Chapter 4

Immunoassay: An Emerging Technology

Contents

Box

Figure

Tables

Chapter 4

Immunoassay: An Emerging Technology

INTRODUCTION

Immunoassay, which use antibodies to detect chemical compounds, are widely used in clinical chemistry but have not been equally applied to the analysis of pesticide residues (4). Yet they seem to have a potentially significant role in analyzing pesticide residues in food. Antibodies can be developed to identify single pesticides or, in some cases, small groups of similar pesticides. Those immunoassay that determine groups of pesticides supply data for the entire group and not the individual pesticides. Immunoassay can also be used to provide quantitative data, similar to that provided by conventional analytical techniques, or they may provide qualitative or semiquantitative data. The latter type can, in some cases, yield results more quickly than conventional techniques.

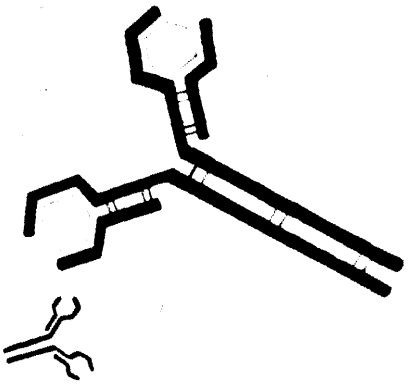
U.S. Federal and State agencies do not currently use immunoassay in their pesticide residue regulatory programs on food. However, FSIS has begun implementing the use of an immunoassay to detect a small group of pesticides, and Canada's Department of National Health and welfare (which regulates pesticide residues in food) will be training laboratory personnel in the fall of 1988 in the use of a specific immunoassay to determine one pesticide (1,13).

THE IMMUNOASSAY

In higher animals, specialized cells (known as B lymphocytes) recognize substances foreign to the body (known as antigens) and respond by producing antibodies that recognize and bind to the antigens (figure 4-1). The introduction of a pesticide can stimulate an animal's immunological system to develop antibodies that will recognize and bind to that specific pesticide. These antibodies can be obtained from the animal's serum and used for the detection of the pesticide. However, antibodies are so specific that it is important to decide upon the purpose of the antibody before development begins. For example, for pesticides that are metabolized quickly, antibodies may need to be developed for the significant metabolizes rather than the original pesticide.

Pesticides are usually made up of molecules too small to induce the production of antibodies. Therefore, pesticides must first be conjugated to a larger carrier molecule, often a pro-

Figure 4-1.-Artist's Conception of Antibody-Antigen Interactions



Antibody-antigen interactions result from a precise f it between a surface feature of the antigen and the correspondingly shaped binding sites in the antibody molecules.

SOURCE: Environmental Monitoring Systems Laboratory Las Vegas, Environmental Protection Agency.

tein. Once attached to the carrier molecule, the pesticide is called a hapten. Where the conjugation occurs will influence the types of antibodies produced. The chemical synthesis of the hapten-carrier conjugate is generally considered to be the most important factor in obtaining useful antibodies for analytical use, and the chemistry involved is a major factor in the cost of immunoassay development (9). The hapten-carrier conjugate is then injected into a vertebrate, e.g., a rodent or rabbit, or for large amounts of antibodies, a sheep or goat. The animal will produce an array of antibodies; some will bind to the carrier molecule, some to the hapten-carrier conjugate, and some to the hapten. Only the last of these is useful for developing an immunoassay to detect the pesticide. These antibodies will be heterogeneous because different B lymphocytes may produce antibodies that bind to slightly different sites on the hapten. These antibodies are known as *polyclonal* because they are produced from a number of different B lymphocyte clones in the animal. They need to be characterized for their affinity for the hapten (the strength of their binding interaction with the hapten) and their specificity (whether they bind only to the hapten or to other related chemicals as well).

For most immunoassays, the greater the affinity, then the greater the sensitivity of the analysis (10). The degree of specificity must be known to determine if the antibody will bind to, or cross-react with, compounds other than the hapten. The mixture of antibodies will vary inside the animal producing them with changes in the number of each type of B lymphocyte; it will also vary between each animal immunized. The changes in the proportion of hapten-specific antibodies and the existence of other antibodies can interfere with the analytical application of a polyclonal-based immunoassay. By analyzing known concentrations of a pesticide along with the unknown concentrations in a sample, these variations can be adjusted for, and successful polyclonal-based immunoassay for pesticides can be developed (10, 11).

The production of *monoclinal* antibodies can offer some benefits over polyclonal antibodies, but some tradeoffs exist. Monoclinal antibodies are produced through a fusion of mouse or rat B lymphocyte spleen cells with myeloma tumor cells to produce hybridoma cells, a small percentage of which will produce the desired antibody. The spleen is normally taken from an animal that has first successfully produced useful polyclonal antibodies. This process takes a minimum of 3 months before large quantities of antibodies can be produced (21). Hybridomas can live almost indefinitely and can produce an unlimited amount of homogeneous monoclinal antibodies without the interfering antibodies that may exist with polyclonal antibodies. And like polyclonal antibodies, hybridomas can be stored in liquid nitrogen and easily distributed between laboratories.

Monoclinal antibodies, however, are not necessarily the better choice for a specific immunoassay. Polyclonal-based immunoassay may be adequate for an immunoassay, and in some cases, they are the more sensitive of the two (3). But for other pesticides, monoclonals may be necessary (3). Production of monoclinal antibodies requires more time, labor, equipment, and training than polyclonal antibodies and can add 25 percent to development costs (7). New techniques now under development may reduce the costs of hybridoma production, however (9).

Polyclonal or monoclinal antibodies are next incorporated into an immunoassay. Immunoassay for pesticides operate by competitive inhibition, or displacement, in which the antibodies are simultaneously exposed to an unknown amount of a pesticide in the sample and to a known quantity of the pesticide separate from the sample. The more pesticide in the sample, the fewer antibodies will bind to the latter pesticide (4).

To allow measurement, some sort of tracer must be attached to either the antibody or the pesticide. Currently, the most widely used tracer is an enzyme that will generate an easily measurable color when an additional substance is added. Other tracers include radioisotopes, fluorescent molecules, and magnetic particles (10). The radioimmunoassay (RIA), while in some ways more effective than the enzyme immunoassay, currently receives less at-

tention for pesticide detection because of the demands and hazards of working with radioactive substances and because enzyme immunoassay have become increasingly practical (13). The fluorescent immunoassay, currently used in clinical applications, potentially may become as, or more, important for pesticide residue analysis in food as the enzyme immunoassay. It can be faster, more sensitive, and more easily automated than the enzyme immunoassay (9).

To determine the amount of pesticide in a sample, a standard curve is prepared. Several different known quantities of the pesticide (called standards) are separately analyzed with the immunoassay. A standard curve is prepared from these results and usually based on the ratio of the amount of pesticide in the standard to the measurement of the tracer (e.g., the intensity of color produced by an enzyme tracer). The measurement of the tracer from an assay of a sample can then be compared against the standard curve to determine the amount of the pesticide in the sample (see box 4-A).

Some extraction and possibly cleanup of the sample may be required before the antibodies can be used. For some aqueous solutions such as juices, immunoassay may be applied directly. Immunoassay for some vegetables and fruits have also been used without a cleanup step (14). However, cleanup is commonly necessary and time-consuming for food containing oil or fat.[1]

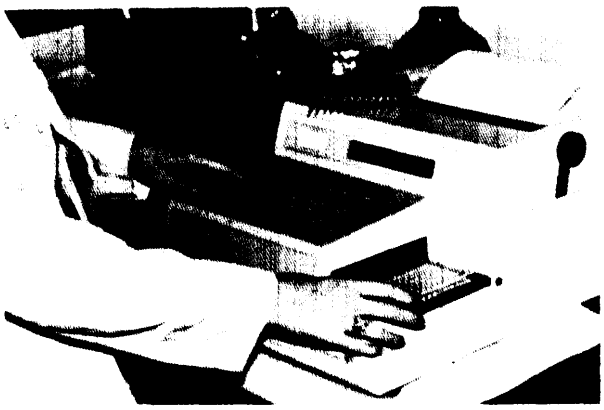


Photo credit: Environmental Monitoring Support Laboratory-Las Vegas, Environmental Protection Agency

A microprocessor-controlled photometer automatically reads samples in the 96-well microtiter plate within 2 minutes and provides results in report form.

Most immunoassay work has taken place in academic and regulatory laboratories (14). The time to develop an immunoassay can vary.

Box 4-A.—Enzyme-Linked Immunosorbent **Assay** (ELISA)

ELISA is a common example of an immunoassay using an enzyme tracer. A test tube or well in a 96-well plastic microtiter plate is coated with a known amount of pesticide (conjugated to the carrier molecule) and so the pesticide is immobilized on the surface of the tube or well.

The sample extract containing an unknown amount of the same pesticide is added to the tube or wells. In separate tubes or wells on the plate, known concentrations of the pesticide (the standards) are added instead of the sample extract. The antibody that recognizes and binds to that pesticide is then added. Some of the antibody binds to the immobilized pesticide and some to the pesticide in the sample extract or the standards. How much antibody binds to the immobilized pesticide depends on how much pesticide is in the extractor standard,

The extract is washed away, and the amount of antibody bound to the immobilized pesticide will next be measured using the enzyme tracer. A tracer enzyme may be already attached to the antibody or may be attached by adding a second antibody (that binds to the first) conjugated with the enzyme. If the latter is done, then any unbound second antibody is washed away. A solution of colorless subtrate is added, which will be changed by the enzyme to a colored product (2 I).

The amount of antibodies bound to the immobilized pesticide is shown by intensity of the color; the greater the intensity, the less pesticide is in the sample. The intensity of the color can be measured through the use of a microspectrophotometer, which may be linked to a computer with data-analyzing software. This measurement is then compared against a standard curve, derived from the standards, to give the amount of pesticide in the sample.

[1] For a more detailed description of the technology see ref. 10.

polyclonal-based immunoassay generally require 9 months or longer to develop (13), and monoclinal immunoassay may take a year or more (21). However, commercial laboratories having abundant resources and personnel may be significantly faster than smaller laboratories in developing an immunoassay, sometimes as much as 4 to 5 months faster (7).

Another potential application of antibodies is the biosensor, which theoretically can provide real-time, continuous monitoring of pesticides in a matrix. The biosensor uses biological molecules, such as antibodies, to recognize and bind to the desired pesticide and a mechanism whereby the binding generates an electrical signal that can be measured and converted to give the concentration of the antigen (10). Compared to immunoassays, the application of biosensors for the detection of pesticide residues in food is uncertain.

STRENGTHS AND WEAKNESSES

Immunoassay are particularly suited for polar, water-soluble pesticides and their degradation products that are generally difficult to analyze using conventional analytical methods. Because immunoassay can determine biorational pesticides (such as Bacillus *thuringiensis),* they could be important if use of biological pesticides increases (10). They also can be significantly faster than certain conventional methods. Comparisons of quantitative immunoassay with conventional single residue methods using gas or liquid chromatography to analyze specific pesticide/food commodities show that immunoassay can analyze four to five times as many samples in a given time period (15, 16, 17).

The rapid nature of immunoassay is based on a number of factors. The cleanup step can be avoided or abbreviated for aqueous samples (such as juices and milk) and for many fruits and vegetables. The detection step can be faster than in conventional methods. For qualitative and semiquantitative immunoassays, the detection step may take no more than 5 minutes. For quantitative immunoassays, the use of a 96-well microtiter plate and plate reader allows detection and quantification of a large number of samples at one time. Quantitative immunoassay take approximately 4% to 6 hours to perform on food, from sample preparation to detection. Liquids can take significantly less time. At Health and Welfare Canada, one person can analyze 12 to 16 fruit and vegetable samples in triplicate (along with controls) in one day. This work has been for research, not for regulatory application, and Health and Welfare Canada believes the number of samples could be tripled for regulatory application (14). Therefore, although the quantitative immunoassay procedure may take as long as a conventional method, more samples can be analyzed at one time.

The use of automation and robotics could further increase the number of samples analyzed. The principal steps of an ELISA that can be automated include coating of the wells or tubes with the immobilized pesticide; washing; addition of antibody, standards, and samples; and color reading. Systems are available for automating one or more of these steps. For example, unattended, automated spectrophotometers can read 10 to 25 microtiter plates and record the results in report form. However, because most enzyme immunoassay have long incubation periods, automation of the entire procedure by a single unit is not practical yet (9).

In addition, immunoassay can be simpler to use than conventional techniques, require less skilled personnel, and require minimal instrumentation time and comparatively inexpensive equipment. Technicians can be trained within 2 weeks (8, 13). And given that immunoassay can be more portable and simpler to use, they may be adaptable to field use for food. The actual costs of an immunoassay used on food for pesticide analysis versus a conventional method have not been compared (13). But the costs of analyzing a sample in general and for specific nonfood matrices with an immunoas-

say are lower than for conventional techniques (9, 22).

Despite these advantages, the use of immunoassay for monitoring pesticide residues in food has been constrained by a number of factors. Immunoassay may not be as sensitive for some compounds as conventional methods, and they can have lower levels of reproducibility. Because immunoassay are compound-specific, they are not suitable for multi residue analysis. Therefore, while they may analyze more samples in a given time than multiresidue methods, they can detect fewer pesticides.

Characteristics of the food or the pesticide, in some cases, may also preclude the use of immunoassays. For food samples and pesticides requiring considerable cleanup work, immunoassay may be no faster than conventional techniques. In addition, immunoassay may not work well in certain foods. For some pesticides, e.g., those of very small molecules or having nonrigid structures, it may not be possible to develop antibodies. Or if the pesticide has little aqueous volubility, it may not be possible to use an immunoassay.

Not enough is known about possible cross-reactivity of specific antibodies with other chemicals present in food. Problems caused by cross-reactivity are a concern but can be controlled if the antibodies are first well characterized and if blank samples and samples with known concentrations are analyzed at the same time with the sample in question (4). Cross-reactivity can also be a benefit if an immunoassay is needed for a group of similar pesticides.

Another constraint to the use of immunoassay for pesticide residue analysis in food seems to be the reluctance of some analytical chemists to explore the potentials of immunoassays. This is due in part to analytical chemists' general unfamiliarity with the biologically based technology. This constraint may be overcome by validation of the technique and increased training in its use. The speed of doing so will depend on institutional commitment, however.

STATUS OF REGULATORY USE OF AND RESEARCH ON IMMUNOASSAYS

Antibodies have been developed and reported for at least 30 pesticides, though few have been applied to food (for a listing of immunoassay developed for agrichemicals, see ref. 11). Currently, no government agency has used immunoassay for regulation of pesticide residues in food, but many are supporting research and development for immunoassay determination of pesticides, in some cases for matrices other than food (see table 4-1).

Regulatory agencies' acceptance of immunoassay vary. Health and Welfare Canada is the furthest along in the development of immunoassay for pesticide testing in food, Since 1980, Canada has developed seven immunoassay for use *in* food and is currently developing one for 2,4-D. Canada has focused its work on quantitative polyclonal-based ELISAs for polar compounds in non-fatty foods. Canada's regulatory laboratories are not yet using immunoassays, but a planned fall 1988 training workshop on an ELISA for carbendazim is a first step toward transferring the technology to the field laboratories (13).

FSIS recently has decided that immunoassay can have an important role in its regulatory program. This is in part a response to the National Research Council's recommendations to test more samples and to test for more chemicals using more rapid methods.[2] FSIS is now working on implementing a semiquantitative immunoassay for the rapid detection of a group of five pyrethrin insecticides for regulatory use in 1989 at its Athens, GA, laboratory. Part of

[2]In response to a request from FSIS, the Committee on the Scientific Basis of the Nation's Meat and Poultry Inspection Program, Food and Nutrition Board of the National Research Council prepared in *1985* the report *Meat **and** Poultry Inspection: The **Scientific Basis of the Nation Program*** (12), which included technical recommendations for FSIS's inspection program.

Table 4-1.—Immunoassays for Pesticides Under Development by Regulatory Agencies

Agency	Pesticide	Matrix	Type of assay	Type of antibody[a]	Data provided	Contractor (if one)
FSIS	heptachlor & heptachlor expoxide	meat & poultry	ELISA	M	quantitative	Lawrence Livermore Laboratory
	triazines[b]	meat & poultry	ELISA	P	qualitative	
FDA	paraquat	potatoes	ELISA	M	quantitative	Research Triangle Institute (RTI)
	fenamiphos, fenamiphos sulfone and sulfoxide	oranges	ELISA	M	quantitative	RTI
	benomyl, carbendazim, thiophanate methyl	apples	ELISA	M	quantitative	RTI
	glyphosate	soybeans	ELISA	M	quantitative	RTI
EPA	paraquat	soil & water	ELISA	P & M	quantitative	
	pentachlorophenol[c]	water	ELISA	M	quantitative	
	atrazine & simazine[d]	soil	ELISA	M	quantitative	
CDFA	molinate	water	ELISA	M	quantitative	University of California—
	thiobencarb	water	ELISA	M	quantitative	Davis & Berkeley
	atrazine & simazine	water & soil	ELISA	M	quantitative	EPA—Las Vegas laboratory[e]
Health &Welfare Canada	2,4-D		ELISA	P	quantitative	

ap = polyclonal and M = monoclinal.
[b]FSIS is evaluating a rapid ELISA test for triazines developed by ImmunoSystems Inc.
[c]EPA is evaluating a pentachlorophenol immunoassay developed by Westinghouse Bio-Analytic Systems Company.
[d]EPA is developing the soil extraction technique for the immunoassay under contract with CDFA.
[e]The University of California at Davis is doing the hapten synthesis work and developing polyclonal antibodies. The University of California at Berkeley is developing the monoclinal antibodies. The EPA Las Vegas laboratory is developing an extraction technique for atrazine and simazine in soil samples.
SOURCE: Office of Technology Assessment, 1988.

this work is on completing the extraction and cleanup steps for the immunoassay (l). FSIS has contracted for the development of other ELISAS for heptachlor and a number of animal drugs. In addition to contracting for the development of immunoassays, FSIS also tests commercially developed test kits and is currently evaluating a commercial qualitative immunoassay for triazine herbicides. FSIS's use of immunoassay is made more difficult because it works primarily with fatty commodities—meat and poultry—which normally require significant cleanup.

FDA has no current plans to implement the use of immunoassay for regulatory work. FDA, however, has a 3-year, approximately $500,000 contract begun in September 1987 for the development of six complete, quantitative immunoassay methods based on monoclinal antibodies (2). FDA is taking a somewhat "wait and see" attitude on the results of this research before determining the role of immunoassay in its pesticide regulatory program.

EPA has established a program at its Las Vegas Environmental Monitoring Systems Laboratory on using immunoassay for the detection of hazardous substances, including pesticides, in the environment. The program tests commercially available immunoassay as well as develops immunoassays. EPA does not address food but it has an interagency agreement with FSIS for the development of antibodies of common interest,

The California Department of Food and Agriculture (CDFA) has contracted for the development of three immunoassay for use on environmental matrices: soil, surface water, and groundwater. CDFA has no plans yet to use these immunoassay on food (19).

Agencies have also taken different approaches to the development of immunoassay. FDA, for example, has contracted for the development of entire immunoassays. EPA has cooperative agreements with university laboratories to provide hapten work, antibodies, and in some cases the entire immunoassay. Health and Welfare Canada has developed its immunoassay in-house. It seems that enough outside expertise exists in antibody development for regulatory agencies to tap using contracts instead of hav-

ing to develop the capability to do such work in-house. Agencies, however, would need some in-house expertise, at least to identify the types of antibodies needed, to evaluate the results of the antibody development, and to adapt the immunoassay for use on food. This last capability would also allow agencies to take advantage of antibodies developed by others for nonfood matrices.

Because the application of immunoassay to pesticide monitoring in food is new, a great opportunity exists for agency coordination of research. As noted earlier, EPA and FSIS have an interagency agreement, and some of CDFA's work is done at EPA. But neither FDA nor Health & Welfare Canada seem to be well linked with one another nor with the other agencies. Coordination could be stimulated if agencies jointly listed which pesticides need improved methods and then identified those best addressed by immunoassay. In this way, development of antibodies useful to all agencies could be done without duplication of effort.

Commercial development of immunoassay for analyzing pesticides is also taking place. A number of rapid immunoassay tests have been developed by small private firms (see table 4-2). Many of these test kits were developed for use on water and require adaptation to food. Currently, FSIS is the only regulatory agency doing adaption work. Quantitative immunoassay for pesticides are also being developed privately, but again they are not aimed for use in regulating pesticide residues in food. Identification by Federal agencies of priority immunoassay needs and communicating these needs to the private sector might stimulate private development of immunoassay for use in food. In some cases, private companies have developed immunoassay for internal use, and Federal agencies could investigate the possibility of obtaining and modifying these immunoas-

Table 4-2.—Commercially Available ELISA Test Kits for Pesticides

Pesticide	Claimed limit of detection	Water & food matrices[b]	Firm
Paraquat	100 ppb	water	Environmental Diagnostics Inc. Burlington, NC
Triazine herbicides (atrazine, simazine propazine)	1 ppb	water, milk, soup, and fruit juices	ImmunoSystems Inc. Biddeford, ME
Chlordane-related pesticides (chlordane, heptachlor dieldrin, aldrin endrin, endosulfan)	1 ppb 200 ppb	water beer	
Benomyl	500 ppb	water, orange and grape juice concentrate	
Carbofuran	1 ppb 25 ppb 100 ppb	water grape juice rice	
2,4-D	1 ppb 100 ppb	water beer	
Triazine herbicides	same as triazines above		Westinghouse Bio-Analytic Systems Co. Rockville, MD
Aldicarb	10 ppb	water and watermelon	
Carbofuran	10 ppb	water	
Parathion	10 ppb	water and fruit juice	
Pentachlorophenol	10 ppb	water	

[a] ppb = parts per billion

[b] These food matrices are ones that the firms have tested their immunoassay on. Some Of these immunoassays were developed to analyze nonfood matrices and their modification for use on food may *not* be a *priority* of the firm.

SOURCE: Office of Technology Assessment, 1988

says for agencies' use on food (10). For example, a polyclonal-based quantitative immunoassay for cyanazine (an atrazine herbicide) was developed for soil and water by Shell Oil Company and used to provide data for EPA reregistration of the pesticide. The immunoassay has a detection limit of 0.5 parts per billion and can analyze five times as many samples a day as a conventional method using gas chromatography *(18)*.

As a new technology in the pesticide analysis area, immunoassay require rigorous validation before acceptance by analytical chemists (21). Validation of immunoassay initially could be accomplished through comparisons with established methods, although in time agencies may need new validation and quality control protocols to address the unique properties of immunoassay (3). Standardized methods for using immunoassay and criteria for evaluating data, a general plan for establishing degree of cross-reactivity, and minimum quality specifications for the materials (including antibodies used) would all benefit the validation of immunoassay (9).

As in the case of research, coordinating the validation process for immunoassay among regulatory agencies could be improved, possibly in conjunction with appropriate professional associations such as the Association of Official Analytical Chemists (AOAC). Currently, agencies are conducting validations of immunoassay individually. No official validation studies of immunoassay for pesticide residue detection in food have been submitted to the AOAC. Health and Welfare Canada validated each of its immunoassay by analyzing four to five commodities each with four different concentrations of the pesticide, in duplicate or triplicate, using conventional methods and the immunoassay on each sample (13). EPA used the same process of analyzing each sample by both a conventional method and an immunoassay in its validation of a commercial, quantitative immunoassay for pentachlorophenol. For future evaluations, EPA will prepare individualized evaluation studies based on a statistically significant number of samples analyzed through conventional methods, which will eliminate the need to analyze every sample by conventional methods (20).

THE POTENTIAL ROLE OF IMMUNOASSAY IN THE DETECTION OF PESTICIDE RESIDUES IN FOOD

Immunoassay have a number of potential regulatory roles. The small number of pesticides each immunoassay can detect means that immunoassay will complement or improve multiresidue methods (MRMs) rather than replace them. For example, development of immunoassay could be focused on those polar, moderate-to-high health hazardous pesticides that MRMs cannot address. Current MRMs might also be improved by analyzing a sample extract with conventional techniques as well as immunoassay, thus increasing the number of pesticides that could be detected by the MRMs. Additional work would be required to overcome possible negative effects of extraction solvents on the immunoassay, For all uses of immunoassay, conventional methods will be necessary to confirm violations and ensure that the immunoassay are not giving false negative or false positive results.

Advances in immunoassay technology may result in immunoassay being submitted to EPA during the tolerance-setting process to fulfill the requirement for an analytical method. EPA has not formally decided if such a method would be acceptable, and FDA and FSIS would need to provide input because the submitted methods are to be used for regulatory work. Therefore, EPA's, FDA's, and FSIS's capability to use immunoassay could affect the agencies' decision to accept them as submitted methods. In a worst case scenario, EPA might be faced with the submission of analytically

acceptable, commercially available methods whose acceptance might be denied because a regulatory agency does not have the expertise or equipment to use them.

Quantitative immunoassay could replace impractical conventional single residue techniques, increase the number of samples analyzed for certain pesticides (even those for which practical conventional techniques exist), and increase the number of special surveys for specific pesticides. Increased automation, including robotics, of the immunoassay would further support these activities. Once accepted, quantitative immunoassay may also be used as a confirmatory single residue technique for analysis by conventional methods.

Semiquantitative or qualitative immunoassay could test large numbers of food samples rapidly for specific pesticides that need to be monitored but that have shown few violations in the past. Thus, more time-consuming and expensive, conventional quantitative methods could be reserved for confirming violative samples. Currently, some private sector food manufacturers, such as certain baby food producers, use rapid ELISA tests to ensure that the products they buy do not have illegal residues of certain pesticides (5,6), The use of immunoassay in monitoring programs may require some rethinking of objectives because they would enable a greater number of samples to be analyzed but they do not provide the quantitative data some agencies require.

The ability of immunoassay to analyze large numbers of samples would make them useful when a widespread pesticide residue problem is suspected in a specific commodity or commodities. The large number of samples that need to be analyzed in this situation can overwhelm a regulatory laboratory using conventional methods. Such tests could be used to sort out the violative samples and allow the legal samples to reach the market more rapidly.

In time, the portability and simplicity of immunoassay, especially the semiquantitative and qualitative ones, could provide the opportunity to perform testing outside of the laboratory. Issues such as how to address extraction and cleanup needs, training of the field testers, and how such analysis would fit into current regulatory programs would first need to be addressed before field testing was implemented.

CHAPTER 4 REFERENCES

1. Ashworth, R., Chemistry Division, Food Safety and Inspection Service, Washington, DC, persona] communication, May 13, 1988.
2. Clower, M., Center for Food Safety and Applied Nutrition, Food and Drug Administration, Washington, DC, personal communication, July 12, 1988.
3. Hammock, B., Gee, S., Cheung, P., et al., "Utility of Immunoassay in pesticide Trace Analysis," *Pesticide Science and Biotechnology,* R. Greenhalgh and T. Roberts (eds.) (London: Blackwell Scientific Publications, 1987).
4. Hammock, B., and Mumma, R., "Potential of Immunochemical Technology for Pesticide Analysis," *Pesticide Analytical Methodology,* American Chemical Society Symposium Series 136, J. Harvey, Jr. and G. Zweig (eds.) (Washington, DC: The American Chemical Society, 1980).
5. Harvey, R., Beech Nut Nutrition Corporation, Canajoharie, NY, personal communication, May 20, 1988.
6. Hether, N., Gerber Products Company, Fremont, MI, personal communication, May 13, 1988.
7. Hunter, K., Westinghouse Bio-Analytic Systems Company, Rockville, MD, personal communication, July 1, 1988.
8. Hunter, K., Westinghouse Bio-Analytic Systems Company, Rockville, MD, personal communication, Apr. 15, 1988.
9. Karu, A., Hybridoma Facility, College of Natural Resources, University of California-Berkeley, Berkeley, CA, personal communication, June 28, 1988.
* 10. Mumma, R., and Hunter, K., "Potential of Immunoassays in Monitoring Pesticide Residues in Foods, " OTA commissioned paper, Spring 1988.

*These references are contained in appendix B.

11. Mumma, R., and Brady, J., "Immunological Assays for Agrochemicals," Pesticide Science and Biotechnology, R. Greenhalgh and T. Roberts (eds.) (London: Blackwell Scientific Publications, 1987).
12. National Research Council, Food and Nutrition Board, Committee on the Scientific Basis of the Nation's Meat and Poultry Inspection Program, *Meat and Poultry Inspection: The Scientific Basis of the Nation Program* (Washington, DC: National Academy Press, 1985).
13. Newsome, W. H., Food Research Division, Health and Welfare Canada, Ottawa, Canada, personal communication, May 6, 1988.
*14. Newsome, W. H., and Graham, G., "Pesticide Residue Monitoring in Canada," OTA commissioned paper, Spring 1988.
15. Newsome, W. H., "Determination of Iprodione in Foods by ELISA," *Pesticide Science and Biotechnolology*, R. Greenhalgh and T. Roberts (eds.) (London: Blackwell Scientific Publications, 1987).
16. Newsome, W. H., "An Enzyme-linked Immunosorbent Assay for Metalaxyl in Foods," *Journal of Agricultural and Food Chemistry 33(3): 528-530,* May/June 1985.
17. Newsome, W. H., and Shields, J. B., "A Radioimmunoassay for Benomyl and Methyl 2-Benzimidazolecarbamate on Food Crops," *Journal of Agricultural and Food Chemistry* 29(2):220-222, *1981.*
18. Sharp, J., Agricultural Products Department, E.I. DuPont de Nemours & Co., Wilmington, DE, personal communication, Aug. 2, 1988,
19. Stoddard, P., California Department of Food and Agriculture, Sacramento, CA, personal communication, Apr. 18, 1988.
20. Van Emon, J., Environmental Monitoring Systems Laboratory, Environmental Protection Agency, Las Vegas, NV, personal communication, May 3, 1988.
21. Vanderlaan, M., Watkins, B. E., and Stanker, L., "Environmental Monitoring by Immunoassay," *Environ. Sci. Technol.* 22(3):247-254, March 1988.
22. Wie, S., and Hammock, B., "Development of Enzyme-Linked Immunosorbent Assays for Residue Analysis of Diflubenzuron and BAY SIR 8514," *Journal of Agricultural and Food Chemistry* 30:949-957, *1982.*

*These references are contained in appendix B