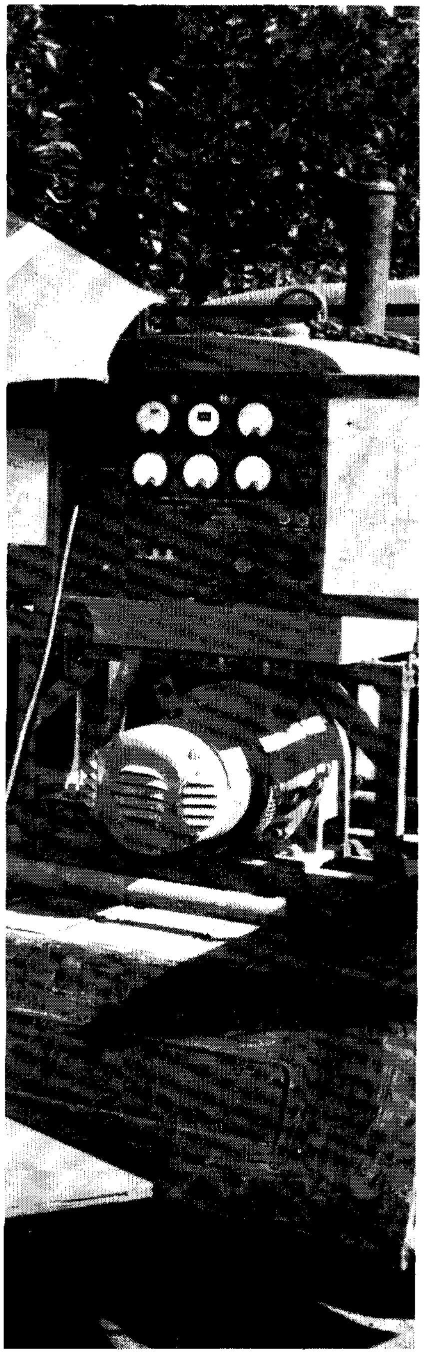

Instruments in

THE PESTICIDES FIELD

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MUCH OF AGRICULTURE'S PROGRESS in production during the past 20 years is attributable to mechanization, improved crops and livestock, efficient marketing organizations, conservation, more efficient use of soil and water resources, and the intensive efforts of the agricultural chemicals industry. Thus, it is generally recognized that a given amount of land is capable of producing more crops of higher quality than was possible prior to World War II. Types of chemicals that have helped this improvement in production include antibiotics, defoliants, dehydrants for hay, fertilizers, fumigants, fungicides, growth regulators, insecticides and acaricides, packaging materials, processing and preserving chemicals, rodenticides, seed disinfectants, soil conditioners and soil fumigants, washing and cleaning chemicals, and weed killing chemicals.

Progress in research and in successful application of this impressive array of chemicals in agriculture has largely been made possible through the use of a wide variety of precision scientific instruments. With pesticides, for example, the intelligent and generous use of precision instruments has permitted many initial understandings of the modes of action of these chemicals in living organisms, the relations of their chemical structure to their toxicity, their metabolic pathways, and their widespread use on a variety of crops against a variety of pests in a manner acceptable and nonhazardous to the consumer.

These four areas of practical investigation of nearly all pesticides are important and interdependent and relate to the current strong interest in persisting residues of pesticides on and in foodstuffs as expressed by the enactment of the Miller Amendment (Public Law 518) in 1955. Thus, the significance of any residue evaluation rests ultimately upon the identity of the total residue in its relationships with *in situ* metabolic pathways as the residue is degraded, with structure-toxicity correlations, and with modes of action both against insects and against warm-blooded animals. Integration of research activities in these areas with systematic residue evaluations is necessary to achieve pesticides truly and reliably nonhazardous to the consumer.

Precise, detailed evaluations such as these require adequate research and routine instrumentation. Modern precision electronic instruments are characterized by ease of manipulation, high initial cost but rapid amortization, reproducibility and standardization, rugged construction, and versatility in that many of them are capable of identifying as well as measuring. Thus, in this as in most other fields, instrumentation is largely analytical, and probably the most important task facing the agricultural chemist today is the development of methods of analysis for the problem generally summarized by the phrase "chemicals in foods."

Basic Instruments

Basic electronic instruments in use in a great many pesticides laboratories in routine but important fashions include colorimeters, flame photometers, manually operated spectrophotometers, standard pH meters, and automatic titration equipment. Several of these instruments may serve dual functions—for example a potentiometric titrator doubling as a pH meter or a spectrophotometer in use as a simple colorimeter.

In addition to what may be considered as the routine and normal uses of these basic instruments, numerous special applications are possible. One such application is the use of a pH meter in the estimation of residues of organophosphorous insecticides. These insecticides act by inhibiting the enzyme cholinesterase and this property is used as the basis for the analytical method. The determination of cholinesterase activity depends upon the measurement of the acetic acid produced by the action of cholinesterase on acetylcholine and this may be conveniently measured with a pH meter in terms of change in pH in a standard buffer solution over a definite time interval. A comparison of the inhibition produced by extractives from treated plant materials with that produced by standard amounts of the organophosphorus insecticide involved is the basis for the enzymatic-inhibition method of analysis.

Colorimeters and Spectrophotometers

Both colorimeters and spectrophotometers are essential in the modern pesticides laboratory. The former are used for rapid, sensitive, and uncomplicated routine analyses, after the adequacy of the analytical method has been established with a precision spectrophotometer. For example, the spectrophotometric identity and homogeneity of standard as well as occasional routine experimental samples should always be established, and in-

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terferences from accessory substances in the residue-containing extractives should be evaluated and subsequently circumvented with spectrophotometric tests.

As an analytical tool the ultraviolet spectrophotometer has not been used to the full extent of its capabilities; ultraviolet analysis has the same potentiality that colorimetry has. Any program concerned with the fate of pesticide molecules should include both ultraviolet and infrared comparisons as a matter of course. Visible, ultraviolet, and infrared techniques have been successfully and repeatedly used in our laboratories, in both qualitative and quantitative applications.

Flame photometers have found little application with insecticide problems other than in plant nutrition imbalances sometimes ascribed to applications of insecticides. Residues of some of the metal-containing fungicides and herbicides, however, can be evaluated with these instruments.

Standard Instruments

The more intricate and complex electronic instruments developed essentially within the past two decades are extremely valuable in the hands of the person who adequately understands their limitations as well as their potentialities. In general and in contrast to the simpler basic instruments, these standard units are not used routinely for long periods but rather are used in combinations in specific research-type problems where a given instrument may afford only a portion of the desired data. Intimate familiarity with instrumental research techniques and equipment is, therefore, essential to the efficient, realistic solution of most problems in the pesticides field. Thus, a given problem may appear solvable from several instrumental approaches; the decision must then be made as to which instrument or combination of instruments and ancillary techniques will afford data of unquestioned reliability and value for the problem at hand.

Instruments of the sort in question include the various recording spectrophotometers, nucleonic equipment, scanners and integrators for paper and other types of chromatograms, electrochromatographic devices, and recording polarographs. Many of these instruments are available as manually operated models, but the time-saving feature of accurate automatic recording permits study of otherwise impractical or impossible sequences of complicated operations.

Recording spectrophotometers are available for the visible and ordinary ultraviolet regions of the spectrum from about 1000 mμ down to about 210 mμ—at least two instruments are functionally capable of attaining about 190 mμ. Most of these instruments are adequately described and explained in the literature available from the manufacturers.

The Beckman model DR recording attachment for the standard model DU spectrophotometer permits the simultaneous, recorded, spectral scanning of three samples and a reference sample, with automatic reference correction. It has been useful in some reaction rate studies and in preparing multiple-concentration curves for pesticide analytical standards in both the visible and the ultraviolet regions.

There are in the literature abundant examples of the use of ultraviolet and infrared spectrophotometric methods for the identification and quantitative determination of currently interesting insecticides and acaricides. A recent infrared study permitted the simultaneous identification and quantitative determination of residues of the acaricide *p*-chlorobenzyl *p*-chlorophenyl sulfide degrading to its sulfone on and in treated pear fruits.

Nucleonic Instruments

Radioactive-tracer techniques are just beginning to be exploited in pesticide research to their full potential. They are particularly useful where other methods are not available, where interfering substances or lack of sensitivity prevent the assured application of either chemical or other physical methods, and in elucidating the dynamic metabolic and physical changes in pesticide residues in plants, animals, and soils.

Nucleonic detection devices are many and varied. They include ionization chambers, proportional, Geiger-Müller, and scintillation counters, and associated scalers or rate meters. The nature and complexity of the measuring devices necessary for use in obtaining adequate and reproducible measurements will be governed by the type, physical state, and activity of the sample and the characteristics of the particular radioactive atom to be measured. The very minimum equipment necessary consists of a Geiger tube, tube and sample holder, and scaler or rate meter. Improvements in instrumentation in this field have been rapid and continuing, such as thin-window and windowless Geiger-Müller counters, automatic sample-changing and recording equipment, and especially more effective proportional and scintillation counters.

Electrochromatographic Devices

The technique of paper chromatography, now an almost universally used tool in separating the components of complex mixtures, is widely and well understood. The closely allied technique of electrochromatography by which charged molecules may be separated on a stabilizing medium, usually paper, by the application of an electric potential has been receiving increasing attention. Its routine application is more specific, and its requirements are more rigorous than those necessary for paper chromatography, however. Based on its usefulness as an analytical, clinical, and chemical tool, numerous commercial electrochromatographic models have become widely available in the past few years. These consist of a regulated power supply, a variety of devices for supporting the moist stabilizing medium including water cooling mechanisms in some, and graphite or other electrodes for contact with the stabilizing medium or electrolyte solution.

Apparatus for the continuous resolution of mixtures by the combination of electrical migration with transverse flow of solution has even more recently become available as well. As yet electrochromatography has been little exploited in pesticide research although it has recently been used to separate the charged insect and animal metabolites of malathion. It is certain to be adopted for many uses as research workers become familiar with its requirements and possibilities.

Chromatographic Detection Devices

Considerable attention has been and is being devoted to detecting and estimating substances separated by paper chromatography or electrochromatography. Although much progress has been made in this regard the extension of isolation-identification to absolute determinations has not been fully realized. Detection of radioactively labeled compounds by direct scanning of chromatograms has been widely used and there are a number of commercial instruments available for the automatic scanning and recording of activities on such chromatograms. Instruments which depend for detection upon physical properties of the separated compounds such as fluorescence, opacity, phosphorescence, and spectral absorption are increasing in availability. These vary from relatively simple, manually operated densitometers to complex instruments which automatically scan the chromatogram, plot the trace of absorbance, and integrate the areas under the trace for quantitative evaluation. Attachments for spectrophotometers for both visible and ultraviolet scanning of chromatograms are on the market as well. In our laboratories the preparation of a radioautograph of a radiotracer-labeled chromatogram and its subsequent evaluation for relative concentrations of the separated components with a densitometer has been especially useful.

DDT, γ-BHC, dialkyldithiophosphates, malathion, parathion, and potosan have all been determined by the technique of polarography, but not as residues.

Special Instruments

Commercially available electronic devices have been rebuilt or otherwise significantly modified for a specific function in the pesticides field. Some of these are described below.

- Most insecticides containing organically bound chlorine can be conveniently and quantitatively determined by conversion to inorganic chloride; the latter can be determined in a variety of ways, but recently a direct determination of anions such as chloride has been developed. It is based upon the general Nernst equation and utilizes sensitized electrodes.
- A new, practical fungicide for certain applications to citrus fruits in cartons is gaseous ammonia. To assist in the establishment of tolerance classification under Public Law 518 it was necessary to determine how much ammonia was sorbed by the fruits. Previously existing analytical methods were not applicable, so a direct spectrophotometric method was developed, utilizing an absorption maximum of ammonia at 204.3 mμ (A_s, 3127) which allows determinations from 7 p.p.m. to 1000 p.p.m. in air at atmospheric pressure. Both the manual Beckman DUS and a modified Beckman DK-2 were used in this problem. The former was incorporated into an assembly to measure sorption of ammonia by fiberboard, and utilized an all-glass flexible cell arrangement to permit shifting either cell into the energy beam without interrupting gas flow through the sample cell. The DK-2 instrument was modified to incorporate a 17-hour time drive and was continuously purged with dry nitrogen, and operated only at 204.3 mμ. With both instrumental assemblies it was possible to determine ammonia concentrations in air streams before and after exposure to the sorbing materials. Differences therefore represented the amounts sorbed, on a time-concentration basis.

Precision pH meter doubles as a millivolt meter for chloride ion determination

- By nature and construction most analytical instruments are laboratory shut-ins. One ultraviolet spectrophotometer, however, was needed for on-the-spot investigations of air contamination in orchards. The prevailing opinion had been that parathion evaporates into the air after application and thus contaminates the environment. Use of the above equipment demonstrated that parathion vapors were not detectable in the air but that small amounts in suspended particulate matter were present.

Instrumentation and a Complex Problem

Most problems which confront the present day research worker in the pesticides field would have been virtually unsolvable a decade ago. Their solution has been made possible principally by the extensive use of modern instruments and instrumental techniques. Such a problem is one involving the systemic insecticide, demeton, and its toxicology and biochemical transformations in plants and animals in relation to the determination and significance of residues in edible plant materials.

Commercial demeton is a mixture of two isomers *O,O*-diethyl *O*-ethyl-2-mercaptoethyl phosphorothionate (thiono isomer) and *O,O*-diethyl *S*-ethyl-2-mercaptoethyl phosphorothiolate (thiol isomer). It is active insecticidally by contact and systemically, being absorbed and translocated throughout the plant. The systemic action of demeton, however, is not principally due to the action of the unchanged isomers but to their metabolites which are toxic to both insects and animals.

Since chemical, biological, and physical techniques for the definitive analysis of demeton were inadequate, phosphorus-32–labeled thiono and thiol isomers were prepared. In addition seven probable oxidation products of the thiono and thiol isomers were synthesized and their properties compared with the metabolic products of the demeton isomers isolated from plant and animal tissues. An important aspect of this study was the verification of structure and initial purity of these compounds by comparative infrared spectrophotometric and paper chromatographic techniques.

By bringing all of the available techniques of radioassay, paper chromatography, enzyme inhibition, radioautography, densitometry, and spectrophotometry to bear on this problem it was possible to determine the metabolic pathways of the demeton isomers in plants and animals. The principal metabolic pathway of metabolism to toxic metabolites for both isomers is the oxidation of the mercaptosulfur moiety to the sulfoxide and sulfone. A secondary but minor pathway for the thiono isomer may involve the oxidation of the thiono sulfur to produce the phosphate and its sulfoxide and sulfone. Both isomers and their toxic metabolites are degraded by hydrolysis of the P-O- or P-S- bond to form the alcohol and acid. The final proof for this metabolic pathway was the identical comparison of infrared spectra of the synthetic compounds with the metabolites isolated from the plant.

It was then possible to assess the effects of these toxicological and biochemical studies upon the estimation and significance of demeton residues in edible plant materials. The metabolism of the thiono and thiol isomers has been shown to take place in the same fashion as described above. Within one or two weeks after application only oxidative metabolites and hydrolyzed degradation products could be found in orange, apple, and walnut tissues. The thiolphosphate sulfoxide and sulfone are the principal toxic plant metabolites resulting from the pesticidal application of demeton since the thiol isomer and its metabolites have been shown to accumulate from five to 10 times as rapidly as the products from equal rates of treatment with the thiono isomer. The presence of toxic oxidative metabolites should not provide any additional concern in assessing residue hazards resulting from demeton applications since the process of metabolism in animals is identical with that in plants. The animals are therefore exposed to the same toxic processes as the plant, regardless of whether the isomers are ingested in their original or metabolized forms. The hydrolytic degradation of the isomers and toxic metabolites to nontoxic phosphoric acid derivatives is a further safeguard. The estimation of the maximum amounts of toxic demeton metabolites which may be present in plant materials treated with standard commercial dosages of demeton was also reassuring. Average residue values at two and four weeks after application were substantially below 0.1 p.p.m. and generally in the range of 0.01 to 0.03 p.p.m. for orange juice, apple peel and pulp, walnut meat, whole pear, and potato flesh.

These residues are of such low magnitude and their chemistry so complex that they could not have been determined by other than tracer techniques and ancillary instrumentation.

Forefronts of Research and Application

Further developments and applications of these electronic instruments are certainly to be anticipated in this dynamic field. Applications of other presently available electronic instruments are also to be anticipated. For example, vapor fractometry or gas-liquid chromatography will have immediately applications in diffusion studies with fumigants, as in grain storage. Because of limitations in sensitivity of standard thermal-conductivity detectors, microapplications of this technique may conveniently require direct coupling of the chromatographic apparatus with a mass spectrometer or with infrared or ultraviolet spectrophotometers for maximum utility in laboratory scale evaluations of pesticide problems. Nuclear magnetic reasonance spectrometers and Raman spectrometers are still around the corner so far as applications in this field are concerned, although both will undoubtedly be used in the many pressing problems at hand.

As mentioned earlier, the availability now of two spectrophotometers capable of attaining about 190 mμ opens many new analytical possibilities. Theoretically, the region from 185 to 210 mμ is especially interesting in that strong absorption from simple and unconjugated double bonds, as well as from the free bonding electrons occurring in many organic compounds containing oxygen, nitrogen, and sulfur, frequently exists here. This region may find most applications with gases, as solvents adequately transparent from 185 to about 205 mμ are scarce. Water is essentially transparent in this region, however.

It is abundantly clear that the complexity and diversity of the chemical and physical problems currently associated with pesticides research and application cannot possibly be solved without precision instrumentation. The advances in this field during the past decade, as illustrated here, can only portend confidence in significantly greater advances in the future.

A bibliography prepared in connection with this article is available from the authors.

Instruments in SOILS AND WATERS

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PRECISION INSTRUMENTS are making it possible to measure the character of soil and to tailor the needed fertilizer and amendments accordingly. Likewise, water supplies for irrigation can be analyzed quickly by instruments, and proper choice can thus be made of suitable water for irrigation. The soil chemist, with the aid of precision instruments, can make diagnosis of soils and waters for a given crop.

The soil chemist's instruments vary from a simple glass electrode pH meter, conductance potentiometer, and photoelectric colorimeter, through emission spectrophotometers of various kinds, to a radiochemical analysis counter, an x-ray diffractometer, and the electron microscope.

The soil chemist employs precision instruments because they are timesavers; they give more precise information; and they give some specific information not available by any other means.

As a result of the time-saving feature, precision instruments are being increasingly adopted for routine laboratory use. Savings in wages quickly repay the first cost of the instruments and increased precision usually comes along as an extra dividend. For example, the increased objectivity of the modern photoelectric colorimeter is highly worth while aside from mere time-saving.

The instruments that give new specific information are mainly used in more advanced soil chemistry re-