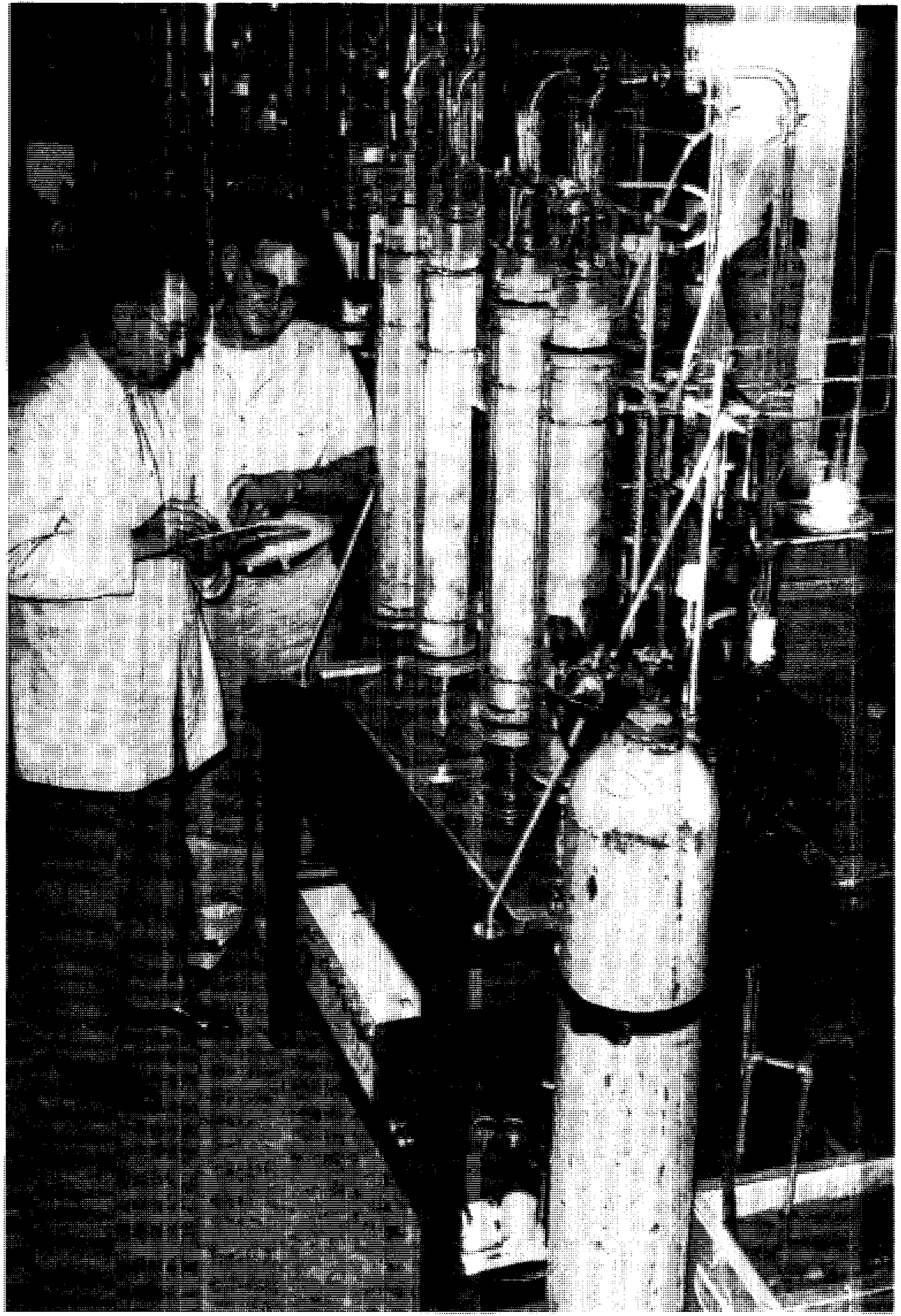

The author (right) believes the major research task in assessing the practicability of any pesticide is a complex and challenging analytical one

Status of Analytical Methods for Residues

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It is not reasonable to expect simple chemistry, equipment, and techniques will give data adequate for appraising all hazards that might exist from pesticide residues

THERE CAN BE NO QUESTION about the urgent need for a large variety of pesticide chemicals to assure crop production adequate for the increasing demands of man and his animals. Even with modern protective measures, it has been frequently estimated that insect damage to crops alone amounts to an annual loss of $4 billion in this country.

Among the closely related problems arising from the now intensive use of agricultural chemicals are possible interferences with biotic potential, the chance of soil sterilization, adverse ecological factors, and persisting residues and their effects. These particular problems are not new, nor are they localized in any one country or area, but the proper attachment of significance to the problem of pesticide residues persisting to the consumer of crops and of foods has been developed during the past decade. Indirect effects of persisting residues could be considered to include the production or induction of off-flavors and of adverse plant physiological responses; a direct effect, of concern to all, is the possible introduction into the foodstuff or feed of resident chemicals toxic to the consumer.

Most contemporary pesticide chemicals seem to possess the common constitutive property of ready penetration into plant parts. Indeed, systemically acting pesticides are deliberately designed for ready ingress into plants. As summarized by Metcalf (5), the greatest barrier to the widespread acceptance and usage of systemic pesticides on food crops is probably the fear of excessive contamination of food products with poisonous residues. With insecticides, however, he points out that harvest-time residues of systemic compounds have been of no greater magnitude than those from conventional materials, all of which have some degree of local systemic action.

It is obvious that penetrated pesticides are not amenable to easy removal from plant parts. Deposits and residues of pesticides in or even on plant parts may be physically displaced or chemically altered, at least in part, by both environmental and controlled mediation. At present, however, the major nonbiological research task involved in the assessment of the practicability of the large-scale use of any pesticide is analytical, in that magnitudes and locales of persisting residues must be established for each of these chemicals on and in each crop or food. As data accrue, this empirical approach may dictate establishment of the natures of these residues as well. Thus, the evaluation of the significance of any residue rests ultimately upon the identity (7) 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 the pest involved and against warm-blooded animals (*3*). Integration of research activities in these areas with systematic residue evaluations is obviously necessary to attain pesticides that are—whether in their original or metabolized forms (*3*)—truly and reliably nonhazardous to the consumer.

Important analytical problems such as these are complex and therefore challenging. The fact that ultimately all of them also demand the skillful use of chemical as well as instrumental micromethodology increases this challenge to the art and to the ingenuity of the residue analyst. The modern residue analyst can and should employ every useful tool and device known to biology, to chemistry, and to physics for assistance in isolating, in measuring, and in identifying these persisting or degrading residues located usually *within* a plant part contacted by the parent pesticide or intermediate metabolite.

The value of this pragmatic approach to the problem of pesticide residues in crops and in foods is manifest from the recent literature on residue methods involving biochemistry, chromatography, electrode reactions, radiochemistry, selective organic reactions, spectrometry, and both direct and indirect biological assay. At present there are no broadly applicable residue techniques that will isolate, measure, and identify in one or even in several standardized operations. Combinations of techniques can be adequately standardized for a specific problem, however, if the analyst is sufficiently familiar with the tools and devices available. But it is not reasonable to expect simple chemistry, equipment, and techniques to afford residue data adequate for the necessary appraisal of any hazard that might exist from persisting pesticide residues.

Any attempt to summarize and reevaluate the residue problem in terms of recent developments in the field must recognize four implicit facets: the residue, the necessity for residues, the factors involved in deposition, and the practical factors in persistence. The analytical chemist is common to all these facets, but is most directly concerned with the actual analytical methods and closely associated analytical responsibilities.

The Residue Program

According to the Miller Pesticides Amendment, petitions for establishing tolerances for pesticide chemicals shall contain data showing, among other things, "the results of tests on the amount of residue remaining, including a description of the analytical methods used." This law does not specify whether the analytical method shall be simple or complex, manual or instrumental, but obviously it must be adequately sensitive and reproducible to justify the tolerance under petition.

These analytical methods are scrutinized and judged by competent chemists both in the Food and Drug Administration and in the Pesticides Regulation Section of the USDA's Agricultural Research Service, often in consultation with one another. Evaluations and opinions as to the adequacy of these methods for the particular problems under petition are therefore matters of considered judgment by the responsible chemists in these two organizations. A petitioner's objections to unfavorable consideration of a particular method of analysis, *per se* or *in toto* in its application to a specific pesticide on a specific crop, thereby become matters for debate between petitioner and representatives of the government agencies. Apparently there has not yet been a situation of this sort where legal recourse has been required. Borderline cases have been considered, however, and arbitration has at times undoubtedly been necessary.

To be adequate, pesticide residue programs must therefore be planned carefully and conducted with scientific acumen to anticipate the following analytical questions for Section 408. (*d*) (*1*) (*D*) of the Miller Amendment, under conditions of usage that reasonably reproduce the anticipated commercial conditions of usage:

- What is the residue?
- Where is the residue located in the plant or plant part?
- How much of the residue is present per unit weight of plant or plant part?
- If the terminal residue is not the same chemical species as the initial residue, what is the chemistry of the transformation?
- Will the terminal residue be transferred in significant amounts from the crop to a domestic animal and thence to marketed animal products, with or without secondary metabolic alterations?

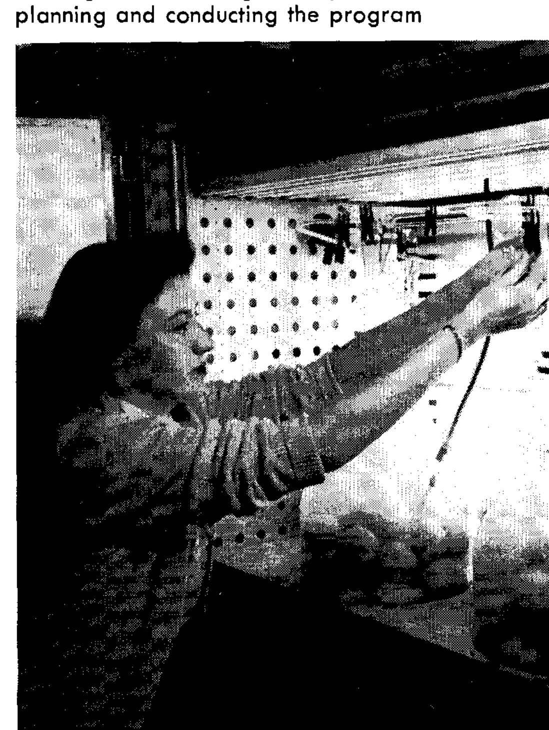
Obviously, the answers to all five questions must ultimately be mediated by a toxicologist or pharmacologist supplied with the factual background requisite for intelligent conclusions. It has not always been so obvious, however, that the accrual of reliable analytical information of this sort absolutely requires the intelligent utilization of the training and knowledge of a competent analytical chemist in every phase of the planning and conduction of the residue program. These phases include:

Presampling. As Lykken (*4*) has repeatedly emphasized, diminutive plots, insufficient replicates, disregard of uniform application procedures, and lack of attention and understanding with regard to sampling techniques can give analytical data with no value to the investigator and, more important, can frequently give rise to misleading and erroneous conclusions. Residue data should not be accrued to supplement entomological performance records; rather, residue data should be the primary objective of the experimental design in question (*1*).

Sampling. Samples (*1*) selected for analytical investigation must be truly representative; yet in many residue experiments sampling is performed with little or no regard for acceptable sampling practices. Indeed, there is often little thought as to whether the sample should be collected to define limits (implicit range) or to represent the arithmetic mean (average); rarely, even, is the residue sampling technique evaluated as to reliability or variability. Though this establishment of reliability may occasionally be impracticable or unwarranted, a large number of samples *will* provide an average value approaching the true value as a limit.

Sample Processing. By sample processing (*1*) is meant the transfer

Getting reliable residue information requires intelligent use of the analyst's training and knowledge in all phases of planning and conducting the program



The residue analyst can and should employ every useful tool and device known to biology, physics, and chemistry for help in isolating, measuring, and identifying residues

of the pesticide by mechanical or physical means from the plant part into a suitable solvent. This process usually represents a concentration operation and always results in some purification since those plant parts and constituents insoluble in the solvent are eliminated as residue diluents; frequently, these insolubles represent the bulk of the sample, such as the cellulosic components. For practical purposes, this transfer or equilibrative extraction of the pesticide from the plant part to the solvent need not be quantitative, but it must be reproducible within about 5%. The efficiency of this processing operation must be established for each pesticide material, for each solvent, and for each substrate.

Cleanup. The most difficult and exacting phases of the analytical investigations of pesticide residues are encountered in attempts to eliminate the interfering substances present in the solutions obtained from the processing operations. This preparative treatment or cleanup (*1*) includes all operations designed to yield the desired analytical constituent in a form suitable for measurement by a particular method or tool.

An efficient pesticide may occur on or in the substrate in quantities less than 1 p.p.m., or about 0.5 mg./per pound. During the stripping operation there may be obtained simultaneously as much as 200 g. of extractives per pound of substrate, as with avocados, nut meats, and olives. Despite this formidable contamination of the pesticide there are at least four logical approaches to this cleanup or isolation requisite to the final analytical determination:

- Cleanup required when the pesticide is evaluated by direct measurement. This is selective measurement and calls for only superficial cleanup. Biological methods may be included here.
- Cleanup required when the pesticide can be measured only after all interfering substances have been removed or converted to reaction products that will not interfere.
- Cleanup required when the pesticide can be measured after being converted to a suitable derivative and then isolated.
- Cleanup required when methods of compensation can be used.

Every foodstuff to be considered for pesticide residue studies must be individually investigated and quantitatively evaluated as to performance in the final analytical method.

The Analysis. Analytical methods (*1*) are usually typed according to their discriminatory capabilities, although the ultimate means of measurement is always physical. The distinguishing feature is that the significant interaction is between the pesticide molecule and living matter, energy, or another chemical. The selection of a means of measurement is always influenced by the presence or absence of interfering extractives in the analytical samples as received for the ultimate determination after cleanup, as well as by the sensitivity requirements of the problem.

Most, if not all, organic pesticide chemicals in residue form behave similarly in that they seem to obey certain fundamental laws of physical chemistry (*2*). This situation makes available to the analyst the confirmation of predicted residue behavior and thus permits the ready establishment of a "confidence factor" for the total program from field application to final analytical operation.

Discussion and Conclusions

Quantitative analytical methods have been conveniently classified by Strong (*6*) as physical, electrical, thermal, optical, chemical, or biological, with 20 major subclasses and with 63 specific means of measurement. The residue analyst should be prepared to exploit any combinations of these.

By law, the residue analyst is required to describe his detailed method as used for promulgation of tolerance classification; by implication of law he may have to defend it in its application to a particular problem pertinent to the licensing, registration, and tolerance classification of a particular pesticide chemical applied to a particular crop or food. Contrary to some common beliefs, however, it is not mandatory for the residue analyst to restrict his efforts to the elaboration and utilization of simple, noninstrumental, or nonbiological residue methods. Perpetuation of the idea that analytical problems as delicate and complex as those involved in modern residue investigations can be unravelled with simple equipment and techniques is not to be condoned. A relic of the days of arsenic and lead, this idea is contrary to and incompatible with accepted practice and progress in every field of science.

In broadest terms, the residue analyst should recognize and accept the responsibility of using techniques and procedures that are sufficiently reliable and sufficiently sensitive to assure recognition and acceptance under the Miller Pesticides Amendment, and thus lead eventually to successful commercial applications of a new and useful pesticide chemical. Where the responsibility lies for providing simple—albeit questionable—residue methods for attempted rapid and routine policing of marketed crops and foods could well form the basis for a separate symposium.

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