PESTICIDES[1]

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INTRODUCTION

A great number of living creatures, including microorganisms and plants may, under certain circumstances, become noxious, destructive, or otherwise troublesome, so that they may properly be regarded as pests. Adequate control of such pests is necessary for Man's survival. There are many different ways in which a particular pest might be controlled. This review is primarily concerned with the use of chemical pesticides for this purpose.

The choice of the most appropriate method of pest control depends upon many factors. In general, it calls for an evaluation of the likely benefits and risks. The benefits of pest control, in such terms as increased yields, improved quality of products, reduction in waste, more food, cheaper food, and human and animal disease eradication and control, are vast. The pharmacologist is more concerned with the risks and it is this aspect that will be mainly discussed in this review. Chemical pesticides are generally easier to apply, more certainly efficacious, and economically more competitive than many other methods of pest control. The main disadvantages arise from the introduction into the environment of chemicals that may have unwanted effects on other living creatures. These unwanted effects must be defined, detected, and, if necessary, prevented or controlled. Many of the problems that may arise in the course of pest control, such as the development of resistant pests, are not peculiar to the use of chemical pesticides, but may also occur with other methods of pest control. The use of pesticides is a complex field and to facilitate discussion of current problems this article has been arbitrarily divided into four parts: (*a*) pesticides versus pests; (*b*) residue problems; (*c*) toxicological aspects; and (*d*) control measures.

PESTICIDES VERSUS PESTS

New Pesticides

Selectivity of pesticides.—Pesticides are chemicals chosen because they have been shown to have a relatively selective deleterious effect on a pest or group of pests. This selectivity may broadly differentiate between different kingdoms, classes, or families of living creatures. Thus, pesticides may be chosen to be effective against plant pests that have little, if any, effect on animals, or substances may be selected as potent insecticides that are relatively harmless to mammals. In some instances a much more subtle selectiv-

[1] The survey of the literature pertaining to this review was concluded in June 1966.

ity has been sought; for example, an insecticide effective against a pest species may be needed that has no deleterious effect on useful insects, such as bees. Many factors are involved in achieving a selective action. These include the chemical and physical properties of the pesticide, anatomical or metabolic peculiarities of the pest as compared with other creatures, careful planning and timing of the application of the pesticide, or the use of special formulations, methods of application, and appropriate synergistic agents. By these and other ways, every effort should be made to increase efficacy and diminish risks.

New developments may include the discovery of new pesticides, better understanding of their mode of action, or increased knowledge of their metabolism. These studies may also involve the investigation of the development of resistance to pesticides. This is potentially a serious problem, but the existence of resistant pests sometimes provides a valuable research tool to elucidate the significance of metabolic changes or differences in penetration or distribution of pesticides. The development of resistant pests may call for the use of a new pesticide or a combination of pesticides, or some other method of control. More effective changes in strategy or tactics can be made if the mechanism by which resistance has developed is understood.

The need for new pesticides.—There is a continuing need for the development of new pesticides (1). As already mentioned, there is always a risk that pests will develop resistance and one useful way of combatting this may be to change to another pesticide or to use a combination of pesticides. Second, many pests are not yet adequately controlled by any method; thus, as it was with antibacterial agents twenty-five years ago, so it is now with pesticides, there is a need for a great increase in the number of pesticides available and their range of action. Third, many potent pesticides are prohibited or severely restricted in use, because of the risk of causing serious deleterious effects to other living creatures; there is a need for efficacious pesticides that are safer-in-use. Fourth, some pesticides are excessively persistent. Long persistence at the site of application is often advantageous; however, if persistence continues when the pesticide is removed from the site of application, and if this allows recognizable residues to pass from the soil into crops and through the various food chains into a wide variety of animals, problems are bound to arise. It may be true that small residues will do no harm to the plants or animals in which they can be detected. It is, however, difficult, if not impossible, to prove this conclusively. There is no apparent benefit in the indiscriminate distribution of persistent pesticide residues; many people do not regard the widespread occurrence of pesticide residues in soil, water, plant, and animal tissues with equanimity, even if the amounts are small and even if these residues are indeed harmless. Thus, there may be a need to develop pesticides that do not persist for a longer period than that necessary to achieve the agricultural purpose for which they are applied.

The development of new pesticides.—Thirty-seven pesticides in common

use were reviewed by a joint FAO/WHO Committee in 1963 (2) and this report provides a useful baseline for the assessment of any change in the pattern of common pesticides. Organochlorine pesticides were widely used, but restriction in the use of some members of this group has been recommended because of the widespread occurrence of residues (3–5). It is too early yet to determine what major changes in pattern of use may emerge as a result of these recommendations or what new groups of pesticides may become established. So far as can be judged from published work, interest has been shown towards esterase-inhibiting agents, such as organophosphorus compounds and carbamates (6–12), dinitrophenol derivatives (13), fluoroketones and their semithiocarbazones (14, 15), imidazole (16), and nicotine derivatives (17).

Mode of Action, Metabolism, Development of Resistance, and Synergism

Increased knowledge of the mode of action and metabolism of a substance in a wide range of living creatures is likely to help in the search for new pesticides. Recent advances in this field have been reviewed by O'Brien (18). The number of published papers dealing with work on the mode of action or the metabolism of pesticides is relatively small, although it is appreciated that there may be a great deal of work of this nature going on in industrial laboratories, which has not been published at the present time for commercial reasons. It has been a common practice in the past to leave work in this and related fields largely to industrial laboratories with a special interest in pesticide manufacture. While many of these laboratories carry out excellent research studies in this as in other fields, there are difficulties in this policy that have to be faced. The pesticide field is highly competitive and fruitful new ideas are not likely to be freely exchanged; publication may need to be restricted to allow for development of the new pesticide. Some ideas may turn out to be unrewarding; in this case, information that might be of value to other people may be discarded without publication. Furthermore, the ideal highly selective pesticide acting on only one pest species and relatively harmless to other creatures may not be an attractive commercial proposition; restriction of sales due to its selective action may make it impossible to cover the expenses of the research and development needed to put it on the market. For these and other reasons it seems desirable to encourage more fundamental research in university laboratories and appropriate research institutes on the mode of action, metabolism, and selective toxicity of pesticides. It is an appropriate field for investigators in such laboratories. Some of the fundamental aspects of selective toxicity have been discussed recently by Albert (19). It is remarkable that the precise mode of action of such widely used pesticides as chlorophenothane (DDT) and organophosphorus compounds is still unknown. The fatal effect of chlorophenothane on insects seems to be caused by interference with conduction of the nerve impulse along nerve

trunks associated with an efflux of potassium (20–22). Perhaps the recently demonstrated charge-transfer complex formed between chlorophenothane and a component of the axon of the nerve trunks of the American cockroach may furnish an explanation of this effect (23, 24).

The metabolism of chlorophenothane has also been extensively studied. Modification of the chlorophenothane molecule occurred, especially in the liver. The most recent modification reported was conversion of chlorophenothane to DDD in rats and mice, and by yeast and other organisms (25–29). It has been shown recently that chlorophenothane and many of its metabolites stimulated the development of drug metabolizing enzymes in the liver microsomes. Five ppm in the diet caused stimulation. Metabolism beyond the DDE stage diminished the ability to stimulate the liver in this way. With chlorophenothane, the effect was observed in the rat, but not the mouse; chlordane, however, was effective in both rat and mouse (30). Species differences of this description might be important in choosing the most suitable animal for long-term predictive studies. Resistance to chlorophenothane has been largely ascribed to the development of chlorophenothane dehydrochlorinase. However, resistance to chlorophenothane may be more complex than this and may also involve difficulties of penetration or translocation in resistant insects or animals (31).

Organophosphorus compounds and certain carbamates are esterase inhibiting substances. Cholinesterase is inhibited either by competition for receptor sites or by phosphorylation (32). These two properties can be separately assessed and largely predicted (33, 34). Nevertheless, the extent of the anticholinesterase effect correlates poorly with the occurrence and severity of toxic effects. The possibility that the inhibition of some other esterases may be a more significant factor in toxicity has been extensively discussed (35), but no conclusive evidence has been presented.

Synergistic action has been shown between EPN (ethyl-*p*-nitrophenylphosphonothionate), TOCP (tri-*o*-cresyl phosphate), and malathion. This synergistic effect has been attributed to inhibition of carboxyesterase, which readily degrades malathion in the liver of vertebrates (32, 36). EPN also showed a synergistic effect with malathion against resistant houseflies and mosquitoes in which carboxyesterase activity was demonstrated (37–40). EPN also inhibited the degradation of dimethoate in mice (41). EPN synergized the effect of sarin (isopropyl methylphosphonofluoridate), but this effect appeared to be due to interference with the binding of sarin in the tissues, especially in the lungs (42). The synergistic effect has been studied in a wide range of organophosphorus compounds; a number of triphenyl phosphates or phosphonates and tris(alkylthio) phosphate were active as synergists (43). It may well be that the effect of EPN on carboxyesterase is not the complete explanation of its synergistic effect on malathion. A more detailed study of its effect on all the enzymes and factors concerned in inactivation is necessary.

TOCP synergized malathion and also caused demyelination in hens. Various metabolites of TOCP appeared to be involved in the latter effect (44). A group of organophosphorus compounds was studied for synergistic effect on demyelination (43), but the mechanism of this effect remains a mystery.

The mechanism of action of nicotine derivatives has been studied. Pesticidal action depends upon a similarity to acetylcholine in electron distribution and conformation; adequate basicity of the compound is required, but quaternization of the nitrogen results in loss of activity. Metabolic studies on a number of other potentially interesting substances are slowly making their appearance.

Comments on Developments

It is difficult to escape the conclusion that insufficient attention is being given to studies on mode of action, metabolism, synergism, and the mechanism of resistance to pesticides. Studies in this field should form the foundation of new knowledge upon which future developments can be based. Excellent work is being done, but further support is needed.

Survey of the literature also gives another impression. Investigators often seek to explain phenomena, such as resistance to pesticides or synergistic action, on a unitarian basis. Thus, the effect may be attributed to enzymatic degradation, to enzyme inhibition, or to some change in penetration. There are some indications that several of these factors may be involved, so that a broader approach to the problem might sometimes be advantageous. Full use is not always made of appropriate studies in resistant pests; effects that are as easily produced in resistant as in susceptible pests are unlikely to be closely involved in the basic mode of action of the pesticide.

RESIDUE PROBLEMS

Many substances have been introduced as pesticides during the last few years. The persistence of these substances after their application varies. The rate of degradation of the pesticide and the nature of the degradation products are important characteristics that require detailed study. The organochlorine group of pesticides has given rise to residue problems on a large scale. Some others, especially fluoro compounds, and substances containing mercury or arsenic, might also cause difficulties if they were to be more widely used.

Two factors have contributed to the residue problems associated with the organochlorine pesticides; their wide use and the development of extremely sensitive methods for the detection of residues derived from them. The wide use is no doubt due to the fact that these pesticides are extremely efficacious and easy to apply, since the members of the group are not acutely toxic to man or other animals. The benefits derived from these organochlorine pesticides are immense. The widespread use of chlorophenothane in malaria

eradication programs has conferred remarkable benefits on millions of human beings; this is but one example of the benefits derived from the use of organochlorine pesticides.

First, it may be useful to review the developments that have occurred in the analysis of pesticide residues; secondly, the nature of the residues found and their distribution will be considered; thirdly, an attempt will be made in a later section to analyze the present situation with regard to residues in relation to the possible benefits and risks.

Analytical Methods

So many different chemical species have been used as pesticides that a wide range of analytical methods has been needed for their detection and measurement. The perfect analytical method would be a procedure sensitive to microgram quantities or less and specific for the particular pesticide under examination. Such an ideal was rarely possible.

Clean-up procedures.—Normally a clean-up stage was necessary before the residue could be analyzed. The object of the clean-up method was to concentrate all the residue without loss, and reduce to a minimum the substances that might interfere with the final analysis. If this process did not extract all the residue, then the final analytical test, even if completely specific and accurate, gave a lower result than the true value. The efficiency of the clean-up method was usually measured by the recovery of known amounts of pesticide added to a typical sample (45). This gave a measure of the recovery in an artificial mixture, but in practice pesticides might be in a bound form and not extractable under these conditions. Similarly, residues of pesticide metabolites were of great importance, and in the clean-up these also had to be extracted. The only true way of measuring the efficiency of a clean-up method under these more stringent conditions was to feed the plant or animal on radioactive-labelled pesticide (46, 47) and then measure the residual activity in the sample after clean-up.

Many clean-up procedures have been reported for use in the analysis of pesticide residues. Organophosphorus residues in fruit have been cleaned up by fractional vacuum distillation (48), but in all clean-up methods the normal constituents of the sample have to be taken into account. Thus, for the analyses of fruit and vegetables (49) for residues of the organochlorine type pesticide, the sample could be readily cleaned up by extraction with acetone (50) or acetone/benzene mixture (51). For the analysis of similar pesticides in fatty material, extraction with dimethylsulphoxide (52), methylene chloride/hexane (53), or acetonitrile/hexane (49, 54) has been used. McKinley et al. (55) recorded clean-up methods for ten different pesticides in the organochlorine and organophosphorus groups and suggested that a combination might give a general clean-up method. The basic clean-up method developed by Mills et al. (49) for organochlorine residues has been adapted for organophosphorus residues (56), but with variable success. The problem of the

clean-up of multiple residues of organochlorine pesticides in food has been examined by Johnson (57).

The initial cleaned product was sometimes adequately purified and concentrated for final quantitative analysis, but many reported clean-up methods required a second chromatographic purification stage. The particular method employed was usually the most simple one that would do the task efficiently. In the cleaning-up of organochlorine residues, Baetz (58) reported that 13 different pesticides in this group were freed from interfering substance by passage of the initial clean-up extract down a column of acid-washed Norite-A followed by selective elution. Other workers (53, 59) have reported the use of fluorosil columns to remove lipid material from the initial methylene chloride extract from fat and dairy samples containing organochlorine residues. Using the same column material, but with a different elution solvent, organophosphorus pesticides (60) have been successfully purified and concentrated. Arbor-Celite (61) and silica have also been used as column absorbents in pesticide residue clean-up procedures. Column chromatography was useful in that large sample sizes could be used, but with the increasing sensitivity of paper, thin-layer, and gas-liquid chromatographic methods, column techniques have tended to be less used.

Paper chromatographic methods.—Paper chromatography has been widely employed in the field of residue analysis. It was particularly useful in the separation of polar substances, especially phosphate ester. The application of paper methods to organophosphorus residues was examined by Getz (62), who gave several different methods for organophosphorus analysis and suggested that a combination of the reported methods might give a general separation and clean-up procedure. Bates (63) considered that paper chromatography was useful as a general analytical method for organophosphorus residues in foodstuffs. Organochlorine residues have been detected by paper chromatography (64), but thin-layer and gas-liquid methods were preferred.

Thin-layer chromatography.—Thin-layer chromatography was found to be more rapid and often a more sensitive technique than paper chromatography. With a careful choice of absorbent, both polar and nonpolar substances were separated. With the introduction of cellulose as absorbent, many paper methods were transposed on to thin-layer. Thin-layer chromatography has been successfully used to resolve most pesticide residues (65, 66). Walker & Norton (67) have described a single thin-layer procedure for the analysis of 62 different pesticides and quote the R_F values in 19 different solvent systems. Methods for carbamates have been recorded by Chiba & Morley (68). Thin-layer methods have been made even more rapid with the use of microplates, and methods using these have been described for both organochlorine (69) and organophosphorus (70) residues.

Detection of spots on paper and thin-layer chromatograms.—Many detection methods were applicable to both paper and thin-layer chromatograms, but because of the absorbents used in the latter, more violent detection

methods were appropriate. Organochlorine residues were readily detected by their reaction with 5 per cent 2-toluidine (71), or with silver nitrate 2-phenoxyethanol reagent. The latter has been examined by Mitchell (72) who recorded its sensitivity for a number of organochlorine pesticides. The overall problem of detection of organochlorine residue spots has been examined by Katz (73). A number of methods have been reported for the detection of organophosphorus pesticides on paper and thin-layer chromatograms (74, 75). Probably the most simple detection method involved the oxidation (76) or hydrolysis (77) of the organic moiety of the pesticide to leave inorganic orthophosphate which was detected by the standard molybdate-blue method (78). Very low levels of organothiophosphate pesticides were detected by the sensitive and specific tetrabromophenolphthalein ethyl ester/silver nitrate/citric acid reagent (79), whereas other organophosphates were detected with 4-(*p*-nitrobenzyl) pyridine reagent (80).

Gas chromatography.—This technique was undoubtedly the most sensitive of the chromatographic methods available to the pesticide analyst. The limit of the method was set by the vapour pressure (81) of the pesticide at the column temperature; by temperature programming (82), many different residues have been determined simultaneously in the same sample. If the pesticide was insufficiently volatile, as was often the case with carbamate pesticides, then volatile derivatives (83), such as those formed by reaction with trimethylsilyl chloride (84), were examined and analyzed. Many detection methods have been developed for application to pesticide analysis. Organochlorine residues were determined with the electron capture detector (85–87). This was very sensitive to molecules containing chlorine and essentially insensitive to others. Thus, organochlorine residues caused a large response, whereas the normal constituent of a sample gave hardly any. This method for organochlorine analysis has been applied to residues in all types of samples, from human fat (88) to vegetable products (89). Microcolorimetric detection methods have been used (90, 91) to detect organophosphorus pesticides on gas chromatography. Again, this was a sensitive test and less than microgram amounts were easily detected. Most of the other available gas chromatographic detection methods have been applied to pesticide analysis. Hydrogen flame detection (92) has been used to determine pesticides containing sulphur or chlorine, while for phosphorus-containing pesticides the sensitive and highly specific sodium thermionic method has been employed (93). Flame ionization, a nonspecific detection method, has been used (93) to measure total substances extracted in a particular clean-up method, while the actual pesticides were detected by a more specific technique.

Limitation of chromatographic detection methods.—Pesticides were usually characterized in chromatographic techniques by their R_F values or relative retention times. Unfortunately, many substances had the same R_F value or relative retention time when run under a particular set of conditions. Thus,

the fact that a spot occurred on a chromatoplate, or a peak appeared in a particular position relative to a standard substance on a gas chromatogram, did not provide an absolute indication that a pesticide was present. For identification of pesticide residues, the spot had to be removed and extracted (94), or the peak trapped and the residue thus obtained examined by a positive identification method. This problem was often made intractable by the fact that destructive methods were used or the original detection method was far more sensitive than the available positive method; in such cases it was important to run the sample under more than a single set of conditions. If the R_F's or retention times were found to coincide with the corresponding values for the standard pesticide, this was regarded as strong evidence for the positive identification of the residue.

Positive identification methods.—Mass spectrometry has been applied to the positive identification of pesticide residues. This technique proved especially useful in the detection of carbamates (95) which were difficult to analyze by other methods, while other workers found that mass spectrometry was useful in the analysis of organophosphorus compounds (96). High resolution mass spectrometry was a sensitive test, and with some machines the molecular weight was given sufficiently accurately for the empirical formula to be deduced. Few pesticide laboratories were equipped for mass spectrographic analysis, so positive pesticide analysis had to be done largely by alternative methods.

Absorption spectroscopy.—Spectrophotometric absorption was useful in the identification and analysis of pesticide (97) residues, since the pattern of light absorbed by the molecule was specific and the amount of light absorbed was proportional to the concentration of the substance. Thus, by reducing the sample area while keeping the optical path as long as possible, very small amounts could be examined. Infrared and ultraviolet visible absorption have been used for residue analysis. Infrared absorption was usually more complex, but less intense than ultraviolet visible absorption, thus infrared was used mostly for identification of residues while ultraviolet visible absorption was used for quantitative analysis. Infrared spectra have been reported for the identification of organophosphorus (98), organochlorine (99), and carbamate (100, 101) pesticides. However, great care was needed in the identification of a residue by comparison of its infrared spectrum with that of a known standard pesticide (102). The spectrum of the unknown had to be run under identical conditions to those used for establishing the standard. The ideal state for infrared examination was a solution in which conditions were readily reproduced. In the past, some difficulties were encountered with the infrared examination of samples containing appreciable quantities of water, but these have been largely overcome with the introduction of attenuated total reflectance techniques which have been applied (92) to organophosphorus (98) residues. Spectra occurring in the ultraviolet visible region of the electromagnetic spectrum were usually a series of overlapping broad intense

bands which were ideally suited to quantitative analysis. Some pesticides were detected and determined by virtue of their own absorption spectra. This was possible with nicotine (103), triazine-based (104), and several nitropesticides (105), but many exhibited either weak or featureless spectra unsuitable for spectrophotometric analysis. This problem resulted in the development of certain specific chemical reactions that have been so chosen that the products were intensely coloured and hence suitable for this method of analysis. Organophosphorus pesticides were detected and determined colorimetrically by hydrolysis or oxidation of the residue to give inorganic orthophosphate which was analyzed as the molybdate-blue complex. Reaction with 4-(*p*-nitrobenzyl) pyridine (80) was used in the colorimetric analysis of organophosphorus residues, but this method was subject to interference. Chlorophenothane residues were determined as their tetranitro derivative (106), while many of the pesticides based on 1-napthol have been analyzed by hydrolysis to the parent phenol followed by coupling with a suitable diazotized amine (107, 108). Other colour tests have been reported for triazine (109) and carbamate residues (110, 111).

Spectrofluorometric analysis has been applied to certain organophosphorus residues (112), and MacDougall (113) has examined the potential of fluorescence for pesticide analysis. This technique was doubly specific, since absorption and fluorescence spectra were characteristic of the substance under examination and the technique was usually more sensitive than straight absorption methods and was less subject to interference.

Polarographic analysis.—Polarography has been used to analyze pesticides which had either reducible or oxidizable groups in them (114, 115). The technique had a sensitivity which was comparable with colorimetry, but it was more rapid, less subject to interference, and specific. The half-wave potential for the redox reaction was characteristic of the substance, and the wave height was proportional to the concentration of the solution. Polarographic methods have been reported for microamounts of zinc (116), lead (114), antimony (114), and compounds such as chlorophenothane (117), DDD (114), organophosphorus (118, 119), carbamate (120), and nitropesticides (121, 122). Thompson (104) has investigated the use of derivatives of triazine for polarographic analysis and reported extremely high sensitivity.

Bioassay of pesticide residues.—Some of the many compounds used as pesticides were so potent that trace amounts killed fish (123) or insects (124), or altered the relative enzymatic levels within a plant, animal, or tissue (8). The use of fish to detect residue of organochlorine pesticides in lakes was reported by Terrière et al. (125), while their more general use to detect pesticide residues was examined by Weiss (123). Usman (124) has detected organochlorine phosphorus and carbamate residues on actual plant leaves by their effect on *Aedes aegypti* larvae, but isolated tissue systems were used in most bioassay methods (126). The effects of pesticides on many tissues have been extensively examined and developed into sensitive assay methods (127–129).

This technique was applied to the detection of organophosphorus compounds on paper (130, 131) and thin-layer chromatograms (132), and the quantitative analysis of these compounds as residues in water (133), water plants, and milk (134). The acetylcholinesterase detection method was utilized in an automated method for organophosphorus residue analysis (135).

Other methods of pesticide analyses.—Some applications of radioactive analyses have been reported in pesticide analyses. γ-Hexachlorocyclohexane has been detected by isotopic dilution techniques (136). This was an ideal method for pesticide analysis, since any deficiency in the initial clean-up was eliminated. Other workers (137) assayed residues containing organochlorine and bromine pesticides by neutron activation analyses. This was claimed to be extremely sensitive and not subject to so much interference as other methods. Certain methods have been developed for the determination of specific pesticide residues. Thus, traces of organochlorine pesticides have been determined by degradation with metallic sodium (138) or titrimetric analysis (139). Parathion (140) and malathion (141) were detected by oxidation with chloramine-T, while dithiocarbamate residues were readily analyzed by assaying the carbon disulphide evolved on acid hydrolysis (142).

Comment on the Development of Analytical Methods

There has been a remarkable increase in the availability and sensitivity of analytical methods, and further developments and improvements will no doubt be made. In each year, about half of the published papers in the pesticide field deal with methods of analysis or the occurrence of residues. This may indicate some imbalance in the overall scientific effort in this field. It is important that studies of the biological significance of pesticide residues should keep pace with their detection and measurement. However, many more graduates in chemistry are produced every year than biochemists or biologists, and methods of analysis and assays of residues are more quickly developed and completed than the analysis of complicated biological effects. It is a matter of some importance that more work should be developed on the biological aspects of these problems; this calls for better training facilities, more encouragement and opportunities for those who can tackle the biochemical and toxicological problems involved. There is little point in gaining more and more detailed information about residues if their biological significance remains unknown. Nevertheless, adequate toxicological and metabolic studies are largely dependent on the availability of satisfactory methods of analysis. The great achievements of chemists in this field should be of immense value to biologists; the biological investigations should not be allowed to lag too far behind.

There are some other aspects of these chemical developments that call for comment. While analytical methods need to be of sufficient sensitivity, increased sensitivity is not the only important objective for research in the analytical field. There is a need for more adequate identification and mea-

surement of metabolites, more detailed study of the factors concerned in degradation and persistence, and more methods of sufficient sensitivity for the study of blood and tissue concentrations of a wider range of pesticides, to mention a few of the pressing current problems. Research in the analytical field needs to keep in close touch with requirements on the biological side.

The Occurrence of Residues

The existence of some form of dose-response relationship for every substance is a fundamental concept of pharmacology and toxicology. This carries the implication that there is always a level of intake at which the chance of any effect occurring is negligible. In practice, the no-effect level can be assessed in animals by experiment. The limitations are failure to detect an effect and the possible existence of species differences between the animals studied and man. These difficulties will be further discussed in the section on toxicology. With suitable safeguards, the method is considered to be acceptable and a margin of safety of at least 100 is commonly used. The concept of acceptable daily intake of pesticides for man has been thoroughly discussed by Stoner (143).

Many of the substances that may give rise to persistent residues have relatively low toxic potential as judged by short-term studies. Thus, the presence of such pesticide residues in the parts per billion range or less is unlikely to have any short-term toxicological significance. In the past, this problem of trace residues did not arise; they were ignored on the basis of a "zero tolerance," which implied the acceptance of the limit of detection as being equivalent to zero. The development of much more sensitive methods of analysis has made it necessary to abandon the "zero tolerance" concept. It may be possible to lay down some fixed daily intake level which could be applied to residues that only occur in minute quantities. Some form of tolerance level that ensures reasonable safety-in-use should be established for each pesticide, or some alternative control measures should be instituted.

Residues of pesticides have been widely demonstrated (144); thus, they occur in almost every part of the environment; for example, in soil (145, 146), from which they may pass into water; ordinary water purification methods do not remove all pesticide residues (147, 148). Residues have also been demonstrated in air and rain (149). As might be expected, residues can be demonstrated both in and on plants and forage crops (45, 50, 150–152). The latter has been shown to be an important source of residues found in carcass meat. Many other foods have been shown to contain detectable residues (153–159). Since food chains are involved, pesticide residues will also be found in the tissues of many different animal species (160–163) and in human adipose tissue (164–166). Many of these residues are present in extremely low

concentration; in human fat the mean concentration of dieldrin was 0.15 ± 0.02 ppm in the United States (165); this corresponds closely with the British figure (164). Other residues vary considerably from country to country. In general, the levels in the United States seem to be somewhat higher than those found in Britain.

While most attention has been directed towards residues of the organochlorine pesticides, there is now a greater awareness of the need for a more detailed study of degradation and persistence of pesticides. Residues have been reported of members of several other groups of pesticides, including organomercury and organophosphorus compounds and carbamates (167–170).

Comment on the Occurrence of Residues

The main features of the pesticide residues reported are first, that they are widespread throughout the environment and present in most animal and plant tissues; secondly, that they are generally present in extremely low concentrations; thirdly, that local concentration in particular animals or types of tissue, such as adipose tissue, may occur. The toxicological significance of these residues will be discussed in the next section.

The precise nature of the residues and the rate and pattern of further degradation is often unknown. There is a need for more detailed investigation of the factors concerned in the degradation and persistence of all new pesticides and of many already in use. Some of the pesticides at present regarded as nonpersistent might be found to form residues if more sensitive methods of analysis were available.

The effective replacement of over-persistent organochlorine pesticides calls for further research. Methods may be found that will cause effective degradation of unwanted residues, new pesticides may be discovered, or existing pesticides may be successfully applied as alternatives to those organochlorine compounds that need to be replaced. Significant progress has been made in field trials, using appropriate alternative pesticides against such pests as wheat bulb fly, cabbage root fly, carrot fly, bean seed fly, onion fly, pests of brassica, pea moth, various fruit pests, and sugar beet aphid (171).

TOXICOLOGICAL ASPECTS

Mechanism of Toxic Action and Assessment of Toxic Potential

The mechanism of toxic action is closely related to mode of action and metabolism of the pesticide, both of which have already been discussed in an earlier section. However, the importance of the relationship between chemical structure of a substance and its toxic effects has been recently discussed in a review by Medved & Kagan (172). Since small changes in chemical structure may profoundly affect toxicity, it follows that metabolic modifica-

tion may cause no alteration to, may promote, or prevent toxic effects. It is important to consider toxicology in molecular terms; this calls for a chemical and biological approach.

The assessment of the toxicological potential of a pesticide is based on consideration of its chemical and physical properties, the results of biochemical and toxicological studies in experimental animals, and information, if available, on toxic effects in man. The toxicological potential of 37 commonly used pesticides, including 12 organochlorine and 11 organophosphorus compounds, has been evaluated by an FAO/WHO Committee (2). Other published toxicological studies include metabolic and toxicological work in the organophosphorus compounds, fenthion (173), trichlorometaphos (174), Delnav (175, 176), haloxon (177), DDVP and Dibrom (2,2-dichlorovinyldimethyl phosphate), and the corresponding bromo compound (178), on carbamates (6, 179), on milon (3,5-dimethyltetrahydro-1,3,5-thiadiazine-2-thione) (180), on ovex (*p*-chlorophenyl *p*-chlorobenzenesulphonate), a miticide (181), and on a group of pyrethrum derivatives (182–184).

Methods of Assessment

Whole animal.—Studies involving the whole animal commonly form the starting point of a toxicological investigation. The criteria used are commonly rather gross changes, such as death of an animal. By using a range of doses, a level of intake can be determined at which no ill effect is observed. Methods of this type are relatively insensitive and the results may only apply under the experimental conditions used to the particular strain and species of animal studied.

Sensitivity can be considerably increased by the use of signs and symptoms instead of mortality. However, these are not easily elicited or measured in experimental animals, but the difficulty may, perhaps, be overcome by the study of the animal's behaviour. The effects of toxic substances on conditioned reflexes have been studied (185). Alteration in behaviour studies has also been carried out in rats as a means of investigating combined toxic effects. (186). There is considerable scope for development in this field.

Comparison has also been made of the effects observed in newborn or young animals with those observed in the adult. Since the pattern of enzymes is often different in newborn or young as compared with older animals and enzymes play a vital role in the metabolism of chemical substances in the body, considerable differences in toxicity may be observed when the same substance is given to a newborn or to an adult animal of the same species. Since metabolizing enzymes may not affect, may increase, or decrease the toxicity of the substrate, it is not possible to predict what differences, if any, will be found. Newborn rats have been shown to be more sensitive than older rats to the toxic effects of malathion, but less sensitive to DDT or dieldrin (187). Difference in toxicity of pesticides to weanling and adult rats

has also been observed (188). Further comparative studies on newborn, young, and adult rats should be encouraged.

Special preparations.—The metabolism and effects of pesticides may be studied in isolated preparations of cells or tissues. Recently, white blood cells and their contained enzymes have provided useful material for the study of certain toxic agents (127, 189). The chick and duckling embryo have also been used (190–192). As already discussed, there is plenty of scope for further development of enzyme studies in toxicological investigations.

Toxicological Observations and Occurrence of Poisoning

In man.—Toxicological studies in man are of three types: experimental investigations, accidental poisoning, and epidemiological studies. There is a need for metabolic studies at low dosage level in human subjects to establish the pattern of metabolism; such studies should not be done, however, until sufficient animal tests have been completed to give a clear indication of likely risks. A number of cases of poisoning have been reported from several different countries. It is important that such patients should be as fully studied as possible. It has been suggested that a mobile combat team, which could investigate cases of alleged poisoning on the spot, would be of value in this connection (193). Recent cases reported have included poisoning with various organophosphorus compounds (194–196), mevinphos (197), parathion (198), Diazinon (199, 200), mercaptophos (201), fluoro compounds (202), *p*-dichlorobenzene (203), thallium (204), and arsenic (205). The important points about this list are: first, the reports were from several countries; secondly, a wide range of pesticides was involved; thirdly, the number was relatively small if the vast number of people at risk is taken into account.

Epidemiological studies are difficult to undertake. They do, however, represent an important way of gaining more information about possible toxic effects in man. Those concerned with the manufacture, distribution, or application of pesticides should be kept under observation. Some studies of this type have been reported (206–210) and more are needed. An analysis of a group of 161 pesticide deaths revealed the fact that more than 50 per cent of them occurred in children; inadequate control of pesticides or carelessness in the disposal of containers is mainly responsible for the high level of poisoning in children (211).

In animals.—Farm animals are often at risk; a recent report described pentachlorophenol poisoning in swine (212). Effects on wild animals continue to receive attention. In a recent study on pheasants, chlorophenothane was administered in doses of 10, 100, and 500 ppm in the diet. Residues were found in the brain and this appeared to be a useful index of exposure. A similar correlation has been reported in rats (213). A dosage level of 500 ppm was toxic to the breeding birds, but the lower levels were not. At the 500 ppm level, all eggs contained residues, but egg production, fertility, and hatch-

ability were not affected. Chick mortality was highest in the group receiving 500 ppm of chlorophenothane. These observations indicated that the levels of chlorophenothane normally encountered were unlikely to harm pheasants or pheasant breeding (214).

Selective toxicity.—As already described, the ideal pesticide that is effective against one pest or group of pests and innocuous to all other living creatures is not likely to be a chemical. It is possible that the development of viruses against pests might provide a highly selective pesticidal agent. However, it should be remembered that the problem of resistant pests may still arise; the necessary range of viruses still has to be discovered; and this type of pesticide will not eradicate the pest, but reduce the pest population to a new point of equilibrium (215). Chemical pesticides usually have some broad specificity; thus, they are commonly categorized as herbicides, insecticides, or fungicides, indicating the group of pests against which some selectivity of action has been demonstrated. It should not be assumed, however, that herbicides are necessarily harmless to animals. Several studies have been published illustrating the toxic effects that some herbicides, such as 2–4D, may have on animals (216–219). Similarly, insecticides are not necessarily harmless to plants; a number of organophosphorus insecticides have been shown to have a phytotoxic effect.

Alteration in toxic potential may occur. Enhancement of toxicity has been described, under certain circumstances, on storage, although a decrease in toxicity is to be expected (220). This emphasizes the importance of more detailed study of the factors involved in degradation and metabolism. The other main modifying factor is synergism. Synergistic effects between some organophosphorus compounds have already been discussed (43). Synergism has also been described between phenothiazine and certain organophosphorus compounds (186, 221, 222). Such effects indicate the possible importance of controlling drug treatment in those likely to be exposed to toxic chemicals; it may also be relevant to the treatment of those who have been accidentally poisoned.

Toxicity of Certain Groups

Organochlorine pesticide residues.—It is most unlikely that the residues at present reported in the environment and animal tissues have any acute toxic significance. Considerably higher concentrations have been observed in experimental or occupational exposure without demonstrable ill effect. The danger of sudden mobilization of fat might arise if the residue concentrations were much higher than those so far demonstrated; the complete release of the trace amounts usually present does not present an acute toxic hazard.

So far as long-term effects are concerned, the evidence is less reassuring. The number of investigations so far carried out is few. In one life-span study in rats, a no-effect level could not be established and a significant general in-

crease in tumour incidence was observed. The pesticides administered were aldrin and dieldrin at levels of 0.5 ppm up to 150 ppm in the diet (223). While this study alone cannot be said to incriminate these residues, it is apparent that more work of this sort is needed before anyone can justifiably conclude that the risk of long-term effects from low concentrations of pesticide residues in the food chain and body tissues is negligible.

Toxicity of arsenicals.—Further information has become available on arsenical compounds. Arsanilic and acetylarsanilic acid have been shown to be poorly absorbed by hens and no evidence was obtained of any conversion to inorganic arsenic (224). Distribution and metabolism has also been studied in mouse, rat, cat, hen, and man. Species differences in toxicity were observed, the cat being the most sensitive animal (225). Detailed study failed to reveal any carcinogenic potential in the mouse and rat (226). The alleged human-species-specific carcinogenic action of arsenic, which is based on doubtful premises, was not supported by a statistical study of the causes of death in a group of individuals occupationally exposed to arsenious oxide (227). Any risk to sheep from arsenical dips can be satisfactorily controlled (228).

Comment on Toxicological Information

A considerable amount of toxicological information on pesticides from studies in laboratory animals is made available in the scientific literature. The collection and evaluation of this information by an international organization, such as the World Health Organization, is useful. The evidence of poisoning in human subjects tends to be scattered through a great many different journals. It would be helpful if this information could also be collected and critically evaluated from time to time.

CONTROL

Principles

As already discussed, the fundamental principle upon which the use of any pesticide should be based is evaluation of the balance of benefits and risks. If it is decided that a pesticide should be used, control measures may be introduced that aim to enhance the benefits and minimize the risks.

Enhancing benefits.—Many factors are involved, such as correct choice of pesticide, appropriate formulation, effective application, careful planning of the concentration used, and the timing of application both in relation to the ecological pattern of the area concerned and the subsequent use of the product. Much has been written about the possible replacement of chemical pesticides by so-called biological methods of pest control. These are usually taken to include such measures as breeding for resistance against pests, the use of

pest diseases or predators, and chemical and other methods of sterilization. With regard to the use of chemical sterilants, many of these substances present toxicological problems not very different from those arising with chemical pesticides; their indiscriminate use is potentially dangerous. As far as other biological methods are concerned, there appears to be broad agreement that any major shift from chemical to biological methods of pest control is impracticable in the foreseeable future. However, an intelligent approach to pest control should always be encouraged; biological methods may be suitable for certain pest problems and it may be possible to integrate chemical and biological methods to achieve more effective pest control (229).

Minimizing risks.—The first essential for effective control of toxic hazards is adequate knowledge of the chemical, physical, biological, and toxicological properties of the pesticide (172). Consideration of this information should provide a clear picture of the possible hazards, the groups within the community that might be at risk, and the most appropriate measures of preventing any significant toxic effects. Other aspects of toxicity must also be considered, such as toxicity to domestic and farm animals, damage to useful insects or other creatures, phytotoxic effects on other plants, and injurious effects on wildlife. It is not appropriate here to discuss all the measures that may be adopted to bring about effective control; it may, however, be emphasized once again that effective control must always depend upon adequate metabolic, biochemical, and toxicological knowledge.

SUMMARY

To conclude this review, some of the outstanding problems affecting the development, use, and control of pesticides today will be briefly summarized.

(*a*) Not enough work is being done on the biochemical, metabolic, and toxicological aspects of these problems. This work is necessary to provide a sound basis for effective control and safer use. (*b*) The great advances in analytical methods are not being fully used to press forward more biological studies and to gain a more detailed understanding of the factors concerned in degradation and persistence of pesticides. (*c*) Greatly increased sensitivity of analytical methods has resulted in an intensification of residue problems. Extremely low concentrations are unlikely to give rise to any acute toxicological risk; long-term risks are more problematical and call for more intensive study. (*d*) The development of new pesticides depends on fundamental studies on the mode of action, metabolism, and mechanisms of resistance to pesticides. A great deal of work is undoubtedly going on in this field in industrial laboratories; this field of study deserves wider attention. (*e*) More extensive epidemiological studies and more detailed investigation of patients accidentally poisoned with pesticides are needed. (*f*) Pesticide poisoning would probably not occur if everyone concerned read and obeyed

the instructions for proper use; in view of the large proportion of children that appear to be victims of pesticide poisoning, more attention should be paid to safety measures that are not so dependent upon intelligent human cooperation. The situation with regard to the use of pesticides is not so alarming as many would have us believe; there is a continuing need, however, for more research in this field, especially on the biochemical, metabolic, and toxicological aspects of pesticide problems.

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