Chapter 6

Pesticide Analytical Methods

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INTRODUCTION

The regulatory responsibilities of FDA and FSIS influence the type of methods these agencies use to monitor pesticide residues in food. Methods must provide results that are cost-effective, timely, reliable, and verifiable. The agencies also need methods that can identify as many pesticides as possible in a range of food commodities because they are charged with monitoring all foods for all pesticides. In addition, these methods should use instruments, associated hardware, and reagents that are readily available in the regulatory laboratory or are commercially available and inexpensive.

Regulatory agencies need methods that can give reliable results rapidly—within 24 hours—if violative products are to be kept from reaching the market, Neither FDA nor FSIS has the authority to detain commodities routinely monitored for pesticide residues, but both agencies can detain imports suspected of illegal residues and FSIS can detain suspected domestic meat until the results of analytical testing are received.

Methods must also be able to detect pesticides at, and often below, tolerance levels. They must endure interfering compounds such as other pesticides, drugs, and naturally occurring chemicals and be insensitive to such environmental variations as humidity, temperature, and solvent purity. Chemists with varying levels of training and expertise must be able to use them. There also should be some other means of confirming that a method is accurate.

EPA provides guidance for methods as part of the tolerance-setting process that involves many of these points. According to its Subdivision O Guidelines, submitted methods should 1) take 24 hours, 2) require readily available equipment or reagents, 3) identify the residue in the presence of other residues, and 4) detect the residue at or below the tolerance. EPA's guidelines do not include an emphasis on multiresidue methods (MRMs) or the submission of a confirmatory method.

FSIS also has criteria for methods suitable for its regulatory use: 1) methods must take no more than 2 to 4 hours of analytical time per sample, 2) they must have a minimum proficiency level at or below the tolerance, 3) there must be a quality assurance plan developed for the method, and 4) the method must be successfully validated through an interlaboratory study (6).

FDA does not have a formal listing of guidelines for its methods, but it uses many of the same criteria in evaluating them (13).

TYPES OF METHODS

Muitiresidue Methods (MRMs)

MRMs come closest to meeting the method needs of the regulatory agencies. They are designed to identify and quantify a number of pesticides and their toxicologically significant metabolizes simultaneously in a range of foods. Their usefulness is based on a combination of three factors:

- determining abroad spectrum of pesticides and their toxicologically significant metabolizes in an array of food,
- being sensitive, precise, and accurate enough to be useful for regulatory purposes and acceptable to the scientific community,
- being economical or at least affordable for those laboratories using them.

No single method can optimize each of these three factors; as a result, the MRMs used are a compromise of these elements (see ref. 20 for further discussion of this point).

MRMs have two other advantages. An MRM may be able to detect, but not measure, a particular pesticide or metabolize in food. The MRM, in such cases, signals the presence of the compound, which can then be analyzed with a single residue method (SRM) (16).

Second, MRMs record the presence of unidentified chemicals, known as an unidentified analytical response (UAR). Once observed, the chemical's identity can be determined by matching its result to a known chemical with a similar chromatographic result or by other techniques such as mass spectrometry. In this way, MRMs can identify the presence of possibly hazardous chemicals that were not expected to be residues in food and might have been overlooked. For example, polychlorinated biphenyls (PCBs) were discovered in meat and animal feed after appearing as UARs on the chromatograms of samples analyzed for the chlorinated hydrocarbon pesticides.

MRMs contain the steps of preparation, extraction, cleanup, chromatographic separation, and detection (as described in chapter 3). All MRMs used today in the United States are based upon either gas chromatography (GC) or high performance liquid chromatography (HPLC) as the determinative step. of the 10 MRMs routinely used by FDA and USDA, 8 rely on GC as the determinative step (see table 6-l). Thin layer chromatography is still used by several agencies in Europe, but it has lost favor in this country because of its semiquantitative nature (1)$_0$

The FDA and USDA have geared much of their pesticide methods research to developing

Table 6-1.–Multiresidue Methods Routinely Used by FDA, USDA, and CDFA

Agency	Method[1]	Food type analyzed	Pesticide groups detected
FDA	GC-multiple detectors (Luke method)	nonfatty	organochlorines organophosphates organonitrogens
	GC-multiple detectors (Mills method)	fatty	organochlorines organophosphates
	GC-multiple detectors (MOG method)	nonfatty	organochlorines organophosphates
	GC-multiple detectors (Storherr method)	nonfatty	organophosphates
	HPLC-fluorescence (Krause method)	non fatty	N-methyl carbamates
USDA-FSIS	GC-ECD	fat	chlorinated hydrocarbons
	GC-ECD (western method)	liver and fat	chlorinated organophosphates
	GC-NPD (eastern method)	liver	organophosphates
	HPLC-fluorescence	liver	carbamates
USDA-AMS[2]	GC-ECD	fatty (raw egg products)	chlorinated hydrocarbons
CDFA[3]	GC-ECD	nonfatty	organochlorines
	GC-NPD or FPD	non fatty	organophosphates
	HPLC-fluorescence	nonfatty	carbamates

l Methods are identified by the combination of the instruments used for chromatographic Separation and detection. Abbreviations for the these instruments are as follows:
GC: gas chromatography
HPLC: high performance liquid chromatography
ECD: electron capture detector
NPD: nitrogen-phosphorus detector
FPD: flame photometric detector
In some cases, a method may also have a name and these are noted in parentheses.
2AMS: The Agricultural Marketing Service
3CDFA: The California Department of Food and Agriculture

SOURCE Office of Technology Assessment, 1988

MRMs over the years. FDA's MRMs appear in Volume I of the Pesticide Analytical Manual (PAM I), and they are considered of high quality and capable of providing data that will withstand challenge during court litigation (for details on the development of FDA's MRMs see ref. 19).

The primary weakness of existing MRMs is that they cannot detect every pesticide. For example, of the 316 pesticides with tolerances, only 163 of them could be analyzed with FDA's five routinely used MRMs. A second weakness is that some MRMs require a great deal of time to perform, thereby reducing the number of samples analyzed and the speed of analysis. For example, certain foods, such as those with high concentrations of fats and oils, are difficult to analyze in a timely manner.

Single Residue Methods (SRMs)

A large number of methods exist that are designed to analyze a single pesticide and, in many instances, its metabolizes or degradation products. Although less efficient than MRMs, the use of SRMs is necessary to monitor those pesticides, including a number of high health hazard ones, that cannot be detected by MRMs.

SRMs depend on a number of different techniques and vary widely in terms of reliability, efficiency, throughput (samples per day), degree of validation, and practicality for regulatory use. SRMs are primarily developed by the private sector for submission to EPA as part of the tolerance-setting process, Therefore, a method exists for every pesticide with a tolerance, although methods for some pesticides (primarily the older ones) may not be effective.

Most SRMs, like MRMs, are based on GC using the full array of element-specific detectors. Volume 11 of the Pesticide *Analytical Manual* (PAM II) consists solely of SRMs, both those that have undergone EPA review and possibly EPA *laboratory evaluation,* and those that have appeared in a peer-reviewed journal of high quality (these methods are normally similar to ones approved by EPA but adapted for other commodities) (10). In PAM II, those methods reviewed by EPA are listed with Roman numerals and those not reviewed are lettered.

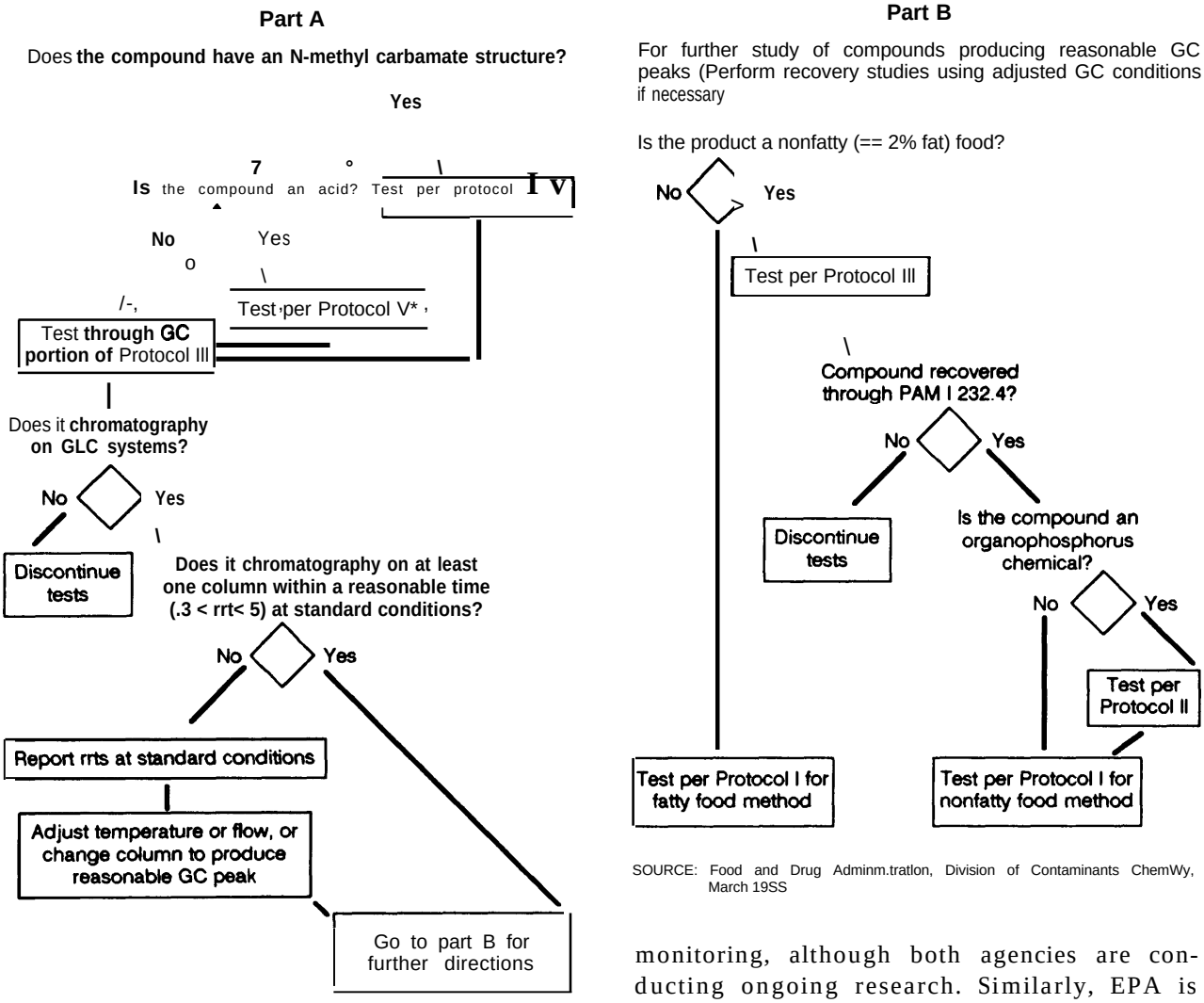
SRMs are not considered adequate for routine monitoring by the regulatory agencies, although FDA uses them. To monitor one pesticide with an SRM is considered inefficient when an MRM can measure many pesticides using the same resources. In addition, SRMs vary widely even for chemicals of the same class, so a laboratory needs a wide array of glassware, evaporative devices, chromatography, and detectors to use the SRMs available. There is also dissatisfaction with the performance of some SRMs (24). Some chemists feel they are better served sometimes by 1) going to the scientific literature for methods, 2) borrowing methods from State laboratories, or 3) going directly to the registrant for the newest method. Others feel it is better to develop their own methods or adapt existing methods developed for pesticides of similar structure. SRMs are also not as capable of identifying UARs as MRMs.

In an attempt to reduce the need to use SRMs, EPA now requires that all pesticides requiring a new tolerance be evaluated to see if they can be detected by FDA and USDA MRMs. Only FDA has developed the testing protocols to support such testing. FDA has also devised a "decision tree, " showing the order in which the FDA MRMs should be tested using the new pesticide to minimize research time (figure 6-1), The results confirm or deny whether that particular pesticide can be recovered through one of the MRMs. It has not yet been decided whether the EPA will still require development and submission of an SRM if the pesticide can be analyzed by an MRM (23).

Semiquantitative and Qualitative Methods

Semiquantitative and qualitative methods range widely in their ability to quantify the chemical present in a sample. Semiquantitative methods indicate the range of pesticide residue concentration in a sample; qualitative methods show whether or not a particular pesticide exists above some predetermined concentra-

Figure 601.– Decision Tree for Testing Pesticides Through FDA Multiresidue Methods



SOURCE: Food and Drug Adminm.tratlon, Division of Contaminants ChemWy, March 19SS

tion. In this way they differ from the majority of conventional MRMs and SRMs, which fully quantify the amount of pesticide in a sample. (See also box 6-A.)

The benefits of these methods maybe their low cost, speed, or ease of use. These benefits can contribute to an increase in the number of samples that could be analyzed, although tradeoffs may exist in the number of pesticides that can be analyzed. Currently, neither FDA nor FSIS is using these methods for pesticide monitoring, although both agencies are conducting ongoing research. Similarly, EPA is conducting research on the use of these methods on nonfood matrices. Given that the majority of these methods have been developed for pesticides in nonfood matrices, significant adaptation research may be necessary for their use on foods.

Semiquantitative and qualitative methods make use of such technologies as thin layer chromatography (TLC), enzyme inhibition, and immunoassay, These three technologies can be moved from the laboratory into the field without losing their ability to detect pesticides. And because sophisticated instrumentation is not required, they are relatively inexpensive compared to quantitative methods.

Box 6-A.—The Concepts of Screening and Rapid Testing

Screening and rapid testing are two terms commonly used when discussing improvements in methods that will determine in a short time whether or not a pesticide or group of pesticides is present in food. The use of these terms often is confusing because they have different meanings to different people, and the confusion can be compounded when the two are used together, i.e., "rapid screen. "

The term screening, in general, can be applied to two different types of methods. The distinction between the two methods depends on what is being screened--either a large number of pesticides or a large number of samples.

First, screening can mean a method that can detect a large number of pesticides in a sample, that is, the method screens for pesticides. The multiresidue methods (MRMs) used by FDA and USDA are screening methods under this definition. These "MRM screens" analyze for a large number of pesticides at one time and therefore are the most cost-efficient approach when data on pesticide application are lacking or when a number of pesticides are known to have been used. Also, they can uncover the presence of residues not expected to be in the food. This type of screening method may also be labeled "rapid test" for a number of reasons. These MRMs are faster than single residue methods (SRMs) because they can analyze for more pesticides in a given time period. Some MRMs are considered rapid because they are relatively faster than other MRMs. For example, the Luke MRM used at FDA's Los Angeles laboratory can detect some 200 pesticides and metabolizes in *30* samples each day and therefore is considered a rapid screen.

Second, screening is also used to mean a method that can analyze a large number of samples often for one pesticide or a small group of pesticides in a relatively short period of time, that is, the method screens samples. This type of screening method supports efficient identification of violative samples when a known pesticide/commodity problem exists or where a pesticide/commodity combination is known to have a low violation rate. When a method is used in this manner, the speed of analysis in terms of the number of samples that can be analyzed per unit time is emphasized and, in this context, it would then be considered a "rapid screen. " Application of such a rapid screen to a large number of food samples thought to contain violative samples would allow nonviolative food to reach the market more quickly and reduce the number of samples that need to be analyzed by more time-consuming and expensive conventional methods. Those samples with positive results would be analyzed by a conventional method.

This type of screening uses technologies that are less expensive and more rapid to use than conventional methods, such as thin layer chromatography, immunoassay, and enzyme inhibition. The lower cost of these techniques stems from their relative speed of analysis and use of less expensive and more simple equipment. These techniques often are called "rapid tests, " because of their speed. ' The tradeoffs of using these techniques are noted in chapters 4 and 6. Neither FDA nor FSIS uses such screening methods for pesticide monitoring in food, although FSIS is actively researching its use.

IFor more detail on this type of screening, see Ellis 1988 in appendix B.

A drawback of semiquantitative methods is that they do not provide the degree of accuracy necessary for enforcement action, e.g., for use in a court of law. Violations found by a semiquantitative method would have to be verified by a quantitative analytical method—or maybe two. And with the possible exception of thin layer chromatography, none of the semiquantitative techniques provide data that can be used to address UARs.

TLC is used sometimes in Europe for regulatory purposes (see ref. *1* for a bibliography of TLC applications). Thin layer chromatography-based methods have the advantage of an ability to analyze several pesticides simultaneously. As many as 20 pesticides can be tested at once if chromatographic conditions are properly chosen. TLC has been used successfully by FSIS to analyze the drug sulfamethazine in animal tissues; field use by inspectors relatively un-

skilled in analytical chemistry was also successful. An attempt to use TLC for analysis of chlorinated hydrocarbons in animal tissues has been unsuccessful, however, because of problems in achieving the desired sensitivity and an overly complicated sample extraction procedure for nonlaboratory use (3). Several potentially useful TLC methods are described in chapter 4 of PAM I. They are carryovers from early work at FDA and USDA and require sample cleanup by conventional Florisil or alumina columns. Both FDA and FSIS have ongoing research on TLC applications.

Enzyme inhibition-based color reactions are a means of making the spots and bands of pesticide residues on thin layer chromatographic plates visible in order to measure the pesticide residue either visually or with instruments. Such techniques have been developed for cholinesterase-inhibiting insecticides and photosynthesis-inhibiting herbicides.

In addition to working with TLC, enzyme inhibition may also be used for a "stand alone" test kit. Currently, one such qualitative kit is commercially available for the detection of cholinesterase-inhibiting insecticides (organophosphates and carbamates). The kit is inexpensive and can detect a large number of pesticides in concentrations of parts per million. The kit has been privately used for analyzing food extracts and for analyzing water used to wash skins of fruits and vegetables for pesticide residues (9). This type of kit is not specific unless information about the history of the sample is available. For example, it will give a positive response for a large number of compounds without being able to identify the specific compounds. This type of kit also may suffer from interferences produced by extraction solvents.

Immunoassay have been developed for semiquantitative and qualitative tests, although much immunoassay research has focused on quantitative assays (17). If needed, quantitative immunoassay based on color reactions could be adapted to semiquantitative assays with visual interpretation of the results. Such tests could then be more easily used outside the laboratory. Several qualitative tests are commercially available. Immunoassay-based methods have the advantage of speed since many tests can be performed simultaneously and some analyses take less than a few minutes if extractions are not necessary. They also have the advantage of being extremely sensitive, detecting some pesticides far below their tolerances, and they are usually specific, although sometimes cross-reactions occur that give false positives (17). A drawback is that immunoassay provide analyses for individual or small groups of pesticides.

Current Needs in Methods Development

improving Existing Analytical Methods

Considerable time and resources have already been invested in developing analytical methods. Rather than devoting resources exclusively to developing new analytical methods, existing methods also can be improved through changes in technologies to reduce analysis time and to increase the number of pesticides that can be analyzed. For instance, improvements could be made in the following ways:

1. simplifying cleanup
2. improving extractions with supercritical fluid extraction (SFE)
3. miniaturization with solid phase extraction (SPE)
4. capillary columns
5. increased use of high performance liquid chromatography [HPLC)
6. use of immunoassay as a detection technique
7. increased automation
8. mass selective detection (MSD)

(1)Simplifying cleanup. Simplifying a method by eliminating sample manipulation in the cleanup step would shorten analysis time, eliminate opportunities for pesticide loss, reduce solvent and consumables usage, and reduce overall analytical costs.

Two FDA MRMs and the three used by the California Department of Food and Agriculture (CDFA) use food extracts that have not undergone any type of sample cleanup. Approximately 80 percent of the food samples analyzed

by FDA are examined with the Luke method, and CDFA conducts more analyses with its own three methods than any other State. The trend toward less extensive sample cleanup in these methods has been a result of improving capabilities of element-specific detectors (NPD, FDP, ECD, and Hall).

As sample cleanup is reduced or even eliminated, increased stress is placed on the determinative step. As a result, the chromatographic separation begins to suffer from the presence of large amounts of sample coextractives. Such coextractives may produce a loss of resolution of pesticides in the sample, a loss of pesticide on the chromatographic column, and fouling of the detector. For these reasons, the chemist must weigh the need to shorten analysis time with the instrumentation "down time, " that is, time required to clean, repair, and regenerate the instrument to its original operating specifications.

However, because reduction of cleanup steps pays high returns in time saved for a typical analysis by reducing analytical costs and increasing sample throughput, efforts should be made to explore it fully.

(z) *Improving extractions with SFE.* As more efficient hardware (particularly miniaturized valves, pumps, ovens, and refrigeration devices) becomes available for SFE, the technique may become more practical for extracting pesticides in foods, possibly in the field, e.g., the slaughterhouse. SFE can be coupled to capillary column gas chromatography or supercritical fluid chromatography (see ch. 3) to provide an on-line extraction/determination, although validated methods have yet to be developed using this approach. Since extraction time can be shortened, then selectivity can be gained by leaving potential interferences behind and thermally unstable chemicals can be dealt with. The technique has become attractive for consideration in the future. It may ultimately shorten analysis time while expanding the array of pesticides and metabolizes that can be extracted,

Carbon dioxide, a relatively inert gas, has been used as an SF for the extraction of many types of organic compounds. Straight chain hydrocarbons have been selectively extracted from other chemicals present (8). More than 85 percent of such hydrocarbons were extracted in 5 minutes. Extractions can be even more efficient and faster as well as applicable to more polar chemicals by modifying the carbon dioxide with small amounts of polar organics.

(3) *Miniaturization with SPE.* An opportunity to reduce analysis time, solvent consumption, and overall costs might be through use of miniaturization **(20).** The philosophy of present miniaturization focuses on the use of small solid phase extractions (SPEs). These cartridges are now commercially available, are inexpensive ($2 to $3 each), and are disposable. Use of SPEs has not been demonstrated yet for MRMs, although they have been successfully used in SRMs (for such pesticides as aldicarb and paraquat). Problems associated with larger adsorption columns, such as the Florisil columns, may still exist with SPEs in MRMs. For example, pesticides may not exit the SPE in distinct groups but may instead be scattered among several fractions. In addition, there still may be a problem of pesticide loss on these extraction columns, depending upon the elution conditions and the pesticide under analysis. Associated with miniaturization are the problems of taking a truly representative sample, so that analytical results will reflect the average concentration of the pesticide in the food (2).

Miniaturization of MRMs might assist in adapting robotics to MRMs (see ch. 5). Present robotic modules handle samples of ***1*** to 10 grams better than heavier ones of **25** to ***100*** grams, like those used for conventional MRMs. Similarly, robots dispense and manipulate 5 to 25 milliliters of solvents more easily than the 100 to 250 milliliters typically used in conventional MRMs (12).

Other spinoffs of miniaturization might be that sample preparation could be done in the field (20), as is now commonly done for water samples, Extending this approach to milk, juices, and other fluid foods might be feasible. If some sort of solid sample extraction could

be devised in the field, this approach could be extended to other foods.

In addition to miniaturization, the further development of SPE extractions will reduce or eliminate the need to use hazardous solvents. present use of such solvents creates a health hazard for the chemist and produces a dilemma for their disposal.

Another spinoff of SPEs is that once the pesticide is on them, they can be stored more easily. The requirement for refrigerating potentially explosive solvents is removed, making the storage more safe and economical.

(4) *Capillary columns.* with the exception of cost, essentially all objections to capillary column chromatography for analysis of pesticides in foods have been removed. Fifteen-meter-long capillary columns of the wide bore variety cost about $250 compared with about $80 for a packed column, a small difference considering the potential savings in analysis time (see table 3-2), the availability of guard columns, and the reusability of columns following a solvent wash (7).

More important, capillary columns usually provide lower limits of detection because the chromatographic peaks are sharper. Lowering the limit of detection also means that smaller food samples (100 grams or less) can be analyzed. Once smaller food samples are used, then analysis becomes more adaptable to robotics.

For capillary columns to become accepted by many regulatory agencies, additional examination and standardization of columns will-be needed so that the relative retention time concept of identifying pesticides can be extended to them. Relative retention time will change when compared to packed columns, but selection and detailed specifications of capillary columns and resources to characterize them fully should relieve this problem. However, costs in terms of equipment and time could be great and would have to be considered in light of existing monitoring activities.

(5) *Increased use of HPLC.* Since pesticide metabolizes usually are more polar than their parent molecule and since HPLC is more adept at dealing with polar compounds than GC, it seems that HPLC has potential for analyzing both the parent and metabolizes simultaneously. Recent examples include HPLC methods used for benomyl (fungicide), glyphosate (herbicide), and metabolizes of fenvalerate (insecticide). All of the sulfonyl urea herbicides are analyzed by HPLC with the photoconductivity detector.

The trend toward more polar pesticides among those under development also makes HPLC worth examining in the development of MRMs and SRMs. Detectors are the constraining factor in applying HPLC to pesticide residue analysis in food. While columns are now available for almost any conceivable type of pesticide, there is a lack of effective detectors when compared to those available for gas chromatography. Particularly lacking are the element-specific detectors for pesticides containing atoms such as phosphorus, sulfur, nitrogen, and the halogens chlorine and bromine, although the photoconductivity detector works for some sulfur- and chlorine-containing pesticides.

(6) *Use of immunoassay as a detection technique.* Using immunoassay for detecting pesticide residues can have several advantages over conventional methods: They can analyze an increased number of samples in a given period, are simpler to use, require less skilled personnel and comparatively inexpensive equipment, and can analyze samples for less cost than conventional methods. However, widespread use is constrained by several factors, indicating that immunoassay will complement conventional determinative steps for MRMs but are unlikely to replace them. They may also offer a means to improve SRMs. (See ch. 4 for a detailed discussion of immunoassay.)

(7) *Increased automation.* Continued improvements in analytical methods are possible through automation. Improvements in automation have focused primarily on the cleanup and detection stages of pesticide residue analysis. Although automating the sample preparation and extraction steps would generate the greatest time savings, these steps are also the most difficult to automate because of the many types of food samples requiring different preparation (15).

Automated equipment including robots involves high capital costs, and many Federal regulatory laboratories may have difficulty purchasing such equipment. Manual procedures may still be faster than automated ones, although automation may provide other benefits, e.g., freeing up analysts' time or reducing analyst exposure to hazardous solvents. Therefore, decisions to increase the use of automated equipment must consider the goals of monitoring programs and the moneys available. Robots are not a cure-all for those regulatory agencies now inundated with food samples, However, robots can measurably improve the overall operation of the analytical laboratory. (See ch. 5 for a detailed discussion of automation.)

(8) MSD. The mass selective detector (MSD) may have an increasingly important role as a GC detector in developing MRMs; it is the only GC detector able to detect any pesticide that can be volatilized that has a molecular weight of no more than 650 atomic mass units (20). This may become an important factor in detector selection because it would not be constrained by the need to have a particular atom, such as sulfur, in the molecule. Although still considered a confirmatory tool, MSD has potential as a programmable GC detector that can be set to provide a relatively large degree of selectivity for pesticides at the trace level. The degree of applicability of this detector to samples of unknown pesticide application history—the sample types for which the MRMs are designed—will depend greatly upon improvements in the number of ion programs that can be used during a chromatographic run.

At present, only eight sets of ions can be programmed into the instrument during a chromatographic run, making its usefulness limited for MRM work. As use of this type of detector grows, its purchase cost ($40,000 to $65,000 per unit, depending upon accessories), should drop accordingly. Some laboratories have difficulty justifying such an expensive detection device, particularity when it is dedicated to quantitative work; typical element-specific detectors cost about one-tenth this much. Such an MSD can be used for full mass-spectral scans, however, giving it the capability of being a quantitative and confirmatory tool. MSD devices have been reduced in size compared with mass spectrometers of the 1970s and can be placed on desk or table tops, requiring little more room than the gas chromatography itself. Space considerations become important when the high costs of supplying a cool, safe, dust-free environment for contemporary analytical instrumentation are taken into account.

Photo credit: Food Safety and Inspection Service Laboratory, Athens, GA

Bench-top gas chromatograph/mass selective detector combinations are used to confirm a violative residue level in a food sample.

Developing New Methods

(1) New MRMs. Research needs to focus on the potential for incorporating more pesticides into existing and emerging MRMs. Significant metabolizes of these pesticides—often more difficult to detect than the parent compound–must also be addressed (see box 6-B). As additional data become available, it may become apparent that existing MRMs need to be modified or new methods developed.

It maybe more advantageous to develop new MRMs for small numbers of chemically similar pesticides; this has been done for the phenoxy herbicides (PAM I); for the pesticides captan, folpet, and captafol (25); and for the benzimidazole-related fungicides (25). A new MRM is being proposed for collaborative study for analyzing the urea herbicides (11). Restricting new MRMs to such small groups of pesticides would probably not be as efficient for monitoring purposes as adding to existing MRMs or developing new comprehensive ones, but it may provide an interim solution to the ques-

Box 6-B.—Ongoing Challenges to Methods Development: Metabolizes and New Pesticides

Greatly complicating the issue of developing analytical methods for pesticides in foods is the task of addressing not only the parent compound but also significant metabolizes. Since metabolism or degradation of a parent compound generally occurs through cleavage, hydrolysis, conjugation with sugars or other polar compounds, or oxidation, the products so formed are usually more polar than the parent and thus more difficult to detect using conventional multiresidue methods (MRMs).

New pesticides may also pose problems for analysis. Forecasts for emerging pesticide types indicate molecular structures that are similar to those seen today; therefore, current MRMs seem adaptable to many new chemicals. However, analytical difficulties may result from lower applications rates (grams or ounces per acre) of some new pesticides (e.g., the sulfonyl urea herbicides and synthetic pyrethroid insecticides). While the use of this type of pesticide results in low residue levels, its use will require more sensitive analytical methods for detection. In addition, many new pesticides have reduced environmental persistence and therefore rapidly metabolize or breakdown into more polar products. Also, increased use of non-conventional chemical pesticides, such as microbiological and genetic and behavioral biochemical, will pose difficulties for analysis and require methods development (18).

Analytical chemists are then faced with the current and growing problem of detecting metabolites. In order to provide a method for determining such metabolize residues, a method that is satisfactory for the parent pesticide may have to be altered; such alterations may include chemical derivatization, changes in the nature of the extracting solvent, changes in the chromatographic determinations such as different columns and detectors, and sometimes even going to a different mode for the determinative step. Many metabolizes cannot be analyzed by MRMs and will require special procedures (18).

One potential solution to the parent compound metabolize dilemma may be to agree on the use of “indicator compounds”; these maybe parent compounds or toxicologically significant metabolizes that have been shown by metabolism studies to exist in a predictable manner under certain environmental conditions. Previous studies may also have shown that the relative amounts of the other associated compounds fall within some quantitative boundaries. Knowing the amount of indicator compound present can therefore provide a semiquantitative idea of the amount of other associated compounds present. The use of indicator compounds may, in many instances, obviate the need for using multiple analytical methods to provide information on both parent compound and a list of metabolites (18). Another potential solution would be to develop inexpensive methods to rapidly test whether such residues exist and will need more difficult analysis performed (18).

tion of how to handle pesticides of widely different chemical and physical characteristics.

(2) New SRMs. Developing functional SRMs is a balance between the use of innovative approaches and the use of techniques that are practical for regulatory chemists. A successful SRM should be capable of analyzing any of the toxicologically significant metabolites—as defined in Subdivision O of EPA’s Guidelines (4)–without separate extractions, cleanup steps, or analytical columns, certainly without incorporating another type of detector. Since most metabolizes are more polar than the parent pesticide, this is a challenge for the method developer and will slow the development process. Since the method will be used for enforcement only when MRMs are not available, it should use the same glassware, solvents, reagents, and instrumentation as the MRMs. This is currently not a requirement of EPA’s Subdivision O Guidelines, but it makes the best use of available resources, obviating the need for having infrequently used equipment sitting around the laboratory.

Frequently, little similarity seems to exist between SRMs presented by tolerance petitioners for individual pesticides with similar structures. This situation might be improved if petitioners made efforts to design “mini-MRMs,” that is, methods that would apply to more than one pes-

ticide, This could be accomplished by making only slight modifications to one SRM.

Cost Considerations of Methods Development Research

The costs of sample analysis and research reflect a number of factors. There are the housing and associated upkeep costs for a laboratory that must store toxic and possibly explosive materials and at the same time must maintain an environment suitable for sensitive equipment. A range of glassware and solvents, which must be pure, are required. The sophisticated instruments are a substantial cost, both in the initial purchase and in upkeep. GCS when equipped with detectors and autosamples can cost $32,000 apiece and HPLCS can average $25,000 to $30,000. Together they also require high purity gases or solvents. Service contracts per instrument can cost $2,000 or more a year. The other major cost is analyst time, which accounts for a large part of the cost of each analysis. The cost to analyze individual samples has not been calculated by regulatory laboratories. The closest approximation may be the price charged by a private laboratory, where a single MRM analysis may cost hundreds of dollars.

Over the last 30 years as the sensitivity of instruments has improved, their purchase and upkeep costs have increased; therefore, improvements in analysis are often accompanied by increased costs of analysis (20). New instruments to improve methods also require a high initial capital expenditure although improvements in manufacturing help control cost. Such expenses may slow Federal regulatory agencies from investigating the use of new instruments for improved methods. A further difficulty is if such instruments are used to improve methods, field laboratories will also need to purchase them if these methods are to be used for routine regulatory analyses.

Methods research involves costs beyond those for sample analyses. First are the tradeoff costs of doing research. Equipment and personnel spent on research mean less equipment and analyst time available for sample analyses. Therefore, requirements for more research need to address requirements for current sampling programs, given that changes in one area can adversely affect the other. Second are the research resources spent unsuccessfully. In the process of improving a method or developing a new one, the analyst attempts to improve the steps involved in the method. Failures in each step or in the entire process use up resources but do not produce results apparent to others. Third are the costs of validating that an improved method is accurate.

Another factor determining the cost of research will be the goals for improving the regulatory programs. Improving MRMs to analyze more pesticides and commodities may be carried out in conjunction with regulatory analysis work. But if the focus is on high health hazard pesticides that cannot be analyzed by existing MRMs, development of new MRMs or practical SRMs maybe needed. Longer-term research, high capital costs, and validation costs may be required for introducing new technologies for either improving methods or developing new ones. The same may be true for introducing technologies, such as automation and robotics, that can improve the use of methods. Goals must be set before the level of research resources can be determined.

Needs for Adoption and Use of Methods

Vaiidating Methods

Before any analytical method can be used routinely in the laboratory, it must be validated. Validation is the process whereby one or more individual chemists test the suitability of a particular method for collecting analytical data (21). The suitability of a method will depend in part upon the circumstances of the application. For example, a method that will be widely used will require more validation than one whose use is more confined. The effort expended in validating analytical methods serves to validate the results of sample analyses. Consequently, method validation at several levels (e.g., intralaboratory, interlaboratory, and AOAC collaborative study) is considered inherent to the methods development process.

Intralaboratory validation is the lowest validation level. It requires the developer to demonstrate that the method is reproducible, sensitive, specific, and contains all the qualities needed to meet the method's analytical purposes. The developer then hands the evaluation of the method to someone else within the laboratory for further validation.

Interlaboratory validation is the next level. This level is usually required before a method is used by other laboratories. The laboratory developing the method must find another laboratory to test the method and its written description by analyzing samples with unknown residues and levels. Successful performance of the method by an analyst other than its developer must be provided before the method can be sanctioned for use in monitoring.

A more rigorous validation is undertaken for methods intended for widespread and continuous use. Collaborative study, under the auspices and rules of the Association of Official Analytical Chemists (AOAC), is a major effort involving six to eight laboratories and is usually performed for methods that an organization expects to continue using for many years. Methods are usually not studied collaboratively until they have been in use in several laboratories over an extended period of time and results indicate that they are worth the considerable effort involved. Collaborative studies are far too expensive to be conducted for all methods. If the residue measurements produced in a collaborative study meet the statistical requirements for accuracy and precision, the method is declared official by the AOAC and published in the *Official Methods of Analysis* of the AOAC.

The degree of validation required by FDA and USDA will differ depending upon the application of the method. For the majority of methods, both agencies require an interlaboratory validation involving at least two laboratories. FDA and FSIS encourage the use of AOAC official methods where possible because they are most widely accepted. All five of FDA's routinely used MRMs have received AOAC collaborative study for some commodities and pesticides. In some cases, however, methods validated by an AOAC collaborative study or interlaboratory study may not be available, for example, for pesticides not used in the United States but found in imports or for applying a validated method to a new commodity. A lesser form of validation, such as intralaboratory study, can be used in such cases. As long as the analytical results follow well-accepted principles—sample custody, sample stability, no false positives in control samples, adequate recoveries from fortified samples, and confirmation of results—the method can be used for regulatory enforcement action.

The success of an MRM or group of MRMs is not necessarily guaranteed by the degree of formal validation undertaken. For example, the MRMs used by the California Department of Food and Agriculture (CDFA) have been developed in-house over the past 20 years as a result of information from one of the chemical apparatus supply houses (22). No collaborative studies have ever been done by CDFA (though one is under consideration now) on their in-house MRMs, although they have split samples with FDA laboratories; these split-sample analyses have produced results comparable to those generated in FDA laboratories (22).

Confirmation of Results

When an analysis leads to the finding of a violation, regulatory agencies require that the violation be confirmed by a different technique or method. The most common approach to confirmation is to re-analyze the sample after modifying the original method, for instance, after changing the detector, column, or sometimes both (5, 14). In these cases, confirmation does not require the development of completely new methods.

A second approach to confirmation has been identifying a suspected pesticide residue by its mass spectra. Regulatory agencies are increasingly using mass spectrometers, including the smaller bench-top types, as detectors for GC, and in some cases HPLC, for the confirmation of violations.

When violations are found, confirmatory analyses need to be performed only on certain samples. That is, if numerous tentative positives are uncovered in a group of samples, confirmation is required on only a representative part of these samples.

EPA does not require that pesticides receiving new tolerances have confirmatory methods in addition to the method required for monitoring, Consequently, a second battery of confirmatory methods does not exist. This suggests that some SRMs might not have confirmatory methods and thus can not be used for regulatory purposes.

Overall, however, it appears that confirmatory methods will, for the most part, take care of themselves, assuming that adequate MRMs will be forthcoming for future pesticides and that growth in technologies continues,

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