PESTICIDE RESIDUES
Basic Principles for Quantitative Determination

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The widespread use of synthetic organic pesticides has emphasized a number of analytical problems previously not significant. One of these problems involves the establishment of the magnitudes of persisting residues of these pesticides on and in foodstuffs. Present approaches to this residue problem are largely empirical. In the present paper, the basic analytical approaches to residue determinations are considered to be evaluation by direct measurement (selective) and isolation followed by measurement (nonselective). The latter approach interrelates physical separation with reaction separation. These logical approaches to devising analytical methods for pesticide residues are illustrated from the field of insecticides. Even though every foodstuff containing pesticide residues must be individually investigated as to performance in the final residue method, there is real promise of systematization and standardization of efforts in new applications.

THE WIDESPREAD USE of synthetic organic pesticides during the past decade has emphasized problems not previously significant. These include all aspects of quantitatively establishing magnitudes of persisting residues of these pesticidal substances, with special reference to foodstuffs.

Although pesticides are subject to weathering and other losses in the field, many of them persist on or in the commodity in decreasing amounts for remarkably long periods even under drastic temperature conditions. To the analytical chemist, therefore, falls the challenge of establishing quantities and locales of perhaps a few micrograms of an organic molecule within an orange, an ear of corn, a sugar beet, the liver of a cow, the Malpighian tubes of an insect, or other complex substrates. Such substrates frequently yield several grams of solvent-extractable materials containing varying combinations of organic acids, hydrocarbons, sugars, alcohols, esters, waxes, terpenes, pigments, small protein molecules, amino acids, and others, from which the pesticidal molecules must essentially be freed before they can be assayed properly. In addition, there may be present fragments or other metabolic derivatives of the parent pesticidal compound.

Heretofore, emphasis in residue considerations has been placed upon the development or adaptation of specific colorimetric reactions for quantitatively estimating the pesticide present. Arguments advanced to justify this emphasis include general ease of applicability and manipulation, reasonably rapid analytical techniques, and presumed specificity. Yet the successful adaptation of a given colorimetric method to a new substrate nearly always represents a major research endeavor. Frequently the method is applied blindly, then grossly misinterpreted, with little attention to accuracy, sensitivity, reproducibility, varying composition of substrate, and similar fundamental aspects of the new application. Thus, the usual techniques of pesticide colorimetry could most judiciously be expressed in units of quantitative "estimation" rather than "determination" or "analysis."

The current importance of establishing magnitudes and locales of pesticide residues is undisputed. There are now available many accurate techniques for the ultimate measurement of the pesticide present once it has been isolated, including ultraviolet, infrared, and x-ray spectrometry, polarography, and the many devices and procedures for functional group analysis. It is clear that more attention should be focused on developing isolation procedures adequate to the use of these precision techniques.

There is little reason to attempt general use of a color reaction by applying it blindly to a variety of substrates, and it also is illogical to attempt to develop specific isolation techniques for the benefit of a particular color reaction responding to a single, simple functional group. Development and exploitation of a variety of basic types of concentration and isolation techniques would provide more adequate methods of more general applicability, when used either alone or in combination. Precise methods could then be used to analyze natures and magnitudes of residues, without recourse to single-function color reactions.

Careful study of published methods of pesticide estimations and determinations and the many modifications developed to overcome difficulties encountered with specific substrates will afford better understanding of the diverse problems involved and furnish clues to approaches and techniques of general utility. Ordinarily, an efficient pesticide may occur on or in a plant part in quantities less than 1 p.p.m., or 0.5 mg. per pound. During extraction of the pesticide from the substrate there may simultaneously be obtained as much as 30 grams of extractives per pound of plant part, as with avocados and olives. Despite this formidable contamination of the pesticide, there

are at least three logical approaches to the cleanup or isolation required before the final analytical determination, as outlined below with typical examples from the field of insecticides. These basic considerations also apply to residue evaluations with the other classes of pesticides.

Analytical Approaches For Residue Studies

In some residue investigations it may be possible to establish the amount of pesticide without recourse to any isolative procedure other than the quantitative measurement itself. This situation rarely obtains with plant extractives except perhaps when biological means of assay are employed. With these selective measurement techniques some unique physical, chemical, or biological property of the pesticide itself is quantitatively measured, which must necessarily be immune to interference by any accessory substances present.

The other two approaches require isolative procedures prior to the analytical measurement. The first involves the physical separation of the intact pesticide from the bulk of extracted material by physical, chemical, or biological means; this is called the "physical separation" technique. The second involves subjecting the pesticide to a discriminatory reaction which will facilitate the separation of the resulting pesticide derivative from interfering materials; this is called the "reaction separation" technique.

Combinations of physical and reaction separation techniques may be necessary to achieve quantitative or consistent recovery from a given substrate. Final measurements are performed by physical-chemical means (such as spectrophotometry) or by biological means: inhibition of biological activity (such as cholinesterase inhibition by parathion) or bioassay (such as housefly assay of dieldrin). Thus, for example, most colorimetric procedures for the quantitative determination of pesticide residues are combinations of techniques such as chromatography, followed by color development and extraction of the color body into a suitable solvent and spectrophotometric evaluation. It must be emphasized again, however, that an analytical cleanup procedure or combination of techniques providing satisfactory results when used on one plant or plant part may prove unsatisfactory when used on another. This means that every foodstuff to be considered for pesticide residue studies must be individually investigated and quantitatively evaluated as to performance in the final analytical method.

In this paper, the basic techniques involved in these three analytical approaches are discussed, and, where possible, illustrated with procedures currently used for the determination of insecticide residues.

Evaluation by Direct Measurement (Selective)

The purpose of the selective measurement technique is to utilize some unique property of a pesticide for its measurement in the presence of substrate extractives. When such a property can be utilized, this approach affords the advantages of simplicity and rapidity in determining large numbers of samples.

Physical and Chemical Means When a pesticide has absorption characteristics markedly different from those found in extracts of the plant part under investigation, spectrophotometric measurements may suffice for determination. In general, such methods nearly always require extensive cleanup. Pesticides which are colored or can be converted directly to colored complexes or compounds are most easily determined in this way because background (plant extractive) interferences are generally less serious in the visible spectrum. Crude extracts of most plant parts are nearly always opaque to ultraviolet and infrared energy, but usually contain only yellow or green, and occasionally red pigments. Yellow and red pesticide color bodies or "dyes" (*4*) are ideally suited to this type of spectrophotometric assay.

The method for Dilan [a mixture of 2-nitro-1,1-bis(*p*-chlorophenyl) propane and 2-nitro-1,1-bis(*p*-chlorophenyl) butane] (*31*), which is red ($\lambda_{max.}$ 490 mμ) in alkaline solutions, is a good example of the facility of this procedure. Usually, little cleanup is necessary for the successful application of such procedures, although background variations should be carefully evaluated. Many other insecticides yield colored solutions upon treatment with complexing reagents. An excellent example of such color production from the parent insecticide is the Stiff-Castillo (*48*) method for DDT [1,1,1-trichloro-2,2-bis(*p*-chlorophenyl) ethane], where a red color ($\lambda_{max.}$ 520 mμ) is developed by treating the DDT with xanthydrol, pyridine, and potassium hydroxide.

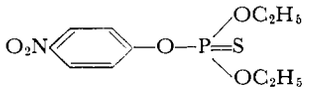
An example utilizing ultraviolet spectrophotometry is that described by Davidow and Woodard (*11*) for benzene hexachloride (1,2,3,4,5,6-hexachlorocyclohexane) in the presence of biological tissue. Their procedure is based upon the alkaline conversion of benzene hexachloride to 1,2,4-trichlorobenzene, which is measured by the change in transmittancy in the ultraviolet ($\lambda_{max.}$ 284, 286, and 290 mμ).

There are several analytical procedures using infrared spectrophotometry for establishing magnitudes of residues, but because of substrate complexity, it is impossible to use infrared methods without meticulous cleanup. The method of Garhart *et al.* (*16*) for aldrin (1,2,3,4,10,10-hexachloro-1,4,4a,5,8,8a-hexahydro - 1,4,5,8 - dimethanonaphthalene) and dieldrin (1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro - 1,4,5,8 - dimethanonaphthalene) illustrates the techniques and problems involved for such methods. These workers developed infrared procedures with sensitivities of 0.0005 and 0.0007% for aldrin and dieldrin, respectively, with a probable error of ±0.001%. Using cleanup techniques described elsewhere in the literature, they carried out a large scale program of field residue studies on these two insecticides.

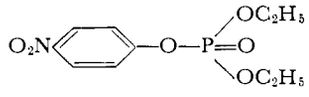
The only spectrometric methods of genuine promise in these isolation-by-measurement techniques are those of spectrophotometry. There are other promising physical means, however. At present, polarographic methods have been developed for the production control of benzene hexachloride (*12, 50*) and of parathion (*O,O*-diethyl-*O*-*p*-nitrophenylthiophosphate) (*5*), for example. These methods apparently have not been adapted to residue studies involving plant or animal substrates, but an adaptation to soil analysis (gamma isomer of benzene hexachloride) has been reported (*19*). Wiesmann (*55*) reports that for the detection of traces of DDT in foodstuffs a polarographic method shows some promise. Cryoscopic (*51*) and mass isotope dilution (*52*) techniques have also been reported for benzene hexachloride, but, again, were not successfully adapted to residue studies. Labeled insecticides are being used in many laboratories for pharmacological studies; applications to plant residue problems have not been reported.

Biological Means One of those distinctive properties of a pesticide that differentiate it from most substrates is its capacity to inhibit some biological activity. If this biological activity is conveniently measurable in the laboratory under controlled conditions, the degree of inhibition of this activity by an evaporated extract containing the pesticide is a measure of the quantity of pesticide present; background interference (masking) effects can be significant in such an application, however, and may require scrupulous evaluation.

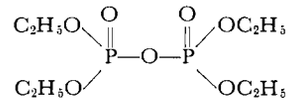
Giang and Hall (*17*) describe such a procedure using the enzyme cholinesterase, which is inhibited by several of the organic phosphorus insecticides such as parathion, paraoxon, TEPP, sulfo-



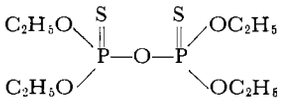
Parathion



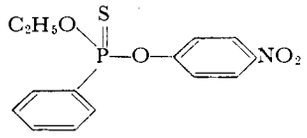
Paraoxon



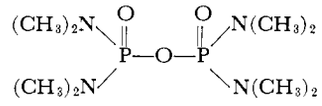
TEPP



Sulfotepp



EPN



OMPA

tepp, EPN, OMPA, and others. They measured the enzyme activity by pH determinations before and after contact with the insecticide residues; it might be more precisely determined colorimetrically by Metcalf's method (*39*). This colorimetric method determines the acetylcholine remaining after exposure to enzyme action, rather than the acetic acid formed, and is especially suitable for determining very low degrees of cholinesterase activity. The reactions involve the interaction of unhydrolyzed acetyl choline with alkaline hydroxylamine to form acethydroxamic acid, which yields a purplish brown ($\lambda_{max.}$ 540 mμ) complex with ferric ions.

Another interesting technique involving enzyme inhibition is that of Keller (*34*) for determining small amounts of DDT by carbonic anhydrase inhibition. He claims a sensitivity of 0.2 microgram of the insecticide. Adaptation to residue studies was not reported.

For trace amounts of the many pesticides which are difficult to detect by chemical means, biological assay has proved of value. In this general method, the insecticide-containing extract is prepared and concentrated by the techniques used for chemical assay. A test population of insects is then exposed to this concentrate, usually as a residual coating on a glass surface, for assay with the housefly, *Musca domestica* L., or as a suspension in water, for assay by mosquito larvae such as *Aedes aegypti* L. The mortalities produced under uniform conditions are compared with those obtained using a range of known concentrations of the insecticides concerned, and the results are then interpolated in terms of parts per million of residue. These techniques have been described in detail in the entomological literature (*6*, *9*, *26*, *49*).

Isolation Followed by Measurement (Nonselective)

The purpose of any cleanup technique is to isolate the pesticide from accessory substances as quantitatively and cleanly as possible. The techniques involved are usually physical, chemical, or biological attacks upon the extract from the substrate which allow the pesticide molecule to retain its identity. Complete isolation of the pesticide would probably require a combination of several such attacks upon the substrate extract. In practice, a partial cleanup of the substrate permits satisfactory measurement of the pesticide by some selective method. Some of the techniques now used are listed.

Physical Separation

Physical Means. STEAM DISTILLATION. Many natural oils and waxes are to some extent steam-volatile, and can be separated from a nonsteam-volatile pesticide. Steam-volatile pesticides which form salts or complexes can also be treated in this manner. While there is no published procedure for this particular technique with intact insecticides, the method of Kutschinski and Luce (*38*) as modified by Gunther *et al.* (*23*) for compound K-6451 (*p*-chlorophenyl-*p*-chlorobenzene sulfonate) on citrus illustrates its versatility. The insecticide is hydrolyzed to sodium *p*-chlorophenate, with the subsequent elimination of citrus oils and waxes by steam distillation; the boiler mixture is then acidified and again steam-distilled to afford essentially uncontaminated *p*-chlorophenol for colorimetric assay. This technique might profitably be used with such insecticides as the DN (dinitrophenol) series. A converse procedure is the steam distillation of the intact insecticide from the substrate, as used by Koenig *et al.* (*35*) to remove aldrin from minced alfalfa extractives.

DISTILLATION OR EVAPORATION. A simple distillation or, more commonly, evaporation of volatile extractives from a nonvolatile pesticide will result in its significant concentration. The pesticide must possess an extremely low vapor pressure, or significant losses may arise. Koenig *et al.* (*35*) used this technique in the isolation of dieldrin from citrus extracts for infrared analysis. They allowed the citrus oil (mostly limonene) to evaporate spontaneously from open Petri dishes, to achieve a 95% enrichment of dieldrin in orange oil.

PARTITION DISTRIBUTION. Most pesticides have chemical structures foreign to most of the types of compounds present in the substrate, and, in effect, possess some physical and chemical properties unique to the immediate environment. Such grossly distinctive nature would favor segregation by favorable partition into one of two suitable immiscible solvents, with the substrate extractives largely favoring the other solvent. Both solvents should be low-boiling. Jones and Riddick (*32*) describe such a procedure for several insecticides in the presence of extractives from animal tissues, milk, green beans, cucumbers, and apples. They used acetonitrile and *n*-hexane as the solvent pair, and encountered little or no interference by standard methods of assay.

A related example of partition distribution is the procedure of Davidow (*10*), who isolated DDT from butterfat by passing a carbon tetrachloride solution of DDT and fat through a column of concentrated sulfuric acid on Celite, thereby eliminating interference from microgram quantities of DDT in 5 grams of butter. In the Schechter *et al.* (*46*) procedure for DDT in milk, chloroform solutions of DDT and butterfat were washed with concentrated sulfuric acid to remove all but a small residue of the fat, which was probably hydrocarbon in nature. Gunther *et al.* (*21*) found that dieldrin in commercial orange waxes will partition favorably into acetonitrile from Skellysolve B in the ratio 1.9 to 1 and into nitromethane from Skellysolve B in the ratio 2.1 to 1, with the orange waxes favoring the hydrocarbon solvent in the ratios 1 to 3 and 1 to 36, respectively.

CHROMATOGRAPHY. A technique related to the above partition distribution is chromatography, which achieves isolation of a compound by means of its selective sorption on an inert solid material. This procedure has been generally used for removing interfering substrate material such as the highly adsorptive plant pigments. In the Averell and Norris (*2*) method for parathion, for example, possible interfering colored materials are removed from the original extract solution by filtration through Attapulgus clay. Koenig *et al.* (*35*) have successfully used the chromatographic technique for removing interfering substances from various substrate extracts in preparing dieldrin samples for infrared analysis. Norton and

Schmalzriedt (*43*) used alumina to clean up alfalfa extracts for DDT determinations.

There are no published procedures for using paper chromatography in insect residue determinations. However, Metcalf and March (*42*) have recently obtained excellent separations of various phosphate and thionophosphate esters by reversed phase paper chromatography using silicon-impregnated paper and a mixture of ethyl alcohol–chloroform–water as solvent. Using essentially this technique and S^{35}-labeled compound 1059 (*O,O*-diethyl-*O*-β-thioethyl ethyl thionophosphate) these workers (*41*) have been able to establish residue levels of this systemic insecticide and of its metabolites in bean leaves. Successful use of this paper technique requires that the total residue of insecticide and substrate extractives be present in less than milligram quantities, and that there be some method of locating the position of the insecticide on the chromatogram. There conceivably is a definite utility for this technique and its many modifications in conjunction with one or more of the other isolation techniques for establishing natures and magnitudes of pesticide residues.

CRYSTALLIZATION. The fact that a pesticide is present in only minor quantities in a substrate extract encourages the use of crystallization techniques for reducing the proportion of the substrate extractives while the pesticide remains in solution. Fairing and Warrington (*15*) have recently described such a procedure for allowing fats to crystallize from acetone, leaving the ethylene derivative of methoxychlor [1,1,1 - trichloro - 2,2 - bis(*p* - methoxyphenyl) ethane] in solution and thus reducing interference to below 3% transmission. An interesting modification was utilized by Gunther and Miller (*24*) for estimating DDT in avocados. Benzene extracts of avocado pulp were cooled to 0° C. to allow the benzene to crystallize from a benzene–avocado oil mixture, the DDT preferentially remaining with the benzene. The liquid avocado oil was then filtered from the mush of benzene crystals and retreated with fresh benzene. This procedure resulted in the nearly quantitative transfer of the DDT into an essentially oil-free benzene solution suitable for analytical examination.

Chemical Means. OXIDATION. Many solvent-extractable compounds occurring in plant parts are readily oxidizable to alkali-soluble products. Hence, a complex substrate extract may be converted by oxidation to relatively few types of unreactive, colorless compounds less likely to interfere with the subsequent measurement of the pesticide, or more easily separated from the pesticide than were the many components of the original mixture. The pesticide, of course, must be resistant to oxidation or else oxidizable reproducibly and in good yield to readily identifiable fragments. This technique has been applied by Kolbezen *et al.* (*36*), who used potassium permanganate in acetone solution to oxidize commercial oil of orange containing dieldrin. A 99% enrichment of dieldrin in oil of orange was achieved. Other stronger or weaker oxidizing agents might be used according to the natures of the insecticide and the substrate. Excellent cleanup can be obtained if the insecticide contains a phosphorus atom oxidizable to inorganic phosphate. Thus, Metcalf and March (*40*) have successfully oxidized orange extracts containing P^{32}-labeled OMPA and its metabolites with nitric acid, and measured the resulting phosphate.

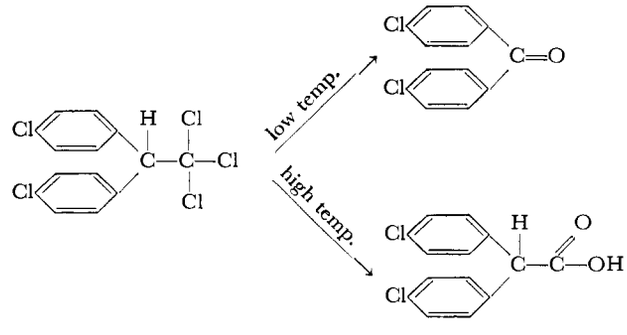
SAPONIFICATION. Plant parts nearly always contain large amounts of solvent-extractable alkali-unstable compounds which can be saponified and thus separated from many pesticide residues. This hydrolysis of the substrate extractives is possible only in the presence of alkali-stable pesticides.

Such drastic hydrolytic conditions were used by Perry *et al.* (*44*) to determine aldrin in the presence of extractives from peanuts and from corn. The fats were saponified with alcoholic potassium hydroxide, and the unchanged aldrin was extracted into hexane; when combined with chromatographic treatment to remove unsaponified materials, the final aldrin-containing residue from 200 grams of plant part weighed less than 100 mg.

Biological Means. HYDROLYSIS. Hydrolysis can be utilized as a cleanup technique in the presence of many alkali-unstable pesticides as well. An ingenious variation of this technique was proposed by Clifford (*8*), who used the lipase from hog pancreas to hydrolyze fat interfering with DDT determinations; he was able to reduce 5 grams of butterfat to less than 100 mg. in a reaction buffered to pH 8. Papain has been used (*23*) to remove interfering proteinaceous substances from aqueous solutions of the hydrolysis products from compound K-6451.

Reaction Separation In the reaction separation techniques, the chemical structure of a pesticide is altered to aid its isolation through the use of some functional group of the pesticide that is not common to the plant extractives involved. This approach will be most valuable when the only possible source of the desired reaction product is the parent pesticide. If this product is one of the natural, normal *in situ* degradation products of the pesticide, or if it can result from this reaction with such degradation or other metabolic products, the procedure will not be selective. Even under these circumstances, however, these procedures may prove of value in estimating the maximum possible hazard from a pesticide residue. This reaction separation approach is the one most widely encountered in the field of pesticide residues, usually with supplementary cleanup procedures. The following examples of the basic procedures will include the isolative steps for the desired product, even though they are similar to those in the preceding section.

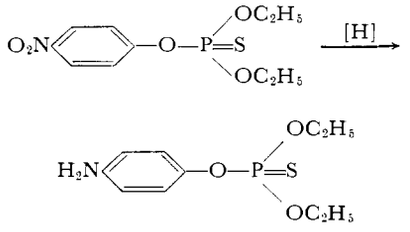
Specific Chemical Means. OXIDATION. Oxidative techniques offer much promise for cleanup purposes. Some pesticides under carefully controlled oxidizing conditions will yield acidic or ketonic fragments that can be readily separated from the bulk of the oxidized plant extractives. For example, under strongly alkaline oxidizing conditions *p,p'*-DDT would be converted into 4,4'-dichlorobenzophenone at low temperatures, or to 4,4'-dichlorodiphenylacetic acid at higher temperatures (*13*, *53*).



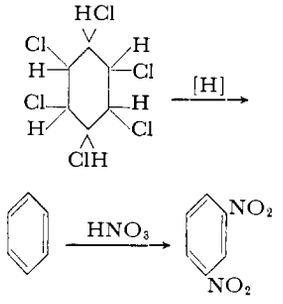
Claborn and Patterson (*7*) suggested this scheme for residue determinations as a two-step degradation, with the DDT-ethylene as an intermediate, and conversion of the final ketone to the 2,4-dinitrophenylhydrazone.

REDUCTION. Subjecting a pesticide to reducing conditions may likewise alter its physical or chemical properties to facilitate isolation from the substrate extractives. Illustrative of the potentialities of this procedure is the method

of Averell and Norris (*2*) for parathion. They reduce an aromatic nitro to an amino group, thus changing a water-insoluble compound to one soluble in dilute acids, and which can therefore be freed from all water-insoluble materials.



The procedure of Schechter and Hornstein (*47*) for benzene hexachloride illustrates a different type of reduction for isolation purposes. They reduce benzene hexachloride to benzene with zinc and acetic acid, and free the resulting benzene carefully from extraneous materials in a special apparatus. Additional specificity is provided by nitrating the benzene for subsequent colorimetric determination.



HYDROLYSIS. As with reduction, hydrolysis may change inert compounds to reactive ones or result in products with physical properties more favorable for isolation.

An excellent example utilizing both acidic and basic hydrolysis for isolative purposes is provided by the acaricide Aramite [2-(*p-tert*-butylphenoxy) isopropyl-2-chloroethyl sulfite]. In the procedure of Gunther *et al.* (*22*), the concentrated residue extract containing Aramite is hydrolyzed with alkali to liberate ethylene oxide which, in turn, is swept from the reaction mixture and absorbed in cold diethylene glycol for subsequent colorimetric determination. Aramite residues can also be determined (*25*) through acid hydrolysis to release sulfur dioxide, which is then isolated and determined.

In the procedure devised by Kutschinski and Luce (*38*) for the acaricidal compound K-6451, the concentrated residues are hydrolyzed under alkaline conditions to sodium *p*-chlorophenate, which can then be freed from aqueous insoluble material by extraction, filtration, or, most neatly, by steam distillation of volatile material from the aqueous alkaline solution, followed by acidification and steam distillation of the *p*-chlorophenol (*23*) for colorimetric determination.

DEHYDROHALOGENATION. Because many organic pesticides possess labile chlorine groups, simple dehydrochlorination will liberate easily determinable chloride ions. The procedure for DDT residues proposed by Gunther (*20*) illustrates the utility of this approach. He dehydrochlorinated with alcoholic potassium hydroxide to liberate essentially one chloride ion per DDT molecule. An interesting modification (*3*) involves the use of a dehydrochlorinating solution of ammonia gas in methanol to avoid the production of sulfide ions when DDT and sulfur are used in admixture.

The method of Fairing and Warrington (*15*) for methoxychlor employs dehydrochlorination to obtain the ethylene derivative, which is isolated by a two-stage selective solution process. The ethylene derivative is then determined colorimetrically.

Specific Biological Means. This technique has hardly been exploited, yet it shows considerable promise. For example, Metcalf and March (*40*, *42*) have been able to demonstrate the conversion of parathion to paraoxon by incubation with insect tissue or with sliced mouse livers. Kearns (*33*) has recently extracted in crude form an enzyme from DDT-resistant houseflies which will dehydrochlorinate DDT in the presence of glutathione. Applications of both these developments to residue studies are indicated.

Nonspecific Chemical Means. COMBUSTION. Organic chlorine compounds do not commonly occur in plants. Plant extracts containing pesticides with chlorine components may therefore be subjected to combustion and the released chloride ions precisely determined. While this process minimizes the usual total-chlorine interferences introduced by the substrate, the parent pesticide and all its degradation or other metabolic products containing chlorine are also determined. As stated previously, this does not preclude the usefulness of this technique. A detailed combustion procedure for precisely determining chlorine in organic compounds has been described by Peters *et al.* (*45*) and adapted for chlorine in insecticide residues by Agazzi *et al.* (*1*). The combustion is accomplished either in a horizontal tube or in a wick-type lamp, the choice depending largely upon the burning characteristics of the extractives, and has proved successful for aldrin, Aramite (*21*), DDT (*21*), dieldrin, compound K-6451 (*21*), and compound R-242 (*p*-chlorophenyl phenyl sulfone) (*21*) residue analyses. Krauze *et al.* (*37*) had previously used a microcombustion technique for determining gammexane (lindane) residues in wheat flour.

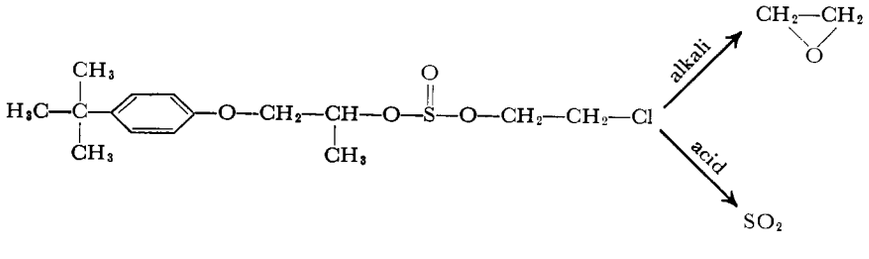
Final ionic chloride may be determined in a number of ways, including amperometric (*1*, *45*), colorimetric (*18*), nephelometric (*37*), and potentiometric (*14*) analyses, although the amperometric technique is most frequently employed.

The very high sensitivity of this combustion total chlorine method implies unusually careful and thorough cleanup. Full characterization of the interfering substances occasionally encountered in check samples has not been proposed.

OXIDATION. An interesting application of microdiffusion techniques to the isolation of chloride liberated from chlorinated insecticides has been made by Gordon (*18*). Chlorine is liberated from the biological extractives by direct permanganate oxidation.

REDUCTION. Organically bound halogen may also be determined by reduction, as in the numerous modifications of the sodium–isopropyl alcohol total-chlorine method (*54*). Such methods may involve simple alkaline hydrolysis as well, although they are usually classed as reductive operations. In general, these methods involve such large quantities of inorganic salts that decreased sensitivity results from the ensuing chloride contamination.

Nonspecific Biological Means. The well known action of parathion and EPN upon cholinesterase to form biologically inactive complexes illustrates this type of technique. In addition, Jansen and coworkers (*27–30*) have studied the inhibition and complex formation of a number of enzymes, including the acetylesterases and the chymotrypsins, by several of the insecticidal phosphate esters. Applications



of these investigations to residue studies could be made via these complexes.

Conclusions

A thorough study of the chemical and physical properties of a pesticide is the first consideration in devising an analytical method for its determination on and in plant parts or in soils. An analytically useful definitive property should either allow measurement of the pesticide in the presence of these substrate extractives, or permit its isolation from them either as the unchanged pesticide or as an altered product thereof that is obtainable only from the parent substance. Physical, chemical, and biological means are available for measuring the amount of pesticide in the residue by isolation (selective techniques) or after isolation (nonselective techniques). The applications of these techniques to a new problem are as yet largely empirical maneuvers, but there is promise of systematization and standardization of efforts in such new applications.

Acknowledgment

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