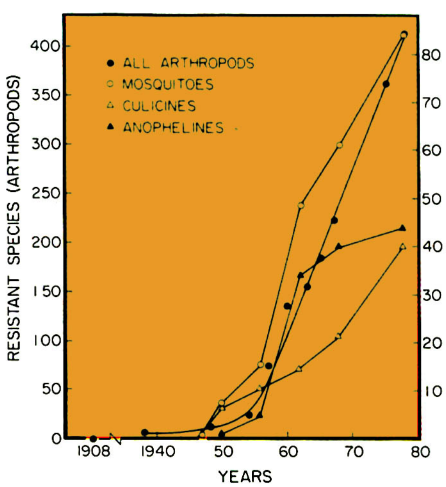
New chemicals

Because of widespread resistance, new chemicals must be evaluated not only against susceptible mosquitoes but also against strains that represent specific resistance mechanisms. This laboratory maintains several strains of mosquitoes, each possessing one or more of the known mechanisms of resistance, such as DDT-dehydrochlorinase, DDT-nerve insensitivity, organophosphorus-degrading esterases, carbamate-degrading oxidases, pyrethroid-insensitivity, juvenile hormone resistance, and organophosphorus- and carbamate-insensitive cholinesterase. Every new insecticide received from universities, from industry, or by agreement through the World Health Organization is thoroughly tested against these strains to determine the impact of already existing resistance mechanisms on its effectiveness. Several hundred new chemicals have been subjected to this crucial test during the past decade.

Successful compounds are subsequently studied for their propensity to induce resistance. A representative field population is established in the laboratory, and successive generations are subjected to intensive selection by the new chemical. The resultant resistance is then characterized biochemically and genetically, providing vital information on the future of the chemical.

Such studies in recent years have yielded useful information on potential resistance to methoprene in *Cx. tarsalis*, resistance to pyrethroids in *Cx. quinquefasciatus*, insensitive cholinesterase in *Anopheles albimanus*, and organophosphorus resistance in *Cx. tarsalis* and *Cx. quinquefasciatus.* They have also revealed a limited response to selection by propoxur and diflubenzuron in *Cx. tarsalis*, and to selection by chlorphoxin in *An. albimanus.*



Chronology of appearance of resistance to insecticides in mosquitoes, in relation to resistance in all arthropods.

Counteraction of resistance

To remain effective, an insecticide must be used in ways that minimize selection pressure toward resistance. To achieve this goal it is necessary to develop means of quantifying the "propensity for resistance" in a given situation, and to devise sophisticated control techniques that keep selection below a permissible level. Progress has been achieved toward both objectives.

The genetic and biological parameters that influence the evolution of resistance have been identified and their contribution quantified by simple models on a computer. Population isolation, reproductive fitness of resistant individuals, and dominance of resistant genes were found to be strong determinants of the propensity for resistance. Likewise, significant operational parameters included the dosage of chemical applied, the size of the treated area, and the environmental persistence of the residue. This information was used in constructing a computer simulation model to predict population densities and evolution of resistance in *Cx. tarsalis.* This research and similar studies in other laboratories should eventually make it possible to forecast the course of resistance in insects.

Research on chemical approaches for countering resistance has focused on the principle of synergism, or utilizing interacting chemicals, and on the employment of chemicals in mixtures or in rotations. A thorough knowledge of the types of resistance already present in the target population is required, and new chemicals are needed with distinctly different modes of action and pathways of detoxication.

The principle of mixtures assumes that genes for resistance to each member chemical are so rare that they are not present together in the same mosquito. Thus, an individual capable of surviving one insecticide will be killed by the other insecticide in the mixture. The principle of rotation is based on the premise that resistance to a given chemical declines in the absence of selection pressure. Thus, when insecticides are used in rotation it is expected that resistance to each member chemical will decline during the time intervening between successive uses of the same chemical. Recent discoveries of compounds of disparate chemical structure and mode of action have prompted us to initiate long-term experiments aimed at testing these approaches to forestalling the development of resistance.

George P. Georghiou is Professor of Entomology, University of California, Riverside, CA 92521.

Immunochemical methods to detect pesticide residues

For the safe, effective use of a pesticide, the residue of the pesticide in food, fiber, and the environment after application must be known. Gathering residue data on a new pesticide is a major expense in its development, and this expense may be prohibitive when registration of a pesticide is being considered for use on minor crops or as a mosquito larvicide. To insure the future availability of safe new compounds, especially for minor uses, the cost of residue analysis must be reduced.

Gas liquid chromatography has been the most commonly used residue analysis technique. More recently, high-performance liquid chromatography has proved useful, especially with nonvolatile or heat-unstable compounds, such as benzoylphenyl ureas and carbamates. However, these techniques often require extensive sample cleanup before the assay, which is costly both in time and money. These analytical techniques also have some deficiencies and—for various reasons—rapid, sensitive, reproducible, and economical residue technology is not presently available for some compounds including the benzoylphenyl ureas, juvenile hormone analogues and some pyrethroids.

Immunochemical methods of residue analysis may solve some of these problems. Immunochemical analysis is not new; it has been used successfully for many years in several fields including clinical chemistry. In fact, Rosalyn Yalow received the 1977 Nobel Prize in medicine for her pioneering work on immunochemical analysis. In many cases, the chemical structures now assayed by immunochemical methods are not fundamentally different from those of

Keith D. Wing □ Bruce D. Hammock

Antibodies obtained from the blood serum of animals such as rabbits aid in immunochemical analyses to detect pesticide residues.

pesticides. Immunochemical methods offer many advantages including high specificity and sensitivity, which lead to few cleanup steps before analysis and thus rapid, inexpensive assays.

The key reagents for immunochemical analyses are antibodies obtained from the serum of animals such as rabbits or goats. Although animals provide the reagent, only their serum in highly dilute form is used in the assay. To raise pesticide-specific antibodies in an animal, the pesticide is attached to a protein, and after injection, this conjugate stimulates the animal's immune system. Following immunization, the animal makes antibodies not only to the protein and the attached pesticide, but also to the pesticide alone. These serum antibodies bind tightly and specifically to the pesticide if they contact it.

Many immunochemical methods are available for the analysis of small molecules, all based on the principle of competitive binding. Thus, immunoassays are physical rather than biological assays. In our laboratory we use radioimmunoassay (RIA). For this procedure a known, constant amount of radiolabeled (hot) pesticide and specific antibody is added to each of a series of tubes. Known amounts of nonradiolabeled (cold) pesticide are then added and the components allowed to equilibrate. Because the antibody cannot distinguish between the hot and cold pesticide molecules, the presence of increasing amounts of cold pesticide causes displacement of increasingly greater amounts of hot pesticide from the antibody. The antibody-bound and unbound hot pesticide can be separated by many methods, and the bound/unbound ratio indicates precisely how much cold pesticide was added to the system. By this procedure one can construct a standard curve that relates decreasing hot pesticide bound to increasing cold pesticide added. One can then assay for unknown amounts of cold material in residue samples by an identical process followed by comparison with a standard curve. Many such analyses may be performed simultaneously.

Immunochemical assays have already been developed for a few pesticides, such as cyclodienes, parathion, and, in our laboratory, S-bio-allethrin. These assays compare favorably with conventional assays in sensitivity and reproducibility, but much less time is required per assay, because fewer cleanup steps are needed.

The studies with allethrin demonstrate another important advantage of immunochemical assays in that, after injection with a single isomer, the assay was most selective for this most insecticidal of the eight possible isomers of allethrin. Since pesticide manufacturers and regulatory agencies are becoming more interested in the isomeric composition of pesticides, this power of immunoassays to distinguish compounds based on stereochemistry may shortly become very important.

Other immunochemical techniques that may prove useful include enzyme-linked immunosorbent assay (ELISA), in which a spectrophotometric assay provides a measuring device. Radial-immunodiffusion or other precipitation techniques may permit rapid field determination of pesticide residues or deposits, because they require no sophisticated equipment. Such applications of immunochemical technology could include determination of pesticide drift and problems associated with applicator safety and worker reentry.

We are now working on development of RIAs for the insect growth regulators diflubenzuron and BAY SIR 8514, as well as several pyrethroids. All of these compounds show good larvicidal activity and are excellent candidates for registration against mosquitoes and other insect pests. In addition, we will perform a comprehensive study of the time, cost, and labor involved in classical residue techniques as opposed to immunochemical techniques.

There are no panaceas in analytical chemistry, but immunochemical methods promise to solve some problems of pesticide analysis. Reducing the cost of residue analysis may reduce the final cost of pesticides and will possibly increase the number of new compounds available to mosquito control districts and to other small-volume users.

Residue analysis is currently so expensive and equipment-intensive that it is used only to obtain pesticide registration and for subsequent enforcement and monitoring. The speed, economy, and simplicity of immunochemical analysis may allow these procedures to be used to monitor pesticide application methods and coverage. Such new uses may provide valuable information when assessing the efficacy of a compound or when tailoring pesticide use to integrated pest management programs.

Keith Wing is Research Assistant, Department of Entomology, and Bruce D. Hammock is Assistant Professor of Entomology and Entomologist, Division of Toxicology, and Physiology, University of California, Riverside, CA 92521.