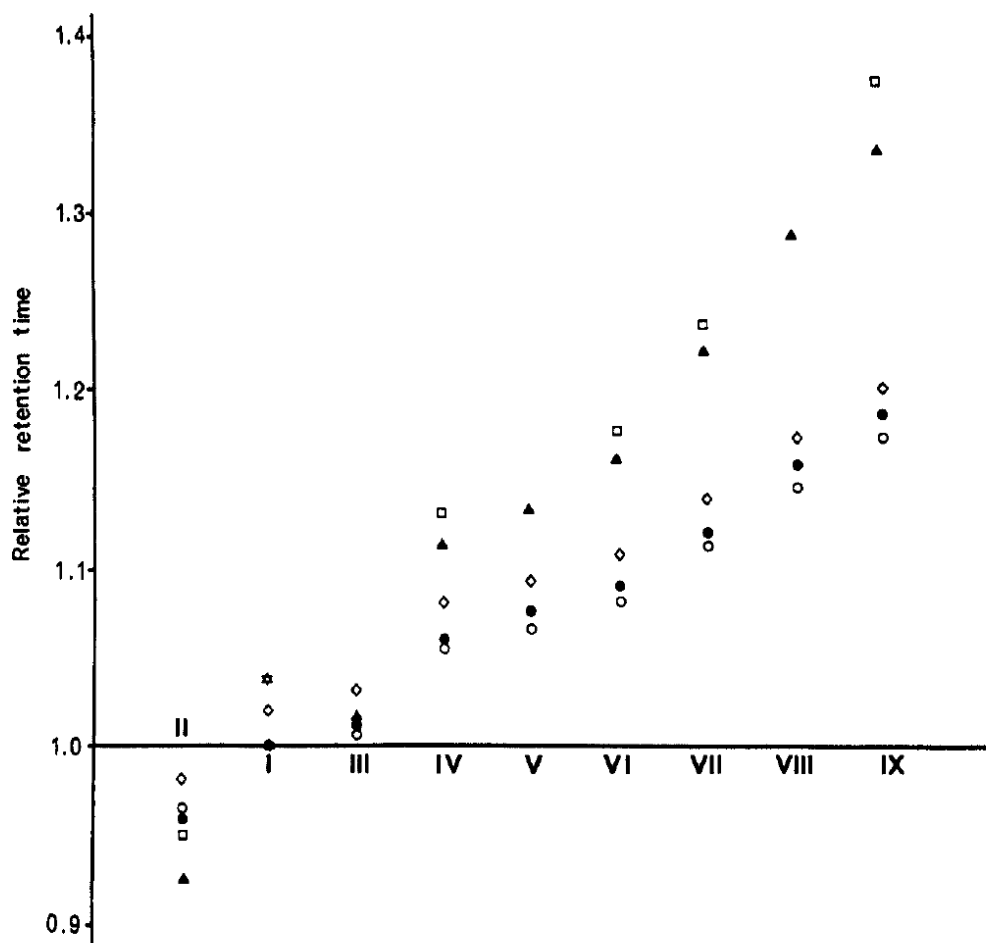


Fig. 2. Diagrammatic presentation of the relative retention times of the trans-esterification products of malathion (malathion=1.000): (○) series A; (●) series B; (▲) series C; (◇) series D; (☆) series E; (□) series F.

compared with all the other series A and B isomers, which is consistent with the general observation that the isomer with the larger substituent group at R², compared with that at R¹, has the longer retention time. Data for series F compounds are included in Fig. 2, but are not included in Table 1 because the concentration of these products was too low for accurate measurement of their mass spectra.

Malathion and series A, B, C and E compounds, whose molecules contain one chiral centre, eluted as sharp, single g.c. peaks. Series D and F compounds which contain two chiral centres appeared to elute as broad, single g.c. peaks but their low concentration made accurate observation of peak shapes impossible. Iso-malathion $[(\text{CH}_3\text{O})(\text{CH}_3\text{S})\text{PO}.\text{SCH}(\text{COOC}_2\text{H}_5)(\text{CH}_2\text{COOC}_2\text{H}_5)]$ which also contains two chiral centres, was resolved into two sharp g.c. peaks of similar size with relative retention times of 1.106 and 1.108. These observations for compounds with two chiral centres are presumably due to the partial or complete separation of the two diastereoisomeric pairs of enantiomers. After very long reaction times many solutions were found to contain tetra-alkyl thiodisuccinates.

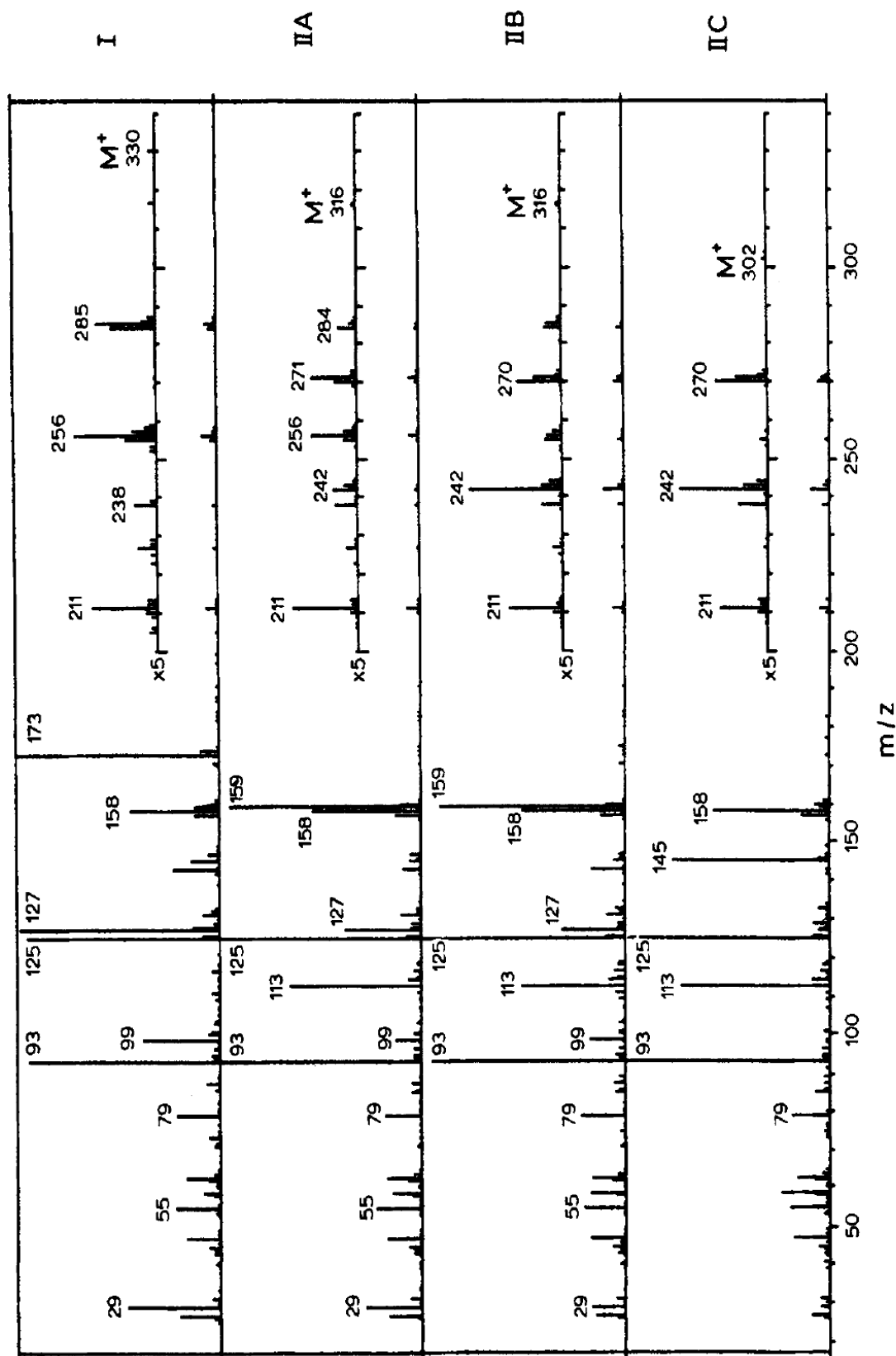


Fig. 3. Electron impact mass spectra of compounds I, II A, II B, and II C.

The diastereoisomers of these compounds were more readily resolved. The two g.c. peaks due to tetraethyl thiodisuccinate (the resolution of which has been used to check g.c. column performance during the analysis of malathion formulations)¹⁰ had relative retention times of 1.136 and 1.142. The relative retention time of malaoxon was 0.976.

3.4 Mass spectrometry

The mass spectral data for malathion and thirty-four of its trans-esterification products, isomalathion and malaoxon are given in 'Eight Peak Index'⁸ format in Table 1.

The mass spectra presented by Hansen *et al.*³ for malathion, 'methyl ethyl succinate analogue of malathion', and 'ethyl butyl succinate analogue of malathion' are very similar to the spectra of **I**, **IIB** and **VIIIB**, respectively, but as they were using packed column g.c., they were unable to resolve the minor isomers **IIA** or **VIIA**.

The spectra of **I**, **IIB**, and **VIIIB** have characteristic ions at m/z 173, 159 and 201 respectively, which are due to the intact dialkyl succinate moiety $[\text{CHCOOR}^1.\text{CH}_2\text{COOR}^2]^+$. Analogous characteristic ions are observed in the spectra of the other compounds reported here, for example; **IIIA** and **IIIB**, **IVA** and **IVB**, m/z 187; **VIIIA** and **VIIIB**, m/z 203; and **IXA** and **IXB**, m/z 217.

The ion characteristic of the intact phosphorodithioate moiety $[(\text{R}^3\text{O})(\text{R}^3'\text{O})\text{PS.SH}]^+$ is observed at m/z 158 for malathion, and this ion is also found in the spectra of other series A, B and C compounds. For series D and F compounds, this ion is observed at m/z 172, and for series E compounds (of which only **IE** was observed), it appears at m/z 186. The measurement of the retention times of series D and F compounds, which were present in low concentrations, was often dependent on the observation of the ion at m/z 172. Another ion characteristic of series D and F compounds is observed at m/z 111, due to $[\text{CH}_3\text{O.PS.OH}]^+$ produced by a rearrangement reaction involving the loss of ethylene.³ A related ion, m/z 97 $[(\text{HO})_2.\text{PS}]^+$, is observed in the spectrum of **IE**.

Generally, the differences between the mass spectra of pairs of series A and series B isomers are quantitative rather than qualitative, and are not easily interpreted in simple stereochemical or mechanistic terms. Thus the isomers of these pairs could not be identified unambiguously on the basis of their mass spectra. This was mainly because there is no evidence in their spectra of the fission of the α/β C-C bond under the conditions used here, which might have given rise to diagnostic ions. The complete mass spectra of **IIA** and **IIB** are presented in Fig. 3 (with those of **I** and **IIC** for comparison). The small differences apparent between them were reproducible. For example, in the mass spectrum of **IIA**, the most intense ions of higher mass than m/z 220 are at m/z 256 and 271, whereas for **IIB** they are observed at m/z 242 and 270 (as in the spectrum reported by Hansen *et al.*² for 'methyl ethyl succinate analogue of malathion' mentioned above). As can be seen from Table 1, the differences between the spectra of most other pairs of series A and B isomers are greater. For example, the ion at m/z 143 is more abundant than that at m/z 145 in series A compounds, whereas the contrary is true for series B compounds.

4 CONCLUSIONS

The β -carboxylate group of malathion, under acid-catalysed conditions, undergoes trans-esterification reactions more readily than does the α -carboxylate; the phosphorodithioate moiety is much less reactive than either of the carboxylate groups. The ease of production of the malathion trans-esterification products, demonstrated here, indicates that formulations of malathion containing alcohols will be liable to undergo such reactions. The amount of conversion will presumably depend upon the concentration and type of alcohol(s) in the formulation, the pH of the formulation, the time spent in storage and the temperature at which it is stored. The previously reported³ 'environmental alteration' product of malathion (ID) may therefore have been a formulation alteration product.

The data presented should allow other g.c. detectors, such as nitrogen-phosphorus or flame photometric detectors, to be used for the determination of malathion trans-esterification products, although mass spectrometry, because of its specificity, would generally be the preferred means of determination. The technique described affords a means of determining trace levels of malathion α - and β -monoacids, after their conversion to suitable esters. These monoacids are important biological detoxification metabolites of malathion.⁶

The insecticidal properties of a malathion formulation may be modified by the changes to its composition caused by the trans-esterification reactions described. The environmental and toxicological implications of the presence of these malathion analogues in formulations is unknown. This type of reaction may occur when any pesticide whose structure contains an ester group is formulated with an alcohol, possibly causing changes to its efficacy and toxicity.

ACKNOWLEDGEMENTS

The advice and encouragement of Donald Lee and Alan Hill are greatly appreciated. The authors also thank Stuart Pattinson for technical assistance, Dr Norman Janes and Diana Johnson of Rothamsted Experimental Station, Harpenden, Herts for the ¹³C-n.m.r., and Dr John Chambers of MAFF Slough Laboratory, for the proton n.m.r.

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Rationalisation of the Mass Spectrometric and Gas Chromatographic Behaviour of Organophosphorus Pesticides: Part 1—Substituted Phenyl Phosphorothioates

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ABSTRACT

The mass spectrometric and gas chromatographic behaviour of a series of O,O-dialkyl O-(substituted phenyl) phosphorothioates, and some related compounds is reported. Several of these compounds are widely used as pesticides. Appraisal of these data has permitted an increased understanding of the influence of phenyl substituents on the appearance of the mass spectra of these compounds. In particular, the nature and extent of mass spectrometric ortho effects, which are associated with weak molecular ion intensity, and/or the generation of rearrangement ions are reported. Examples in which an understanding of these effects has been helpful in the identification of related compounds, such as technical impurities and degradation products, are given. Published mass spectrometric data for some of these compounds are critically appraised.

1 INTRODUCTION

Many pesticides, and particularly insecticides, are organophosphorus compounds. Unequivocal determination of contaminants in formulations and of residues of these compounds and their biologically active metabolites in food and the environment is most important. The use of mass spectrometry for the identification of pesticides is becoming increasingly important.¹ The enhanced reliability of the data which may be generated using mass spectrometric (MS) techniques (as compared to those produced by less specific detection systems such as flame photometry or electron capture) has ensured that mass spectrometry is usually the preferred means of detection following gas chromatographic (GC) separation,

though its expense and technical complexity have, until recently, limited its widespread application. The increasing availability of relatively low-cost 'bench-top' MS instruments has overcome these limitations to some extent.

Electron-impact ionisation (EI) mass spectrometry is almost invariably the ionisation mode used in initial GC-MS investigations. It affords high sensitivity, and interpretation of the spectral data can reveal structural information. The reproducibility of EI data on a given mass spectrometer, and between different mass spectrometers using similar conditions, is generally good, so that rapid identification of spectra by comparison with mass spectral databases is possible. A disadvantage of EI is that it does not always provide molecular weight information, as the molecular ion species (M^+) of many compounds is too transient to be observed in the mass spectrum. The milder ionisation conditions of chemical ionisation (CI) mass spectrometry can be helpful in these cases, as they may be used to generate spectra with strong pseudo-molecular ions (but it must be borne in mind that these do not necessarily indicate unambiguously the molecular weight). Conversely, absolute sensitivity is usually lower, and the reduction in fragmentation means that CI spectra contain less structural information. There is also much greater possibility for variability in CI spectra because, apart from differences due to the reagent gas used, they are sensitive to differences in instrument design and performance, source temperature and reagent-gas purity, so they are best used in conjunction with, and normally following, EI measurement.

The EI-MS fragmentation pathways of organophosphorus esters have been extensively investigated,^{2,3} but most authors have concentrated upon the behaviour of the phosphorus-containing moiety. Examination of the mass spectra of a number of organophosphorus pesticides, particularly the three isomers of chlordiophos,⁴ had indicated that there were strong isomeric influences to be observed. However the structures of organophosphorus pesticides are very diverse, and provide little opportunity for systematic investigation of such effects. In the present study the influence of aromatic substituents on the mass spectra of a series of groups of isomers with identical organophosphorus moieties has been explored. The *O,O*-dimethyl, *O*-(monosubstituted phenyl) phosphorothioates (Fig. 1) were selected for study, for several reasons; primarily because they are representative of one of the largest classes of organophosphorus pesticide, the *O,O*-dialkyl *O*-aryl phosphorothioates (see Table 2 for 21 examples); and because a wide range of synthetic precursors, the monosubstituted phenols, was available.

In general *O,O*-dialkyl *O*-aryl phosphorothioate pesticides are readily amenable to GC separation, a factor which greatly facilitates their identification. The capillary GC retention data for the series of synthesised compounds are presented (Table 1) and the influence of isomer-substitution on elution order discussed.

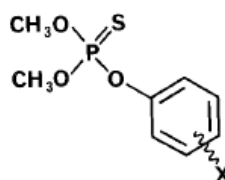


Fig. 1. *O,O*-Dimethyl *O*-(monosubstituted phenyl) phosphorothioates.

2 EXPERIMENTAL METHODS

2.1 Materials

Samples of pesticides were obtained variously from the Laboratory of the Government Chemist (London, UK), the United States Environmental Protection Agency (Pesticides and Industrial Chemicals Repository, Research Triangle Park, NC, USA), and Promochem Ltd (St Albans, Herts, UK). Dimethyl chlorothiophosphate, 97%, was obtained from Aldrich. The analytical grade phenols were obtained from a variety of commercial sources. The 3-methylmercaptophenol, which was not readily available, was prepared from 3-methylmercaptoaniline (Aldrich, 97%) by formation of its diazonium salt and reaction with 0.1 M aqueous sulphuric acid (identity of product confirmed by GC-MS).

2.2 Synthesis of *O,O*-dimethyl *O*-(monosubstituted phenyl) phosphorothioates

A series of *O,O*-dimethyl *O*-(monosubstituted phenyl) phosphorothioates with *ortho/meta/para* fluoro-, chloro-, bromo-, iodo-, nitro-, methyl-, methoxy-, methylthio-, trifluoromethyl-, cyano-, phenyl-, amino- and hydroxy- substituents (see Table 1) was prepared by reaction of the appropriately substituted phenol with dimethyl chlorothiophosphate.⁵ To the monosubstituted phenol (0.5 mM), dissolved in ethanol (5 ml) and potassium hydroxide (0.7 mM; added as 500 g kg⁻¹ aqueous solution), was added dimethyl chlorothiophosphate (0.06 mM) at room temperature. The reaction mixture was warmed to 70°C for 1 h with constant stirring. A crystalline white precipitate of potassium chloride indicated that reaction had occurred. Water (20 ml) was added to the cooled reaction mixture, the phosphorothioate product was partitioned into ethyl acetate, dried with anhydrous sodium sulphate and analysed by GC-MS. (Several other polysubstituted analogues were also prepared using this procedure.) The bis-substituted *O,O*-dimethylphosphorothioyl [(CH₃O)₂P=S.O⁻] derivatives were generated as by-products of the hydroxy-substituted compounds.

Addition of potassium hydroxide solution to 4-aminophenol produced a black tar, so the 4-amino-substituted product was prepared (albeit in low yield) using potassium carbonate. The compounds containing methyl sulphoxide (CH₃SO-) and methyl sulphone (CH₃SO₂-) groups were obtained by peracetic acid and permanganate oxidation respectively of the methyl sulphides.⁶

2.3 Gas chromatography and mass spectrometry

Capillary GC separations were performed on a 25 m × 0.3 mm i.d. CPSil-19 CB column (Chrompack UK Ltd). The temperature programme was 40°C for 1 min, then 10°C min⁻¹ to 270°C, held for 20 min; injector 230°C. Helium was used as carrier gas, with a flow of *c.* 1 ml min⁻¹. The column was directly coupled to a JEOL DX300 double-focusing mass spectrometer. Electron-impact ionisation mass spectra were obtained with an electron energy of 70 eV at 1000 resolution and a source temperature of 200°C. This mass spectrometer was also used for the direct insertion and accurate mass (performed at 10 000 resolution) measurements. For comparison, and evaluation of mass spectral reproducibility, data were also

TABLE 1
 Capillary GC Retention Times (Obtained Using a 25-m CPS1119 Column) and Characteristic Ions in the EI Mass Spectra of the *O,O*-
 Dimethyl *O*-(Monosubstituted Phenyl) Phosphorothioates Investigated (All Spectra Exhibited m/z 47, 62, 63, 79 and 93)

Phenyl substituent	MW (daltons)	RT (s)	m/z 109 (%)	m/z 125 (%)	m/z 217 (%)	M^+ (%)	($M-110$) ⁺ (%)	Other ions [m/z (%)]
F-	<i>o</i>	904	100	47	1	66	2	83(15)
	<i>m</i>	855	100	50	0	70	0	173(9)
	<i>p</i>	896	100	45	0	63	0	173(5)
Cl-	<i>o</i>	1034	8	20	100	0	1	202(7)
	<i>m</i>	1030	100	65	2	50	3	189(7)
	<i>p</i>	1052	100	58	1	50	3	189(5)
Br-	<i>o</i>	1095	7	18	100	0	1	202(15)
	<i>m</i>	1096	100	50	3	34	0	169(12), 171(12)
	<i>p</i>	1116	100	42	2	31	0	169(10), 171(11)
I-	<i>o</i>	1172	5	16	100	0	1	172(7), 202(20)
	<i>m</i>	1178	86	58	27	100	2	
	<i>p</i>	1203	94	43	18	100	2	
NO ₂ -	<i>o</i>	1192	21	40	100	0	0	106(10), 199(7), 202(17)
	<i>m</i>	1223	93	100	5	68	1	200(4), 233(12)
	<i>p</i> ^a	1260	100	83	2	54	2	200(7), 233(3)
CH ₃ -	<i>o</i>	962	22	50	3	100	43	91(50), 199(32), 200(19)
	<i>m</i>	974	87	42	2	100	6	105(58), 91(30)
	<i>p</i>	989	94	47	2	100	6	105(52), 91(23)
CH ₃ O-	<i>o</i>	1077	17	30	5	20	100	
	<i>m</i>	1093	72	45	3	100	4	120(22), 121(46)
	<i>p</i>	1113	55	45	2	100	4	121(15), 123(21), 139(28)

CH ₃ S-	<i>o</i>	1187	43	41	100	55	87	202(18), 249(76)
	<i>m</i>	1209	42	40	3	100	12	231(40)
	<i>p</i>	1228	53	45	2	100	2	155(26), 232(7)
CH ₃ SO-	<i>o</i>	1370	13	25	100	0	1	202(20), 265(2)
	<i>m</i>	1418	44	67	3	43	3	234(30), 263(93), 265(100)
	<i>p</i>	1456	32	100	2	28	2	233(7), 263(1), 265(87)
CH ₃ SO ₂ -	<i>o</i>	1394	6	17	100	0	1	172(7), 202(17)
	<i>m</i>	1450	87	100	5	61	1	122(43), 233(12)
	<i>p</i>	1498	93	100	8	65	2	122(37), 233(8)
CF ₃ -	<i>o</i>	886	42	100	5	67	1	158(90), 267(3)
	<i>m</i>	853	100	70	1	72	1	158(33), 223(10), 267(9)
	<i>p</i>	878	100	63	1	58	1	158(14), 223(10), 267(5)
CN-	<i>o</i>	1134	100	98	0	83	5	149(17), 180(10), 211(12)
	<i>m</i>	1162	100	55	0	56	2	180(9)
	<i>p</i> ^b	1188	100	58	0	52	2	180(6)
C ₆ H ₅ -	<i>o</i>	1316	5	21	1	38	100	152(12), 139(5)
	<i>m</i>	1394	65	29	1	100	6	152(21), 167(40)
	<i>p</i>	1431	54	29	1	100	5	152(13), 167(16), 185(15)
NH ₂ -	<i>o</i>	1104	11	52	1	100	5	200(37), 201(44), 80(38)
	<i>m</i>	1233	32	42	6	100	3	106(42), 201(11)
	<i>p</i>	1146	19	35	1	100	2	106(7), 108(44), 124(99)
HO-	<i>o</i>	1026	28	100	2	96	3	139(65), 201(35), 202(77)
	<i>m</i>	1261	65	50	1	100	3	107(27), 171(9), 202(6)
	<i>p</i>	1257	100	65	0	90	3	107(24), 171(3), 202(8)
(CH ₃ O) ₂ PSO-	<i>o</i>	1401	10	48	100	12	1	202(9), 249(38)
	<i>m</i>	1441	48	99	12	93	15	93(100), 231(35), 250(9)
	<i>p</i>	1472	45	100	2	90	4	231(17)

^a Parathion-methyl.^b Cyanophos.

obtained on a packed-column GC-MS system incorporating a VG 7035 mass spectrometer, as described previously.⁴

3 RESULTS AND DISCUSSION

3.1 *O,O*-dimethyl *O*-(monosubstituted phenyl) phosphorothioates

3.1.1 Synthesis

The synthetic procedure using potassium hydroxide solution as base was successful (*c.* 70–100% yield) with all phenols except 4-aminophenol, which produced a black tar. In this case a weaker base (solid potassium carbonate) was successfully used, though the yield was substantially reduced.

Unreacted *O,O*-dimethyl chlorothiophosphate was sometimes found on analysis of the extracted product (capillary GC retention time 433 seconds; MS data (expressed as *m/z*(%)) M^+ 160(100), 162(35), 130(93), 132(34), 125(42), 97(90), 99(30), 79(17), 63(16) and 47(60)). *O,O*-dimethyl *O*-ethyl phosphorothioate, produced by reaction between the chlorothiophosphate and ethanol, was invariably observed as a by-product (GC 508 s; MS M^+ 170(100), 142(40), 112(60), 93(85), 79(93)). *O,O*-dimethyl *O*-phenyl phosphorothioate was occasionally observed, arising from reaction of unsubstituted phenol present in the phenolic reagent as a contaminant (GC 910 s; MS M^+ 218(100), 155(13), 125(45), 109(97), 91(48), 79(31) and 77(30)).

In addition to the desired products, the reaction of the dihydroxybenzenes also generated small quantities of diphosphorylated by-products. Increased yields of these materials were obtained by use of a 2:1 molar ratio of dimethyl chlorothiophosphate to dihydroxybenzene. A small quantity (*c.* 2% relative to the 2-hydroxyphenyl phosphorothioate) of the cyclic phosphorothioate ester (*O,O*-*ortho*-phenylene *O*-methyl phosphorothioate, analogous to the pesticide dioxabenzofos [‘Salithion’] without the benzylic methylene group) was detected in the 1,2-dihydroxybenzene reaction products (GC 873 s, MS M^+ 202(80), 139(100)).

Theoretically, the phosphorylation of each of the aminophenols can produce three products: the two monophosphorylated species (aminophenyl phosphorothioate and hydroxyphenyl phosphoramidothioate) and the diphosphorylated compound. Two monophosphorylated products were obtained with both the 3- and 4-aminophenols, but only one was obtained from the 2-aminophenol. It was not possible to determine unambiguously which isomer(s) were present from their mass spectra, so the required amino-substituted phosphorothioates were alternatively generated from the corresponding nitro-substituted compounds by reduction (Wilkins, J. P. G., paper in preparation) for retention time comparison. Generally, it was found that the yield of the hydroxyphenyl phosphoramidothioates was enhanced if a weaker base, such as potassium carbonate, was used instead of potassium hydroxide. It was also possible to verify that the sole monophosphorylated product obtained from 2-aminophenol retained a primary amino group, by its reaction with acetone to produce the corresponding imine (MS M^+ 273(60), 164(45), 125(100)).

3.1.2 Evaluation and interpretation of MS data

As found previously with other organophosphorus esters,³ the reproducibility of the EI mass spectra of the compounds here described, when obtained under similar conditions on different instruments, was good. For comparison, some spectra were obtained with an ionisation energy of 30 eV as well, as this has been used in the past. They were found to differ little from those acquired at 70 eV, except that the ions at low mass (<100 daltons) were slightly weaker relative to those at high mass, and slightly better absolute sensitivity was obtained (*c.* 2 ×).

Ions characteristic of the *O,O*-dimethyl phosphorothioate moiety were present in the mass spectra of all these compounds.³ Their compositions are as follows:

<i>m/z</i>	Ion
47	CH ₃ S ⁺
62	CH ₃ OP ⁺
63	CH ₃ OPH ⁺
79	CH ₃ O . P . OH ⁺
93	(CH ₃ O) ₂ P ⁺
109	(CH ₃ O) ₂ PO ⁺
125	(CH ₃ O) ₂ PS ⁺

Of these, the ions at *m/z* 109 and 125 are most prominent (and their relative intensities are included in Table 1); the other ions listed above were generally of the order of 5–30%. Details of more compound-specific ions, such as M⁺, (M-110)⁺ (the relevance of which is discussed later) and (M-*ortho*)⁺ (*m/z* 217), are also given in Table 1.†

It can be seen that most of the mass spectra exhibit intense molecular ions; for several the molecular ion is the most intense ion. However the spectra of those compounds which have Cl-, Br-, I-, NO₂-, CH₃SO- or CH₃SO₂-*ortho* substituents differ very markedly from their *meta* and *para* substituted analogues; the molecular ion intensity in the spectra of these compounds is negligible, and an ion at *m/z* 217 is the base peak. The full mass spectra of the three chlorinated isomers presented in Fig. 2 vividly illustrate these differences. The mass of the *m/z* 217 ion indicates that it is formed by loss of the *ortho* substituent from the molecular ion species. The likely mechanism of this fragmentation is attack of the >P=S⁺ group (the primary ionisation site³) at the *ortho* position to displace an appropriate substituent and generate the cyclic ion illustrated in Fig. 3. The energetic favourability of this fragmentation pathway is demonstrated by the dominance of the *m/z* 217 ion at the expense of the *m/z* 109 and 125 ions which dominate the spectra of the *meta*- and *para*-substituted isomers (especially the *m/z* 109 rearrangement ion). Factors influencing the extent of this reaction, which is an excellent example of a mass spectrometric '*ortho* effect',⁷ include the stability of the cyclic ion produced, the substituent-phenyl bond energy, and the stability of the substituent radical (X[•]). A very similar *ortho* effect, found with a synthetic phosphonothioate containing a nitroaromatic moiety, has been reported.⁸

† Complete spectral data have been deposited with the British Lending Library at Boston Spa, Wetherby, West Yorkshire, UK, as Supplementary Publication No. SUP 11019 (2 pages).

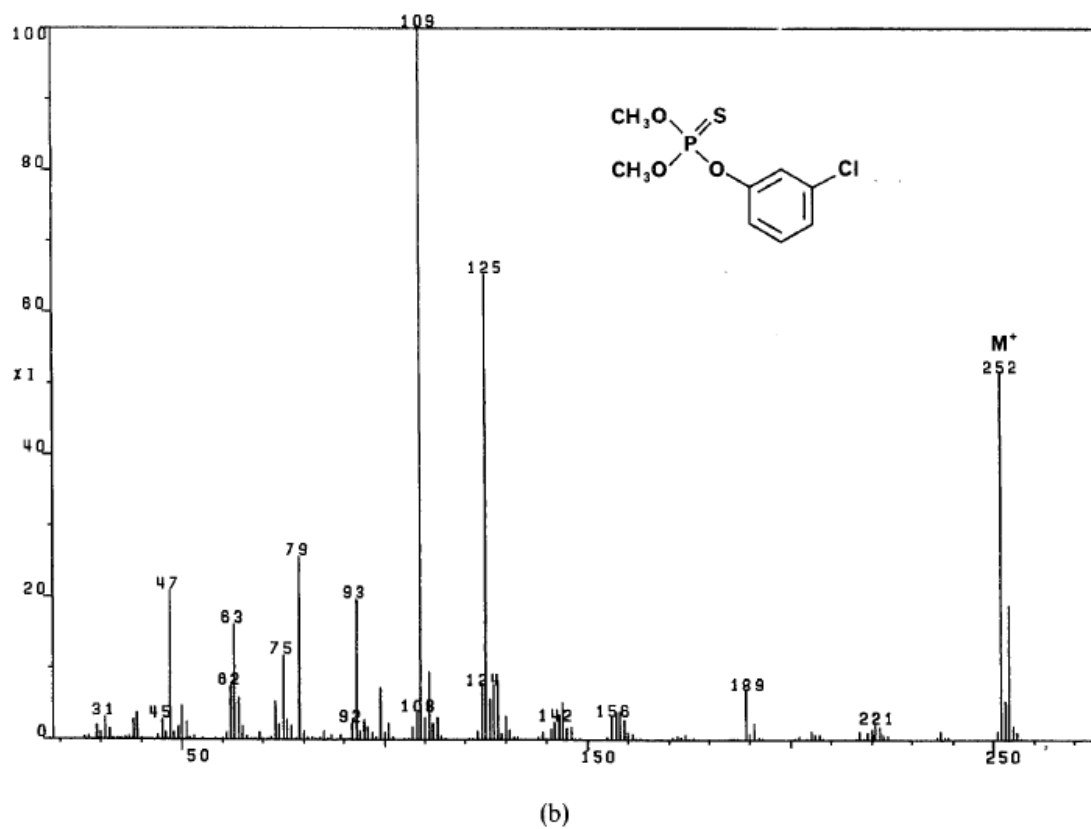
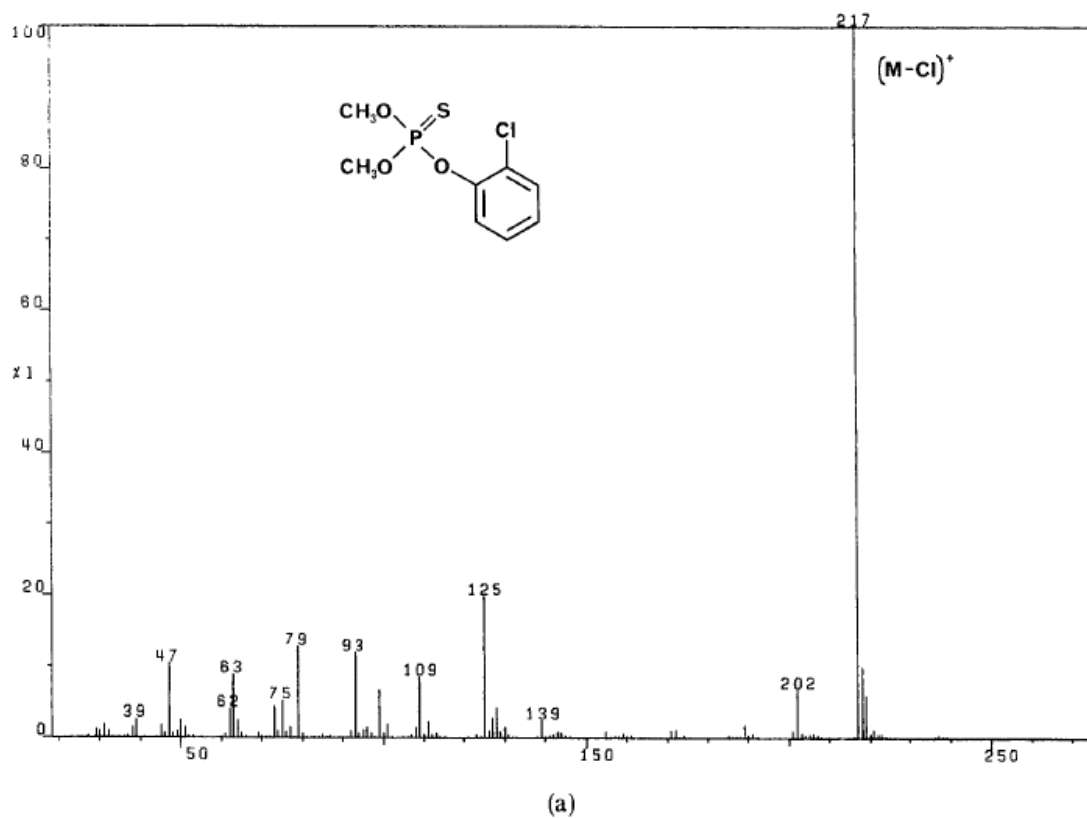
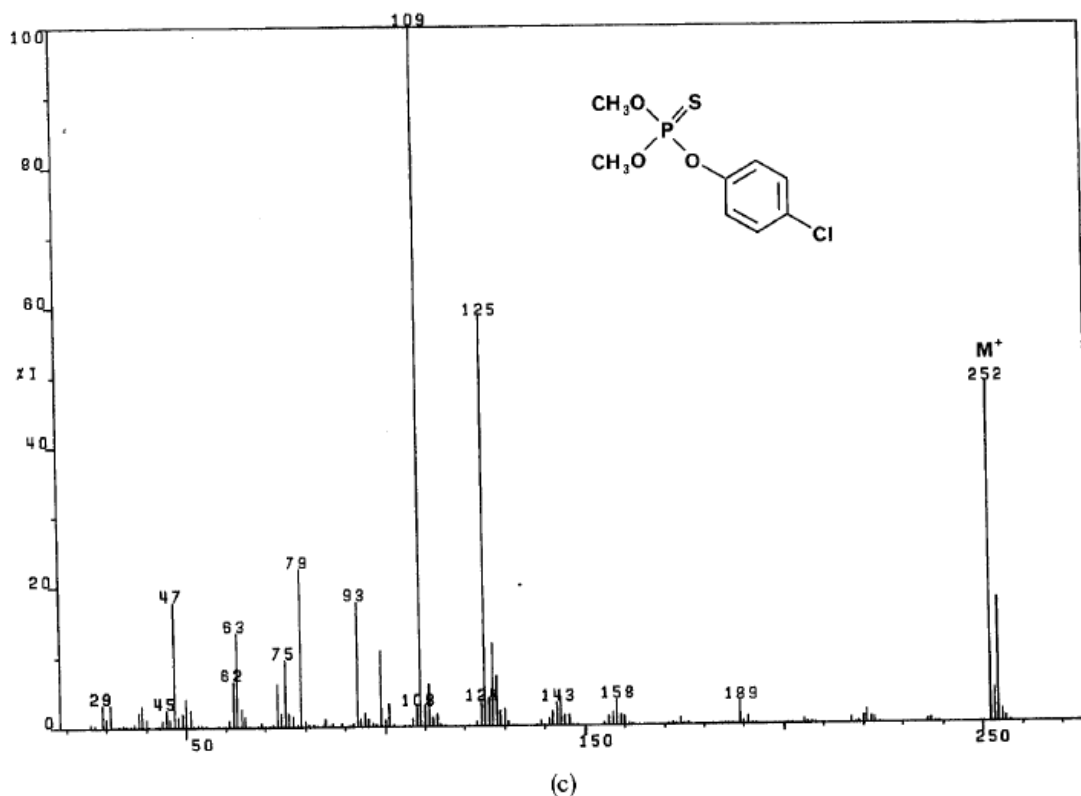


Fig. 2. EI-mass spectra of the three isomers of *O,O*-dimethyl *O*-chlorophenyl phosphorothioate. (a) *ortho*, (b) *meta*.

Fig. 2—contd. (c) *para*.Fig. 3. MS fragmentation of *ortho*-substituted compounds to produce m/z 217 ($X = \text{Cl, Br, I, NO}_2, \text{CH}_3\text{SO or CH}_3\text{SO}_2$).

The m/z 217 ion is also base peak in the spectra of compounds with CH_3S - and $(\text{CH}_3\text{O})_2\text{PS}$. *O*-*ortho* substituents, though in these the molecular ion intensity is not insignificant. All the spectra examined which have m/z 217 as base peak also exhibited an ion at m/z 202 (relative intensity 5–20%) produced by subsequent loss of a methyl group from one of the phosphoryl methoxy groups.

A more complex effect is to be observed in the spectra of the *ortho*-substituted CH_3O -, CH_3S - and C_6H_5 - compounds. The molecular ion intensities of these *ortho* compounds are lower than those of their *meta* and *para* analogues, and a rearrangement ion is observed in their spectra, at m/z 138, 154 and 184 respectively, corresponding to $(\text{M}-110)^+$; this is equivalent to the loss of dimethyl phosphite $((\text{CH}_3\text{O})_2\text{POH})$. The intensities of $(\text{M}-110)^+$ ions for the synthesised compounds are given in Table 1. The production of $(\text{M}-110)^+$ must involve initial thionate/thiolate isomerisation of the molecular ion $((\text{CH}_3\text{O})_2\text{P}=\text{S}\cdot\text{OAr})$ to $(\text{CH}_3\text{O})_2\text{P}=\text{O}\cdot\text{SAr}$, followed by abstraction of a hydrogen atom, most probably

from the *ortho* substituent by the $>P=O$ moiety, and expulsion of dimethyl phosphite to form a stable aromatic ion; for instance, the m/z 184 ion of the *ortho* C_6H_5 - compound is equivalent to the M^+ of dibenzothiophene, as confirmed by accurate mass measurement. For this rearrangement to occur, it appears that there has to be a labile hydrogen atom that can be abstracted from the *ortho* substituent. Thus, the *ortho* CH_3 - compound undergoes this fragmentation (but unlike the compounds above, its M^+ is as intense (100%) as those of its *meta* and *para* analogues), though interestingly the *ortho* NH_2 - and HO - compounds do not.

The greater intensity of the m/z 125 ion in the spectrum of the *ortho* CF_3 - compound, as compared to that found in the *meta* or *para* isomers, is perhaps a manifestation of yet another form of *ortho* effect. The abundant ion at m/z 158 (M-128), which is also observed in the spectra of the *meta* and *para* isomers at lower intensities, corresponds to loss of $(CH_3O)_2POF$ (*cf.* dimethyl phosphite). The $(M-F)^+$ ion is also observed in all three spectra (at m/z 267).

Curiously, the most disparate spectrum of the three NH_2 - compounds is not the *ortho* but the *para* isomer. The spectra of the *ortho* and *meta* compounds are not very different, but that of the *para* isomer is dominated by the rearrangement ion $(M-109)^+$, (m/z 124). The abundance of this ion (which was assigned the structure $NH_2-C_6H_4-S^+$ following accurate mass determination) is perhaps due to its enhanced stabilisation by conjugation and delocalisation with the NH_2 - group in the *para* position (manifestation of a *para* effect!). (A related ion at m/z 108 ($NH_2-C_6H_4-O^+$) is also observed in this spectrum.) This difference is particularly noticeable, as the *O,O*-diethyl analogue has a much weaker m/z 124 ion (35%). These compounds, which are sometimes called 'aminothion-methyl' and 'aminothion' or 'amino-parathion', are of special environmental relevance, as they represent the reductive metabolites of parathion-methyl and parathion.⁵ The reaction of these metabolites with carbonyl compounds to form imines may be useful (or misleading) analytically: the rapid reaction with acetone (described above for the *ortho* NH_2 - compound), to give $(CH_3)_2C=N$ - compounds is an example (the spectra of these products are dominated by strong diagnostic M^+ and $(M-CH_3)^+$ ions).

Surprisingly, the mass spectra of the three CH_3SO - compounds display an apparent concentration-dependence. When these compounds are introduced into the mass spectrometer ion source at rates greater than *c.* 5 ng s^{-1} , the spectra produced are largely as would be expected; that of the *ortho* CH_3SO - compound is dominated by m/z 217, and those of the *meta* and *para* isomers exhibit abundant M^+ and $(M-CH_3)^+$ (see Table 1), but when the rate of introduction is reduced, the relative intensity of the ion observed at m/z 264 (M-16), which is low (< 5%) at high introduction rates, is found to increase (becoming base peak at low source concentrations). The production of this ion corresponds to loss of oxygen from the molecular ion, and the conditions of its observation indicate that it is not produced by mass spectrometric fragmentation (though such losses have been widely reported), but arises from reduction of a proportion of the CH_3SO - compounds to their CH_3S - homologues in the source (Wilkins, J. P. G., paper in preparation).

An explanation for the presence of m/z 263 (M-17, due to loss of OH) in the spectrum of the *meta* CH_3SO - compound, and m/z 231 (M-33, loss of SH) in that of

the *meta* CH₃S- compound (ions which are not found in the spectra of their *para*-substituted homologues) remains unclear (perhaps this is a form of *meta* effect!). The behaviour of the *meta* and *para* CH₃SO₂- compounds, the spectra of which are very similar, is more in keeping with the general trend.

In summary, all the compounds synthesised exhibited ions produced by fragmentation of the phosphorothioate moiety, and of these the ions at *m/z* 109 and 125 were usually the most intense. Those with *meta* or *para* substituents tended to give easily observable molecular ions (> 25% relative intensity), and generally rather similar mass spectra (though the CH₃SO- and NH₂- compounds were interesting exceptions). The effect of an *ortho* substituent on molecular ion intensity can be classified into three groups:

- (i) For the *ortho* F-, CH₃-, CF₃-, CN-, NH₂- and HO- compounds, their molecular ion intensities are not very different from those of their *meta* and *para* analogues.
- (ii) For the *ortho* CH₃O-, CH₃S-, C₆H₅- and (CH₃O)₂PSO-compounds, their molecular ion intensities are significant (> 10%), but two to ten times less than those of their *meta* and *para* analogues.
- (iii) For the *ortho* Cl-, Br-, I-, NO₂-, CH₃SO- and CH₃SO₂-compounds, their molecular ion intensities are negligible (< 1%).

With the *ortho* CH₃O-, CH₃S-, C₆H₅- and CH₃-compounds, an intense ion, formed by loss of dimethyl phosphite from the molecular ion, is observed at (M-110)⁺ (relative intensity 40–100%).

3.1.3 *S*-methyl isomer and phosphate contaminants

Low concentrations of *S*-methyl (or 'iso') compounds were detected on analysis of several of the synthesised materials (particularly the halogen and cyano compounds). They are produced by thermally induced isomerisation during synthesis or storage, (CH₃O)₂.P=S.OAr becoming (CH₃O)(CH₃S).P=O.OAr. Their spectra have many features in common with those of their parent compounds. The diagnostic difference is that *m/z* 109 is absent. Other differences include slightly weaker M⁺ intensity, more abundant *m/z* 125 ((CH₃O)(CH₃S)PO⁺), and they also tend to exhibit a significant (M-CH₃)⁺.

Traces of phosphates related to the synthesised materials were also sometimes found (general structure (CH₃O)₂.P=O.OAr). Their spectra tend to have abundant *m/z* 109 ions, and *m/z* 125 is absent. Knowledge of the MS behaviour of such compounds (combined with the GC behaviour rationalisation given below) could be of value in identifying impurities formed by isomerisation or oxidation in technical phosphorothioate pesticides. The presence of the *S*-alkyl isomer, for example, may be important because such compounds have very different biological activity from their *O*-alkyl precursors.⁵

3.1.4 Evaluation of the GC retention time data

Gas chromatographic retention times are sensitive indicators of molecular structure, reflecting the molecular weight, polarity, potential for hydrogen bonding and other intra- and intermolecular interactions of the analyte with the stationary

phase. With isomeric compounds, the differences in physical properties caused by different patterns of substitution may result in easily recognised elution behaviour. This is the case with the *O,O*-dimethyl *O*-(monosubstituted phenyl) phosphorothioates. The elution order of the three isomers for most of the types of substituent studied (11 out of 16) was *ortho*, *meta*, *para* (see Table 1). The exceptions were: the Cl- compounds, which eluted *meta*, *ortho* then *para* (though the *ortho* and *meta* retention times were rather similar, as they were for the Br- and I- compounds); the F- and CF₃- compounds, which had shorter retention times than would have been expected from their molecular weights, and whose elution order was *meta*, *para*, *ortho*; and the NH₂- and HO- compounds, whose elution orders were *ortho*, *para*, *meta*, with the *ortho* HO- compound eluting much more rapidly than the other two hydroxy compounds. Steric influences on polarity or hydrogen-bonding capacity appear to be important in these cases.

The *S*-methyl isomer contaminants described above (Section 3.1.3) have longer retention times than their *O,O*-dimethyl analogues (under the GC conditions described consistently *c.* 100 s). As they have the same molecular weight, this must be due to their greater polarity. The phosphate analogues are observed at shorter retention times than their parent compounds⁴ (although of higher polarity, they have lower molecular weight).

3.2 *O,O*-dimethyl/diethyl *O*-(substituted phenyl) phosphorothioate pesticides

The occurrence of the effects observed in the synthesised monosubstituted phenyl phosphorothioates was assessed in the mass spectrometric data obtained for analogous mono- and poly-substituted pesticides and their metabolites. (*NB* Two pesticides, cyanophos and parathion methyl, were also members of the series of synthesised compounds.) As with the synthesised monosubstituted phenyl compounds, the mass spectra of the pesticides may be greatly influenced by the presence of an aromatic *ortho* substituent, as can be seen from the data presented in Table 2. The presence of *meta* and/or *para* substituents generally has less effect on fragmentation behaviour (the exceptions, famphur and cythioate, are discussed later). A particularly marked example is to be found in the spectra of the isomeric chloronitrophenyl compounds 'Chlorthion', dicapthon and a synthetic isomer of these compounds with an *ortho* NO₂-group. 'Chlorthion', lacking *ortho* substituents, has a prominent molecular ion; the other two, having Cl- and NO₂- *ortho* substituents, have negligible molecular ion intensities, and base peaks at *m/z* 262 (M-Cl⁺) and *m/z* 251 (M-NO₂⁺) respectively (and, unlike 'Chlorthion', more abundant *m/z* 125 than *m/z* 109 ions (not shown in Table 2)).

Unlike the spectra of the other pesticides examined which lack *ortho* substituents, that of the insecticide famphur exhibits a weak molecular ion. This is because the presence of the *para* (CH₃)₂NSO₂- substituent encourages the formation of a rearrangement ion at *m/z* 218 (accurate mass measurement suggests that this ion is (M-C₂H₅NSO₂)⁺, equivalent to M⁺ of *O,O*-dimethyl *O*-phenyl phosphorothioate) which dominates the spectrum. Interestingly, the spectrum of cythioate, the analogous compound with an unmethylated NH₂SO₂- substituent, does have a strong M⁺. However, its base peak is the rearrangement ion *m/z* 127, [(CH₃O)₂P(OH)₂]⁺ (confirmed by accurate mass measurement), an ion usually

observed in the spectra of dimethyl phosphates. Further, the intermediate homologue *N*-dimethyl famphur, a metabolite of similar toxicity to that of famphur itself⁵ (alternatively described as *N*-methyl cythioate), was found in a sample of cythioate that had been treated with diazomethane. Mass spectrometrically it behaved more like famphur than cythioate, exhibiting a base peak at m/z 218, though the intensity of its molecular ion (m/z 311), at 25%, was intermediate between the two. Polarity appears to be the dominant factor in determining the GC behaviour of these three compounds as they elute in descending molecular weight order. Investigation of the behaviour of the *ortho* and *meta* analogues of these compounds would be most interesting, but was not undertaken because the phenolic starting materials necessary for their synthesis were not available.

The *O,O*-diethyl ester pesticides in Table 2 exhibit similar behaviour to that of the *O,O*-dimethyl compounds, though where (*M-ortho*)⁺ ions are present they are not usually as dominant. This is because further fragmentation of the (*M-ortho*)⁺ species occurs, involving successive elimination of two ethylene molecules from the phosphoryl moiety. This produces ions 28 and 56 daltons lighter, so with dichlofenthion, for example, *M-Cl*⁺ (85%) is accompanied by ions at m/z 251 (35%) and m/z 223 (85%).

The *ortho* effect is nicely demonstrated in the spectra of the three isomers of chlorthiophos. The spectra of isomers **I** and **III**, with *ortho* Cl⁻, are rather similar (exhibiting intense ions at m/z 325 (*M-Cl*)⁺, m/z 297 (*M-Cl-C*₂*H*₄)⁺ and m/z 269 (*M-Cl-2* × *C*₂*H*₄)⁺), whereas that of isomer **II**, with *ortho* CH₃S-, is markedly different (m/z 313 (*M-CH*₃*S*)⁺, m/z 285 (*M-CH*₃*S-C*₂*H*₄)⁺ and m/z 257 (*M-CH*₃*S-2* × *C*₂*H*₄)⁺). A prominent rearrangement ion is also observed at m/z 222 (100%) (*M-138*)⁺. This ion is formed by loss of diethyl phosphite and is analogous to the dimethyl phosphite loss described above.

The presence of a CH₃S- substituent appears to confer unexpected stability on the molecular ion species of chlorthiophos **I** and **III**. From the behaviour of the monosubstituted model compounds, the presence of an *ortho* Cl- substituent would be expected to result in negligible molecular ion intensities for these compounds, but significant intensities are found. The CH₃SO- and CH₃SO₂- substituents are less effective in this respect, as the molecular ion intensities of the sulphoxides and sulphones of chlorthiophos **I** and **III** are much weaker.

3.2.1 Application of these generalisations to compound identification

Three examples of the use of this rationalisation of the GC-MS data obtained for phosphorothioates in the identification of impurities found in technical pesticides are the following.

3.2.1.1 Fenitrothion. A contaminant found in technical fenitrothion (*c.* 1%, at shorter GC retention time than fenitrothion) was tentatively identified as an *ortho* NO₂-isomer of fenitrothion, by its base peak at m/z 231 (i.e. 277 - 46, *M-NO*₂⁺) and the absence of a significant molecular ion. Its identity was confirmed as the *O,O*-dimethyl *O*-(3-methyl-6-nitrophenyl) phosphorothioate by comparison with a synthesised sample (MS data: m/z 79(15), 93(25), 109(15), 120(20), 125(25), 216(25), 231(100) and *M*⁺ m/z 277 < 1%). This compound, referred to as 'iso-fenitrothion', has been reported⁹ as a minor contaminant of fenitrothion, but no MS data were

TABLE 2
 Data for Molecular Ion [M^+] and the Ion Produced by Loss of an *ortho* Substituent (if present) [$(M-ortho)^+$] for Some *O,O*-Dimethyl and *O,O*-Diethyl *O*-(Substituted Phenyl) Phosphorothioate Pesticides, Their Oxidative Metabolites and a Synthetic Analogue

Pesticide	Phenyl substituents			M^+		$(M-ortho)^+$	
	<i>ortho</i>	<i>meta</i>	<i>para</i>	<i>m/z</i>	%	<i>m/z</i>	%
<i>O,O</i> -dimethyl compounds							
Cyanophos			CN	243	52	—	—
Parathion-methyl			NO ₂	263	100	—	—
Fenitrothion			NO ₂	277	55	—	—
Methylnitrophos ^a				277	0	231	100
Fenthion		CH ₃	CH ₃ S	278	100	—	—
Fenthion sulphoxide		CH ₃	CH ₃ SO	294	58	—	—
Fenthion sulphone		CH ₃	CH ₃ SO ₂	310	94	—	—
Chlorthion ^a		Cl	NO ₂	297	40	—	—
Dicapthion	Cl		NO ₂	297	0	262	100
Dicapthion isomer	NO ₂		Cl	297	0	251	100
Cythioate ^a			NH ₂ SO ₂	297	70	—	—
Tolclofos-methyl	2 × Cl		CH ₃	300	0	265	100
Fenchlorphos	Cl		Cl	320	1	285	100 ^b
Famphur		Cl	(CH ₃) ₂ NSO ₂	325	2	—	—
Bromophos	Cl		Br	366 ^c	1	331 ^c	100 ^b
Iodofenphos	Cl		I	412	2	377	100 ^b
Temephos ^d	Cl		SAr ^e	466	100	—	—
Temephos sulphoxide ^d			SOAr ^e	482	14	—	—
Temephos sulphone ^d			SO ₂ Ar ^e	498	51	—	—

O,O-diethyl compounds								
Parathion						NO ₂	291	75
Fensulfothion sulphide						CH ₃ S	292	100
Fensulfothion						CH ₃ SO	308	55
Fensulfothion sulphone						CH ₃ SO ₂	324	100
Dichlofenthion ^a						Cl	314	2
Chlorthiophos I						CH ₃ S	360	44
Chlorthiophos II						Cl	360	51
Chlorthiophos III						CH ₃ S	360	16
Chlorthiophos I sulphoxide						Cl	376	0
Chlorthiophos II sulphoxide						CH ₃ SO	376	0
Chlorthiophos III sulphoxide						Cl	376	2
Chlorthiophos I sulphone						CH ₃ SO	392	0
Chlorthiophos II sulphone						Cl	392	0
Chlorthiophos III sulphone						CH ₃ SO ₂	392	6
Bromophos-ethyl						Br	394 ^c	0
								279
								85 ^b
								80 ^b
								25
								90 ^b
								85 ^b
								49
								100 ^b
								91 ^b
								50
								93 ^b
								49 ^b

^a Not a BSI/ISO approved name.

^b Substituent(s) similar to that at the *ortho* position also present at the *meta* and/or *para* position(s).

^c Most intense ion in cluster.

^d Not amenable to GC under the conditions described.

^e The temephos substituent Ar is -C₆H₄-*p*-O . PS . (OCH₃)₂.

NB. The intensities of ions attributable to (M-*meta*)⁺ or (M-*para*)⁺ were insignificant, except where they could also be due to (M-*ortho*)⁺

given. In the USSR, the product called methylnitrophos, which is prepared from the unpurified mononitration products of 3-methylphenol (i.e. the 4- and 6-nitro isomers), is a mixture of fenitrothion (70–75%) and the 3-methyl-6-nitrosubstituted compound (30–35%).¹⁰

3.2.1.2 Fenthion. A contaminant (c. 2%) was detected in an aged sample of technical fenthion, with a longer retention time than fenthion itself (MS data: m/z 79(16), 93(32), 109(21), 125(35), 214,215,216(100,16,15), 262(15), 277,278,279(45,7,5), 309(17), M^+ 324,325,326(95,13,12)). The spectrum of the contaminant exhibits all the characteristic ions of an *O,O*-dimethyl phosphorothioate, and it has a molecular weight 46 daltons greater than that of fenthion, equivalent to the presence of an additional CH_3S - substituent (in accord with the relatively abundant $(M+2)^+$ ion, which is indicative of the presence of three sulphur atoms). The abundant m/z 277 ($M-47$) betrays the presence of an *ortho* CH_3S - substituent, indicating that the likely structure of the contaminant is *O,O*-dimethyl *O*-2,4-di(methylthio)-[3 or 5]-methylphenyl phosphorothioate. The base peak at m/z 214 ($M-110$)⁺, provides additional evidence of the *ortho* position of the CH_3S - substituent. Such a contaminant, termed 'bis-methylthio-fenthion'¹¹ has been assigned the phenyl substitution pattern 2,4-di(methylthio)-3-methyl-, though it is more likely that the methyl group is at the 5- position for stereochemical reasons (unfortunately no MS data were reported by Boyd). The presence of this technical impurity is not unexpected, being due to a contaminant in the phenolic starting material, which is produced by mercaptomethylation of *meta* cresol.

3.2.1.3 Fenchlorphos. Technical fenchlorphos ('Ronnel') was found to contain several contaminants on GC-MS. Two, with molecular weights of 316 but very different mass spectra, were tentatively identified as methoxy analogues of fenchlorphos, with the structure *O,O*-dimethyl-*O*-(dichloro-methoxyphenyl) phosphorothioate. Both elute after fenchlorphos—that at the shorter retention time exhibited M^+ 316,318,320(6,4,2) and $(M-\text{Cl})^+$ m/z 281,283(100,37), and the other M^+ 316,318,320(25,18,3), $(M-110)^+$ m/z 206,208,210(100,67,13), and $(M-\text{CH}_3)^+$ m/z 301,303(10,8), in addition to the characteristic organophosphorus ester ions. This clearly indicates that the first has an *ortho* Cl-, because of the weak M^+ and base peak $(M-\text{Cl})^+$ ion, and that the second has an *ortho* CH_3O - because it has the dimethyl phosphite loss $(M-110)^+$ ion (m/z 206), observed in the monosubstituted *ortho* CH_3O - compound.

3.3 Appraisal of some published data

The observations and findings reported in this study were compared with published data from a variety of sources. Though some degree of variation in the reported MS data is to be expected, on the whole the level of agreement was good. In some cases it has been possible to re-interpret reported data in the light of this study, though, as described below, the omission of sufficiently comprehensive data often hindered such attempts.

A study of the photodegradation products of iodofenphos by Walia *et al.*¹² includes GC-MS data for several *O,O*-dimethyl *O*-(substituted phenyl) phosphorothioates (and phosphates), but most of these are not in accord with the observations reported here. For example, their photoproduct **II**, the 2,5-

dichlorophenyl compound, has a reported M^+ intensity of 90% and an $(M-Cl)^+$ of 95%; because of the *ortho* effect, a negligible molecular ion intensity would be expected, and this was confirmed (by synthesis and GC-MS: M^+ 0% and m/z 251 100%). For comparison, the 3,5-dichlorophenyl isomer was also synthesised; having no *ortho* Cl- this gave a significant M^+ (30%), and a weak $(M-Cl)^+$ (2%). The identity of photoproduct II remains unclear. The assignments given by Walia *et al.* for m/z 109 ($CH_2OP(S)O^+$) and m/z 93 ($CH_2OP(S)O^+$) are incorrect. The data given for iodofenphos oxon also differ from those obtained at this laboratory, in particular, the unaccountably intense M^+ (m/z 396), m/z 234 and 94 ions. Unfortunately, MS data for iodofenphos itself, which would have been useful for comparison, are not included. The molecular weights reported for several compounds are incorrect. The GC characterisation is also suspect, for example iodofenphos oxon and a dechlorinated iodofenphos analogue (VI) are both reported to have longer retention times than iodofenphos, when shorter retention times are to be expected under the GC conditions described.

Chukwudebe *et al.*¹³ report data for a number of compounds described in this paper, which they found during photodegradation studies of fenthion. These include *O,O*-dimethyl *O*-3-methylphenyl phosphorothioate (MS data similar to those reported here, but m/z 105 and 91 omitted), fenthion, fenthion sulphoxide, and a compound (apparent M^+ 294) which was tentatively identified as fenthion oxon sulphone. This last identification is unlikely to be correct, as the prominent m/z 125 ion reported ($(CH_3O)_2PS^+$) is not produced by phosphates. The m/z 215, which has been reported to be an abundant ion,⁴ is also absent.

The mass spectrum of *O,O*-dimethyl *O*-3-methylphenyl phosphorothioate (and several other UV irradiation products of fenitrothion) has also been described by Greenhalgh *et al.*,¹⁴ who confirmed their identification by synthesis. Their data for this compound ('denitrofenitrothion') were in good agreement with those presented here.

Spectral data for some 200 pesticides and related compounds, in full scan pictorial format, are given in a compilation by Skinner and Greenhalgh;¹⁵ fenitrothion (and several of its photodegradation products) and some other phosphorothioate pesticides are included. Those described here include dicapthion, fenchlorphos, fensulfothion, iodofenphos, parathion and parathion-methyl, and though generally similar, the ions at high mass are often weaker than those reported here (e.g. iodofenphos m/z 377 $(M-Cl)^+$ *c.* 3%, *vs* 100% here). This apparent discrimination in favour of the low mass ion intensities may result from the use of a quadrupole analyser.

The spectrum of *O,O*-dimethyl *O*-4-methoxyphenyl phosphorothioate given by Desmarchelier *et al.*³ is similar to that reported here, but the ions at high mass are of reduced intensity (again this may be due to the use of a quadrupole instrument). The ion at m/z 109, which is reported to be the base peak, is incorrectly identified as being due to $C_6H_5O_2^+$.

Spectra for many of the pesticides discussed in this paper are reported in the NIST Standard Reference Database (MSDB).¹⁶ Generally there is a high degree of correspondence between these data and those presented here, though the molecular ion intensities reported in the MSDB are usually lower. This is particularly evident

in the spectra of temephos and its sulphoxide (M^+ 0% and 1% respectively, *vs* 100% and 14% reported here). The only other notable disparity was found in the MSDB spectrum of fenitrothion; though its M^+ intensity was not very different (59.4% *vs* 100% reported here), it includes an ion at m/z 127 (100%) not observed at this laboratory; the presence of other additional ions at m/z 164, 192 and 224 indicates that the pesticide mevinphos was present when this spectrum was acquired. The spectra of famphur and cythioate, which are difficult to rationalise, are corroborated by the MSDB data.

The collection of mass spectra of environmental contaminants by Hites¹⁷ includes full scan data for parathion-methyl, fenitrothion, fenthion, chlorthion, dicaphton, fenchlorphos, bromophos, cythioate, famphur and temephos. These are generally in good agreement with those presented here, though the spectrum of cythioate lacks the 'controversial' m/z 127 ion.

4 CONCLUSIONS

The data presented provide an increased understanding of the mass spectrometric and gas chromatographic behaviour of substituted phenyl phosphorothioates, which should be widely applicable in the field of organophosphorus pesticide analysis. In particular, it should now be possible to develop a more predictive approach to the mass spectra of organophosphorus pesticides, their isomeric contaminants and metabolites. Further work is being carried out at this laboratory to expand the applicability of this approach to a much wider range of pesticides.

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RCM
Letter to the Editor

To the Editor-in-Chief
Sir,

Reactions of perfluorotri-*n*-butylamine fragment ions in the quadrupole ion trap: the origin of artefacts in the perfluorotri-*n*-butylamine calibration spectrum

Perfluorotri-*n*-butylamine (PFTBA, commonly known as FC43 or heptacosane) is used widely as a calibration compound in EI mass spectrometry. The spectrum of PFTBA obtained using a quadrupole ion trap mass spectrometer shows the characteristic $[C_nF_{2n+1}]^+$ and related EI fragment ions used for routine calibration purposes, but also often contains a number of ions, the most prominent being at m/z 197, which cannot be derived from PFTBA by EI fragmentation processes. These ions are not generally observed in the PFTBA calibration spectra recorded on magnetic sector or quadrupole instruments, so their origin presumably lies in ion/molecule chemistry occurring in the ion trap between fragment ions derived from PFTBA and residual water or other neutrals present in the mass spectrometer.

The PFTBA EI fragment ion, $[CF_3]^+$ (m/z 69), has been shown to undergo electrophilic addition with a variety of compound classes including halogenated aromatics,¹ pyrrole,^{2,3} furan,² thiophene,² alkyl benzenes,⁴ aromatic carbonyls,⁵ hydroxy and alkoxy benzenes⁶ and oxygen and nitrogen bases.^{7,8} This electrophilic addition is followed by molecular elimination, for example, loss of HF or CO. The perfluoroacyl ion, $[CF_3CO]^+$, has also been shown to react with oxygen-centred nucleophiles, water and trifluoroacetic acid to yield $[CF_3OH_2]^+$ and $[CF_3C(OCF_3)_2]^+$, and with benzene and $CD_3C_6H_5$ to yield $[C_6H_5CO]^+$ and $[CD_3C_6H_4CO]^+$, respectively.⁴ The

ion/molecule chemistry in the ion trap of EI-derived PFTBA ions with oxygen- and nitrogen-centred nucleophiles has not been reported to our knowledge. In this communication we describe the results of an ion trap mass spectrometric study of the reactions of ions of the type $[C_nF_{2n+1}]^+$ with water vapour and PFTBA, and discuss the origin of the ion observed at m/z 197 in the PFTBA calibration spectrum.

A quadrupole ion trap mass spectrometer (ITMS, Finnigan MAT, San Jose, CA, USA), operated at 120 °C in positive ion mode, was used to investigate the reactions of the perfluoroalkyl ions. Some additional experiments were carried out on a Finnigan GCQ ion trap and a Micro-mass Quattro I triple quadrupole spectrometer. The ITMS electron multiplier voltage was typically set to +1.7 kV with the conversion dynode voltage at -3 kV. Helium buffer gas was introduced into the ion trap via the gas chromatograph (Varian 3400) at a pressure of 4×10^{-5} Torr. PFTBA was obtained from the Aldrich Chemical Co. and Purite water (Purite Limited, Thame, UK) was used in all experiments. The fluorinated fragment ions investigated using the ITMS spectrometer were generated by electron ionization of PFTBA, which was introduced into the vacuum manifold via a leak valve

(Meggitt Avionics, Portsmouth, UK) at 1×10^{-6} Torr. Water vapour was also introduced into the vacuum manifold via a leak valve (Meggitt Avionics) at 3.8×10^{-5} Torr. All pressures are uncorrected, as measured using an ionization gauge (Granville-Phillips, Boulder, CO, USA) mounted on the vacuum chamber. Electrons were gated into the trap for 300 μ s, with the low mass cut off set to 10 Th, and during this period a supplementary radiofrequency (RF) field (filtered noise waveform)⁹ was applied to the end-cap electrodes to retain the appropriate mass-selected PFTBA fragment ions in the trap. The isolated PFTBA ions were allowed to react for periods up to 100 ms, before the products were analysed using a mass-selective instability scan.

A typical EI calibration spectrum recorded in a quadrupole ion trap (Fig. 1(a)) shows the characteristic EI fragment ions together with a weak m/z 197 ion. The effect of introducing a partial pressure of water vapour into the ion trap during the ionization stage is shown in Fig. 1(b). The intensity of the PFTBA fragment ion at m/z 219 ($[C_4F_9]^+$) decreased, whilst the ion at m/z 197 increased in intensity in the presence of the water vapour. The relative intensities of the other PFTBA fragment ions did not change significantly. The m/z 197 ion therefore appears to originate from

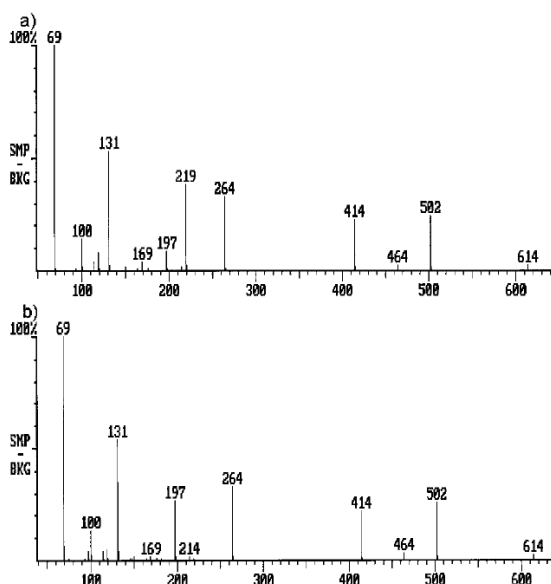


Figure 1. EI spectra of perfluorotri-*n*-butylamine in a quadrupole ion trap mass spectrometer (a) typical calibration spectrum and (b) spectrum observed in the presence of a higher partial pressure of water vapour.

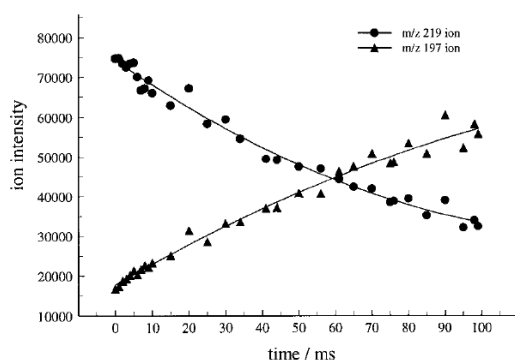
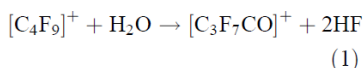


Figure 2. Variation in ion intensities of the $[C_4F_9]^+$ (m/z 219) and $[C_3F_7CO]^+$ (m/z 197) ions with reaction time for the reaction of $[C_4F_9]^+$ with water vapour (3.8×10^{-5} Torr) in a quadrupole ion trap.

Table 1. Ion/molecule reactions of perfluoro-*n*-butylamine derived ions with water vapour in a quadrupole ion trap

Ion	m/z	Product ions
CF_3^+	69	No reaction
$C_2F_5^+$	119	CF_3CO^+ (m/z 97)
$C_3F_7^+$	131	No reaction
$C_3F_7^+$	169	$C_2F_5CO^+$ (m/z 147), $C_2F_5^+$ (m/z 119), CF_3CO^+ (m/z 97)
$C_4F_9^+$	219	$C_3F_7CO^+$ (m/z 197), $C_3F_7^+$ (m/z 169)
$C_5F_{10}N^+$	264	No reaction
$C_8F_{16}N^+$	414	No reaction

the reaction of $[C_4F_9]^+$ with residual water in the quadrupole ion trap, by electrophilic addition and HF elimination, to form $[C_3F_7CO]^+$ according to Eqn (1).

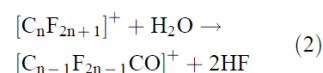


The structure of the $[C_3F_7CO]^+$ product ion was supported by collisionally activated dissociation, which yielded fragments at m/z 169 ($[C_3F_7]^+$), by loss of carbon monoxide, m/z 131 ($[C_2F_5]^+$) and m/z 69

($[CF_3]^+$). The reaction scheme in Eqn (1) was also confirmed by isolating the m/z 219 ion in the ion trap and allowing it to react with neutral water vapour. The expected m/z 197 ion was present in the product ion spectrum. The formation of $[C_3F_7CO]^+$ was dependent on the reaction time in the ion trap as well as the partial pressure of water, and the effect of increasing reaction time on the intensity of the ions at m/z 219 and 197, at a constant partial pressure of water, is shown in Fig. 2. The intensity of the m/z 219 ion declines rapidly and the

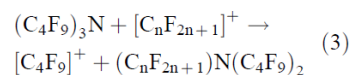
intensity of the m/z 197 ion increases until it becomes the dominant ion at reaction times greater than 70 ms.

In order to further study this ion/molecule chemistry, selected fragment ions from perfluorotributylamine were isolated individually using a supplementary RF field and allowed to react with neutral water vapour for 100 ms. The results of these reactions are summarised in Table 1. Ions of the type $[C_nF_{2n+1}]^+$ ($n=2-4$) react with water to form $[C_{n-1}F_{2n-1}CO]^+$ product ions at m/z 97, 147 and 197 (Eqn(2)), but the nitrogen-containing fragment ions (e.g. $[C_5F_{10}N]^+$, m/z 264 and $[C_8F_{16}N]^+$, m/z 414), $[CF_3]^+$ (m/z 69) and $[C_3F_5]^+$ (m/z 131) do not react.



Reactions of perfluoroalkyl ions with water are highly exothermic. For example, the enthalpy change, ΔH_r , for the reaction between $[C_2F_5]^+$ and water is -288 kJ mol^{-1} , taking ΔH_f for the perfluoroethyl and $[CF_3CO]^+$ ions to be 180^{10} and $192^{11} \text{ kJ mol}^{-1}$ respectively, and the heats of formation for water and HF as -242 and -271 kJ mol^{-1} .¹² A lower estimate for ΔH_f ($[CF_3CO]^+$) of 150 kJ mol^{-1} has been reported,¹³ but this does not significantly alter the calculated exothermicity of the reaction. The corresponding $[CF_3]^+$ addition/HF elimination reaction with water is also expected to be exothermic by 213 kJ mol^{-1} ,⁷ but no reaction was observed in the ion trap. In this case back-dissociation of the adduct must predominate over HF elimination, presumably as a result of entropic constraints. This is supported by the experimental observations and theoretical calculations reported by Speranza and co-workers in a Fourier transform ion cyclotron resonance (FT-ICR) study.⁷

Substitution reactions between PFTBA fragment ions of the type $[C_nF_{2n+1}]^+$ ($n=1,2,3$) and neutral PFTBA were also observed in the ion trap (Eqn 3).



The $[C_4F_9]^+$ ion (m/z 219) is formed in all three cases ($n=1-3$) and this ion then reacts with residual water in the ion trap, according to Eqn(1), further enhancing the intensity of the $[C_3F_7CO]^+$ (m/z 197) ion. The $[CF_3]^+$ ion has previously been

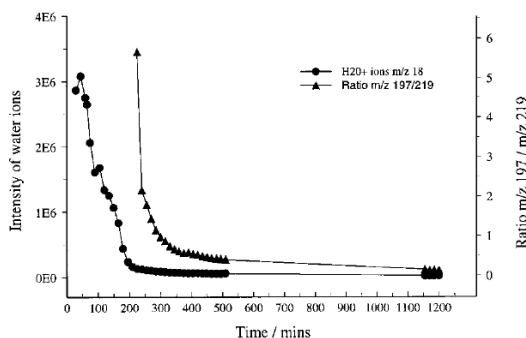


Figure 3. Variation in the H_2O^+ (m/z 18) ion intensity and the $[C_3F_7CO]^+/[C_4F_9]^+$ (m/z 197/219) intensity ratio with time during a typical quadrupole ion trap pump-down sequence.

shown to react with a variety of amines by attack at the nitrogen centre of the amine.⁸

Residual water may be present in the ion trap during pumping down after cleaning and maintenance of the spectrometer, or as a result of a vacuum leak. This determines the $[\text{H}_2\text{O}]^+$ (m/z 18) ion intensity and the relative intensities of the m/z 197 and 219 ions, as shown in Fig. 3 for a typical pump-down sequence. Initially the m/z 18 and 197 ions are prominent in the spectrum, since the partial pressure of water vapour is high as a result of outgassing of the trap and vacuum housing components. The intensities of these ions fall rapidly during the initial stages of the pump down. The m/z 219 ion is first detected above the baseline after 200 min has elapsed and the m/z 197/219 ratio then decreases rapidly at a point when the $[\text{H}_2\text{O}]^+$ ion intensity has begun to level out. Evacuation of the trap overnight (18 hours since pumping started) was required to reduce the concentration of water vapour in the trap to a level where m/z 219 became the dominant ion (ratio m/z 197/219 = 0.13). The m/z 197 ion was also observed in the EI spectrum of PFTBA obtained using a triple quadrupole mass spectrometer shortly after pump-down, when the first quadrupole was set to transmit all ions from the EI source to the collision cell and the final quadrupole was used to scan the mass spectrum. The reaction of the m/z 219 ion with residual water in the collision cell was thought to be responsible for the formation of the $[\text{CF}_3\text{CO}]^+$ product,

since the spectrum recorded by scanning the first quadrupole did not contain the m/z 197 ion.

These observations establish that the prominent m/z 197 ion in the ion trap EI calibration spectrum of PFTBA originates from the reaction of the PFTBA-derived $[\text{C}_4\text{F}_9]^+$ ion with residual water vapour. The PFTBA fragments $[\text{C}_3\text{F}_7]^+$ and $[\text{C}_2\text{F}_5]^+$ also react with water vapour to yield low abundance product ions at m/z 97 and 147. The relative intensities of the m/z 197 and 219 ions are dependent on the partial pressure of water and monitoring this ratio provides a more sensitive indicator of the presence of low partial pressures of water vapour in the ion trap than the $[\text{H}_2\text{O}]^+$ ion intensity.

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CHAPTER 4. CONCLUSIONS & RECOMMENDATIONS

Pesticides continue to be used effectively in the service of mankind. Used wisely they can continue to help increase food production and prevent wastage. Used responsibly, they should not cause significant harm consumers, wildlife and the environment.

This thesis contains observations, recommendations and a comprehensive GCMS data collection, which should be of use to those undertaking MS analysis of pesticides and related compounds. Many of the observations will find application in related analytical applications.

Regarding future work, the most helpful and beneficial immediate priority would be the acquisition of further accurate mass MS data, in order to confirm and extend the ion structure assignments.

The next priority would be to evaluate alternative and/or complementary analytical techniques, such as ion mobility spectrometry.

And finally, to monitor changes in agrochemical science and practice, in order to keep abreast of the latest developments.

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