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The Analysis of Pesticide Residues: New Problems and Methods

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Public attention is currently centered on the widespread use of pesticides. Chlorinated hydrocarbons are continuously reported as residues in birds, fish, water, and other substances. Adverse ecological effects from the use of defoliants in Vietnam, the suspected link between birth defects in rats and mice and ingestion of 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) herbicide, and numerous other consequences from the use of chemicals have effectively motivated public concern. The results of this concern have necessarily changed the course of pesticide research and the associated analytical problems. Ecological samples are being analyzed, particularly for chlorinated hydrocarbons, almost to the extent that raw agricultural commodities were examined in the past. Also, many samples are at present being analyzed to again check the true safety of older chemicals, such as mercury and arsenic, which have been in use for many years. Finally, persistent chlorinated hydrocarbons are being phased out, to be replaced by new, effective compounds of much shorter life. These latter compounds must then also be analytically reckoned with.

The analytical difficulties encountered in these new research areas may not be evident. Analysis of wildlife samples for chlorinated hydrocarbon pesticides is usually complicated by a lack of uncontaminated controls. Reliable identification of the toxicant usually requires more than one method of determination. Possible contamination of these samples by other, interfering pesticides or industrial compounds such as the polychlorinated biphenyls must be dealt with. The determination of chlorinated hydrocarbons in water or air is exceedingly difficult, and at present the results are grossly inaccurate, owing to the fact that the pesticide is present in the sample in extremely low concentrations (typically, one part per trillion) and variously combined in the sample, as well as to other difficulties.

To illustrate the analytical and other experimental difficulties, let us suppose that an ecologist and an analytical chemist propose an experiment to study the uptake and metabolism of DDT by fish in water to which DDT is to be added. What questions must be asked to assure that samples for analysis are truly representative and the experimental results valid? They might be as follows.

How will the DDT be added to the water, since it is highly insoluble?

Will emulsifiers be used, which may introduce artifacts?

Is the insecticide to be added as pure, recrystallized *p,p'*-DDT or as a technical grade DDT containing other isomers?

If an aquarium is to be used, how will possible losses of insecticide by adsorption on glass walls be reckoned with?

How will possible losses by codistillation with surface water be accounted for?

Water analysis will be required, and do the investigators know that DDT may exist in water in several forms: truly dissolved, adsorbed on clay particle surfaces, dissolved in the lipid layers of innumerable aquatic microorganisms, and in other forms?

How will these combined forms of DDT be related to the extent of absorption by fish, and how will analysis be accomplished? One would commonly first pump many gallons of water through a charcoal filter to concentrate the toxicant, then, with Soxhlet extraction remove the insecticide from the filter. It has been found, however, that charcoal may remove only about half of the compound from water and that Soxhlet extraction may remove only part of the adsorbed DDT from the charcoal.

Regardless of the method of extraction, how will a few nanograms of DDT be isolated from simultaneously extracted concentrated interfering substances?

If fish are to be held for any length of time in a limited volume of water, how will the excreta and other metabolic products that they rapidly produce be removed (normally the water is cycled through charcoal to accomplish this) without removing the DDT to which the fish are to be exposed?

Are the investigators aware that several controls must be included to correct for other factors? For instance, a control experiment is needed in which DDT is added to water containing no fish, to measure the possible effects of light on the production of DDT metabolites in water. Another control experiment is needed in which DDT is added to water containing the metabolic waste products of the fish but not the fish themselves, in order to study possible microbiological and chemical reactions of the insecticide with these components.

What is the DDT (and metabolite) content of the fish prior to the experiment? (Most species already contain some DDT.)

Will all the fish used be of the same age? The common method of judging the age of a fish by examining its scales is very inaccurate. Yet this knowledge is essential, since older fish contain considerably more oil than

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younger fish and their ability to store DDT is greater.

Do the investigators know that the past history of the fish (the temperature at which they were reared, the type of food they ate, and other factors) can greatly affect the degree of hardness of their fat and therefore the solubility of DDT in the fat?

If gas chromatography is to be used for analysis, does the chemist know that thermal degradation of DDT to certain of the very metabolites formed in biological systems is not uncommon?

One could go on and on, but it is questions of this type that must determine the experimental design if the results are to be meaningful.

Newer substitute pesticides, such as carbamates, which may rapidly degrade in ecosystems also present problems for the analytical chemist. They disappear so rapidly following application that, to prove that they have not been taken up by samples, the sensitivity limits of the analytical method are often strained. Many of these compounds either do not contain the usual heteroelements (sulfur, phosphorus, halogens, and so on) or contain them in amounts too small to be determined by existing sensitive, element-selective gas chromatographic detectors. Some of these new compounds, such as carbamates, are easily degraded thermally and therefore are very difficult to chromatograph. Federal requirements for analytical information concerning metabolite formation are becoming ever more stringent, and this presents another problem. Finally, whereas the metabolism of older compounds has undergone intensive study, relatively little is known about many of the newer compounds. Certain of those of exotic structure may degrade by way of pathways hitherto unknown.

New Gas Chromatographic Detectors and Refinement of Existing Ones

New instrumentation and methodology have been developed during the past few years which facilitate analysis of many compounds. Among all the analytical techniques, gas chromatography and its ancillary developments remain, so far, the most important. Much progress has been made in the design of new detectors and in the refinement and modification of existing ones. Owing, in part, to the current emphasis on analysis of chlorinated hydrocarbons in ecological samples and to the routineness of operation of the technique, electron affinity detection is probably still used for pesticide analysis more than all other detection systems combined. Its unparalleled detection limits, often in the picogram range, and its uncanny response to a wide range of compounds which, almost without exception, are toxicants (halogenated, nitro, heavy-metal, and polynuclear compounds, for example) also account for its popularity.

The detector contains a source (usually isotopic) of slow-moving electrons (low-energy beta rays) which, following reaction with nitrogen carrier gas to form negative molecular ions, are captured by positive sites on pesticide molecules which contain functional groups or structures possessing electron affinity. A resulting decrease in current between the detector's cathode and anode is amplified and recorded as the peak signal of the eluting molecule.

The radioactive source in most earlier electron affinity detectors was tritium; a smaller number of these sources contained strontium-90 or radium-226. Tritium's upper operating temperature limit of about 225°C, and therefore the need for frequent cleaning of the detector, was its main limitation. Strontium and radium foils, although less sensitive than tritium, were operable at much higher temperatures. Unfortunately they emit gamma radiation and are therefore of concern to the operator. Nickel-63 is now available as a foil (*1*); although more expensive than tritium, it is operable at temperatures up to 300°C and emits pure beta radiation. Since nickel-63 emits beta radiation almost as soft (0.06 million electron volts) as tritium (0.02 million electron volts), there is little sacrifice in sensitivity.

The alkali thermionic detector, first reported by Giuffrida (*2*), for analysis of residues of organophosphorus insecticides has been variously modified and continues to be the most widely used detector for these compounds. This detector involves the addition of a wick impregnated with alkali metal salt (for example, potassium sulfate) to the flame of the standard flame ionization detector. Chromatographed phosphorus compounds entering the flame elicit a greatly enhanced response, the mechanism of which is still a matter of dispute. The high sensitivity and good selectivity of the device and the fact that it is possible to rapidly and cheaply convert flame ionization detectors to the alkali thermionic mode account for its popularity. A thermionic detector incorporating rubidium sulfate and sensitive to nanogram quantities of nitrogen compounds has been reported (*3*). Proper geometry is especially important for optimum performance of this detector.

The flame photometric detector (*4*) is rapidly becoming a standard device in residue analysis. Its highly specific response to either sulfur or phosphorus compounds makes it ideal for such analysis, a minimum of preliminary isolation being necessary. When the detector is in operation, separated sulfur and phosphorus organic compounds enter a flame which excites sulfur and phosphorus band emission at 526 and 394 nanometers, respectively. The emitted radiation is isolated by appropriate filters and measured by a photomultiplier tube detector. Its sensitivity is about 0.1 and 1 nanogram of phosphorus and sulfur, respectively. Bowman and Beroza (*5*) reported use of this detector in the dual-channel mode that permits simultaneous detection of phosphorus and sulfur in chromatographed compounds.

The microwave-powered emission detector reported by McCormack *et al.* (*6*) and applied to residue analysis (*7*) has been modified. This device is similar in principle to the flame photometric detector. The chromatographed compounds are subjected to strong electron bombardment and thermal degradation in an intense microwave-powered argon plasma. Atomic emission of phosphorus and iodine in the respective compounds is resolved and monitored spectrometrically to measure the peak emission of chromatographed pesticides. More recently a low-pressure helium plasma has been used which is more intense and excites emission of atomic sulfur, chlorine, and bromine as well as elemental emission of phosphorus and iodine in organic compounds (*8*). The helium plasma device has been applied in trace analysis of many residues in a variety of samples (*9, 10*).

Use of the microcoulometric detector is well established in gas chromatographic analysis of sulfur and halogenated organic pesticides (*11*). This detector has been adapted to specific analysis of nitrogen compounds (*12*). The eluted compound is catalytically reduced in hydrogen to ammonia, which is titrated by coulometrically generated hydrogen ions. As little as 1 nanogram of ammonia yields a detectable response. Selective analysis of chromato-

graphed nitrogen compounds is also possible, through use of the newly developed electrolytic conductivity detector (*13*). The compound is similarly reduced to ammonia, which is then determined conductometrically. Quantities of ammonia in the range of 5 to 10 nanograms are detectable. To date, these devices are the most generally sensitive and specific detectors of nitrogen compounds.

Column Substrate Materials

Although innumerable column substrate materials are commercially available for gas chromatography, most separations are carried out with only a few. Initially DC-200 and SE-30 were used as nonpolar substrates for most analyses. The combined substrate column incorporating DC-200 and QF-1 was first devised to separate dieldrin and *p,p'*-DDE and is now frequently used for general insecticide separations. Semipolar substrates such as Carbowax 20M, DEGS, and Apiezon L have been useful for chromatographing particular compounds.

Similarly, several polar substrates have been valuable for specific separations. FFAP is useful for the separation of keto compounds (*14*). Ucon Polar is appropriate for the separation of certain compounds such as the methylated alkyl phosphate hydrolytic metabolites of organophosphorus insecticides (*15*). The cyanosilicone substrate XE-60, is sometimes used for special separations. The OV series of phenyl-substituted silicones are a new addition to the available substrates. Many applications have been reported. OV-17 is particularly versatile as a stationary phase for the chromatography of pesticides (*16*). The recently introduced porous polymer beads will probably also be used in more analytical problems with pesticides and their metabolites. These beads have been shown to be appropriate for analysis of the fumigant Nemagon (1,2-dibromo-3-chloropropane) in soil (*17*) because they markedly increase the retention time of this normally rapidly eluting compound. The great number of investigators using gas chromatography has made it profitable for several manufacturers to market these substrates in very pure form, and as coated support materials showing good analytical reproducibility. This has greatly advanced the successful application of this analytical technique.

Derivatization Procedures

The application of derivatization procedures in the analysis of pesticide residues continues. There are several reasons for forming derivatives of a compound to be analyzed. These include (i) reducing its polarity or increasing its volatility or stability to permit chromatography; (ii) converting the compound to a new product which is more responsive to a particular detection system; and (iii) altering its structure in order to change its retention time to separate it from interferences or to aid in its characterization.

Much effort has been applied to the selection of organic reactions for forming pesticide derivatives. For facilitating the chromatography of pesticide compounds, reactions have included methylation of herbicide acids (*18*), formation of herbicide phenol methyl ethers (*19*), and decarboxylation of a herbicide acid (*20*). These reactions reduce polarity and increase volatility. More successful chromatography of pesticidal carbamates and ureas is possible through the formation of trimethylsilyl derivatives (*10, 21*) which increase volatility and possibly thermal stability.

The array of reported derivatives to accentuate detector response has been particularly diverse. For increasing the electron affinity of molecules the following examples might be used: bromination of diphenylamine (*22*) and 1-naphthol (*23*), nitration and methylation of herbicide acids (*24*), formation of 2,4-dinitroaniline derivatives of amines derived from carbamates and certain other pesticides (*25, 26*), trichloroacetylation (*27*) and chloroacetylation of pesticide phenols (*28*), and halomethyldimethylsilylation of pesticide acids and phenols (*29*). The method described (*25*) for formation of 2,4-dinitroaniline derivatives of amines derived from carbamate insecticides is rapid, reliable, sensitive, and versatile and should provide an exceedingly valuable screening procedure for these important compounds. Thiophosphorylation of carbamate phenols has been used to increase response to the flame photometric detector (*30*). As an example of derivatization to obviate interferences prior to gas chromatographic analysis, dieldrin has been treated with hydrobromic acid and acetic anhydride to open the epoxide ring and yield two peaks with a longer retention time in order to separate dieldrin from *p,p'*-DDE (*31*).

Gas Chromatographic Auxiliary Techniques

In conjunction with gas chromatography, a very useful technique to aid in the characterization of pesticides was developed by Beroza and his co-workers (*32*). They devised a simple procedure for determining the partition coefficient ("p" value) of a pesticide between two immiscible solvents. Following gas chromatography of a given pesticide and peak-height measurement, a measured volume of the remaining solvent containing the compound is partitioned with an equal volume of a second immiscible solvent. The original pesticide solution is again chromatographed, and from measurement of the new reduced peak height the "p" value is computed. These investigators have determined and published a large number of "p" values for numerous pesticides in several common solvent combinations. All aspects of this technique have now been described in a single reference (*33*).

The research of a number of investigators (*34*) has led to the commercial availability of a sweep codistillation apparatus for preliminary isolation of pesticides prior to gas chromatographic analysis. A volume of a concentrated solvent extract representing up to 2 grams of sample is injected into a heated tube containing glass beads and quartz sand. The sample is swept through the tube by means of a rapid flow of nitrogen and several portions of injected solvent. Many extraneous materials remain in the tube. Emerging vapors containing the pesticide are condensed in a Teflon coil cooled in an ice bath. These condensed materials are redissolved and analyzed by gas chromatography. The method has been successfully applied to the analysis of various pesticides in agricultural samples (*35*).

Other Chromatographic Methods

For isolation and separation of toxicants from endogenous interferences or from each other, thin layer chromatography continues to be a major tool. Many procedures in which new substrates, eluting solvents, and sensitive, specific chromogenic agents are used have been published. Several developments either have found or probably will find increasing application in pesticide analysis. The use of glass or polyester sheets (*36*) coated in advance with

the desired adsorbant substrate greatly facilitates application of the technique and yields more uniform results. The introduction of channel layer chromatography (*37*) increases the capacity of the plate, permitting the use of larger samples, and eliminates edgewise diffusion of compounds during development or elution of plates. Also, the recent availability of double-beam ratio-recording spectrophotometric scanners should greatly aid in the quantitative measurement of toxicants and metabolites.

Another technique in which significant advances have occurred recently is that of liquid-liquid chromatography. The application of this method to analysis of insecticides was reported by Lambert and Porter in 1964 (*38*). These workers built an automatic chromatographic system, employing a column of hexane–carbon tetrachloride as the mobile phase and water–ethylene glycol as the stationary phase adsorbed on siliconized firebrick. They used a refractometer detector. Several companies now offer automatic liquid-liquid chromatography systems which accept any of a number of column packings and use any of several detectors. Among the newest developments in column materials are controlled surface porosity supports (*39, 40*). These stable materials may be operated at high pressures in narrow columns for rapid analysis, with negligible loss of resolution (*40*). The method has been applied to pesticide analysis (*39, 40*). As little as 0.5 nanogram of organic compounds has been detected, by means of an ultraviolet absorption detector (*40*). A good description of available detectors, including, among others, refractometers and detectors based on electrolytic conductivity, heat of adsorption, spectrophotometry, and flame ionization, has been published (*41*).

Automated Pesticide Residue Analysis

The automation of pesticide residue analysis is a recent development largely pioneered by Gunther and his co-workers (*42*). The equipment most often used is that marketed by Technicon Corporation. The automated analysis of organophosphorus insecticide residues by initial combustion (*43*) or wet oxidation (*44*), followed by colorimetric analysis of orthophosphate, has been reported. Ott (*45*) reported the automated analysis of organophosphorus insecticide residues by two simultaneous procedures for verification. One was based on the colorimetric analysis of orthophosphate following wet oxidation of the insecticide. The second involved colorimetric analysis of thiocholine released from acetylthiocholine by excess cholinesterase following inhibition of the enzyme in human blood plasma by the insecticide. Winter (*46*) has also automated a method based on cholinesterase inhibition for analyzing organophosphorus insecticide residues. Levine *et al.* (*47*) have reported an automated micro method for determining serum cholinesterase. An automated system of injection for performing unattended analysis of organochlorine and organophosphorus insecticides by gas chromatography has been described (*48*). Methods for performing automated ultraviolet absorption analysis of the fungistat biphenyl (*49*) and colorimetric analysis of aziridine (*50*) in chemosterilants have also been reported.

Mass Spectrometry

Mass spectrometry is a sensitive technique which is being increasingly applied to analysis and elucidation of the structure of pesticides and metabolites. Under ideal conditions, quantities as small as 10 nanograms will produce a usable mass spectrum. Mass spectrometry in combination with gas chromatography has been used to advantage for separating and identifying the polychlorinated biphenyls in ecological samples (*51*). The mass spectra of organophosphorus insecticides (*52*) and carbamate pesticides (*53*) have been determined by means of time-of-flight mass spectrometry. Organochlorine or phosphorus insecticides have been identified in mixtures by high-resolution mass spectrometry (*54*). The high-resolution mass spectra of several organochlorine pesticides have also been determined (*55*). Various chlorinated phenoxycarboxylic acid herbicides have been identified by mass spectrometry (*56*). Negative ion mass spectrometry could presumably be used for determining chlorine isotope ratios (*57*), although it appears that this method has not been applied to studies of pesticide metabolism. Spark source mass spectrometry (*58*) is also an exceedingly sensitive method for trace element analysis, which is being more and more frequently applied to the analysis of biological materials. It has been used for detecting various metals in lung tissue (*59*) and in hair (*60*) and for detecting mercury in apples (*61*).

A new and promising device is the plasma chromatograph (*62*). This instrument, when used in conjunction with a mass spectrometer, offers a powerful means of analyzing and elucidating the structure of pesticides and pollutants (*63*). In principle, a mixture of organic compounds is injected into an ionizer section where ion molecules are formed. These species are next separated by drifting at different rates through a tube in a strong electric field. The ion molecules arrive at a collector as ion peaks at times determined by their structures. A portion of the components emerging from the tube may be directed into a quadrupole mass spectrometer, simultaneously producing a mass spectrum. The sensitivity of the method is in the range of a fractional part per billion.

Other Approaches

Nuclear magnetic resonance spectrometry is another analytical tool being used for the identification of pesticide structure. Its main limitation is lack of sensitivity, but the valuable structural information obtained often makes any preliminary isolation and concentration steps worthwhile. The method has been applied in the analysis of chlorinated (*64*) and organophosphorus insecticides (*65*) and carbamate drugs (*66*).

Many other approaches are currently being used for the analysis of pesticide residues. These include ultraviolet, visible, infrared, and fluorescence spectrophotometry; neutron activation analysis; oscillographic polarography; and isotope techniques. The application of these methods to residue problems has been discussed elsewhere (*67*). One may expect that laboratories investigating these problems will also use methods such as atomic absorption and emission spectrometry as they enter the field of analyzing toxic metals in agricultural or ecological samples, such as mercury in fish.

Still more elegant analytical instrumentation for detecting ever smaller quantities of toxicants and their metabolites will undoubtedly be developed in the future. The isolation and proof of structure of minute traces of many of these compounds will, however, remain a formidable challenge.

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Human Injury Inflicted by Grizzly Bears

The chance of human injury in the national parks can be reduced to a minimum through improved management.

Stephen Herrero

Within recent geological time the grizzly bear (*Ursus arctos horribilis*) was at the top of the North American food pyramid wherever the species was found. Storer and Tevis (*1*) claim that in California before the arrival of the Spaniards the grizzly and certain Indian tribes competed for such foods as acorns. The bear fed as he pleased. The Indian took what was left. Then European man arrived with firearms and by the mid-19th century had established his supremacy. Since then, the demise of the grizzly has been dramatic.

The range of the species in North America, until shortly before the arrival of European man, extended from the western coast to the Mississippi River, south into Mexico, and north to the Arctic Circle, reaching northeast to the Arctic coast near Simpson Strait, Mackenzie District (*1*, *2*). Today both range and numbers have contracted to a fragment of what they were originally (*1*). Grizzlies previously favored foothills, brushlands, and river valleys, rather than the high mountains (*3*). Grizzlies now inhabit mountainous parts of Montana, Idaho, and Wyoming, centering about Glacier National Park and Yellowstone National Park. Farther north, populations exist in parts of Alberta, British Columbia, Yukon Territories, Northwest Territories, and Alaska. As man disperses into these northern areas, further encroachments on grizzly habitat and numbers will occur. The last home for this one-time monarch of

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