Appendix B
OTA Workshop Papers

The Development of Analytical Methods for Pesticide Residues

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Contents

Abstract

The U.S. Food and Drug Administration's (FDA) objectives of enforcing tolerances for pesticide residues in foods and feeds and of determining incidence and levels of pesticide residues in the food supply are driving forces in FDA development of residue analytical methods. In turn, such method development is influenced by development of analytical instrumentation, by changing chemical characteristics of new pesticides, and by the constant need to analyze additional food samples for more and varied potential residues.

FDA's chronology of methods development is therefore presented as an example of how the availability of appropriate technology can either advance or hinder development of a needed method, Evolutionary development of five multiresidue methods is discussed, starting with recognition of an analytical need through effects of available instrumentation or determinative systems, development of extraction and cleanup procedures, verification of overall method performance, and extension of such established methods to additional residues and commodities beyond those in the original method study. Reference to interlaboratory validation of each method is also included.

Laws and regulations have affected the limits of determination at which analytical methods must be valid and have dictated coverage for metabolizes as well as parent compounds, Future methods development will continue to be driven substantially by such forces and will include new demands for efficiency in application. The search for improved efficiency will dictate exploration of such new approaches as immunoassays, rapid cleanup techniques, improved instrumentation, and automation. Incorporation of these techniques will depend on the degree to which they prove beneficial in a given laboratory situation,

The Food and Drug Administration (FDA) samples and analyzes a wide variety of raw agricultural food and feed products (hereafter referred to as food) to enforce maximum limits, or tolerances, established for pesticide residues. Commodities sampled do not include meat and poultry, which are the province of the U.S. Department of Agriculture (USDA). Residue levels detected, even though they may be below tolerance levels, are quantified and recorded in a central data base. Results of analysis may provide the basis for regulatory actions or may serve the Environmental Protection Agency (EPA), World Health Organization and other groups that

have an interest in the historical incidence and levels of pesticide residues in food.

Over the past 25 years, the number of samples analyzed annually by FDA has varied between about 7,000 and 30,000. In fiscal year 1987, about 15,000 samples were analyzed. Also during this year, FDA responded to 26 separate requests from EPA and USDA regarding levels and incidence of residues for 95 chemicals (l). Additionally, FDA's Center for Food Safety and Applied Nutrition responded to 28 Freedom of Information Act (FOI) requests for pesticide monitoring data (1). FDA field offices also routinely respond to a number of similar FOI requests each year.

Since the early 1960s, the FDA has also monitored dietary intake of pesticides in a "market basket" of selected food items (incIuding meat and poultry) that are purchased at the retail level and then prepared ready-to-eat prior to analysis. Results from this Total Diet Program provide the only information available in the United States on types and amounts of pesticides that remain in or on food as it is consumed. These results are compared with Acceptable Daily Intakes and serve as a measure from which food safety judgments can be made. The data also provide a means to identify trends and detect isolated contamination sources. In addition to pesticide residue data, the program also provides dietary intake information for radionuclides, toxic elements, essential minerals, and several types of industrial chemical contaminants. Emphasis, however, has been on pesticide residue data. Bennington and Gunderson (2) and Lombardo (3) provide in-depth discussions of the history and significance of this program. Reed et al. (4) discuss the design and purpose of all FDA pesticide monitoring activities. These references should be consulted for more details on FDA program goals.

This paper addresses FDA's analytical methods development history and does not attempt, in the brief space allotted, to review the entire field of pesticide residue methods. Analytical methods used in its regulatory activities include those with the capability to detect and measure several residues in a single analysis as well as those that detect a single residue or a limited number of related residues.

In most cases, single residue methods are supplied to EPA by the registrant of a pesticide during the process of approval for food use. Applicability of such methods need only be demonstrated on food items for which a residue tolerance is set. These methods are published in Volume II of FDA's Pesticide Analytical Manual (PAM) (5). They often involve complex and time-consuming analytical manipulations. Therefore, resources dictate that single residue methods are generally used only when it is known that a pesticide chemical, not recoverable by a multiresidue method, has been used or when information on its potential residue level is needed. In these situations, PAM II methods if applicable are used in selected surveys. A recent example is the use of a PAM II method for daminozide in apples.

On occasion, residue information and regulation are needed for a pesticide for which no adequate method exists, e.g., ethylene dibromide (EDB), Resources are then diverted to developing and validating a single residue method. Overall, single residue methods are employed, out of necessity, to analyze for selected residues by the FDA and other organizations seeking to test for suspected residues in a given food or category of foods. Judicious use of such methods is an important part of the FDA pesticide monitoring program.

Since spray history or environmental background of most samples is unknown, FDA method development efforts have concentrated on multiresidue methods. This work has continued for approximately 30 years and has sought to take advantage of advancements in analytical technologies as they have become available. Consultation of the scientific literature and contact with other researchers has allowed FDA to stay abreast of new approaches to analysis. Continuing interaction among analysts in FDA's 16 field laboratories and in headquarters has led to refinements that have improved reliability and efficiency.

Analytical method studies usually fall into one of the following categories: development of a new method or technique; expansion of an existing method's applicability to additional analytes and sample types; integration of new technology into an existing method; and validation of a method, technique, or modification. This paper provides an overview of the historical development of five multiresidue methods and illustrates how FDA's research in these categories has been applied to evolution of the methodology.

FDA investigators developing residue methods publish their findings in the scientific literature. Multiresidue methods most commonly used by FDA, along with associated supporting information, are compiled in Volume I of the PAM (5). Once analytical methods are developed, published, and proven reliable by a number of laboratories, a more formal process of validation usually occurs. Collaborative study under the auspices of the Association of Official Analytical Chemists (AOAC) is un-

dertaken for selected methods and analyte/food combinations. Successful collaboration results in the method's adoption as "official" and publication in the AOAC's *Official Methods of Analysis* (OMA) (6).

Hill and Corneliussen (7) have published a comprehensive discussion on the needs and considerations related to official pesticide residue method validation. They emphasize that validation of methods and changes in methods area long standing regulatory policy. Aside from being a usual laboratory practice, validation is mandated to ensure that analytical results will withstand scrutiny resulting from public overview and possible legal actions that may occur. By regulation (8, 9), both the OMA and PAM contain official validated methods for regulatory use. However, the degree of validation for inclusion in the OMA is more stringent than that required by the PAM.

Space in this paper does not permit extensive discussion of the application of methods. Two recent publications should be consulted, however, for discussions of why FDA develops analytical methods. Reed et al. (4) describe the goals and strategies of FDA pesticide programs, and McMahan and Burke (10) describe the application of analytical methodology in those programs. Topics critical to the valid application of the methods are covered in the latter; this includes discussions on limits of quantitation and quality assurance in the FDA laboratories.

Muitiresidue Method Development: Background

Analytical methods for pesticide residues generally require a procedure for extracting residue(s), "cleanup" procedures to isolate residues of interest from other components, and techniques to measure residue level and confirm its identity. In a review article by Dewey (l), reference is made to use of pesticide bioassay as early as 1933 (12) for measuring residues of rotenone and its breakdown products. Though this may not be the genesis of pesticide residue analysis, it provided precedence for extensive research and application of bioassay techniques that continued until about 1960. This means of determination was both highly sensitive and multiresidue in scope. It also demonstrated good accuracy and sensitivity if a single pesticide residue were present and its identity known. However, for samples of unknown spray history, it could only be used to indicate whether a toxicant(s) was present. This type of information is of little use for regulatory purposes or for gathering exposure data. Consequently, research activities were initiated to adapt sample extracts to other sensitive determinative techniques that would simultaneously offer qualitative and quantitative information. This need was fulfilled by chromatographic separation followed by a detection step.

Applied research in chromatographic separation and detection of multiple pesticide residues provided the greatest impact in evolution of current methodology. Initial work with paper and thin layer chromatographic (TLC) systems provided semiqualitative and semiquantitative information. Relatively poor chemical separations were achievable with a single chromatographic development and quantitation relied on visual estimations. These procedures were rapidly replaced with gas chromatographic (GC) systems that provided improved qualitative separations and quantitation capabilities with sensitive (and selective) electronic detectors. TLC continued to be used but primarily to confirm the identity of residues first detected by GC.

Ever since GC applications became commonplace, a continual growth has occurred in detector and column technologies. Many advances have been incorporated into FDA multiresidue applications only to be replaced by still newer refinements. It is expected that many of the current GC applications will again by replaced by capillary column technologies that currently provide greatly increased separation capabilities, once the latter are validated or defined to the extent needed for FDA regulatory purposes.

Multiresidue methods generally include single or multiple steps to extract, purify (clean up and concentrate) and detect several potential residues simultaneously. During the early developmental stage, each individual step is tested to illustrate and document its applicability and limitations. This testing is done with all, or at least several, representative chemicals and sample types for which the method is intended. Continual refinement then follows during routine applications as additional sample types and chemicals are tested. During this time, changes, additions, or minor modifications in the originally proposed steps can occur to expand the method's applicability.

The continual changes that have occurred in multiresidue methodologies are reflected by numerous revisions issued for PAM I. This manual was issued in 1963 and was updated with yearly changes until 1967, In 1968, it was completely revised, and the second edition was published. Twenty-five major and minor revisions have been issued since that time.

In the following discussions, specific examples of the evolution of the five principal multiresidue

methods will be presented. Each method is published in PAM I and the OMA. The OMA version reflects applicability of the method as it was collaborated. The PAM version offers guidance to additional applications and options. Methods to be discussed are commonly referred to by the name(s) of the researcher(s) that is (are) recognized as the developer(s) of the originally proposed extraction and purification steps. The discussions will follow this convention.

Mills and Mills, Onley and Gaither methods

These two methods are discussed together because several similarities exist in both their analytical principles and historic development. Collectively, they have been studied more than all other FDA multiresidue methods combined, and knowledge gained benefited development of later methods.

The Mills fatty food (13) was originally developed for determining residues of organochlorine (OC) pesticides in both fatty and nonfatty food products. Published in 1959, the method used paper chromatography in the determinative step. The nonfatty extraction steps were refined and resulted, in part, in the Mills, Onley and Gaither (MOG) nonfatty food method (14) in 1963. The extraction and cleanup steps described for fats, oils, cheese, milk, and animal tissue in the 1959 Mills paper are basically the same as those currently used for these products. Both the fatty food and MOG methods use a similar solvent partitioning step and an adsorption (Florisil) chromatographic purification step to clean up the extract. The original MOG method provided two determinative techniques, paper chromatography or GC.

The extensive expansion of these two basic procedures has been reviewed by Burke (15). In his article, Burke chronologically details, with supporting precedent data, the development and evolution of FDA's multiresidue methodology from its inception in 1959 to 1970. Most of the paper's 103 references are related to these two methods and include 21 different studies on variables in the method (e.g., Florisil quality, effect of moisture content of sample), 19 method extension reports, and nine AOAC collaborative studies that eventually led to recognition and expansion in the OMA. There were 24 reports describing GC applications, 11 describing related identity confirmation tests, and 19 for other reference purposes,

During this period, the number of chemicals that were known to be recovered by the original Mills fatty food method had expanded from 11 to 59 OC pesticides. Additionally, recoveries of nine organophosphorus (OP) pesticides were documented. The MOG procedure was initially published with a demonstrated ability to recover 5 representative OC pesticides from 11 products. By 1970, the recoverability of 84 pesticide (and related) chemicals was documented; 15 of these were OP pesticides. The combined methods were known to be applicable to about 450 different food products.

By 1970, the determinative step for both procedures had evolved from semiquantitative paper chromatography to quantitative GC determinations with an ever-expanding selection of element-specific or element-selective detectors. During 1959-1970, the following detectors were developed and applied to pesticide analysis: microcoulometric, electron capture (EC), alkali flame ionization (KC1TD) and its simultaneous operation with EC, flame photometric (FPD), and electrolytic conductivity. Mass spectrometry was also applied for confirmation of residue identity. Also during this period, GC behavioral characteristics of many pesticide chemicals (at specified conditions) were determined and tabulated to aid in identifying GC responses. This was accomplished primarily with two general purpose GC columns, but other specialty columns began to be developed for difficult separations and difficult-to-chromatograph polar chemicals.

Since 1970, expansion of the methods' proven capabilities has continued with five AOAC collaborative studies (16-20) and recovery information for additional pesticides and commodities. New chromatographic technologies continue to be introduced and older ones replaced. The methods have also been expanded for use in determining residues of industrial chemicals (e.g., polychlorinated biphenyls). Currently, PAM I Appendix I lists approximately 160 chemicals that are partially or completely recovered by the Mills fatty food method and approximately 215 by the MOG.

In 1987, of the 15,592 food and feed samples (21) analyzed by FDA laboratories, approximately 18 percent (2,827) were analyzed by one of these two methods. Usage and expansion are expected *to* continue, particularly with feed materials and fatty foods, These methods, originally designed for nonpolar *OC* compounds, do not recover many of the currently used pesticides and their metabolizes. This limitation led to development of the Storherr method for the OP class of pesticides.

Storherr Method

As noted earlier, some of the OP class of pesticides are recovered quantitatively by the MOG method. However, many are polar or reactive and consequently are not recovered through the partitioning and/or Florisil cleanup steps of that procedure. Also, because of their polar or reactive nature, the OP pesticides are more difficult to determine by GC than the nonpolar OC pesticides.

The Storherr method is applicable to low and high moisture nonfatty foods (e.g., fruits, vegetables, grains) and, like the MOG method, it evolved from previous procedures designed for fly bioassay, paper chromatographic, and TLC determinative steps. Although method development for OP pesticides was being conducted concurrent with that for OC pesticides, researchers lacked selective GC detectors that were available for OC pesticides in the early 1960s. In 1964, Giuffrida (22) introduced the KCITD, which was both sensitive and selective to OP chemicals. In the same year, Storherr et al. (23) published a method for OP determinations using this detector. The method demonstrated the detector's utility, but it did not extend recoverability to any chemicals beyond that achievable by the MOG procedure. Consequently, the detector was connected with the EC detectors used for OC analysis so simultaneous determination of some OP pesticides could be made. Thus, used in this way, the early Storherr method was an extension of the MOG method.

As Storherr et al. (23) noted, GC determination of the more polar OP pesticides was not possible at that time without development of different types of GC columns. In two separate studies in 1966 (24) and 1968 (25), GC columns containing diethylene glycol succinate (DEGS) were demonstrated to be compatible with polar OP pesticides. Storherr and Watts (26) investigated chromatographic properties of more than 60 OP and metabolize chemicals with DEGS and the commonly used silicone liquid phase columns. In a companion paper (27), the DEGS column was described for determining recoveries of highly polar OP chemicals in a method that used an ethyl acetate extractant and a charcoal column cleanup.

In 1971 (28) Storherr et al. changed the extraction step of the previous method so that it was identical to that used with the MOG. This improved overall analytical efficiency by enabling analysis for a wider variety of OP pesticides from a portion of the same extract prepared for MOG analysis. This method was collaboratively studied in 1974 (29) and is published in the OMA. The collaborative study also demonstrated equivalent performance of KC1TDs and the newer FPDs that have been introduced for phosphorus selective detection in 1966 by Brody and Chancy (30). Unfortunately, determinations with DEGS columns could not be included in the collaborative test of the method because this material was not manufactured in a uniform manner; consequently its chromatographic performance proved extremely variable.

Prior to development of this method, other developments occurred in OP methodology that are still of interest. A study of the variation in different charcoals (27) set precedence for the cleanup step used in the Krause(31) method for N-methyl carbamates and an ancillary cleanup step in the Luke et al, (32) method. A distillation method of sample cleanup (sweep-codistillation), was developed (33) and collaboratively studied (34). The method was also investigated for use with OC pesticides (35, 36) and is of current interest because of recent commercial development and claimed efficiency (37). The commercial system, Unitrex®, is undergoing evaluation for FDA applications in multiresidue analyses.

The Storherr method had its most extensive use in FDA's Total Diet Program after modifications (38) were made to achieve lower limits of quantitation. Its application in the Total Diet Program and other FDA pesticide programs for high moisture products has now been essentially replaced by the Luke method. The method was referenced for use in only 13 analyses by FDA in 1987. The Luke method has also essentially replaced use of the MOG procedure for analysis of fresh fruits and vegetables.

Luke Method

This method, in one variation or another, was used in approximately 80 percent (11,922) of the 15,592 1987 FDA pesticide residue analyses. The evolution of this method's applicability and general acceptance has been in direct relationship to advances in GC technology since 1975.

The method (32) was proposed by FDA's Los Angeles pesticide analytical group and was designed to recover essentially all nonionic pesticides in the OC, OP, organonitrogen (ON) and hydrocarbon (HC) classes. The approach uses an acetone extractant, minimal cleanup and various GC systems with element-selective and element-specific detectors. The initial method determined residues of the OP and ON classes in a crude extract obtained after a solvent transfer step. These classes were to be determined with the KC1TD detector and use of two GC columns with methyl silicone and DEGS liquid phases. Separate portions of the extract were cleaned up with a modified MOG Florisil step prior to OC

and HC determinations by GC with EC and flame ionization detectors, respectively. This approach could recover 15 OP, 9 OC, 5 ON, and 2 HC pesticides.

The major advantage of the Luke method when it was first proposed was an increase in efficiency of sample work up. Most chemicals initially studied could be recovered by existing multiresidue methods of Storherr, MOG, and Holden (39). The improvement in efficiency resulted from the modified MOG Florisil cleanup and substitution of acetone for acetonitrile (common to Storherr and MOG methods) as the extractant. Acetone eliminated the exhaustive concentration steps necessary for removing traces of acetonitrile if a KC1TD (acetonitrile sensitive) was used.

The Luke method was not immediately adopted outside the Los Angeles laboratory, however. Since FPDs were replacing KC1TDS in general use for OP determinations, residual acetonitrile was of diminishing concern, and efficiency claimed for the method seemed minimal. There also was an initial reluctance among chemists to subject GC systems to the crude sample extracts obtained by the method.

By 1977, several FDA laboratories realized the potential of this approach, and in 1978 the method was published in PAM I. However, the GC determinative steps were not well defined or rugged. Later in 1978, the first of several interlaboratory studies was initiated to standardize GTC conditions for use with this procedure. The first study addressed the troublesome DEGS chromatography (discussed in the Storherr method) with FPD detection. Satisfactory reproducibility was obtained with an improved quality of commercially available DEGS. Other studies with fortified samples in 1979, 1980, and 1981 showed that overall interlaboratory performance of the procedure was acceptable.

This method was further refined in 1981 when Luke et al. (40) reported that a satisfactory substitution of the EC detector could be accomplished with a newly designed Hall electrolytic conductivity detector for OC pesticide determinations. This refinement eliminated the need for Florisil cleanup and further increased the efficiency of analysis along with the potential for expanding recovery to additional compounds. After a successful interlaboratory study (41) of this detector's performance was completed, the method was successfully collaborated in 1983 (42) and was published in the OMA. This AOAC study included six pesticides that represented both OC and OP classes of pesticides. These are the only broad classes of chemicals for which the GC determination has AOAC official status, but the method is adaptable to any number of specialized determinative steps. The extraction and cleanup steps of this method have recently been proven adaptable to the multicarbamate detection of the Krause method (31).

Krause Method

This method is unique among the other multiresidue methods mentioned. It introduced high performance liquid chromatography (HPLC) for separation and fluorescence spectroscopy for detection. The HPLC method was developed after several GC approaches were investigated and considered inadequate for analysis for this class of pesticide chemicals.

FDA began monitoring for residues of one highly used carbamated insecticide (carbaryl) in the mid-1960s. The method was a semiquantitative TLC procedure (43) that also determined one carbaryl metabolite. In 1973, Holden (39) published a multiresidue method with a GC determinative step that recovered 13 chemicals of the carbamate class. It used the same extraction step as the MOG procedure, and GC conditions were basically those used for OC pesticide determinations; however, it required that residues be derivatized in order to be detected by the GC system. The method was officially collaborated in 1974 (44) and published in the OMA. For the most part, method performance was satisfactory. However, it was lengthy and interferences were common, A purified derivatization reagent was needed, and proper GC conditions were difficult to maintain. It also failed to recover some metabolizes and two of the most widely used pesticides of this class, benomyl and methomyl, These two pesticides are thermally unstable and not amenable to GC analysis.

To overcome these inherent problems, Krause (31) adapted the HPLC approach pioneered by Moye et al. (45) for the determinative step. Besides HPLC separation and fluorescence detection, this approach featured a unique two step, in-line chemical reaction and derivation process. In developing the total method, a modification of a partitioning step used in Holden's procedure and a charcoal column cleanup based on the Storherr method were included. The extraction step was extensively studied and validated (46, 47) with ^{14}C labeled *carba*mate pesticides that were field-incurred. Another feature of the method is a refrigerated rotary evaporation step, which minimized losses attributable to thermal degradation.

After this method became generally available, it was 3 years before the method could be collaboratively studied. This time was needed so that a sufficient number of laboratories could obtain needed equipment and develop necessary expertise. The collaborative study was completed in 1984 (48) and the method is in the OMA.

This method is capable of recovering approximately 16 parent and metabolize chemicals of the N-methyl carbamate class. It also has shown the ability to recover certain other chemicals (49). In 1987, FDA analyzed only 34 samples by this method in its entirety, but the HPLC detection step was used with 588 other samples. Currently, the faster Luke sample work up is usually used in place of that initially researched and collaborated. A recovery study that supports the validity of this combination of methods has been completed (50). The primary use of the complete Krause method is to confirm levels of regulatory significance when found by the rapid approach.

Multiresidue Method Deveiopment: Summary

These necessarily brief discussions of the most widely used FDA multiresidue methods exemplify the constant evolution that has occurred, and is occurring, as new technologies are made available and experience with method performance is gained. They also illustrate the historical time that has elapsed from the first proposal of a method to completion of a successfully collaborated official method, about 10 years. By the time the Storherr, Holden, and Krause methods had gained official status, they were already being modified or preferentially replaced by more efficient procedures. The popular Luke method has been modified for use in FDA's Total Diet Program (51). [Note: Total Diet multiresidue methodology development and evolution have roughly followed those of the general methods, but this methodology is specialized enough that it warrants a separate discussion, which is not included here. The previously referenced (z) review article of the 26-year history of this program should be consulted for further details.]

Expanding the number of compounds recovered by multiresidue methods provides FDA with improved coverage of potential residues within existing monitoring programs. For this reason, FDA has committed resources every year to testing additional chemicals through existing methods. A computerized system, called Pestrak, has been developed to track the current status of data about compounds known to be recovered through each of the methods discussed here (10).

The constant hybridization of methods has made it difficult to describe which chemicals are recovered through any particular methods. Certain variations in all the basic methods can be, and are, employed to address particularly difficult analyte/food combinations. This may be accomplished through variation in any of the steps of the method, such as changing the extraction, modifying the cleanup, use of special GC columns or detectors, etc. Validation of the resulting method variation is an integral part of the process. FDA currently defines analytical method codes for 59 individual extraction/cleanup variations and 23 determinative steps for recording multiresidue method analysis results in its residue data system. Up to 20 of the extraction/cleanup codes apply to the MOG procedure alone. Specific knowledge of the capabilities of each of these steps and of exactly how they were applied determines the recovery capability of an analysis, not of a method per se.

Multiresidue methods are often criticized for their inability to produce rapid regulatory answers for samples collected for monitoring purposes. In reality, these methods, with modifications, are readily adaptable to provide this type of information when a specific pesticide/commodity problem has been identified or is suspected. In these situations, it is also not uncommon to utilize less formalized methods such as those found in FDA's Laboratory Information Bulletins or the scientific literature to facilitate rapid analyses. Much of the analytical data generated under these circumstances is semiquantitative. Examples of such rapid testing occurred in two recent widely publicized misuse situations: aldicarb in California watermelons and heptachlor metabolizes in milk from an Arkansas dairy shed.

Application of such techniques, as used by FDA laboratories in the above instances, greatly increased sample throughput. However, this practice fails to detect other potential residues present in legal or illegal amounts. Since illegal residues occur in only a small percentage of samples, and other residues are routinely detected, classical multiresidue analytical approaches provide a better measure of the total pesticide residue burden in the food supply. Usage of such methods is applicable for those specific pesticide/commodity situations in which there is an identified need for rapid analysis and such analyses are carried out on a planned and coordinated basis to allow proper interpretation of the findings.

Multiresidue Method Development: The Future

Method development for pesticide residues is expected to continue evolving as it has in the past; researchers will apply and adapt technology, as it becomes available, to meet the needs resulting from pesticide usage and environmental contamination. Multiresidue methods are still the most effective way to examine food samples of unknown treatment history and so they will be used where applicable. Existing methods will continue to be used and expanded wherever practical. Special attention will be given to use of new determinative techniques.

However, new methods for residues not amenable to existing methods must be developed, and these will be multiresidue methods wherever possible. The method proposal by Clower for determination of a number of volatile fumigants (52) is an example.

New methods will be applicable to fewer residues than most of those described here because they involve chemicals whose structures vary widely and preclude easy separation and detection by today's technology. Method development for chemicals not recovered by existing methods may well follow the approach taken in developing the Krause method, in which a very selective determinative step was developed to focus on a relatively small group of chemically related residues.

Current examples of this approach include the Hopper method for chlorophenoxy acetic acid residues (53), the Luchtefeld method for phenylurea herbicides (54), and an ongoing effort within FDA's Pesticides and Industrial Chemicals Research Center to develop methods for compounds with benzimidazole structures, for the "quat" family (paraquat, etc.), and for organic tin compounds. Within the Division of Contaminants Chemistry, work continues to develop methods for residues with substitute aniline and nitro aromatic structures.

Technologies currently available and being tested for adaptability in multiresidue methodology include selective HPLC detection using photoconductivity and electrochemical detectors, capillary column chromatography, and simplified cleanup steps such as solid phase extraction and distillation (Unitrex) techniques. Attempts continue to find a stable and reproducible GC detector that is selective for ON compounds. Other *technologies* yet to be applied broadly in residue monitoring include supercritical fluid chromatography and immunoassay techniques.

Certain analytical techniques that have been available for many years are still not used routinely in residue analysis. Mass spectrometry is used extensively for identification and confirmation of residue identity, but it has not been adapted to routine analysis because of its cost and the degree of expertise required to maintain the system. More routine use of mass spectrometry is expected in the future, however.

Portions of methods can be routinely automated. Equipment that is manufactured with microprocessor control units, such as automated injectors for chromatography, is one example. The likelihood that complete methods will be automated within the next 10 years is small because of the diverse sample types that are encountered and the individual challenges that each poses.

A commonly acknowledged disadvantage of existing multiresidue methods is their "macro" design, which is based on analysis of a 100 g portion of sample. This analytical portion is larger than those used in recently developed methods and results in increased analytical expense from greater volumes of solvents required. This macro scale approach was initially validated with the MOG procedure and subsequently copied in other methods to assure that the size of the analytical portion would be representative of the amount of food collected (10-20 lbs.). A current FDA study is statistically addressing analytical sample size and homogeneity issues to establish a basis for reduction in sample and solvent volumes. Findings of this study should have a major impact on future method development efforts as well as future usage of current methods in "scaled down" versions. The ultimate goal is to achieve more rapid and efficient methodology without sacrificing analytical integrity.

The cleanup step is often a limitation in residue methods because it generally consumes a large amount of the total analysis time and restricts the number of pesticides that are recovered. Development of new, more effective or efficient approaches to removing unwanted materials in sample extracts, while minimizing the restrictions on number of residues recovered, would significantly improve analytical capability. Automation of cleanup procedures offers a partial solution in that it frees the analyst for other tasks. Application of automated cleanup procedures is itself severely limited however, since efficient use of automation requires that a large number of predictable analyses be planned for similar samples. As noted throughout this paper, development of determinative procedures that can

tolerate extracts with less stringent cleanups will be a dominant factor in considering the cleanup issue.

Most of the above focuses on enhancement and adaptation of the type of methodology most widely employed in residue monitoring. Screening methods, e.g., immunoassay methods, may provide a useful extension to residue monitoring activities in the future. Although the concept of screening is not new, screening is defined and used in a number of different ways by regulatory agencies and others. One type of screening is aimed at providing rapid "yes/no" answers for one or more selected residues at specified levels, usually levels of regulatory interest in a compliance situation. A positive result would trigger reanalysis by more conventional and time-consuming quantitative methods. Although this screening would permit analyses of more samples, the time savings could be reduced or eliminated if followup analyses had to be conducted on a large proportion of the samples. The real gain in efficiency will thus need to be considered before screening analyses are applied.

Coverage for certain selected residues might increase with addition of screening methods. However, designing the monitoring program to incorporate these methods will require careful planning. The need to be able to summarize and evaluate data from diverse methods will remain a dominant factor.

Because residue analysis is so challenging and its successful application relies so heavily on the expertise of the analyst, development of new personnel is of critical importance to FDA. Within the next 10 years, the majority of today's FDA pesticide experts will have become eligible for retirement; recruitment and training of their replacements are vital considerations to the agency.

Impacts of Laws and Regulations

The laws and regulations governing the use of pesticides on foods in the United States have had a necessary impact on the development of the analytical methods used to enforce those laws. In turn, the capabilities and limitations of the methods have sometimes indirectly caused changes to be made in the regulations.

Two amendments to the Food, Drug and Cosmetics Act originally provided the basis on which the requirements for pesticide residue analytical methods depend: the Miller Pesticide Residue Amendment of 1954 and Food Additives Amendment of 1958, These laws established the concept of tolerances to describe the maximum residue limits of individual chemicals that would be permitted on specified foods. These limits in turn established the analyte concentration levels at which analytical methods would be required to function reliably.

The practical imperative for multiresidue analytical methods was also provided by these two amendments because they permitted more than one pesticide on a single food commodity. (Unknown spray histories for most foods and inadvertent pesticide contamination of nontarget foods provide other reasons for the reliance on multiresidue methods,)

Early laws established zero tolerances for certain pesticides in certain commodities. The abandonment of this concept was dictated by advancements in analytical methodology which permitted determination of ever-diminishing quantities of residue and made the zero tolerance concept impractical. In a similar way, practical analytical capabilities are taken into account when reducing tolerances or action levels for pesticides whose uses have been suspended, and in setting action levels for unavoidable contamination from environmental sources.

In actual practice, FDA's analytical methods are applied at limits of quantitation sufficiently below the tolerance levels to provide data on incidence and levels of residues (both above and below tolerances) in the food supply, while still being realistic in terms of the effort required for each analysis, These data are vital for evaluation of pesticide regulations. Typical examples are the following: (1) FDA data for DDT findings from 1964 to 1969 were used in 1970 to reassess tolerances and resulted in cancellation of registration for certain uses and lower tolerances for other uses; (2) FDA's historic findings of aldrin and dieldrin were pertinent in the cancellation hearings of these pesticides; (3) FDA's lindane findings from 1964 to 1980 were used by EPA in its Special Review (56) to calculate the changing dietary exposure to lindane residues; (4) FDA data on methomyl residues from 1978 to 1987 are being used by EPA in its tolerance reassessment for re-registration of this pesticide; and (5) FDA data are used to assist in setting action levels for a pesticide when its use is suspended and the corresponding tolerance is no longer applicable.

FDA's monitoring program also directs agency laboratories to maintain uniform limits of quantitation below tolerance levels since levels for a given pesticide are not the same for all commodities. Tolerances for permethrin, chlorpyrifos and dimethoate in apples and peaches illustrate this complexity. Tolerances for these pesticides on apples are 0.05, 1.5, and 2.0 ppm. respectively. The correspond-

ing tolerances on peaches are 5,0, 0.05, and none since dimethoate is not registered for use on peaches. Analytical procedures for both sample types are identical and cannot be readily adjusted for a given tolerance/commodity combination. In most cases, such adjustment would not result in significant savings in analytical cost or time.

EPA regulations have long required that registrants provide an enforcement method for each tolerance being requested. As mentioned earlier, these methods constitute the bulk of PAM II. Since PAM II is the reference of first choice when a single residue method is needed, it is important that the methods be reliable. Registrants must be encouraged to adhere to the spirit of this requirement and provide methods usable by regulatory laboratories without excessive adaptation,

A more recent EPA requirement (56) has permitted the expansion of FDA multiresidue methods to newly introduced pesticides. Registrants must determine analytical behavior of a new pesticide through these methods. This additional information provides FDA, state governments, and the food industry with better tools to inform consumers about pesticide residues in food. Availability of this information also frees research resources of these institutions to concentrate on development of methods for the more difficult compounds.

Certain recent situations have demonstrated that establishment of different acceptable residue levels by different government bodies have a profound impact on regulatory decisions, which in turn affect the development and application of residue methodology. The international organization, Codex, is seeking to remedy the international level of this dilemma by proposing pesticide maximum residue limits for adoption by member countries. This effort is expected to become more important as the level of international trade increases.

The Food and Agriculture Organization (FAO) of the United Nations and the World Health Organization (WHO) cooperate in a program that collects information from 34 countries on levels of pollutants in foods and other environmental samples. This Food Contamination Monitoring Programme is designed to assess human exposure and allow estimates of health threats caused by such pollutants. One of the main objectives of this portion of the Global Environment Monitoring System is to provide Codex with levels of pesticide residues in food to assist that organization in its determination of maximum residue limits. FDA's data base of quantitative residue data has permitted the United States to contribute requested data to this program throughout its history.

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Pesticide Design: Outlook for the Future

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Contents

Figures

Tables

Abstract

The need to analyze pesticide residues in food and drinking water for regulatory purposes creates increasingly complex analytical problems because so many diverse molecular types must be determined in a large variety of crops or foods. Multiresidue procedures (MRPs) are important because a method must detect as many pesticides as possible and it must be applicable to samples of unknown treatment history.

For a number of reasons, including the high costs of development and safety tests, reduced success in screening programs, and pest resistance, the number of pesticides entering the U.S. market has decreased in recent years. However, there continues to be a strong demand for pesticides, partitularly herbicides, and this is likely to continue into the future. Despite rapid progress in fundamental aspects of biotechnology, its widespread application to pest control technology will proceed at a steady pace because many questions of safety must be answered.

New biochemical and biological knowledge is important in developing new leads for synthesis, and quantitative structure activity relationships are guiding the optimization of promising, active molecules, Complexity may increase as products of microbial metabolism (such as the avermectins) are found to be pest control agents.

Extremely low rates of application result in low residue levels, and the detection and quantitation of such low levels presents a considerable challenge to analytical chemists and designers of instrumentation. The sulfonylureas and the pyrethroids are two examples of classes of pesticides that may be used at very low rates of application and, in consequence, require analytical methods capable of determining residues at the parts-per-billion level.

Novel methods of analysis (such as biosensors) are being developed, and immunoassay techniques are increasing their range of applicability and sensitivity. The latter are beginning to meet the need for simple and rapid screening procedures that may simplify the task of the analyst.

A consequence of the increased complexity and the potent biological activity of new pesticide molecules is the need for more sensitive methods of detection and improved methods of separation. Multiresidue methods will continue to be adaptable to many new compounds. It is recommended that information concerning their applicability to new compounds be made readily available.

Introduction

There is increasing awareness among consumers of the potential of man-made chemicals to contaminate sources of food and drinking water. There is particular concern over the implications of food contamination by pesticide residues. The capability of analytical techniques to detect extremely low levels of trace contaminants has continually expanded. However, knowledge of the toxicological significance of such contaminants has not progressed at the same rate. It is also important to bear in mind that the study of the effects of individual compounds on biological organisms does not provide satisfactory information concerning the biological effects of several interacting compounds.

Residue analysis may be conducted for several purposes. The registration and use of a pesticide is preceded by supervised trials to determine the rate of disappearance. Residues on raw agricultural commodities must also be determined.

For regulatory and monitoring purposes, residues in food for human consumption and residues in environmental samples must be determined in samples that do not have a known treatment history. Therefore, procedures must be employed that can detect as many pesticides as possible in the most economical way. Multiresidue procedures (MRPs) are used for this purpose, and these are usually limited to the parent compound and closely related compounds. An excellent survey of the scope and capabilities of MRPs appeared in a recent International Union of Pure and Applied Chemistry report (3).

Rapid developments in analytical technology contribute to, but cannot be equated with, improved ability to determine the presence and amounts of contaminants in food. Much progress has been limited to the analysis of specific analytes or groups of analytes. Before beginning an overview of developments in agricultural chemicals, it should be stressed that the problem of analysis requires for its solution that we consider both analyte *and matrix*. The former is the compound of interest, a definition that is often extended to cover not only the parent compound but also its metabolizes and transformation products; the latter refers to the particular crop or food type for which the information is desired.

The magnitude of this problem can be gauged by considering the efforts of the Codex Alimentarius Committee on Pesticide Residues (CCPR), which has established minimum residue levels for 150 compounds and more than 2,500 pesticide/commodity combinations over a period of 20 years (10), Multiresidue procedures are essential if it is necessary to determine as many pesticides as possible in various types of matrices. The complexity of the problem will increase in future years as new classes and types of pest control agents are introduced in response to a variety of constraints.

Economics

There is little likelihood that agricultural production and pest control will abandon their prime reliance on chemical methods of pest control in the coming decades, although there will be greater emphasis on the use of biological controls and technology that will contribute to the reduction of pesticide use. The market for agrochemicals continues to grow but more slowly than in the past. In the United States, there was an 8 percent decline in cropland from 1986 to 1987, and it was predicted that pesticide use would decrease 9 percent during that period (30). A market study predicted that herbicide growth would be most rapid primarily for corn and soybeans with a growth of about 5.3 percent per annum to a value of $3.47 billion. Expansion would emphasize new compounds (such as the imidazolinone and sulfonyl urea herbicides) that possess new modes of action and are used at extremely low rates. The synthetic pyrethroids that now account for about one-third of world insecti-

cide use (27) would lead the way in insect control, and new fungicides, primarily the ergosterol biosynthesis inhibitors, would be emphasized, Table 1 indicates the herbicides and insecticides that are currently most widely used in the United States.

Economics of pesticide production is a major factor in change. The end of patent protection for a number of compounds of major importance means that there will be a shift to a commodity market with a more competitive approach. Pesticide manufacture is also becoming consolidated. Foreign buyers have now acquired many U.S. businesses. The agrochemical market is international, but the developing countries' market has not materialized to the extent anticipated. New compounds are slow to appear on the market. Successful introductions have dropped from 60 new compounds between the 1950s and '60s to 21 between the 1970s and '80s. Because the costs of research, development, and market introduction have increased to about $40 million per compound according to data developed in 1982 (22), the market will be largely restricted to major international companies who will emphasize the needs of major world crop markets. Profitability continues to be limited by the time that elapses between discovery, market introduction, and patent expiration. About 13 years is needed to reach a break-even point after discovery.

The food producer must also cope with major constraints because the cost of pesticide development is passed on unaccompanied by any increase in farm prices. Thus, the farmer must continually review the cost of all chemical inputs to adjust pesticide and fertilizer use to maximize his return. To attain this goal in part may be practicable if substantially lower rates of application can be achieved by using pesticides of greater biological effectiveness and by using better application technology,

Screening

Although there is little doubt that synthetic chemical pesticides will continue to be the main weapon in our crop protection arsenal in the next 10 to 20 years, the rate of new compound introductions has dramatically decreased in recent years. This decrease is largely due to the reduced number of companies engaged in agrochemical research and development, to the difficulty in discovering viable new pesticides by the process of empirical synthesis and screening, and to cost and safety considerations.

The rate of commercial success from screening to market development has fallen from 1 in 1,800 in 1956 to 1 in 15,000 in 1979 (21), and this adverse ratio is expected to increase in the coming years. From the 1950s through the 1970s, the majority of insecticides were neurotoxicants represented by chlorinated hydrocarbons, carbamates, and organophosphorus esters. These pesticides have similar modes of action in insects and vertebrate species, including humans. Today, the largest class of insecticides in use are the synthetic pyrethroids, which are also neurotoxicants. Representatives of this class are shown in figure 1.

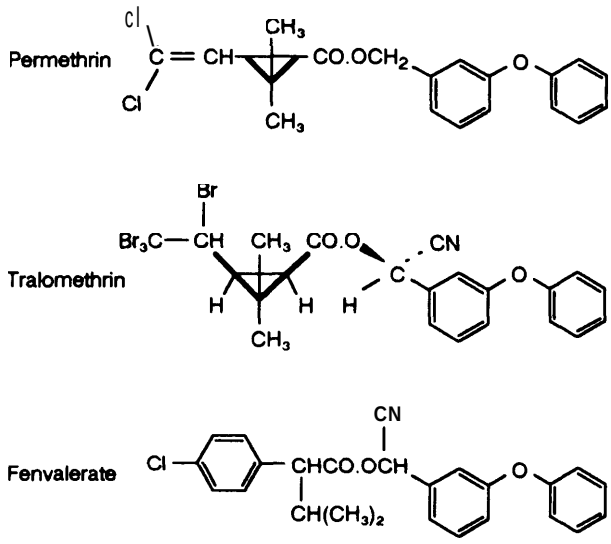
At present, the major agrochemical companies are directing greater efforts and resources toward more fundamental research to discover novel classes of pesticides. Morrod (23) discussed approaches to current and future directions for discovery involving the following: novel synthesis, speculative biological chemistry, directed synthesis, natural product ana-

Table 1.—Ten Most Widely Used Herbicides and Insecticides in the USA

Herbicides		Insecticides	
Common name	Trade name	Common name	Trade name
alachlor	Lasso	aldicarb	Temik
atrazine	Aatrex	carbaryl	Sevin
butylate	Sutan	carbofuran	Furadan
cyanazine	Bladex	chlorpyrifos	Dursban
2,4-D	many	malathion	Cythion
glyphosate	Roundup	methyl parathion	Penncap
metolachlor	Dual	parathion	Folidol
metribuzin	Sencor	phorate	Thimet
propanil	Stare	synthetic pyrethroids	many
trifluralin	Treflan	terbufos	Counter

SOURCE: P.C Kearney, A R. Isensee, and J.R. Plimmer, "Contribution of Agricultural Pesticides to Worldwide Chemical Distribution," *Toxic Contamination in* Large Lakes, vol. III, N.W.Schmidke, Lewis, Chelsea, MI, 1988.

Figure I.-Structures of Representative Synthetic Pyrethroids



SOURCE: C R Worthing ed., *Pesticide Manuel,* 8th edition, British Crop Protection Caned, Thornton Heath, UK, 1987

log synthesis, and greater reliance on quantitative structure-activity relationship (QSAR) methods.

Safety

An important part of the high cost of a pesticide is the continual increase in the cost of safety tests. Environmental consequences and health effects of pesticide use continue to be major topics of public and regulatory concern. Testing for acute and latent toxicity is a substantial portion of the cost of pesticide development. These costs and the regulatory implications of such factors as the production of oncogenic responses in test animals substantially influence the current directions of chemical innovation. A recent National Academy of Sciences study on the issue of pesticide residues in food has addressed some controversial issues involved in pesticide regulation, especially as they pertain to the Delaney Clause (26).

Because the environmental behavior of a pesticide is largely determined by its chemical structure, the constraints on the selection of structural classes continue to be more pressing. For example, the contamination of groundwater by pesticides may result from agricultural use under certain conditions depending on soil, crop, method of application, etc. Although the amounts reaching groundwater may be well below the limits deemed as potentially harmful to human health, the fact that such trace amounts are present was not predicted on the basis of existing knowledge at the time of registration. Aldicarb, alachlor and atrazine are among the compounds detected in groundwater, and a number of survey programs are planned or in progress to determine the scope of the problem.

Studies are being undertaken to detect precise conditions under which contamination occurs and to limit its occurrence by changes in pest management practice. However, the recognition that the major contributing factors are the soil environment (e.g., soil fractives; channels; agricultural practices; microbial activity; moisture; clay, mineral, and organic matter content; etc.) and the structure and physical properties of the pesticide molecule (e.g., rate of degradation in soil, water volubility, vapor pressure, partition coefficient between water and soil, organic matter, etc.) lead to the conclusion that pesticide design must take into account leachability or the potential for biologically active materials to move vertically in soil to groundwater.

The regulatory foundation for safety issues will continue as a major factor in the design of chemicals. The emerging issues include the U.S. Environmental Protection Agency (EPA) actions to bring the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA) into compliance with the Endangered Species Act in the United States, beginning in 1988. This action will prohibit pesticide use in specified areas that are the habitats of endangered species. The issues of health and safety are not only of concern to the consumer. Farmers, formulators, applicators, and field workers are by their occupations exposed to pesticides. Home and garden use is also an important safety consideration. Thus, the scope of regulation extends over a wide range of activities.

Thus, only a limited number of structural types will be considered for development as they emerge from the elaborate program of safety testing. Analytical considerations will follow these dictates in so far as they are part of the accountability needs during the process.

Resistance

A further constraint on molecular design is the problem of pest resistance to pesticides. Insecticides are particularly susceptible, and reliance on chemical classes that possess closely related modes of action or similar target sites hastens the obsolescence of particular classes of compounds. The response of the manufacturer is to broaden the chemical screen to search for new modes of action, and several classes of insecticides and herbicides introduced in recent years reflect this consideration. As

an example, diflubenzuron may be considered the first of the commercially introduced chitin synthesis inhibitors. Chitin, the skeletal material of insects, is absent in man and vertebrates. Innovative pesticide design and the search for alternatives will continue to challenge the potential development of resistance,

In summary, many factors have combined to create pressure to reduce the use of pesticides in food production. These include economic and regulatory pressures at the producer and farm level. In addition, changes in agricultural management (for example, adoption of conservation tillage, change in land use, and new irrigation and application systems) and formulations have contributed to the evolution of new agrochemicals. Although biological control and developments in biotechnology will contribute to decreased reliance on chemicals, it is probable that at least a decade will elapse before a substantial contribution can be expected from such sources. These changes will then be accompanied by shifts in regulatory emphasis. The current problem of analysis of pesticides may be relatively less complex than the problem of satisfying the safety concerns arising from new technologies.

Design of New Pesticides

As discussed previously, more rational approaches are needed to improve the odds for developing a successful, marketable pesticide chemical.

In recent years, there have been considerable advances in the understanding of basic biology, biochemistry, and the physiology of host and target species. In addition, there is improved understanding about the site of action and effects of pesticides at the molecular, cellular, and whole organism levels. These have contributed substantially to rationalization of approaches to molecular design of pesticides. Research discoveries as related to bioactivity have been greatly aided by the development of regression functions that form the basis of QSAR.

QSAR combines elements of quantum chemistry, biodata, and computerization to fit parameters predefined by biochemical processes. Knowledge derived from this methodology should provide a better foundation for the rational design of novel, highly active, and environmentally sound crop and livestock protection chemicals. More detailed aspects of QSAR in pesticide design were reported in a symposium on this topic (17),

The following examples illustrate the utility of QSAR in optimizing synthesis and bioactivity.

Nakagawa et al, **(24)** described the optimization of quantitative structure-activity of benzoylphenylurea larvicides with reference to substituents at the aniline moiety against the major rice insect pest, the rice stem borer (Chilo *suppressalis* Walker).

Table 2 shows the empirical formula for a series of N-2,6-difluoro and N-2,6-dichlorobenzoyl-N'-(4-substituted phenyl)ureas and the regression equation parameters used in the QSAR analysis to predict optimal insect (chitin synthesis inhibition) activity. The analysis was performed with each compound synergized with piperonyl butoxide (PB) to reduce metabolic degradative effects in the insect.

Also included in table 2 is the resultant analysis for four compounds in the series. Activity is enhanced by electron withdrawing (op) and hydrophobic substituents ( ) and reduced by bulky groups (ΔB).

Plummer **(28)** succeeded in designing a novel series of highly active biphenylmethylpyrethroids through the QSAR approach. His success was specially significant since it came when the field appeared to be already saturated with synthetic pyrethroids.

From these studies, Plummer concluded that where X = F or CH3, activity was optimal resulting from the confirmational preference of these compounds for a twist angle at about 500 involving ring B. The latter is most likely involved as a ligand of the active site, involving a specific fit (figure 2).

In a comprehensive QSAR study of terpenoid and non-terpenoid insect juvenile hormone mimetic compounds (juvenoids), Nakagawa *et al.* **(24),** through regression analysis and correlation equations formulated for 85 compounds on two insect species, developed hypothetical "mode of action" models involving overall similarity as well as species differences at the receptor site showing structural conditions necessary for activity. Without such quantitative calculations, it would have been difficult to predict similarity in the mode of action of such diverse compounds as terpenoids and N-alkyl-N,N-ethylenebis (thiocarbamates).

The QSAR approach to design of candidate compounds offers a great deal to the analytical chemist who shares the need for much of the physiochemical data, such as the octanol/water partition coefficients that must be generated for the calculation of regression functions. Analytical schemes could benefit by close cooperation at the pesticide design stage.

Undoubtedly, greater structural diversity is in ***store*** for the future as biochemically inspired **targets** in insects, weeds, and fungi are better understood and exploited.

Such new bioactive models include insect neuropeptides **(15, 22),** which provide potential new vistas in insect control by which insects' native

Table 2.e Quantitative Structure Activity Relationahips of Some Benzoyl Phenyl Urea Larvicides

1A	**Cl**	6.32	6.64	0.23	0.60	0.49	0.79
IIB	**CF**	**6.86**	**6.92**	**0.54**	**1.60**	**0.93**	**1.42**
lll	CH_3	5.10	4.60	-0.17	1.04	-0.02	-0.03
IV	CH_3	3.47	4.30	-0.27	2.07	-0.41	-0.73

Y=2, 6-D (ly=O). **A. Diflubenzuron B. Penfluron**

Larvicidal activity of piperonyl butoxide (PB) synergized compound against larvae of rice stem borer (*Chilo suppressalis* Walker)

$$pLD(PB) = 5.690 + 0.748\sigma_p - 0.398\Delta B_5 + 1.695\,\Sigma\pi - 0.179(\Sigma\pi)^2 - 1.172\,I_\gamma$$

σp = Hammett sigma factor for parasubstituent (inductive component of electronic effects of substituents). Strong electron-withdrawing groups increase activity. Electron donating groups ineffective, e.g., lll and IV.

$\Delta\beta_5$ = Volume factor (Verloop's STERIMOL parameters). Activity increases as bulk decreases.

π = Hansch's constant. Hydrophobicity (solubility) parameter of the anilide constituent, derived from partition coefficient of substituted anilides in octanol/water system. Activity increases as hydrophobic character increases.

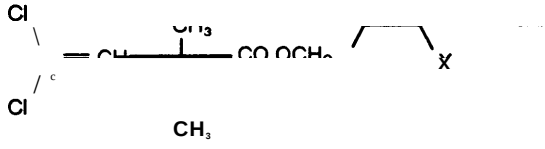
$\Sigma\pi$ = Sum of π values.

pLD_{50} = -Log LD_{50} (Larvicidal activity).

I_γ = Indicator variable represents effects of structural modifications in the benzoyl moiety.

SOURCE: Nakagawa *et* al., "Quantitative Structure-Activity Studies of Benzoylphenylurea Larvicides," *Pestic. Biochem. & Physiol.*, 21:309-325, 1984

Figure 2.- Biphenylmethylpyrethroid Series (Plummer)



X= F or CH_3

SOURCE: C R Worthing ad, *Pesticide Manual*, 8th edition, British Crop Protection Council, Thornton Heath, UK, 19137

biochemical serve as prototypes for lethal peptide agonists and antagonists (19).

It is likely that in the next two decades, increasing resources will be directed toward pest management technologies that involve the use of microbiologicals, natural products, genetic and behavioral biochemical, and transgenic plants (20).

The question of the impact of biotechnology on pest control presents difficulties because the future direction of expansion is not clear and techniques are in the exploratory stage at present. For exampie, a technique that appears promising is the potential control of insects that attack corn roots by

infecting corn with a vascular, endophytic microorganism that carries the gene capable of expressing the bacillus thuringiensis endotoxin. This technique and some others that rely on gene transfer to plants or microorganisms depend on the expression of toxins to achieve insect control. Safety considerations in biotechnology are viewed quite differently from considerations of food contamination by residues of synthetic pesticides, although some of the same questions must ultimately be asked, and it seems likely that the analysis of bacterial toxins or other complex molecules of biological origin will become more important in future. Because MRPs exclude biological macromolecules during the cleanup stage, methods of study or assay that have been developed for biological or clinical studies will probably be more appropriate in this field and immunoassay would appear to be a logical technique.

These new developments will pose increasingly more difficult challenges to the analytical chemist in the quest of accessible and practical residue analytical methods.

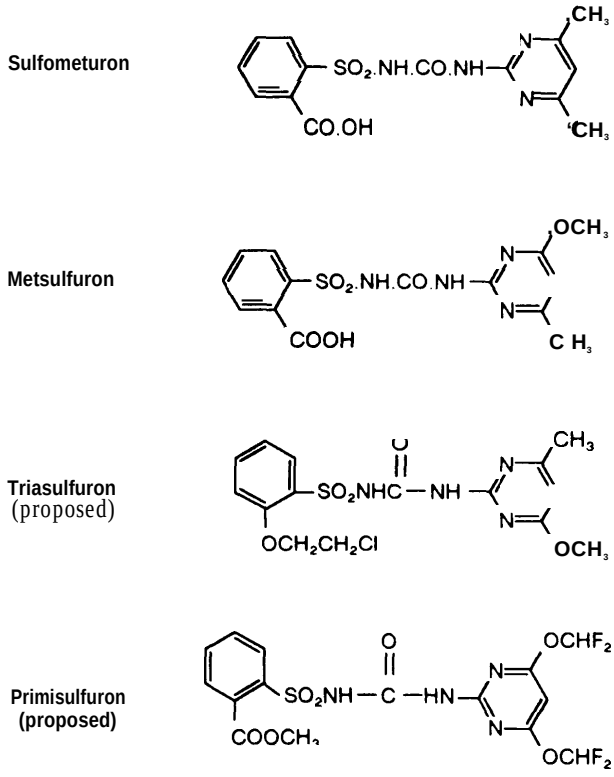
Emerging Classes of Compounds: Examples of Specific Analytical Problems

A number of newer herbicides are active at extremely low rates of application. For example, fluazifop applied at 4 to 8 oz. per acre will control annual grasses and perennial weeds. Sethoxydim is active at 3 to 7.5 oz. per acre, chlorsulfuron at 0.17 to 0.5 oz. per acre, and chlormeturon at 1 to 5 oz. per acre.

Several manufacturers are involved in the development of these compounds. Chlorsulfuron and sulfometuron (figure 3) are the active ingredients of GleanR and OustR, respectively, both introduced by DuPont. Other manufacturers have introduced similar herbicides. The activity of these and other new herbicides currently being developed is extremely high, and as application rates will be low, the residue levels in soils and plants will also be extremely low.

Residue levels in agricultural products will be so low as to challenge the ingenuity of the analyst. Since chlorsulfuron and sulfometuron are both thermally unstable, they cannot be directly determined by GLC. Chlorsulfuron was analyzed by gas chromatography after conversion to the methyl derivative. Residues in agricultural runoff water were determined at the parts-per-trillion level (z). The earliest method for analysis in soil at the parts-per-billion level relied on HPLC separation combined

Figure 3.- Sulfonyl Ureas



SOURCE: C R Worthing ed., *Pesticide Manual*, 8th edition, British Crop Protection Council, Thornton Heath, UK, 1987

with photoconductivity detection. Because extraction procedures normally used for soil liberate quantities of ultraviolet-absorbing material, there is considerable interference with the operation of the UV detector (33). The procedure was used because no chemical methods were available when field evaluation was conducted. A 5-day incubation period gave the most satisfactory data. Groves and Foster (11) described a bioassay for chlorsulfuron in soils that was based on the inhibition of corn root growth after a 7-day period of development in soil containing chlorsulfuron. The benefit of such bioassays is their reliance on simple techniques and their potential accuracy, For such highly active herbicides, simple bioassays may offer some advantages. Disadvantages are the length of time needed to conduct the bioassay and the need to conduct the test in a greenhouse or growth chamber.

Chlorsulfuron is a water-soluble compound, and a scheme for extraction and separation of the compound and its metabolizes from treated plants was proposed by Bestman et al. (5) using aqueous ex-

traction of plant tissue. Subsequent chromatography on a reverse-phase column and elution with aqueous formic acid / methanol gave an average recovery of 94 percent (based on 14C data). The use of reverse-phase solid-phase extraction for analysis of aqueous environmental samples has been advocated as a general method for trace organics, and this appears to work well in the case of the sulfonylurea herbicides (31). The paper contains useful suggestions for the development of procedures for a solid-phase extraction method and discussion of the potential value of this technology for extracting of organic compounds from aqueous solutions. Confirmatory procedures for the identification of sulfonylureas include combinations of gas chromatography with liquid chromatography (29, 18). An immunoassay analysis for chlorsulfuron can be used to determine chlorsulfuron in unfiltered soil samples at nanogram levels (16) and the technique appears promising. The authors comment that the method is relatively specific in contrast to the bioassay method, and interferences with the HPLC method may raise detection limits considerably.

Analytical methods for the new herbicides are thus in an evolutionary stage. The extremely low levels at which residues are to be expected contribute to the analytical problem, but these low levels represent a desirable factor in future pesticide design.

Bioassay is useful for the determination of chlorsulfuron, as well as for dichlofop acid and sethoxydim residues in soil at very low levels. The test involves measurement of the root length of pregerminated oat or corn seedlings (13).

An example of the trend in insecticide development can be found in the class of compounds known as synthetic pyrethroids. The synthetic pyrethroids are derived from the structures of natural pyrethrins, a series of chrysanthemic acid esters extracted from chrysanthemum flowers. Beginning with allethrin in 1949, both the acid and alcohol moieties of the ester have been replaced, modified, or substituted to produce a family of insecticides having greatly enhanced activity and stability. The original pyrethroids could only be used indoors as sprays in homes and greenhouses due to short residual activity. However, the discovery that halogenation of the vinyl moiety of the chrysanthemic acid increased photostability and enhanced insecticidal activity led to the modern pyrethroids that can be used as field insecticides on crops.

The application rates of some of the current products are measured in grams / acre instead of the traditional pounds / acre of other pesticides (table 3). Relative mammalian toxicities are shown in table 4. The lower application rates of the synthetic pyrethroids are due to their greater toxicity to insects, but not to mammals. For example, permethrin and carbaryl have about the same mammalian toxicity, but permethrin can be used at rates about 10 to 20 times lower than carbaryl (tables 3 and 4). Similarly, deltamethrin and chlorpyrifos have similar mammalian toxicities, but deltamethrin rates average about 100 times less than chlorpyrifos. Therefore these lower application rates also imply that the potential health hazard is reduced. Low application rates and consequent low residues and the lipophilicity imparted by the halogen functional groups determine the approaches used in developing multiresidue methods of analysis. Residues can be extracted by methods already developed for the organochlorine insecticides such as DDT, etc. Fortunately, the group is characterized by fairly high melting and / or boiling points, which permit their separation from other halogenated compounds by high-temperature gas chromatography and sensitive electron-capture detection. The lipophilic properties also result in accumulation in animal fat when treated grains, forage, and other crop products are fed to animals.

However, the general structure of this family of compounds results in both cis / trans isomers and optical isomers, which complicate the chromatographic

Table 3.—Recommended Application Rates of Selected Pyrethroids

Fenpropathrin decreasing Compound	Rate. lb./Acre
Permethrin	0.050-0.200
Fenvalerate	0.050-0.200
Fluvalinate	0.025-0.100
Flucythrinate	0.025-0.080
Cypermethrin	0.020-0.075
Tralomethrin	0.013-0.024
Cycloprothrin	0.009-0.180
Cyfluthrin	0.009-0.045
Deltamethrin	0.008-0.024
Alphamethrin	0.0045-0.027
Karate	0.0045-0.027
Phenothrin	0.004-0.016
Fenpropathrin	0.002-0,010
Other insecticides for comparison:	
Chlordane OC	1.0-10.0
Aldicarb carbamate	0.5-10.0
Carbaryl carbamate	0.5-4.0
Malathion OP	0.5-3.0
Diazinon OP	0.25-2.0
Chlorpyrifos OP	0.10-5.0
Parathion OP	0.10-1.0
Diflubenzuron IGR	0.02-0.14

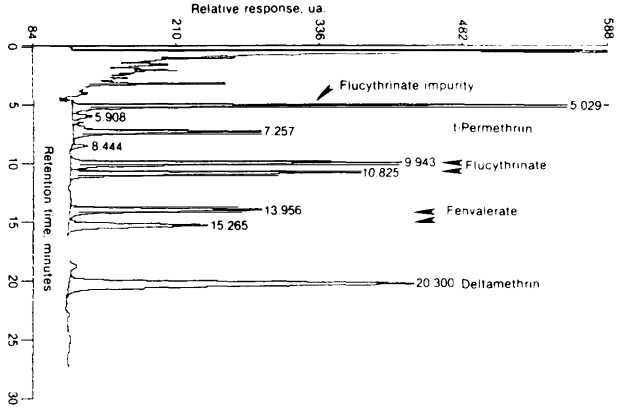
SOURCE: Agricultural Chemicals, Book 1, Insecticides, W.T. Thomson, (cd.) (Fresno, CA: Thomson Publications, 19S6).

Table 4.—Relative Mammalian Toxicities of Selected Pyrethroids

Compound		Increasing Toxicity LD (rat, oral), mg/kg body wt.
Phenothrin		10,000
Cycloprothrin		5,000
Tralomethrin		1,070
Cyfluthrin		500
Fenvalerate		451
Permethrin		450
Fluvalinate		261
Cypermethrin		200
Deltamethrin		128
Alphamethrin		79
Flucythrinate		67
Karate		56
Fenpropathrin		54
Other *insecticides for comparison:*		
	Class	
Diflubenzuron	IGR	4640
Malathion	OP	1375
Carbaryl	carbamate	500
Diazinon	OP	300
Chlordane	OC	250
Chlorpyrifos	OP	135
Dichlorvos	OP	56
Parathion	OP	3
Aldicarb	carbamate	0.79

SOURCE: Agricultural Chemicals, Book 1, Insecticides, W.T. Thomson, (cd.) (Fresno, CA Thomson Publications, 1986).

Figure 4.–Separation of Pyrethroids on GLC



SOURCE: Hill, Kenneth, Agriculture Research Service, U S. Department of Agriculture, Beltsville, MD.

determination step. If resolution of the isomers is desired, then high-quality capillary column GC must be used. Some success along this line has already been achieved with special large-bore capillary columns as demonstrated by a typical gas chromatogram showing the separation of four pyrethroids in a fortified animal-fat extract (12). The success of the pyrethroids as agricultural insecticides will likely lead to new structural variations in the future with even more enhanced stability and activity (figure 4).

The Utility *Of* Pesticide/Pest Chemical interactions

Many pesticides act by inhibition of an important enzyme system. In those cases where the mode of action is well defined and the activity of the enzyme can easily be determined, a method of analysis based on enzyme inhibition may be very useful as a screening technique. For example, organophosphate esters or carbamates inhibit the enzyme cholinesterase, which is responsible for the hydrolysis of the neurotransmitter acetylcholine. Rapid assays have been based on calorimetry or radioactivity as a measurement of the extent of reaction. For assay, acetylcholine chloride is used as a substrate to determine the activity of cholinesterase in a sample (blood, tissue, etc.) The reaction produces acetic acid, which can be detected by an indicator dye or, using 14C-acetylcholine chloride, by determining residual radioactivity in the sample after removing acetic acid by evaporation. Indicator papers are commercially available for field tests of insecticides that inhibit cholinesterase. Such tests are useful for screening and indicate the presence of one or more compounds of the general class, Although other types of enzyme inhibition may be common to classes of pesticides, and methods of analysis based on these reactions are feasible, they have not been widely exploited or passed into regular analytical use for pesticide determination.

A method of analysis for chemicals affecting insect behavior involves the detection of pheromones by isolated insect antennae. Since this method offers unique selectivity, it has been used as the basis of gas chromatographic detection (4). The method depends on specific recognition of a complex organic compound by a biological receptor site. Recognition of an organic molecule by a specific receptor is also the basis for the immunoassay techniques, which depend on the interaction between a pesticide and a complex antibody. The production of antibodies capable of recognizing individual pesticides or groups of pesticides is being rapidly exploited, and immunoassay techniques are currently available for qualitative analysis and quantitative determination of pesticides. The ability to recognize a class of pesticides renders this tech-

nique extremely suitable for screening. Its advantage is that it relies on some degree of correspondence between the biological site (on the antibody) and the pesticide, whereas many enzyme systems function in *situ* and the site of action of a pesticide in a linked series of processes may be difficult to define or isolate for use as a basis for an analytical technique.

A further example of a system that may be useful for screening purposes is the ability of many herbicides to inhibit photosynthesis. This activity may be correlated with inhibition of the ability of cell-free plant extracts to catalyze a light-dependent evolution of oxygen in the presence of an acceptor such as ferricyanide, a process known as the Hill reaction (8). A variety of herbicides inhibit the Hill reaction (ureas, triazines, uracils, dinitrophenols, diphenylethers, pyrimidones, carbamates, anilides, etc.), and such a reaction may have analytical utility as a screening tool.

Pesticide/pest chemical reactions may be useful in the future as part of a screening system to indicate the presence or absence of one or more of group of analytes. A procedure that demonstrates the presence of one or more of a very wide range of compounds could provide a useful screen to indicate which samples should be selected for further analysis.

The value of cholinesterase inhibition as a rapid field method is well accepted, but new approaches are needed to combine biochemical and analytical thinking in devising procedures that will provide potential for both broad screening and quantitative, specific detection of analytes. There is an indication that some biosensor techniques can meet the latter need, but at present, biosensors are primarily developed to address specific problems of substrate analysis. A sensor that could respond to each individual member of a group of analytes still remains beyond practical limits.

Methods based on biological properties (immunoassay and enzyme inhibition) are likely to find application in rapid screening of samples in order to eliminate negative samples prior to instrumental analysis in a laboratory. Biological methods will be unlikely to provide satisfactory multi residue methods for the following reasons: 1) They are not sufficiently selective to distinguish members of family groups (enzymatic methods); or 2) They maybe too selective (immunoassay) and therefore will require a separate reagent for each of the thousands of possible pesticides, degradation products, and metabolites. However, highly automated procedures would permit rapid screening for perhaps a few hundred selected compounds.

Conclusions

A number of major concerns have dominated the design of new pest-control chemicals. Predominant among these is the combination of optimized biological activity against target species with minimal acute or latent toxicity toward other organisms. To accomplish this and reduce the possibility that pest resistance may rapidly render the product worthless, approaches to the discovery of pest control chemicals now proceed with a greater understanding and regard for modes of action and metabolism. In recent years, the major advances in techniques by which structure-activity relationships are investigated and interpreted have also been reflected in product chemistry, where the producers now offer new chemicals that may be pure isomeric or optically active forms. Progression from the use of relatively crude materials containing many isomers or related impurities (e.g., toxaphene) applied at rates of several pounds per acre to chemicals that are highly purified and are active at a few ounces per acre has significant impact on the work of the analyst because, in the future, residues from field use will generally be much lower.

Initially, the major problem for the regulatory analyst is the question of tolerance, and it is to be expected that reductions in rate of use will be beneficial if corresponding median lethal dose (LD50) values remain high. Although absolute sensitivity is not a factor in the analysis, it is to be expected that tolerances will be reduced as methods improve.

There are many new approaches to control of pests, and these will continue to gain ground at the expense of chemical control. However, pest-control chemicals are significant in an international market. Their use will continue, and there will be increasing diversity of chemical structures as molecular architecture is varied and refined to combine biological activity with the reduction of adverse effects on nontarget organisms and the environment. Although the range of structural types is increasing, compounds currently being developed do not appear to present insuperable analytical difficulties. The major problem is the increase in number and variety and the proliferation of structures that differ only in detail (for example, the pyrethroids), thus calling for more sophisticated separation techniques.

Multiresidue procedures appear to be adaptable to many new chemicals, and it is now required that the performance of new chemicals in standard MRPs be investigated as part of the registration process. This information is of great assistance to

the regulatory analyst and it is important that it be made readily available. However, as the number of potential matrix/compound interactions increases, so the difficulties of the regulatory analyst will also increase. The parent compounds can usually be recognized by MRPs, but the type and variety of metabolic products from a single pesticide may obviate their determination in a single MRP, or even in any MRP. To simplify this problem, Frehse (10) has proposed that a single indicator compound should be selected to represent the residue of a certain pesticide and its metabolizes. It was suggested that the concentration of the indicator compound should bear a known relationship to the concentration of the toxicologically significant residue; in addition, the indicator compound should be available as a standard, be recoverable in MRPs, and sufficiently stable for reproducible analysis. The concept of indicator compounds is a useful one and is one that could be included in the framework of efforts to bring about international harmonization of maximum residue limits.

The simplification of approaches to the problems of the regulatory analytical chemist was also advocated by Frehse (10), who described a three-step system proposed by Westlake and Gunther (32). The first stage involves screening for given constituents to previously established limits of detectability. The second stage consists of screening to discriminate samples that are above tolerance from those below. The third stage is that of quantitative analysis. Clearly, current screening methods for groups of compounds, such as immunoassay, are capable of providing much information and could form part of a tiered analytical procedure.

The major obstacle to improved multiresidue methods is still the labor intensive extraction and cleanup procedures required. The initial stages of analysis involving the selection of a representative sample, extraction, and cleanup of the extract are critical and also time-consuming. Much more research is needed in automation and robotics to increase throughput and reduce per-sample costs for the conventional approaches. Not much research effort has yet been expended on techniques for eliminating cleanup steps, although direct injection of extracts without extensive cleanup was reported as long as 17 years ago for organophosphorus compounds (6, 7).

The introduction of synthetic organic pesticides was followed closely by the rapid development of gas chromatography in the early 1960s. Element specific detectors simplified residue determination for such compounds as the organochlorine, organophosphorus ester, and carbamate insecticides. Procedures for extraction, solvent partitioning, purification, and determination have evolved, but in the past two decades there have been remarkable advances in the performance of columns for gas or liquid chromatography. Identification of specific residues has been made easier by combination of liquid or gas chromatography with mass spectrometric detection. Nevertheless, a variety of compounds remain outside the capability of MRPs and the analyst must resort to special procedures. Highly polar or water-soluble materials often present difficulties and must be converted to lipid-soluble derivatives. Unfortunately, many metabolites belong to this category and cannot be included in general MRP procedures. There is no simple generalization to describe new compounds appearing on the market, and there may be difficulties if polar or thermally unstable compounds must be analyzed. Lower rates of application are to be expected in the future because the design of biologically active molecules can more readily be optimized. If toxicity is extremely low, there may be fewer residues of significance, but analytical needs will still exist. Simple, rapid, and sensitive screening methods will be essential to indicate whether further analysis of samples should be undertaken, There is a critical need for such methods to reduce the burden on the regulatory analyst.

There is little reason to believe that the necessity to continue to develop and apply MRPs will decrease in the next two decades. Agricultural chemicals will continue to be used worldwide and it is important to protect the quality of produce reaching the consumer. However, it is important to increase effectiveness and reduce costs of current methods and some priorities should be allocated; among these the following may be considered:

1. Current MRPs will probably be adaptable to many new chemicals entering the market. However, analysis of closely related isomers will require improved separation techniques, and the potential requirement to determine residues at the parts-per-billion level will demand more sensitive detectors.
2. Sampling, extraction, and cleanup procedures are generally time-consuming and expensive in terms of solvents, etc. The application of automated techniques may avoid some labor costs, but new technology is needed.

3. Rapid methods for screening that require no processing or minimal processing of the sample would be extremely valuable, particularly if they were applicable in the field.
4. Methodology is needed that is applicable to separation and analysis of macromolecules or complex molecules of biological origin that might be involved as new active principles in future pest-control technology.

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Validation of Analytical Methods for Pesticide Residues and Confirmation of Results

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Contents

Abstract

Decisions by governmental agencies based on analytical data on pesticide residues in food can have a significant impact on public health and other socioeconomic factors. It is therefore essential that this data be of the highest quality and generated through the application of validated methods inconjunction with a well-designed quality assurance (QA) program.

Details are given of the varying degrees of validity achievable for analytical methods. These can range from validation within a single laboratory up to the demonstration of satisfactory performance in a collaborative study conducted and evaluated according to the guidelines established by international standards-setting organizations such as the AOAC.

The main problems associated with the development and utilization of collaboratively studied methods in pesticide surveillance and compliance activities relate to the plethora of possible pesticide/commodity combinations and the daunting task of devising and conducting collaborative studies of methods to handle such situations. Some details of the QA program in effect in the Canadian Health Protection Branch to ensure the production of valid analytical data are also presented.

Among a variety of other responsibilities, the Health Protection Branch, Health and Welfare Canada, is accountable for ensuring the safety of the Canadian food supply–one aspect of which is the control of pesticide residues in food. The branch fulfills this responsibility by *(1)* establishing maximum residue limits (MRLs) for pesticides (and their metabolizes) in foods, and (2) establishing monitoring programs to ensure compliance with these MRLs and to assess the presence of pesticide residues for which no provision exists in the Canadian Food and Drug Regulations,

The analytical data generated in these programs form the basis of decisions regarding compliance that can have a considerable socioeconomic impact. It is therefore important that the data be of the high-

est quality. To this end, an intensive quality assurance (QA) program is in place for pesticide residue analysis throughout the branch (12). Similar QA programs are in place in other Federal agencies (Canadian Department of Agriculture, U.S. Department of Agriculture, and U.S. Food and Drug Administration) with responsibilities for ensuring the safety of the food supply in North America. The Association of Official Analytical Chemists (AOAC) has also recently published a handbook in this area (15) that has drawn extensively on the procedures and practices of the aforementioned and other organizations.

Although there are many critical elements in these QA programs, there are two that refer to the analytical aspects of the validation process:

1. Development and/or use of appropriate validated methods.
2. Use of appropriate quality control systems to ensure the production of valid data.

It is particularly important to note that the use of a validated method, although necessary, is not in itself sufficient to ensure the production of valid data. Quality assurance of the measurements on an ongoing basis is also required.

Development of Validated Analytical Methods

Validation has been defined (17) as the process of determining the suitability of methodology for providing useful analytical data.

There are several steps in the process of developing and of demonstrating the validity of an analytical method. These steps can be conveniently broken down into the three stages outlined in figure 1.

As one proceeds from stage 1 to stage 3, the degree of confidence that one can ascribe to the validity of a particular method increases. Stage 3 represents what is generally accepted (2, 3, 8, 16) to be the highest degree of method validation, i.e., successful performance in a collaborative study conducted according to the guidelines of recognized international standards-writing organizations, such as the AOAC (9).

Figure 1.—Stages in Method Development and Validation

Stage 1. Estimation of acceptable performance parameters within a laboratory.
Stage 2. Demonstration of successful performance in limited interlaboratory studies.
Stage 3. Demonstration of successful performance in recognized collaborative study.

The main parameters, referred to in stage 1, that should be taken into account have been identified in several papers (2, 8) and include accuracy, precision, specificity, limit of detection, limit of determination, linear range, and scope. While these parameters have also been thoroughly discussed in these publications, it is considered important to reiterate them here, particularly with reference to the determination of pesticide residues.

(i) Accuracy–a measure of how closely the determined value (generally expressed as the mean of several determinations) approximates the true value of the analyte. This is best supported by the analysis of standard reference materials; however, the availability of such materials, especially for pesticides in foods, is generally extremely limited. Normally the recovery of added analyte to "blank" samples of the commodity in question, over an appropriate range of concentrations, is taken as an indication of accuracy. For pesticide compliance work, the concentration range chosen should certainly bracket the MRL. It should also be recognized that analyte added to a field sample may behave differently (typically showing higher recovery) from field-incurred residues. For analysis at the ppb/ppm level, recoveries of 70 to 120 percent are generally considered acceptable.

(ii) Precision–the total interlaboratory precision, or reproducibility, is the most important aspect of precision because it is a measure of how much allowance should be made for between-laboratory variability in interpreting results produced by different laboratories. It is possible, however, to have a measure of one component of this, the within-laboratory precision, or repeatability, by multiple analyses of samples in the same laboratory over a short time-period. The reproducibility coefficients of variation (CVS) should fall within the range estimated by Horwitz et al. (7) with the repeatability components being somewhat lower, generally one-half to two-thirds of the former. For example, at a pesticide residue level of approximately 1 ppm, the expected reproducibility CV is approximately 16 percent and the repeatability CV, approximately 10 percent. Similar values have been found by Smart (16) in an examination of UK collaborative studies on pesticide residues.

(iii) Specificity-the ability of the method to measure only what it is intended to measure. In any method, it is absolutely *essential to* run *reagent and* field blanks to ensure no interfering compound, or indeed none of the analyte itself, is present. These blanks should be run for each commodity examined. To verify the identity and amount of an analyte, it

has been suggested that the ideal approach is to utilize two entirely different analytical methods, based on different analytical principles (1). However, the availability as well as the characteristics of such methods often place a practical limitation on the application of this suggestion. Thus in the pesticide area, advantage has been usually taken of the following confirmatory techniques:

a) Mass spectrometric confirmation of identity.
b) Use of different detector, i.e., operating under different principles such as Coulson vs. Electron Capture.
c) Chromatography using different systems.
d) Chemical reaction followed by analysis.

More detailed descriptions of such techniques can be found in reviews by Cochrane (5) and by Lawrence (13).

In the past, these confirmatory techniques have been generally qualitative in nature and have been used by the analyst to give reassurance that the validated method was in fact measuring the residue that it was intended to measure. Since only the qualitative aspects were sought, such techniques were not required to be subjected to the same rigorous assessment as were the validated methods.

Now, with the availability of the smaller, more affordable benchtop-type mass spectrometers, the emphasis is moving toward quantitative mass spectrometric confirmation. This necessitates much more detailed study of the confirmatory technique.

(iv) Limit of Detection–the lowest concentration of an analyte that the analytical process can be reliably differentiated from background levels. This has been defined as the level (background level) measured in the field blank plus 3 standard deviations (2, 3).

(v) Limit of Quantitation (LOQ)–The lowest concentration of an analyte that can be measured with a stated degree of confidence. This has been defined as the level measured in a field blank plus 10 standard deviations; however, it is recommended that this value be established in the laboratory by repeated analysis of appropriate samples (spiked or endogenous). In collaborative studies, the LOQ of the method should be considered as the lowest level successfully analyzed in the study. Collaborative studies have in fact been used to establish the LOQ (14).

(vi) Linear Range–this is generally taken as the range over which the procedure has been demonstrated to give a linear response, A reproducible non-linear response, which is the case with certain immunological procedures, can also be acceptable.

(vii) Scope–the scope of a method refers to the number of substrates and the number of analytes to which the procedure can be successfully applied.

Which of these seven factors is the most important depends on the purpose for which the data will be used. In the Canadian Food and Drug Regulations, there is a general MRL of 0.1 ppm to cover pesticides for which MRLs have not been established. Thus, in the Health Protection Branch, in selecting methodology for surveillance and compliance programs, a major effort is directed toward the development and validation of methods with acceptable values for accuracy and precision down to the 0.05 ppm level.

A higher degree of validation (stage 2) for an analytical method can be obtained by participation in internal (to the organization) or external check sample programs.

Within the Health Protection branch, certain procedures have evolved over the years to validate the methodology. These have included the exchange and analysis of individual (generally violative) samples among branch laboratories, and the distribution and analysis of a variety of check samples. The latter have usually been distributed in connection with the emergence of certain contentious issues, such as the recent ethylene dibromide problem, but plans are underway to increase the frequency of check sample distribution during normal monitoring programs, For example, a check sample program underway at present involves the distribution of two commodities, each containing two different levels of 1,1-dimethylhydrazine (UDMH) to three Health Protection Branch laboratories. This study will serve to validate the methodology recently developed (18) for UDMH.

Undoubtedly the major external check sample program in which branch laboratories participate is the Federal Interdepartmental Committee on Pesticides check sample program, details of which have been given in a recent paper (6). The present program outline is shown in table 1.

As mentioned previously, the highest degree of validation (stage 3) for an analytical method is the demonstration of its performance in a successful collaborative study. Current AOAC guidelines (9) require the successful analysis of at least five samples in six laboratories. The collaborative study approach not only demonstrates that the method can be applied successfully in several laboratories but that it can also withstand an objective, rigorous peer-review process,

However, a collaborative study generally demonstrates validity for only those commodities and those analytes included in the study—a fact that presents a major problem in the area of pesticide residues because of the large number of pesticide/commodity combinations possible. This, together

Table 1.-FICP Check Sample Program Outline

Sub- Program	Substrates	Distribution [a]	Pesticides
Soils	Soil	3	2,4-D
			Picloram
			Atrazine
Foods	Tallow	4	Captan
	Strawberries		Iprodione
	Potatoes		Carbofuran
			Chlorophenols
Water	Standards		Common OCs
	Sediments, Water		Phenoxy Acids
Fish	Fish, Eels, Cod Liver Oil	6	DDE, Mirex
			PCBs
Forest Substrates (Insecticides)	Fish, Soil		Pirimicarb
	Balsam Fir Needles		Aminocarb
			Mexacarbate
			Carbaryl
Forest Substrates (Herbicides)	Soil		Hexazinone
Wildlife	Herring Gull Lipids and Homogenates		DDE, Mirex, PCBs
			Heptachlor Epoxide
			Chlordane
			Oxychlordane
			Dieldrin
Feeds	Grains		Trial late
			Malathion
			Carbathion
			Permethrin
			Lindane
			Chlorpyrifos

[a] Numbers of check sample projects conducted in last 5 years

with the wide range of MRLs, would render the design and conduct of collaborative studies to cover all possible combinations a most formidable task that cost alone would surely doom to failure.

An excellent example of these difficulties and the approach taken to resolve them can be obtained from consideration of a recent AOAC collaborative study conducted by Krause (10) of a multiresidue method for the determination of N-methyl carbamate insecticides and related metabolizes in crops, and a subsequent publication by the same author (11).

The collaborative study involved the determination of seven methyl carbamates and two carbamate metabolizes at two levels in two crops: grapes and potatoes. This study proved extremely successful and was adopted Official First Action by AOAC. Nevertheless the collaborative study had only included two commodities and therefore the method was only validated for these commodities.

To extend the scope of an Official AOAC Method, a mini-collaborative study can be required demonstrating that the performance parameters generated in the main study can be met with the additional commodities and/or analytes. In Krause's study, the method had been initially studied successfully by four laboratories in an interlaboratory trial on lettuce, in effect a mini-collaborative study, thus permitting the scope to be extended to include lettuce.

The main question relates to what is required to extend the scope of the official carbamate method to include other carbamates and other commodities. In his subsequent publication (11), Krause describes recovery values obtained over a 3-year period in four FDA laboratories for seven parent carbamates and five carbamate metabolizes added to 14 crops. These data were obtained as part of the in-laboratory quality assurance programs. In many cases, the recoveries obtained were similar to those obtained in the collaborative study. Whether this data is sufficient to further extend the scope of the Official Method depends on its evaluation by the relevant AOAC committee. In the author's opinion, some form of interlaboratory study would be preferable for this purpose.

Similar situations exist with the other multiresidue screening methods for pesticides.

The stage to which validation should be taken depends to a large extent on the use to which the data will be put, on the urgency with which the data is

required and, indeed, on the operational structure and philosophy of the organization involved.

In general, regulatory agencies, when faced with important compliance decisions, wish to have data of the highest quality. There is therefore a preference for fully collaboratively studied methods (stage 3) or, at a minimum, methods that have been subjected to some form of interlaboratory study (stage 2).

However, if the objective of a survey is simply to assess if a problem exists, a method in the stage 1 category can readily be used. Even in such cases, agencies with several field laboratories involved in generating the data generally undertake limited interlaboratory assessment (2 or 3 check samples) prior to the survey,

The main drawback to the collaborative process is the length of time required from initiation of the study to the stage where the method is given official approval. At present, within AOAC, this takes a minimum of one year. Thus in situations where the data is required on an urgent basis, and collaboratively studied methods do not exist, many agencies resort to the use of methods validated to a lesser degree.

Quality Assurance of Data

It cannot be stressed enough that the adoption of, and strict adherence to, a sound quality assurance program is essential toward the production of valid analytical data. Within the Health Protection Branch, an important part of this whole QA program is the use of appropriate quality control systems in conjunction with validated methods to ensure the production of valid data. The quality control systems, which include the confirmation of results and the reporting limits required, vary depending on the nature of the program. The national surveillance and compliance programs generally have the highest level of quality control.

The national surveillance program is designed to determine the state of compliance of selected food commodities in the marketplace with respect to specific pesticides. The pesticides are divided into high, medium and low priority groups, and emphasis is placed first on the high priority group. All laboratories involved must ensure that these pesticides can be determined by the general screening methodology, or by specific methods, (4) by analyzing samples spiked with a mixture of pesticides at a minimum frequency of 1 in every 20 samples. Commodities used as the spiked sample are required to be varied throughout the year, and all high priority pesticides must be included in the spiking mixtures at least once per year at, or below, the MRL. If these recoveries are less than 70 percent or if the limits of quantitation are greater than one tenth the MRL, or 0.05 ppm (in the case of the 0.1 ppm MRL), it is concluded that the particular chemical/commodity cannot be handled by the methodology and/or the laboratory in question, and steps are taken to investigate and correct the situation. The medium and low priority groups of pesticides are included as time permits.

The confirmation techniques and reporting limits for the surveillance program, together with the corresponding requirements for the compliance program for comparative purposes, are summarized in table 2.

Additional confidence in the quality of the data is obtained through continued (and, it is hoped, successful) participation in the internal and external check sample programs.

Table 2.—Confirmation Techniques and Reporting Limits for HPB Surveillance and Compliance Programs

Program	Concentration of residue (ppm)	Report	Confirmation technique
Surveillance	<.01 (or quantitation limit, whichever is higher)	no report required	
	>=.01 - < 1/2 MRL	1 significant figure	1/10 of specimens by level 1• or by GC/MS
	>/112 MRL - MRL	2 significant figures	level 2** • or GC/MS
	> MRL	2 significant figures	GC/MS
Compliance	< 1/1O MRL	no report	
	> 1/10 MRL (or 0.0 ppm, whichever is higher	2 significant figures	GC/MS

"level 1 = quantitative agreement between 2 columns/2 detectors
" ● level 2 = level 1 plus an additional column or detector, derivative, or other technique

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Conventional Pesticide Analytical Methods: Can They Be Improved?

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Contents

Figures

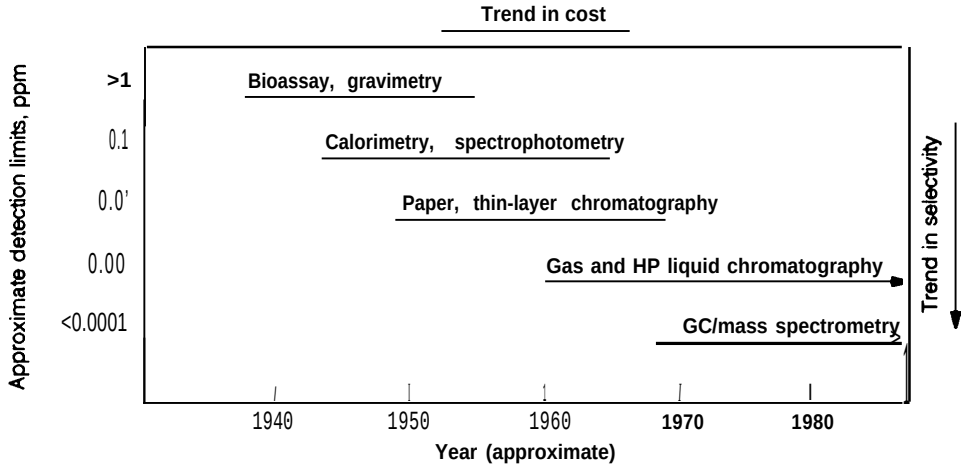
Abstract

Pesticide multiresidue analytical methods have been continually improved and expanded over the years. Further improvements are possible based upon recognition of the limitations of existing methods and their modification with new sample-handling and instrumental techniques. For example, the use of solid phase extraction (SPE) cartridges in place of liquid-liquid extraction and, perhaps, Florisil column fractionation might allow for miniaturization, smaller solvent volumes, extended breadth of applicability, and greater throughout when integrated into existing multiresidue schemes. Wide-bore capillary gas chromatography columns can eliminate the need for some derivatizations and, when interfaced with autoinjectors and integrating data systems, can improve throughput and data quality. High performance liquid chromatography can be used for fractionation and also for determination of compounds (including some new classes of pesticides) that can not be gas chromatographed without derivatization. Mass-selective detection (GC/MS), particularly in the selective ion mode, can improve detection limits and the accuracy of analytical results. These types of potential improvemerits will require coordinated research involving academic, industrial, and regulatory laboratories, including new levels of funding for the academic and regulatory sectors. The importance of academic involvement can not be overemphasized because of the need to attract a new cadre of well-trained young scientists into the residue analytical field.

Introduction

The field of trace analysis, including pesticide residue analysis, has made tremendous advances in terms of selectivity and detection limits (figure 1) (9). In the 1940s and early 1950s, gravimetric and bioassay techniques were the mainstays in ''trace" analysis, extending detection limits to the then-frontier levels of about 1 ppm. These were time-consuming methods, lacking in compound selectivity but broad-based in terms of responding to whole classes of chemicals. Calorimetric and spectrophotometric methods held sway through the 1950s and early 1960s, providing improvements in both detection limits and specificity. Many of these,

Figure I.-The Evolution of Analytical Methodology for Organic Toxicants in Environmental Samples



SOURCE: J N Seiber, *1982 Analysis of Toxicants in Agricultural Environments*, Genetic Toxicology, R A Fleck and A Hollaender (eds.) Plenum, NY, pp 219-234

such as the Sheeter-Hailer method for DDT and Averill-Norris method for parathion, involved extensive derivatization because they required that a visibly colored product be formed even when the parent compound was colorless (as was the usual case), The inroads of chromatography began roughly in the 1950s with paper and thin layer chromatography (TLC), and for the first time, chemists were able to resolve in a given sample several individual chemicals using a single technique without extensive sample preparation-derivatization. Paper chromatography (PC) and TLC were essentially qualitative techniques, best used to screen samples for the presence or absence of specific compounds. Clinical chemists interested in drug analysis, natural-products chemists interested in plant secondary products, and pesticide residue chemists quickly adopted these chromatographic techniques. At present, gas liquid (GLC) and high performance liquid chromatography (HPLC) techniques have largely supplanted (but have not completely eliminated) PC and TLC. These are resolution techniques par excellence, with the added dimension of quite precise quantitation made possible by very sensitive and often highly selective detectors. A few of these, such as the thermionic detector, were developed by pesticide residue chemists while others (electron capture, microcoulometric) were popularized just for the field of pesticide analysis. The now common use of mass spectrometry (MS) coupled with GC provides detection limits to 1 ppb routinely, and it adds the dimension of near-absolute confirmation of residue identity when somewhat higher residue levels are encountered. These achievements in sensitivity and selectivity have been costly, such that equipping a modern laboratory for a broad spectrum of trace analyses requires considerable capital—several GCS and LCS plus mass spectrometry capability. Figure 1 shows the interplay, tradeoff, and trends in a very general way for the analytical transition from the 1950s to 1980s. Figure 1 omits the important point that many analyses are now possible that were not possible in the 1940s and 1950s, examples being provided by volatile halogenated organic compounds (VHOC) in drinking water and pesticide multiresidue analysis in foods.

While these sophisticated methods have revolutionized trace analysis in many respects, the analytical process itself has not changed materially in that all analyses conform to basic steps, or unit processes, which vary little regardless of the application. These steps include extraction to remove the analyte from the bulk matrix, cleanup of the extract to remove potentially interfering coextractives, modification or derivatization to change the analyte to a more readily determinable form (an optional step), and resolution to separate analyte or a derivative from other chemicals remaining in the prepared sample. The elements of concentration, removing a few micrograms of analyte from several grams or kilograms of sample substrate to a small volume of solvent, and purification, isolating one or a few specific compounds from the thou-

sands present in the raw sample, run through these steps. The determination phase includes detection —obtaining a response related to the structural feature and amount of analyte; measurement—relating the response to a reference standard of the chemical of interest or a close relative; and confirmation—assuring that the measured response is indeed due to the analyte and not an artifact or imposter. This time-honored strategy takes advantage of physical and chemical properties unique to the analyte or analyte class: properties of volubility, polarity, volatility, reactivity, and interaction with electrochemical, optical, ionization, or other detectors. Generally, the more properties built in to the analytical scheme, the more selective and sensitive the analysis. A corollary is that short-cut methods are often less selective and sensitive, and thus they place more demands on the detection and confirmation instrumentation.

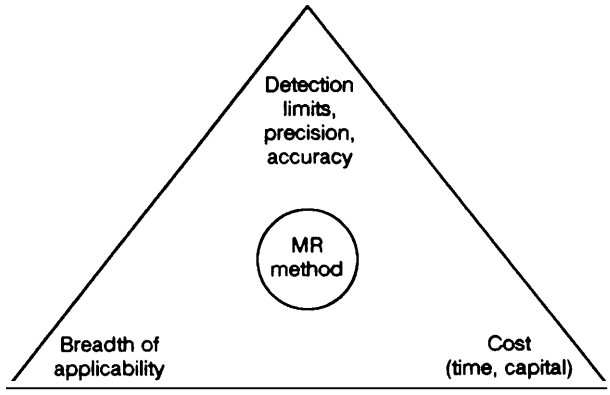
The tradeoffs involved in selecting methods can also be seen in the following types of analysis:

Qualitative (Screening)–What is present or absent.
vs.
Quantitative–How much is present.
Multiresidue—Capable of measuring many chemicals in a given sample.
vs.
Specific—Tailored to just one or a few chemicals (e.g., a single parent pesticide plus its major metabolizes).

Regulatory agencies will require and routinely use multiresidue pesticide screening techniques because they need to ascertain the presence (or absence) of many chemicals in a given sample of unknown origin. Chemical companies, on the other hand, need specific and quantitative methods to determine the residue distribution and dissipation of their own specific pesticide products in a given sample set, in connection with EPA registration requirements or their own need to know.

The dilemma in multiresidue methods may be stated as follows: The method must cover a broad waterfront of chemical types and matrices. In so doing, however, the science of the method, reflected in the analysts' prime quality control characteristics—detection limits, precision (reproducibility), and accuracy—becomes diluted. Very often, costs (both capital and labor) increase as breadth increases. These tradeoffs, summarized in figure 2, occur with present-day methodology and thus are responsible, at least in part, for the compromise nature of existing multiresidue methods. But must it be this way? Are there approaches yet to be found that will combine low costs and broad applicability with good science? If so, will they involve modifying methods that now exist (based largely on GC and HPLC with selective detection) or instituting whole new technologies (MS-MS or immunoassay)?

Figure 2.-Tradeoffs in Multiresidue Methods



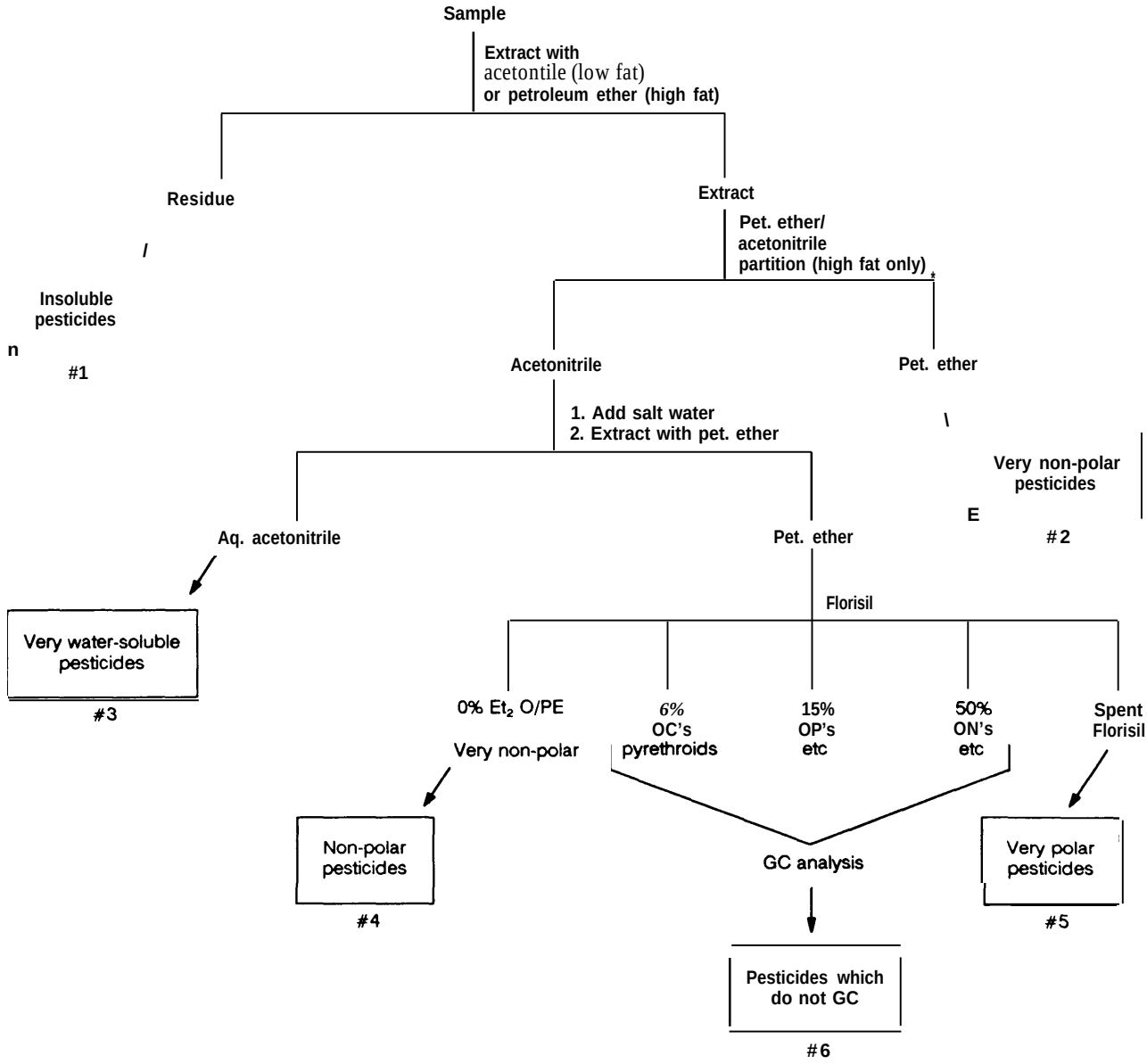
SOURCE: James Seiber, University of California, Davis, CA, 1998

In order to answer these questions it is useful to look at a few leading multiresidue methods in terms of their advantages and limitations; in essence, to find their "pressure points" that are amenable to incremental (or drastic) improvement.

Pressure Points in Multiresidue methods

Every analytical method has inherent limitations that may or may not be amenable to manipulation. As an example, the PAM (Section 201) Mills procedure (figure 3) represents a "middle of the road" method optimized to provide quality data on pesticides of intermediate polarity and volatility. It has at least six points where losses of individual pesticides may occur. For example, paraquat is insoluble in acetonitrile and petroleum ether and is thus lost in the first step (#l). Aldrin has an unfavorable petroleum ether / acetonitrile partition coefficient and is thus partially lost in the discard of the petroleum ether when high fat samples are processed (#2). Very water-soluble pesticides, such as some organophosphates, maybe lost in the back extraction from aqueous acetonitrile to petroleum ether (#3). Very nonpolar and very polar pesticides, which survive to the Florisil cleanup, are further lost because they elute prior to (#4) or after (#5) the three prime ethyl ether / petroleum ether fractions. Of course, some pesticides, including several N-methylcarbamates, degrade on Florisil, adding another limita-

Figure 3.- Mills Procedure (PAM)



SOURCE: James Seber, University of California, Davis, CA, 1988

tion to the Mills procedure. Finally, success in this procedure depends on gas chromatography of the cleaned residues, so that non-volatile materials that do not elute or degrade upon GC (#6) as well as those showing poor response to the common selective detector will not be determined.

Thus, in essence, only pesticides within a range of polarity, volatility, and stability characteristics will survive the Mills determination. In fact, that number is about 200 (in fatty foods) or 274 (in non-fatty foods) for pesticides, transformation products, metabolizes, etc. (5). Some pesticides are not recovered at all, or only partially, because of failure in one or more of the steps described above. Limitations exist in all multiresidue analytical methods, so that several pesticides simply "fall through the cracks" in Federal/State regulatory monitoring programs (l). How can the Mills method be expanded?

One approach is to alter or eliminate the limiting steps. For example, loss in the discarded aqueous acetonitrile phase (#3) could be minimized by using a stronger solvent (ethyl acetate or methylene chloride) than petroleum ether in the preceding partition. The tradeoff here could be the appearance of more interfering material in the final extract, which might raise detection limits. Alternately, this entire partition could be eliminated and the acetonitrile concentrated directly for Florisil cleanup. The tradeoffs here might be increased detection limits and lower recovery of some volatile pesticides (v.p. > 10-3 Torr) because concentrating acetonitrile is more difficult than concentrating petroleum ether. Finally, one might substitute a solid phase extraction (SPE) cartridge for the liquid/liquid partition, providing recovery of a slightly broader range of compounds and reduced volatility losses—the latter because the SPE elution solvent volume is much less than that used in liquid/liquid partitioning. Whether the SPE raises or lowers the detection limit would need to be determined experimentally.

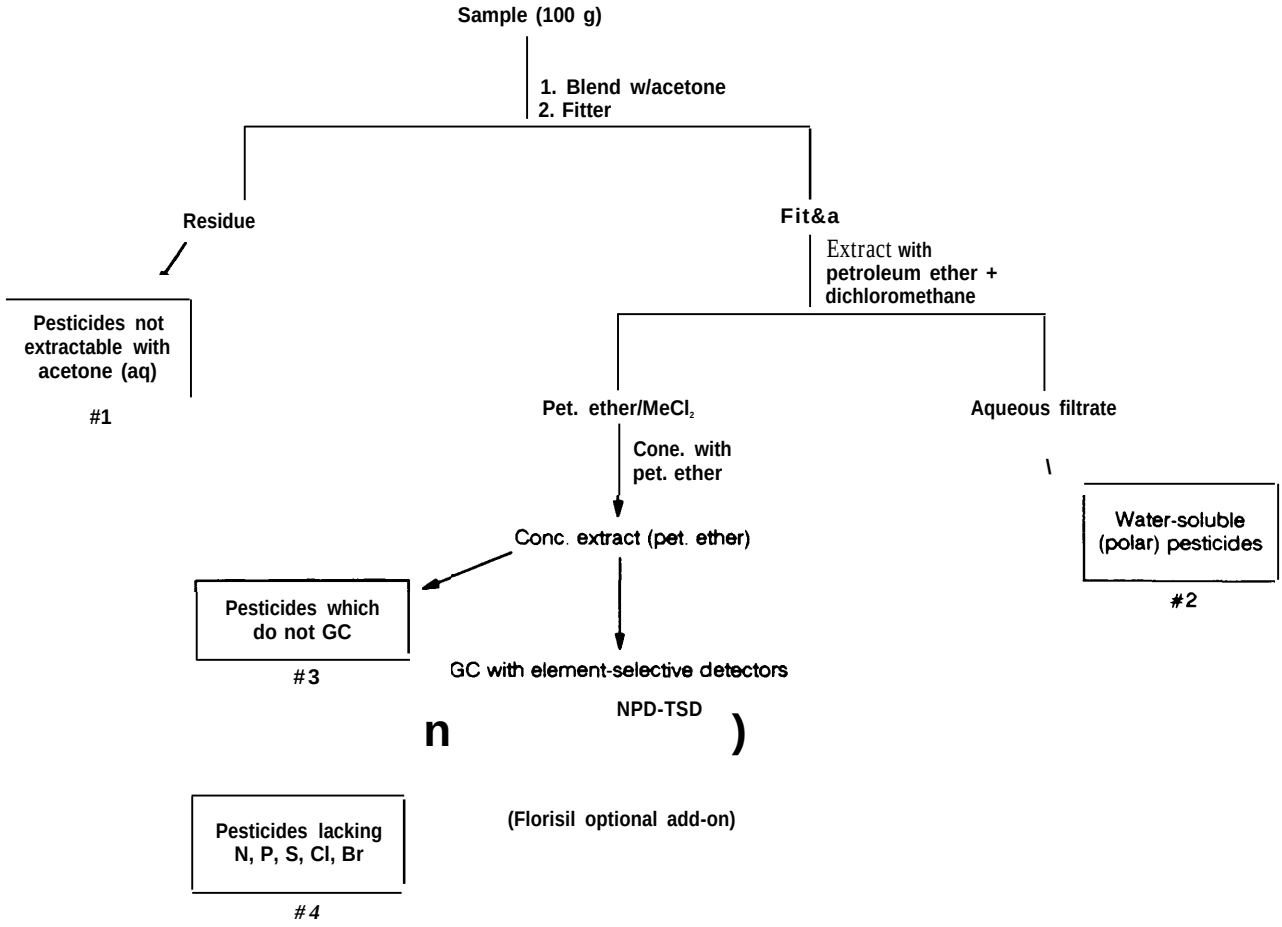
One could thus critique each step in the Mills procedure, optimize to enhance breadth of applicability while maintaining acceptable detection limits, and in some cases (such as SPE substitution) perhaps achieve savings in time. Our own experience with the use of SPEs to isolate metabolizes of organophosphorus pesticides from urine (13) would tend to support the time-savings notion, particularly because several samples (up to 10) maybe extracted simultaneously by SPE. Manufacturers are offering gadgets built upon the SPE concept that facilitate simultaneous extraction and elution as well as concentration of eluate, providing overall savings of considerable time and amounts of solvents.

The Florisil cleanup can be attacked in several ways; miniaturized columns are already in wide use for relatively clean substrates (many non-fatty foods), thus reducing solvent volumes, and elution and evaporation times. Deactivated silica gel can be substituted for Florisil to alleviate the breakdown of certain compound classes on Florisil, HPLC cleanup methods could be substituted, allowing for expansion of the range of polarities that could be accommodated, minimizing solvent volumes, and alleviating breakdown. HPLC also has the ability to be automated, which is difficult with gravity column chromatography. For example, gel permeation column cleanup has been automated and commercialized for use with fatty foods (PAM). Finally, the Florisil cleanup step could be eliminated entirely so that the extract normally entering this cleanup would go directly to GC determination. The tradeoff here is increased detection limits (because more "garbage" enters the GC), decreased GC column life (same reason), and increased chance for misidentification and misinterpretation (because the Florisil fractionation works to simplify chromatograms and help in result interpretation). However, the large time-saving provided by Florisil elimination, in such procedures as Luke (PAM Section **232.41,** Figure 4), has led to reduced use of it for multiresidue screening. Note also that the Luke method still provides opportunities for loss of pesticides, but fewer than in the full Mills procedure. As might be expected, the Luke method is applicable to non-fatty foods and can not be used with electron-capture or flame ionization GC detection without prior cleanup. It should also be apparent that various hybrid techniques can be devised in which the extract prior to Florisil cleanup is injected on GC to screen for some chemicals or chemical classes (OPs are most successfully handled) and then subjected to Florisil before looking for other classes (OCs, for example) or for GC/MS confirmation.

As a matter of fact, there are many hybrids and variations of the PAM version of the Mills (and Mills-Onley-Gaither) and Luke procedures, most of which are not published. This is good in that innovations are continually possible, but it introduces some uncertainty in knowing what is the best procedure for a given analysis and in comparing results from one laboratory to another. An in-depth study of innovations already in practice might yield clues that could improve the PAM versions of the multiresidue methods.

For alternate pesticide multiresidue approaches, it might be useful to see what types of extraction and fractionation schemes are used for chemical pollutants other than pesticides, HPLC-based cleanup methods include the silica column used by Wehner et al. (12) for analyzing pesticides in air, which has also been applied to water (14) including fog water (3), The column has a long useful lifetime and maintains its resolution characteristics. It does require periodic calibration with standards to show where fraction cuts should be made, but this is a simple matter of injecting a mixed standard. The polarity range was successfully extended to include polar glycosides (11) and derivatized glyphosate in plant extracts (10) by adding a methyl-butyl ether (MTBE]-THF gradient after the hexane-MTBE gradient. On the negative side, HPLC cleanup requires sample concentration to a very small volume (<0.5 ml) prior to injection on the HPLC, does not tolerate suspended particulate matter, and has a sample throughput of only 1 sample/hr/column, There

Figure 4.-Acetone Extraction Method (Luke)



SOURCE: James Seiber, University of California, Davis, CA, 1988

may be ways of increasing the throughput considerably (very short columns) but these remain to be proven. Also, the 1 sample/hr/column limitation is somewhat misleading because 24-hour operation might be possible if an autosampler were used.

A second HPLC cleanup approach used a cyano column with hexane to acetone gradient. This procedure was developed by Crowley et al. (3) for separating shale oil extracts, and it was used by our group (4) to separate mutagenic constituents of smoke particulate matter. It could probably be used for some pesticide residue analyses requiring fractionation. Other columns/mobile phases could be used as well.

It *is* somewhat interesting to note the diverging paths taken by pesticide residue chemists who have concentrated on column chromatography for cleanup and fractionation, and chemists involved with priority pollutant and drug analyses who have used acid-base partitioning against organic solvents for cleanup and fractionation. For priority pollutants in water, the total scheme uses purging with air or nitrogen to remove volatile pollutants (benzene, chlorinated solvents, etc.) and then acid-organic solvent extraction to separate acids (phenols) from base-neutral chemicals. This scheme would probably not be of general utility for pesticides in foods because (1) few pesticides are volatile or acidic, (2) some pesticides are not stable to acid conditions, and (3) the final base-neutral fraction (which would contain most common pesticides) might not be clean enough for low-level GC or HPLC analysis. The common drug schemes use a more complex acid-base partitioning system, which serves drug analyses well because so many of these agents are bases (alkaloids) or acids (barbiturates, salicylates,

etc.). Thus, the cleanup technologies in these schemes are not applicable to the problem of multiresidue pesticide analyses in foodstuffs, where essentially neutral compounds need to be handled.

Other Stages of Pesticide Analyses

Once a residue-containing extract is provided, with or without cleanup, the steps of resolution, detection, measurement, and quantitation are performed, occasionally after derivatization. The gas chromatography equipped with selective detection based upon the heteroatom (halogen, P,N,S) content of the pesticide analytes is the most common resolution-detection system employed.

Choices here include the following:

Column type —packed vs. capillary, phase choice
Detector type–Flame photometric (S,P)
Thermionic (N,P)
Hall electrolytic conductivity (Cl, Br, N, S)
Electron capture (halogens)

Voluminous literature exists on the applicability of each combination. Suffice it to say, the packed vs. capillary issue is still debated, with more converts to capillary following the introduction of reproducible splitters and megabore columns. These columns provide greater resolution and greater efficiency than packed columns, both of which generally lower detection limits and increase confidence in the results. They also minimize breakdown and irreversible adsorption of the more thermal-labile and polar pesticides, thus increasing breadth of applicability. They do not, however, have the capacity to accept very dirty extracts that might be chromatographable on packed columns. This represents another tradeoff, although in spite of it the technology is clearly leaning toward more capillary and less packed column use.

Regarding column phases, analysts already have a large selection (summarized in PAM Section 301), with the only new developments occurring in adapting conventional phases (or mimics) to fused-silica capillary columns.

Summarizing for columns, high load, bonded-phase megabore columns will suffice for virtually all GC systems and, through improvements in technology that are rapidly emerging, will extend the applicability of GC to even broader ranges of pesticide types, They will also minimize the need for derivatization (a time-consuming and error-prone procedure best avoided if possible) of some phenolic, carbamate, and polar metabolize chemical classes, More work in proving these points will pay rich dividends in improving conventional methods.

In GC detection, many improvements in virtually all detectors have occurred in the past 5 years, and these are being rapidly adopted by residue chemists. They include the following:

1. FPD detection limits have been improved almost tenfold. This is still the most reliable system for OP and S-containing pesticides,
2. Hall-type electrolytic conductivity detectors have improved dramatically and are now clearly the first choice for organohalogen compounds and near first choice for organonitrogen compounds.
3. The thermionic NP-TSD shows steady improvements and represents a viable choice for OP and ON analyses.
4. The pulsed-mode Ni63 EC is a vast improvement over earlier EC detectors and is still useful for some organohalogen compounds, particularly the more volatile ones resolved by capillary GC.

Newer detectors that may supplement the above improvements include the following:

1. Photoionization, particularly for aromatic compounds lacking heteroatans and for some polysulfur and polyhalogen compounds. There is now more than one supplier of this promising detector,
2. Mass selective (MS) detectors, for virtually all compounds,

The mass selective detector, or MS, is worth special note because of its universality, confirmatory power, rapidly improving detectability (particularly in the selective ion or SIM mode), and a healthy trend to lower priced, user-friendly systems of increasing ruggedness.

Many analysts have shied away from MS, including the mass selective detector (MSD) Ion-Trap version, and other GC/MS systems, feeling that it is more suited to dedicated analyses for a single analyte or small analyte clusters than for the range of analytes potentially present in a multiresidue sample of unknown origin. This is becoming a less valid objection because new MSs can be programmed to shift rapidly between pre-selected masses as the chromatogram develops, thus covering the broad range needed for many applications. Some industrial and contract labs have moved more to MS, to the point of replacing element-selective detectors. This is a very healthy trend and should be encouraged by increasing research funding in the area of tailoring multiresidue schemes to be compatible with the MS.

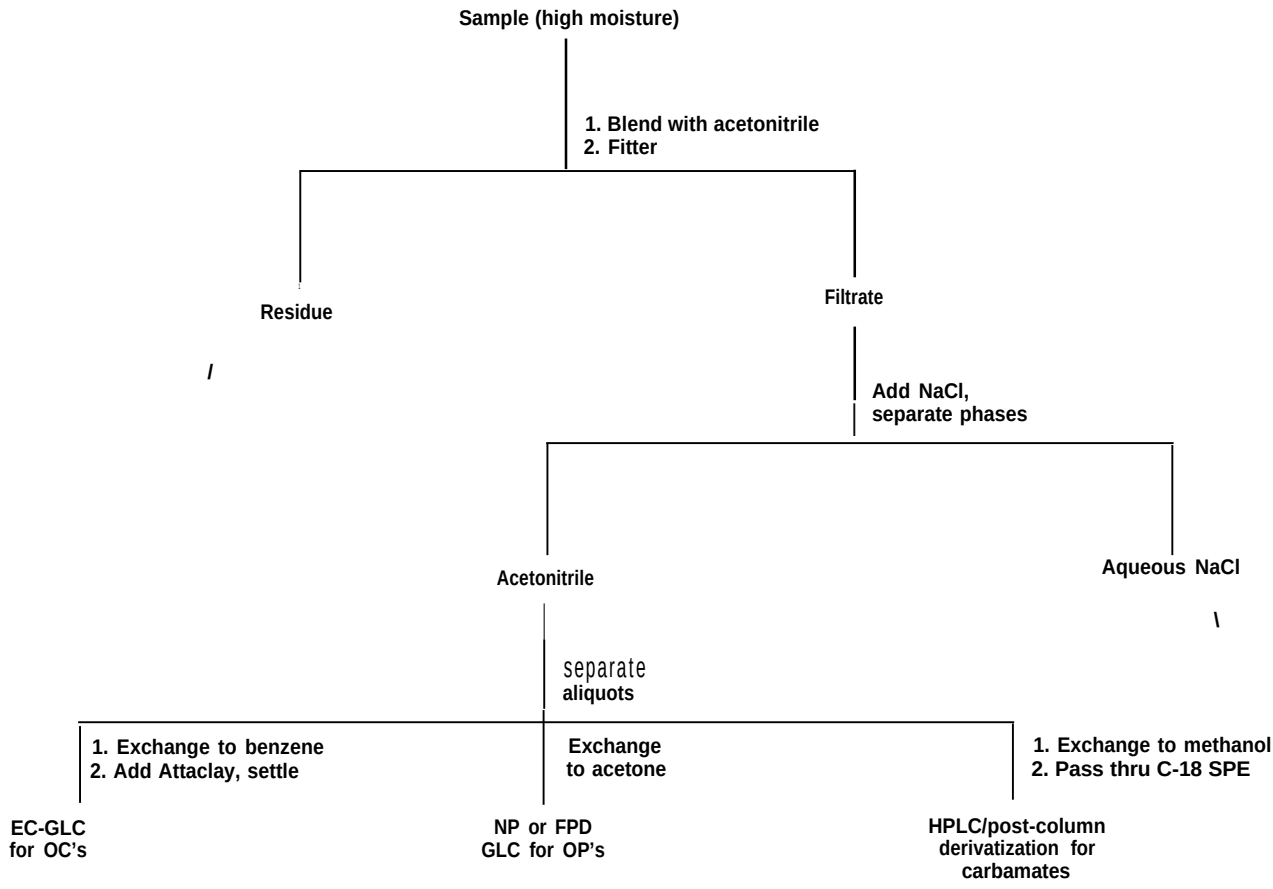
Aside from much improved versions of traditional pesticide analytical methods in the areas of capillary columns, improved selective detectors, and the

GC mass spectrometry-based systems, other conventional techniques and a few relatively new approaches are finding increasing applications in pesticide multiresidue methods. Chief of these is HPLC, which has become close to routine in handling analysis of some pesticides that are not amenable to GC, or for which HPLC provides an alternative to derivatization for GC. For example, some N-methylcarbamate insecticides (and their metabolizes), for which derivatization and GC represented the only viable approach just a few years ago (8), are screened by HPLC using either direct UV/fluorescence detection, or detection following automated postcolumn derivatization. The multiresidue procedure of the California Department of Food and Agriculture (2) (figure 5), for example, integrates the use of SPE isolation with postcolumn derivatization HPLC, with detection limits for eight carbamates in the range of 0.2-0.5 ppm. Moye (6) provided other examples, including for glyphosate, phenoxy acids, and substituted ureas. Once again, technical improvements in HPLC columns and detectors have provided increased resolution, detectability, and reliability. Capillary column HPLC and supercritical fluid chromatography promise further advance: capillary columns in extending resolution and detectability further, and SFC in the ability to interface with the selective GC detectors (an area where HPLC is normally at a disadvantage relative to GLC). HPLC-MS is also improving, particularly with thermospray and other new interfacing systems, but is not yet competitive with GLC-MS in detectability and confirmatory power.

Still other instrumental advances may find future use in multiresidue analysis, including the following:

- *Headspace* GC—Volatile pesticides (methyl bromide, ethylene dibromide, phosphine)
- *GC-Fourier Transform* 1nfrared—Semivolatile-

Figure 5. -CDFA Multiresidue Screen



SOURCE: Jamas Seiber, University of California, Davis, CA, 19S8

volatile pesticides; can be interfaced with GC-MS (GC-IR-MS)

- *Multidimensional* GC—More rapid screening for varieties of pesticides in single extracts.
- *Tandem mass spectrometry* (MS-MS)—Screening samples for classes of chemicals; confirmation.
- *High resolution mass spectrometry* (HRMS)—Screening samples for classes of chemicals; ultra-low level detection of specific chemicals; confirmation.
- . Immunoassay—screening samples for selected classes of chemicals, particularly those not amenable to low-level GC or HPLC analysis.

Finally, a few comments should be made on automation, and other time- and labor-saving approaches. Autoinjectors for GC and autosamplers for LC are commercially available in much improved versions over early devices introduced in the 1970s. Both can be considered routine and have extended sample throughput to 24-hour operations. A necessary adjunct to autoinjectors is a programmable integrating data system for data compilation, eliminating the need to have all peaks on-scale for quantitation. Another convenient adjunct is the use of internal standards (nonpesticide surrogates that chromatography similarly to pesticides), bypassing the need for extensive standard curve preparation and reinjection of "out of range" samples.

Thus, microprocessor-controlled GLCs and HPLCs with autoinjectors and computer data systems are seen in increasing frequency in pesticide analytical laboratories and, combined with internal standards, can make large improvements in the time and costs of residue analysis. This trend will continue, as "smart" systems that identify (based on retention time) and quantitate suspected residues with less operator involvement are further refined and utilized. The data systems of GLC and HPLC instruments have the added advantage of providing records, which can help fulfill good laboratory practices requirements.

The use of SPE cartridges was mentioned previously as a sometimes more rapid and generally solvent-saving device in the extraction-cleanup phases of analysis. SPEs also provide an opportunity to conduct some sample preparation in the field. For example, we extracted water samples for pesticide residues by pumping water through C-18 SPEs immediately after the samples were taken in the field; very little extra time was added to that required for sampling and only the small SPEs needed to be transported to the lab for completion of analysis by GLC. This approach could perhaps be extended directly to milk, juices, and other fluid foodstuffs, and perhaps even to solid foods if a solvent extraction operation were set up close to the point of sampling.

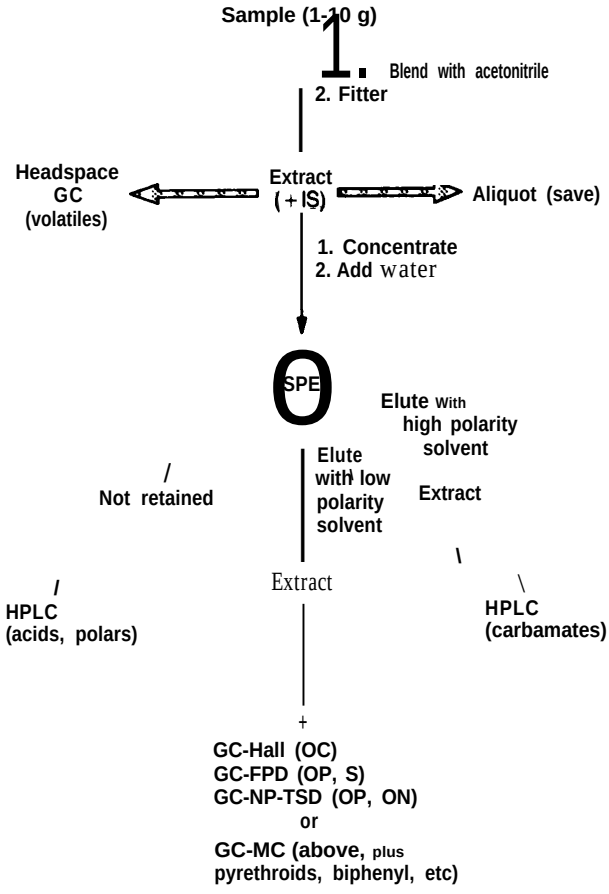
Improved gadgetry for solvent concentration is gradually replacing the very clumsy, labor-intensive rotary evaporators and Kudera-Danish-Snyder column steam concentrators. For example, the N-Evap proved useful in simultaneously concentrating many samples of small solvent volumes when first introduced in the 1970s. A recently introduced programmable sand bath evaporator should also find use, particularly for concentrating aqueous samples. In fact, any move by technology toward smaller sample sizes and extract volumes should save time and decrease chances for in-house contamination because of the smaller glassware requirements.

Putting It All Together

Taking what appears to be the best of existing MR schemes, and extending to sample miniaturization and SPE cleanup-fractionation, leads to the hypothetical "method" in figure 6. The hypothetical method is broad-based in terms of handling pesticides over a range of volatilities and polarities, and it is quick because of lower volumes handled and the elimination of liquid/liquid extraction and solvent evaporation.

In this approach, all GCs are equipped with megabore capillary columns, autoinjectors, and data systems. LCs are equipped with short, small particle columns, autosamplers, and UV and fluorescence detectors with and without postcolumn derivatization. The GC/MS is programmed in the selective ion mode to search for ions diagnostic for individual pesticides. Quantitation is done vs. internal surrogate standards added to the first extract. Recoveries are calculated for the internal standards by occasional external standardization. Fluid samples, high fat, and low fat samples could potentially be accommodated with some modification of the first extraction step. All steps would need research and developmental optimization, using a variety of substrates and pesticide types. The point here is that most pesticide residue chemists could come up with a scheme that, conceptually, improves on existing methods by instituting newer technologies and miniaturization. Whether these conceptual schemes could extend multiresidue methodologies to new levels in the parameters in figure 1 is a question worth asking—and perhaps worth investing of public funds to answer.

Figure 6.- Hypothetical MR Scheme



SOURCE: James Seiber, University of California, Davis, CA, 19SS

Conclusions

Can existing analytical methods be improved? The answer is certainly yes, and in fact they are continually undergoing improvement as new GC, HPLC, and MS systems are introduced and as new chemicals are included in the existing schemes.

How can they be improved? The following are offered as potential elements to improvement:

1. *Miniaturization—Offers* savings in time by processing smaller volumes, increases the possibility of automation, and might extend breadth (yet to be proved) through the use of commercial cleanup columns (HPLC or SPE cartridges).
2. GC Technology—Wide bore capillary columns interfaced with element-selective detectors will minimize the need for derivatization and provide more efficient resolution, thus lowering detection limits. When used with auto-injectors and integrating data systems, throughput and data quality will improve.
3. HPLC Technology—Will focus on those compounds that cannot be determined by GLC; critical need for selective HPLC detectors that go beyond UV, fluorescence, and electrochemical (SCF improvements will help here); if detectors were available, promises major improvement on breadth of MR technology. Even without super-selective detectors, HPLC will find specialty use in removing problem interferences and in postcolumn derivatization for specific classes of chemicals.
4. *MSD* Technology—This represents a real opportunity because this is an affordable, here-and-now technology that only needs a few well-designed studies to show applicability, particularly in the SIM mode with programmed ramping of ion masses through the chromatogram. MSD can be used as a detector (SIM mode) and also for confirmation (Scan mode).

Who Should Do It?

Industry has a responsibility for fitting new compounds into multiresidue schemes, but not for the development of the schemes themselves. Federal labs should take the lead, set the goals, and conduct the validation (with AOAC or other assistance). But they should not shoulder the burden of discovery and development alone.

A well-conceived, extramural funding program is needed, allowing for participation by academic institutions, research institutes, and state lead agencies. New funds in the range of $10 million would be needed, with half devoted to upgrading the equipment and scientific expertise in the residue labs of state agencies and the four regional Leader Laboratories of the USDA- CSRS - State Experiment Station's Minor Use Registration Program (IR-4), and half to competitive funding on a Request for Proposal (RFP) basis. Categories for the RFP would be the four mentioned above, in this section, and also a fifth dealing with extending multiresidue methods to new classes of pesticides (sulfonylureas, pyrethroids, etc.). Academic involvement is crucial, both to supply new ideas and also to stimulate involvement of graduate students and post-doctorals who will provide the invigoration needed for a longer-term, sustaining program of residue analytical excellence, which is needed over the long-haul.

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Techniques for and the Role of Screening Pesticide Residue Analysis

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Contents

Figure

Abstract

Analytical testing for residues has progressed from relatively insensitive bioassays to highly technical procedures combining attributes of computer science, electronics, materials sciences, and biotechnology. Consumers, consumer action groups, the National Academy of Sciences, Congress, and others have highlighted public health concerns about the detrimental effects of pesticides residues in food, water, and other parts of our environment. Regulatory programs are designing improved statistical-based sampling programs to ensure a safe and wholesome food supply. These programs highlight the magnitude of testing required to accomplish that goal. Testing procedures, once the domain of relatively complex quantitative and confirmatory techniques run in well-equipped laboratories with trained personnel, are not adequate to meet analytical demands with available resources. Rapid test systems employing advanced technology are being produced that can make residue testing programs responsive to this need. Regulatory statutes require a preponderance of information to support enforcement responsibilities. Thus, integrating rapid test procedures with quantitative and confirmatory methods is needed. Rapid testing procedures may be used by personnel nonexperienced in analytical sciences. This requires strong quality-assurance and quality-control programs to ensure proper design and use of rapid test methods. Regulatory-agency policy development on roles for these screening methods as well as other technological developments for testing procedures will influence development and application for improved public health analytical testing programs now and in the future.

Background

Modern agricultural production uses commercially available pesticides to combat a variety of weeds, insects, fungi, and other agricultural pests. These pesticides contribute substantially to the high level of agricultural production we currently enjoy. As a result, consumers are exposed to pesticides, usually in minute quantities, in several food groups including meat, dairy products, fruits, vegetables, dried food goods, most processed foods and many other household staples, Some pesticides, however, are considered as either acutely or chronically toxic to humans and other segments of the environment, and they pose potentially serious health risks to nontarget organisms and species. This presents a significant regulatory responsibility to public health-related agencies as well as to Congress.

The magnitude and complexity of the regulatory responsibility is well developed by the Congressional Research Service 1986 report and the National Academy of Sciences report, “Regulating Pes-

ticides in Foods." Regardless of the number of pesticides registered for use on agricultural products, the number is small compared to the more than 8,000 food tolerances listed in the Food, Drug and Cosmetic Act, Section 408 and 409 (2). From data supplied by EPA, 53 of these pesticides have active ingredients identified as oncogenic or potentially oncogenic (2). This does not include some of the chlorinated hydrocarbons considered as oncogenic in animals or humans. Further, FDA has classified 81 compounds through its surveillance index as warranting residue monitoring because of their potential health hazard. Based on the GAO Domestic Food Report (3), 30 of these received little or no residue testing between 1979 and 1985, and several pesticides included in the two highest classes as potential health hazards are not covered by the five current FDA multiresidue methods, although these residues may be analyzed by other methods or other programs. Recognizing the universe of pesticides used on foods, there is agreement that more residue methods are needed for monitoring purposes. This becomes more important as higher levels of sampling are called for to improve confidence that regulatory agencies are providing greater assurance of a safe food and water supply to consumers.

Screening Method Concepts

Analytical methods play an important role in food production inspection systems to protect public health. A universal analysis scheme that can simultaneously quantify the presence of all compounds or classes of compounds of interest in foods, animal tissue, or fluid with acceptable accuracy and correctly identify the analyte or analytes would be a desirable, unified methods approach for regulatory control agencies. Yet at present, there are very few analytical procedures available to regulatory agencies that simultaneously quantitate and confirm the identity of such residues. Until universal methods are available, regulatory programs will have to employ methods with individual attributes of presumptive presence, quantitation, and positive identification. To accomplish this goal, methods with different attributes must perform in concert for a highly effective residue program regardless of individual regulatory mandates.

Terms such as confirmatory, reference, quantitative, semiquantitative, screening, rapid, and presumptive methods are well known. An alternative to the potential difficulty of categorizing methods, and the stigma associated with these descriptive terms, is to define the methods independent of intended purpose, according to the attribute or qualities of method performance. Attributes and qualities of three levels of analytical methods are relevant to support regulatory programs. Though the focus will be on screening methods, a brief description of the method types is needed to understand their interrelationship.

Level I methods incorporate the ability to quantify the amount of specific analyte or class of analytes and positively identify their presence in a single analytical procedure. These are assays with the highest level of credibility and are unequivocal at the level of interest. They maybe single procedures that determine both the concentration and identity of the analyte, or combinations of methods for determining and confirming a residue for definitive identification, These methods are most commonly identified as confirmatory methods.

Level II methods are those that are not unequivocal but are used to determine the concentration of an analyte at the level of interest and to provide some structural information. For example, these methods may employ structure, functional group, or immunochemical properties as the basis for the analytical scheme. These methods are often reliable enough to be used as reference methods. Level II methods provide a quite acceptable approach for residue testing.

They may be used to corroborate the presence of a compound or class of compounds. Thus, two Level II methods may provide information suitable for Level I attributes, providing they employ different chemical technologies. The majority of analytical methods now available and used by regulatory *con*trol agencies are Level II methods, These methods are usually the quantitative analytical methods used in laboratories for regulatory control programs.

Level III methods are those that generate imperfect, though useful, information. These testing procedures detect the presence or absence of a compound or class of compounds at some designated level of interest and often are based on non-instrumental techniques for analytical determination. Results on a given sample are not as reliable as Level I or II methods without corroborating information. Level III methods may, for example, provide reasonably good quantitative information but poor compound or class specificity or identity, or they may provide strong or unequivocal identification with very little quantitative information. Level III methods are not poorly described or sloppy methods; rather, they must have defined operating characteristics of reliable performance. Many microbiological assay procedures and immunoassay test

systems may fall into this category. They are used because of convenience and potential suitability to non-laboratory environments, analytical speed, sample efficiency through batch analysis, portability to non-laboratory environments, sensitivity, and the ability to detect classes of compounds. The hallmark of Level III-type methods is that action based on individual positive results require substantiation using Level I or II methods as required by the uncertainty of an individual result. However, epidemiological information may provide substantive data, reducing the uncertainty of individual results. These are typically screening or rapid test methods and may offer several advantages to a regulatory control program.

The reliability of Level III methods should be measured in part by their performance characteristics as well as their ability to handle relatively large numbers of samples within a given timeframe. Two key characteristics requiring definition include their percent of false positives (reporting a positive response when no analyte is present) and percent of false negatives (reporting a negative response when the analyte is present) when measured against a validated quantitative assay in a statistically designed protocol to derive the test method operating characteristics. When the operating characteristics are defined for false negative and false positive results, the operating range of the screening method may be established. Individual programs may select those false negative and false positive values to suit their particular program needs. The percent of false negatives must be quite low at the levels of interest (less than 5 percent), while slightly more flexibility may be acceptable for false positives for screening tests. A minimum level of residue detection can be described based on a balance between these two parameters.

Attributes of Screening Methods

Methods suitable for regulatory purposes must be reliable. To ensure analytical reliability, performance characteristics of a method must be determined by multilaboratory evaluation. In most cases, minimum standards should be set, designed to fit the needs of specific program requirements. By consensus with public health standard-setting organizations or agencies, the principal attributes of analytical methods are specificity, precision, systematic error, and sensitivity. Other attributes relevant to screening methods will be described as well.

Specificity is the ability of a method to respond only to the substance being measured. A residue control method must provide for unambiguous identification of the compound being measured. One set of measurements of specificity is the percent of false positives and false negatives. This characteristic is often a function of the measuring principle used and the analyte functionality—key factors for rapid test methods. Methods should be able to qualitatively differentiate the analyte from analogues or metabolic products of the compound(s) of interest under the experimental conditions employed.

Precision is a measure of the variability of results when the method is applied to separate portions of a homogeneous sample. Precision is usually expressed as standard deviation. This term is sometimes used to describe other method characteristics such as limit of detection, limit of decision (4), and limit of reliable measurement (5). Another useful term is the relative standard deviation because it is relatively constant over a considerable concentration range (an order of magnitude, for example), ideally covering the level of interest.

Systematic error is analytical method bias, the difference of the measured value from the true, assigned, or accepted value (mean 8 value). It is commonly expressed as the percent recovery of added analyte to a sample blank. At relatively high concentrations, recoveries are expected to approach 100 percent. At lower concentrations and particularly with methods involving a number of steps, recoveries may be lower. Regardless of what average recoveries are observed, low variability is a desirable feature. Commercial rapid test systems should be designed so that parallel curves for standard solutions of the analyte and sample extracts of analyte added to a sample are routinely achieved.

Accuracy refers to the closeness of agreement between the true value and the mean result. The accuracy requirements of different types of methods will vary with the use being made of the results. For screening methods, characteristics of false positive results and false negatives define a methods operating range.

The sensitivity of a method is a measure of the ability to discriminate between small differences in analyte concentration. A common practice is to define sensitivity as the slope of the calibration curve with known standards at the level of interest.

Beyond these method characteristics are a number of collateral criteria particularly suitable for screening methods for regulatory control programs. Methods should be rugged or robust, cost-effective, relatively uncomplicated, portable and capable of handling a set of samples simultaneously in a time-effective manner. Ruggedness of a method refers

to its capability to be relatively unaffected by small deviations from the established values in the use of reagents, quantities of reagents used, time factors for extractions, and reaction or temperature. This does not, however, provide latitude for carelessness or haphazard techniques. Cost-effectiveness refers to use of relatively common reagents, efficient use of resources, and using instrumentation commonly used for trace environmental analyses. A method of being relatively uncomplicated refers to use of simple, straightforward mechanical or operational procedures throughout the method. Portability is the characteristic of the method that enables it to be transferred from one location to another without loss of established performance characteristics. The capability to analyze a set of samples simultaneously reduces the analytical time requirements of sample analysis. This is particularly important for screening methods in which large numbers of samples are to be analyzed in short or fixed timeframes.

The importance of establishing the attributes and performance criteria cannot be overemphasized. It provides the necessary information to allow regulatory control officials to develop and manage programs responsive to their public health responsibilities. Performance criteria for analytical methods also provide a basis for good management decisions in future planning, evaluation, and product disposition.

Quality Assurance

Regulatory control agencies responsible for monitoring foods are routinely made aware that any analytical discrepancy may require the inevitable defense of our analytical programs. A principal objective becomes one of assuring we have a well planned and executed quality-assurance program. Quality assurance is an important part of all regulatory control programs. With screening methods, or any rapid test system, established policies and procedures are needed to ensure that these methods are being properly conducted and the testor is evaluating the test response in the appropriate manner. The implications of poor performance of rapid test methods would be difficult for regulatory control agencies to deal with.

Quality assurance begins with the method development process. Activities include experimental optimization of each procedural step or manipulation to determine the critical control steps—those having a substantial impact on method performance. Other activities include identifying when an analytical method may be stopped without adversely affecting the results; determining the ruggedness or process variability that maybe employed in any particular method step without reducing the method's performance; and determining the sample requirements necessary to ensure reliable, interference-free results. Instrument parameters should be optimized and a mechanism to test instrument performance established if instruments are required. Mass transfers in the procedure should be minimized, Lastly, the method must be written in thorough, concise, unambiguous language. These factors will facilitate method transfer and training for end-users of a method into a regulatory program. The focus on quality assurance cannot be overemphasized. In the long-term, it is less expensive to do it right the first time. It ensures credibility to a regulatory program and esprit de corps among analysts.

Detection Systems for Screening Methods

Two important reasons for using screening methods are 1) their capability to analyze a relatively large number of samples in a given unit of time, and 2) their robust nature. This latter characteristic allows latitude for using screening methods in non-laboratory surroundings. In these instances, methods will often be used by individuals not necessarily experienced in analytical chemistry techniques. This places a constraint on certain types of methodology. It limits use of certain types of equipment, instruments, and reagents. Further, methods need simple, unambiguous test instructions that will enable a testor to correctly prepare the test material, conduct the analysis, and interpret and report the test findings. Process controls defining critical steps in the test procedure are very relevant to the success of such a testing program.

Thin layer chromatographic procedures satisfy a significant number of desired attributes for screening methods. The capability to analyze a set of samples in a given timeframe is usually higher than other common chromatographic systems. There is a-wide variety of absorbents, chromatographic solvents, and reagents facilitating residue detection. In addition, residue detection is a static process rather than a dynamic one; quality assurance is easier because control samples and reference standards can be analyzed simultaneously with the test samples. A comprehensive review on thin layer chromatographic systems and procedures has been published recently (6). It describes an extensive array of systems for pesticide analysis. One that has

been reduced to practice for a regulatory program consists of thin layer chromatography for separation of 12 organophosphate pesticides using cholinesterase enzyme inhibition for residue identification (7). A recent project with Food Safety and Inspection Service (FSIS) for screening chlorinated hydrocarbon pesticides employing thin layer chromatography with a variety of detection systems for chlorine (including many in reference 6) was not successful because the sensitivity at the level of interest was not attainable (8). A further complication was the sample extraction procedure from animal fat being too complicated for use in a nonlaboratory situation. Detection systems focused on the chlorine atom because of the relatively high chlorine content in the compounds of interest. A successful application of thin layer chromatography for a rapid field test has been developed for sulfamethazine by FSIS. Although these are limited applications for regulatory programs, this technique offers promise for the future as new reagents improve sensitivity and thin layer chromatography systems provide new approaches for effective sample purification and analysis.

The detection limits of most color-producing or fluorescent-generating reagents provide sensitivity at low microgram per gram (µg/g, ppm) concentrations. Reagents using enzyme inhibition allow detection limits in some systems at low picogram per gram (pg/g, ppb) concentrations. For example, many herbicides employing photosynthesis inhibition as a mechanism of action have been detected at picogram (10-12g) levels using plant chloroplasts and a reduction-oxidation chemical indicator. In corn, potatoes, and carrots, detection limits without purification of the sample extract were less than 10 pg/g (ppb) (9). Classes of herbicides adaptable to this detection system include triazines, phenylureas, phenylcarbamates, 13 uracils, and acyl anilides. This suggests the possibility of broad-spectrum screening tests suitable to nonlaboratory use.

Immunobased assays are emerging as promising screening test methods. Test systems for a wide variety of organic residues in soil, water, food, plant, and animal tissues are being developed by a number of companies in the United States. These tests are being developed in rapid, very sensitive, easy to use, and usually highly specific formats. They show promise for rapid onsite testing as qualitative assays while some are now being designed for fast, quantitative laboratory tests. Their designed specificity, which is commonly very high, generally allows use of relatively crude samples as a test material and makes them attractive for use in nonlaboratory environments. Generally, the cost of these assays is lower than traditional analytical laboratory methods. However, they are still dependent on sample preparation. Nevertheless, potential per-sample cost for such assays is less than $15,00, including administrative costs. Instrumental methods are usually $50.00 or more for similar analyses. The major constraint of these assay systems is their relatively high cost of development. It is estimated that they become practical economic investments by economy of scale, when 100,000 tests per year are run (10).

Within our current regulatory and statutory environment it is not reasonable to expect registrants of pesticides or other chemical entities used in food production to voluntarily provide screening methods. There is little interest in developing multiresidue methods, in particular, that may be capable of either identifying or quantitating residues in food products that may include a competitor's product. Where a residue control problem exists or is likely to exist, Federal agencies commonly take the initiative for developing these methods. Because of the costs involved, prudent decisionmaking on priorities is essential. It must be understood that in certain instances, other metabolism or metabolic research may be needed to provide a basis for developing an analytical system responsive to regulatory control needs.

Opportunities do exist to stimulate methods development in the private sector. Examples include the recent legislation allowing commercialization of Federal government supported patents, federally supported research contracts and grants, and advertisement for commercially available analytical technologies. Within the Food Safety and Inspection Service, the last two have been extensively explored with measurable success. It is likely other Federal agencies have similar and possibly other opportunities to stimulate private sector interest. A known long-standing or highly publicized residue problem often generates heightened interest.

The big advantage of rapid test systems is their simplicity allowing tests to be performed by testers that are not highly experienced in diagnostic or analytical procedures. A disadvantage on occasion is that they are designed specifically for only one compound and require separate test systems for a class of pesticides. In some instances, sufficient cross-reactivity to a class of pesticides will allow other compounds to be detected, usually at higher concentrations in a sample matrix. Thus, there is some tradeoff for development by laboratories and use in regulatory programs.

It is often possible to develop effective quantitative methods using the same technology. These assays require state-of-the-art instrumentation and being performed by analysts in fully equipped laboratories. Adoption of qualitative or quantitative immunochemical assays is likely to take time before confidence and recognized legal status for such methods is attained. It may require considerable experience and familiarization with the technology by regulatory agencies to use test systems containing unknown reagents ["black box" test systems) to develop procedures assuring themselves that public health protection is not compromised.

Experience and familiarization with rapid testing systems such as immunochemistry based "card tests" is best accomplished by hands-on experience with them and supplemented by appropriate training materials prepared by experts in theory and technology of the rapid test systems. This is comparable to the education analysts had to acquire when chromatography and associated instrumentation was introduced into regulatory programs. This basic understanding enables regulatory programs and analysts to properly diagnose and evaluate test results and serves as a foundation for developing quality assurance plans and subsequent training for regulatory control programs.

Occasionally, in the development and design of ready-to-use products such as these tests, reliability and consistent performance of the assay from lot-to-lot production can vary. Quality control for production will likely improve with gained experience. Nevertheless, users of these systems must employ good quality-control and quality-assurance protocols to ensure method performance. Developing criteria for manufacturers of such systems either by the industry itself or by regulatory agencies planning to use such methods would be a step in the right direction to facilitate their acceptance.

A concern facing regulatory agencies is that some of these assays are more sensitive than the traditional quantitative and confirmatory assays, so that these qualitative results cannot be confirmed. This may limit further regulatory action and force technology to develop new quantitative and confirmatory methods to match the sensitivity levels. Regulatory agencies need to confirm what they have the capability to detect, particularly at the level of interest. This level of interest is usually either an action level or tolerance established by EPA or FDA.

It is important to recognize that analytical programs designed to detect potential residue problems must have the capability to provide quantitative values and structure identity at or below the level of interest. For example, within the Contamination Response System in FSIS, an analytical result for a pesticide or environmental contaminant that is at or above 80 percent of the tolerance or action level will trigger a set of specified actions, including directed sampling programs if a significant residue issue emerges. Without having the needed quantitative and confirmatory assays to support results from a rapid test system, inappropriate regulatory actions may occur. For enforcement purposes, for residues above an established action level or established tolerance, confirmatory methods must be capable of unambiguously identifying the analyte of interest at these concentrations. In situations where a tolerance or action level is established with a zero residue limit, confirmatory and quantitative methods must work at sub parts per million (ppm, µg/g) to parts per billion (ppb, pg/g) concentrations based on the approved analytical method for the analyte.

Another limitation is the heavy reliance on using aqueous media for performing the test. For certain food types, this may be of little consequence, but for others it maybe a measurable deterrent. For example, most chemical-based assays rely on use of organic solvents to release the analyte of interest from the test sample matrix. This requires developing solvent systems providing sufficient transfer from the organic extraction solvent to the test system while not denaturing or deactivating the immunochemical reagents. Progress is being made in this area. For example, Immunosystems has developed an assay for chlorinated triazines (Res-I-Mune®) (11) that allows low rig/g (ppb) detection using select aqueous organic solvent systems. This system is currently being evaluated by FSIS for meat products.

Today, immunochemical assays are available not only for chlorinated triazines but also for paraquat, chlordane (heptachlor, dieldrin, endrin, aldrin, and endosulfan are detected via cross-reactivity), 18 pentachlorophenol and polychlorinated biphenyls (PCBs) at levels of interest. FSIS has method-development contracts for developing immunochemical assays for heptachlor-related organochlorine pesticides; ivermectin; synthetic pyrethroids (permethrin, cypermethrin, and deltamethrin); and nitroimidazoles in meat and poultry tissue (12). These are expected to provide improved laboratory analytical capability for these analytes. Development of qualitative screening assays is possible.

A commercial pesticide detection system based on cholinesterase enzyme inhibition has been developed by EnzyTech, Inc. (13) The enzyme ticket

system detects common insecticides that account for about 85 percent of all insecticides used in the United States at concentrations in the low rig/g (ppb) range. Shelf stability for the test system is estimated to be several years. The design of the system allows for a two-tier analytical scheme that will allow differentiation of organic sulfur containing organophosphate insecticides from their oxygen analogs. This advantage reduces some of the options of further analysis to quantify and confirm these analytes. Research is being done to enable analytes from an organic extract to be analyzed with the test system. Development of quantitative and confirmatory analysis using other analytical technologies may be needed to support these qualitative methods.

New column chromatography packing materials have simplified many sample purification analysis procedures. These solid phase extraction materials allow many solvent-to-solvent extraction and purification systems to be eliminated from traditional methods. Future applications may become the basis of rapid test systems requiring only solvent elution to isolate analytes of interest.

integrating Screening Methods Into Multiresidue Regulatory Programs

Applications of screening methods for pesticide-residue regulatory programs to some extent depend on residue violation rates. The first scenario covers instances when data indicate a low incidence of an above-tolerance residue for approved pesticide use. The second scenario applies to situations with a relatively higher percent of residue violation incidence for approved pesticide use. A third scenario would be for detecting and confirming pesticide residues from unapproved pesticide use. The first two may be influenced depending on whether or not agreements exist for residue avoidance programs between a regulating agency and a food producer. Where such agreements are available, one incentive to such programs would be to reduce sampling of such producers, assuming a history of good quality-control in their production systems.

Integrating rapid test methods into regulatory programs does not imply reducing emphasis on reporting quantitative values below tolerances or action levels. These quantitative values both below and above these levels of interest are important for analyzing trends and designing future residue control programs. However, integrating rapid test methods for regulatory control programs implies an intelligent design using rapid screening methods (commonly, Level III methods) with quantitative methods (analogous to Level II methods) and confirmatory methods (Level I methods) to optimize the limited resources available to regulatory control programs. With a low-level violation incidence from statistical-based random-sampling programs, screening methods are particularly attractive for field or in-plant use because they allow for methods with broad versatility to test large numbers of food products and related samples, Data indicate that with statistical-based random-sampling programs, the large majority of samples contain non-detectable and below level concentrations of residues (14). This provides programs with the opportunity to clear products with non-detectable residues or detectable below a tolerance, while retaining suspected positives for more definitive analytical procedures. This generally provides for more effective use of expensive laboratory facilities and resources as well as for reducing the significant costs involved in sample collection and shipping all samples to a designated laboratory. Data management systems have to be appropriate to the regulatory need in all cases.

For instances in which there is a known or high-residue violation incidence, quantitative immunochemical, enzyme-inhibition assays and thin layer chromatographic systems designed for rapid testing in laboratory environments become very attractive. In this scenario, where large numbers of samples are expected to give results in residue violations, the advantages to regulatory programs for reduced analytical costs for sample collection and shipping are diminished. The level of effort needed for field or in-plant personnel to use and follow-up on results from rapid tests could result in an increased workload for additional sample collection and shipping. Using another laboratory analytical method (another Level H method), in these situations provides an independent assay for the analyte of interest and is generally suitable for verifying initial results. This may require developing and validating new methods using improved analytical detectors, more sensitive color-forming reagents, fluorescent-generating reagents, or biochemical and color-forming reagent systems that match the sensitivity, specificity, and other screening method performance characteristics.

Another option is to allow well-defined sample-compositing schemes to be employed for laboratory analysis. This is particularly attractive when no known incidence of a residue problem exists. It is somewhat less attractive under the second scenario with known or high-residue violation rates because it calls for reanalysis of individual samples within

the composite sample when an actionable finding is indicated by analysis of the composite sample.

In the third scenario (detecting residues of unapproved pesticides or pesticide use), residue screening tests are very attractive because detection of any amount of pesticide residue in specific products is a residue violation. It normally requires support by a confirmatory procedure. This assumes, as in all other cases, performance characteristics of methods are well defined. In this scenario, quantitation is not a specific requirement, although administrative level may be defined by an agency before initiating other regulatory action. Under any of these scenarios, epidemiological information should be incorporated to design effective subsequent laboratory-analysis programs. To facilitate design of an integrated residue control program, a decision-tree process may be a suitable objective. This will require some preliminary activities. The mission statement of the regulatory agency and the objectives of residue analysis must be clearly defined and adopted. Figure 1 is a suggested approach. Others may be developed based on specific needs or other regulatory and statutory considerations.

Consraints on Use of Rapid Test Methods

A perceived constraint in some programs with screening tests is that they are not specific and consume too large a portion of valuable resources to identify the residue of interest. A possible resolution to this would be to encourage development of more selective screening assays to improve the selection process of a multiresidue or single-residue analysis method. That is, develop a hierarchy of test methods based on current and emerging technology, focusing on the concept of simplicity in design and application, automation technologies, and commercially available systems and equipment with potential for broad application.

Rapid testing systems may be compromised by other constraints. A rate-limiting factor for application of rapid tests is, or may become, preparing an appropriate sample for analysis. Encouragement is warranted for research to develop improved sample preparation techniques amenable to rapid test systems in nonlaboratory and laboratory environments. For example, supercritical fluid technology may be a valuable research avenue for laboratory based analysis. An alternative could be to focus on physical or chemical properties of pesticide residues. A third possible approach would be research on pharmacokinetic or metabolism studies to correlate pesticide residues in food and tissue matricies to fluid extracts. Incentive is needed to encourage commercial organizations to develop rapid test procedures. It is primarily through the economy of scale and return on investment for manufacturers that tests will become available and provide regulatory agencies with sufficient potential low-cost onsite, laboratory onsite, and laboratory test procedures. Development of rapid tests for regulatory use can be fostered by offering contract funding to the relevant regulatory agencies. Recent examples of this method development process have shown promise, particularly with colleges and universities.

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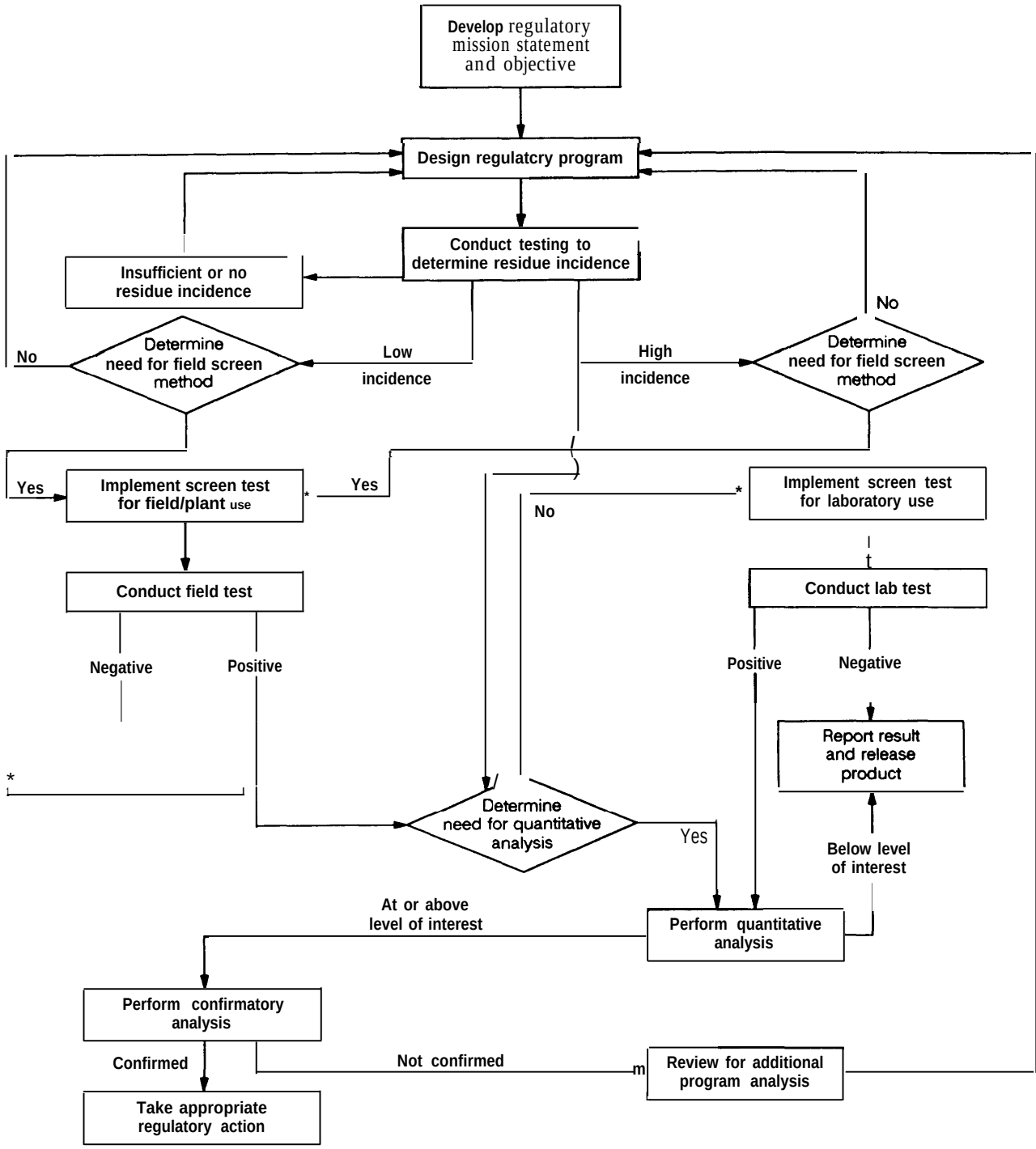
Figure I.-Decision Tree for Regulatory Control Program



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The Role of Robotic Automation in the Laboratory

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Contents

Abstract

Rapidly increasing numbers of samples for pesticide residue analysis have forced the analysts in analytical laboratories (governmental, industrial, and private) to look for ways to increase sample throughput. This trend has provided a need for the development of a variety of automated equipment for the analytical laboratory.

In support of the workshop on ''Technologies to Detect Pesticide Residues in Food'' sponsored by the Office of Technology Assessment (OTA), this paper will l) provide an overview of laboratory automation, 2) assess the emerging robotic technology for the analytical laboratory, 3) review the current status of automation in pesticide residue analysis, and 4) identify research needed for expanded automation in the analytical laboratory.

Introduction

Scarcely an aspect of modern life has be enunaffected by automation, yet defining automation is difficult without using automatic or automated in the definition. Consider Webster's definition: "the technique of making an apparatus (as a calculating machine), a process (as of manufacturing), or a system (as of bookkeeping] operate automatically" or the condition of being automated(l). Automation implies the integration of a self-governing system. Unlike mechanization, which is defined as simple replacement of human labor by machines, true automation must have the ability for feedback control and the ability to regulate. Four key elements of automation are 1) a source of power, 2) sensing mechanisms, 3) decision elements, and 4) control elements (2). For all practical purposes, laboratory automation is the use of devices that perform repetitive tasks. Reviews of large annual trade and equipment shows, such as the Pittsburgh Conference & Exposition on Analytical Chemistry and Applied Spectroscopy, provide an excellent overview of current automated equipment.

Laboratory automation can be divided into four basic categories:

1) Dedicated, single-task
2) Dedicated, multi-task
3) Computers
4) Robotics

The simplest type of automation is a dedicated, single-task instrument. These instruments may be commercially available or custom manufactured and perform just one independent function. Examples of single-task automated equipment include autoinjectors, electronic balances, timed shakers, centrifuges, switching valves, etc. Dedicated, single-task devices are well-established and widely used in most laboratories.

The next category of automation is the multi-task device. Instruments in this category perform multiple tasks such as diluting, mixing, filtering, solid phase extraction, chromatographic separations, etc.

Examples of dedicated multi-task automation include automated sample preparation devices that combine adding solvents, mixing, extracting, filtering, evaporating, etc. and multiple-step auto samplers that can heat or cool, add internal standards or derivating agents, serially dilute, etc. Most dedicated multi-tasking devices are highly specialized. For example, automated cleanup and extraction devices facilitate rapid processing of a highly selective number of repetitive, routine samples but remain subject to obsolescence and represent isolated, stand-alone equipment. The number of commercially available, multi-tasking devices is markedly less than its single-tasking counterpart. Additional multi-tasking devices include hyphenated technologies such as gas chromatography-mass spectrometry (GC/MS), liquid chromatography-mass spectrometry (LC/MS), and gas chromatography-infrared spectrometry (GC/IR), etc., which couple established, analytical technologies to enhance the automated detection and measurement of compounds.

Computers differ from the first two types of automation since they automate data handling and calculations instead of physical and mechanical manipulations. This category of automation includes hand-held calculators, integrators, personal computers for control and data handling, and networked laboratory information management systems (LIMS). Computers and associated microchip technology have developed into a modern "Industrial Revolution" that is extremely vast and important but beyond the scope of this paper.

Robotics evolved as hybrid systems incorporating technology from both mechanical and computer (microprocessor) automation. Robotics uses a computer-controlled, mechanical manipulator to incorporate both single- and multi-tasking automated devices into an integrated system. Since its commercial introduction and implementation in the early 1980s, laboratory robotics has provided reprogrammable, multi-tasking, computer-assisted automation in the laboratory for a variety of pesticide and non-pesticide applications. The cost of a laboratory robotics system typically ranges from $40,000 to $60,000. The return on investment depends upon the application but is typically 6 months to 3 years. Before discussing robotic automation, a working definition of a robot must be established.

Robotics Definition

A definition of a robot is in itself a formidable task, since there are so many misconceptions and preconceived ideas of what a robot is. A definition of a robot that has been adapted from the Robotic Industries Association's definition is as follows: A robot is a reprogrammable, multifunctional manipulator designed to move materials, parts, and specialized devices through a variety of computer-controlled motions for the performance of a variety of tasks (3). The key words are reprogrammable, multifunctional, and computer-controlled.

Misconceptions and preconceptions of robots are difficult to overcome. Most every equipment manufacturer wants products that are associated with the latest technology. An example of equipment that does not fit the definition is a "robotic" autosampler. The autosampler transports vials from a tray to an injector with precise timing, performs multiple injections, and varies the size or speed of injections. The autosampler is *not* a robot: it is an automated instrument that performs a single-task of repetitive, precision injections. In addition, most toys or mechanized trade-show mannequins do not fit this definition of a robot.

Several types of robots that do fit the definition include the following: 1) industrial, 2) research and development, and 3) laboratory robots. The first industrial robot in the United States was sold by Unimation to General Motors in 1961. Currently, industrial robots perform such tasks as welding, painting, parts assembly, and material handling. Research and development robots cover such areas as education, cybernetics, and space exploration. In 1982, another type of robot was introduced, a laboratory robot. Its major function has been automated sample preparation. The remainder of this paper will limit its scope to assessing the emerging technology of the laboratory robot, discussing its advantages, constraints, current applications, and future prospects.

Advantages of Robotic Automation

The advantages of robotics include many of the same advantages as conventional automation and are summarized in five categories:

1) cost-effectiveness
2) reproducibility
3) versatility
4) safety
5) automated documentation of procedures.

Robotics can be cost-effective. Like conventional automation, robotics provides a competitive advantage in that the robots can work extended hours and increase the use of existing equipment. Robots free personnel from performing repetitive tasks and devoting constant attention to minute details, thus

allowing additional work or research to be performed. The robot is not subject to hunger, boredom, fatigue, or illness. Robots do not need promotions, pay raises, or medical benefits. However, robotics does not preclude the need for well-trained, competent analysts.

Reproducibility is a major attribute of a robotics system. Performing tasks with the exact timing and uniform sample handling, the robotics system separates the actual chemistries involved from variations in techniques, which may in turn lead to improved precision and accuracy in methodology. This is very useful in method validation, where conditions and parameters can be systematically varied to optimize new methodology during the validation process. Transfer of robotics technology could improve intra- and interlaboratory reproducibility.

Robotics is versatile automation and thus less subject to obsolescence. The reprogrammability of the robotics system allows method optimization or a complete change of application to meet changing needs in a laboratory. Robotics technology also bridges the gap between what is commercially available in dedicated automation and what is actually needed for a specific application (e. g., custom sample preparation, instrument interfacing, specialized autosamplers, etc.).

The use of robotics reduces human exposure to hazardous chemicals, extreme temperatures, and other undesirable conditions such as pinch points, defective glassware, and sharp objects.

Finally, the computer portion of the robotics system can provide automated documentation of the procedure. The entire program as well as sample weights, dilutions, timing, calibrations, etc., can be printed or transferred directly to a host computer, vastly reducing transcription errors. Computerized documentation thereby can establish an audit trail for the entire procedure.

Constraints of Robotic Automation

The present technology of laboratory robotics has several constraints, which are grouped into the following categories:

1) New technology
2) Mechanical and computer failures
3) Spatial and physical limitations
4) Safety
5) Associated technology lagging behind automation

Laboratory robotics is still an emerging technology. The world market for laboratory robotics in 1985 was estimated at 30,000 to 50,000 units (4), but this market has not been reached because there seems to be a general reluctance to change and a lack of wide acceptance of any new technology. As of December 1987, laboratory robotics systems numbered about 1,3oo, and only a limited number of personnel were trained in the operation and repair of robotics systems. To date, Zymark Corporation has more than 85 percent of the present laboratory robotics market. Other laboratory robotics companies include Lerkin, Fisher Scientific, and Hudson Robotics.

The majority of robotics systems have required significant cost and time to be fully programmed and functionally implemented. Newer robotics systems, such as the PyTechnology introduced by Zymark Corporation in 1986, have reduced start-up cost and time by providing systems that are preprogrammed and pre-positioned for basic laboratory operations.

Robots are hybrid systems that combine mechanical equipment with computers. This results in a combination of problems associated with machines (such as physical wear, mechanical failure, etc.) and with computers (electrical power, noise spikes, “glitches”).

Spatial constraints also limit the robotics system because the robot is confined to its working envelope, typically less than a cubic meter. Exact positioning, spatial orientation and readily accessible work areas are necessary for the robot to interact with peripheral modules and support equipment, Modules and equipment are usually bolted on the table or placed in a rack to assure proper positioning. As a result, samples and solvents are limited in size (0.1 to 50 ml) and weight (less than 3 lbs.) to maximize the use of the working envelope.

A human has extensive systems of intricate sensors and feedback mechanisms that provide information about the environment (e.g., touch, pressure, temperature, hearing, sight), A human also possesses an extensive memory from which to recall and process that information. In comparison, a robot has a limited memory, limited feedback mechanisms, limited dexterity, and limited artificial intelligence. Thus safety, which was listed as one of the advantages of robotics, can become a liability when errors occur that require logical decisionmaking to recover from an unanticipated situation for which it was not designed or programmed,

Associated technologies (disposable supplies, glassware, ancillary equipment, etc.) are lagging behind the robotics technology. The associated technologies are not ready for a blind analyst with

limited dexterity (4 to 5 degrees of freedom compared with more than 40 degrees of freedom in the human upper limb). A robotics system must be viewed as an integrated **system** consisting of the robotic arm, peripheral work-station modules (such as vortex mixers, centrifuges, evaporators, etc.) and supporting equipment (such as test tubes, pipet tips, extraction columns). Since robotics systems are integrated systems, they are only as good as the weakest component. Modules with moving parts such as shakers, centrifuges, and vortex mixers need to be manufactured for computer control and must be designed to return to an exact position to compensate for the blind analyst. Disposable items (such as pipet tips that are bowed, screw-capped bottles that will not seal, etc.) can produce spills, malfunctions in laboratory operations, or in the worst case, cause catastrophic failure in unattended operations. Although these types of laboratory supplies have been around for years, the specifications and quality control in their manufacture did not become an issue until they were used in conjunction with robotics systems. A failure is a failure whether it is caused by a $2 tip, a $7 centrifuge tube, or a $50,000 robotics system.

Key Elements to Implementation

At the Third International Symposium on Laboratory Robotics, Frank Zenie, president of Zymark Corporation, summarized the key elements to implementation of robotics. "Once adequate funds and people are available, the following four requirements are key to all successful automation projects: 1) motivated people, 2) proven chemistries, 3) disciplined planning, and 4) creative implementation" (5).

Robotics is an emerging technology and as such needs development from key, dedicated personnel. The technology is new enough that systems will fail from short-term problems (lack of time, lack of resources, lack of key people). There are many obstacles that can make a robotics system fail, but with motivated, dedicated people these problems can be overcome.

Automation without valid, proven chemistries is useless. If the application is not based on sound, reliable chemistry, robotics systems will only be automating the generation of meaningless numbers and useless results. On the other hand, robotics with its exact timing and uniform sample handling can be used as a research tool for separating the chemistries involved from the variability in the manual techniques.

Disciplined planning is another key element in successful implementation. Robotics systems have a great amount of versatility but actual applications are well-defined and limited in **scope.** The goal, tasks, and laboratory unit operations (LUOs) need to be well defined and focused to allow automation, Examples of well-defined applications versus open-ended applications are as follows:

Well-defined	*versus*	*Open-ended*
machine, tool and die; writing a calculations program; production of goods;		sculpting writing poetry basic research
analysis of organochlorines in corn using the Luke screening method	*versus*	determination of all pesticides in all food groups

Creative implementation is necessary since the robot can not emulate the human in task performance. "The analogy between the marvelously dextrous human hand and the robot hand is extremely crude, as is the analogy between human learning and robot programming" *(6).* The good news is that the robot does not have to emulate the human in task performance. The human hand has more than *50* distinctively different movements. That degree of dexterity is not necessary to pick up a test tube and move it to a balance, mixer, etc. In developing conventional laboratory methodology, humans incorporate unit operations that maximize their strengths and minimize their weaknesses. As a result, manual methods are validated with procedures, timing sequences, and specific laboratory tools that are most efficient and convenient for the human. If a manual application is to be performed by a robot, tasks must be modified, then optimized, and finally programmed for the robot's capabilities. Robotics systems can be used for automating method validation as well as for routine sample preparation. Methods can be developed and optimized by systematically varying parameters, eliminating the need for first developing manual methods that need to be modified for use with robotics.

Robotics Applications

Laboratory robots and workstations have automated a variety of laboratory unit operations (LUOs) including weighing, pipetting, diluting, filtering, centrifuging, evaporating, solvent dispensing, mixing, etc., which can be sequenced for specific applications. More than 100 applications have been developed in the pharmaceutical, chemical, biologi-

cal, environmental, biotechnical, and food industries. More than 45 percent of those robotic applications are in the pharmaceutical industry. Currently more than 200 companies in the United States are using laboratory robotics. As of December 1987, the seven largest customers of laboratory robotics were The Dow Chemical Company, DuPont, Eastman Kodak, Eli Lilly, Merck, Monsanto, and Procter & Gamble, totalling almost 20 percent of the current robotics systems *(7).*

Current Status of Automation in Pesticide Residue Testing

The presence of several hundred registered pesticides has led to the development of multiresidue screening procedures. The FDA's pesticide analytical manual (PAM) describes screening procedures that are distinguished by both the type of food group and type of pesticide residue that are probably present. [8] All these methods have four basic, common operations: 1) obtaining a representative sample, 2) sample cleanup, 3) chromatographic analysis, and 4) data reduction and reporting.

Methodologies begin with procurement of a representative food material, preparation (peeling, grinding, homogenizing, etc.), and extraction with an organic solvent (acetonitrile, acetone, petroleum ether, etc.). The extraction step is usually a manual operation with large variations in sample and solvent sizes depending upon the food and levels of pesticides. This step is very labor-intensive, time-consuming and difficult to automate.

The sample cleanup isolates the pesticide from the rest of the matrix. This may be accomplished by techniques such as liquid/liquid partition, gel permeation chromatography, or solid phase extraction (SPE) columns (i.e., Florisil columns).

Sample cleanup is very labor-intensive and time-consuming. Developments in automating sample cleanup have been reported by several authors. For example, Stallings et al. applied automated gel chromatographic cleanup to the analysis of pesticides in fatty-food materials (9). Gretch and Rosen reported a cleanup procedure for multiresidue testing using automated solvent partitioning extractions interfaced with a column chromatography module for automated solvent partitioning extractions (10). Other automated gel permeation and chromatography systems addressed the cleanup step by automating collection of eluent fractions, evaporation, and solvent substitution as well as injection into a gas chromatography (11, 12). A review of papers from the *Advances in Laboratory Automation-Robotics* indicated robotic automation of the cleanup step using solid-phase extraction (SPE) columns of a variety of pesticide and non-pesticide compounds from a variety of matrices (13, 14, 15).

The third step involves the actual chromatographic analysis. The three most common techniques are gas chromatography (GC), liquid chromatography (LC), and gas chromatography-mass spectrometry (GC/MS). Types of gas chromatography detectors used for pesticide residue analysis include electron capture, flame ionization, or Hall conductivity. High performance liquid chromatography (HPLC) with ultraviolet or fluorescence detection is also widely used for pesticides that are not readily analyzed by GC. Autosamplers coupled with chromatographic instrumentation facilitate the automation of the analysis step.

Finally, the data reduction and summary reporting step has been largely automated with the use of computers,

Due to the newness of automation technology and the timeframe required for method development, review, and publication, the literature does not reveal much in the way of published information on the specific application of robotics to multiresidue testing. It is very difficult to breakdown the percentage of work that is being done using automation versus manual preparation since there is so much variation from laboratory to laboratory in the type and amount of equipment, funding, and personnel. Private inquiries as to the status of automation by the authors of this paper indicated a considerable amount of research being done.

Automation of preparation, cleanup, and detection of pesticide residues in foods has typically focused on single, discrete operations. Specific tasks have been automated with single-task, dedicated equipment such as autoinjectors, which make repetitive, precision injections into chromatographic instruments. Reducing the variables in injection techniques allows more unattended operations and higher use of the analytical equipment. Automation in the final analysis step with the use of autosamplers and computerized data systems was a major accomplishment in increasing sample throughput in the analytical laboratory and has been incorporated in most of the laboratories, yet it did not address the labor-intensive extraction or cleanup. Dedicated, multi-task instruments have been developed for automated fraction collection, and **solvent** exchange can process several dozen pesticide residue samples sequentially. The $20,000 to $40,000 capital investment has prevented the incorporation of this equipment into some laboratories. Laboratories with large numbers of the same sample type

and limited personnel resources may derive the most benefit from dedicated automation. On the other hand, a $50,000 robotic system may provide a better investment for these laboratories since the flexible, automated system may be used to process different types of samples as needed to meet the varying demands and optimize current methodology. As pesticide technology changes, the system can be upgraded to avoid obsolescence and reprogrammed to meet changing analytical needs.

At the Third International Symposium on Laboratory Robotics, Tinier et al. reported on their determination of deltamethrin (active ingredient in Decis insecticide) and its metabolize in milk and vegetables at the 50 ppb level (16). Their robotics system was used as a tool for optimization of various LUOs that are commonly used in screening techniques such as centrifugation, multiple solvent extraction, mixing, and solid phase extraction cleanup.

Applications using robotics for the automation of the Luke multiresidue screening procedure has been recently reported by Grady and Lento (17). Test results on several food matrices (tomatoes, corn, peas, and carrots) using several different organic, chlorinated pesticides gave similar results to those obtained by the manual assay. They predict continuing developments in the application of robotic techniques to other types of multiresidue screening tests, particularly in assays of fatty-type foods, organic phosphates, and methyl carbamates.

Constrains to the Use of Robotics for Multiresidue Testing

Regulatory and environmental samples are not readily automated because of some of the following factors:

1) Diverse type of samples and matrices
2) Widely varying classes and concentrations of pesticides
3) Large quantities of samples and reagents handled
4) Varying complexity and multiple sequences of extraction, cleanup, analysis
5) Lack of large series of similar samples
6) Need to show equivalency to official methodology

Samples requiring residue analysis have many variables (constraints 1-5), which interfere with the total automation process. In addition, many present day procedures were developed using equipment that is not suitable or compatible with other types of automation. The research cost for the development of new equipment to replace existing equipment is also a formidable problem.

The need to show equivalency to established methodology has placed a serious constraint on the practicality of automating current methods. Often it is cheaper for companies to stay with the manual techniques than to spend the time and effort on evaluating and implementing any new automation. Efforts need to be continued in the government and private industry to research and develop automated technology, particularly in the following areas:

1) robotics
2) laboratory information systems
3) automated cleanup apparatus
4) new, specific detectors
5) artificial intelligence/expert systems

Existing techniques and equipment need to be networked and integrated into working systems, not used solely as isolated workstations.

To test specifically for each of several hundred pesticides registered for use would be costly and extremely inefficient. Adoption of screening techniques (which could be automated) in conjunction with official methods (used for specific confirmation of over-tolerance samples) could allow for a rapid throughput of large numbers of samples without sacrificing regulatory methodology. The small number of suspect samples maybe reanalyzed using the more lengthy, time-consuming, and specific methodology. A tolerance assessment system should be maintained with methodology that is shorter and instrumentation that is sensitive and specific. By narrowing the scope of the problem (i.e., from measuring for all types of pesticides in all types of foods to development of a series of screening techniques for specific classes of compounds in specific groups of foods), the possibility of automation then becomes more a reality.

Once a method is modified for the robotic system, subsequent method validation is not a constraint due to the exact timing, uniform sample handling, and reproducibility that is inherent in robotic systems. Transfer of robotic technology can easily be accomplished, making the multi-validation procedure required for official validation easier.

Future Prospects for Automation

Future prospects for robotic automation include improvements in the following areas:

1) robotics
2) computers
3) sensors
4) associated technologies

Although pre-programmed robotics technology is still not fully developed, advances in that technol-

ogy could have the same positive impact that preprogrammed software packages had for the microcomputer systems, allowing an analyst to use the instrumentation without requiring an extensive knowledge of programming or theory of operation. Research and development is needed for improvements in existing electronics and mechanics of the robot. Expanded sample and solvent sizes (micro to macro-semi prep) are also needed for a variety of robotic applications.

Advances in computer technology will improve robotics, instrumentation, and data handling. The robots will need more memory, auxiliary control functions, graphics software, smaller physical size, and fewer hardware/software problems. Instrumentation must be able to communicate with computers as well as other dissimilar equipment without the need for extensive programming or additional interface modules. Computer technology must allow further integrated, networked automation in the laboratory.

Sensory technology such as sight, touch, hearing, temperature, and pressure need to be developed, miniaturized, and enhanced for the laboratory robot. Research and development of sensors will aid in the safety, performance, and feedback mechanisms of the new robotics systems.

One of the biggest opportunities for future improvement in robotics technology is with the associated technologies. Providing improved quality control and new designs in disposable (glassware, plasticware, etc.) can reduce the potential errors in unattended operations. Research and design of specific workstations (such as cappers, mixers, evaporation stations, etc.) that can be easily implemented with the existing robotics architecture will provide new application opportunities for the automated analyst.

"Laboratory automation is not based on a single technology, but rather on several technologies that can be focused on different parts of a lab operation. Some of those approaches are mature, others are evolving, and others are still experimental. Thus you should not attempt to implement a system in one grand stroke, but rather consider the options and plan a stepwise implementation" (8). If an advantage is to be gained using automation, all facets of the methodology must be examined. For example, while great strides have been made in automated analysis and data reduction, the sample preparation still involves much manual labor in many of the laboratories surveyed. Reviewing the prospects for future automation, several factors must be considered before choosing automated equipment such as amount of funding, training, and availability of personnel; numbers and types of current and anticipated samples; and where the strategic advantage would be gained using a particular type of automation.

Summary

Robotics allows reprogrammable, multifunctional, computer-controlled automation for a variety of laboratory unit operations up to and including complete applications. Although robotics is an emerging technology, it has made great strides in laboratory automation by addressing the need to link isolated workstations with one another and has provided a means for an integrated, laboratory network with other automated systems. Robotics should continue to' develop as a fundamental, integral tool for sample preparation, automation, and research and development in analytical laboratories.

Acknowledgment

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Potential of Immunoassay in Monitoring Pesticide Residues in Foods

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Contents

Figures

Abstract

The principles of immunoassays are presented, and selected applications of these assays for analysis of pesticide residues are cited; the advantages and disadvantages of immunoassays are compared with conventional analytical methods. The constraints and opportunities of immunoassay are discussed in light of regulatory and legislative influences.

Introduction

Analysis of agricultural commodities for agrochemical residues is usually time-consuming and performed by highly skilled chemists utilizing expensive analytical equipment. Consequently, costs of analyses are high. Five commonly used multiresidue methods detect 203 different pesticide residues, but this is less than half of the pesticides that the Food and Drug Administration (FDA)

claims may occur in foods. A number of the pesticides not detected in the multiresidue methods are classified as high health hazards and must be analyzed by single residue methods. Again, the cost of analysis is a major limiting factor in how many analyses can be performed. Regulatory agencies responsible for ensuring the safety of agricultural commodities, both grown at home and abroad, do not have limitless finances and can only analyze a fraction of the samples that should be analyzed, A small percentage of samples possess illegal levels of pesticides, but unfortunately, we have to analyze all the samples to determine the few bad ones. Not only are Federal and State regulatory agencies concerned about pesticide residues, but the general public is taking an ever-increasing interest and demanding greater assurances of the safety of food and water. United States' farmers also have raised questions about the importation of agricultural products from countries that fail to regulate pesticide usage, for the use of high levels of pesticides can certainly increase a farmer's yield per acre and provide a competitive edge in the marketplace.

There is a need for simple, rapid, cost-effective screening techniques for pesticide residues in raw and processed foods. Since most foods do not contain illegal residues, inexpensive semiquantitative techniques could screen large numbers of samples, and only those few samples found in violation could be further analyzed by more conventional means.

Immunoassay offer many of these advantages. They have been routinely used for many years in clinical and forensic laboratories for analysis of small molecules such as hormones and drugs. The procedures are becoming so simple that they are now being conducted in doctors' offices and even in private homes (e.g., pregnancy tests). Immunoassay should be equally applicable for the analysis of pesticide residues.

This article will briefly introduce the principles of immunoassays, present selected applications of these assays for analysis of pesticide residues, and compare the advantages and disadvantages of this technique to conventional analytical methods. We will also discuss our prejudiced views of the constraints and opportunities for adoption of these techniques and how the regulatory and legislative branches of government can and do influence evaluation and acceptance of immunoassays.

Immunoassay for Agrochemical Analysis

The use of antibodies to identify and quantify agricultural chemicals grew out of the clinical use of antibodies in infectious disease diagnostics and therapeutic drug monitoring. The immunologic principles behind the technology have been known for some time, so the relatively late onset of antibody-based analysis of agrochemicals was probably due to a failure to recognize its potential outside the medical arena. Even after the publication of the first chemical immunoassay in the scientific literature, no commercially available or regulatory agency-approved chemical detection system based on anti-chemical antibodies was seen until recently.

Preparation of Anti-Chemical Antibodies

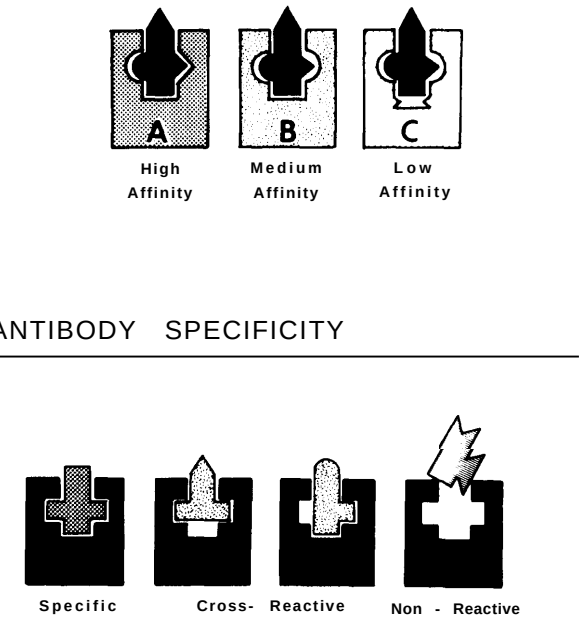
Antibodies are produced and secreted by plasma cells, the end-stage differentiated cell of the B-lymphocyte series. Plasma cells can be thought of as antibody-producing factories. The immunological rule of "one cell, one antibody, " means that only one kind of antibody is made and secreted by one plasma cell (27). The antibody molecule evolved as one of an animal's major lines of defense against foreign substances such as pathogenic microbes. Antibodies are proteins whose primary amino acid sequence dictates a tertiary or three dimensional structure that bears a site into which a distinct chemical structure can bind (26). Some of the structures that can induce and interact with antibodies include sugars on the capsules of bacteria, viral glycoproteins, or glycoopids on tumor cells, but nearly any chemical structure, if presented to the immune system in the proper configuration, can induce and bind with a particular antibody. The analytical capabilities of antibodies have been appreciated for a long time (2, 17). Indeed, to measure the amount of a particular substance, one can inject the substance into an animal, isolate the antibodies to the substance, and in one of many modifications of immunoassay, detect and quantify the substance. Such is also the case with antibodies to small organic chemicals, but another tenet of immunology must first be considered before these chemicals can induce antibodies. The small size of nearly all agrochemicals forestalls their ability to induce the production of anti-chemical antibodies. Nevertheless, by attaching the small chemical to a larger immunogenic carrier molecule such as a foreign protein, the immune system of an animal can be coerced into producing an anti-chemical antibody. A chemical structure too small to induce an antibody by itself, that when conjugated to a larger carrier molecule induces a specific antibody, is termed a hapten (17).

When injected into an animal, a chemical hapten-carrier complex induces an array of different anti-

bodies. There are antibodies to the carrier molecule in abundance, antibodies that bind to the hapten-carrier combination, and antibodies that bind only to hapten. It is only those antibodies that recognize the haptenic structure alone that are of value for the development of immunoassay, However, the antibody response to a particular hapten is extremely heterogeneous. Again, another tenet of immunology is based on the clonal selection theory (5). This theory states that foreign substances, such as the haptens, do not instruct the immune system to manufacture an antibody with structural complementarily to the hapten. Rather, there pre-exists in a mammalian immune system a B lymphocyte that is programmed to produce and secrete, upon stimulation by the proper chemical structure, an antibody with binding affinity to that structure, In essence the hapten selects from a pre-existing repertoire of B lymphocytes. In a typical immune response to a hapten, even with the limited size of a typical haptenic molecule, dozens or even hundreds of B lymphocytes with surface receptors capable of interacting even weakly with the hapten are stimulated to undergo proliferation and subsequent differentiation into end-stage, antibody-secreting plasma cells. Each of these clones of plasma cells secretes an antibody that recognizes in some way the haptenic structure. The family of antibodies that accumulate in the plasma of an animal following immunization with hapten-carrier are termed polyclonal in that they issue from many clones of plasma cells. The serum of such an immunized animal can be used as a source of anti-chemical antibody, and this antibody can be manipulated in ways to be discussed later for the quantification of the chemical.

The property of antibody binding to a chemical can be described by two closely related terms. The first is affinity, a term used to describe the strength of the interaction between chemical and antibody. Affinity is determined by the sum of all operant non-covalent chemical interactions (i.e., hydrogen bonds, van der Waals forces, hydrophobic and electrostatic interactions). A schematic representation of affinity is shown in figure 1. In this illustration, three antibodies are shown, all of which bind to the hypothetical chemical. The one on the left shows a perfect fit with the chemical and is thus a high affinity antibody. The middle antibody has one region of non-complementarity and is therefore of medium affinity, and the antibody on the right has only one complementarity region and is described as low affinity. The concept of affinity is important because, for most immunoassays, the higher the affinity of the antibody for the target chemical, the greater the sensitivity of the immunoassay.

Figure 1.—Antibody Affinity and Specificity

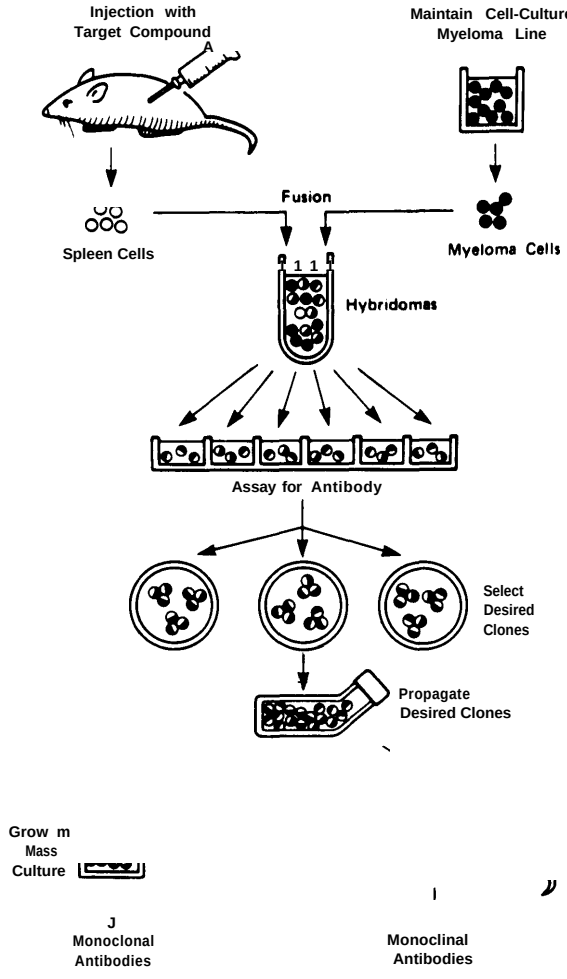


SOURCE: Ralph Mumma, Pennsylvania State University and Ken Hunter, Westinghouse Bio-Analytic Systems, Co., 1988.

The specificity of antibody-chemical interactions is illustrated in figure 2. Specificity and affinity are closely related terms, but they can be differentiated for illustration. Here we have one antibody and four chemical structures. On the left is a very specific interaction between antibody and chemical, and on the far right there is no interaction at all. The two chemicals in the center show a degree of cross-reactivity with the antibody due to a degree of structural homology with the chemical on the left. Specificity is of considerable importance because an immunoassay, as any analytical method, must be able to distinguish between related chemicals.

As mentioned earlier, the serum of an immunized animal can serve as a useful source of anti-chemical antibodies for the development of chemical immunoassay. However, there can be drawbacks to the use of serum polyclonal antibodies, First, the population of antibodies in serum is dynamic with respect

Figure 2.—Monoclonal Antibody Preparation



SOURCE: Ralph Mumma, Pennsylvania State University and Ken Hunter, Westinghouse Bio-Analytic Systems, Co., 1988.

to concentration and quality. Secondly, the presence of an array of qualitatively different antibodies may in some cases obscure the analytical capability of the serum. Finally, the serum often bears unwanted antibodies that bind to the carrier molecule or the spacer unit between carrier and hapten; these antibodies, whether of natural origin or induced, may confound the analytical application. The unwanted antibodies often can be moved by purification steps (e.g., affinity, chromatography). Notwithstanding these problems occasionally encountered with polyclonal antibodies, many excellent immunoassay have been developed using these biological reagents. However, a recent technological advance now allows a single form of anti-chemical antibody to be produced in unlimited quantities.

Hybridomas and Monoclonal Antibodies

In 1975, two British scientists, George Kohler and Cisar Milstein, discovered that somatic hybrids between B lymphocytes and myeloma cells could produce antibody of "predefined" specificity (16). That is, the donor animal could be immunized with a target substance and immortal clones of these hybrids that secrete one particular antibody (monoclinal) with binding affinity for the target substance could be isolated. The hybrid tumors are known as hybridomas, and the monoclinal antibodies secreted by these cells have certain advantages. A generalized schematic of the hybridoma production procedure is shown in figure 2. Briefly, the spleen from an appropriately immunized mouse is removed and dissociated into a single cell suspension. These cells, some of which produce antibody to the target substance, can be maintained in nutrient medium (tissue culture] outside the mouse, but only for a few days. Although some specific antibody can be identified in the culture medium, it is too little to be of practical value. However, these short-lived cells can be physically fused in the presence of an agent such as polyethylene glycol (10) to myeloma cells, tumors of B lymphocyte origin that can live indefinitely in tissue culture (28). The resulting hybrids are heterokaryons, bearing the combined genetic information or genotype of both parental cells. Of paramount importance, the hybrids express two critical phenotypic characteristics, one derived from each parental cell; they secrete the antibody of the parental B lymphocyte, and they have unlimited growth potential, a trait of the parental myeloma. An elegant biochemical selection system is used to isolate the hybridomas (18), which are subsequently cloned to insure homogeneity. The cloned hybridomas can be grown in mass culture where the secreted antibody accumulates in the culture medium, or they can be adapted as ascites tumors in the peritoneal cavities of mice where very high levels of antibody accumulate in the ascites fluid. In either case, the product of the cloned hybridomas is a monoclinal antibody, a homogeneous reagent. The hybridomas can be cryopreserved and stored indefinitely in liquid nitrogen, and the monoclinal antibody is stable indefinitely under a variety of storage conditions. Therefore, the hybridoma technology can produce an unlimited, stable, and homogeneous supply of monoclinal antibodies.

Principles of Chemical Immunoassays

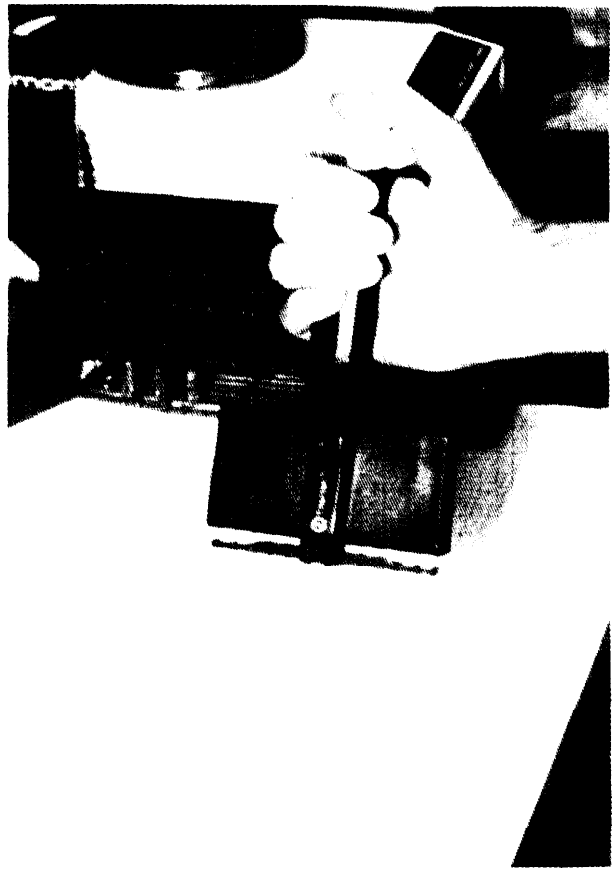
An enormous variety of immunoassay configurations have been developed, and a thorough review is beyond the scope of the present paper. However,

as all immunoassay obey the same laws of mass action and thermodynamics, some general statements can be made. Immunoassay for small molecules such as pesticides must operate by competitive inhibition or displacement in which the binding of the free pesticide molecule to the antibody competes or displaces a tracer molecule, By way of example we will briefly discuss enzyme immunoassay, the most widely used method that employs an enzyme as the tracer and generates a color reaction as the read-out. Other tracers include radioisotopes, fluorescent molecules, magnetic particles, electron spin labels, etc.

The enzyme immunoassay, a modification of the original enzyme-linked immunosorbent assay of Engvall and Perlmann (7), is conveniently performed in a 96-well plastic microtiter plate (see figure 3), but it can be done with tubes or test strips. Prior to the first step in the enzyme immunoassay, the surface of the microtiter wells is coated with an optimal concentration of target chemical-protein conjugate (figure 4). Another popular configuration uses surface immobilized antibody, but the basic principle of both assays is the same. The conjugate adsorbs to the plastic surface by hydrophobic interactions, and following an incubation to assure maximum binding, the nonadsorbed conjugate is removed by washing with buffer containing a mild nonionic detergent. The first addition to the coated plate is a mixture of anti-chemical antibody and a known concentration of target chemical. If no target chemical was added to the antibody, most of the antibody would bind to the target chemical-protein conjugate adsorbed to the solid surface of the plate. The higher the concentration of target chemical added with the antibody, the lower the number of antibodies that bind to the solid phase due to the competitive inhibition of their binding sites through interaction with free target chemical in the fluid phase. After an incubation period, the reactants are washed away, leaving only the antibodies bound to the target chemical-protein conjugate on the plastic.

The second step of the procedure involves the addition of a tracer to detect the surface-bound antibodies from step one. In the case of the enzyme immunoassay, the tracer is a second antibody to which an enzyme is attached. This second antibody-enzyme conjugate binds to the surface adsorbed anti-chemical antibodies, and following an incubation, unbound second antibody is removed by washing, The third step in the enzyme immunoassay involves the addition of a solution of colorless enzyme substrate, which is converted by the enzyme into a colored reaction product, the concentration of which is a direct measure of the concentration of antibody-enzyme tracer bound to the anti-chemical antibody on the plastic surface.

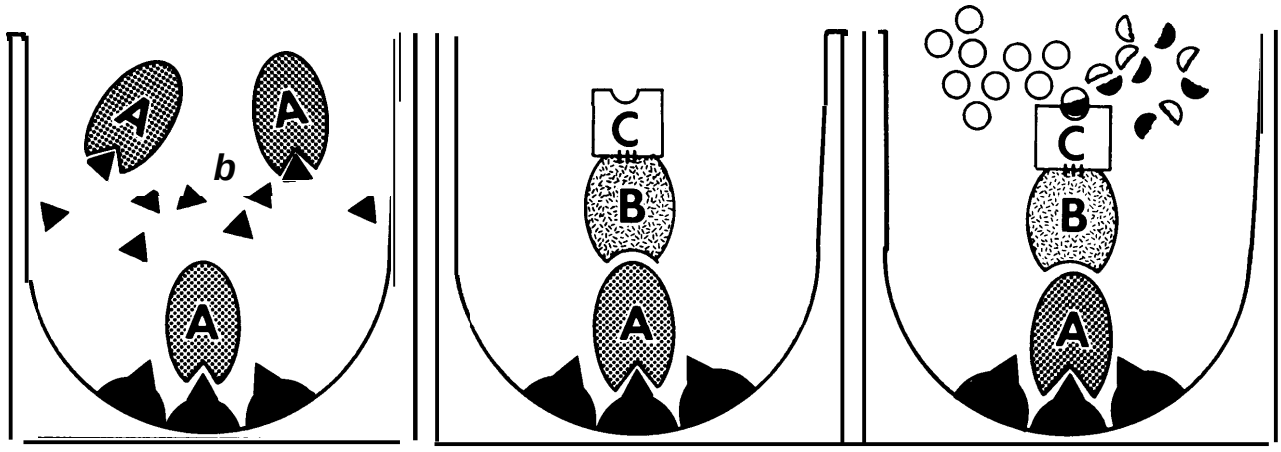
Figure 3.—Applying Reagents to 96 Well-Plate



SOURCE: Ralph Mumma, Pennsylvania State University and Ken Hunter, Westinghouse Bio-Analytic Systems, Co., 1988.

Because the color reaction is directly proportional to the number of anti-chemical antibodies bound to the plate, it is inversely proportional to the concentration of free target chemical. The higher the concentration of target chemical, the lower the color reaction. By running a series of known concentrations of target chemical, one can create a standard curve such as that shown in figure 5. A plot of optical density (color) versus target chemical concentration yields a curve with a linear portion often extending over several orders of magnitude. The enzyme immunoassay becomes an analytical tool when unknown samples are run at the same time and their optical density values compared with the standard curve. The apparatus for analyzing the

Figure 4.-Competitive Inhibition Enzyme Immunoassay



STEP I **STEP II** **STEP III**

SOURCE: Ralph Mumma, Pennsylvania State University and Ken Hunter, Westinghouse Bio-Analytic Systems, Co., 1988.

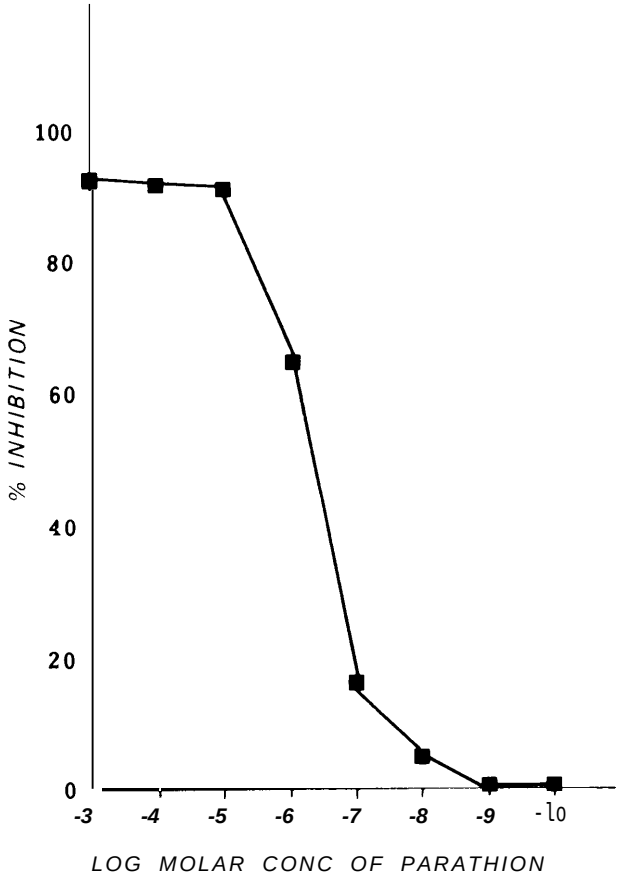
color reaction consists of a commercially available automated spectrophotometer that can evaluate the color in each of the 96 wells in less than one minute, a microcomputer interfaced with the spectrophotometer, and a software program for analyzing the data (see figure 6).

Enzyme immunoassay such as the one described earlier require 2 to 4 hours to perform, and most of this time is devoted to incubation. This assay format is highly quantitative, but other formats such as tube and test-strip enzyme immunoassay can be performed in minutes, and the results can be quantitative or semiquantitative. The criteria of sensitivity, specificity, accuracy, precision, and ruggedness, all critical to any treatment of conventional analysis, must also be addressed in chemical immunoassay. The enzyme immunoassay and similar assays that use a standard curve for comparison and determination of values in unknown samples are very amenable to statistical treatment. Although there is variation between particular chemical immunoassay due to the nature of the target chemical and the idiosyncratic properties of each anti-chemical antibody, chemical immunoassay can generally be as sensitive as conventional analysis techniques. Specificity is an inherent property of the antibody and is defined as the spectrum of cross-reactivities with related chemicals. It is not unusual, however, to see discriminatory capability at the single atom level, or even stereochemical selectivity (4). Accuracy and precision are more related to the performance of the immunoassay than the properties of the antibodies, and for such assays as the enzyme immunoassay, these criteria are comparable to most conventional analysis methods. The variation between assays and between laboratories running the same immunoassay is also comparable. Immunoassay have component parts just as conventional assays, and these components must be standardized. For antibodies, this means that large batches of purified reagents must be prepared, stored in a way that preserves their integrity, and tested in standardized assays to ensure their quality.

Practical Applcations of Chemical Immunoassays

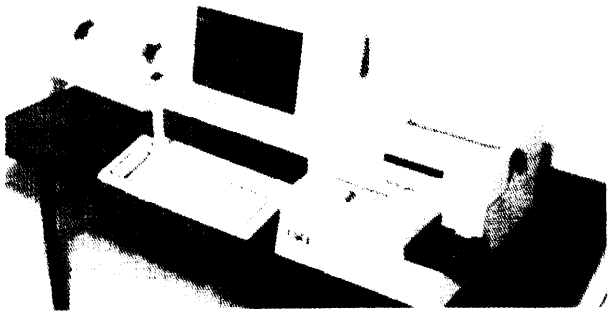
Ercegovich (1976) was one of the first persons to recognize the potential of immunoassay to pesticide residue analysis. His students and colleagues (one of the authors, ROM) pioneered work in developing immunoassay for the organic phosphate insecticide, parathion (1, 9, 30). Similarly, Bruce Hammock's laboratory was actively developing immunoassay for pesticides (32), and Ken Hunter and colleagues (13, 14) developed antibodies to paraoxon and other organic phosphates recognized as war gasses. Subsequently, antibodies have been developed and reported for more than 30 pesticides, and a number of papers have appeared reviewing this

Figure 5.—Standard Curve: Parathion Immunoassay



SOURCE Ralph Mumma, Pennsylvania State University and Ken Hunter, Westinghouse Bio-Analytic Systems, Co , 1988

Figure 6.—Computer, Printer, and Spectrophotometer



SOURCE: Ralph Mumma, Pennsylvania State University and Ken Hunter, Westinghouse Bio-Analytic Systems, Co., 1988.

progress (11, 12, 21). Industrial companies, some with commercial interest in mind, have developed many more immunoassay for pesticides, plant growth regulators, antibiotics, and other xenobiotics, but these data have not and may never be published.

Antibodies have been developed for various classes of pesticides, e.g., organic phosphates, carbamates, triazines, halogenated hydrocarbons, chlorophenoxy herbicides, pyrethroids, chitinase inhibitors, and biorational insecticides (21). Immunoassay also exist for a number of fungicides that cannot be grouped easily into a chemical class, such as Benomyl, Iprodione, maleic hydrazide, Metalaxyl, and Triadimefon. Interestingly, the antibodies for the pyrethroid S-bioallethrin exhibited chiral specificity (32), which cannot be achieved by any approved conventional method. An important potential use is with the biorational insecticides, such as the exotoxins from Bacillus thuringiensis, which can be quantified using immunological techniques. In the future, many biological agents derived through molecular biological techniques may be targeted for pest management practices, and immunoassay may be the only practical method to quantify these organisms or agents. Two environmentally sensitive chemicals, dioxin and pentachlorophenol, can also be analyzed in this manner, and an EPA-approved immunoassay for the latter compound is expected shortly.

Most of the early developmental work of immunoassay has been performed in academic institutions and with polyclonal antibodies. Unfortunately, very few examples of practical applications are documented in the literature. An exception is that of the contribution of W.H. Newsome from the Food Research Division, Bureau of Chemical Safety, Health and Welfare, Canada. Newsome has developed immunoassay for several fungicides and comparatively evaluated these with conventional methods (22, 23, 25, 24, 33). Van Emon et al. (31, 1987) have also compared immunoassay and conventional techniques in worker exposure studies with the herbicide paraquat. The authors of this article feel that many more application experiments need to be performed before we can thoroughly understand the influence of the matrix on immunoassay results.

The detectional limits of currently developed immunoassay for pesticides usually range from 0. I to 1,000 parts per billion. Pesticide tolerance limits on many raw agricultural commodities are in the order of parts per million, and thus, immunoassay

are sensitive enough to immediately make a contribution. With aqueous products such as water, fruit juices, and milk, immunoassay can be directly performed without any cleanup steps (3, 11).

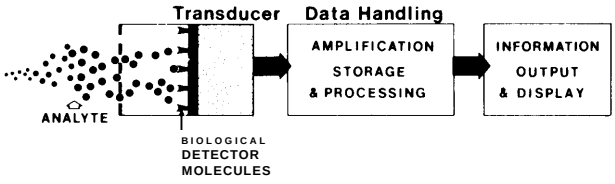
Real-Time, On-Line Agrochemlcal Monitoring

Almost all of modern analytical chemistry deals with discrete measurements. Unfortunately, chemicals often present dynamic problems, concentrations waxing and waning over time (e.g., groundwater contamination with pesticides). Even the immunoassay cannot provide real-time (instantaneous), on-line (continuous) monitoring of these chemicals, However, a revolution in sensor technology is upon us that may provide both capabilities and may do so utilizing the same biological molecules generated for immunoassay.

The interface of biologic molecules like antibodies with microelectronic sensor systems to create hybrid devices known as biosensors promises to provide analytical capabilities beyond those now available. A biosensor is defined as a microelectronic device of one kind or another that utilizes a biologic molecule as the sensing or signal-transducing element. The structural requirements of a biosensor are shown in figure 7, and they include the following: a means of introducing the sample matrix to the sensor surface; an antibody or other biological molecule with binding affinity for a particular analyte in the matrix; a transduction mechanism whereby the binding event generates an electrical signal; appropriate amplification, processing, and storage of the generated signal data; and a means of outputting the information in a usable format.

A review of the many potential biosensors and the principles upon which they are based is beyond the scope of this report, and the reader is referred to recent reviews (6, 19, 20, 15, 29).

Figure 7.—Generalized Biosensor



SOURCE: Ralph Mumma, Pennsylvania State University and Ken Hunter, Westinghouse Bio-Analytic Systems, Co., 1988.

Comparison of Immunoassay With Conventional Methods of Pesticide Residue Analysis

Conventional Methods versus immunoassay Methods

Before we compare conventional analytical methods to immunological assays, we must first summarize the steps used in both procedures. In a traditional analysis the raw agricultural commodity or processed food is first subsampled and extracted or homogenized with an organic solvent to remove the pesticide residue from the insoluble debris, The organic extract is concentrated to small volume, and then an aliquot is analyzed by gas or high performance liquid chromatography. Pesticides are usually identified by the relative time it takes them to come through the chromatographic column (retention time) and their response to selective detectors. Chemical specific detectors are usually used with gas chromatography, and these detectors indicate the presence of halogens, nitrogen, phosphorous or sulfur atoms, which may be components of the pesticide residue. Although these detectors are highly specific, many compounds contain these atoms, and analyses can be confounded by such interfering compounds. Ultraviolet detectors are often the preferred method of detection with high-pressure liquid chromatography, but these detectors are also sensitive to all ultraviolet-absorbing substances. Because of these interference problems, organic extracts of food and raw agricultural commodities usually have to be partially purified using organic-solvent partitioning steps and time-consuming column chromatography prior to analysis by gas or high pressure liquid chromatography. This partial purification is often referred to as a cleanup step, Conventional analytical techniques are time-consuming, require environmentally sensitive and ultrapure organic solvents, utilize expensive chromatographic equipment, and require highly trained technicians. This results in expensive analyses for pesticide residues even for the most simple compounds.

However, when all procedures are followed, conventional analytical techniques are reliable, repeatable, and sensitive, Gas chromatography using atom-specific detectors usually can detect residues at the 1 to 100 picogram (10-9fl grams), level but only a small amount of sample (1 to 5 microliters) can be analyzed in this manner. With high pressure

liquid chromatography, a much larger aliquot of sample can be analyzed (25 to 50 microliters), but ultraviolet detectors usually require at least 1,000 picograms of residue, Gas chromatography separations require the chemicals to be volatile enough for separation as a heated gas, but unfortunately, many pesticides and their degradation products are not volatile and cannot be identified by this method. This is particularly true of the more polar, water-soluble pesticides and their metabolizes. Also many pesticides do not absorb strongly in the ultraviolet and cannot directly be quantified with high sensitivity by this technique.

Immunological assays for pesticides may also involve many of these basic procedural steps. For the purpose of this presentation, we may think of an antibody as a very selective detector that is particularly useful for polar and water-soluble materials. In fact, immunological techniques are superior to conventional techniques in the direct analysis of pesticides in water. As for conventional methods of analysis, immunoassay require that processed food and raw agricultural commodities be extracted prior to analysis. However, the cleanup steps may be much abbreviated with the immunological technique. Also with liquid products, such as fruit juices, milk and soups, immunological analysis can be performed directly (3, 35). A potential drawback to immunoassay is that they are compound-specific and therefore most useful for single residue analysis. In contrast, conventional multiresidue procedures can detect and quantify many pesticide residues simultaneously. However, an antibody's great specificity does not always have to be an issue since antibodies can be selected that detect several chemicals of related structure, and different compound-specific antibodies can be combined in one analysis. Alternately a number of aliquots of food extract can each be analyzed with antibodies selective for a specific compound or for classes of compounds.

Immunoassay can be as reliable and repeatable as conventional methods of analysis, but usually the more highly quantitative immunoassay require more time to run than less quantitative immunoassay, Other immunoassay configurations can be quicker and simpler (1 to 10 minutes), but they are usually semiquantitative. However, if the emphasis is only on pesticide levels in food that exceed a certain concentration requiring regulatory action, the immunoassay are superior screening techniques. Immunoassay are also readily automated, while conventional analytical procedures are not,

Advantages and Disadvantages of Immunoassays

From an application standpoint, most immunoassay for pesticide residue analysis are simple and rapid, and in some cases, they may be used without any cleanup step. They are particularly useful for polar or water-soluble pesticides and their degradation products, and often immunoassay can easily be developed for compounds that are difficult to analyze by conventional methods. Since regulatory laboratories do not routinely analyze for pesticides not included on their multiresidue procedures list, immunoassay has the potential of filling this important void.

On the negative side, we should cite that the more rapid versions of immunoassay are usually not as sensitive and probably not as reproducible as conventional analytical techniques. Immunoassay may not be useful in a broad multiresidue procedure, even though several antibodies can be combined in one analysis. Other disadvantages include the lack of extensive commercialization, the lack of personnel with immunoassay experience, and the lack of knowledge and practical applications to raw and processed food.

Constraints, Opportunities, and Recommendations

Regulatory Agencies

Regulatory agencies at both the Federal and State levels are too slow to adopt or encourage modern methods and immunoassay is no exception. They seem to be spending most of their time on validation testing rather than funding or conducting state-of-the-art analytical methods development. To cite some examples, use of capillary column gas chromatography is only now starting to be accepted—it has been a routine procedure in most academic laboratories for years. Solid phase extraction (SPE) or concentration is rapidly being utilized by industry and private laboratories but it is not being emphasized by regulatory agencies. SPE is particularly attractive, since it often eliminates the need for expensive and environmentally sensitive solvents; this alone should be reason to encourage their use.

Immunoassay for pesticides have been demonstrated for more than 10 years, and regulatory agencies should have been taking a lead role in the development of these new techniques. Since many

agrochemical industries and private laboratories have utilized these techniques for several years, regulatory agencies should encourage these organizations to make their data public so we can get a better feeling for the capabilities of these new methods. The agrochemical industry would not be using immunoassay unless they felt they were useful and reliable for their desired goals. Why has not the EPA or FDA sought information on these analyses so they could make more knowledgeable decisions? Additionally, why do they expect all analytical methods to meet the same strict requirements when perhaps only a screening procedure would be sufficient? By making quantitative analyses so difficult and expensive, regulatory agencies are actually reducing our knowledge of environmental pollutants because present methods can analyze only a fraction of the samples that should be analyzed.

There is at least one bright light in this dim world. The State of California has contracted for the development and testing of a number of immunoassay for pesticides. It is a pleasure to see a State regulatory agency take the lead in developing and evaluating this potentially important tool for the future.

Legislative Actions

Both State and Federal legislatures have constrained the regulatory agencies because they have asked them to do too many things and have not provided the financial backing to perform these tasks, Legislatures could take the lead by encouraging development and applications of the new methods of analysis. They should require the regulatory agencies to set aside a reasonable fraction of their budget moneys for developing the methods of the future. They should support grants and contracts to companies willing to pursue developments of new methods like immunoassay. Many new technologies such as immunoassays, enzyme assays, biosensors, solid-phase extractions, and affinity scavenging are now emerging, and much work needs to be done to determine their capabilities. We do not envision these techniques as replacing conventional methods, but rather as supplements to these methods. Such legislative action would stimulate development of these areas, and the well-being of the general public would greatly benefit.

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Federal Pesticide Monitoring Programs: Analytical Methods Development

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Conents

Figure 8

Abstract

This paper provides a brief overview of the needs of the U.S. Environmental Protection Agency (EPA), Food and Drug Administration (FDA) and United States Department of Agricuhure (USDA) in the area of analytical methods for monitoring pesticide residues in food. The importance in developing effective methods for tolerance enforcement that are rapid, sensitive, and inexpensive is discussed. The congressional mandates and agency approaches related to food residue monitoring, tolerance enforcemerit, and methods development are also described.

The effects of (l) changes in agricultural use practices that increase the extent of pesticide residues in the food supply and (2) limited tolerance data on the methods development process are noted. The acquisition of adequate metabolism data is the single most important chemistry contribution to the methods development process. Without full knowledge of the chemical identity of significant metabo-

lites that occur as residues in food, it is impossible to develop monitoring methods for all residues of concern. While the primary focus for tolerance enforcement is on analytical methods, of equal importance is the need for readily available analytical reference standards.

The contribution analytical methods have in providing monitoring feedback for tolerance enforcement and tolerance-setting, in addition to their role in reducing the uncertainty in risk assessment, is also noted. Finally, suggestions are made for improving analytical methods for monitoring pesticide residues in food,

Background

Prior to any discussion on analytical methods to improve the monitoring and enforcement of tolerances for pesticide residues in the food supply, a brief overview of tolerances and related terminology is needed. Since tolerances depend on the state of scientific and technical knowledge (including analytical methods) at the time they are established, any limits in the existing data used will impose a corresponding limit in the analytical method used. Without an understanding of the key data elements that lead to a tolerance, it will be difficult, if not impossible, to significantly improve the analytical methods or the method development process for better tolerance enforcement (l).

Tolerances

A tolerance is the legal maximum residue concentration of a pesticide chemical allowed in a food or feed. Tolerances minimize uncertainty about food safety with regard to those pesticide residues. If a pesticide is detected and residues exceed the tolerance or no tolerance is established, the crop may be considered adulterated and be seized by the Food and Drug Administration (FDA), the United States Department of Agriculture (USDA), or a State enforcement agency. EPA establishes tolerances for pesticides, while FDA, USDA, and the States carry out tolerance enforcement in foodstuffs. EPA also provides the analytical standards used intolerance enforcement (2).

Tolerances are set under authority of the Federal Food, Drug, and Cosmetic Act (FFDCA). Section 408 of the FFDCA applies to residues on raw agricultural commodities (RACs) and Section 409 applies to processed food or feed. Section 409 includes the Delaney Clause, which specifically prohibits the use of cancer-causing agents as food or feed additives.

There are three types of residue chemistry data that are essential for establishing tolerances:

1. Qualitative Data on Metabolism and Degradation
2. Quantitative Data on Magnitude of the Residue
3. Analytical Methods

The purpose of these chemistry data is to answer two basic questions. First, what is the chemical residue? Second, how much residue is there? Analytical methods are essential in providing answers to these two fundamental exposure questions. The "what" and "how much" information is used by EPA toxicologists to determine whether the dietary exposure is acceptable. The first half of EPA's tolerance-setting job is completed when EPA has concluded what and how much residue is present and that this level of residue is safe. The other half of EPA's job is to be sure adequate enforcement methods are available to check that the residue levels in the food supply do not exceed the tolerances.

@qualitative Data on Metabolism and Degradation

In order to answer the "what is the residue" question, qualitative data are required to determine the identity of the pesticide residues resulting from the transformation in plants and animals. EPA refers to these transformation studies that include both pesticide degradation and metabolism as metabolism studies.

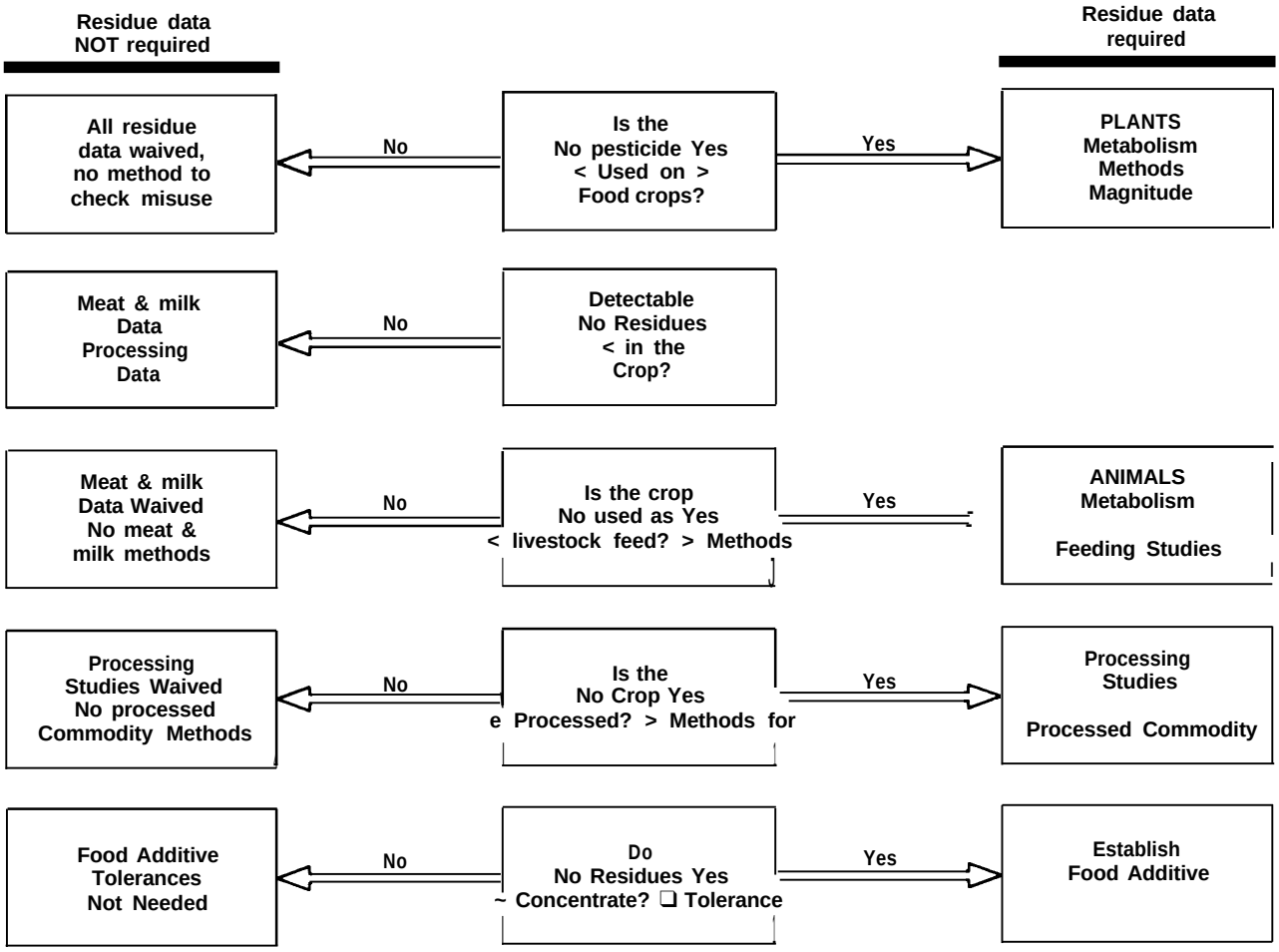
Metabolism in Plants

Plant metabolism data characterize the identity of the residue that occurs in crops intended for consumption as a food or animal feed, These data identify the pesticide residues that remain in agricultural crops as the result of environmental transformation processes (degradation and metabolism). The resulting residue at harvest may be different than the chemical applied, due to breakdown or metabolism of the applied pesticide.

Metabolism in Animals

Whenever use of a pesticide results in residues in a livestock feed, or when a pesticide is applied directly to livestock, animal metabolism studies are required. The resulting data identify the pesticide residues to look for in the edible tissues of livestock or milk and eggs that result from transformation processes in the animal. If feed items are not involved or if this exposure pathway is blocked by label restriction, these data are not required (see figure 1).

Figure 1 .—Residue Chemistry Data Requirements



SOURCE: Charles Trichilo and Richard Schmitt, Environmental Protection Agency, 198S

Quantitative Data on Magnitude of the Residue

Magnitude of the Residue

After the metabolism data have indicated what residue to look for in plants and animals (if applicable), methods are developed to measure these residues, Actual residue field trials are carried out to quantify the residues. These are the studies in which the pesticide is applied to crops at known application rates, in a manner similar to the use directions that will eventually appear on the label. The residue field trial studies result in residue data for the raw agricultural commodity (RAC) as it travels in interstate commerce.

Processing Studios

Processing studies are required to determine whether residues in raw commodities can concentrate or degrade on processing. If residues concentrate on processing, food or feed additive tolerances must be established. If residues do not concentrate on processing, the tolerance on the RAC applies to all processed food or feed derived from the RAC, It should be noted that the current EPA legal opinion on the Delaney Clause is that it applies to food

and feed additive tolerances but not to RAC tolerances.

feeding Studies

Livestock feeding studies are required whenever residues result in or on crops that are used as feed items. These studies provide data on the quantitative transfer of residues to meat, milk, poultry, and eggs. These studies are also required if a pesticide is applied directly to livestock.

Analytical Methods

Analytical methods serve two important purposes. The first is to generate residue data on which the dietary exposure assessment is based. The second is to enforce the tolerance after it is established. It should be noted that plant and animal metabolism data are the most critical data that precede the development of analytical methods. Without proper and complete metabolism studies to indicate which residues to look for, the development of analytical methods for all residues of concern may not be possible (3, 4).

Since risk assessment depends on exposure, analytical methods can have a significant impact on reducing the uncertainty in risk assessments by providing needed exposure data.

Analytical methods also serve an important role in providing feedback for tolerance enforcement and tolerance-setting procedures. Routine monitoring provides the obvious feedback on whether tolerances are being exceeded, or on whether tolerances have been set too high. However, sometimes the results of tolerance enforcement can lead to needed changes in the tolerance-setting process. For example, FDA monitoring uncovered over-tolerance residues of malathion in grain dust. Grain dust is now routinely collected at grain storage sites to prevent dust explosions and has become a disposal problem. Recently, the industry began pelleting this dust and using it as an animal feed. Due to the high concentration of pesticide residues in the dust, feeding of grain dust could lead to detectable residues in meat or milk. Furthermore, feeds formulated with grain dust as an ingredient are subject to seizure by FDA. As a direct result of the monitoring by FDA, EPA established a 135 ppm tolerance for malathion on grain dust (5). EPA also is revising its tolerance-setting procedures to routinely establish tolerances for grain dust to ensure that any potential residues in meat and milk are covered by tolerances and are safe.

Effect of Limited Tolerance Data on Analytical Methods

Any limits in the data that are used to establish tolerances will have a profound impact on the analytical methods. From EPA's perspective, there are two areas that can have a significant effect on the current state of the adequacy of analytical methods: 1) incomplete metabolism data, and 2) missing or impractical label restrictions that do not block exposure pathways,

Analytical methods can only be developed for those components of the residue that are identified in the metabolism studies. If metabolism studies do not fully identify the residues present, important components of the residue may remain undetected. Older chemicals whose metabolism studies fail to identify the significant residues present constitute the largest problem here. From EPA's experience in reviewing older chemicals as part of the re-registration process, it is not uncommon for 50 to 80 percent of the ^{14}C residues in radiolabeled metabolism studies to be unidentified. These limited data have an important effect on the ability to develop analytical methods, The development of analytical methods for chemicals with significant metabolism deficiencies will be delayed until the needed residue identification work is completed.

It should be noted that the complete set of residue data are not always required, particularly if the exposure pathways that lead to residues moving further into the food chain can be blocked by practical label restrictions. Determining what is practical is subject to much judgment and is further complicated by the dynamics of changing customary agricultural practices; this includes both economic and weather conditions that may affect the supply and demand of food or feed items.

In general, label restrictions are considered practical if three criteria are met: 1) the crop is under the direct control of the grower; 2) the economic value of the crop as a feed item is low; and 3) the U.S. customary practice is not to use the item as a feed. For example, label restrictions against feeding corn forage to prevent residues from moving into meat and milk commodities are not practical. Even though corn forage is under direct grower control, the high value of the feed item and the overwhelmingly common practice of feeding this commodity makes the restriction impractical.

What was practical at a certain period of time can change as use practices change. For example, until recently EPA considered the feeding directive, "Do not feed sugar beet tops" to be a practical restric-

tion. Accordingly, data on metabolism, magnitude of the residue, and analytical methods were waived, since the feeding restriction was expected to prevent residues from moving into meat and milk. Therefore, analytical methods for determining residues in meat and milk were not available, since no pesticide residues were expected in these environmental media. In recent years, sugar beet tops have increased in economic value so much that California growers have changed their customary practice and now sell the beet tops for livestock feed. In this case, EPA was aware of the change and required data, including analytical methods, to cover any residues that could be expected in meat and milk.

However, EPA is not always aware of changes in use practices that result in residues moving further into the food chain than originally expected. The EPA Re-registration/Registration Standard process is one systematic scheme to identify such a problem area and call in the needed data. Again, however, until all chemicals are given a current review, the potential will exist for changes to occur in use patterns that result in more residue in the food supply with no corresponding analytical methods for enforcement.

It is important to note that no residue data are required for all nonfood uses. For nonfood uses, analytical methods are not required for detecting pesticide residues in food or feed crops, since residues are not expected in the food chain. Some older uses, previously considered as nonfood uses, may now actually be food uses that require residue data. Until these situations are identified, monitoring for food residues may not be possible because analytical methods are lacking. For these previously classified nonfood-use chemicals, analytical methods may not be available to FDA, USDA, and the States to check for accidental contamination or illegal use in food and feed.

Importance of Analytical Standards

Up to this point, the importance of analytical methods for monitoring pesticide residues in foods has been the primary emphasis of the Office of Technology Assessment workshop. Of equal importance, however, are the analytical standards that are used in the laboratory by those chemists conducting monitoring or enforcement activities. An analytical standard is a high purity reference standard used to calibrate the detector response of an analytical method. Chemists use a known analytical method together with a known analytical standard whose behavior and response is very predictable under laboratory conditions. Use of inappropriate standards or standards of low purity will lead to erroneous methods results, If analytical standards are not of sufficient purity, enforcement becomes more time-consuming and difficult as predictable behavior cannot be obtained. If analytical standards are not available, enforcement becomes difficult, if not impossible.

When EPA is aware that analytical standards are not available, the agency can act under its authority under FIFRA *3(c)2(b)* to require the pesticide registrant to submit additional quantities of the analytical standards. Failure of a registrant to provide or maintain analytical standards in the EPA repository can result in cancellation of the U.S. registration, EPA cooperates with FDA by providing analytical standards for those pesticides not having U.S. registrations that FDA needs to monitor imports. It should be noted that for pesticides used on imported foodstuffs that are not registered in the United States, there is no similar mechanism to obtain standards if the foreign registrant does not wish to cooperate.

Summary of Key Inputs to Food Monitoring

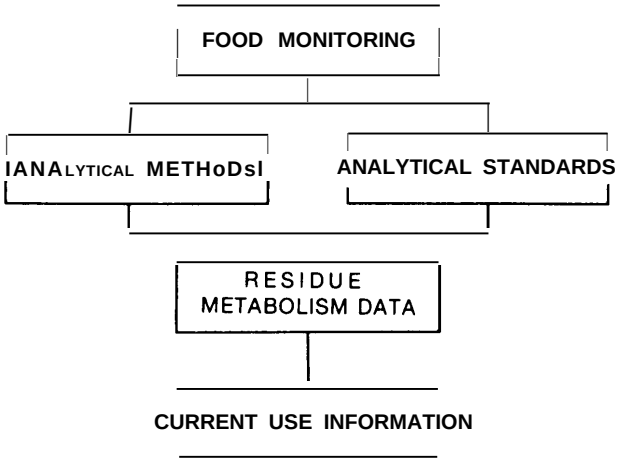
The importance of(1) changes in agricultural use practices that increase the extent of pesticide residues in the food supply, (2) limited tolerance data on residue identification (metabolism studies), and (3) the availability of analytical reference standards to food monitoring methods is depicted in figure 2. Without full knowledge of the chemical identity of significant metabolizes that occur as residues in food, it is impossible to develop monitoring methods for all residues of concern. Similarly, the absence of analytical standards or the lack of knowledge about any increase in the extent of pesticide residue involvement of the food chain due to changes in agricultural use practice severely hampers the methods development process.

Overview of FDA, USDA, and EPA Needs

FDA

The Food and Drug Administration's (FDA) congressional mandate for enforcing tolerances is contained in the Federal Food, Drug and Cosmetic Act (FFDCA). The FDA is responsible for monitoring and enforcing tolerances for pesticide residues in all foods and feeds except meat and poultry. They need rapid, inexpensive methods for a wide variety of food matrices. FDA relies primarily on mul-

Figure 2.— Key Input to Food Monitoring



SOURCE: Charles Trichilo and Richard Schmitt, Environmental Protection Agency, 1988

tiresidue methods that they have developed to handle the bulk of their monitoring efforts. The driving force for FDA to develop these methods is the great economic savings associated with methods capable of determining many pesticides at one time, The five multiresidue methods used by FDA detect approximately 60 percent of the pesticides with tolerances in food. FDA also uses single-chemical methods for monitoring pesticides of special concern when these pesticides are not detected by the multiresidue methods. The FDA also carries out market-basket surveys to determine the level of residues of many pesticides in ready-to-eat food. The FDA compiles the results of these quantitative analyses for pesticide residues in food, Data including incidence and levels of pesticide residues are available to EPA, the World Health Organization (WHO), and other interested parties (l). FDA also publishes their enforcement methods in the Pesticide Analytical Manual (PAM), Volume I (PAM I) (6) of this manual includes sampling procedures and a description of the multiresidue analytical methods. Volume II (PAM II) (8) of this manual includes methods for detecting individual pesticides.

USDA

The U.S. Department of Agriculture's (USDA) congressional mandate for enforcing meat and poultry tolerances is included in the Meat Inspection Act and the Poultry Inspection Act. The USDA enforces pesticide tolerances for meat and poultry. USDA is also responsible for enforcing drug residue tolerances established by FDA. They also need rapid, inexpensive methods for meat and poultry products, (These include meat and poultry muscle, tissue, fat, liver, kidney, and processed meat products.) While USDA relies on multiresidue methods for chlorinated hydrocarbons (9), they also use individual methods for specific pesticides and animal drugs in their enforcement programs. These methods used by USDA are also developed by USDA. Recently, USDA has begun developing and utilizing rapid screening methods for specific compounds so that the more expensive laboratory methods will only be used on samples likely to be contaminated.

EPA

EPA's congressional mandates come from the FFDCA and the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA). EPA sets tolerances under the FFDCA and registers pesticides under the FIFRA. EPA is responsible for establishing pesticide tolerances for all foods and feeds. Although EPA has no direct tolerance enforcement responsibility, the agency shares in the need for practical methods that are readily available. Practical methods need to be rapid, inexpensive, and reproducible, and they must involve equipment and reagents that are commercially available (10).

EPA does not normally develop analytical methods for tolerances. Rather, EPA requires the registrant of the pesticide chemical to develop methods necessary to enforce tolerances (11), EPA has provided written guidelines for the details on how this work should be carried out (12, 13, 14). EPA laboratories carry out method trials to assure that these written methods can actually be used to enforce tolerances.

To facilitate food monitoring and tolerance enforcement activities, EPA includes a methods availability statement in each Federal Register (FR) tolerance notice so that Federal and State enforcement agencies and other interested parties can more readily obtain copies of the methods. EPA also sends copies of methods for enforcing tolerances to FDA for publication in Volume II of the Pesticide Analytical Manual (7). In cases where no methodology exists for a pesticide of concern, EPA has taken the lead and developed methods such as was done to quantify dietary exposure to unsymmetrical dimethyl hydrazine (UDMH), a degradation product of daminozide (15). EPA's goal is to assure that a method suitable for enforcing tolerances is available before a pesticide tolerance is established as well as for all existing tolerances.

EPA accepts single chemical methods as being suitable for enforcement. In 1984, EPA regulations were revised to require data on whether existing FDA and USDA multiresidue methodology will detect and identify the pesticides (16).

To improve the quality of single chemical methods, EPA has encouraged more collaboration by method users, and it has encouraged petitioners to conduct an independent method check by a second laboratory prior to submitting the tolerance enforcement method to EPA. More recently, EPA (17, 18) has formally proposed independent laboratory confirmation for tolerance methods (19).

Analytical Methods Development Program

FDA

The FDA's primary method development efforts are in the area of multiresidue analytical methods (20). If multiresidue methods are impractical or impossible, single residue methods are developed to insure that residues can be determined. FDA uses information in the Surveillance Index to arrange in order of importance the methods development for pesticides used domestically. FDA has ranked pesticides according to the importance of generating monitoring data. This ranking scheme is called the Surveillance Index. The FDA Surveillance Index for pesticides was developed as a result of a recommendation of an FDA study group (21). The study group felt that selection of chemicals for monitoring should be based on potential health risk rather than analytical method availability.

FDA also uses a data base (22) on pesticide use in foreign countries to identify pesticides used outside the United States, for which methods must be developed. FDA has five major goals in the area of analytical methods development for pesticide residues:

Expansion of Existing Multiresidue Analytical Methods to Additional Pesticides and Alteration Products. Five multiresidue methods are regularly used by FDA, and each is undergoing study for expansion to additional chemicals. Multiresidue methods for groups of certain pesticides (e.g., triazine herbicides, chlorophenoxy herbicides, fumigants) are also available and used on occasion.

Extension of Methods to Different Food or Feed Commodities. This is a continuing activity dealing primarily with multiresidue methods. Modifications to existing methods are often required before the method can be used on additional commodities due to different physical or chemical composition or limits of detection.

Validation of Analytical Methods. It is the general practice to conduct a limited interlaboratory trial among a few FDA laboratories of a new method prior to introducing it for field use. The ultimate goal is collaborative study of a regularly used method for AOAC acceptance as an official method,

Adaptation of Newly Available Analytical Techniques for Integration into Existing Methods. Advances in instrumentation and sample preparation have the potential to allow for modification of existing methods so that the methods become cheaper and faster. FDA currently has programs on high performance liquid chromatography (HPLC), capillary column gas chromatography (GC), computer-assisted instrumentation, and a new residue extractor for fatty foods.

Development of "New" Analytical Methods or Techniques. This includes the development of immunoassay residue method capabilities (materials for use in FDA monitoring not now commercially available) being undertaken via contract.

In general, the methods development research could be divided into two broad types: 1) that which deals with the immediate program needs, and 2) that which is directed to future goals of greater scope to solve particular problems or to improve overall effectiveness or efficiency. Most of FDA's effort is forced into the first type.

USDA

Development of residue analytical methods by USDA comes under the purview of the Food Safety and Inspection Service (FSIS). The FSIS method development program is currently emphasizing the development of multiresidue screening methods, many of which are based on immunoassay techniques. Secondary emphasis is being placed on conventional chemical qualitative/confirmatory procedures. Methods are developed both in-house and under contract. USDA finds the meat and poultry methods developed by pesticide producers (PAM II methods) to be too long and expensive to be practical in a large-scale monitoring program. All methods used by USDA are subjected to collaborative studies prior to being used in FSIS laboratories. USDA does in-house collaborative studies and cooperates with the Association of Official Analytical Chemists (AOAC) in carrying out collaborative studies.

EPA

Since EPA has no direct responsibility for enforcing tolerances, methods development for residues in food is not generally carried out in EPA labora-

tories. Methods development at EPA is carried out primarily by the Office of Research and Development (OR D). ORD does not have a specific program to develop methods to detect pesticide residues in food; however, it supports such efforts by providing analytical reference standards and technical information through its Pesticides and Industrial Chemicals Repository (2). As noted previously, the availability of analytical standards areas important as analytical methods in tolerance enforcement. ORD does develop methods to meet specific congressional mandates under a number of laws:

Clean Air Act (CAA)
Clean Water Act (CWA)
Safe Drinking Water Act (SDWA)
Resource Conservation and Recovery Act (RCRA)
Comprehensive Environmental Response, Compensation and Liability Act (CERCLA, Superfund)
Toxic Substances Control Act (TSCA).

In some cases, these methods can be used as a starting point for detecting residues in food. The EPA organizations responsible for administering or implementing specific environmental laws request ORD to develop methods through research committees consisting of ORD and program office representatives. ORD laboratories are then directed to perform the requested work, which they perform internally or by contract, grant, or cooperative agreement. Most analytical method development activities are conducted by the Office of Acid Deposition, Environmental Monitoring and Quality Assurance (OADEMQA) in ORD.

As pesticides become of concern to the program offices, they are sometimes included in multiresidue methods. For example, six multiresidue methods have been developed by the Environmental Monitoring and Support Lab (EMSL) to detect approximately 120 pesticides and degradation products in ground water. Pesticide methods are also developed to monitor pesticide residues for specific projects requested by the Office of Pesticide Programs. In most cases, existing methods available from the literature, the FDA, or a pesticide manufacturer are modified for the matrix of interest.

The Office of Pesticide Programs has laboratories at Beltsville, MD, and Bay St. Louis, MS, that are primarily responsible for carrying out method validations to assure adequate methods are available to enforce tolerances. These laboratories have, on occasion, developed single chemical methods for pesticides or their metabolizes when existing methodology was unavailable for important chemicals of concern, such as those chemicals involved in the EPA Special Review Process.

Sharing Information Among Agencies

Information on analytical methods is shared through implementation of Memorandums of Understanding (23) and periodic meetings. Currently EPA, FDA, and USDA meet quarterly to discuss specific problems associated with analytical methods. Past results from these meetings have included the following:

Protocols to be used by the pesticide registrants to determine whether pesticides would be detected by FDA multiresidue methodology (24).

User response sheets included in PAM II so that problem methods can be identified and better methodology required of registrants (25).

Identification and prioritization of problem methods so that better methodology can be developed.

Current projects under consideration at these meetings include the development of a protocol for determining the acceptability of a method and writing specific criteria on the acceptability of methods for enforcement purposes.

FDA and EPA cooperate on the Surveillance Index project. EPA provides FDA with pertinent exposure and toxicology information for those pesticides with tolerances so that FDA can rank the pesticides in order of priority for monitoring. Similarly, EPA representatives sit in on USDA's surveillance advisory team meeting to provide input on priority pesticides to be monitored in meat and poultry.

EPA has recently initiated procedures to make analytical methods submitted by pesticide tolerance petitioners more readily available to FDA, USDA, the States, and other interested parties. EPA now sends FDA and USDA copies of new chemical methods and method modifications for use on additional crops upon receipt of these methods. This provides the enforcement agencies an opportunity to comment on the suitability of these methods early in the tolerance-setting process and prior to approval of the tolerance. EPA also includes, in each published Federal Register notice for every tolerance, a specific statement on the availability of the analytical methodology. If the method has not yet been published in the PAM II, the FR notice includes the address of the EPA/FOI Office from which the method can be obtained.

Accessing Technology in the Private Sector

EPA requires registrants of pesticide chemicals to submit analytical methods as part of the data necessary to register a pesticide. These methods, developed by the agrochemical producers, are made available by publication in PAM II by FDA and released by EPA under the FOI Office. The publication of environmental matrix methods is done by the National Technical Information Service (NTIS). Since these methods must be made available to enforcement agencies and interested parties, EPA no longer accepts methods that are claimed to be Confidential Business Information (CBI).

Much residue data is generated by the food processing and distribution industry. EPA is currently working with the National Food Processors and the Grocery Manufacturers of America to make these residue data available for use by Federal agencies. EPA/FDA/USDA chemists are also meeting with technical committees of these organizations to suggest areas for monitoring pesticide residues and new methods development.

Dealing with Hazardous Pesticides

EPA evaluates potentially hazardous pesticides that appear to meet or exceed certain risk criteria through its Special Review process. Under the Special Review process, all available data on toxicity and exposure are reviewed. In addition, data essential to the determination of risk of a pesticide are required from the registrants when appropriate.

When necessary for Special Review decisions, EPA requests special monitoring programs from FDA and USDA to determine the level of residues in food. EPA also notifies the Grocery Manufacturers of America (GMA) and the National Food Processors of America (NFPA) so that residue data from the food industry can be made available. Increased cooperation in this area will improve the government's ability to deal with hazardous pesticides.

Areas for Improvement

Each agency should review its current regulations and guidelines with the goal of improving or modifying them if needed, so that analytical methodology needs can be better addressed. The EPA has issued regulation modifications involving multiresidue method protocols (27) and is considering second-lab validation of enforcement methods. Both of these changes were initiated for the sole purpose of improving the capability of enforcement agencies to monitor for pesticide residues in food.

Recommendations for Improving Methods Development Programs

The major need among Federal and State agencies in the area of pesticide food monitoring is the development of quicker, more comprehensive multiresidue programs. The following are suggestions for improving methods development for food monitoring:

- Closer coordination between EPA, FDA, and USDA in methods research and prioritization. Agencies should identify lead organizations for each area of methods research and attempt to minimize overlap.
- Congress should consider providing incentives to industry, academia, and the States to develop methods for pesticide residues in food and to monitor for pesticides in food.
- The pesticide producers and the food Production "industry should increase their efforts at monitoring for pesticide residues in food and should share monitoring results with Federal agencies.

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Pesticide Analytical Methods Development at the State Level

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Contents

Abstract

The role of state regulatory agencies in the enforcement of pesticides residue tolerances and the development of new analytical technologies are determined by Federal policy and State legislative intent. State programs are focused to complement the regulatory activity of the various Federal agencies, but also to meet the state's sometimes more stringent regulatory requirements. This paper discusses the states' role in the national food protection program and highlights the differences between the state and Federal programs.

Introduction

The U.S. Department of Health and Human Services, Food and Drug Administration (USFDA) is the Federal agency responsible for monitoring pesticide residues in food. Over the years, a number of states have developed their own pesticide residue monitoring programs in response to specific needs identified by the states, perceived limitations in the Federal program, and perhaps most important, in response to the increased consumer concern regarding toxics in the food supply. The size and goals of these state programs vary, depending on the perceived need in the state and the funding available. This paper will examine the pesticide residue monitoring programs of several selected states in comparison with that of USFDA; discuss the kinds of analytical methods most needed, including a discussion of the applicability and potential for emerging analytical techniques; provide an overview of present analytical methods development in the states; discuss local regulatory initiatives that have placed special analytical requirements on the state laboratories; identify the present role of the state in analytical methods research and development; and make suggestions for what that role may be in the future.

Pesticide Residue Monitoring Programs

In order to present information that would be more representative of the national status of states' pesticide residue monitoring programs, examples are provided from several states with programs of

different sizes and objectives. The states included are Florida, Montana, Massachusetts, and California.

U.S. Department of Health and Human Services, Food and Drug Administration

The U.S. Department of Health and Human Services, Food and Drug Administration (USFDA) analyzes approximately 10,000 samples of fresh fruits, nuts, and vegetables each year (7). Samples are taken of imported produce, as well as domestic produce destined for interstate shipment. The USFDA program consists of two major components: compliance monitoring and surveillance monitoring. In this paper we will deal with the surveillance monitoring component because it has the most applicability for comparisons with state monitoring programs. The objective of the surveillance monitoring component is primarily to enforce U.S. pesticide residue tolerances established by the U.S. Environmental Protection Agency (EPA). The tolerances are established by Federal regulation and published in Code of Federal Regulations 40 Part 180.

Sampling of imported produce is based on the regional import sampling plan, on headquarters-directed assignments, and on special emphasis surveys. In developing regional sampling plans, districts consider the dietary significance and production volume of the commodities, the compliance history of the country of origin, and pesticides used at origin identified through use of the Battelle World Agrochemical Data Bank. Because of resource limitations, USFDA headquarters places some restrictions on the commodities to be sampled based on the commodities' significance in dietary intake. For example, very few samples are taken of spices and herbs.

Headquarters-directed assignments are aimed at obtaining residue data for commodities or pesticides that have not sufficiently covered during previous years. Also included in these assignments are pesticides or commodities that, on a national level, are of increasing concern or interest. Examples of headquarters-directed assignments proposed for the 1988 Federal fiscal year include imported fresh cucumbers to be analyzed for organohalogen, organophosphorus, and carbamate residue; and imported fresh apples to be analyzed for organohalogen, organophosphorus, Ethylene Bisdithiocarbamates (EBDC), benomyl, thiophanate-methyl, Methyl 2-benzimidazdecarbamate (MBC) and daminozide residues,

Special emphasis surveys are based on selected high volume imports and on commodities treated with pesticides that are not allowed for food use in the United States. Each district is required to select and conduct a minimum of two of these surveys with priority given to country/commodity combinations not covered by previous monitoring in the district (3).

For domestic samples, USFDA districts prepare annual sampling plans based on local conditions such as pest problems, amount of production, past compliance history, or coverage. Headquarters specifies the minimum number of samples to be taken by each district and the resources to be expended on pesticide monitoring. In its annual guidance to the districts, the headquarter's office also specifics coverage of certain pesticides and commodities for each district. This special-survey element normally focuses on pesticides that are of potential health concern and that require analyses by single residue analytical methods, or it monitors the level of specific pesticides of importance to the EPA. For example, for several years a special survey was performed of EDB in grains and fruits. EPA needs information on the extent to which EDB residues were occurring because of carcinogenic concerns.

Other than specific surveys, districts are given considerable latitude in developing annual plans for domestic sampling. Most plans are designed to cover crops of local dietary importance, pesticides with high usage within the district, growers or commodities with past compliance problems, and commodity/pesticide combinations in which misuse is suspected.

Normally, samples are analyzed by one of five multi-residue techniques that detect from 24 to 123 pesticides. Single residues, or specific analyses, is performed during special surveys on specific pesticides, to confirm levels detected by multi-residue techniques, or when misuses of the pesticide is known or suspected (7).

Florida Department of Agriculture and Consumer Services (FDACS)

FDACS began monitoring raw agricultural commodities for pesticides residues in 1960. The Bureau of Chemical Residue Laboratory, under the Division of Chemistry, FDACS, is responsible for the analysis of pesticide residues in food and feed products produced or marketed in Florida. It is also responsible for the enforcement of Federal tolerances and guidelines adopted by the state. Each year the Bureau performs more than 10,000 determinations

on approximately 4,000 food and feed samples. Each routine sample is analyzed by the chlorinated hydrocarbon and organophosphate multi-residue procedures. Samples of fresh fruits and vegetables are also analyzed by the carbamate screening procedure. Single residue analyses are performed on an "as needed" basis. Analyses of the majority of routine food samples are completed within **48** hours.

Since the late 1970s, the program has been setup to target the most probable problem areas in order to direct limited resources. Samples are taken of commodities throughout the channels of trade, including airports and docks. Samples may be of Florida-grown or imported produce, depending on the time of year. Florida's program is a combined crop-pesticide index. That is, in selecting samples for analysis, FDACS considers the propensity of the commodity to retain significant levels of pesticides, and the characteristics of the pesticides applied to the crop. According to W. George Fong, FDACS, the classification of crop groups from the standpoint of potential pesticide exposure of consumed plant parts is based on the book *Food and Feed Crops of the United States,* by J.R. Magness, et al. (2). Considerations of the characteristics of the pesticides applied to the crop include the following: acute oral toxicity, persistency in the crop, toxic metabolizes formed, current EPA special review, systemic property of the pesticide, and human dietary exposure (2).

Massachusetts Department Of Public Health (MDPH)

MDPH has been performing pesticide residue surveillance for about the past four years. Approximately **500** samples are taken each year that can be analyzed for about **30** different pesticides. Recently, MDPH has made a rather major change in its program direction. The department's program is tailored to identify and assess specific potential pesticide-related health risks. Potential risks are identified either through risk assessment analysis or through laboratory results. For example, if the laboratory detects significant levels of a chemical in food, risks assessments associated with that level will be initiated; conversely, if risk assessment demonstrates a concern for a particular chemical, the program will be directed toward analyzing commodities on which that pesticides may be used.

The objective of the program is to identify and assess the pesticide residues that may pose the greatest risk using information and criteria from the FDA surveillance index, data on file with the EPA and with other states (for example, from the FOODCONTAM program, a federally sponsored data-sharing program that collates pesticide residue analytical data from the laboratory. Emphasis is placed on the diets of those subgroups of the population determined to be most at risk from exposure. Samples are taken at the wholesale and retail level for both domestic and imported commodities (1).

Montana Department of Food and Agriculture (MDA)

MDA has been taking pesticide residue samples for about **13** years. An average of **250** samples are taken each year as part of agricultural pesticide misuse investigations. An additional **50** samples per year are specifically collected for residue monitoring (or tolerance enforcement) in food commodities. For misuse investigations, the pesticide analysis is normally limited to the specific suspect pesticide. For the monitoring program, any or all of the major pesticide groups are requested, e.g., carbamates, organophosphates, etc.

The majority of samples consist of agricultural commodities produced and marketed in the state that are known to have been treated with a specific pesticide. This normally occurs after a pest outbreak that has required extensive applications of the target pesticide. Samples are taken at the farmgate or retail level. The analytical laboratory is capable of analyzing for 70 to ***100*** different pesticides both through multiresidue and specific analyses. Analyses requested are dependent on the situation triggering the sampling.

California Department of Food and Agriculturo (CDFA)

CDFA has had a pesticide residue program for more than **60** years. CDFA's pesticide residue monitoring program is organized into four major components: state routine, preharvest monitoring, focused monitoring, and processing foods monitoring. Altogether, the California program results in more than **43,000** determinations on approximately 13,000 samples each year. These samples are in addition to samples analyzed during misuse investigations, which account for an additional **4,000** samples per year. The state routine component is a commodity-based, tolerance-enforcement function consisting of approximately 6,500 samples of fresh fruits, nuts, and vegetables taken from throughout the channels of trade. Both domestic and imported commodities are included. Analysis for the majority of these samples is by multiresidue screens, capable of detecting approximately ***100*** pesticides, Analyses per-

formed through multiresidue screens are normally completed within 4 to 6 hours from the time the sample is submitted to the laboratory. For this component, single method analyses are made on an "as needed" basis, with a turnabout time of generally less than 24 hours. The selection of 75 percent of the commodities sampled in this component is based on a statistical formula that takes into account the amount of consumption and historical residue data. Specialists are allowed to use their discretion in selecting the remaining 25 percent. Factors influencing discretionary sampling include knowledge of pest problems and pesticide usage within the production areas, data from the USFDA program, etc.

The preharvest monitoring component consists of approximately 2,500 samples taken from fields, prior to harvest. These samples are normally analyzed by the multiresidue screens. Specific analyses are requested on an "as needed" basis. Commodities to be sampled are determined by the amount of production in the county of origin, pest problems, pesticide usage within the production area, and by compliance history of the grower. Early detection and deterrence of pesticide misuse is one of the major goals of this program.

The focused monitoring component is a pesticide-based, rather than commodity-based, program. Each year, CDFA medical toxicologists identify pesticides of priority health concern. Commodities known to have been treated with those pesticides are sampled and analyzed for the specific pesticide. As with the Massachusetts program, emphasis is placed on the diet of those subgroups of the population determined to be most at risk.

The processing foods monitoring component consists of approximately 1,500 samples of raw commodities destined for processing. Samples are taken in the field, shortly before harvest or after harvest; at grading stations; and at processing plants prior to processing. These samples are analyzed by multiresidue screens. An important goal of this component is to provide information to the California Department of Health Services (CDHS) to assist them in designing their processed-foods-products pesticide monitoring program. The number of samples to be taken of each commodity is based on California production figures.

As can be determined from the previous discussion, there is quite a variety in the types of samples, types of pesticide analyses performed, and sizes of state programs. The objectives of state programs also vary dependent on resources and public concerns. However, similarities also occur. For example, the Massachusetts program, which is limited to 500 samples per year, has chosen to focus its sampling on specific pesticides as they relate to dietary risk. Though similar in size, this program is similar to California's focused monitoring program. The same theme can be seen in USFDA's headquarters-directed and specific emphasis assignments.

In Montana program, resource limitations have caused this state to restrict its monitoring solely to those situations in which the possibility of over-tolerances is the highest. The Florida program, though larger, has also directed its program in this way.

Most of the programs are, at least partially, developed to act as a deterrent to pesticide misuse. The California program, however, is the only one that routinely takes samples of commodities in the field prior to harvest, as well as in the channels of trade. The Massachusetts program appears to be based more on public health concerns than on deterrence.

All program have multiresidue screening capabilities. There is variation, though, in the number of pesticides that can be analyzed in this manner. Further discussion on analytical capabilities will follow.

All states contacted have the authority to adopt their own residue tolerance levels; however, all of them currently use those set by EPA. USFDA and some states also use "action levels" and "regulatory analytical limits" in determining whether or not to take enforcement action. The use of action levels and regulatory analytical limits is not uniform.

A recent decision by the U.S. Court of Appeals for the District of Columbia stated, in essence, that action levels set by USFDA were legislative rules rather than general statements of policy and, therefore, must be adopted according to the Administrative Procedures Act. The court found the Federal action levels to be invalid because they were not adopted according to this procedure. At best, action levels are useful as a guide and do not require or prevent USFDA from taking action (8).

The results of this 1987 decision have yet to be fully addressed. In Florida, where no tolerances or action levels exist for a pesticide in a particular commodity, a regulatory analytical limit is applied (9), Action levels are treated the same as tolerances. In cases in which no tolerance or action levels exists, Florida set its own regulatory analytical limited based on the lowest residue level the laboratory is able to reasonably detect, measure, and confirm with existing analytical methods (2). Historically,

California has acknowledged those Federal action levels as published in 40 CFR 180, but it has not acknowledged regulatory analytical limits set by Federal policy. California is currently re-examining whether or not action levels can continue to be used because of the Court of Appeals decision.

Analytical Methods Needed

In the past ten years, the need for and ability of pesticide residue laboratories to identify, quantitate, and confirm the presence of trace levels of pesticides in or on food crops has increased dramatically. California regulations require that the pesticide-residue analytical method submitted in support of a California registration for food-use pesticides not exceed 24 hours. The Florida enforcement program's mandate allows for food samples to be completed within 48 hours (2). The EPA currently has a *guideline* for analytical methods that emphasizes the desirability of a 24-hour method, but it is not mandatory.

Multiresidue screens currently being used by states are useful; however, they are not inclusive. Some pesticides do not lend themselves to a screening procedure because of their chemical constituents. Others, though they can be detected in water samples, require extensive preparation time for detection in the various crop matrices. When there is need for data on a non-screenable chemical, the slower single residue analytical method(s) submitted by the registrant or a PAM method must be employed.

There is a need for more multiresidue procedures that detect metabolizes as well as the parent compound. For tolerances enforcement programs, time is of the essence, and analysis should be completed within a normal working day, making multiresidue screens ideally suited for this type of work. Many single residue methods also meet this criteria, although in some cases laboratories must modify submitted methods to achieve this time frame. Built-in quality-assurance features are needed, and methods should not require specific instrumentation that only a few state laboratories have or can afford (2).

Performance characteristics of the *ideal* analytical methods for pesticide residue in food crops would have the following minimum characteristics:

1. Methods would be validated on every crop type for which the pesticides is registered. As new registered food crop uses are approved, the analytical methods would be updated to reflect the new crop matrices. For example, an analytical method may be acceptable in selectivity and sensitivity for head lettuce, but when the same analysis is performed on green onions or parsley, the crop matrix interferences may reduce the analytical sensitivity to an unacceptable level.
2. All new analytical methods would be validated in a series of independent laboratories. This procedure would test the method to evaluate its reliability and reproducibility under various operating and management systems.
3. New methods or analytical regimes would have to include the ability to detect, identify, confirm, and quantify and and all metabolizes included in the 40 CFR 180 tolerances. Ideally, this process should not exceed seven hours from the time the sample is received in the laboratory.

In addition to developing methods for new chemicals, review should be completed on the older chemicals, especially those with potential dietary impact. For example, the current approved methods for EDBC's are not product specific, and there are no known confirmational techniques. The only approved method is wet chemistry and involves CS2 evolution and calorimetric quantification. Besides the obvious shortcomings of these types of methods, different tolerances exist for the various members of this family of chemicals on the same crop. There is no way, short of field investigation, to determine which tolerance applies and if an over-tolerance has occurred.

The needs of the pesticide regulatory programs for accurate data demand that the laboratories monitor their ability to provide accurate, timely, and *reproducible* analytical results. In order to assure these results, use of a well-managed quality-control or quality-assurance program is needed. In most states, such a program has been initiated. However, there is a need for development of new analytical quality-control methods with internal provisions, These internal checks could alert the analyst to developing problems and the need to effect timely corrective action. Such a system could greatly reduce the time currently being spent to investigate the causes of inaccurate analytical results, thereby reducing the analytical cost per sample.

Many of the newer pesticides being used on food crops are thermally liable and not easily analyzed by the high temperature GC systems. The other major analytical tool widely available for use is HPLC. The HPLC, however, lacks easy or reliable analytical confirmation. New methods that will provide quick, reliable, and cost-effective confirmation that will also be legally defensible are needed.

For pesticide residue enforcement, analytical methods that are specific for the parent chemical are needed. Currently, EPA does not require specific analytical methods for the parent compound. Metabolizes included in the tolerances listed in 40 CFR 180 need to be identified. Currently, there are tolerances that state "and cholinesterase-inhibiting metabolizes" or "and its metabolizes" (40 CFR ed: 1980). Confusion exists regarding what parts of a commodity must be included in the analytical procedure. It is imperative that EPA of FDA take action to relieve this confusion. Suggestions would include publishing a single-source document such as that included in CODEX that provides this information or establishing a toll-free telephone number to an information officer to answer questions. This information center should be staffed around the clock to be of service to all states.

To facilitate detection of pesticide misuse, there is a need for development of residue analytical methods for various agricultural and environmental media, and for crops for which the pesticides in question are not necessarily registered. According to Laszlo Torma, Montana Department of Food and Agriculture, the methods in the PAM II are inadequate because they are not collaborated, and they are designed only for those commodities for which the chemical is registered. Companies and Federal laboratories with the assistance of state laboratories could set up and collaborate multiresidue methods for these compounds, and special consideration of a region could be acknowledged to meet these methods and regulations. For example, in Montana a relatively large number of the population consumes meat from wildlife on a regular basis; however, there is no official collaborative analytical method or established tolerance available for these foods. Another area that could be addressed is the pesticides that are not registered in the United States, but are registered in Canada. Frequently, these products enter Montana and other bordering states but when residue analyses are required, there is no method available (6).

Emerging techniques such as immunoassay and biosensors have potential for pesticide residue analysis. The initial impact of these new techniques is expected to be in the area of rapid screening of produce samples for a wide range of specific pesticides. Under this approach, the confirmation of the screening results would be via traditional GC, GC/MS, LC/MS, or other appropriate separation and confirmation systems. As the new techniques are proven to be accurate, dependable, and have internal quality-assurance checks, the classical confirmational steps could be reduced.

The major advantage of these new technologies would be their improved sensitivity and selectivity. CDFA is currently evaluating three ELISA techniques for use in the pesticide residue program. Two of the ELISAs are for the triazine class of compounds and one is for paraquat. The paraquat ELISA is of interest because it is potentially superior in sensitivity, selectivity, and reproducibility to the existing battery of available calorimetric methods.

Evaluation at CDFA indicates that the new technologies are rapid, reproducible, and inexpensive to use. These factors open the possibility of regulatory programs being able to perform more analyses per sample and to run more samples for selected pesticides. This would enhance the regulatory data base and provide statistically valid residue trends and dietary loads.

These new technologies appear to be "user friendly", and the amount of time and money to train staff to utilize these systems appears to be minimal. These procedures are "turn-key", and any laboratory could improve its capability without a massive infusion of funds.

At this time, however, most of these methods are qualitative, or at the most semiquantitative procedures. They do not promise, however, for being used as a preliminary screen (2). Research and field testing should be given high priority to make these tools available within the next few years.

Analytical Methods Developed as State Level

Analytical methods development at the state level varies with the objectives of the various state programs. Most state programs are primarily focused on enforcing Federal tolerances. To be effective, this type of enforcement requires rapid turnaround time. This often necessitates modifying existing analytical methods or developing a new analytical method, For example, CDFA laboratories have adapted a more rapid GC method for EBDC's in place of the Federal wet-chemistry method to be compatible with states' regulatory needs.

States that actively investigate pesticide misuse and pesticide illness incidents often must modify residue methods to meet their needs. For example, CDFA has modified analytical methods that were developed for food crop analyses to be applicable for different analytical uses, such as farmworker exposure monitoring or environmental drift and contamination of non-target areas.

Florida's laboratory has developed an HPLC-UV screening procedure for several families of herbi-

cides, for example, triazines, uracils, phenylureas, etc., in water samples. When the sample preparation technique is worked out, these procedures can be used for vegetable and fruit samples as well (2).

In Massachusetts, EPA's decision not to ban the use of daminozide caused concern at the state level, and Massachusetts decided to take independent action. In order to perform the analytical testing of raw and processed apple products necessary to complete their risk assessment, this state's laboratory developed analytical methods to improve the sensitivity levels (l).

CDFA's resources for analytical methods development are devoted to modifying existing methods. Currently, the State of California may have the largest state-funded pesticide analysis program among states, CDFA's Chemistry Laboratory Services branch has a methods-development group staffed with one principal agricultural chemist (Ph.D.), two agricultural chemists III, which is the highest technical pay-grade in the state's system, and one experienced technical assistant. As part of the methods-development group, an in-house quality-control and quality-assurance program has recently been initiated and maintained by an agricultural chemist III and a technical assistant.

CDFA is involved in the evaluation of new analytical technologies such as the applications of super critical fluid chromatography in pesticide residue chemistry, ELISA, and tandem mass spectrometry through the methods-development group. Due to the geographical location and the past close working relationship with the University of California at Davis (UCD), CDFA is exploring the possibility of a state-funded collaborative effort for analytical methods-development research with UCD. This effort could include the following: (1) improvement, modification, and unique application of conventional analytical methods, for example, GLC, LC, and wet methods; (2) nonconventional analytical methods development, for example, ELISA, alternative detection of pesticides, novel separation science; (3) confirmation of analytical results through shared advanced instrumentation facilities, for example, HR MS, MS MS, Foumer Transfer Infrared Spectrometer (FTIR), and Nuclear Magnetic Spectrometer (NMR); and (4) training of appropriate personnel and technology transfer.

In the area of instrumentation review, California is currently looking for a better and more reliable confirmational technique for GC and HPLC systems. Currently, California is evaluating a GC/MS/LC for both GC and HPLC work. A GC Mass Selective Detector (MSD) will be purchased for evaluation along with further work with photodiode array detectors and supporting work stations. This type of work is very expensive for a state to fund and it is, therefore, limited in scope. The work conducted in California's laboratories is focused on addressing California's needs and may, therefore, not be of any utility to other regulatory or commercial pesticide residue laboratories.

In Florida, methods-development work has traditionally emphasized modification of existing techniques. To augment existing methods, this state is now extensively using Solid Phase Extraction (SPE). According to W. George Fong of FDACS, "SPE technique for sample preparation requires less sample and solvents and can be completed in much less time. It reduces the health hazards in the laboratory and generates less solvent waste. SPE also provides limited specificity" (2). FDACS has developed SPE techniques for carbamate analysis and adapted the techniques for most HPLC analyses. Some limited preliminary studies are also being done of SPE for gas-liquid chromatography. There are two chemist positions devoted to methods development and quality-assurance work in this state.

In Montana, methods-development is limited to determining the accuracy of a published method or adapting a published method for a given commodity to another commodity. Local needs further limit methods development primarily to areas of new herbicides (glyphosate, sulfonylurea herbicides, triazin, substituted ureas, etc.). This state has three chemists who expend approximately 70 percent of their times on residues analyses.

In Massachusetts, methods-development work has been on a case-by-case basis. This has involved pesticide-specific surveillance and compliance testing for chemicals that have been designated as public health priorities and have required state-level regulatory action. During 1987, activities include risk assessment and policy development for alachlor and 2,4-D, methods development and compliance testing for daminozide in apple products, and screening for heavy metals and organochlorine pesticides in bottled drinking water.

There is undoubtedly some duplication of analytical methods-development efforts by states and Federal government agencies when the objectives of the programs are similar, for example, monitoring for tolerances enforcement. In the past, little or no information was exchanged between state laboratories and Federal agencies regarding research or methods-development work being conducted. Currently, CDFA and USFDA Region IX are developing a memorandum of understanding (MOU) that

will include a residue analytical section. Methods-development and quality-assurance procedures are being considered to be included in this MOU.

Impact of Local Regulatory Initiatives

State laws and regulations can place special analytical requirements on pesticide laboratories. Several examples from California illustrate this point. Historically, California law has provided that CDFA may seize a lot of produce if it is suspected of carrying excess pesticide residue. By statute, the lot may only be held for 24 hours unless laboratory analysis confirms the existence of the illegal residue. This has caused CDFA to modify or replace methods that take longer than 24 hours to complete, Proposition 65, passed by the California voters in 1986, provides that no person shall expose any individual to a chemical known to be a carcinogen or reproductive toxin without giving prior warning. While all of the ramifications of this law are yet to be understood, it is conceivable that the pesticide regulatory laboratories will have to modify or replace existing residue methodology to shorten turnaround time or to lower limits of detection for pesticides that are oncogenic or have adverse reproductive effects.

Recently, commercial laboratories in California have begun to conduct pesticide residue testing on produce for grocery stores. A bill has been introduced in the state legislature that would require such laboratories to be accredited by the state and to participate in a state-sanctioned quality-assurance program. Accreditation programs may place additional responsibilities on state laboratories to provide oversight, oversee and qualify control of uniform analytical methods.

Neither Florida nor Montana reported having legislation similar to Proposition 65, though Florida does have a regulation that suspends the use of aldicarb in an area where it has been found in well water in excess of 10 parts per billion. Massachusetts, with program emphasis on pesticides with potential chronic risks, may well have occasion to employ modified residue methods.

Role of States in Analytical Methods Development

Pesticides analytical methods-development at state laboratories has a different focus than that of academia, private industry, and Federal government agencies, Rather than the development of basic new analytical methods, state laboratories emphasize methods-application and subsequent methods-modification. This difference in emphasis has arisen from differences in the overall objectives of the various laboratories. Traditionally, academia has contributed in the aspects of basic, novel analytical methods-development, while industries have emphasized analytical methods for applications of a particular chemical. In general, state laboratories' needs for pesticide analytical methods are to monitor, regulate, and enforce the uses of pesticides within a given state, in accordance with federally-established standards, However, there are still differences in analytical needs between Federal and state laboratories.

The historical and current role of California in analytical methods research and development has largely been limited to modification of existing methods to meet our criteria of performance acceptance. There have been instances when new methods have been developed for residue analysis because existing approved methods were not specific, rapid, or sensitive enough to meet regulatory needs. For example, in 1985, contamination problems resulting from the misues of aldicarb on watermelons resulted in a recall of all California watermelons. In order to allow continued sales, California established a certification program that included sampling and analysis of melons, from all fields prior to shipment, for aldicarb and aldicarb sulfoxide. The original method was judged too time-consuming, as each of the four CDFA district laboratories was attempting to "clear" 20 fields per day, and each field required a minimum of five composite samples. With the single goal of certification in mind, an HPLC method was developed to provide the accuracy speed, and precision required (5).

In addition to ongoing methods modification, CDFA's methods-development group is addressing the use of new instrumentation technology in various residue applications, The pesticide registration laboratory works with pesticide registrants in order to resolve operational problems with their analytical methods. This activity is restricted to methods-modification, not conducting research, which is the responsibility of the pesticide registrant. Work conducted in CDFA's laboratories focuses on addressing California's needs and may, therefore, not be of any utility to other regulatory or commercial pesticide residue laboratories.

In the area of methods-development, Federal agencies should seek states' input to determine what the state's analytical needs are. Collaboration is nec-

essary between state, Federal, and private laboratories. Additionally, states with smaller programs would benefit from a more vigorous training program provided by Federal agencies (6).

Massachusetts sees a need for FDA training programs for pesticide analytical laboratories. Training is necessary for comparability and accuracy of data, including analytical support and guidelines for data interpretation for a variety of analytical procedures and instrumentation. Also necessary, from this State's viewpoint, is the establishment of minimum criteria by which a laboratory would be considered a certified FDA laboratory. Such a program would include quality-control and quality-assurance, possibly including specified recovery rates and detection limits,

In order to provide uniform regulatory analytical results and assure consumer protection, consideration should be given to an EPA/FDA/NBS/state-managed laboratory accreditation program for all pesticide residue regulatory laboratories. As part of this program, EPA/FDA should conduct quarterly regional meetings with the state laboratories and a national meeting for all state chemistry managers. These meetings at the local and national level would provide state input into the national programs.

Many new pesticides are on the horizon which will require very different types of analytical methodology than are currently utilized in state programs. What role will states play in developing/modifying methods to analyze these pesticides? Will state laboratories be able to maintain the efficacy of their programs through modification of existing techniques? State programs have no role in developing analytical methods of support the registration of a pesticide. The responsibility to provide an acceptable analytical method at the time of product registration is clearly that of the registrant, whether that method is a modification of existing techniques or development of a completely new type of methodology, The role of states will continue to be working on published methods to improve their sensitivity, expanding the types of sample matrices, and optimizing registrants' methods for use in the state's analytical system.

The role of states in developing/testing new methods such as immunoassay, automation, and screening will be different depending on the size of the state's programs and the available funding. CDFA has defined its role in the development and testing of newer methods to include the identification of analytical needs, both short-term and long-range; and contractual support for development, or cooperation in product evaluation, such as with private immunoassay product suppliers. The use of automation and the development of expanded or new multiresidue pesticides screens are part of an ongoing process in California's program. CDFA is actively engaged in reviewing its analytical procedures for incorporation into an automated system, and expects to test a robotic system within the next 18 months. The expansion of current multiresidue pesticide screens and the development of new screens are priorities for CDFA's method development group.

The role of state pesticide residue monitoring is to supplement the broad Federal program, while focusing activities on crops produced within state boundaries. Cooperation is essential to minimize duplication of analytical methods-development. The Federal agencies responsible for food safety must provide the leadership in any cooperative effort. This leadership role must be open to address the real needs of the states and be sensitive to local conditions. The cooperative effort should include the development of a national set of methods-development goals that, in turn, could be monitored to ensure efficient use of resources. The technology sharing would reduce duplicative work and encourage state involvement in problem resolution,

Each state may have different analytical requirements and resources; however, there is common ground. On role that states could play would be in an advisory capacity to the Federal government. The establishment of a methods research and development advisory committee to the appropriate Federal agency should be encouraged. Such a committee would include representatives from the state's chemistry laboratory programs, along with representatives of consumers, production agriculture, academic institutions, pesticide registrants, and Federal programs. This advisory group could be composed of two subgroups: one to deal with policy issues, which would include the chemistry program administrators; and one to deal with scientific issues, which would include the principal chemists. This advisory group could be mandated to advise the Federal government on current problem areas, results of on-going state-sponsored projects, and recommend areas of research.

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Pesticides Analytical Methods Development in the Private Sector

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Contents

Abstract

As a result of questions sparked by the ability to measure chemicals at increasingly minute levels, there has been an increased interest in the development of analytical methods for the detection of pesticide residues in foodstuffs. Among those in the private sector, most laboratories involved in pesticide method development have typically been university, industry, and contract laboratories. Food producers, food processors, and distributors also have an interest in analytical methods.

This discussion will focus on the objectives, the driving forces behind development, and the ramifications of these analytical methods. In addition, assessment of existing and emerging technologies will be performed from a private-sector viewpoint. Viewed constraints and opportunities will beaddressed together with possible approaches to enhancing multiresidue method development, This multiresidue screening approach is necessary from an expedient and cost-effective perspective.

Introduction

Pesticides have evolved over the decades from persistent, long-term control, broad-spectrum efficacious chemicals toward short-term control, biodegradable chemicals used with integrated pest management practices. The resulting agencies have required the manufacturers to do extensive screening for toxicological and ecological concerns in the development of any new pesticide, Beyond requirements, each segment of the agricultural industry, whether it be grower, food producer, distributor, manufacturer of agricultural chemical, or regulating agency, has increased interest in the issue of pesticide residues in food.

A tremendous amount of expertise for analytical methods development exists in the private sector. The value of this actual experience in developing methods for the analysis of pesticide residues is often underestimated. The goal of this residue methodology development effort, whether the laboratory is a university, pesticide industry, food producer, food processor, or consulting contractor, is basically the same: to answer the question of how much residual pesticide is contained in the matrix of interest, The incentives and extent of participation of the different types of laboratories vary.

Overview of the Private Sector

The university laboratory may perform method development for the sake of knowledge and achievement, while the food producers and food processors are only concerned that the screening methods used, assure their products contain less pesticide residues than the corresponding tolerance (maximum allowable) levels, These tolerances, which are granted under the Federal Food, Drug and Cosmetic (FDC) Act, are established from su-

pervised field trials at locations representative of each of the major crop-growing areas. The residue field trials are conducted by the pesticide registrant under the most extreme conditions of proposed use, such as the maximum application rate, the maximum number of applications, and the shortest interval from application to harvest. This measurement process ensures that the tolerance levels, established under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) and regulated by the Environmental Protection Agency (EPA), are not exceeded. The Food and Drug Administration (FDA) conducts a program of monitoring for pesticide residues primarily in raw, unprocessed food moving in the commercial channels of trade. Thus, the obvious interest on the part of the food producers is to ensure compliance.

Section 409 of the FDC Act, adopted in 1958, established the procedure for tolerances for processed foods and animal feed when pesticide residues on the raw agricultural commodity (RAC) concentrate in a processed fraction of the RAC. For instance, when raisins are processed from grapes, if a concentration of a residue occurs, then a food-additive tolerance is required for the pesticide in raisins. Conceivably, this concentration could make an undetectable residue in the RAC, detectable in the processed food. Some pesticides of toxicological concern concentrated in processed food would trigger the Delaney Clause. Food processors perform analyses to assure that their processed products contain undetectable residue levels or levels less than these food-additive tolerances.

Several contracting laboratories were surveyed (1, 2,3, 4,5) to determine their involvement in pesticide analyses and the level of methods development. Contract laboratories obtain analytical procedures from their clients, peer-review organizations, literature references, or when not available, develop a procedure from innovative research. In the cases of FIFRA registration projects, analytical methods are generally provided by the registrant. At best, research will be limited to adaptation of a method for additional sample matrices. Those laboratories concerned with minor-use pesticide registration, such as the regional IR-4 laboratories, respond in much the same way as a contracting laboratory. A method is provided by the company sponsor and used to acquire registration data (6). These IR-4 laboratories are usually affiliated with universities. These university laboratories have the analytical residue and method development experience and perform very cost-effective residue analyses. Other university laboratories have not shown a consistent interest in residue analysis except as an application for specific analytical techniques.

The contracting laboratory strives for a competitive edge by analytical method development for a purely monetary interest, while the industrial pesticide laboratory has the weight of economic and social responsibility to comply with the regulatory requirements for EPA registration. In addition to the development of residue field-trial data for estimation of the tolerance level, the pesticide registrant conducts reproduction and long-term animal feeding studies, using various species of test animals to establish the safety of the tolerance level. These toxicity studies determine the No Observable Effect Level (NOEL), the level at which the pesticide has no harmful effect on the most sensitive test animal. This NOEL is divided by a safety factor of up to 100 or more to set the Acceptable Daily Intake (ADI). The ADI represents the amount of pesticide residue that can be ingested by an average person every day for a lifetime without ill effect, Thus, the ADI usually is less than the sum of the normalized tolerances of the pesticide residue levels for all registered uses on crops.

The first step in the process of developing the crop residue and the environmental fate data base for a pesticide is the development of the analytical method. Subdivision O Residue Chemistry Guidelines (7), developed by the EPA, state that the pesticide registrants need to develop methods for residue analyses that serve two functions: 1) they must provide the residue data upon which judgments are made as to the identity and magnitude of residues from the proposed use, and 2) they must provide a means for enforcement of the tolerance. Sometimes, these two functions are best served by development of two separate methods. The initial role of developing analytical methodology justifiably belongs to the pesticide manufacturer. In addition to the production of the parent active ingredient of the pesticide, the manufacturer has had to synthesize the degradants or metabolizes for identification purposes *as well as* for reference standards for the residue method development. The manufacturer is in the best position to develop data on the product chemistry, physical properties, and means of analysis of the pesticide.

As is inferred from the EPA guidance document, there are two driving forces in the development of analytical methodology. One is to develop an analytical method to provide data to quantitate the magnitude of residues from the proposed use to establish the residue tolerance, Toward this goal, there is a certain amount of pressure on the industrial

chemist to develop methodology as quickly as possible, given that it takes approximately 5 to 7 years to perform the necessary toxicological, environmental fate, metabolism, and residue studies to fulfill regulatory requirements to ensure registration of a product to allow it to be sold. Thus, any time saved in this process or timeline can result in market entry advantage and greater profitability. During this rapid development, the optimal method speed and universality is not always addressed. The developing chemist is concerned mainly with quantitation of all required substances, with method sensitivity in that the method must be capable of detecting very low levels (i.e., an acceptable low level in food and feed matrices would be 10 to 50 ppb) and with method selectivity in that there are no interferences that would result in false-positive detections with use of the method. Thus, to achieve this high degree of sensitivity and selectivity in the most rapid fashion, the developer is compelled to use the most powerful state-of-the-art analytical techniques and instrumentation available. Additionally, the relatively large number of analyses needed to support a registration submission further serves as validation of this analytical methodology.

From a contract laboratory standpoint, the pressure to stay on schedule analytically with the various ecological, environmental fate, and residue chemistry studies is overwhelming. The registrant can be a very demanding sponsor as a result of the timeliness desired or imposed by the EPA. Methods are sent to laboratories for validation in both tested and untested matrices. Quite often, these methods have not undergone ruggedness testing to identify the critical steps of the procedure. Ruggedness testing through collaborative interlaboratory study determines the reliability of each step of the method by performance by several different analysts. Most laboratories have experienced, to the detriment of the analytical method, undesirable levels of method variability with different lots of reagents, absorbents, and column materials.

The other driving force is to develop a method that can be used to enforce the established tolerances. This methodology is usually different from the previous method because it has to be as simple as possible to minimize the cost of monitoring for pesticide residues. The EPA required enforcement method is expected to be rapid (less than 24 hours to completion), sufficiently sensitive in relation to the tolerance, interference-free, free of blanks or internal standards, and unencumbered by exotic equipment or reagents. The use of multi-detection methodology is extremely desirable. However, the method must measure the “total toxic residue”, as determined in the metabolism studies [171-4(a), Nature of the Residue]. This total toxic residue includes the parent molecule and all metabolizes of toxicological concern. Since most metabolizes are not tested for toxicity, this means all metabolizes isolated in sufficient quantities to be identified. This requirement greatly increases the level of difficulty in the development of multiresidue methodology and will be explained later in this discussion.

Pesticide Residue Analysis

Pesticide residue analyses can be classified into three groups for the purpose of examining applicability to multiresidue analysis:

1. compounds that do not degrade or metabolize quickly,
2. compounds that do degrade or metabolize quickly, and
3. compounds that degrade or metabolize at a rate that falls between these two groups; they are degraded to only a couple of additional compounds.

Compounds that are not degraded or metabolized either rapidly or significantly in the various environmental compartments (i.e., air, soil, water, plants, or animals) offer the best opportunity for multiresidue analysis. Only the parent molecule has to be isolated from the matrix for quantitation. Four general multiresidue methods for pesticide residues have been published by the Association of Official Analytical Chemists (AOAC) (8). These methods analyze for organochlorine, organophosphorus, fumigant, and carbamate pesticides. Examples of organochlorine pesticides that are addressed by the multiresidue method are as follows: dieldrin, heptachlor, DDT, lindane, methoxychlor, perthane, aldrin, endrin, and mirex. Organophosphorus pesticides that are addressed include the following: diazinon, ethion, malathion, methyl parathion, parathion, and fenchlorphos. Several fumigants are addressed: trichloroethylene, ethylene dibromide, chloroform, and carbon tetrachloride. And carbamate pesticides that are addressed are as follows: carbanolate, carbaryl, carbofuran, and propoxur. Most of these compounds are very stable, quite persistent, and tend to bioconcentrate in biological media or exhibit cholinesterase inhibition. Unfortunately, these descriptors coincide with what many feel to be environmentally obnoxious properties. From an environmental standpoint, one would prefer a pesticide that would degrade or metabolize quickly to naturally occurring compounds.

Compounds that are extensively degraded or rapidly metabolized in the various compartments (i.e., no parent molecule remaining) offer the least opportunity for multiresidue analysis. For these pesticides, the most prevalent method-development approach is to convert the multiple degradates or metabolizes to a common chemophore for quantitation. An example of this is substituted aniline-based products such as diuron, neburon, and linuron, in which analysis is achieved by hydrolyzing metabolizes to the common 3,4 dichloroaniline moiety. Thus, a multiresidue method using this approach could not address these three pesticides, since this procedure could not distinguish which one of the three produced the residue. Additionally, these types of conversion methods need specific optimization of each reaction (i.e., acid, base, or enzyme hydrolysis, oxidation, reduction, etc.). For instance, in the previous hydrolysis example, reaction conditions must be developed to maximize the yield of 3,4 dichloroaniline from diuron residues in a crop matrix to achieve the EPA minimum acceptable recovery of 70 percent. These conditions may be different for the reactions needed to obtain an acceptable recovery of 3,4 dichloroaniline from linuron residues in the same crop matrix not to mention in different crop matrices. Thus, pesticides that are extensively degraded or metabolized would probably not be suitable for multiresidue methods. As a general rule, the larger the number of metabolites, the more difficult the residue method development and the less likely the method would be able to measure many different pesticides.

Some compounds fall in between the two previously mentioned categories; they are somewhat degraded or metabolized to only a couple of additional compounds. These pesticides offer some hope for multiresidue analysis provided, that they are similar enough to other pesticides and they do not have common degradates or metabolizes. For future convenience, metabolizes will be referred to as degradates. These pesticides and degradates may not be amenable to direct detection because the degradates usually contain more polar functional groups, which require a modified analytical approach, than those used with the parent molecule. In these cases, the chemist uses chemical derivatization of the degradate(s) to convert them to a more measurable moiety. Derivatization reactions such as esterification, *acetylation, acylation,* silylation, and many others are used to improve the sensitivity, selectivity, or chromatographic behavior of the compound. Part of the difficulty in development of this type of method is in the isolation of the components from as much of the matrix as possible. This goal is important in order to have the derivatization reaction more closely approach the optimized "neat reaction" with standard materials. The remaining matrix components could be considered to quench or in some cases compete with the derivatization reaction and thus lower recovery (yield). Thus, pesticides that require derivatization don't seem to fit as nicely with the concept of multiresidue methods due to the potential presence of competing reactants both from the matrix and other pesticide residues. This is not to say that with considerable developmental effort a multiresidue method could not be developed, but many parameters would have to be explored in such an endeavor.

Existing methods for detecting pesticide residues in foodstuffs can perhaps best be explained by breaking the method into two parts: 1) isolation from the food or crop matrix, and 2) the detection of the pesticide residue. Isolation of the residue is begun by solvent extraction of a solid food or by liquid partition extraction of a liquid food with a solvent for which the pesticide residue has a greater affinity, Thus, the residue is removed from the majority of the matrix components. However, numerous chemical compounds that are components of the matrix itself are co-extracted, and this is usually the most difficult part of the analytical method commonly known as the cleanup. These co-extracted compounds in fact possess properties similar to those of the pesticide residue and thus are more difficult to remove. Some of the usual cleanup techniques employed in analytical methods are the following: filtration, solvent-partitioning, absorption chromatography, ion exchange chromatography, solid-phase extraction, gel permeation, dialysis, and distillation. These techniques are all aimed at the removal of coextracted matrix materials from the sample extract. After the cleanup in many cases, chemical reactions have been used to convert residue components to a chemophore for enhancement of detectability, specificity, or improved separation from remaining components.

Two of the most common analytical instruments for the detection and quantitation of pesticide residues are the gas chromatography (GC) and the high-pressure liquid chromatography (HPLC). These instruments provide final separation of the pesticide residue from remaining components on a column of absorbent via several different mechanisms. The instruments also provide identification and a degree of confidence that the compounds eluting from the column at the same retention time as standard materials are indeed the pesticide

residues. This is not always a certainty, however; it would be impossible to test every variety of every crop grown in every soil type and treated with every herbicide, insecticide, and fungicide for interferences in the residue method.

For each of these two types of instrumentation, there are numerous types of detectors. For instance, the gas chromatography may be equipped with flame ionization, electron capture, alkali-bead flame, photoionization, flame photometric, Hall electrolytic conductivity detectors, or combinations thereof. These detectors operate under different principles and have the ability in some cases to detect only certain classes of chemicals. Residue chemists use this detector specificity to great advantage in method development and residue analyses.

High-pressure liquid chromatography can have ultraviolet absorption, fluorescence, photoionization, photodiode array, or electrochemical detectors. Some researchers have developed specific reactions that are employed on-line after the column separation but prior to detection. This difficult type of in-situ derivatization, regardless of whether ultraviolet, visible absorption or fluorescent detection is used, is known as a post-column reaction detector. Symptomatically, this points out the great lengths the chemist is willing to go in order to achieve selective and sensitive analytical methods for the measurement of pesticide residues.

Emerging Technologies

Two of the emerging technologies for detecting pesticide residues in foodstuffs are the mass spectrometer and the immunoassay. The mass spectrometer, whether it is coupled with the gas or liquid chromatography, can provide a positive identification of a pesticide residue component by virtue of its peculiar mass-fragmentation pattern. The specificity of the mass spectrometer is the real advantage, although for many compounds it also has great sensitivity. For difficult to detect compounds, there is always the option of derivatization, as with the other quantitation techniques. The mass spectrometer can also utilize several different ionization modes such as chemical ionization, electron impact, field resorption, or fast atom bombardment.

It is feasible to use the Luke-acetone extraction procedure (19) to isolate pesticide residues from the crop matrix, provide a gross cleanup with gel permeation chromatography (24, 25, 26) or florisil column absorption, and then proceed to GC-MS for detection. Coupling the resolving power of capillary chromatography with the specificity of GC-MS would allow screening a large number of compounds through its spectral library. Mass-fragmentation patterns matching particular compounds could be reanalyzed by selective ion monitoring (SIM) for confirmation and quantitation. By analogy, these techniques are now being used to analyze approximately 165 compounds in water and sediment for priority pollutants (26) Sensitivity of detection may in some cases be a severe disadvantage of this technique. As described in 40CFR180, crop tolerances in RACs vary widely by compound and crop type, which could result in some samples in violation being undetected due to the differences in tolerance levels of pesticides. For instance, one pesticide may have a tolerance of 50 ppm in corn grain, and another pesticide may have a tolerance of 0.05 ppm. An analysis screen set for the high-level tolerance would miss the low level and thus the sensitivity of the screen must by approached with knowledge of the tolerances. These tolerance levels could be easily identified by tabular presentation of compounds and RACs.

The use of mass spectrometry as a tool for analysis of pesticide use has been dramatically increasing, although instrument size and expense are a drawback. University and other small laboratories may not be able to justify the expense of dedicating a mass spectrometer for residue analysis. Contamination of the source with large amounts of chemicals from non-residue level use is an additional potential problem. Bench-top models with smaller price tags are being developed and could ultimately have significant impact for use as a multiresidue screening tool. One area for vigilance is that some classes of similar pesticides and degradants could conceivably yield the same fragment ions.

The other area of emerging method technology is the development of immunoassay for pesticide analysis. Immunoassay are generally applicable to pesticide chemistry, and these immunochemical techniques are highly specific, sensitive, rapid, cost-effective analytical methods (9,10). They owe their great sensitivity and specificity to biological systems that can produce the reagent antibodies that bind with high affinity to compounds of interest. Immunoassay are very sophisticated and require a certain proficiency to develop. Each intended use of the immunoassay has to be carefully considered prior to initiation of development efforts. Choice of the hapten, preparation of the conjugate, generation of the antibodies, and incorporation of the antibodies into an assay all have to be carefully and thoughtfully worked out prior to the very important demonstration of method viability by analysis of samples (11).

The most frequently mentioned concern with immunoassay results is the nagging possibility of unsuspected interactions with unknown components in the sample. This doubt is somewhat magnified because of the absence of color development in positive results, which is the opposite of the traditional color development in positive findings of derivatizational spectrophotometric methods. Thus, additionally colored solutions also create a concern (12). Since nearly all pesticide immunoassay are competitive binding experiments, any interferences that inhibit "complexation" of the antibody with the tracer yield incorrect positive findings.

Pesticide residues of regulatory concern in foods are often composed of mixtures of the parent and degradates (i.e., total toxic residue). In such cases, the extreme specificity of an immunoassay method may actually be a handicap. Knowledge of the ability of the antibodies to recognize the degradates is critical. In some cases, degradates could be substantially more sensitive than the parent. Therefore, a positive result above the tolerance level in a multiresidue screen may also be a false positive. In some literature studies, only 30 to 50 percent of the positive occurrences actually contained the analyte as confirmed by GC/MS(l 1). Thus, use of immunoassay for multiresidue screening has to have the potential for confirmation by other analytical techniques and should be evaluated to determine if the potential level of false positives is acceptable. In general, the immunoassay technique appears to offer excellent opportunity for use as a multiresidue method especially because of the low incidence of false-negative detections. Using this technique, the analyst can screen many more samples than previously possible with conventional techniques. However, more research directed toward field validation will be required to evaluate the immunoassay's reliability.

Multiresidue Technologies

The major advantage of a multiresidue method for the analysis of pesticides is that the method allows the analyst the opportunity to look for the presence of many pesticides at once. EPA could further encourage the development of these multiresidue methods by focusing on development of enforcement methods, not on the total toxic residue but on the identification and selection for analysis of the most significant analyte of a pesticide whether it be parent, metabolize, or degradate on a case-by-case basis. This would of course direct method development toward chemical classes or functionalities, as with the existing multiresidue methods, The private sector, especially the food producers, food processors, and contracting laboratories, would benefit greatly from the ability to screen a raw agricultural commodity or processed food for multiple pesticides.

Companies who are processing food for distribution are conducting quality-control analyses. The National Food Producers Association (NFPA) uses PAM 212-2 (Luke) acetone extraction procedure. Four aliquots are taken to analyze for chlorinated hydrocarbons, organophosphates, carbamates, and substituted ureas. If they have special monitoring interests, they revert to specific methodology (13). Campbell Soup Company uses a similar ploy in using the 212-2 extraction procedure and expanding the florisil-elution parameters to include a large number of compounds. Analytical chromatographic conditions are also expanded to include the analytes of interest (14). The Dried Fruit Association in California operates under similar procedures **(15).** The NFPA also conducts research on the effects of pesticide residues in food processing **(13).**

Food processors generally conduct residue analyses (16,17) but they are not involved in methods development. They are contractually requiring pesticide-use history from growers to assure that pesticide label requirements were followed. Classical methods are then used for additional quality control (17).

The California League of Food Processors provides growers information on pesticides that may legally be used in California, that is, the tolerance, maximum-use rate, and the frequency of application for a particular crop. It also provides several forms dealing with pesticide treatment and crop history including guarantee forms, report forms, notices to growers, and refusal forms (18).

Quality assurance and litigation samples lead the analyst of a contract laboratory to the more general or screening procedures. In these cases, qualitative identification of the analyte can be as important as quantitation. The analyst will rely on methods from Federal regulating agencies such as EPA, FDA, and USDA, or from peer-review organizations, like AOAC or ASTM (19).

Actually, the regulating agencies are in the best position to coordinate multiresidue method development, especially EPA, ***since*** they are in possession of all the pesticide registrants' information on the physical properties, product chemistry, metabolism data, and means of residue analysis.

The pesticide registrants are required to submit an analytical method that is not designated as "com-

pany confidential" for enforcement purposes. Additionally, EPA is requiring registrants to report the behavior of pesticides in the four FDA multiresidue protocols (20). However, some judgment is needed with respect to requiring the fit in these multiresidue methods for a parent molecule when the parent has been demonstrated in radio-labeled crop metabolism studies to rapidly and extensively metabolize. Whether expending a great amount of effort for multiresidue method development of pesticides with low toxicity (Category D or E) is worthwhile or not is also an issue to consider. Thus, the starting point for decisions on multiresidue development is in the hands of the regulating agencies.

Federal Interaction

Federal agencies should upgrade their technical approach to multiresidue technology. Industry seems to have the opinion that these techniques are antiquated, but in fact major gains can be made through modernizing the analytical step. Registrants are required to validate residue methodology used to develop the tolerance database by analysis of endogenous residues from the radio-labeled metabolism studies. This validation certainly identifies the solvent system needed for extraction in the enforcement method, as well as the potential selected analyte for multiresidue methodology. Standardization of chromatography materials with respect to size, surface area, moisture and absorptivity, for instance, would be beneficial to the analyst and help reduce inter-laboratory variability. Capillary and wide-bore capillary columns have become very practical in the laboratory since the advent of fused silica-bonded phase columns and they offer substantial increase in resolution from packed columns. Analytical detection systems in multiresidue methods are basically reduced to electron capture and thermionic specific for bas chromatography and UV-VIS and fluorescence detection for HPLC. Multiresidue technologies need to be expanded to include selective ion monitoring of mass spectrometry, especially for those pesticides or degradates that do not have a heteroatom to allow selective identification.

Perhaps the most visible item to be improved is presentation of the methodology in the Pesticide Analytical Manual Volume I (PAM-I). A wealth of information is harbored in its chapters if the analyst masters the system. Improvements could be made by using clear block-letter headings describing procedures instead of relying on the numerical codes. Methods should be presented in complete form to allow a more concise flow of information, as in the style of the AOAC Manual **(8).** References to supplemental methods should be presented in the appendix to PAM-I while listing pesticide and cleanup procedures.

The most innovative step one could undertake would be the computer indexing of the PAM data base. Each pesticide entry file should have all the chromatographic conditions, approved methods, supplemental methods, and data on chemical structure. These data made available as public information in the form of a personal computer (PC) disk would give the analyst easy access to the analytical data. In those instances in which a class of compounds are to be analyzed, this would be a starting point in determining universal conditions and the development of multiresidue methods. The key to acceptance is ease of use *(21)*.

An analyst could further narrow the scope of the multiresidue investigation by consulting an information center or database consisting of agronomic practices, crop registrations (22), pesticide fate, and toxicological significance. For example, Dr. Phil Kearney of the USDA-ARS developed a list of pesticides used on corn by consulting a half-dozen databases such as EPA information and state surveys **(23).**

Similar use patterns could be developed for vegetables, fruits, nuts, and other field crops based on regional use patterns. From these data, optimal selections of analytes would be made for the analyst. For example, the Arizona Department of Agriculture (ADA) requires certified applicators to register the compound being applied, the location of the field to be sprayed, and the registration form be filed with ADA. These data could also be compiled and made available to interested parties on a PC disk for easy access.

If the Federal government wanted to stimulate analytical method development, and particularly multiresidue methodology in the private sector, two things could be done to affect pesticide industrial development. The first would be to expedite the EPA review process. Currently, pesticide registrants are asked to internally review their petitions for registration package completeness. Still the EPA takes 12 to *18* months to respond to a submission. Expeditious review, whether by additional staffing or improvements in efficiency of the review process, could shorten the registration cycle.

The enforcement method seems to be the avenue that the agencies could use to encourage the pesticide industry to enhance multiresidue technology. Conditional registrations could be granted based on the submission of scientifically credible crop-

residue studies with the proviso that the industry chemists attempt to fit the enforcement methods to the multiresidue scheme. Since it takes a while for a product to achieve widespread distribution and a "dent" in the marketplace, by this time the multiresidue enforcement method could be in place for screening purposes.

The pressure on the industrial chemist and the pesticide industry in general could be somewhat relieved by prolongation of the patent life to partially compensate for the 5 to 7 years spent in regulatory clearance studies. Since multiresidue method research is complex, time-consuming, and expensive, allowing industrial chemists greater freedom without delays in the pesticide registration could produce the desired results. Prompt evaluation of registration data would result in a longer market life, more profitability, and a propensity on the part of industry to provide resources toward developing methods for surveillance. In other words, if EPA reviews were expedited and patent-life prolonged, industry would not object to additional methodology requirements. Other options require the higher risk, and less advantageous, pouring of funds into long-term contracts or grants.

Governmental agencies should combine their resources to provide analytical pesticide residue training, similar to the Advanced Pesticide Residue Analysis workshop jointly sponsored by the US EPA National Enforcement Investigations Center and the New Jersey Department of Environmental Protection. The concept of this workshop could be enlarged to include analysts from the private sector. Recent demands in the analytical support of registration studies have resulted in qualified personnel being the limiting factor in laboratory expansion. Training sessions that reviewed the techniques of the PAM-I would be of value to the method development chemist as he researches for a sensitive and selective analytical scheme. Education of the researcher developing methods that coincide with EPA and FDA requirements would go a long way in attaining these goals.

Conclusions

The organization and direction of multiresidue methodology seem to rest with the EPA and FDA. The pesticide registration database complete with metabolic or degradate information is known by the EPA, and the pesticide monitoring techniques are known by the FDA. Incentives for development of these methods, whether in the form of appropriations, grants, or conditional registration, should be fostered by these agencies.

Industry should be requested to develop enforcement methods focused on analytes that are most likely to be present based on compound half-life calculations and the metabolic degradation pathway. FDA is facing an impossible task when asked to screen a crop for a few hundred compounds, as well as all their degradation products. It would be preferable to select one or two representative moieties as a biomarker to be incorporated into a multiresidue screen. If residues and the incidence of violation warrant further analyses, the total residue method should be used as supplied by the registrant.

The applicability of enzyme inhibition and immunoassay should be evaluated for pesticide residue analyses. Federal appropriations should be used to evaluate the number of classes of compounds that can be screened by these techniques. How broad are their applications with respect to sample matrix? Classical methods should be used simultaneously with the screening techniques to validate their accuracy. If these questions and conditions are satisfied, EPA could further promote promising techniques such as immunoassay, by acceptance as enforcement methodologies,

Multiresidue techniques are important and can be improved through a concerted effort. Revision of the PAM-I format, a PC disk database access, and upgraded analytical procedures including capillary chromatography and GC-MS-SIM would encourage this development.

Agency and private sector meetings or training sessions should be promoted to advance discussions for solutions to problems. Ultimately, the greatest step in solving the technical problems of pesticide residue analyses will be the enhancement of communication by all parties involved in the agricultural arena.

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Pesticide Residue Monitoring in Canada

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and

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Contents

Figure

Table

Abstract

The Health Protection Branch of the Department of National Health and Welfare establishes maximum residue limits for pesticides in food in Canada, and it is responsible for ensuring that commodities offered for sale comply with these limits through surveillance and compliance programs. In addition, data are gathered on the levels of residue in a variety of foods to determine the dietary exposure of the population to these chemicals.

Approximately 3600 samples are analyzed annually through four programs designated as surveillance, compliance, data gathering, and total diet. The surveillance program is divided into regional and national components; the former generates data on residues and foods that are of local concern while the national component determines the state of compliance of selected foods in the marketplace across the country. The compliance program investigates and solves problems identified in the surveillance project. The data gathering program conducts analyses of a specialized nature in response to concerns arising from new toxicity data or gaps in the residue database. The total diet study provides data on the actual dietary intake of pesticides from foods prepared as for consumption,

Methodology used for monitoring relies heavily upon a multiresidue procedure capable of determining 155 compounds. An additional 37 compounds predetermined by specific methods. Current research is directed toward increasing the efficiency and scope of monitoring methods using immunochemical, degradation to a common fragment and robotic approaches. The characteristics and applicability of these methods are discussed.

Pesticide Residue Monitoring in Canada

Monitoring for pesticide residues in food is conducted at the federal level by the departments of Agriculture, Fisheries and Oceans, Canadian Grain Commission, National Health and Welfare, and in the provinces by the laboratories of the ministries of Agriculture and Food. The objectives of the various projects differ substantially—from providing assurances to farmers that pesticides used according to label directions will not result in residue problems and approving shipments for export to enforcing compliance with maximum residue limits (MRLs) established under the Food and Drugs Act. The Health Protection Branch of the Department of National Health and Welfare establishes the MRLs and is responsible for their enforcement through surveillance and compliance-type projects carried out in five regional laboratories situated across Canada. In addition, data are collected on the occurrence of residues in order to determine the dietary intake and to ensure that the lowest possible exposure consistent with effective pest control is attained.

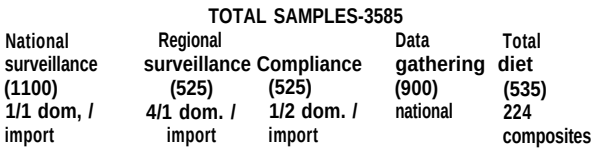
Programs

Monitoring programs conducted by the Branch are divided into four categories: data gathering, total diet, surveillance, and compliance. A summary of these and an indication of the proportion of samples directed to each is given in figure 1.

The objectives of the Canadian program are very similar to those described for the United States (19) and the types of program also resemble each other closely, although the numbers of samples analyzed in Canada are smaller than those outlined for the United States (8). Thus, the total diet, data gathering, and regional surveillance projects in Canada are comparable to the total diet, selected survey, and district option in the United States. However, Canadian national surveillance and compliance projects do not have U.S. equivalents. Another major difference between the two countries' approach is that Canada does not have a separate program for imports but includes these items in most surveys in proportion to consumption.

Data gathering projects are designed to collect information on the occurrence of specific pesticides in the food supply, and they often involve biased sampling. Specific methodology, as compared to multiresidue methods, is often required, and projects are initiated as a result of new information on the probable occurrence of compounds or their

Figure 1.—Monitoring Programs



metabolizes or in response to concerns arising from new toxicity data. Approximately 900 samples are analyzed annually in this program. The total diet program is similar to that conducted in the United States and, in its present form involves the preparation of 161 food items as consumed. One hundred and twelve composites of these items are then made, representing 99 percent of the Canadian diet. Samplings are conducted twice annually from different cities representing the five regions across Canada, such that all regions are covered in a two and a half year cycle. All composites are analyzed by the multiresidue method (9), and six additional compounds are determined by specific methods. The resulting data reflect actual pesticide intake that remains after trimming, washing, and cooking of foods.

The surveillance program consists of national and regional components. The national surveillance component is designed to determine the state of compliance of selected food commodities in the marketplace with respect to selected pesticides. Approximately 1,100 samples are analyzed annually, approximately one half of them by multiresidue methodology. A smaller number of samples (525) is analyzed in the regional surveillance project. Regional surveillance is planned separately by inspection and laboratory staff in each region and is designed to emphasize commodities and pesticides that are of local, rather than national, importance. This program uses information gathered on local pesticide usage, infestation problems, and crop conditions. Commodities are included where pesticide misuse is suspected.

Data obtained from surveillance projects are compiled each year, but are not published or stored in a computerized database. They are used internally to direct future surveillance and compliance projects and are available on request to other interested parties, including international agencies such as WHO/FAO.

The national surveillance component has only been in place in its present form since 1985. Eleven out of fourteen commodities tested have shown a state of compliance of at least 99 percent. The three

exceptions are now included in the compliance project.

The compliance program is designed to investigate and solve residue problems identified by the national or regional surveillance components. This project is instrumental in preventing the sale of foods containing residues in excess of the MRL. Historically, a wide variety of pesticides have been found at violative levels. For example, in 1986/87, thirty-one different compounds were involved, with fungicides and organophosphates being the most numerous. Because the use of pesticides that are registered in other countries but not in Canada often results in residues that exceed our MRLs, residue problems have occurred more frequently with imported products than with domestic ones. Therefore, compliance efforts are concentrated on importers, who, under Canadian law, are responsible for ensuring the products they import comply with the Food and Drugs Act. Actions taken if violative residues occur range from refusal of entry to prosecution of importers who repeatedly import violative products.

Sampling Strategy for National Surveillance

The food supply is divided into 14 commodity classes such as vegetables, meat, dairy products, and fruit. These classes are ranked according to consumption, pesticide application, potential for residues, and data available from other agencies. Of these classes, fruit and vegetables represent approximately 35 percent of the Canadian diet and have the highest potential for residues. Thus, they are designated as constituting a minimum of two thirds of the surveillance samples. Thirty-five items from these commodity classes, representing 90 percent of the apparent consumption are selected for analysis once over a 10 year period. The highest consumption items representing 50 percent of the diet are analyzed twice over the 10 year period. For each pesticide-commodity combination, 100 samples are analyzed, so that a 4.8 percent violation rate would be detected with 95 percent confidence (2). These samples are procured in proportion to the geographic distribution of their origin, i.e., by province of production for domestic commodities and by country of origin for imports.

In Canada, 235 pesticides are registered for use on food. Those recommended in provincial spray calendars are considered to represent those in actual use and are ranked using such factors as volume of use, persistence, and toxicity. The selection of pesticides to be determined on imported foods is based upon the existence of a tolerance in the country of origin and weighted by the frequency of occurrence of previously detected residues. The FDA surveillance index (20) is also heavily relied upon to furnish a criterion for priority, Thus, a list of commodity-pesticide combinations is constructed. For those compounds that can be determined by multiresidue methods, pesticides can be included down to a low level of priority, while those requiring specific methods must be selected from the high priority portion of the list.

Current Analytical Methodology

As indicated previously, a multiresidue method (9) is used for surveillance wherever possible. This procedure, which is capable of determining 155 compounds in a variety of fatty and non-fatty foods, involves cleanup of an acetone extract by automated gel permeation chromatography followed by determination by capillary gas-liquid chromatography using at least two detectors—usually a Hall detector in the halogen mode and a thermionic nitrogen/phosphorous detector. HPLC with post-column derivatization as described by Krause (5) is used for methyl carbamate pesticides. An additional chromatographic cleanup on Florisil is required with some commodities.

Specific methods are used for an additional 37 compounds that cannot be determined by the multiresidue procedure. These compounds are examined as the need arises and, on average, each consumes the same resources as the multiresidue procedure. Often, members of this group of pesticides are insufficiently volatile for gas chromatography and do not contain chromophoric groups necessary for detection after HPLC. Examples are the ethylenebis(dithiocarbamates) fungicides and daminozide. Others such as maleic hydrazide, glyphosate, diquat, paraquat, and the organotin compounds cyhexatin and fentin require unique cleanup steps due to their polar nature. A complete list of these compounds is given in appendix 1.

Research and Development

In an effort to improve the efficiency of the monitoring process, as well as to identify new compounds such as metabolizes and degradation products, research is conducted in the Health Protection Branch into the development of analytical methods. The private sector does not conduct method development as such but relies on methods published in

the scientific literature or in manuals developed by Federal departments. The methods developed by the branch may classified as four types and will be discussed individually as: 1) TLC-enzyme inhibition, 2) degradation to a common fragment, 3) immunological, and 4) robotics.

TLC-Enzyme Inhibition

Two rapid screening techniques, qualitative in nature, have been studied in our laboratory to determine rapidly, and with as little sample purification as possible, whether a sample contained violative residues. Both rely on separation of the pesticides by thin-layer chromatography, followed by treatment of the developed plate with an enzyme or enzyme system and substrate. The first of these methods has been reviewed by Mendoza (10) and consists of detection of carbamate and organophosphate insecticides with an esterase preparation followed by a chromogenic substrate. Zones of inhibition indicated the presence of cholinesterase inhibitors, the identity of which could be indicated by the R_f. Several factors affected the sensitivity of the assay, including source of enzyme, substrate, and pretreatment of the plate with an oxidant to convert thiophosphates to their oxygen analogues. The disadvantage of the method, which prevented its routine use, was the presence of a number of naturally-occurring inhibitors in food extracts.

A more promising development is that of TLC-photosynthesis inhibition, which is applicable to those herbicides that inhibit photosynthesis such as phenylureas, phenyl carbamates, and triazines (6). After chromatographic separation, the plate is sprayed with a suspension of chloroplasts, followed by the redox indicator 2,6-dichloroindophenol and exposure to light. Photosynthetic inhibitors appear as blue spots of unreduced dye. The method requires little sample workup other than initial sample extraction and partitioning into dichloromethane, and it is relatively immune to interference. Detection limits are satisfactory for compliance purposes.

Common Fragment

This approach involves the conversion of pesticides with different properties, such as polarity and vapor pressure, to a common entity, permitting the determination of several compounds in a single analysis. The technique has been used for the detection of phenyl urea herbicides in a variety of sample types (4), and it provides information as to the total burden of a class of compounds in a food. This concept may also be used to determine alachlor, diethatyl, and their 3,5-dichloroaniline-containing metabolizes by hydrolysis to 3,5-dichloroaniline and determination of 3,5-dichloroaniline by GLC (15). Similarly, iprodione, vinclozolin, and procymidone are also determined by alkaline degradation to 3,5-dichloroaniline (18). The obvious disadvantage of this approach is that if excessive residues are encountered, individual determinations must be made to identify the offending compound,

Immunochemical Methods

The widespread successful use of immunoassay techniques (3) in clinical laboratories prompted us to evaluate its applicability to pesticide residues in foods. Both radioimmunoassay (RIA) and enzyme-linked immunosorbent assays (ELISA) have been developed for compounds ranging from the nonpolar polychlorinated biphenyls (11) to the water-soluble fungicide carbendazim (12, 15). Either immunochemical approach resulted in methods that correlated well with conventional chemical analyses for all compounds we have studied. The nonpolar PCBs posed the greatest problem, requiring sample purification as extensive as that required for gas chromatography if false-negative data were to be avoided. In contrast, carbendazim could be determined in crude ethyl acetate extracts without prior cleanup and was not subject to interferences. Similarly, methods for the fungicides metalaxyl (13), iprodione (16), and triadimefon (14) did not require any sample preparation other than initial extraction.

The specificity of immunochemical methods is generally sufficient for screening purposes but, as the data summary in table 1 indicates, varies greatly with the assay and is probably a reflection of the structure of the pesticide. For example, the selectivity of the assay for thiabendazole is very high, with low cross-reactivity for related compounds such as 2-benzimidazoleurea or carbendazim. In contrast, vinclozolin and procymidone react with antibody directed toward iprodione to a higher degree than does iprodione itself. Similarly, the herbicides metolachlor and diethatyl have considerable cross-reactivity with metalaxyl antiserum, further emphasizing the screening nature of the analysis.

The merit of ELISA compared with RIA lies in the relative safety and availability of reagents and in the simplicity of associated counting equipment. However, RIA is often more rapid, requiring fewer incubation steps and produces steeper inhibition curves, which result in greater sensitivity, A larger number of samples may be processed at one time with ELISA, resulting in low unit cost. Compared with conventional specific analyses, ELISA is ca-

Table 1 .—Characteristics of Immunochemical Methods Developed at Health Protection Branch

Compound determined	Assay type	Quantitation limit (ppb)	Major Cross reactions	Sample
Aroclor 1260	RIA	2	Aroclor 1254	milk
Carbendazim	RIA	50	2-benzimidazole urea	cucumber
Metalaxyl	ELISA	100	metolachlor, diethatyl	tomato
Thiabendazole	ELISA	30	nil	potato
Carbendazim	ELISA	350	2-benzimidazole urea	apple
Iprodione	ELISA	100	vinclozolin, procymidone	tomato
Triadimefon	ELISA	500	triadimenol	apple

pable of producing four to five times the number of determinations per day.

Robotics

Robotics is being studied as a means of reducing the labor-intensive component of conventional multiresidue analyses. Two implementations of this technology are being evaluated—one that carries out the liquid-liquid partition step in the Luke et al. procedure (7), and another that prepares milk samples for the determination of a number of organochlorine compounds by gas-liquid chromatography.

The system used for partitioning (1) consists of a Cyberfluor Labotix robot arm, stirrer, and liquid handling apparatus under control of a microcomputer. Aliquots of sample extract are manually added to a flask where partitioning is carried out by a series of stirring actions with dichloromethane. After phase separation, the dichloromethane is recovered by the robot for concentration prior to cleanup. Recoveries of standards added to several commodities were comparable to those obtained with the manual partitioning procedure.

For milk analysis, the entire extraction and cleanup procedure was automated using a Zymark Corp. arm and custom-built series of workstations. This apparatus permits the weighing of sample, extraction with organic solvent, centrifugation, column chromatography, and collection and evaporation of three fractions prior to gas chromatographic analysis. Thirty-two compounds, in addition to PCBs are determined using an autosampler and data acquisition system. When evaluated against manual sample preparation, the robot was found capable of doubling the weekly output. The coefficient of variation at the 1 ppb level was 15 percent for the automated system compared with 8 percent manually. Accuracy was equivalent for both systems.

Method development is now being conducted into the further application of immunochemical and robotic procedures, as well as such techniques as solid phase extraction for inclusion in the multiresidue method. In addition, new pesticides are continually being tested for inclusion into the existing multiresidue method. Since the analytical problems encountered in monitoring the food supply are common to both the United States and Canada, both countries benefit from new developments arising from research in North America or abroad. An excellent mechanism for communication and exchange of this technology is the Association of Official Analytical Chemists. This international organization of scientists from government, industry, and academia disseminates new findings at its annual meetings and through publication in a journal. In addition to being a forum for discussion of new approaches, methods are validated through a process of collaborative study in several laboratories.

Appendix 1

Pesticides Determined by Single Residue Methods: aldicarb, amitraz, *benomyl, bentazon, biphenyl, *chlorophenols, “daminozide, desmedipham, dichlone, diquat, *diuron, dodine, *EBDC, *ethylene dibromide, ethephon, ethylene thiourea, fluazifop-butyl, glyphosate, imazalil, iprodione metabolizes, *maleic hydrazide, methiocarb, methomyl, *methyl bromide, napthalene acetic acid, naptalam, “organotin compounds, oxamyl, oxydemeton-methyl, “paraquat, o-phenyl phenol, pyrethrins, terbutylazine, thiabendazole, triallate, triforine, vinclozolin metabolizes

Those compounds marked with * have been identified by GAO as needing single residue methods.

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Pesticide Monitoring Program in Mexico

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The main objective of the Animal and Plant Health Law of the Mexican Republic is to protect animals and plants from pests and diseases. In addition, the law provides the Secretary of Agriculture and Water Resources the facilities to exercise control over the quality of biological and chemical products applied to animals and vegetables as well as to prevent agrarian activities from originating health risks and environmental contamination. This is carried out through the Plant and Animal Health General Direction, responsible for pesticide registration and control, This office takes care of setting tolerances and checking the quality of the formulations available for the growers.

The system to control these chemical compounds in Mexico involves separate aspects; the law requires the registration of import, manufacturing, development, and distribution firms. It maybe considered that great advances have been made in the regulation of these firms in the past 18 months.

Equally, the registration of compounds sold in Mexico has kept a very acceptable level, as well as the registration of technicians who supervise the quality control in factories and who are responsible for usage recommendations in their own firms.

Pesticides, companies, and consultants are registered in the main offices in Mexico City; number registration of sales and distribution is done through the Agriculture Department officer in the Mexican states. In 1974, the construction of a network of laboratories was begun to bring about quality control of product formulas as well as to determine residue levels in affected crops. There are now 12 regional laboratories for pesticide analysis and one central reference laboratory for pesticide residues analysis of animal products. Five of these laboratories are able to conduct residue analysis as well, and the Vegetables Growers Union has built a laboratory for the same purpose.

Some colleges and universities in Mexico are making efforts to develop analytical methods, but the task is centered most often upon the pesticide industry and in the official laboratories. In both cases, it may be said that more important than the development of new methods is the implementation and verification of those methods developed by benchmarks or published in the literature.

Some efforts have been made with respect to pesticide residue analysis with the objective of modifying some methods to make them more economical, but no conclusive results have yet been reached.

This year, the program involves the analysis of 2,200 samples of vegetal origin, specifically of the following crops: chili peppers, green peppers, tomatoes, tomitillos, and strawberries. This is done using the FDA Pesticide Analytical Manual procedures already discussed and those modifications applicable to the country conditions.

The selection of the products to use against a pest problem should be made on the basis of the manual of Authorized Pesticides, which SAPAF edits and reviews each year and which lists those compounds that have complied with the requirements specified by the law. Also it includes information about pests, crops, and dosages that may be applied, safe intervals of application, and the residue limit that should be observed.

Let's use PAM procedures because the United States is the main consumer of our agriculture exports, thus we check both the domestic and foreign consumption.

However, during past years, economic factors have had the following negative effects on our work:

—lack of proper maintenance of the equipment
—no new equipment
—loss of training technicians and inability to contract replacement personnel.

This situation is aggravated by the problems of inflation and daily devaluation of the Mexican currency. This is reflected in the number of analyses that can be carried out, reducing it a considerable degree each year and thereby reducing the established capacity that the Secretarial de Agricultural y Proteci–opecuaria y Forestal (SAPAF) once had. During 1987, residue analyses were carried out on 970 samples of fresh agricultural produce. Since the tasks of analysis is very specialized and practiced by a small number of technicians, there is no interest in the reagent and solvent industry in Mexico in maintaining a quality product that satisfies the requirements of these analyses. For these reasons, the reagents and solvents are imported. In addition, much of the equipment and glassware is imported, so the prices for these materials have increased.

Considering that the main problem with pesticides is misuse, we are implementing the use of a prescription for selling pesticides. This means that in order to purchase a pesticide, the grower must

present a paper written by a registered professional indicating the crop, the pest, product, and dosage.

As I mentioned before, residue analysis is carried out with the U.S. methodology whenever possible, but we don't eliminate the possibility of using those recommended methods from international agencies such as Codex Committee on Pesticide Residues.

We recognize the importance of fruit and vegetable trade between Mexico and the United States, and we are very concerned about it. To this end, we have instituted and analytical quality-control program between FDA and my office to assure that the pesticide residues in commodities involved in trade are below tolerance levels.

Nowadays the Agriculture Department has the tendency to implement pest control programs that are part of an integrated pest management program that that takes advantage of pests' natural enemies; and includes extending the use of old technologies such as biological control. Last year we had spectacular results on the soybean crop, reducing the use of pesticides by more than 50 percent.

Another technique is the use of sterile insects in the campaign against the Medfly and the cattle screwworm. Other pest control methods have been developed that originated from the EDB ban: hydrothermic treatment for the elimination of larvae in mangoes for export and the use of low temperatures as an agricultural sanitation treatment in citrus exportation.

The biological pesticides *Bacillus thuringiensis* is widely used for forestry pests in ecologically protected areas; we are conducting investigations on the use of fungi against soil pests.

Despite all these efforts, it is recognized that pesticide use will continue to be the extensively used means of pest control. Actions are being directed toward making adequate use of pesticides in which different government agencies, as well as the pesticide industry and professional organizations, participate by means of campaigns, qualification courses, refresher workshops, and symposia, all oriented toward keeping pesticides a useful tool without excessive risk.

Strong efforts are being made to get economic support from international agencies in order to let us continue this task.

Having the opportunity to participate in events such as this undertaking and others in which new technologies are expounded and revised, or at least having access to the information generated, can help Mexico maintain an acceptable level of technological development in this area. Visitation by specialists who might, upon observing conditions in the nation, be in a position to offer a more practical and acceptable assessment, would be equally useful. Nevertheless, the main problem is, and according to our perspective will continue to be, the lack of economic resources that permit us to incorporate innovations in day-to-day work.

It is necessary to identify sources of financing to continue the program and the technical assessment that allow research into other analytical possibilities,

Developing Pesticide Analytical Methods for Food: Considerations for Federal Policy Formulation

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contents

Abstract

Technologies available to detect, identify and quantify pesticide residues in food have played a key role in defining the structure and effectiveness of Federal pesticide monitoring programs. Monitoring programs are used to assess public exposure to pesticides and as a basis for enforcing pesticide laws by detecting residue violations. Because of the volume of samples that must reanalyzed and the dearth of information on the pesticide treatment history of food samples collected for analysis, Federal monitoring programs have been designed around the use of multiresidue methods. However, multiresidue methods are unable to detect all residues of interest, including a number of pesticides of high priority because of their widespread use and toxicity levels. This gap in detectable residues has served as a point of departure in recent policy debates concerning the appropriate direction of research in methods development.

This report examines Federal pesticide monitoring programs and how analytical technology has shaped them. It also considers program limitations that have been identified in recent policy studies, and raises questions about the role of analytical technology in addressing these needs. This report concludes that new analytical technology may offer an opportunity to address not only the gap in detectable residues, but to help achieve even more fundamental improvements in pesticide monitoring programs.

Introduction

Public health policymakers have long been concerned about the health implications of dietary exposure to pesticide residues. They have considered analytical methods capable of detecting and quantifying pesticide residues basic to any program designed to control such exposure. Even before a Federal pesticide monitoring program was developed under modern pesticide laws, this need for methods was recognized. For example, Dr. John Kerfoot Haywood, head of the Federal Insecticide and Agricultural Water Laboratory in 1905, was disturbed about possible health effects of pesticides and stated, "[i]t is essential that these [pesticide] compounds be analyzed by exact. . . uniform methods. . . ."(l).

During the past 3 years, several critical evaluations of Federal monitoring programs directed at pesticide residues in food have advised that improved analytical methods were needed to enhance the effectiveness of the programs. These recommendations have helped to focus public and congressional attention on analytical methods and have fueled the reevaluation of Federal policies currently underway inside the Federal agencies and by the Congress.

However, the interest of the public and of policymakers in analytical methods has been borne of a larger concern—that the government has been unable to supply to the public sufficient data to allay concerns about the safety of pesticide residues in the food supply. Some consumers have construed

the gaps in residue data as indicative of a grave and unknown risk to public health (2). Some policymakers are desirous of more pesticide residue data because they believe it will more clearly show that the food supply is safe, and will help to restore public confidence in the effectiveness of the programs already in place (3).

This report examines Federal pesticide monitoring programs and the relationship between analytical technologies and the design and limitations of current monitoring programs. It considers recent recommendations regarding program needs to improve the analytical methods used in the programs. It also raises questions regarding how the goals and design of pesticide programs in the future may influence the analytical methods policies developed and implemented today.

federal Pesticide Residue Programs and Analytical Methods

Pesticide analytical methods are scientific techniques used to detect, identify, and quantify pesticide residues. The technology generally used for pesticide detection is gas-liquid chromatography (GLC) and high-pressure chromatography (HPLC) (4, 5). There are two general types of pesticide analytical methods in use today: multiresidue and single residue methods. Multiresidue methods are capable of detecting a number of pesticides having similar chemical and physical properties in a test of a single sample. Single residue methods are capable of identifying only one pesticide residue in a food sample. In general, multiresidue and single residue methods require comparable time and resources to conduct per sample. Therefore, multiresidue methods are considered more time and resource efficient than single residue methods (6). The advantages of multiresidue methods over single residue methods have helped to make them the basis of current pesticide monitoring programs. In addition, multiresidue methods capable of detecting large numbers of residues are useful in testing samples when reliable information about the pesticides used on the commodity is lacking.

The Federal pesticide program is actually a patchwork of programs administered by the Environmental Protection Agency (EPA), the Food and Drug Administration (FDA), and the U.S. Department of Agriculture (USDA). Analytical methods play a key role in the programs of each agency.

Environmontal Protection Agency

The EPA has central authority for the regulation of pesticides under the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA). Under the act, EPA is required to grant a license (registration) for pesticide chemical uses for which the applicant (registrant] has demonstrated, among other things, that "when used in accordance with widespread and commonly recognized practice it will not generally cause unreasonable adverse effects on the environment" (7). In determining whether a pesticide can be registered, among the factors EPA considers is whether residues that result from pesticide use pose a dietary hazard to humans or animals. For pesticides that will be used on a crop that will serve as human or animal food, EPA is required to grant a tolerance level for residues under the Federal Food, Drug and Cosmetic Act (FDCA). A tolerance level defines the maximum amount of pesticide residues that may remain in food (8).

A condition of registration for any pesticide for which a tolerance is granted is that the registrant supply to EPA a pesticide analytical method(s) capable of detecting and quantifying the active ingredient and the products of its degradation (9). An EPA laboratory tests the method (validation) to assess the validity and reliability of the method (10). The goal of the requirement is that the registrant supply to the agency the analytical means to enforce the pesticide tolerance. However, the requirement has been ineffective in achieving this goal largely because the methods supplied are often not practicable for regulatory purposes. Registrants generally fulfill the requirement for a method by supplying a single residue method. Because of the limitations of single residue methods when compared with multiresidue methods, as noted above, they are not feasible for routine use in monitoring programs, except in limited circumstances where some reliable information is available on the pesticide treatment history of the sample.

Federal Monitoring Program

Once a pesticide has been approved, the responsibility for monitoring residues in foods belongs to FDA and USDA. FDA is responsible for monitoring all domestic and imported foods for pesticide residues except for meat and poultry, which are monitored by USDA. FDA and USDA enforce the

pesticide tolerances established by EPA. Foods containing illegal pesticide residues are considered "adulterated." FDA and USDA each have authority to inspect food to determine if it is adulterated and to prosecute those who are involved in interstate commerce of adulterated products.

FDA is also the Federal lead agency for the development of pesticide analytical methods for food products. Most of FDA's research focuses on the development and modification of multiresidue methods. FDA developed the surveillance index beginning in 1979 to classify pesticides according to potential health hazards based on toxicity, prevalence of use, and persistence in the environment. The index was designed to plan monitoring programs and has helped to prioritize research on methods for pesticides not detected by multiresidue methods.

USDA also conducts some research on analytical methods, but most of this work focuses on adapting multiresidue methods for special characteristics of meat and poultry samples (11).

Food and Drug Administration

FDA's pesticide monitoring program has been designed to accomplish FDA's legislative mandate under the FDCA within available resources to enforce EPA pesticide tolerances, and enforce the adulteration provisions of the Act (12). The two primary objectives of the pesticide monitoring program are 1) to enforce pesticide residue tolerances established by EPA, and to determine the incidence and level of pesticide residues in the food supply. FDA's monitoring program has two major components: general commodity monitoring and the total diet study. Only multiresidue methods are used for routine testing in these programs. Single residue methods are reserved for work targeted for a specific pesticide.

The general commodity monitoring program component involves sampling on an "as shipped" basis, raw agricultural commodities, processed foods, and animal feeds. The samples are analyzed for the purpose of enforcing tolerances established by EPA, and for determining the incidence and levels of residues (13). Although an express purpose of the FDA commodity sampling program is to determine the incidence and levels of residues in commodities, the program is incapable of providing data that can be used to estimate the general rate of residues that violate pesticide tolerances. This is because there is no statistically valid plan by which samples are collected. In fact, a sampling plan that would make such an estimate possible has not been studied or compared with the plan in use. Some observers of the program have suggested that the sheer size, mobility, and decentralized nature of the U.S. food supply would make it impossible to collect a random sample of the food supply for pesticide analysis, even if the means were known (14).

FDA's sampling plan is made up of a set of guidelines to help officials in the district offices determine where to direct their inspection resources, some guidelines provide commodity-specific quotas, e.g., "collect 12 egg shell samples, " others are more general, "based on local usage, collect agricultural products for malathion analysis" (15). The plans specify that the sampling plan should remain flexible so that resources can be shifted to meet special needs that arise (16). Ultimately, the number of samples collected and analyzed for pesticide residues in a district is determined by available resources in that district. Pesticide monitoring must compete for resources in the districts with other significant public health functions, and sampling plans are sometimes derailed by emergency situations (e.g., a product tampering incident). In addition, even when resources are available, some guidelines are difficult to implement because of inadequate data. For example, the guideline noted above which directs testing pesticides based on local use patterns, is reliant on "detective work" done at the local level because little data is available on pesticide use patterns.

The total diet study (TDS) involves collecting a "market basket" of food samples several times per year in several geographic regions of the country, then analyzing the foods in a ready-to-eat form. The TDS is used to estimate dietary intake of selected pesticides by various U.S. age-sex groups (17). The design of the TDS provides a "snapshot" estimate of public exposure to those pesticides detected by the analytical methods used in the study. FDA relies on the TDS to make judgments about the public health risk presented by pesticide exposure through food.

U.S. Department of Agriculture/Food Safety and Inspection Service (FSIS)

Unlike the pesticide program of the Food and Drug Administration, the legislative mandate of USDA is not just one of enforcing pesticide tolerances in food (meat and poultry) or prosecuting those who engage in commerce of adulterated products. Instead, Congress has prescribed a system of ante-(18) and postmortem (19) inspection whereby

meat and poultry products are affirmatively certified by USDA to be wholesome and in conformance with residue limits (20), The program is sometimes described as "continuous inspection." An explicit goal of both the meat and poultry antemortem inspection provisions is to prevent the entry of adulterated meat or poultry into commerce *(21).* USDA has implemented its inspection program by stationing Federal inspectors in meat and poultry slaughtering and processing facilities. USDA inspectors visually inspect animals and carcasses and collect tissue samples for analysis of chemical residues, including pesticide residues.

The USDA pesticide program is part of its National Residue Program, which also targets residues of animal drugs and environmental contaminants in meat and poultry. The program has been revamped in recent years to focus monitoring activities on pesticide residues according to hazard and estimated exposure (i.e., risk) (22). The program has three components: monitoring, surveillance, and exploratory projects. The focus of the monitoring program is to profile information on the occurrence of pesticide residue violations in specified animal populations on an annual national basis and to form the basis of enforcement actions **(23).** Samples are selected on a statistically random basis. Pesticides selected for analysis are based on an assessment of risk and the availability of an analytical method that is suitable for regulatory purposes **(24).** USDA tests for only those compounds that can be detected and quantified, and for which all metabolizes can be identified by a practical analytical method. USDA has acknowledged that because of the large number of potential residues that may occur in the food chain, practical methods are not available for many compounds of interest. USDA has defined "practical methods" to be those that 1) require no more the 2 to 4 hours of analytical time per sample, 2) require no instrumentation not customarily available in laboratory devoted to trace drug or environmental analyses, 3) have a minimum proficiency level at or below the established residue limit (e.g., tolerance), 4) have a quality assurance plan, and 5) have undergone an interlaboratory validation study. Like FDA, USDA relies on multiresidue methods.

The surveillance program is designed to investigate and control the movement of potentially adulterated meat and poultry products. Samples are collected in a non-random, selective fashion directed at carcasses believed to be adulterated because of information obtained through investigation or through the monitoring program. The surveillance program is sometimes activated to follow the product of a particular supplier who was responsible for violations in the past. The program gives USDA the ability to trace problems to their source and take steps to prevent recurrence.

Exploratory projects can be likened to a research effort designed to examine a particular problem. Exploratory projects are sometimes used to evaluate new methods of monitoring or to study the occurrence of residues for which no acceptable limit (e.g., tolerance) has been established.

The design of the USDA program has enabled the agency to sample a statistically representative sample of the U.S. meat and poultry supply for pesticide analysis. This contrasts distinctly with FDA's commodity monitoring program, in which the actual sampling decisions are made on an ad hoc basis by inspectors in the field within the broad guidance of the pesticide sampling plans and within resource and information limitations. However, because the regulatory burdens placed on both agencies limit the resources that can be devoted to pesticide analysis, both agencies have opted to rely on multiresidue methods almost entirely. Therefore, those pesticide residues not detected by multiresidue methods generally escape without detection by any method. This contrast illustrates that in two agencies, with vastly differing legislative mandates, the capabilities of existing methods have been key determinants of the scope, limitations, and effectiveness of monitoring programs,

Federal Pesticide Monitoring Program Evaluations

Because analytical methods significantly influence the very nature of pesticide monitoring programs, improving methods has been viewed as a critical requisite of the programs in general. In the late **1970s,** the Subcommittee on Oversight and Investigations of the House Committee on Interstate and Foreign Commerce, as well as the General Accounting Office, investigated the Federal pesticide monitoring program administered by FDA. Among the recommendations the subcommittee made was that FDA develop analytical methods to detect more pesticides, and to focus on methods that could be performed more quickly (25).

In FDA's own landmark study of ways to improve the pesticide program **(26),** FDA emphasized the importance of a "strong, continuously well-supported and closely coordinated analytical methods development program" (27) to the overall effectiveness of pesticide monitoring. The FDA study group high-

lighted the need for practical analytical methods, so that they could be used to handle the volume of samples necessary in a regulatory program, The group also suggested that research efforts focus on pesticides not detected by available methods, yet of concern because of toxicity and prevalence of use in agriculture (28).

The study group emphasized that for pesticides not detected by multiresidue methods" . . . research is needed on other kinds of surveillance analytical methodology to reduce the overall time and complexity of analyses,'' Among the specific research projects suggested was the study of rapid bioassay screening tests that would indicate whether further residue analysis was needed by a more complex GLC method (30).

Although FDA reprogrammed resources to focus on these objectives, budgetary constraints and agency commitments to other public health needs curtailed the reprogramming possible (31).

Several recent studies of the Federal pesticide programs have stimulated interest in pesticide analytical methods. Among the most important of these was a 1986 study of the General Accounting Office (GAO) (32), which reported that there was a significant gap between the number of pesticides that could potentially be found in food and the number that could be detected practicably with the multiresidue methods being used in the pesticide monitoring program operated by the Food and Drug Administration (FDA) (33). FDA was found to rely on five multiresidue methods. The scope of coverage of each method ranges from 24 to 123 different residues. Together, the tests are capable of detecting 203 different pesticide residues.

GAO reported that the most serious limitation of the methods was that they could detect only 40.9 percent of the estimated 496 different pesticides that potentially could be found in food (34). Furthermore, the methods could detect approximately 64 percent of the estimated 316 pesticides for which EPA has established food tolerances and are either currently registered for use on food products or persist in the environment and appear in food despite cancellation or suspension of food uses (35). Although single residue methods maybe used to detect the estimated 59.1 percent of pesticides not detected by the multiresidue methods, as a practical matter, they are not used because the inefficiency of the methods cannot be absorbed in the program given resource constraints. GAO reported that pesticides not detected by the five multiresidue methods are not routinely monitored.

Although multiresidue methods detect a substantial number of pesticides of health concern, among those not monitored because of the limitations of existing methods are 33 of 81 of those pesticides identified in FDA's Surveillance Index (36) as being of high priority for routine monitoring (37).

Recognizing the relative cost-efficiency of multiresidue over single residue methods, GAO recommended that FDA expand the number of pesticides that can be detected by multiresidue methods and, until "comprehensive capability" exists to test for most pesticides, conduct more testing of pesticides not detected by multiresidue methods (38).

The manner in which limitations in analytical capability restrict the effectiveness of monitoring was highlighted in two recent studies of the USDA National Residue Program. In a 1985 study, the National Academy of Sciences (NAS) made broad recommendations that the National Residue Program be readjusted to direct inspection to reflect assessments of relative chemical risks and to emphasize residue prevention. NAS also advised, "[t]he analytical methods used must be appropriate to the task. . . .The testing program will require substantial support for research, including the development of more accurate, more sensitive, and less expensive tests as well as tests for new hazards''.

A 1987 GAO report pointed out that, as in the FDA program, a gap existed in the USDA program between the possible residues in food and the scope of practicable testing methods. GAO recommended that USDA systematically assess the status of methods for detecting harmful chemicals in food to provide a basis for deciding on the additional research needed to develop more effective methods (40). In addition, GAO echoed the advice of NAS that greater emphasis be given to new methods development including rapid, inexpensive screening tests to detect an array of hazardous compounds (41).

The issue of pesticide analytical methods was also the focus of a 1987 report of the Congressional Research Service (CRS) (42). That report "considered whether new and relatively inexpensive rapid analytical methods based on such biological reagents as enzymes (e.g., enzyme bioassays) and antibodies (immunoassay) might have applications supplementary to those of the multiresidue methods used in the FDA monitoring program. The report concluded that enzyme bioassays may offer a relatively inexpensive screening method to use in identifying foods free of certain pesticides (negative results). CRS considered the potential applications of immunoassay to include uses as single residue

methods, or chemical class-specific screening methods. Also, immunoassay were believed to hold promise as small-scale multiresidue methods. Some of the tests being designed were considered simple enough to be performed by relatively unskilled persons in the field. However, CRS noted that a policy decision to incorporate rapid test methods into the monitoring program, particularly as screening methods, would have implications for the cost and design of the monitoring program because screening is not a regular part of the current monitoring program (43).

The critical position of analytical methods in pesticide monitoring programs has been recognized repeatedly in evaluations of Federal programs. Each study that has recognized the limited scope of the existing, practical multiresidue methods, has recommended that more research dollars be devoted to expand the scope of practicable methods. Several studies have suggested the need for less expensive and more rapid methods to cover pesticides not detected by multiresidue methods. NAS and CRS have suggested that rapid screening tests may serve a valuable function in pesticide monitoring.

Analytical Technology and Program Deslgn

In focusing on analytical methods as a technical issue bearing on the effectiveness of pesticide monitoring primarily in terms of how many pesticides can be detected, most policy analyses have treated methods as merely tools used to reach a predefined objective. This perspective obscures the fact that analytical technology serves to define program design and goals.

Analytical methods help to define program design and goals in several ways. First, the scope of pesticide coverage and limits of detection of analytical methods define what is and what is not detected in a pesticide monitoring program. Second, the complexity of the method influences who is able to do the testing and what kind of equipment and facilities are needed. Third, the level of confidence in the reliability and validity of the test results influences for what purposes they are suitable. Finally, the resources needed to run the test influence how many tests can be run within fixed resources. The cost of the method is influenced by a variety of factors including its complexity and whether it provides opportunities for economies of scale.

The development of gas chromatography (GC) during the late 1950s, has had a significant impact on the design and goals of Federal pesticide programs. GC technology is the foundation upon which modern GLC and HPLC multiresidue methods were developed. Before GC was available, analytical chemists had to use such relatively unsophisticated pesticide detection methods as calorimetry and paper chromatography, which provided limited quantitative information. The GC technology transformed analytical capabilities because it provided reliable quantitative measures of chemicals and pressed the limits of detection continually lower.

GC was first promoted as a useful method of detecting chemical subunits of fats, known as fatty acids. However, pesticide residue chemists soon adapted the technology for pesticide analysis, and eventually developed the broad scope multiresidue methods currently used in monitoring programs.

The timing of the GC discovery was significant. The period of the 1950s and early 1960s was a watershed period for both the analysis and regulation of chemicals in food (44). The 1954 Miller Amendments to the Food Drug and Cosmetic Act (FDCA) for the first time established in the law the concept of scientifically determined tolerances as a basis for restricting the sale of foods containing pesticide residues (45). The 1958 enactment of the Food Additive Amendments to the FDCA included the precedential Delaney clause (46), which reflected the view prevailing among scientists at the time, that for at least some health risks of chemicals, particularly carcinogenicity, no “safe” level of exposure could be defined. This view accentuated the role of chemical detection and fostered efforts to press the capabilities of analytical chemistry to ever lower limits of detection.

In 1962, as the Miller Amendments were being implemented and the potential of GC being explored, Rachel Carson’s influential book Silent *Spring, (47)* was published. The book highlighted concerns about the health and environmental consequences of the organic pesticides (organochlorine and organophosphorus compounds) developed during World War II. The charges the book made about the accumulation and persistence of such pesticides as dichloro-diphenyl-trichloro-ethane (DDT) in the fat component of foods, including milk, gained the attention of regulators at FDA (48). As regulators made these organic pesticides a monitoring priority, they found GC to be particularly well-suited to detect these classes of chemicals.

The characteristics of GC worked to help shape the design of pesticide monitoring programs. For example, the methods had to be performed in the laboratory, by highly skilled residue chemists. These features meant that food samples collected had to

be sent to equipped laboratories, and results were not available for several days. The complexity of the system caused the analysis of each sample to be relatively costly.

Although improved efficiency was gained through the development of GLC and HPLC multiresidue methods, these are still laboratory methods that must be operated by highly skilled staffs. The limited scope of multiresidue methods also has served to define goals of pesticide monitoring. Organic pesticides have remained a priority in the monitoring of food residues. However, because of the health and environmental dangers presented by the early organic pesticides, many have been phased out of use, Newer pesticides are of more diverse classes and have been designed to degrade more quickly into breakdown products to avoid environmental persistence and accumulation. The changes in pesticide formulation have increased the number and chemical class diversity of compounds to be analyzed in food, significantly increasing the scientific task of monitoring them. Many of these chemicals cannot be detected by the practical multiresidue methods being used in monitoring programs (49). Residue problems have gradually shifted outside the direct focus of multiresidue methods. The very methods that once provided the means by which regulators could rise to meet the challenge of monitoring pesticides in food now limit their ability to do *so*.

The limitations of the methods have, in turn, served to help shape the goals of monitoring programs. As noted above, because of resource constraints that prevent the use of single residue methods, both FDA and USDA have focused their monitoring efforts on those pesticides detected by multiresidue methods. USDA in particular has articulated as a premise of their monitoring programs that a pesticide will not be selected for monitoring in the National Residue Program unless a practical analytical method exists to detect it (50). Multiresidue methods have thus influenced both the design and the goal of the monitoring program. In this sense, the analytical technology of multiresidue methods has ascended beyond the role of a tool to accomplish a policy objective to one that helps define the policy objective.

Of course, analytical technology is not the only variable that influences the design of monitoring programs. The significant differences in the legislative mandates of FDA and USDA are largely responsible for the enforcement focus of FDA's commodity sampling program as distinguished from the certification focus of USDA's monitoring program, as a component of the meat and poultry inspection system (although both programs serve enforcement purposes). In addition, the legislative mandate of an agency may also influence the technologies it adopts into its program by defining certain problems as within the jurisdiction of that agency. For instance, USDA has in recent years incorporated rapid screening tests for certain animal drugs, e.g., the "sulfa-on-site" test into the meat inspection program, These tests are heIpful to USDA in achieving its mandate of not only enforcing drug residue limits, in which case they must be confirmed with a more sophisticated analytical method, but also in preventing the entry into commerce of food containing illegal residues by obtaining test results quickly. FDA may have a similar interest in on-site test results if the mandate of that agency were expanded to require FDA to prevent food containing illegal residues from entering into commerce.

While the studies discussed above (excepting the CRS report) have focused on a range of problems afflicting Federal pesticide monitoring programs, the recommendations regarding methods research have arisen from concern about the gap in pesticides potentially present in food and the coverage of multiresidue methods. Some studies have made vague reference to the costly nature of pesticide analyses (e.g., GAO Livestock Report; NAS Meat Inspection Report) and have implied a need for less expensive and more rapid test methods. However, the program studies have focused little attention on the possible relationship between other fundamental problems in pesticide monitoring programs and the currently used battery of analytical methods.

Some additional problems of Federal pesticide monitoring programs that have been recently documented include the following list.

1. The public is exposed to foods sampled and found to contain violative pesticide residues because the food passes into commerce while the samples are shipped to central laboratories, analyzed, and results reported (51, 52, 53),
2. Time delays and sample backlogs in FDA laboratories expand the time it takes to obtain analytical findings (54).
3. Because of limited program resources, a relatively small portion of the domestic food supply (no estimate available) and approximately 1 percent of imported food shipments are analyzed by FDA (55, 56),

Because most studies of Federal pesticide programs have not focused directly on the possible linkage between analytical methods and monitoring program design, the possible role of analytical meth-

ods in addressing the above problems has not been highlighted.

Conclusion

As the future of pesticide analytical methods development is charted, there is a danger in focusing only on strategies to fill the gap between potential residues in food and residues detectable by multiresidue methods. The so-called gap has been defined using current analytical technology as a reference point, rather than program needs generally. To focus on the gap alone propagates the limitations of the current program and the technology that has helped to define it to the arena of research objectives. Such a focus fails to consider the opportunities analytical technology may offer to improve pesticide programs in more fundamental, structural ways. Rather, it may inform judgments about the direction of future methods research to consider what the future of pesticide monitoring programs should be. The span between that goal and the status of current pesticide programs is a truer estimate of the "gap" that represents current program needs.

Examining the ability of current analytical technology to meet those needs within realistic estimates of program resources will help to suggest an appropriate research strategy.

Questions to Consider as a Research Strategy is Defined

As research priorities are established for pesticide analytical methods development, it maybe useful to consider the following questions.

Studies of Federal pesticide monitoring programs not only have revealed a gap in detectable pesticides but also some structural problems that have been generated by a system that requires food samples to be sent to centralized laboratories for analysis. There, even a relatively small number of pesticide samples can add to and become enmeshed in laboratory backlog and delay. Can analytical methods be designed so that they can be performed in the field?

The studies also reveal a system in which many of even those relatively few foods that are sampled and found to be violative, are nevertheless consumed because there is no rapid way to identify foods containing illegal pesticide residues. Can analytical methods be developed to provide on-site results?

Reports show a system (FDA) in which resources run out after only spot-check sampling is done. Can inexpensive (e.g., screening) methods be developed so that more sampling and analysis can be performed assuming fixed resources? Can more expensive laboratory methods be reserved for confirming results of screening tests?

In addition to considering how methods development might address problems that have been identified inside existing monitoring programs, it also may be instructive to consider the assumptions of the existing Federal program.

Analytical methods have become a limiting factor in the enforcement of laws concerning pesticide residues in food. The premise of the pesticide registration system is that registrants will supply to regulators the analytical means to enforce a condition of registration, i.e., acceptable food residues. As discussed above, the current method requirement imposed on registrants has not fulfilled this objective. This fact spawns several policy questions regarding analytical methods development,

Would a requirement that the method submitted by a registrant be useful to regulators be workable? Would a required contribution by registrants to a Federal research fund for the development of practicable methods satisfy the need for such methods?

What resource commitment to analytical methods development would be necessary to keep pace with the advent of new pesticides?

Does the premise of the method development requirement for registrants expect more from technology than can feasibly be delivered?

Even if the scope of analytical methods were broad enough to detect all possible residues, how meaningful would it be if scaled-up affordable sampling with rapid analytical results were unavailable? From an ideal public health perspective, what should be the food sampling goal? What level of resources would be needed to achieve this goal, given current analytical technology? What would be the impact on cost if inexpensive screening tests could be used in the field to detect residues and laboratory methods reserved for confirmation? Can an ideal level of sampling be achieved assuming the use of screening tests and fixed Federal resources?

If industry (e.g., the fresh produce industry, the food processing industry) obtained private certification of the conformity of their products with pesticide requirements through the use of private analytical testing, what impact would it have on the goals for analytical methods development?

What are the regulatory implications of developing inexpensive, rapid, and simple pesticide analytical methods that may be used by members of the public?

Do the limitations of pesticide monitoring programs and methods suggest a need for enforcement policies that focus more attention on residue control than on residue detection?

References

1. Whitaker, A. H., "A History of Federal Pesticide Regulation in the United States to 1947," p. 49-50 (dissertation, Emory University, 1979). (Dr. Haywood was also head of the insecticide and fungicide section of the Association of Analytical Chemists.)
2. See, e.g., Montgomery, A. America's Pesticide-Permeated Food, Nutrition Action Health Letter, v. 14, June 1987. p. 5 ("Not all pesticides are unsafe. But some can cause cancer, birth defects, heritable genetic mutations, and nerve damage. Clearly, we need to spend more money and devote more resources to improving our testing procedures for detecting hazardous residues.")
3. Mr. Pasquale Lombardo, Division of Contaminants Chemistry, Food and Drug Administration, priviate interview, Wash., D. C., Jan. 26, 1988.
4. Both GLC and HPLC operate on a similar basic principle. A chemical mixture (food sample extract) is injected into the portal of specialized chromatographic equipment that contains a "separating column. " The separating column permits pesticides to pass through it at different rates based on differences in their physical and chemical properties. As each chemical reaches the end of the column, each is in turn sensed by a detector, and a printer displays the chemical as a "peak" on a chromatographic print-out. Chemicals can be identified by comparing the time interval between sample injection and the appearance of a peak with the intervals of known standard chemicals. The amount of a chemical is determined by measuring the size of a peak.
5. The pesticide analytical methods in use in monitoring programs are detailed in U.S. Library of Congress, Congressional Research Service. "Pesticide monitoring Program: Developing New Methods to Detect Pesticide Residues in Food," by Sarah E. Taylor, Apr. 24, 1987.
6. The most costly part of pesticide analysis by GLC or HPLC, whether a single or mult-residue method, is in the perparation of the sample for injection into the chromatography. Consequently, because multi- and single residues methods require similar sample preparation, and a multiresidue method provides methods information on many pesticides, multiresidue methods are considered more resource efficient that single residue methods. However, the cost of a multiresidue analysis costs up to $200 per sample of fruit, vegetable or grain, while fatty foods, including milk and fatty fish cost approximately $320 per sample analysis. Shroff, A. Regulatory Affairs Division, Food and Drug Administration, personal communication, Apr. 16, 1987.
7. 7 U.S.C. Sec. 135a(c)(5)(d). "Unreasonable adverse effects on the environment" is defined as, "any unreasonable risk to men or the environment, taking into account the economic, social, and environmental costs and benefits of the use of any pesticide. " 7 U.S.C. Sec. 136(bb).
8. EPA has failed to company fully with the above described requirements of FIFRA. See, for example, General Accounting Office. Pesticides: EPA's Formidable Task to Assess and Regulate Their Risk. GAO/RCED-86-125. Washington, DC, 1986. p. 92-100. Registration processes are discusses in U.S. Library of Congress, Congressional Research Service, "Pesticide Regulation: Legislative Debate About FIFRA in 1986, " by Aidala, J. May 11, 1987. p. 5. See also Conner, J.D. et. al. Pesticide *Regulation Handbook*. (Washington, DC, 1987.)
9. 40 C,F.F. 158.125.
10. The EPA validation should be distinguished from the formal validation required by the Association of Official Analytical Chemists (AOAC) for a method to gain official status (the "gold standard" for analytical methods). AOAC requires that at least six laboratories validate a method. The purpose of validation by AOAC criteria is to assure that the method is capable of performing as intended and that the results of an analysis are of acceptable accuracy and precision. See Hill, K.R. and P.E. Corneliussen, "Validation of Official Methods, " *Analytical Methods for pesticides and Plant Growth Regulators, v.* XV, Academic Press, New York, 1986. However, the AOAC validation method takes several years and is so resource-intensive, requiring the attention of the limited number of expert residue chemists in the U. S., that many EPA officials consider it an unworkable systems for their regulatory purposes. Dr. Francis Griffith, Residue Chemistry Branch, Environmental Protection Agency, personal communication, Feb. 23, 1988. (557-0826).
11. Discussed in U.S. Library of Congress, Congres-

sional Research Service. "Pesticide Monitoring Program, Developing New Methods to Detect Pesticide Residues in Food," by S.E. Taylor, Apr. 24, 1987.
12. See 21 U.S.C. sec. 342 (a)(2)(C), 374(c)-(d).
13. Reed, D., P. Lombardo, J. Wessel, L.A. Burke and B. McMahon. Division of Contaminants Chemistry. The FDA Pesticide Monitoring Program, *J. Assoc. Off. Anal. Chem., v.* 70, no. 3, 1987. p. 59.
14. Duggan, R.E. and H.R. Cook, "National Food and Feed Monitoring Program," pesticides *Monitoring Journal, 5:37,* June 1971.
15! Food and Drug Administration, Compliance Program Guidance Manual, Pesticides and Industrial Chemicals in Domestic Foods (FY88/89), Oct. 1, 1987.
16. Ibid.
17. CDP Associates. Assessment of FDA's Total Diet Study, Contract HHS-100-82-0076. 1982.
18. Poultry Products Inspection Act, 21 U.S.C. sec. 455(s); Meat Inspection Act, 21 U.S. C. sec. 603.
19. Poultry Products Inspection Act, 21 U.S.C. sec. 455(b); Meat Inspection, 21 U.S.C. sec. 604.
20. See Poultry Products Inspection Act, 21 U.S.C. sec. 455(b) (The Secretary, whenever processing operations are being conducted, shall cause to made by inpsectors, post mortem inspections of the carcass of ***each*** bird processes.") Federal Meat Inspection Act, 21 U.S.C. sec. 604 ("[A]ll such animals found to be not adulterated shall be marked, stamped, tagged or labeled as 'inspected and passed'. . . .").
21, Federal Meat Inspection Act, 21 U.S.C. sec. 603 ("For the purpose of preventing the use in commerce of meat and meat food products which are adulterated. . . .") Poultry Products Inspection Act, 21 U.S.C, sec. 455(a) ("For the purpose of preventing the entry into or flow or movement in commerce of, or the burdening of commerce by, and poultry product which is . . . adulterated.)
22. 1987 National Residue Plan, p. 3. A.3.
23. Food Safety and Inspection Service, United States Department of Agriculture. Compound Evaluation and Analytical Capability National Residue Program Plan 1987, Jan. 1987, p. 2.1.
24. 1987 National Residue Plan, p. 5.1.-5.2.
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27, 1979 FDA Study Group Report, p. 58-59.
28. 1979 Study Group Report, p. 58-59. (FDA developed the surveillance index system as a response to this identified goal.)
29. 1979 FDA Study Group Report, p. 57.
30. Ibid.
31, See, e.g., U.S. Department of Health and Human Services, Food and Drug Administration. Public Health Service. Report on Proposals to Improve Control of Pesticide Residues in Imported Foods, Mar. 10, 1982. p. 9.
32. U.S. General Accounting Office, "Pesticides: Need to Enhance FDA's Ability to Protect the Public From Illegal Residues, " Report to Congressional Requesters by the Comptroller General of the United States, GAO/RCED-87-7. Washington, DC, 1986, p. 31-33. [GAO Domestic Food Report].
33. While the GAO report focused on the FDA monitoring program, its conclusions regarding the adequacy of pesticide analytical methods also implicates the methods used in the monitoring program of the USDA because they are similar in nature to those of FDA's program.
34. FDA has disputed the relevance of comparing the scope of coverage of the five multiresidue methods to the reference point of 496 pesticides. FDA contends that the number includes pesticides that are not actually in use because registration is pending or has been cancelled, and it includes pesticides for which all food uses have been cancelled. They agency has implied that 230 may be a more appropriate reference point. Bowen, Otis R., Secretary of Health and Human Service. Letter to Charles Bowsher, Comptroller General of the United States, Mar. 20, 1987.
35. Domestic Food Report, p, 31-34.
36, The surveillance index is a system developed by FDA in 1979 to classify pesticides according to potential health hazard, The classification scheme was developed to help the agency put in priority its monitoring of residues not detected by multiresidue methods and to direct research efforts to develop new methods. Pesticide chemicals undergoing special review at EPA because of special health or environmental concerns have been given priority in being clas-

sified in the surveillance index. The index lists pesticides in Classes I, II, and III. with Class I being of highests priority based upon the toxicity, use pattern (poundage and crop), potential for food residues and persistence in the environment. See U.S. Dept. of Health and Human Services. Food and Drug Administration. FDA Monitoring programs for Pesticide and Industrial Chemical Residues in Food; Study Group on FDA Residue Programs, June 1979, p. 57.

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44. U.S. Library of Congress, Congressional Research Service. Food Safety Policy: Selected Scientific and Regulatory Issues. IB83158 by S. E. Taylor and D.V. Porter (regularly updated) p. 2-5.

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46. The so-called "Delaney," or "anti-cancer" clause was incorporated into the FDCA through the Food Additives Amendments of 1958, the Color Additive Amendments of 1960, and the Animal Drug Amendments of 1968, The essence of each clause provides that a substance shall not be deemed safe and shall not be deemed safe and shall not be approved if it is found to induce cancer when ingested by man or animal, or if it is found after tests that are appropriate for the evaluation of the safety of the substance to induce cancer in man or animal. The standard of safety embodied in the anti-cancer clause is sometimes termed as "zero-risk" or "zero-tolerance" standard. See, U.S. Library of Congress, Congressional Research Service. Food and Color Additives: "De Minimis " and "Delaney," by Sarah E. Taylor. Aug. 5, 1987. The Delaney Clause applies to pesticide residues in foods when residues concentrate during food processing to levels exceeding the tolerance approved on the food commodity. The implications of the Delaney Clause to perticide chemicals was considered in Board on Agriculture, National Research Council. Regulating Pesticides in Food: The Delaney Paradox. Wash., D. C., 1987.

47, Carson, R. *Silent Spring.* (Boston: Houghton Mifflin Co., *1962)* (The book was first published as a series of articles appearing in The New Yorker.)

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49. Discussed in U.S. Library of Congress, Congressional Research Service. Pesticide Monitoring Program: Developing New Methods to Detect Pesticide Residues in Food, by Sarah Taylor, Apr. **24, 1987. p. 16.**

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