J Forensic Sci, Oct. 1972, Vol. 17, No. 4

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A General Approach to Pesticide Residue Analysis

Organic pesticides came into wide use between 1944 and 1960. The chlorinated hydrocarbons including DDT came first, followed by the organic phosphates, the mercurials, and the organic carbamates. In the 1960s there was an awakening to the hazards to human life, to animals, and to other life forms resulting from the continued use of persistent chemicals and also from the extremely toxic ones even though they may not be persistent. There were increasing numbers of accidental deaths from pesticide poisoning among agricultural workers and among children who accidentally ate or drank pesticide formualtions. The primary culprits were the organic phosphates, such as parathion, but there have also been numerous serious or fatal poisonings by some of the others [*1*].

Because of the toxicity and the wide use of pesticides, it becomes important for forensic chemists to be able to identify and measure those present under circumstances where very little background concerning the presence or absence of pesticides is available. There must be a systematic method of analysis for these toxic chemicals that will give reliable results. The analyst must be capable of dealing with a variety of pesticide chemicals in a variety of materials in a straightforward system of analysis. A suitable sample must be chosen. In the case of a death this might be an organ obtained at autopsy, and possibly materials that may have been involved in ingesting the pesticide, such as a drinking glass, a pesticide bottle, or a sample of contaminated food. In practically every case, the first step in the analysis will be the extraction of the pesticide from the sample into a suitable solvent. Finkle, Cherry, and Taylor [*2*] described a system of analysis for blood, urine, or tissue. In their extraction scheme, the pesticides are first extracted into chloroform from a sample with a neutral pH. A portion of their procedure is given in Fig. 1. This scheme gives a separation of the acidic materials from the neutral and basic materials. Most of the neutral and basic materials can be successfully gas chromatographed without further treatment. However, the acidic materials can usually be more successfully gas chromatographed if the acidic functional group is reacted (for example, with diazomethane) to give the corresponding ester or ether. More often than not, it is necessary to give the sample some additional processing ("cleanup") to remove materials such as oils or waxes that would interfere in the gas chromatography of the sample. A variety of cleanup processes, well documented in the literature, are widely used in pesticide residue analysis in foods. These include: liquid-liquid extraction; adsorption on solids, such as alumina, silica gel, diatomaceous earth, or Florisil; distillation; column chromatography; or even low efficiency, large scale gas chromatography.

Presented at the Twenty-fourth Annual Meeting of the American Academy of Forensic Sciences, Atlanta, Ga., 3 March 1972. Received for publication 30 March 1972; accepted for publication 5 June 1972.

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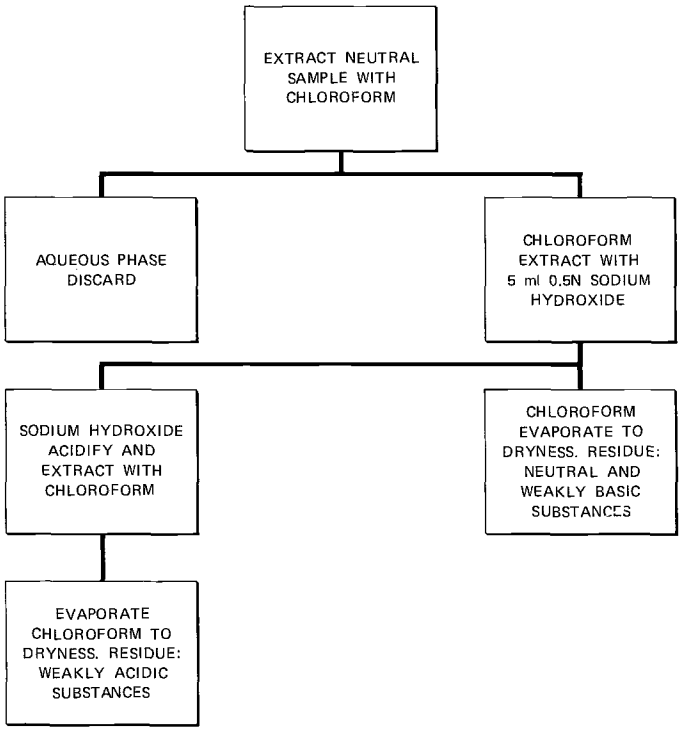


FIG. 1—*Extraction scheme.*

For the qualitative and quantitative determination of unknown pesticides in a variety of matrices, the following approach is proposed.

(1) Extract the sample with a polar solvent such as chloroform.
(2) Separate the acidic constituents from the neutral and basic constituents.
(3) Clean up each of the fractions from (2) above by liquid-liquid extraction.
(4) Make methyl derivatives of the acidic constituents.
(5) Gas chromatograph an aliquot of the two fractions on a relatively nonpolar column, using a variety of element- or compound-selective detectors.

The scientific literature has adequate documentation for the selection of a suitable means of carrying out steps 1 through 5 [*3*]. The remainder of this paper deals in detail with step 5 only.

The choice of a gas chromatographic detector for selective and sensitive detection of organic compounds requires a thorough knowledge of the nature of interfering compounds that may be present and a considerable amount of detailed information about sensors. The problem should be defined as well as possible from the nature and history of the sample so that proper decisions can be made in the selection of a gas chromatographic system, especially the means of sensing. The problem of selective and sensitive detection of small amounts of pesticide chemicals in a complex matrix of materials extracted from natural products became a personal interest about 14 years ago because of the need for rapid screening methods of analysis for pesticide residue in food. This work led to the development of several unique detection systems.

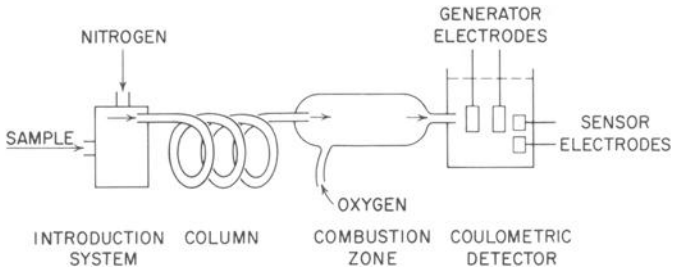


FIG. 2—*Block diagram of microcoulometric gas chromatograph.*

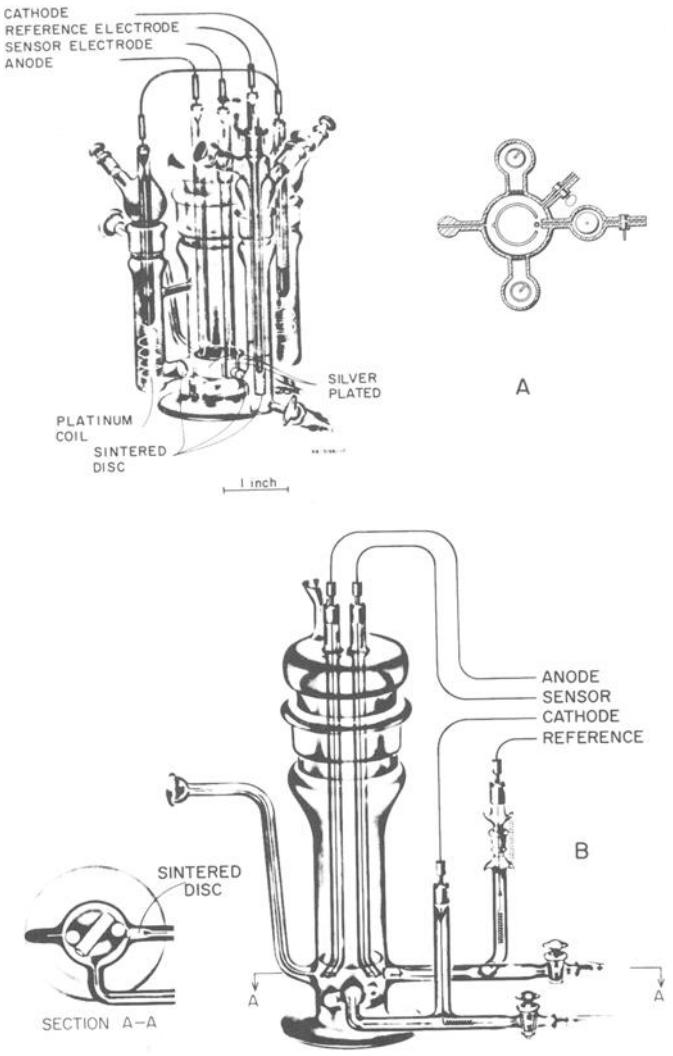
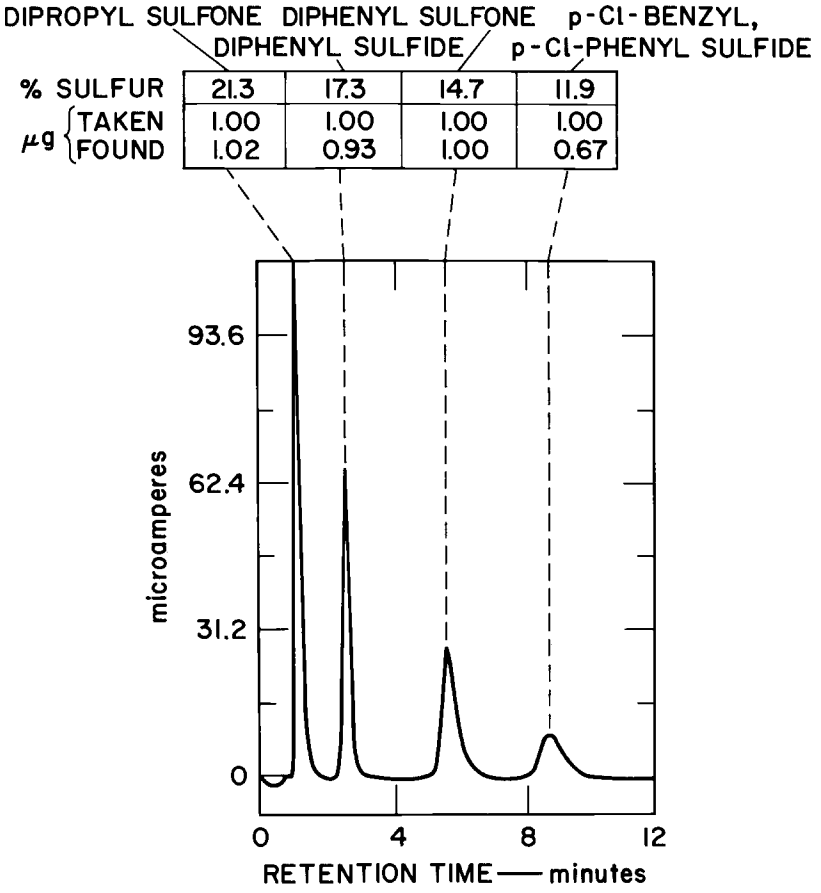


FIG. 3—*Coulometric titration cells.*

The first was a microcoulometric detector that gives a response only to certain halogens or sulfur [4]. Figure 2 gives a block diagram of a gas chromatographic system containing a gas chromatograph, a microcombustion furnace, and the coulometric detector.

A titrant is generated as needed to titrate the products of combustion as materials are eluted from the column. For halides, the titrant is internally generated silver ions generated at a silver anode. For sulfur compounds, which yield SO_2 in the combusiton



COLUMN – 6 FT. x ¼ IN. O.D. PACKED WITH 20% SILICONE GREASE ON 30/60 MESH CHROMOSORB 235°C

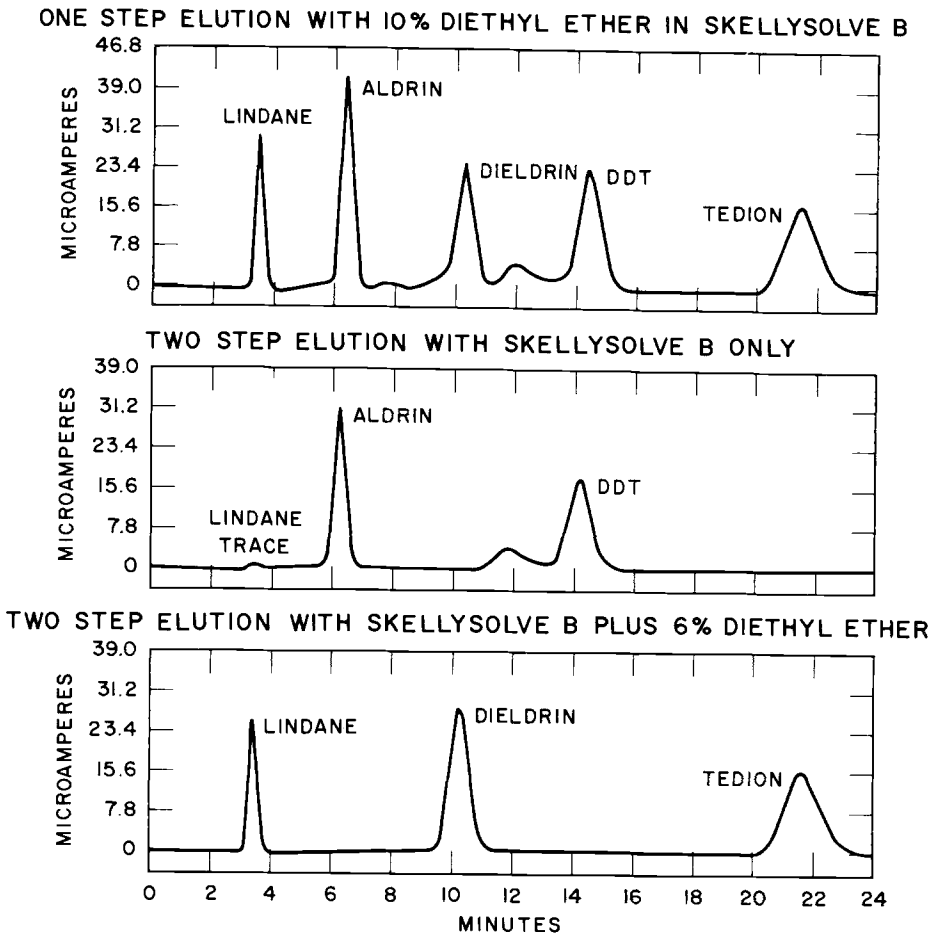
NITROGEN CARRIER GAS – 60cc/min.

BIAS – 110m.v. vs Ag/AgI DAMPING RESISTOR – 6.8meg

SAMPLE – 10 MICROLITERS OF ACETONE SOLUTION THAT WAS 0.01% EACH IN FOUR SULFUR CONTAINING COMPOUNDS

ELECTROLYTE – 0.4% ACETIC ACID AND 0.04% KI

FIG. 4—*Microcoulometric gas chromatogram—SO_2 detection.*



COLUMN - 20% HIGH VACUUM	CHART SPEED - 2 min./in.
SILICONE GREASE ON	NITROGEN FLOW RATE - 47cc/min.
ACID - WASHED CHROMOSORB	COLUMN TEMP. - 238° C

FIG. 5—*Microcoulometric gas chromatogram using aluminum silicate column cleanup.*

furnace, the titrant most commonly used is iodine generated in an iodide solution at a platinum anode.

Figure 3 shows the first commercial coulometric detector cell [5] and also a simpler, but more sensitive, later model. Coulometry is very attractive for quantitative work, since the amount of material detected can be put on a theoretical basis according to Faraday's laws of electrolysis. That is,

$$\text{Micrograms} = \frac{10^6 Q \times \text{Eq. wt}}{96{,}500} \tag{1}$$

where Q is the product of the generator current and time, in coulombs.

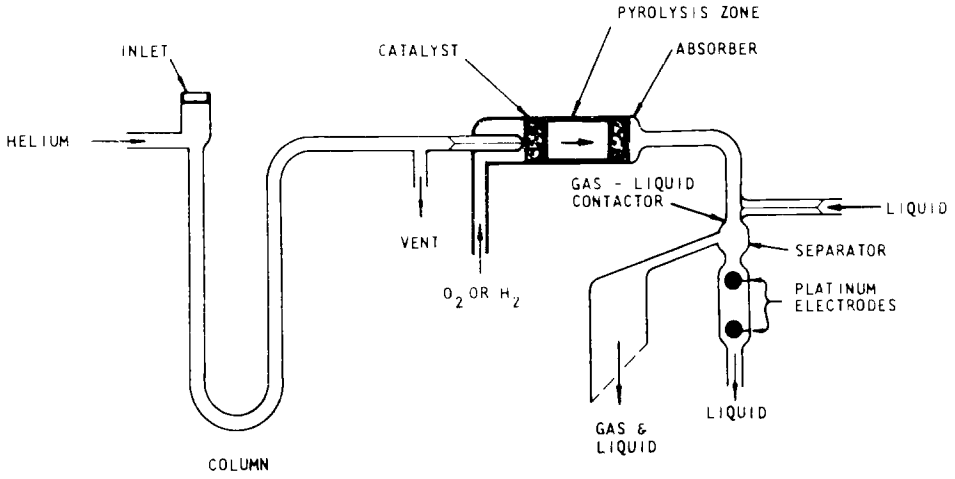


FIG. 6—*Electrolytic conductivity gas chromatograph.*

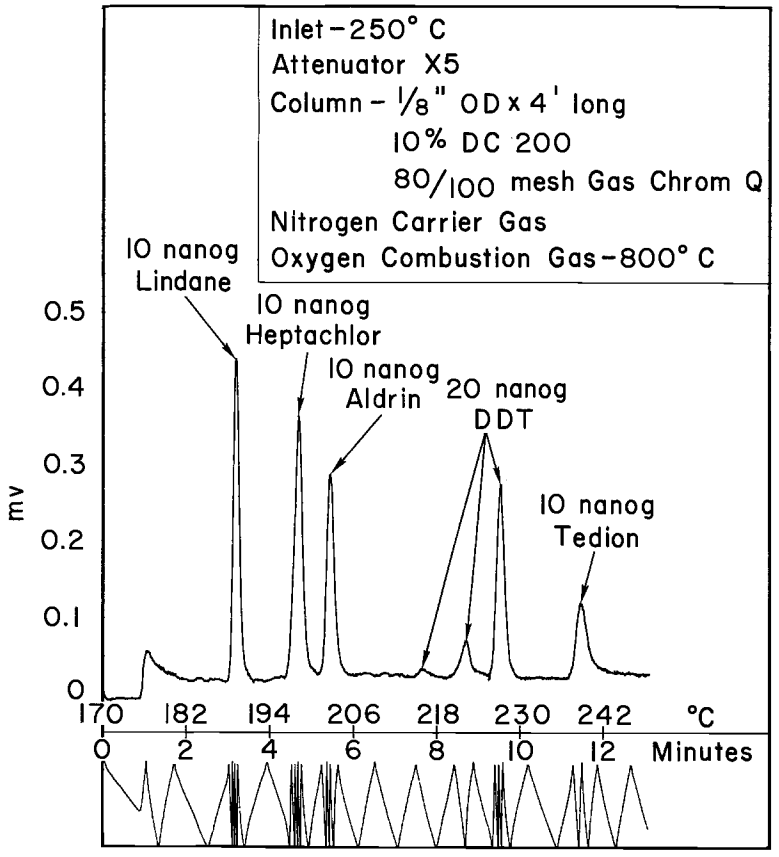


FIG. 7—*Electrolytic conductivity gas chromatogram.*

Figure 4 shows a microcoulometric gas chromatogram of a mixture of sulfur-containing compounds with an iodimetric titration medium in the detector cell. The cause for the low recovery of 69 percent for *p*-chlorobenzyl, *p*-chlorophenyl sulfide was probably losses in the gas chromatographic system.

Figure 5 demonstrates the use of the coulometric detector for specific halide detection using a silver ion titrant. This figure also demonstrates the results of separating chlorinated pesticides into two groups by adsorption chromatography before the gas chromatographic determinations. Such separation can be of great assistance in interpreting the gas chromatographic results.

The second detector developed was the electrolytic conductivity detector [*6*] shown in Fig. 6. This detector is generally used with a pyrolysis furnace on the downstream end of the gas chromatographic column. The selectivity of this detector is based on two factors. First, it detects only substances that form ions when dissolved in water, and second, the nature of the pyrolysis products that are emitted from the pyrolysis zone can be controlled. For example under oxidative conditions, the products of pyrolysis of organic halides are hydrogen halide acids and the product of pyrolysis of sulfur compounds is SO_2. Under reductive conditions, the corresponding pyrolysis products are hydrogen halides and hydrogen sulfide. Hydrogen sulfide is such a weak acid that it is scarcely detectable. However, if an absorber is added to selectively remove certain classes of electrolytes, such as hydrogen halides, or SO_2, or both, the detector can be made very selective.

Figure 7 shows the use of this system for some chlorinated compounds under oxidative furnace conditions without any absorber in the pyrolysis zone. Figure 8 shows a comparison of electron capture and electrolytic conductivity gas chromatograms on an

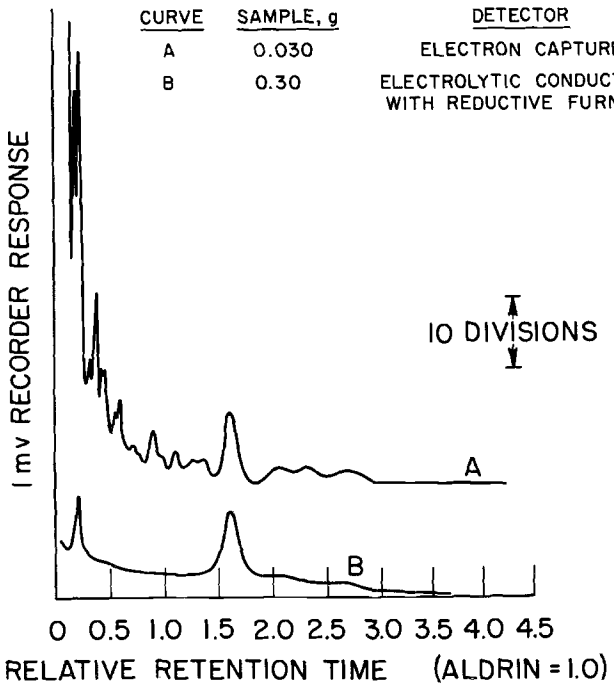


FIG. 8—*Gas chromatograms on extract of rodent*

extract of a rodent. In this case reductive furnace conditions with no catalyst or absorber were used for the electrolytic conductivity gas chromatogram.

The detector can be made selective for halogens with an oxidative furnace and a 800 deg C CaO absorber for SO_2 as shown in the upper curve in Fig. 9. The lower curve in Fig. 9 was obtained on another aliquot of the same solution, but with a 700 deg C silver absorber for halide in the end of the pyrolysis tube. In this case, the SO_2 came through the absorber and was detected. The halide, on the other hand, was collected on the silver absorber, so it was not detected.

Another means, and probably the most exciting one, of using the electrolytic conductivity detector is the selective detection of organo-nitrogen compounds [7]. In this case a nickel catalyst in the pyrolysis zone is used to hydrogenate the organic compounds. This leads to the conversion of organic nitrogen to ammonia. Any acidic products of hydrogenation or pyrolysis are absorbed on a solid strontium hydroxide absorber at the end of the pyrolysis zone. This detector system is extremely selective for ammonia and sensitive to as little as 0.1 ng of nitrogen. Figure 10 gives curves for an organic nitrogen containing compound and for a mixture of chlorinated hydrocarbons. The only element in the parathion that gave a response was the nitrogen. Figure 11 gives a curve for a mixture of nitrogen containing compounds demonstrating the nitrogen mode of detection.

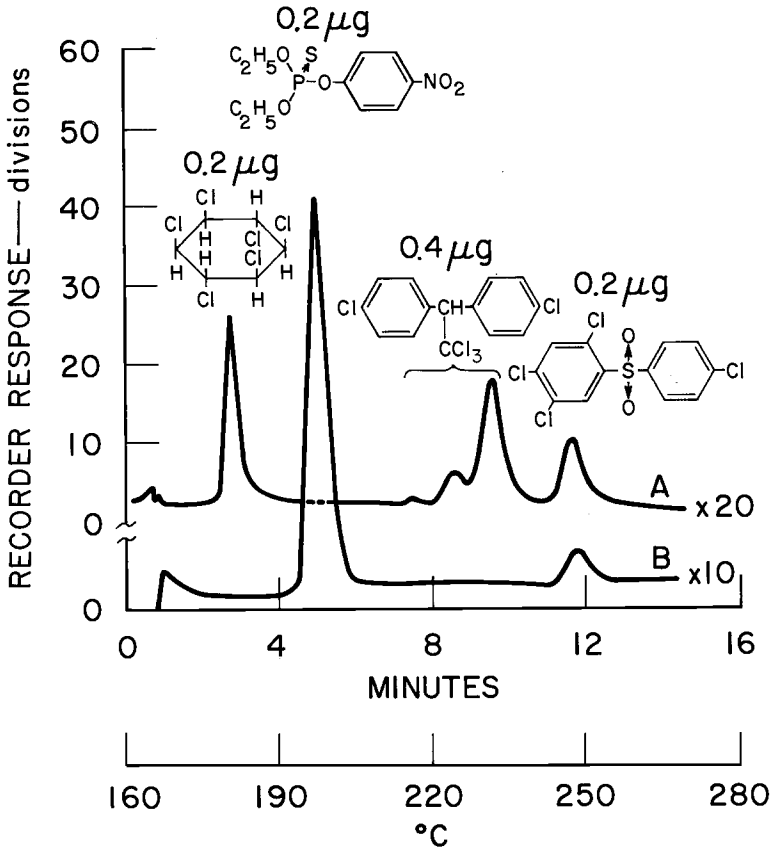


FIG. 9—*Halogen specific and sulfur specific detection gas chromatography.*

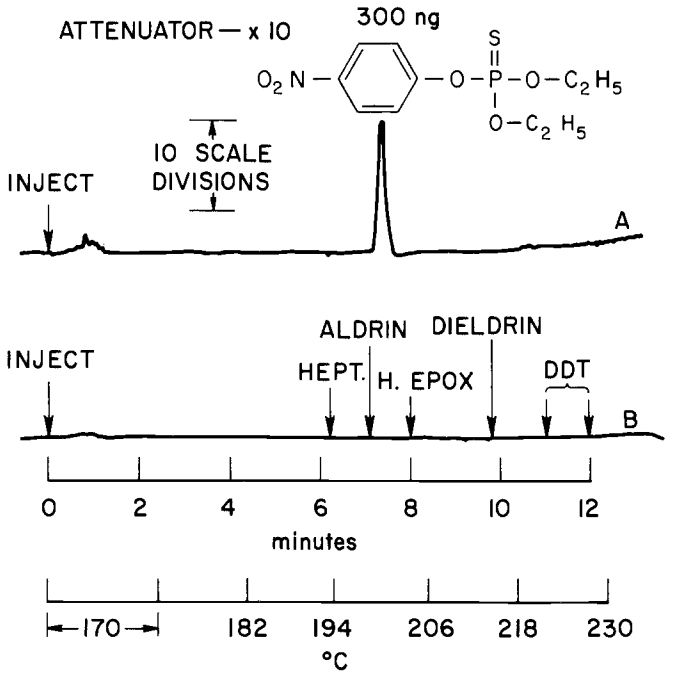


FIG. 10—*Nitrogen specific detection of parathion with no response from chlorinated hybrocargons.*

Using these detectors that are element selective and a simple method of sample extraction and cleanup (as described by Finkle, Cherry, and Taylor [*2*] along with an atlas of gas chromatographic retention time data for a variety of columns, it is possible to obtain useful results in a systematic manner for samples with unknown histories. This approach is by no means limited to pesticide chemicals. It should be successful for the identification of any compound that can be gas chromatographed and that contains nitrogen, halide, or sulfur.

There is an additional approach to selective detection that must be added to bring matters up to date. The use of a mass spectrometer as a gas chromatographic detector is an extremely powerful tool. This is particularly true because of some recent developments in mass spectrometry. The use of field ionization and chemical ionization makes it possible to ionize the compounds without fragmentation, giving the predominant peak at the m/e^+ value that corresponds to the molecular weight or the molecular weight plus one of the compound.

Another mass spectral technique that should find wide usefulness is called mass fragmentography. It is based on ionization by conventional electron bombardment. Finnigan Corporation provides an automatic peak selector accessory for peak monitoring [*8*]. According to Bonelli [*8*] this method can be used to monitor as many as eight preselected m/e^+ values, essentially continuously, with a sensitivity of approximately 1 pg/s for molecules with molecular weights in the 100 to 200 range.

Thus there exists the potential for rapid qualitative and quantitative analysis for pesticides and other organic poisons by combining simple extraction and cleanup procedures with gas chromatography using a variety of selective detectors.

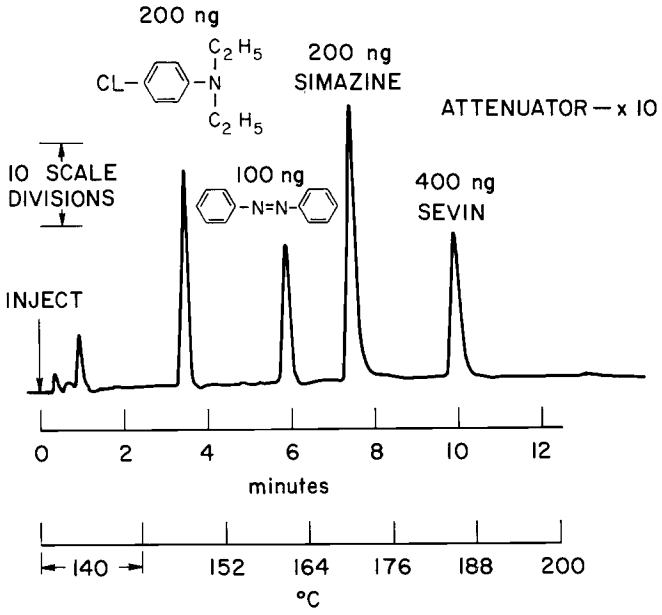


FIG. 11—*Nitrogen specific electrolytic conductivity gas chromatogram for several nitrogen compounds.*

Summary

Pesticide residues or contaminations can be measured by a variety of methods which include the use of a combination of techniques, such as solvent extraction, chromatography, and numerous methods of detection. Good separation methods in sample preparation are especially important in qualitative analysis. Strengths and weaknesses of the various detectors for chromatographic analyzers are discussed in terms of selectivity and sensitivity with particular attention to electrochemical methods.

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