TOPIC 4B

ENVIRONMENTAL AND ECOLOGICAL ASPECTS OF PESTICIDE USE

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EFFECTS OF PESTICIDES ON THE ENVIRONMENT

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ABSTRACT

Although pesticides provide benefits in our battle to control pests that destroy more than a third of all food, the use of pesticides also results in significant costs to public health and the environment. As many as 500,000 humans are poisoned annually by pesticides in the world; the United States alone reports about 45,000 human pesticide poisonings. Pesticides have also caused many detrimental effects on agroecosystems and natural ecosystems. These include: (i) reducing and sometimes eliminating beneficial natural enemies that keep pest populations at low densities; (ii) increasing or decreasing reproduction in various animal populations; (iii) altering decomposition rates of soil organic matter; (iv) developing insecticide resistance in pests, which results in increased pesticide use and additional crop losses; and (v) eliminating honey bee and other bee populations in certain regions thus reducing crop yields and quality. Pesticides also have had major impacts on fish, bird, and mammal populations.

INTRODUCTION

Pests in the world today are destroying about 35% of all potential food crops before harvest (Pimentel and Pimentel, 1979). These losses to pests are primarily due to insects, plant pathogens, and weeds. After the crops are harvested, an additional 10 to 20% of the crops are destroyed by insects, microorganisms, rodents, and birds. Thus as much as 48% of the potential world food material is being destroyed annually by pests, despite all efforts to control pests with pesticides and other nonchemical controls.

The quantity of pesticides applied annually to world agricultural crops for pest control is estimated to be 4 million tonnes (CSM, 1978). Although a large quantity of pesticides is applied, only a small percentage reaches the target pests. Less than 1% of the pesticide generally gets to the target pests (PSAC, 1965; Graham-Bryce, 1983). Most of the pesticide therefore reaches the nontarget sectors of the agroecosystems and/or spreads to the surrounding ecosystem as chemical pollutants.

Pesticides generally are profitable to use in crop production. On average pesticide use on crops returns about $4 per $1 invested for pest control (Headley, 1971; Pimentel et al., 1978). These are direct benefits and do not include the social and environmental costs (Pimentel et al., 1980). The environmental and social costs are significant and include human poisonings, domestic animal poisonings and contamination, impacts on agricultural production, destruction of wildlife, and alteration of natural ecosystems. This paper assesses the effects of pesticides on the environment including human health.

HUMAN PESTICIDE POISONINGS

Human pesticide poisonings are a major concern in using pesticides in agriculture. It has been estimated that annually in the world, there are about 500,000 reported pesticide poisonings (WHO, 1981). What proportion of these are fatalities is unknown, but it could be about 5,000 annually. In only four of the Central American republics (Guatemala, El Salvador, Honduras, and Nicaragua), the estimate was about 3-4000 reported pesticide poisonings annually with about 10% fatalities (ICAITI, 1977). In the United States, the best estimate is 45,000 human pesticide poisonings annually, with about 3,000 of these serious enough to be hospitalized and therefore reported in the conventional channels (Pimentel et al., 1980). An estimated 200 fatalities occur annually due to pesticides, with about slightly more than 50 as actual accidental pesticide poisonings (EPA, 1974).

The humans that are poisoned come in contact with pesticides by various means. Workers are exposed during the production of pesticides and others are exposed when these materials are formulated, often in small formulation plants where safety measures may be more difficult to enforce. Another important means of human exposure is during the application of the pesticides; this includes loading sprayers and the actual application of the pesticide to the crop. Another important means is when workers are exposed to pesticide drift during aircraft application. Also, workers may enter treated crops soon after the treatment. In this situation they obtain pesticide by rubbing against contaminated foliage and poison is deposited on arms, legs, and faces (ICAITI, 1977). This was the primary means of pesticide exposure in field laborers in Central America (ICAITI, 1977).

Other means of humans obtaining semi-lethal and lethal dosages of pesticides is drinking contaminated water, breathing contaminated air, coming into contact with contaminated soil, and/or eating pesticide contaminated foods. In the United States, it is estimated that at least 50% of the foods sampled in supermarkets have detectable levels of pesticides (Johnson and Manske, 1977; McEwen and Stephenson, 1979; Johnson et al., 1981). These levels, however, are said to be "no-effect" levels.

Probably many pesticide poisonings and other health effects from pesticides go undetected because medical doctors are seldom familiar with the symptoms of pesticide poisonings. Consequently, the actual number of pesticide poisonings and health problems that are reported in the United States and world is probably underestimated. There is no question that human pesticide poisoning and other health effects are occurring, and that these are a serious worldwide concern.

EFFECTS OF PESTICIDES ON AGROECOSYSTEMS

When pesticides are applied in agriculture, numerous changes can occur in agroecosystems and adjoining natural ecosystems. Many of these changes can have a detrimental effect on agricultural production and quality of the environment. Some of these impacts are:

Natural enemies destroyed

Parasites and predators attack a great variety of pests and in some cases provide the primary means of control (Debach, 1964; Huffaker, 1980). When insecticides and other pesticides are employed, the poisons not only destroy the pest, but also have a severe impact on natural enemies of the target pest. In some cases the natural enemies that are destroyed are important in controlling certain other pests. When these natural enemies are eliminated, it may result in outbreaks of pests that were previously not a problem in the target crop (Pimentel and Edwards, 1982; Adkisson et al., 1982).

When pesticide use has destroyed natural enemies and resulted in secondary pest outbreaks in the crop, additional insecticide treatments are usually made to deal with these particular pests. In cotton, for example, it has been estimated that at least 4 to 5 additional treatments have been used to control the cotton bollworm and tobacco budworm due to the fact that the natural enemies of these two pests were destroyed when pesticides were applied to control the boll weevil (Pimentel et al., 1977).

Reproductive effects

Organochlorine insecticides can both raise and lower animal reproduction. For example, reproduction of raptorial and other bird species was found to be reduced due to sub-lethal exposure to pesticides. The major effect was egg-shell thinning due to the uptake of organochlorine insecticides, which caused changed calcium metabolism in these birds (Keith et al., 1970; Peakall, 1970). In general, aquatic fish-eating birds were more severely affected than terrestrial predatory birds because the aquatic birds acquired more pesticide via their food chains.

Pesticides have also been found to increase reproduction in invertebrates. For example, sublethal doses of DDT, dieldrin, and parathion increased egg production by the Colorado potato beetle by 50, 33, and 65% respectively (Abdallah, 1968). DDT has also been reported to increase the reproductive rate of spider mites (Hueck et al., 1952).

Species diversity and food chains

Pesticides can reduce species diversity or richness and thus, alter ecosystem functions (Pimentel and Edwards, 1982). In agricultural ecosystems, parasites are generally less tolerant of insecticides than their herbivore hosts (Croft and Brown, 1975). This may affect the function and structure of ecosystems. For example, treatment of cole crop plants with DDT or parathion reduces the number of attacks of herbivores, but reduces the number of parasitic and predaceous taxa even more (Pimentel, 1961). This significantly changes the species diversity and the complex structure of this ecosystem. There are fewer herbivore species and more dominance by even fewer species.

In orchards, where heavy applications of insecticides, fungicides, and herbicides are utilized, a significant reduction in species diversity resulted. This was especially noticeable with phytophagous and predaceous invertebrates (Menhinick, 1962). Some of the saprophytic types of invertebrates in the soil and litter were also reduced.

When carbaryl was applied to a grassland, the species diversity of the aboveground arthropods was reduced soon after treatment (Barrett, 1968). The phytophagous arthropod species populations recovered more quickly than the predaceous species populations.

Bioconcentration of pesticides

Pesticides in the environment may be taken up and concentrated in the tissues of organisms. It is generally assumed that bioaccumulation through the trophic level of food chains is a common phenomenon (Pimentel, 1971; Edwards, 1973a, b; Brown, 1978). Although organisms in the higher trophic levels tend to have higher pesticide concentrations, this concept of gradual concentration of residues through the food chain is heavily dependent upon circumstantial evidence and in some cases is probably oversimplified (Pimentel and Edwards, 1982). Fish and other organisms may obtain large quantities or levels of pesticides up to 100,000 times the concentration directly from their environment without consuming prey that have been contaminated with pesticides (Reinert, 1967, 1972; Benevue, 1972). Thus, one must be extremely careful in making estimates as to whether there is actual bioconcentration or whether the pesticides were obtained directly from the water or other environmental resource.

Organic matter decomposition and nutrient cycling

Most dead organic matter is broken down by the activities of the soil fauna and microflora. These organisms assist in cellulose decomposition and the decomposition of other refractory organic matter. This action incorporates the material into the soil and makes available nutrients for plant growth. Clearly, pesticides that reach the soil and affect the soil microfauna directly or indirectly may alter the decomposition and nutrient cycling in ecosystems (Edwards, 1973a; Thompson and Edwards, 1974).

Pesticides also can directly alter the chemical makeup of plants. These changes are often specific for the particular plants and pesticides that interact. For example, certain organochlorine insecticides have been found to increase the amounts of some macro- and micro-element constituents of corn and beans and reduce the amounts of others (Cole et al., 1968). Specifically, heptachlor in soil at dosages of 1, 10, and 100 ppm caused significant changes in the elements N, P, K, Ca, Mg, M, Mn, Fe, Cu, B, Al, Sr, and Zn measured in the corn and bean plants. Zinc concentrations were significantly higher (89 ppm dry weight) in bean plants treated with 100 ppm of heptachlor than in the untreated controls (55 ppm). Nitrogen levels, however, in the beans were significantly lower (5% in the treated plants) than in the unexposed controls (7%).

Changes in plant constituents caused by pesticides can affect the insects that feed on these plants. For example, when corn plants were exposed to recommended dosages of 2,4-D in the field, the nitrogen content of the corn increased, and this in turn, resulted in nearly a three-fold increase in corn leaf aphid populations (Oka and Pimentel, 1974). The corn was also 26% more susceptible to corn borer attack. The corn borer females were about 33% larger and produced about one third more eggs than the normal female moths. Similarly, caterpillars of the rice stem borer grew 45% larger on rice plants treated with 2,4-D than on untreated rice plants (Ishii and Hirano, 1963).

HONEY BEES AND POLLINATION

Honey bees and wild bees are well known for their essential role in the pollination of fruits, vegetables, forage crops, and other plants (McGregor, 1976). Thus, if pesticides destroy or reduce the populations of honey bees and other wild bees, they may have a significant impact on the agroecosystem as well as natural vegetation.

In some agricultural regions, such as California, where heavy use of pesticides has reduced bee populations and therefore reduced crop pollination, large numbers of colonies of honey bees have to be rented to replace the activity of the destroyed bees. In California, for example, about 700,000 colonies of honey bees must be rented annually at about $8 per colony to supplement natural pollination of almonds, alfalfa, melons, and other fruits and vegetables for seeds (Atkins, 1977).

Estimates of annual agricultural losses from poor pollination because of the destruction of bees by pesticides in the United States range from about $80 million (Atkins, 1977) to a high of $4 billion (McGregor, 1977). Atkins (1977) emphasized that poor pollination will reduce crop yields, but more importantly he points out that it may reduce the quality of crops such as melons and some fruits. In experiments with melons, Atkins reported that improved pollination increased yields by 10%, but that there was a 25% improvement in quality.

DRIFTING PESTICIDES INTO ADJOINING ECOSYSTEMS

Drifting pesticides are known to cause significant environmental problems. Drift occurs with any method of pesticide application, but the potential for problems is greatest when the pesticides are applied by aircraft. This is significant because only slightly more than half of the pesticides applied by aircraft land inside the target area (Ware et al., 1970) and large quantities of pesticides are applied by aircraft (USDA, 1976).

WILDLIFE EFFECTS

Bird and mammal populations in agroecosystems and in natural ecosystems can suffer from exposure to pesticides. The deleterious effects include death from direct exposure to high pesticide dosages, reduced survival, growth, and reproduction from exposure to sub-lethal dosages, and habitat reduction through the elimination of various food sources. Because of the scant data that exists on wildlife kills caused by pesticides, it is difficult to estimate the significant impact that pesticides have had in reducing mammal and bird populations (Pimentel and Edwards, 1982).

Aquatic ecosystems are frequently under stress from pesticides, and pesticides in particular are known to cause a significant loss in fishes. The impacts of pesticides in aquatic ecosystems include high pesticide concentrations in the water, which directly kill fish, and low level dosages, which may kill highly susceptible fish fry or eliminate essential fish foods like insects or other invertebrates (Pimentel and Edwards, 1982). In the United States, reported losses from direct fish kills from pesticides range from 0.2 to about 6 million fish, but these estimates of fish kills are considered to be low for many reasons (Pimentel et al., 1980).

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4B—S2

METHODS OF PREDICTION OF ENVIRONMENTAL EFFECTS OF PESTICIDES. FIELD TRIALS TO ASSESS THE HAZARD PRESENTED BY PESTICIDES TO TERRESTRIAL WILDLIFE

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ABSTRACT

The hazard to wildlife from a pesticide is dependent on the toxicity of the chemical and on the level of exposure. Field tests are often required to refine the preliminary predictions of this hazard which are based on laboratory studies. Field test techniques are reviewed for assessing the hazard to mammals, birds, earthworms and the honey bee and the advantages of the different techniques are compared by reference to field tests that have been conducted with a range of pesticides. It is strongly recommended that in addition to wildlife observations, positive sampling of wildlife is undertaken for biochemical, pathological and analytical studies. Results of such studies can lead to an understanding of the dynamics and toxicology of the compound and its metabolites in wildlife and allow greater confidence in the hazard assessment.

INTRODUCTION

In the United Kingdom, the use of pesticides is controlled under the Pesticides Safety Precautions Scheme (PSPS) (MAFF 1979) which is a voluntary agreement negotiated between Government and Industry. When new pesticides or new uses of existing pesticides are being considered, the risks to the user, the consumer of treated produce and to wildlife are assessed. Such assessment is based on the results of laboratory and field tests and the PSPS allows for the stepwise introduction of pesticides as the results of tests become available to provide the necessary assurance that the proposed use is safe.

The hazard to wildlife from a pesticide is dependent on the intrinsic toxicity of the pesticide and on the level of exposure experienced by wildlife species. The toxicity of the pesticide to a limited range of test species can be determined under laboratory conditions. The decision on whether further testing is required is based on the results of the laboratory toxicity tests and on the maximum level of exposure that can be expected to result from the use of the pesticide. The results of the laboratory toxicity tests may underestimate the toxicity to wildlife because of the presence in the field of species which are more susceptible to poisoning by the pesticide. It has long been recognised that there can be a wide variation in the toxicity of a pesticide to different species and it is not yet possible to extrapolate with confidence between species (Tucker and Leitzke 1979) or from laboratory toxicity data to the toxicity manifested under field conditions.

The prediction of the maximum level of exposure is based on the application rate of the pesticide and its known physical and chemical properties. In some circumstances, as for example with seed treatments or granular formulations, it is possible to do a 'worst case' analysis and predict with confidence the maximum level of exposure. However in many other cases, variable factors such as temperature, rain, soil conditions and wind can influence the actual exposure level experienced in the field. Because of the lack of confidence in the decision based on the laboratory toxicity data and the predicted exposure level, it is to be expected that in many cases further testing will be recommended with the objective of establishing the scale of the hazard to wildlife under field conditions. During the preliminary assessment of environmental hazard, it is sensible to consider the maximum possible hazard or 'worst case' and in many cases field testing may indicate that the actual hazard is less than initially predicted. The sequence of testing that may follow preliminary assessment has been reviewed by Bunyan and Stanley (1979) and can include cage tests with wildlife species, field trials and subsequently environmental surveillance of the commercial use of the pesticide.

In recent years, there have been major advances in the development of field testing techniques and the aim of this paper is to review the current techniques and to discuss their application. The assessment of the hazard to wildlife from a new pesticide for registration purposes is restricted mainly to assessment of the risk to terrestrial wildlife, in particular to mammals, birds, earthworms and the honey bee (*Apis mellifera*) and to aquatic wildlife, in particular to fish. Although the methodology described here has largely been developed for terrestrial vertebrate wildlife, it can be applied to other groups of species.

The assessment of the environmental hazard presented by the use of a pesticide takes place late in the development programme for the pesticide. It is only practical to establish the risks to wildlife when the proposed use of the pesticide is well defined and the target crop, application rate and time of application are known. Although it is often desirable to establish whether the application of the pesticide leads to direct mortality of wildlife, monitoring of exposure levels and positive sampling to enable biochemical and histological studies to be conducted can profoundly affect the interpretation of the results and lead to greater confidence in the eventual hazard assessment.

The design of the field test should take into account the available information on the toxicology, residues and environmental fate of the pesticide and needs to accommodate the characteristics of the field site and the available wildlife. The programme of observations and sampling should be compatible with the various crop husbandry operations that will be carried out and should be flexible to allow for contingencies due to factors such as weather and crop pest and disease outbreaks.

For convenience, the field testing methodology is reviewed for mammals, birds, bees and earthworms but it is assumed that any field test may need to assess the hazard to a combination of these species. Therefore the field test protocol must embrace the range of observations and sampling necessary to provide a complete hazard assessment. The results of tests conducted for registration purposes generally have a commercial confidentiality and are not normally published which inhibits the adoption of new techniques. In this review, where possible, reference is made to examples of field tests on individual pesticides.

FIELD TEST METHODOLOGY TO ASSESS THE HAZARD FROM PESTICIDES TO MAMMALS

The assessment of the hazard presented by new pesticides to mammals is aided by the extensive toxicological testing conducted during the development of pesticides with the rat, mouse, rabbit and other mammals. In particular, the long-term chronic toxicity studies provide a valuable insight into the toxicological significance of continuous exposure to low levels of a pesticide which can be similar to the dynamics of exposure under field conditions. The comprehensive knowledge of the biochemistry, physiology and pathology of species such as the rat and mouse and of their toxicological response to the pesticide often provides a sound basis for designing field tests and may indicate biochemical and histological lesions that can be exploited to monitor exposure in the field.

In addition to being valuable for assessing the hazard to mammals, the presence on field test sites of populations of small mammals and especially mice, voles and shrews can provide the opportunity to monitor the level and duration of exposure experienced by relatively sedentary populations. These species are easily trapped and individually marked and are also suitable for keeping in the laboratory. It is therefore possible to conduct complementary laboratory studies to establish dose-effect relationships to assist in the interpretation of results from the field. There is extensive knowledge of the field biology of these species and it is thus possible to select appropriate species to examine particular environmental hazards. The wood mouse (*Apodemus sylvaticus*) is generally suitable for studies of seed treatments whereas the herbivorous bank vole (*Clethrionomys glareolus*) may be more appropriate for studies on herbicides.

In recent years there has been a continuing research programme at the Tolworth Laboratory to provide the necessary background knowledge on the biochemistry, pathology and ecology of wildlife species to allow their use in field trials. Enzyme profiles have been produced for a wide range of species including a number of mammals (Westlake *et al*. 1983). The wood mouse and the bank vole are particularly valuable species for field testing as they occur in reasonable numbers on agricultural land (Green 1979). Techniques are available for sampling these species and it is possible to collect small blood samples and to release the individuals. Other species such as the rabbit (*Oryctolagus cuniculus*) can be used to assess the hazard to grazing species but studying the larger mammals presents difficulties in terms of the size of the field test site and the necessary resources.

An early field trial used wood mice to monitor the environmental contamination that results from the use of HCH and organomercurial fungicides as cereal seed treatments (MAFF 1978). In this study, it was possible to demonstrate that there is a short-term 'pulse' of residues in wood mice within 14 days of drilling the grain but that the residues are transitory and rapidly decline. Complementary laboratory studies revealed than the maximum levels measured in the mice were substantially less that the minimum levels that produced toxic effects in the laboratory. The results of this field test are consistent with the experience of the commercial use of these seed treatments obtained during widescale surveillance exercises.

The replacement of the persistent organochlorine dieldrin as a cereal seed treatment by the more acutely toxic organophosphate insecticides, chlorfenvinphos and carbophenothion, presented a potential hazard to seed eating species. Two intensive field tests have been conducted to assess their direct hazard to seed eating mammals and birds and to provide information on the fate of residues on grain left on the soil surface after drilling which could present a hazard to wildlife. The study of the potential hazard from the use of chlorfenvinphos and an organomercurial fungicide as a seed treatment on winter wheat involved the trapping of wood mice over 3 ha of a drilled field and in immediately adjacent hedgerow and copse. Residues were monitored on the surface grain and in wood mice tissues and enzyme activities, including a range of plasma and tissue esterases and plasma glutamate oxaloacetate transaminase, glutamate pyruvate transaminase, glutamate dehydrogenase and sorbitol dehydrogenase were measured in the wood mice (Westlake *et al.* 1980). The results clearly demonstrated that wood mice were exposed for at least 30 days after drilling to residues of chlorfenvinphos and this was reflected in decreases in plasma acetylcholinesterase and cholinesterase activity during the first 10 days after drilling. The decrease in esterase activity was directly related to the chlorfenvinphos residue level in the wood mice. However, the residue levels and effects on enzyme activities were transient and were shown in complementary laboratory studies not to present a serious toxic hazard (Westlake *et al.* 1982a). The trapping of wood mice in the hedgerows revealed that the contamination was strictly limited to wood mice trapped on the field and even individuals trapped 1 metre away from the field showed no evidence of exposure.

The protocol for the field test to assess the hazard from the use of carbophenothion as a seed treatment was similar to that used for the chlorfenvinphos study and involved intensive trapping and marking of wood mice and measurement of enzyme activity and residue levels (Westlake *et al.* 1982b). A major difference between the results of the two tests was that carbophenothion proved to be more persistent on the surface grain. Whereas the level of chlorfenvinphos had fallen from the treatment rate of 600 ppm to 10 ppm within 3 days of drilling, the level of carbophenothion was in excess of 200 ppm after 30 days and still measurable after six months (Bunyan and Stanley 1979). Although the wood mice were exposed to carbophenothion residues for at least six months, laboratory studies demonstrated that the level of exposure produced only transient biochemical effects and did not present a risk of mortality. Mammals appear to be more able to metabolise a range of organophosphate pesticides than birds (Brearley *et al.* 1980) and this is often reflected in the higher toxicity of organophosphate insecticides to birds (Stanley and Bunyan 1979). Large numbers of geese died after consuming carbophenothion treated grain (Stanley and Bunyan 1979) and although it is believed that geese are particularly susceptible to carbophenothion poisoning, the persistence of the residue on the surface grain certainly contributed to the problem. Thus the results of the field test with carbophenothion helped to explain and resolve the wildlife problems experienced with carbophenothion.

Bunyan *et al.* (1981) in their assessment of the hazard to wildlife from the use of the carbamate nematicide aldicarb applied 'in furrow' with sugar beet seed, sampled wood mice, bank voles, shrews, rabbits and hares (*Lepus europaeus*). Because of the systemic action of aldicarb, the test was designed to assess the hazard to grazing species from residues in newly emerged sugar beet. Analysis of sugar beet seedlings indicated that the maximum residue level in young plants was less than 6 ppm and although small residues of aldicarb and changes in enzyme levels were detected in some mammals, the conclusion was drawn that the plant residues did not present an appreciable risk to mammalian wildlife.

A recent field test examined the hazard to wildlife from the use of the herbicide diclofop-methyl on spring barley and, in addition to wood mice, herbivorous bank voles were used to monitor exposure and the results interpreted by reference to laboratory feeding studies (Westlake and Tarrant 1983). In the free living rodents, although transient changes were noted in sensitive biochemical parameters there was some evidence of minor liver damage during the four weeks of post-spray sampling but this is not considered to have represented a longer term hazard to the resident animals.

Some pesticides can present a secondary poisoning risk to carnivorous mammalian predators such as the weasel (*Mustela nivalis*) but this is very difficult to assess under field conditions due to the low density of weasels on agricultural land and the practical difficulties of working with this species. At the present time, it is probably more reliable to carry out cage tests with such species to assess the secondary poisoning hazard (Townsend *et al*. 1983).

FIELD TEST METHODOLOGY TO ASSESS THE HAZARD FROM PESTICIDES TO BIRDS

Early field tests to assess the hazard to birds from new pesticides relied on casualty searches to reveal evidence of direct mortality. Such tests could only be expected to identify the most hazardous of pesticides. An alternative approach is to census the birds present before and after application of the pesticide but accurate and practical census techniques for application throughout the year are not available although it is possible to census breeding passerine species on agricultural land during the breeding season (Edwards *et al*. 1979). For field tests outside the breeding season or with non-passerine species it is valuable to establish an index of the species at risk based on the results of transect counts or programmed observations from a fixed point. This approach was employed by Bunyan *et al* (1981) in their study of the wildlife hazard from the use of a granular formulation of aldicarb.

Although birds are more mobile than small mammals, it is still possible to trap and mark individuals and to obtain samples for biochemical, histological and analytical studies. Techniques are available for obtaining blood samples from even small birds in such a manner that the birds can be released. Sophisticated techniques are available including telemetry for following individual birds in order to answer specific questions concerning the hazard presented by pesticides but, whilst such techniques may have occasional applications, the interpretation of results is difficult and more conventional approaches should be considered. Studies in the breeding season can be aided by observations on breeding success of individual species and where appropriate eggs can be collected for chemical analysis. Nest boxes can be used to attract certain species such as the starling (*Sturnus vulgaris*) to breed on the field site although this, of course, requires the test to be planned well in advance.

Background biochemical information is available on a range of British bird species from the research programme at the Tolworth Laboratory (Westlake *et al*. 1983). In addition, there have been a number of studies of the toxicological responses in birds following exposure to pesticides (Westlake *et al*. 1981a & b) and the results of these studies and the results of the toxicological studies conducted on the pesticide being considered may suggest biochemical or other measurements that can be used to monitor exposure in the field.

If during the field test, bird or mammalian casualties are found on the site, then it is essential that the specimens are submitted to a rigorous post-mortem examination to differentiate between an effect due to the pesticide application and background mortality from disease, trauma or pesticides being used on adjacent farmland. Where possible it is recommended that the diagnosis of pesticide poisoning is supported by chemical analysis and biochemical and pathological studies. In the case of organophosphate and carbamate pesticides, esterase reactivation techniques may be used to diagnose poisoning (Martin *et al*. 1981).

Bunyan *et al*. (1981) in their study of the hazard presented to wildlife by the carbamate nematicide aldicarb used 'in furrow' with sugar beet seed , assessed the hazard to birds both from the ingestion of granules left on the soil surface and from exposure to residues through other routes including their food. The study encompassed a risk index for the bird species present on the site, a survey of breeding success, casualty searches and sampling of eggs and birds. The interpretation placed on the results of the test was influenced by the analytical and biochemical studies conducted on the samples. At least one red-legged partridge (*Alectoris rufa*) died after ingesting granules and of the 31 birds sampled, 30% showed evidence of sub-lethal aldicarb intoxication based on the presence of residues and esterase depression. Residues of aldicarb were detected in birds 90 days after application which demonstrates that even pesticides that are not persistent require careful field evaluation. The results of the biochemical and analytical studies allowed greater confidence in the conclusions of the environmental assessment than would have been the case if the study had been restricted to the biological observations and casualty searches.

The Tolworth Laboratory recently conducted a field test to assess the hazard to wildlife, in particular, to birds of the repeated application of the insecticide methiocarb to a cherry orchard (MAFF, 1981). It was necessary to assess the hazard to species both feeding and breeding in the orchard and nest boxes were erected throughout the 6 ha study site in order to establish a resident breeding population of tits, sparrows and starlings. The breeding success of the birds using the nest boxes and of natural nests in the orchard was recorded and birds were trapped and individually ringed in the orchard prior to the study. Methiocarb was applied to the ripening cherries on five occasions with each tree receiving three applications. Regular casualty searches were made and birds were trapped throughout the study and blood samples taken for biochemical measurements. A number of birds were collected to monitor the pattern of residues. Many individual birds were trapped repeatedly and this allowed a time-related record to be produced for individual birds in addition to the more conventional analysis of results using statistically adequate samples. Significant decreases in plasma acetylcholinesterase (AchE) activity were found in house sparrows (*Passer domesticus*) immediately after the five sprays. The records for individual birds which exhibited depressed AchE activity demonstrated that the depression of activity was transient and that the individuals had successfully bred and moulted body feathers, both of which are demanding in terms of energy and nutrition. Therefore it was concluded that the exposure to the pesticide, although producing temporary biochemical changes, did not influence the survival of the birds. Once again casualty searches were of limited value in the hazard assessment.

FIELD TEST METHODOLOGY FOR ASSESSING THE HAZARD FROM PESTICIDES TO THE HONEY BEE

Honey bees are widespread beneficial insects which may be extremely important as pollinators of some agricultural crops. The acute toxicity to bees can be measured in the laboratory by well-established methods (Stevenson 1968, MAFF 1979 document D3) and this allows the identification of compounds likely to present a hazard to bees in the field (Stevenson 1978). Where the toxicity is revealed by laboratory tests to be high and where the proposed use is on or near flowering crops or plants which may attract bees, assessment of the practical risk must be based on the results of a field trial (MAFF 1979 document D4, Int.Comm. Bee Botany 1980).

Artificial tests may be conducted in flight cages where bees are confined on sprayed crops (Gerig 1979) but results may be difficult to interpret and to relate to field usage. Although the results of most trials remain commercially confidential for registration purposes, a number of studies have been recently published which illustrate the field approach.

Experimental hives, fitted with dead bee traps and pollen traps at their entrances, can be sited adjacent to the field trial site. Measurements of bee foraging activity in the crop, mortality at the hives, pollen analysis to identify the crop and monitoring the subsequent development of the brood in each hive provide information to allow adequate assessment(e.g. Shires and Debray 1982). The effects on bees of the application of the candidate pesticide can be compared with those of a toxic insecticide applied to other plots. Biochemical and analytical studies on samples of bees collected during field tests can provide evidence of the scale of exposure and the toxicity under field conditions.

The toxicity to bees demonstrated in the laboratory may not be realised under field conditions for a variety of reasons. The use of granular formulations of organophosphate insecticides presents minimal hazard to bees by reducing exposure compared with spray applications to the same flowering crops (Free *et al.* 1967). Evidence is accumulating that the agricultural use of synthetic pyrethroid insecticides on crops attractive to bees does not present the hazard predicted on the basis of high toxicity in laboratory tests (Shires and Debray 1982, Wilkinson and Bull 1983). This may be due to the repellancy of bees during or after spraying (Gerig 1979, 1981, Atkins 1981) in combination with the low application rates recommended for pyrethroid use (Smart and Stevenson 1982). Another practical example is that while the insecticide diflubenzuron completely inhibits brood production when fed directly to honey bee colonies (Dr. J.H. Stevenson, pers comm.), this has not been dected during field use in apple orchards (Emmett and Archer 1980) suggesting that practical exposure is too low to produce such an effect.

FIELD TEST METHODOLOGY TO ASSESS THE HAZARD FROM PESTICIDES TO EARTHWORMS

Earthworms play an important role in breaking down dead organic matter, aerating and mixing soil thus improving structure. Although laboratory tests can be conducted to determine the toxicity of pesticides, the results of field tests, though more variable, are generally easier to interpret and to relate to the use of the pesticide in the field (MAFF 1979 proposed doc. D6). Large replicated plots are treated at two application rates of the candidate product and a toxic standard (e.g. benomyl) can be used for comparison. Earthworm populations in each plot are sampled at intervals by formalin extraction in quadrats (Raw 1959) and compared with those of control plots. Chemical analysis may be conducted at intervals on cleaned worms to detect any evidence of the accumulation of pesticide residues. Casual observations during other intensive trials may provide useful information on toxic effect on earthworms. With their basic but important place to many food chains, information on the effect of exposure on earthworms is particularly useful during hazard assessment.

CONCLUSIONS

It is now possible to design field tests which can reveal subtle effects of pesticides on wildlife in addition to detecting gross effects such as mortality. The results of these tests will allow greater confidence in the assessment of the hazard presented to wildlife but such tests demand a multi-disciplinary approach to their design, execution and interpretation. Although the tests will require a greater investment of resources, it can be argued that the results may avoid costly errors in the future development programme of the pesticide. It is envisaged that surveillance of the commercial use of new pesticides may occasionally be necessary to provide additional assurance that the risks presented to wildlife are acceptable (Bunyan and Stanley 1979). However post-clearance surveillance can only be undertaken when the pesticide is in commercial use by which stage the pesticide company will have made a considerable financial investment in the development and introduction of the pesticide. It is clearly undesirable to the pesticide company and the environment for unacceptable hazards to be revealed during post-clearance surveillance.

It is recommended that pesticide companies consult registration authorities before preparing detailed protocols for field tests. This will allow the registration authorities to make constructive suggestions based on their experience of the hazards presented by similar compounds or similar applications.

Consideration is being given to the development of further techniques for assessing the hazard presented to wildlife. The use of behavioural studies for the early identification of pesticide intoxication is currently being evaluated. In addition techniques are being developed to enable the environmental hazard to be assessed for a wider range of wildlife species.

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4B—S3

ENVIRONMENTAL EFFECTS OF PESTICIDES IN THE TROPICS

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ABSTRACT

According to information available at present the use of pesticides in the developing part of the world will increase considerably over the next decades. Present and possible future risks to the environment are summarized in the present paper. A number of guidelines are discussed which may help to reduce or minimize these risks. An important conclusion is that risks posed by pesticides will vary largely from region to region according to factors like use pattern of the pesticides, geo-hydrological conditions, climate, life-cycle characteristics of species and the local structure and composition of ecosystems.

INTRODUCTION

Recent estimates indicate that approximately 25 percent of the world pesticide market is used in developing countries, mainly on cash crops (e.g. Anon., 1977, 1980; Balk and Koeman, 1983). However, it is expected that over the next one or two decades the consumption of pesticides by developing countries will increase considerably. FAO has carried out a study entitled, 'Agriculture: Towards 2000 (AT 2000)'(FAO, 1979) with the aim to develop long-term quantitative projections and production strategies in order to create a condition of self-sufficiency as far as food production is concerned in 90 developing countries in the year 2000. This implies that the agricultural output should be raised by about 4 percent per year and as the possibilities for an increase of arable land are very limited (estimated at 28 percent for the total of the 90 developing countries) the major output should come from increased yields per hectare. Strategies for yield increase may vary from rather simple methods to prevent pre- and post-harvest losses to very sophisticated techniques, requiring external inputs and large cash expenditures. Two basic means to increase yields are the use of selected high-yielding varieties of crops and the reduction of environmental constraints that hamper production through the application of pest management (including the use of pesticides), fertilizers, irrigation schemes, improved storage etc. The projections for pesticide consumption (excluding herbicides) in connection with developments in agriculture given in the AT 2000 report are summarized in Table 1.

Herbicide consumption in the developing world is still relatively low and comprises only 20 per cent of the worldwide consumption. As with regard to the other pesticides it is expected that their use will increase considerably (Anon., 1980).

The use of pesticides in other fields will probably increase as well. For instance in connection with the control of vector-borne diseases. According to the programma 'Health for All by the Year 2000' launched by the World Health Assembly in 1981 (WHO, 1981) one of the global targets is, that 'all possible ways will be applied to prevent and control non-communicable diseases'.

It is well documented that the use of pesticides may give rise to undesirable effects on non-target organisms and ecosystems. The main aims of the present paper are to summarize present and future risks pesticides may pose to the environment and to discuss a few guidelines that may help to reduce or minimize these risks.

TABLE 1

Pesticide use (excluding herbicides)

region	total used US $ million a)	US $/ha	total used US $ million	US $/ha	growth rate in total use, % p.a.
year	1980	b)	2000	b)	1980-2000 b)
Developing countries in:					
Africa	344	3.08	890	5.31	4.9
Far East	725	2.54	1908	5.28	5.0
Latin America	749	6.30	1695	8.78	4.2
Near East	265	4.64	607	8.17	4.2
Total of 90 developing countries	2083	3.63	5100	6.41	4.6
Low Income Countries	701	2.49	1949	5.38	5.2

a) The report does not indicate to which year the value of the dollar refers. However, it seems likely that the 1980 US $ is used.
b) Harvested area.
Source: FAO (1979).

THE USE OF PESTICIDES IN TROPICAL AGRICULTURE

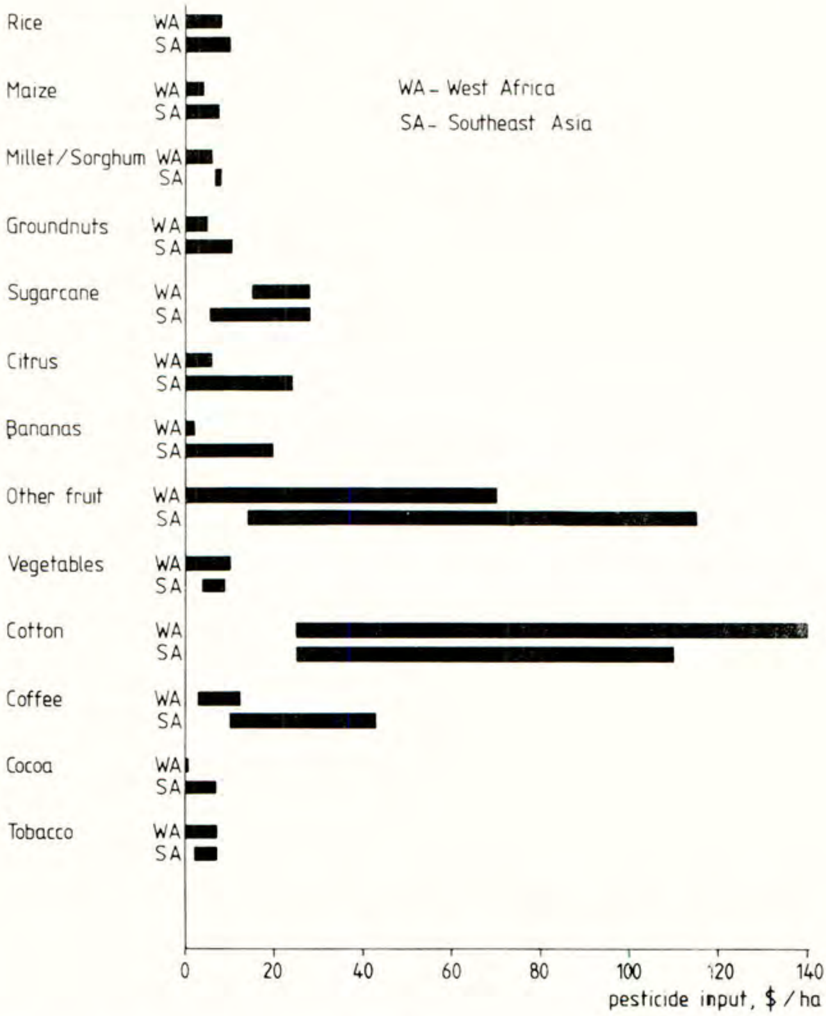
In the scope of the present paper no detailed overview can be presented of the present status of the use of pesticides per country or region. However, on the basis of available reports and publications (e.g. FAO, 1975; 1980; Skaf, 1979 and Wachter and Staring, 1981) a few general comments can be made.

From the available information the conclusion can be drawn that the use of pesticides in developing countries follows a general pattern of development. In an early development stage countries start using small quantities of traditional cheap products, e.g. some of the still commonly available organochlorine insecticides. When after some time the use increases, some legislative and regulatory measures are taken. The range of products widens, herbicides, fungicides and more modern insecticides such as pyrethroids become available and the distribution systems develop to higher levels of sophistication. The driving variable in this process is the general level of agricultural development or intensity of agriculture, as determined by the use of high yielding varieties, the application of fertilizer, and developments with regard to extension, communication, road networks etc. The level of the use of pesticides thus varies with the general level of agricultural development as well as with the general level of economic development. Peninsular Malaysia may for instance be considered as a region where relative high level of development has been achieved. This implies that a wide range of pesticides is used. There are other countries, like some West African countries where agricultural practise is still more traditional and where the use of pesticides is low or even declining.

A second comment concerns the variation in the average pesticide input per unit area in the crops concerned. In Fig. 1. a summary is given of the prospected average pesticide input in thirteen important crops. The figure

FIGURE 1

Pesticide requirements per crop, derived from the global technology matrix of FAOs AT 2000, and the crop yield per country for the year 2000, projected by the AT 2000 normative scenario (calculated for the total acreage of sprayed and unsprayed areas).



Source: Balk and Koeman (1983)

shows that in the year 2000 cotton will need the highest input of pesticides per ha in both areas considered (West Africa and Southeast Asia). Secondary to cotton is fruit. Coffee and sugarcane are also important consumers of pesticides. Likewise rice, groundnuts and vegetables will require a considerable investment per ha and these are, in contrast to the former, basic food crops which are grown almost everywhere. Maize, sorghum, millet, cocoa, tobacco and coconut will be less demanding as far as pesticide consumption is concerned, as a relatively large acreage will still be untreated. The overview given in Fig. 1. does not take into account the possibility that a higher input of biological methods of control in Integrated Pest Management schemes may become more important in future development. At present biological means of control (e.g. large scale release of Trichogramma spp) against different insect pests in cotton are for instance practiced successfully in the Soviet Union besides chemical means of control.

THE USE OF PESTICIDES IN VECTOR CONTROL AND OTHER FIELDS

In some developing countries, the amount of pesticides used in public health programmes may currently exceed the amounts used for the control of agricultural pests and diseases. In this connection one may think about the chemical control of mosquitos (Malaria, Yellow Fever), Simulium larvae (Onchocerciasis), tsetse flies (Trypanosomiasis) and snails (Schistosomiasis). From an environmental point of view it is important to stress the fact that chemical control of the vectors mentioned frequently takes place in more or less natural habitats. This implies that non-target organisms are more liable to get exposed to the pesticides than is the case in various agricultural applications.

As far as the types of pesticides used in the control of insect vectors are concerned, one may observe a shift from the use of organochlorine insecticides to the use of organophosphorous compounds and pyrethroids. Accurate quantitative data on the use of pesticides in public health have not yet been summarized in the literature. The same applies to other uses such as in forestry and livestock protection. The use of pesticides in forestry is not widely practiced in the countries concerned. However, this may change as soon as forest management becomes more intensive.

ENVIRONMENTAL EFFECTS OF PESTICIDES

Environmental effects of pesticides can be classified according to the values one may recognize in the functions of the environment.

Interference with productive functions

With regard to the possible impact of pesticides on the productive functions one may in first instance consider the direct impact on non-target species of economic importance. Fish kills and cases of mortality in prawns and shrimps have for instance been reported from various countries both after the use of pesticides in agriculture (e.g. in rice paddies, Santosa and Rustami, 1976; and vector control, e.g. Dortland et al., 1977; Koeman et al., 1978). The use of pesticides for catching fish should also be mentioned in this connection. Although official reports on fish kills following the use or abuse of pesticides are scarce, personal contacts with people in the field indicate that they occur frequently in many countries.

Other effects on productive functions are damage to pollinating insects and effects on organisms which serve as food for economic species. The pollinators comprise an array of species much larger than bees alone. Many economic tree species (fruit trees) but also forest trees fully depend on insects for reproduction. The presence of pesticides in water systems may depress the populations of aquatic insects, crustaceans and other invertebrates, that form an important source of food for fish and aquatic birds. The possible occurrence of long-term suppressive effects of pesticides on the total biomass has not yet been studied in any detail but certainly deserves further attention, especially in agricultural areas where the frequency of pesticide applications is comparably high.

Interference with regulatory functions

Pesticides may affect various regulatory functions of a natural system. The purification functions of the aquatic environment largely depend on the presence of metabolic activity exerted by a variety of plant and animal species, including primary and secundary producers as well as decomposers. Micro-climatic changes may occur in cases where the plant cover is altered by the use of herbicides. Subsequent changes in soil condition may cause soil degradation, erosion and a decreased water retention capacity of the soil. Again possible effects of this nature have not yet been studied in detail. However, full attention should be given to the subject for instance in connection with the use of herbicides in areas with potentially vulnerable soil conditions.

Pesticides may interfere with populations which play a role in the regulation of populations of other organisms, such as entomophagous insects, and parasites. For instance it was demonstrated in Saudi Arabia (Talhouk, personal communication) that the use of certain contact insecticides in dates may have such a detrimental effect on predatory insects (coccinellid and cybocephalid beetles) that the pest, the parlatoria date scale (Parlatoria blanchardii) becomes a much more serious pest than it would have been without insecticide treatment.

The applicaton of pesticides against one pest or disease may also lead to a shift to non-susceptible pests and diseases (pest resurgence). This has been observed in control operations against root-infecting fungi in cereals. This type of effect is likely to occur in particular with selective pesticides. Selectivity, which generally should be considered as a favourable property from an environmental point of view may thus have disadvantages too.

Interference with carrier and information functions

The carrier function is defined as the support given by the environment to man in connection with amenities like recreational functions and the availability of medicinal plants.

The information functions indicate both the use of information derived form the natural environment for orientation and education and the potential information (especially biotic) that should remain available as a reservoir, such as genetic resources of cultivated plants. Interference by pesticides may imply for instance that recreational functions will impoverish (e.g. loss of sport-fishing facilities; disappearance of attractive species) or that valuable resource information gets lost (such as the wild ancestors of cultivated plants). Under this heading one should also comprise the aspect of ethical values ('the moral right for survivial of a species').

REVERSIBILITY OF EFFECTS

Many environmental effects caused by pesticides have sofar been shown to be reversible, that means that populations of affected organisms restore quickly to their previous condition when a certain treatment has been completed. From observations made in connection to fish kills caused by single applications of endosulfan against tsetse flies in some African rivers the conclusion could be drawn that even in case of massive incidental fish kills repopulation of the affected stretches of river may take place fairly quickly. The repopulation could be attributed largely to immigration from untreated areas. Similar conclusions could be drawn with regard to other species like birds and insects in pesticide treated habitats (e.g. Koeman et al.,1971, 1978; Dortland et al., 1977, Everts et al., 1983).

The possibility for a resilience of affected populations by immigration from reservoirs elsewhere will strongly depend on the local availability of such reservoirs. Still in many agricultural areas and areas where vector control operations are practiced, there are untreated places which may serve as reservoirs for fish, birds, insects and other organisms and hence contribute to the reversibility of effects caused by pesticide treatment. Considering the ongoing activities with regard to land-use development one should realize that reservoirs become increasingly scarce. This could very well imply that environmental effects such as cases of local mortality in a species may become much less reversible in the not too far future.

Reversibility of effect will also largely depend on the frequency of pesticide application and on the persistence of the chemicals concerned. It should be realized that persistence not only results from applications of persistent pesticides. It may also be a consequence of frequent applications of relatively non-persistent compounds.

Irreversible effects are most likely to occur with pesticides which may cause long-term effects on reproductive functions in organisms. Examples are rare in this context. The most obvious example sofar is formed by the long-term reproductive effect demonstrated for DDT in a number of bird species, an effect which is reinforced by the persistant cumulative nature of DDT and some of its metabolites.

REGIONAL ASPECTS OF ENVIRONMENTAL EFFECTS BY PESTICIDES

The environment in the world shows a tremendous regional variability. As a consequence of differences in climate, geochemistry and physiography the ecosystem structure and performance varies as well. This implies for instance that in one location a pesticide is broken down fairly quickly (e.g. high ambient temperature and rich microbial life in soil or sediment) while this does not occur or at a much lower rate in other places. Likewise there may be large differences as far as phenomena like volatilization, adsorption, dilution and mobility are concerned. Other variables are the composition of the ecosystems (e.g. the presence or absence of relatively vulnerably species), differences in life-cycle phenomena (e.g. spawning periods and breeding seasons), the geographical distribution of the biota relative to the agricultural or other locations and the availability of reservoirs as mentioned before in this paper, which are treated with pesticides. Finally there is a large variation in the way pesticides are applied (application method, formulation, dose-rate and frequency of application). It is therefore virtually impossible to extrapolate experience gained with regard to environmental effects of pesticides from one locality to another. A proper assessment will always require that local circumstances are considered in detail. Therefore it is also not possible to make generalizations about environmental effects of pesticides in tropical countries. A pesticide which can be used in a harmless way in one place may turn out to pose unacceptable risks in other places.

GUIDELINES TO REDUCE ENVIRONMENTAL EFFECTS BY PESTICIDES

Sofar the principles outlined in the previous paragraphs are generally not considered in sufficient detail in the planning stage of pest and disease control campaigns. The following guidelines may help to avoid or minimize environmental effects of pesticides.

(1) In an early stage of the planning process an assessment should be made of the ecological characteristics of the region concerned (ecotoxicological valuation, Koeman 1983). This should comprise the following steps:
- a survey of the geography of the area in order to identify potentially vulnerable sites within or in the vicinity of the areas to be treated
- an assessment of the condition of soils, waters and sediments with regard to the possible fate of chemicals in the environment (leaching, dilution, degradation etc.)
- an assessment of the nature of disturbance cycles (scale and frequency of flood, drought, climate and agricultural cycles)
- an inventory of the life-cycle characteristics of economic and other species (fish, shrimps, fish food organisms, entomophagous insects, plants, soil microflora etc.)
- an inventory of general features (e.g. importance for protected and migratory species).

(2) With the ecotoxicological appraisal it is possible to review the proposed uses and to decide if modifications are needed to avoid unacceptable environmental effects. Possible modifications that could be taken into account are:
- the selection of the active ingredient, taking note of the specific toxic properties of the compound (e.g. toxicity for fish and bees)
- the formulation (e.g. granules or spray); environmental exposure concentrations may be less after application of granular formulations while the efficacy of the treatment might be sufficient
- the selection of the dose-rate in connection to the frequency of treatment. In tsetse control repeated low dose rate applications of endosulfan near water-courses did not cause any fish mortality while the efficacy of operations was similar to single high dose-level application which caused mass mortality in fish. (Everts et al., 1978)
- the application techniques chosen; drift contamination of vulnarable aquatic habitats may for instance be prevented by replacing aerial application by hand-spraying in the vicinity of the water bodies concerned.
- the timing of application for instance by not spraying at the time that vulnerable species (bees, birds) are foraging.

The planning of a pest management scheme which follows the guidelines outlined above requires considerable skill as well as collaboration among the individuals and parties responsible for land use development in a region. Finally it should be mentioned here that the guidelines are applicable on a world-wide basis and are certainly not restricted to pest management situations in tropical countries.

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EVALUATION OF METHODS FOR TESTING EFFECTS OF PESTICIDES ON MICROORGANISMS

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Background and objectives

Microorganisms and their activities are important factors in the soil ecosystem as well as in soil fertility. Disturbance of this very complex system, or of parts, may be detrimental to soil fertility. If pesticides reach the soil surface or deeper soil layers, changes in the soil ecosystem cannot be excluded in all cases. In the last 10 years, therefore, several proposed methods for investigating effects of pesticides on non-target soil microorganisms and their activities have been published. We think some of these methods are sufficient to test side effects of pesticides on microbial activities related to soil fertility, and others are not sensitive enough for such special tests. The objective of the investigations presented here, using the herbicide dinoseb acetate as an example, was to establish the ability of some tests to indicate potential disturbance to the soil ecosystem by pesticides.

Material and methods

Two soils were used: loamy sand soil and sandy loam soil. The herbicide "Aretit flüssig" (492 g dinoseb acetate/liter) was used as a standard test substance at field dosage (4 liter/ha). For the laboratory investigations this area-related dosage was calculated to occur in the upper 5-cm soil layer (normal dosage = 1x) or in the upper0.5-cm soil layer (10-fold dosage). In laboratory trials, the soils were incubated at 10 and 20°C with moisture contents of 40 and 60% (max water capacity) for up to 16 months. Field trials were run for 2 years at locations having the same soil types as were used in the laboratory trials. Soil samples were taken from 0-5-cm depth.

To investigate effects on some important transformation activities in the soil, we selected straw decomposition, soil respiration for 2 weeks after addition of straw meal ("long-term respiration"), ammonification and nitrification. As indicators of overall microbial activities or microbial biomass we used dehydrogenase activity, soil respiration for 12 hrs after addition of dextrose ("short-term respiration") and ATP content. Details of methods are given by Malkomes & Wöhler (1983).

Results and conclusions

Under laboratory conditions, dehydrogenase activity, ATP content and short-term respiration were inhibited in the loamy sand soil by normal herbicide dosage, whereas straw decomposition and long-term respiration were scarcely affected. Nitrification was slightly reduced. Higher herbicide dosage caused stronger effects in most cases.

In the loamy sand soil the effects were more pronounced than in the sandy loam soil. Where lucerne meal was added, the herbicide stimulated nitrogen transformations. The herbicidal effects on the different microbial activities were only slightly modified by the temperatures and moisture contents tested.

If, under laboratory conditions, the herbicide affected microbial activities in the loamy sand soil at the beginning of the experiment, the effects then persisted for about 8 months. In the sandy loam soil this occurred only with the higher dosage.

The same activities were sensitive to the herbicide under field conditions as in the laboratory, but in the loamy sand soil the inhibitions were smaller in the field. All effects became less pronounced with time.

The reactions of different biological activities to the herbicide found in our trials lead to the suggestion that at least two of the sensitive methods should be used in future routine testing. They should be accompanied by a test in nitrogen transformation. Using these methods, stimulation of activity as well as inhibition should be considered in interpretation of the results. In addition, we suggest that, if effects occurred under laboratory conditions, the pesticide should also be investigated under field conditions.

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✓

4B—R2

INVESTIGATION OF THE POTENTIAL SIDE-EFFECTS OF AGRICULTURAL CHEMICALS ON THE SOIL MICROFLORA

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Laboratory tests for assessing the potential side-effects of plant protection chemicals on the metabolic processes and populations of the microflora of soils are described. Two tests in the program examine the influence of chemicals on the microbial mineralization of carbon and nitrogen in soils. In their overall design, these tests closely follow the internationally agreed upon recommendations described in a Technical Report of the Agricultural Research Council's Weed Research Organization (Greaves et al. 1980). However, since Guidelines currently being developed by individual countries and organizations (e.g. Fed. Rep. Germany, Netherlands, United States, OECD) have not yet been completely harmonized, a "worst possible case" design has been adopted for the experiments. Using this design, a single set of tests is conducted: because of the design, the results obtained should satisfy most countries requiring registration of plant protection chemicals.

The incubation chambers selected for the C- and N-mineralization tests are simple and inexpensive. Hence, they can be assembled from the equipment found in most chemical or biological laboratories. The techniques for analysis of C- and N-mineralization products in soils (carbon dioxide, ammonia, nitrate, nitrite) can also be simple, or -as in our laboratories- can be fully automated to allow a rapid production of high quality data. Examples of both simple and automated methods for each test are given. The population analyses included in program (which are not included in Guideline drafts) examine the influence of agrochemicals on special soil populations and on the in situ soil microbial biomass. These tests are used to support data from the C- and N-mineralization experiments and provide basic information on the microbiology of the C- and N-test systems. In the biomass experiments, the weight of the metabolically active microflora is indirectly measured. If the total biomass is decreased by a chemical, both the extent of damage to the bacterial and fungal populations and the recovery rates of these populations are investigated. Results from experiments with a fungicide and a herbicide are used to demonstrate the correlations between population analyses and C- and N-mineralization tests. The suggestions of Domsch (see Greaves et al. 1980) are used to interpret the results in terms of their ecological relevance.

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A COMPARISON OF FIELD AND LABORATORY METHODS FOR TESTING TOXICITY TO EARTHWORMS

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Background and objectives

Earthworms improve soil structure, incorporate organic matter and mineralise plant nutrients. They are killed by many pesticides and may accumulate high concentrations in their body tissues. The EEC and OECD ecotoxicology testing programmes have chosen earthworms to represent the soil fauna in toxicity hazard assessment. The relationship between laboratory and field toxicity testing is often uncertain and needs clarification before laboratory methods can be used to predict field effects. This work examines the toxicity of four compounds in five laboratory tests and two field experiments, and attempts to define the relationship between them.

Materials and Methods

The earthworm species chosen for laboratory testing was the compost worm _Eisenia foetida andrei_, which is reasonably sensitive to toxic chemicals and easily cultured in animal waste. The field studies assessed the populations of three species from arable soils, the surface-feeding _Lumbricus terrestris_ and deeper dwelling _Allolobophora longa_ and _Allolobophora caliginosa_. Pentachlorophenol, carbaryl and chlordane were tested in the laboratory and field with chloroacetamide included as a laboratory reference compound.

Eisenia foetida was exposed to toxic compounds in the following tests. 1) 14 day artificial soil. The treatment was applied in a fine spray to a medium that consisted of 70% fine sand, 20% kaolinitic clay, 8% peat and 2% $CaCO_3$, which at 48% moisture content and $pH^{6.5}$, had a C.E.C. of 160 meq/kg^{-1}. 2) 48 hour contact. Airtight glass vials, 8 x 3 cm, were lined with Whatman No. 1 filter paper, then treated and rewetted to maximum holding capacity. 3) 14 day natural soil. The toxicant was sprayed onto sieved Geescroft soil, a pesticide free clay loam with a C.E.C. of 160 meq/kg^{-1}.4) 14 day silica paste. The treatment, mixed with the paste which had a C.E.C. of 158 meq/kg^{-1}, was suspended on a matrix of 2 cm. diameter glass balls. 5) 48 hour immersion. The worms were submerged in an aqueous test solution, suspension or emulsion. The test data was replicated at least twice and analyzed by probit analysis. The mean LC_{50} and standard error of the mean were calculated for each compound.

Field trials were set up at Rothamsted and Sittingbourne on loam soils, both with a C.E.C. of approximately 160 meq/kg^{-1}.The organic matter content was approximately 5% and 7% respectively. Both trials were arranged as randomised blocks containing three replicates of each treatment, which were sprayed at two rates and rotovated during March. Samples were taken one month later by a formalin extraction method on 0.5 m^2 sub plots. The data were analysed for treatment effects on the worm populations by analysis of variance and covariance.

Results and Conclusions

The laboratory tests offered such different conditions that the results could not be compared directly, although the ranking of toxicity shows general trends. All the compounds tested were toxic. Chlordane was very toxic in all tests. Pentachlorophenol had an intermediate toxicity and carbaryl was of consistently low toxicity. Chloracetamide had a variable toxicity dependent upon the testing method. No obvious trends appeared between type and duration of exposure or class of chemical. Standard errors were greatest in the artificial soil and immersion test, and least in the silica test.

The field toxicities after one month were similar in each trial. Carbaryl at 25 kg/ha was very toxic to all species and chlordane at 10 kg/ha slightly less toxic. Pentachlorophenol at 12.5 and 75 kg/ha was toxic to _L. terrestris_ on the low organic matter site, and toxic to _A. longa_ at 12.5 kg/ha on the other site.

The laboratory tests accurately predicted the field toxicity of chlordane and indicated the hazard from pentachlorophenol but gave a poor field prediction for carbaryl. The silica test most clearly predicted the carbaryl effect. The field effect of carbaryl was probably not identified in the laboratory tests because they were too brief. Laboratory tests of longer duration may overcome this problem but until tru ly predictive laboratory methods exist, field testing for toxicity should be retained.

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√ **4B—R4**

EVALUATION OF EFFECTS OF PESTICIDES ON BENEFICIAL SPECIES IN ARABLE CROPS

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Background and objectives

Integrated control programmes involving pesticides and beneficial insects are easier to establish in the glasshouse and orchard than in the field. When insecticides are applied to cereals, their influence in the long term on the ecology of the farm and in the short term on parasites and predators should be studied. This report describes the development of methods to assess the latter.

Materials and Methods

A commercial formulation of demeton-S-methyl was applied to a single square plot of spring barley in 1980 (3.3 ha) and 1981 (0.84 ha). In 1982 a cypermethrin treatment (0.84 ha plots of winter barley) was added.

Sites were arranged along a cross on each plot and sampled using visual counts (12 sites per plot), vacuum samplers (12 sites), pitfall traps (9 sites) and "sticky" traps (5 sites). There were respectively 8, 8, 4 and 4 further sites in the unsprayed area surrounding each plot.

Principle groups collected were cereal aphids, Chloropidae, Syrphidae, Thysanoptera, Chrysopidae, Hymenopterous aphid parasites, predatory adult and larval Carabidae, Coccinellidae and Staphylinidae.

Samples were taken two or three days before application and up to three weeks afterwards. To assess overall population fluctuations, "background" counts were taken at the four unsprayed corners of the field during the growing season.

Results and conclusions

The results and much past experience established the mobility of many of the species involved, justifying the use of large plots at the expense of replication.

Aphid numbers were greatest in 1980 (maximum 1.5 per tiller), but fewer in 1981 (0.15) and 1982 (0.5). Good control was achieved and could be accurately measured, demeton-S-methyl being more effective than cypermethrin.

In 1980 aphid parasites were abundant and numbers were reduced by the insecticide.

Vacuum samples showed reduction of spider populations after all treatments, and of predatory larvae in 1982.

Syrphidae were the most obvious aphid specific predators in 1982 but the sampling methods used for this group are not yet adequate for accurate population estimation.

Slight population changes in other groups were detected immediately after treatment, but were not significant.

These results emphasise that different beneficial groups predominate in different seasons. As measured by these techniques, the short term effects of insecticides on beneficial species were less than expected, but the failure to quantify at least one group adequately (Syrphidae) points to the necessity of improving these methods before they can be considered reliable.

EFFECTS OF STORAGE AND PESTICIDE TREATMENTS ON HONEY BEE BRAIN ACETYL CHOLINESTERASE ACTIVITIES

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Background and Objectives

The importance of monitoring hazards to honey bees during the application of insecticides to oilseed rape and other flowering crops attractive to pollen-gathering insects has led to the inclusion of suspected bee poisoning incidents in routine laboratory investigations of wildlife incidents in which agricultural chemicals are implicated. The interpretation of bee brain acetylcholinesterase (AchE) inhibition has been limited by a knowledge of the enzyme stability and the degree and reversibility of inhibition after storage. During laboratory studies, untreated honey bees were stored under different temperature conditions and periods of time to investigate aspects of AchE stability. Batches of honey bees were dosed with lethal concentrations by contact using either organophosphate, carbamate, pyrethroid or organochlorine pesticides to determine the extent of inhibition and whether any reactivation occurs.

Materials and Methods

Untreated honey bees (*Apis mellifera*) were killed by cold exposure and stored at either -20°C, 4°C or room temperature for periods of up to 26 days in unsealed glass bottles. Further groups of bees were dosed by contact with 2 or 3 times the LD_{50} using azinphos-methyl demeton-S-methyl, dimethoate, phosalone, triazophos, carbaryl, ethiofencarb, pirimicarb, cypermethrin, deltamethrin, permethrin and HCH. Bees which died from pesticide treatments were stored at -20°C for 24 hr and were then divided along with untreated bees into 3 groups for either immediate assay or storage at either -20°C or room temperature for a further 7 days. AchE activity was assayed in bee head extracts using the method of Ellman *et al.* (1961). Chemical reactivation of AchE activity was made using aqueous pyridine 2 - aldoxime methiodide (2-PAM) solution.

Results and Conclusions

This study indicates the instability of the bee head AchE when stored at room temperature for 12-18 days, control enzyme activity showing significant losses after 26 days and apparently some chemical reactivation. Lethal pesticide treatment of bees with the organophosphate and carbamate compounds resulted in significant esterase inhibition (less marked with triazophos) which was not reversible on storage at room temperature. This inhibition showed marked recovery to normal levels on chemical reactivation with 2-PAM with all pesticide treatments. Although the pyrethroids and HCH resulted in no inhibitory effects on the bee acetylcholinesterase, the esterases lost activity on storage at room temperature and this was apparently reactivated using 2-PAM.

The results indicate that diagnosis of the probable involvement of organophosphate or carbamate insecticides in bee mortality incidents using brain esterase values requires a knowledge of the exact storage history of the samples. The measurement of relative AchE activities before and after chemical reactivation would appear useful in differentiating whether apparent low esterase values result from pesticide inhibition or are due to ageing and deterioration of bee samples in the field.

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4B—R6

A FIELD STUDY ON THE EFFECTS OF A NEW INSECTICIDE, FASTAC, ON HONEY BEES

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Background and Objectives

Fastac (WL85871) is a new insecticide from Shell with the proposed common name, alphamethrin. Extensive field trials have shown it to have considerable potential for the control of a wide range of insect pests including those in flowering crops such as oil seed rape. These crops are especially attractive to honey bees and therefore an examination of its effects on their survival and development in field conditions was considered to be important. The study reported here was carried out in order to make a first assessment of the direct effects of this pyrethroid insecticide on bees by making applications to plots of flowering mustard during peak foraging activity. Side-by-side comparisons were made against methyl-parathion [MEP] (generally accepted as being hazardous to bees) and against phosalone (generally accepted as non-hazardous to bees).

Materials and Methods

The study was carried out on a suitable isolated trial site near Beaumont-le-Roger in Normandy, France. Two dose rates of Fastac (10 and 20 g ai ha^{-1}), MEP (500 g ai ha^{-1}) and phosalone (1200 g ai ha^{-1}) were applied to plots (0.3 ha) of flowering mustard on different dates during June and July 1982. Applications were made between 1200 and 1300 h on warm sunny days by a tractor-mounted boom and nozzle sprayer delivering 500 l ha^{-1}. Several days before each treatment 3 bee hives fitted with dead bee traps and 1 bee hive fitted with a pollen trap were placed adjacent to the mustard plots. At intervals before and after each application detailed observations were made on bee mortalities, foraging activity and pollen collection. In addition, the general condition of each hive used during the study was assessed by a professional beekeeper at the end of the season in September.

Results and Conclusions

No increases in the numbers of dead bees collected in the hive traps were observed following the applications of both dose rates of Fastac or phosalone (Table 1). In contrast, the application of MEP resulted in a substantial increase in the numbers of dead bees. Very few dead bees (<10) were found during many detailed searches on the soil surface within the crop following any of the applications.

Table 1 - Mean cumulative mortality per hive of worker honey bees

	Time from treatment in days					
	−1	0	+1	+2	+3	+4
Fastac (10 g)	24	47	52	55	77	90
Fastac (20 g)	40	55	59	65	68	72
MEP (500 g)	6	8	853	1502	1571	1596
Phosalone (1200 g)	22	37	43	55	61	70

Pollen collection was not adversely affected by the applications of either dose rates of Fastac or phosalone. However, a significant reduction was observed in the amount of pollen collected following the application of MEP. Foraging activity in the mustard crop declined after each insecticide treatment. Although in the case of Fastac this only involved a sharp decline immediately after spraying, followed by a return to normal activity within a few hours. With phosalone, a gradual reduction in foraging activity occurred throughout the afternoon following the application and with MEP foraging activity fell rapidly to a very low level and remained so for the remainder of that day and all of the next day.

Only after the application of MEP were significant effects on long-term hive development found.

It was concluded that the application of Fastac at $\leqslant$20 g ai ha^{-1} had no direct effects on honey bee survival and no detrimental effects on hive development.

TOXICITY OF SYNTHETIC PYRETHROID INSECTICIDES TO HONEYBEES

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Introduction

Rather little is known on the toxicity of synthetic pyrethroids to honeybees. Most farmers are, therefore, very careful in applying them on crops where bee visitation could be expected. However, in recent literature there are some indications that the toxicity of pyrethroid products can be different and some of them could be much less dangerous to bees than others. Accordingly, experiments were made to compare the toxicity of different synthetic pyrethroid insecticides to honeybees.

Materials and methods

Five products were tested in laboratory and field studies: Ambush /permetrin/, Chinetrin /permethrin + tetramethrin/, Sumicidin /fenvalerat/, Ripcord /cypermethrin/, Decis /deltamethrin/. Decis was tested in two different formulations.

Direct contact toxicity at recommended field dosages was inspected first at laboratory spraying tower the working performance of which closely resambles to field machines. Afterwards toxicity of field-weathered residues was investigated at the laboratory on samples taken at flowering winter rape and lucerne plots. Finally large scale field experiments were made with honeybee colonies at flowering crops with those products that were less toxic as field-weathered residues.

Results and conclusion

Direct toxicity of each product tested was very strong, except Decis. Some products /Ambush, Chinetrin/ caused high residual toxicity, however, residual toxicity of others /Ripcord, Sumicidin, Decis/ was fairly short.

Large-scale field experiments showed that Ripcord Sumicidin and Decis formulations are usually little toxic on bee colonies if applied at flowering crops. Decis formulations, however, are much more safe, since practically no bee mortality was observed in several experiments on flowering winter rape and lucerne fields. In the contrary, Ripcord and Sumicidin sometimes caused much higher mortality than the normal bee loss observed at untreated fields.

Thus, it is concluded that the toxicity of synthetic pyrethroid insecticides is largely different to honeybees. Some products are strongly toxic both as direct sprays and as residues. Others, however, can be highly toxic as direct sprays but are much less toxic as residues. These do not cause serious bee losses if sprayed on blooming fields when bees are not present. Some formulations /Decis 2,5 EC, Decis ULV/ can be regarded as "safe on bees insecticides" if sprayed on flowering crops when bees are not on wing.

✓ **4B—R8**

AN ORIGINAL NEW INSECTICIDE - NEVIFOSZ^R 50 EC - BEING SAFE ON BEES IF APPLIED OUT OF THEIR DAILY FLIGHT PERIOD

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Introduction

Several crops are attacked by serious pests during the blooming period. However, their control is always a problem because bees are abundant in flowering crops. The Research Institute of Heavy Chemistry has developed original new insecticides which seems to be suitable tools to solve this problem.

The product and its biological efficacy

Nevifosz^R 50 EC contains 50 per cent phosmethilan* as active ingredient. It has a wide range of biological efficacy on pests of various crops. Laboratory and field trial including large-scale farm experiments show that it can be recommended on winter rape, lucerne, sugarbeets, flax, hop, peas, apple, peach, plum and grape-vine against various pests as pollen beetles, weevils, tentredinids, gall midges, seed chalcids, noctuid larvae, flea beetles, fruit moths, pea moth, tortricids, geometrids, leaf miners, American fall webworm.

Methods

The new product was tested on bees to explore its toxicity to honeybees. It was tested first as a direct spray on bees in a laboratory spraying tower the working performance of which resembled widely used field machines. In the second step studies were made on its residual toxicity to honeybees in the laboratory on field-weathered samples taken at different times after application at treated flowering plots of winter rape and lucerne. Finally, farm experiments were made on large winter rape and lucerne fields in bloom with honeybee colonies equipped with dead-bee traps.

Results and conclusion

Results show that the new product has rather a strong direct toxicity to bees and thus, it should never be applied to flowering crops during day-time hours. On the other hand, both field-weathered samples and farm-scale experiments showed clearly that its residual toxicity is not longer than a few hours. Accordingly, this new product can be classified as temporarily toxic to honeybees and it is suggested to be safe on bees if applied during hours when bees are not on the wing, first of all in the twilight period. Its application, therefore, is a new solution to control insect pest in flowering crops without causing any loss to bee colonies placed nearby.

* O,O-dimethil-S [N-/2-chlorphenyl/-N-butiril]-amino-methyl dithiophosphate

A LABORATORY TOXICITY TEST FOR CARABID BEETLES

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BACKGROUND AND OBJECTIVES

In association with the International Organisation for Biological Control (IOBC) work group 'Pesticides and Beneficial Insects' ICI has developed a laboratory test for evaluating the toxicity of pesticides to predatory ground beetles (Carabidae). The objective of the IOBC is to identify pesticides which do not interfere with beneficial pest predators and parasites or can be used for integrated control. The group consists of contributing laboratories from many countries. Each year about 20 pesticides are tested. The carabid test was designed to give realistic levels and routes of exposure. It was checked by comparing laboratory results with those from the field.

METHOD

Beetles were exposed to chemicals by 4 routes which are believed to be important in the field,they were:

1) spray deposit on soil;
2) direct spray on the beetle (diurnal species only are likely to be at risk by this route);
3) spraying a vessel of soil and growing barley in which the beetle had been established for 24 hours. This was an attempt to simulate the field situation closely;
4) spraying the food of beetles.

An additional route was:

(5) exposure of beetles to a dry spray deposit on glass.

Beetles were first tested at the commercially recommended spray dose (N). If there was no or little mortality after 6 days then fresh beetles were exposed to 5 x N dose. If there was a high mortality then 0.2 x N dose was used. This generally provided a lethal threshold concentration to enable a comparison with field data.

Four species, known to be predators in agricultural crops,were tested; Agonum dorsale, Pterostichus cupreus, P. melanarius and Nebria brevicollis,to select 1 species for the proposed method. Three criteria were of primary importance in selection:

1) availability;
2) survival in captivity;
3) susceptibility to pesticides.

Eight pesticide products were used, gamma HCH, dimethoate, cypermethrin, pirimicarb, chlorfenvinphos, carbophenothion, trichlorphon and carbendazim. They were chosen for:

1) availability of field toxicity data;
2) likely range of toxicity to carabids,
3) different types of uptake, ie fumigant or contact.

RESULTS

Agonum dorsale was in general the most susceptible species, followed by the Pterostichus species. Least susceptible was Nebria brevicollis. The results with different chemicals varied with the method of application. Gamma HCH was more toxic when applied to the soil surface; chlorfenvinphos when sprayed directly on the beetles. Dimethoate,cypermethrin, (knockdown followed by recovery) carbophenothion, and trichlorphon had the same activity by both routes. In general applications to beetles in pots of soil containing barley, which combined direct spraying with soil residual treatment, gave results similar to those of the most active single application. However trichlorphon was more active in the barley test than by either single exposure. When the beetles were exposed to dry deposits on glass, toxicity was generally much higher. Where comparisons could be made the laboratory data were consistent with results from the field (displayed in the poster).

CONCLUSION

A laboratory test in which Pterostichus cupreus or Agonum dorsale are exposed directly to spray and to spray deposits on soil for 6 days has promise as an indicator of the toxicity of pesticides to carabids in the field.

✓ **4B—R10**

THE EFFECTS OF DDT AND OTHER ORGANOCHLORINE PESTICIDES ON BIRD POPULATIONS

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Of all pesticides yet used widely, the organochlorines have had the most harmful effects on wildlife populations, especially of predatory birds, some of which have been exterminated over areas up to half the size of the United States. Besides being toxic, these chemicals have three main properties which contribute to their effects. First, they are chemically extremely stable, so that they persist more or less unchanged in the environment for many years. Second, they dissolve in fat, which means that they can accumulate in animal bodies, and pass from prey to predator, concentrating at successive steps in a food chain. Predatory birds, near the tops of food chains, are thus especially liable to accumulate large amounts. Thirdly, at sublethal levels of only a few ppm in tissues, organochlorines can disrupt the breeding of certain birds.

All bird species that have been studied have been found to be susceptible to organochlorine pesticides. The most marked population declines have occurred in bird-feeding raptors, especially the Peregrine Falcon (*Falco peregrinus*), but also the Sparrowhawk (*Accipiter nisus*) in Europe and the Sharp-shinned Hawk (*Accipiter striatus*) and Cooper's Hawk (*Accipiter cooperi*) in North America. Certain fish-eaters have also declined greatly, including the Osprey (*Pandion haliaetus*), Bald Eagle (*Haliaetus leucocephalus*) and Brown Pelican (*Pelecanus occidentalis*) in parts of North America and the White-tailed Eagle (*Haliaetus albicilla*) in northern Europe. Among other animals, various predatory mammals are particularly vulnerable, including otters and several bat species, and various amphibia and fish.

DDT and its various metabolites are not particularly toxic to birds. However, the main metabolite, DDE, causes thinning of eggshells. Such shells often break during incubation, so that the reproductive rate is lowered. Metabolites of DDT also cause embryo deaths in intact eggs, thus further lowering the breeding rate. Adverse effects on reproduction can be so great as to lead to population extinction. Different taxonomic groups of birds vary in their sensitivity to DDE residues. Birds of prey are particularly vulnerable, partly because they are more sensitive than some other birds to a given level of DDE (i.e. they show more shell-thinning), and also because, being predators, they accumulate larger amounts than most other birds. As assessed by shell-thinning, herons and pelicans are also relatively sensitive to DDE, whereas game-birds and songbirds are relatively insensitive.

The more toxic cyclodiene compounds, such as aldrin and dieldrin, cause direct mortality of adult birds, in some cases leading to population decline. These chemicals are held responsible for the elimination of the Peregrine and Sparrowhawk from large parts of Britain in the period 1957-60. Population recovery of these birds has followed reductions in aldrin/dieldrin usage. Throughout the population decline and the recovery in Britain, reproduction has been impaired to some degree by DDE residues.

Not all mortality from cyclodienes occurs at the time of ingestion. These chemicals are stored in body fat, and a bird may die when its fat is metabolised, and organochlorine is released to other, more sensitive, tissues. Thus birds may die during periods of food shortage or migration, from organochlorine accumulated in the body during previous months. The problem has proved particularly acute in some arctic nesting geese, which accumulate fat and organochlorine residues on their wintering grounds, and die months later on their breeding grounds, when their fat is mobilised.

Birds of prey which breed in areas with no pesticide use are not free from organochlorine contamination if they, or their prey species, migrate to winter in areas where these chemicals are used. Thus Peregrine Falcons nesting in arctic North America have recently declined, as a result of increasing DDT usage in Latin America, where these Peregrines winter. Other migrant birds which breed in the northern hemisphere are also likely to decline, as a result of growing organochlorine use in tropical and sub-tropical countries.

Once organochlorine use has been curtailed, these various birds have mostly begun to recover in numbers, and recolonise areas from which they were eliminated. However, certain DDT residues - particularly DDE - are so persistent in soils, that they could well remain a problem for birds of prey and other animals for several decades after use of DDT has ceased. There is as yet no sign that any bird species has developed any degree of resistance to organochlorine residues.

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USE OF FIELD TESTING IN THE EVALUATION OF PESTICIDE HAZARD TO BIRDS

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Background and objectives

The safety of pesticides to birds can normally be predicted from laboratory toxicity studies combined with an assessment of the likely exposure in the field. Where there is doubt over safety field studies, which can examine the risks under realistic conditions, have usually been undertaken with reluctance because experienced personnel are few and there has been a lack of confidence in the results. Outdoor cage tests have been employed, but their results are difficult to extrapolate because the level of exposure to the chemical and the behaviour of the birds may be unnatural.

The poster outlines the methods available for field studies and presents results from one of them, the 'territory mapping' census method. Consultation with technical experts, including those from the relevant registration authority, is strongly recommended before expensive field work is put in hand.

Methods

1. Estimates of exposure may be obtained by
   a) Direct observation of birds feeding on pesticide contaminated material
   b) Collection of live and dead birds for analysis of pesticide residues or for measurement of enzyme levels and autopsy to check for poisoning symptoms.
2. Populations may be monitored by
   a) Census methods eg the 'territory mapping' method (International Bird Census Committee 1970)
   b) Ringing
   c) Radio tracking

Radio tracking, used principally in behavioural studies, has great potential for the assessment of wild life hazards, particularly with the recent advances in transmitter design. It is most appropriate for less abundant, larger species that are difficult to assess by other methods. The species chosen must, however, be sedentary to avoid loss of radio-contact, and even so the number of animals tracked may fall during the course of a study to a level that reduces confidence in any conclusions.

Territory mapping method

During the spring of 1976 and 1980 the precision of the 'territory mapping' census method was evaluated in 2 separate studies, Edwards et al 1979 and Fletcher et al (in press). In one study a known number of birds were caught and removed from their territories. Territories estimated to have been lost from census data were compared with the numbers of male birds removed. In addition the subsequent rate of repopulation was monitored by regular censusing.

In the second study, bird populations were assessed independently by 2 methods, using 'territory mapping' and observations of colour ringed birds.

Conclusions

The 'territory mapping' census method is precise enough to detect population changes of significant magnitude. Experienced personnel are essential. Census workers should be aware of the potential for repopulation from outside the study area.

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4B—R12

AVIAN TOXICITY STUDIES DESIGNED TO ASSESS THE POTENTIAL TOXICITY OF CHEMICALS

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Background and objectives

The safety of chemicals to man and the environment has been reflected in the scope of world-wide regulatory requirements. This poster attempts to demonstrate a typical toxicity programme for an agrochemical product using avian species as the experimental models. In the interests of clarity, most of the studies discussed in this document are based on the United States of America Environmental Protection Agency (EPA) guidelines as these command widespread acceptance.

Studies are undertaken to determine the possible in-use hazard to wild birds and a typical study programme may be summarised as follows:

Avian acute toxicity

This study is based on a single oral dose at different dose levels to groups of 10 birds. The species of choice is normally the Mallard duck as being a typical water fowl or the Bobwhite quail as a representative upland game species. The study design should determine an acute oral toxicity value and a maximum tolerated dose level.

Avian 5-day dietary toxicity

The normal species of choice are the Mallard duck and Bobwhite quail with the birds being below 15-days of age at the start of the test period. Groups of 10 birds are either offered basal diet or basal diet plus test compound at different levels over a 5-day period followed by a 3-day observation period when basal diet only is offered.

Avian reproduction

These studies are required under certain use conditions and where there is evidence of accumulation in crops. The species of choice are the Mallard duck and Bobwhite quail with a control group and two or three treatment groups each of 20 paired replicates. Test compound is included in the diet for 10 weeks prior to the start of egg production and for a 12-week egg production period. Assessment of the results is based on a number of parameters including egg production, fertility, hatchability, viability and egg shell thickness.

Wildlife surveys

This type of study has been required for certain compounds by the UK PSPS for a number of years and is becoming increasingly required by other regulatory authorities. Studies are designed to determine the practical in-use hazard to avian species and small mammals and depends in the first instance on selecting a site which is both typical of the normal use of the compound and contains a satisfactory number and range of species present. A typical site would be of 10 hectares, be planted with the target crop and the compound would be applied following standard farm procedures. The parameters measured include mortality counts, bird behaviour and in certain cases, residues determination may be required.

The results of these studies give a very good indication of the practical hazard to wildlife and have the advantage over laboratory studies in that a wide range of species at risk are included in the observations. Many of these species would not respond well to captivity or laboratory investigations and this is considered to be the most practical way of obtaining information on a range of use conditions and avian species.

Avian palatability

These studies are conducted under laboratory conditions to compare different formulations of granular insecticides or seed dressings. The preferred species for studies of this type are the pheasant as a typical large bird, the finch as a typical small bird and the pigeon as a representative grain eating species. Other species may be used depending on the practical limitations of housing and management.

Studies are designed to compare formulation preference, and thus reduce the possible hazard to wildlife based on a range of factors which may include colour, smell or size.

Conclusions

By selecting studies from those outlined above, it is possible to calculate the potential hazard of an agrochemical to wild birds while at the same time assisting in the development of less toxic chemicals and encouraging their correct use and disposal.

BIOCHEMICAL AND HISTOLOGICAL EFFECTS OF DICLOFOP-METHYL ON MICE AND VOLES

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Background and Objectives

The sub-lethal effects of diclofop-methyl, a foliar herbicide which is applied post-emergence to spring cereals, have been characterised in wild-trapped wood mice and bank voles to establish reliable background data to assist in the interpretation of any biochemical or histological effects observed in field trials. During laboratory studies, chronic dietary administration of diclofop-methyl to wood mice (*Apodemus sylvaticus*) and a limited number of bank voles (*Clethrionomys glareolus*) was made for periods of up to 4 weeks. A range of blood, liver and brain enzyme activities were monitored and liver and kidney sections examined for histological changes.

Materials and Methods

Wild wood mice and bank voles, trapped in a woodland in Surrey were maintained, on untreated wheat and water *ad libitum* until placed on treated diets. Groups of 6 wood mice were fed on wheat treated with technical grade diclofop-methyl at levels of 20, 200, 500 and 1000 ppm for 1 and 2 weeks and at 20 and 200 ppm for 4 weeks. Groups of 5 bank voles were fed on wheat treated with technical grade diclofop-methyl at levels of 200 and 1000 ppm for 2 weeks. Plasma, liver and brain acetylcholinesterase (AchE), cholinesterase (ChE) and nitrophenyl acetate esterase (NPAE), plasma glutamate oxaloacetate transaminase activities and liver cytochrome P_{450} levels and relative liver weights were measured and compared with those of control animals. Liver and kidney sections were fixed using buffered formalin and stained with Ehrlichs Haematoxylin and Eosin.

Results and Conclusions

Following chronic administration of diclofop-methyl to wood mice, plasma NPAE activity significantly increased and this was accompanied by significant elevation in hepatic cytochrome P_{450} and relative liver weight. Bank voles also showed significant increase in relative liver weight and apparent increases in hepatic cytochrome P_{450} and plasma NPAE activity. Liver NPAE activity decreased. Increases in plasma cholinesterase activities have previously been suggested as reflecting hepatic cell proliferation and observed increases in plasma NPAE may be analagous.

Histological evaluation of the livers from wood mice given diclofop-methyl for up to 4 weeks in the laboratory compared with controls showed progressive hepatic degeneration with both increased time of exposure and increased dose.

An initial hepatocyte hyperplasia was replaced by hepatocyte hypertrophy commonly accompanied by single cell necrosis with loss of cytoplasmic eosinophilia; fatty degeneration was also observed. Chronic inflammatory change was observed as increased monocyte infiltration amongst the hepatocytes and monocyte cuffing at the portal triads. In lower dose groups, eosinophilic nuclear inclusion bodies were also observed. Livers from voles exposed to 200 and 1000 ppm for 2 weeks showed similar hepatic degeneration to that observed in mice but at the higher dose rat, acute inflammatory changes and focal necrosis were also observed. Kidneys from treated mice and voles also showed cellular and inflammatory changes when compared with controls.

The results from this study demonstrated that changes in serum enzyme activities in mice and voles exposed to the herbicide diclofop-methyl were accompanied by observable changes in liver morphology. The effects of a field application of diclofop-methyl to spring barley were monitored in free-living mice and voles. Although transient changes were noted in sensitive biochemical parameters, significant histological changes seen at high dosing in the laboratory were not detected in the field samples.

4B—R14

SCREENING OF PLANT PROTECTION AGENTS AND TECHNIQUES FOR WILDLIFE HAZARDS IN HUNGARY

A ZAJAK

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Background and objectives

Pheasant, hare and deer populations are concentrated in the cultivated areas of Hungary. The agricultural pesticide registration system requires, in addition to standard fish and bee toxicity, assessment of the hazards to these species, the protection of which also contributes to the protection of other wildlife. Hazard assessment is based on toxicity data and application methods in the general farming context. Detailed knowledge is needed both of pesticide formulation and application and of the behaviour and ecology of wildlife in various crops. Ultimately, wildlife hazards can only be judged from 'Wildlife toxicity field trials' and from full scale practical use.

Screening scheme

The basic scheme for pesticides intended for large scale farming is as follows :-

1 Analysis of toxicity data in publications and from manufacturers.

2 Acute toxicity studies on pheasants, mallards, Japanese quail (oral LD50) and hares (oral ALD).

3 Chronic toxicity studies including 8 day feeding LD50 values on pheasants and quail and repeated oral dosing (10 days) at zero, minimal, high and lethal doses on hares.

The above are considered sufficient for the majority of pesticides. Further tests are needed for pesticides highly toxic to specific species or where high exposure is likely :-

4 14 day feeding tests on pheasants in the egg laying period involving behavioural observation, pathological examinations and fertility monitoring, eg. seed treating agents for summer crops.

5 21 day feeding tests on pheasants when sexually inactive (for more persistent pesticides).

6 Feeding tests on hares (rodenticides).

7 Simulated field trials using 1000 - 2000 m^2 treated plots and incorporating forced animal contact in cages.

8 Large scale field trials (50-100 ha) are carried out when neither the above tests nor the recommended application methods preclude the possibility of harmful effects. Wildlife population and behaviour is observed for 7-14 days before and after treatment and samples are collected for pathology studies.

9 After registration, national organisations report wildlife incidents arising from practical use to the centre, which investigates them and proposes modifications to the registrations if necessary.

An integrated approach

The assessment of wildlife hazards for registration in Hungary excludes dangerous pesticides. The Hungarian system also attempts to integrate plant protection methods, using both prediction of pest numbers and biological control methods which have only a moderate environmental effect. It is hoped to prove that plant protection is compatible with wildlife management.

The poster session will illustrate the wildlife hazard screen using a current pesticide example.

WILDLIFE POISONING INCIDENTS FROM AGRICULTURAL PESTICIDES IN ENGLAND AND WALES

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Background and Objectives

Before a new chemical or the new use of an existing chemical is cleared for agricultural use in the UK, pesticide manufacturers are required to notify for clearance under the Pesticides Safety Precautions Scheme (PSPS) so that safety can be assessed to the operator, the consumer of any treated crop and to wildlife. Environmental hazard assessment is based on both laboratory toxicity data and information generated in intensive field trials. Post-clearance surveillance of the commercial usage of pesticides is considered to provide an important check on the assessment of potential hazard to wildlife species. This poster outlines the procedures conducted by the Ministry of Agriculture, Fisheries and Food for investigating the possible involvement of agricultural pesticides in suspected wildlife deaths.

Materials and Methods

Since the late 1950's when the use of cyclodiene insecticides and cereal seed treatments resulted in casualtines amongst birds, wildlife incident investigations have been carried out by the Tolworth Laboratory. This co-ordinates the results of both field and laboratory studies.

When an incident involving wildlife casualties is reported and is thought to involve an agricultural chemical or has occurred on agricultural land, a field investigation is conducted by experienced MAFF regional staff to record relevant field information including details of local pesticide use. Specimens are taken to the nearest local MAFF Veterinary Investigation Centre (VIC) for post-mortem examination to establish whether disease or trauma has contributed to the cause of death. Tissue samples are forwarded to the Tolworth Laboratory where chemical analysis and biochemical studies are carried out to diagnose whether a pesticide was involved in the reported incident.

Most incidents involve vertebrate species but recently the incident scheme has been extended to include suspected bee poisoning incidents. Bee mortality, initially reported to the National Beekeeping Unit at Luddington Experimental Horticultural Station, is then investigated in the field and samples, having been screened for disease at Luddington, are forwarded to the Tolworth Laboratory for biochemical and residue measurements.

Results and Conclusions

The number of incidents involving the deaths of wildlife that can be attributed to the cleared use of agricultural chemicals has been small, reflecting the substantial reduction in hazards to wildlife brought about by the measures introduced under PSPS since the early 1960s. The results of the incident investigations are regularly submitted to the Advisory Committee on Pesticides which under PSPS has the capability to review and modify the use of any pesticide in the light of new evidence of adverse effects. Continuous surveillance is necessary if a high standard of environmental safety is to be achieved to ensure that the use of existing pesticides and the introduction of new pesticides does not have environmental impact.

4B—R16

SIDE EFFECTS OF RICE HERBICIDES IN KILLING MOSQUITO LARVAE

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Background and objectives

Statistical data in Japan show that the decrease of the sleeping illness (Japanese encephalitis) and the increase of application of diphenylether herbicides to paddy fields have a close relationships. This human disease is mediated by mosquitoes, especially *Culex tritaeniorhynchus*. On the other hand, some of the diphenylether herbicides, nitrofen (2,4-dichlorophenyl *p*-nitrophenyl ether) and chloronitrofen (2,4,6-trichlorophenyl *p*-nitrophenyl ether) show a high larvicidal activity. Then it was supposed that these herbicides applied to flooded paddy fields at a suitable time had a possibility to prevent the first emergence of the mosquitoes.

As herbicides, these chemicals require for light to kill weeds, but even in the dark they are active as the larvicide. Then the mode of action of these diphenylether compounds against mosquito larvae is different from that against weeds. In this report some results as to the mode of action of diphenylether herbicides on mosquito larvae will be presented.

Materials and methods

Larvicidal activity of nitrofen was assayed with mosquito larvae at several growth stages in water with a deep cylindrical glass vessel having a diameter of 9 cm and a height of 6 cm. The behaviors of larvae until their death after treatments with several insecticides were also compared with that of nitrofen treatment. In order to survey the adsorption of nitrofen by the larvae, the number of them in a test vessel was changed. If necessary, the amount of nitrofen in the water was assayed by a gass chromatography with an ECD detector. The effect of nitrofen on acethylcholinesterase was tested by the method of Ellman *et al.*(1961). As a matter of convenience to rear the larvae, *Culex pipiens molestus* was used for the experiments in place of *C. tritaeniorhynchus*.

Results and conclusions

In the diphenylether herbicides, nitrofen and chloronitrofen showed high larvicidal activity; while those with a substituent adjoined the nitro radical, e.g. chlomethoxynil (2,4-dichlorophenyl 3-methoxy-4-nitrophenyl ether) and oxyfluorfen (2-chloro-4-trifluoromethylphenyl 3-ethoxy-4-nitrophenyl ether), or with 3,5-substituents, e.g. 3,5-dimethylphenyl *p*-nitrophenyl ether (DMNP) showed very low or no activity. The larvae at the first instar stage were the most susceptible to nitrofen, and the older showed the less susceptibility. Nitrofen did not show any effect on the acethylcholinesterase of mosquito larvae. In the behaviors of the larvae until their death after treatment with the following insecticides: γ-BHC, p,p'-DDT, diazinon, nereistoxin and rotenone, nitrofen resembled somwhat to rotenone.

The increase of larva number in the test vessel kept at a constant concentration of the chemicals had no effect on the larvicidal activity in the case of γ-BHC, diazinon, nereistoxin and rotenone, but it decreased the activity in the case of nitrofen and p,p'-DDT. However the higher activity in the lower temperature shown with p,p'-DDT was not the case with nitrofen.

From several observations, the phenomenon of adsorption of nitrofen on the larva body was found to be very important. Then the leakage of endogenous substances from larva bodies was surveyed, but any increase in the leakage of ions, protein or nucleic acids was detected after the treatment of nitrofen.

In order to clarify the adsorption status of nitrofen on the larva body, the effects of lipids, proteins and amino acids on the larvicidal activity of nitrofen were tested. Among them, both bovine and human serum albumins decreased the activity very much. From these results, nitrofen will be adsorbed on a special protein of mosquito larva body and cause the death of a larva after the absorbed amount will increase over a limited one.

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ENVIRONMENTAL STUDIES ON FISH USING THE TWO INSECTICIDES RIPCORD AND FASTAC.

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Background and Objectives

The acute toxicity of pesticides has often been used to assess hazard in aquatic environments. However, an adequate assessment of hazard can only be made if information is available on both toxicity and exposure in the field. The synthetic pyrethroid insecticides developed during the last 10 years have a relatively low acute toxicity to birds and mammals but a high acute toxicity to fish. As a result of this, environmental concern has focussed on their potential hazard to fish. RIPCORD* (a.i. cypermethrin) and FASTAC* (a.i. proposed name alphamethrin) are synthetic pyrethroid insecticides developed for use throughout the world in crop and non-crop outlets. The experiments described were carried out to determine their toxicity in laboratory tests and to assess their hazard in the field.

Materials and Methods

Acute toxicity tests in the laboratory were either static water tests with daily renewal or continuous-flow tests, and the results are expressed as 96 h LC50 values. In the field three types of study were undertaken. In the first, rainbow trout (*Salmo gairdneri*) were held in stainless steel enclosures (1 m^3 capacity and with air/water and water/sediment interface of 1 m^2) in a 1 m deep pond whilst a range of application rates of RIPCORD (100 g l^{-1} e.c.) or FASTAC (100 g l^{-1} e.c.) was applied to the water surface by hand-held sprayer. Secondly, in two experiments RIPCORD (400 g l^{-1} e.c.) was applied by hand-held boom-and-nozzle at 100 g a.i. ha^{-1} to the surface of 1 m deep ponds which contained populations of fish. Finally, the effects on fish of spray-drift from commercial applications of RIPCORD were monitored.

Results and Discussion

The laboratory tests confirmed the high acute toxicity of RIPCORD and FASTAC to fish.

Chemical	Species	96 h LC50 (µg l^{-1})
RIPCORD	*Salmo gairdneri**	2.8
	Cyprinus carpio	0.9-1.1
	Scardinius erythrophthalmus	0.4
FASTAC	*Salmo gairdneri**	2.8

*Static water renewal tests.

The field studies, however, showed that at dosages within and above commercial application rates (typical commercial rates for RIPCORD and FASTAC are 20-70 g ai ha^{-1} and 7-20 g a.i. ha^{-1}) neither spray-drift nor direct over-spray of either RIPCORD or FASTAC resulted in fish deaths.

Test system		Application rate at which no toxic effects were seen (g a.i. ha^{-1})	Species
Direct overspray	- Enclosures	RIPCORD 50	*S. gairdneri*
		FASTAC 50	*S. gairdneri*
	- Ponds	RIPCORD 100	*S. erythrophthalmus*
			Tinca tinca
Spray-drift	- Ponds	RIPCORD 70 (tractor)	*S. erythrophthalmus*
	- Ditches	RIPCORD 25 (aerial)	*C. carpio*

The lack of hazard, despite the high inherent toxicity, is explained by low commercial application rates, particularly for FASTAC, and the physico-chemical properties of these insecticides. These properties limit their penetration into water and cause a significant proportion of that entering the water to be adsorbed onto surfaces (e.g. suspended solids, sediment, vegetation).

*A SHELL Registered trade mark.

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4B—R18

ADSORPTION REDUCES ACTIVITY OF PESTICIDES IN SOIL AND WATER

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Introduction

Following application, pesticides partition between the solid, liquid and gaseous phases. The biological activity of a pesticide in soil or aquatic environments is likely to be proportional to the concentration of the chemical in the water and/or gaseous phases.

The effect of soil adsorption on the toxicity of paraquat to plants and cypermethrin to aquatic invertebrates has been investigated. Both chemicals have negligible vapour pressures; therefore, only their partition between solid and water phases need to be considered.

Methods

Solutions of paraquat dichloride, equilibrium solutions from soil slurries treated with paraquat, and paraquat-treated soils were bioassayed with wheat (Triticum aestivum).

Daphnia magna, Cloeon dipterum nymphs and Asellus aquaticus were exposed to cypermethrin in water, water over a layer of undisturbed soil, and water with suspended soil. In the systems containing soil, acetone solutions of cypermethrin were applied direct to the soil. After equilibration for 4 hours the soil was flooded and test animals were introduced after a further 1 hour.

Results

For paraquat in solution bioassays of root length were the most sensitive indicator of phytotoxicity, whereas in the soil bioassay there was no major difference in the sensitivity of roots and shoots. In solution about 0.01 µg paraquat/ml reduced root growth by 50%. The concentration in soil required to produce the same effect was 5,000 - 100,000 times higher. Effects on plants in the equilibrium soil solutions were very similar to those on plants grown directly in the soil, showing that adsorbed paraquat has no effect on plants.

In the cypermethrin study the presence of soil reduced toxicity to Daphnia, Cloeon and Asellus about 200, 250 and 350-fold, respectively. There was no significant difference between the undisturbed and suspended soil treatments.

Toxicity of cypermethrin to Daphnia, Cloeon and Asellus in the presence and absence of soil; 72-hour EC50 values expressed as µg cypermethrin/l assuming cypermethrin was evenly distributed throughout total water volume.

Organism	Water	Water plus undisturbed soil	Water plus suspended soil
Daphnia	1.6	310	250
Cloeon	0.012	2.9	3.3
Asellus	0.009	2.8	3.1

Conclusions

1. Adsorption by soil or aquatic sediments greatly reduces the biological activity of pesticides.
2. Solution toxicity data must be adjusted to take account of the effects of adsorption when predicting effects under field conditions.

EVALUATION OF LEACHING OF ALDICARB RESIDUES FROM SOIL TO GROUNDWATER

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Behaviour of the oxidation products in soils and subsoils

The insecticide and nematicide aldicarb is mixed into soil in spring to protect potato crops against harmful soil nematodes. Some humic and peaty soils in the eastern Netherlands have comparatively low pH and the transformation rates of the toxic oxidation products of aldicarb (sulphoxide and sulphone) are low. About 15 % of the dose may remain as toxic residue in soil late in autumn. The oxidation products are only weakly adsorbed onto soil and may leach to groundwater with excess precipitation in winter.

The transformation and movement of aldicarb and its oxidation products was simulated with computer models, introducing physico-chemical data from the laboratory. The computed behaviour in soil was similar to the measured behaviour.

The rate of transformation of the oxidation products in aquifers presumably ranges from fairly high to very low, with geochemical conditions. Fairly high rates have been found in some anaerobic subsoil materials, especially at pH about 7 or more. However in aquifers in some areas of the United States and the Netherlands, aldicarb sulphoxide and sulphone seemed to be only slowly degraded.

The concentrations of the sulphoxide and sulphone in the phreatic aquifer were calculated on the basis of the following assumptions : (a) dressing rate of 3 kg of aldicarb per hectare in spring; (b) leaching of 3 % of the dose as sulphoxide and 12 % as sulphone in winter; (c) precipitation surplus of 250 mm/year; (d) treated area 5 % of total land area; (e) no further degradation in subsoil.

Toxicological evaluation

Limiting values for groundwater pumped for human consumption were calculated by the procedure of Train (1979). Starting from the no toxic effect level, a safety factor of 0.01 is used to calculate the acceptable daily intake (ADI). A fraction of 0.2 of the ADI is reserved for water. A man of 70 kg is assumed to consume 2 l of water per day. The calculated concentrations of the sulphoxide and sulphone in groundwater were somewhat below these limiting values.

Conclusions

In areas with fairly low transformation rates in soil and subsoil, the area treated with aldicarb should be restricted. In areas with fairly high rates, the effect on groundwater quality seems small. However further research on the transformation of the residues in divergent subsoils is needed.

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✓ **4B—R20**

DISTRIBUTION AND BREAKDOWN OF DDT IN AN ORCHARD ECOSYSTEM

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Background and objectives

Knowledge of the persistence and behaviour of DDT and its breakdown products in different compartments of the environment is inconclusive. This is because either (a) the study period was too short, or (b) experimentation was done during the era of DDT spraying when further inputs of DDT masked the true behaviour pattern.

A nine-year study was done in an orchard ecosystem in Herefordshire, UK, from 1972 - 1980; DDT applications had ceased in 1969. Results were compared to those of workers (Stringer et al, 1970), who sampled the same orchard during the period when DDT was being used regularly.

Materials and Methods

The orchard studied (10 ha) was planted in 1930 with Worcester Permain bush apple trees at 6 m x 6 m spacings. The orchard soil (density 1.47 g cm^{-3}) was a freely drained sandy loam (pH 7.8 - 8.2). There was a small reservoir (c ½ ha/x 2 m deep) adjacent to this area which received some of the drainage water. Soil samples (10 cm depth) were taken from under tree canopies and in alleyways on several occasions during the study period. Soil fauna, rain water, reservoir water, aquatic plants, plankton and fish were also sampled. Quantitative analyses were done by glc and qualitative confirmation achieved by tlc, gc - ms, hplc and other methods.

Results and conclusions

Soil In 1979 soil residues were 1.2 mg/kg pp'DDT, 0.1 mg/kg op'DDT, 0.05 mg/kg pp'TDE and 1.52 mg/kg pp'DDE; in the era of DDT spraying (1960-1969) the corresponding figures (1968) were 2.3, 0.3, 0.4 and 0.6 respectively. Residues of pp'DDT, op'DDT and pp'TDE decreased whereas those of pp'DDE increased; the decrease of pp'DDT approximately matched the increase of pp'DDE. The op'DDT isomer was less persistent than pp'DDT, with no obvious build up of the equivalent pp'DDE. A soil profile analysis showed that 87% of all DDT residues were contained in the top 10 cm layer.

Calculated decay times (Dt_{50}) over the period of study were 12 years for pp'DDT and 7 years for op'DDT, four times greater than those reported by earlier workers (Stringer et al 1974, Edwards, 1966). These differences probably reflect the rapid initial losses from the sward and soil surface by volatilisation; thereafter the residues penetrate and become strongly adsorbed into the top 10 cm of soil, and volatilisation is impeded. Regression analysis showed that the amount of total residues of DDT and related compounds is not decreasing with time, and in approximately 100 years only pp'DDE will be present.

Soil fauna In the post DDT period the major metabolites found in soil fauna were pp'DDE and pp'TDE similar to those reported in the DDT spray period. All earthworms species studied in the spray era contained much DDT residues (13 - 16 mg/kg) due to ingestion of residues on surface vegetation and in detritus. In the post spray period, the shallow dwelling species (eg. *Allolobophora rosea*) had greater concentrations (6 - 14 mg/kg) compared with deep dwelling species (eg. *Lumbricus terrestris*) (2 - 3 mg/kg); this was due to their continuous contact with and ingestion of the DDT residues present in the top 10 cm soil layer. In all species of slug, DDT residues gradually decreased with time.

Reservoir Total residues in reservoir water sampled in the post DDT spray period (1980) were 22 ng/l, half those found in the earlier spray period but twice those found in rain-water; all these values although very small compared to soil, were highly significant because of the biological magnification shown in different compartments of the aquatic system. Typical biological magnification factors found in reservoir samples during the post DDT spray period were 2×10^4, 12×10^4, and 1.2×10^6 for plankton, pond-weed and trout brain respectively.

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ROLE OF FORMULATION ON PESTICIDE DYNAMICS AND FATE IN A FOREST ECOSYSTEM

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Background and objectives

The presence of volatile components in a formulation results in spray evaporation in flight, providing small droplets at the target site and reducing its impaction efficiency. Droplet evaporation is influenced by relative humidity (RH), wind speed and temperature (temp) of the surrounding air and droplet size spectrum. Formulation ingredients are known to have a marked influence on spray atomization characteristics and to affect the droplet size spectrum at the target site. This study reports data on the role of formulation ingredients on spray droplet spectrum, dynamics and fate of aminocarb in aqueous (FW) and oil-based (FO) mixtures in the four compartments of the forest ecosystem, namely biosphere (conifer foliage), atmosphere (air), hydrosphere (water, sediment, aquatic plants and fish) and lithosphere (forest litter and soil).

Materials and Methods

Matacil® 180F (flowable) contains finely divided aminocarb in a heavy oil base and it was formulated in two liquid media: Matacil® 180F, Atlox® 3409F and water; Matacil® 180F and a volatile light petroleum oil. The mixtures were aerially sprayed over conifer forests at the rate of 2 x 70 g AI in 1.5 L/ha, to give a total dosage rate of 140 g AI in 3.0 L/ha, using a TBM Avenger aircraft equipped with 1010 Flat Fan Teejet ® nozzles. To measure the relative droplet sizes produced during atomization, the formulations, containing a tracer dye, were sprayed in the laboratory at the temp and RH of the field study (for FW, 10°-15° C, RH, 87-90%; for FO, 15°-19°C). Droplet stains were collected on Kromekote® cards, sized by microscopy and were converted into their corresponding aerodynamic drop sizes using spread factor data. Aminocarb residues in various compartments of the forest ecosystem were measured by gas-chromatography after solvent extraction and the necessary cleanup.

Results and conclusions

Formulation ingredients exerted a marked influence on spray droplet spectra and deposition on targets. Under the temp and RH of aerial spraying, the emulsion formulation provided larger droplets than the oil-based mixture on the Kromekote® cards, resulting in c. 50% higher initial deposits in all matrices of the forest ecosystem. However, pesticide loss was generally curvilinear and was fairly rapid in all components with both formulations. Residues in balsam fir foliage ranged from 1080 to 1340 ppb and varied according to the formulation sprayed. In all cases, the 12 d post-spray samples contained only 10% of the initial value. The max. air concentration was 1200ng/m^3 but decreased to 150 ng/m^3 at 24 h. In stream waters, aminocarb residues were 3 ppb at 1h with the oil-based mixture and disappeared to undetectable limits in 12 h. With the aqueous mixture however, the residues were 23 ppb at 1 h and disappeared more slowly to 0.62 ppb at 12h. Aquatic plants acted as sinks for aminocarb. The watercress showed rapid uptake and accumulation of the chemical (max. 880 ppb) beyond 24 h. On the other hand, peak residues in the moss were only 174 ppb and decayed to 60 ppb at 24 h. Stream sediments contained only traces (10 ppb) and were not quantifiable. Fish samples collected from streams contained c. 10 ppb and bioaccumulation was low. The emulsion formulation resulted in a noticeable but small bioaccumulation as compared to the oil formulation. However, no mortality or sublethal effects were observed in fish during the study. The forest floor is generally a major receptor of aerially applied materials. However, in the present study, residues in forest litter and soil were extremely low, c. 40 to 70 ppb at 1 h post-spray and decreased to undetectable limits within a few hours. The mechanism of disappearance of aminocarb from the various forestry substrates is primarily through processes such as volatilization, leaching, photolysis, dilution, oxidation and hydrolysis and probably to a lesser extent by biodegradation. The conclusion of the study is that formulation ingredients markedly influence the spray atomization characteristics, in flight droplet evaporation and deposit concentrations of aminocarb on targets, and to some extent, affect the rate of disappearance from various components of the forest ecosystem.

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✓ # 4B—R22

THE ROLE OF SEDIMENT IN THE DEGRADATION OF BENDIOCARB IN WATER

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Background and objectives

There is increasing concern over the fate of pesticides in the aquatic environment since contamination of natural waters may occur as a result of normal pesticide usage. Consequently, a number of tests have been proposed, by Regulatory Authorities, which have been designed to assess a chemical's potential for biodegradation in water. Since these evaluations invariably use simple laboratory "screening" tests it follows that the results may not necessarily reflect the true behaviour of the pesticide in the aquatic environment. Generally the tests involve the incubation of the chemical, as the sole carbon source, in filtered natural water, with either low nutrient status or with minor nutrient amendments. Occasionally they include either a small sediment inoculum or less commonly, a sediment layer with overlying surface water.

In order to assess the role of sediment, this report describes a study to compare the degradation of the insecticide bendiocarb (2,2-dimethyl-1-3-benzodioxol-4-ol methylcarbamate) in water in the presence and absence of sediment.

Materials and methods

Three laboratory tests were initiated in which $[^{14}C]$-radiolabelled bendiocarb was incubated in filtered river water, non-filtered water and a sediment/water system for up to 2 months. The experiments were carried out under 'enclosed' conditions in order to obtain a balance of radioactivity, and any evolved radioactive products were collected in solvent 'traps'.

At intervals during the incubation period, samples from each of the three systems were removed for analysis. The distribution of radioactivity in the sediment (where present) and water, was separately analysed and $[^{14}C]$-products were characterised by radiochromatographic techniques.

Results and discussion

From the results obtained, the characteristics of bendiocarb degradation can be determined in each of the test systems.

This should allow discussion of the relevance of the sediment/water system as compared with the other two methods, based on the proposal that such a system more closely resembles the natural relationship between sediment and water in the environment.

A HISTOLOGICAL TECHNIQUE FOR THE IDENTIFICATION OF POISONING IN WILDLIFE BY THE RODENTICIDE CALCIFEROL

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Background and Objectives

As part of the field assessment of potential environmental hazard from pesticides and in particular to monitor studies of the commercial use of rodenticides, it is necessary to identify and confirm the cause of death of any non-target casualties thought to arise from their use. Chemical confirmation of the rodenticide calciferol, although possible in baits, has not been successful in animal tissue or stomach contents obtained from field casualties. The toxicological action of calciferol leads to hypercalcaemia which may in turn cause the deposition of calcium, principally in the cardio vascular system and kidneys. This study was carried out to determine whether this calcium deposition arising from calciferol ingestion could be detected histologically in heart and kidneys of dosed rats and quail.

Materials and Methods

Laboratory bred rats and quail were given the standard rodenticide test regime of two days on 0.1% calciferol diet. Animals were then killed from 0-4 days after removal from the diet. Heart and kidneys were removed into buffered formalin, processed to paraffin blocks which were sectioned at 6 μ and stained using the Alizarin Red S pH 4.1-4.3 method. Heart and kidneys from rats were also removed, up to seven days post-mortem, and processed and stained in order to determine the stability of the tissue calcium deposition after death. Field casualties may not be examined until several days after their death.

Results and Conclusions

The Alizarin Red S pH 4.1 to 4.3 stain showed that severe calcium deposition occurred from 0 to 4 days in the hearts and kidneys of male rats and quail exposed to 0.1% calciferol in their diet. Female quail showed a lower initial calcium deposition but after day 2, the calcium deposition was similar to that in the male. Calcium deposition in the heart and kidneys of male rats were detected up to 7 days after death indicating the stability of the tissue calcium deposition. The Alizarin Red S pH 4.1-4.3 staining method differentiated between kidneys from animals exposed to 0.1% calciferol diet and controls fed normal laboratory diet. The Alizarin Red S pH 4.1-4.3 staining method may provide a rapid and easily reproducable method for detecting extensive calcium deposition occurring in tissues from wildlife poisoning cases thought to involve calciferol.

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✓ **4B—R24**

LABORATORY ECOTOXICITY TESTS: HOW REPRODUCIBLE NEED THEY BE?

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INTRODUCTION

The hazard a pesticide presents to wildlife depends on both:

(a) the inherent toxicity of the pesticide and

(b) the degree of exposure of the organisms.

Laboratory toxicity tests are chiefly designed to measure (a), although they sometimes take into account one aspect of (b); namely the effects of soil adsorption on toxicity. When assessing the reproducibility required from laboratory tests it is necessary to examine the variations of (a) and (b).

VARIATION OF TOXICITY AND EXPOSURE.

When a laboratory bioassay is done by one person, under one set of conditions it is not unusual for the 95% confidence interval for an LD50 or EC50 value to cover only a 2-fold range. However, ring tests using standard methods on plants, earthworms and Daphnia have shown that at best there is a 5-fold range in LD50/EC50 values from different laboratories and more typically the range is 10-fold. Thus the toxicity values for a particular pesticide, measured under a moderate range of conditions, for a few similar species, cover at least a 10-fold range. This is illustrated in the poster with data for the bipyridylium herbicides and pyrethroid insecticides. Under field conditions climate, growth stage and health of animals are much more variable. Also one is using laboratory data for a small number of species to predict toxicities to a wide range of species.

Degree of exposure to a pesticide in the field is extremely variable. For example, when a crop is sprayed with a pesticide a moderately sensitive species severely exposed at the top of the crop might be killed while a sensitive species living at the bottom of the crop may be unharmed. The persistence of a chemical can greatly affect the length of exposure,and persistence of a chemical, e.g. in soil, can vary at least 10-fold. The degree of adsorption of some chemicals, and thus their bioavailability, varies at least 10-fold. Movement of a chemical in the environment, e.g., systemic movement in a plant, volatilization and leaching also have a major influence on the exposure of organisms to the chemical.

CONCLUSIONS

1. The uncertainties in the degree of exposure of an organism to a pesticide and uncertainties in the effect of variable field conditions on the toxicity of the pesticide are much greater than variations in results from standard laboratory toxicity tests.

2. The reproducibility of a laboratory toxicity test is adequate if LD50/EC50 values from different laboratories do not differ by more than one order of magnitude (10-fold).

3. Effort on assessing field exposure levels should be increased, relative to that spent on improving the reproducibility of laboratory toxicity tests.

ENVIRONMENTAL AND ECOTOXICOLOGICAL PROPERTIES OF THE FUNGICIDE BENALAXYL

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Background and objective

Benalaxyl [D,L-alanine,N-(2,6-dimethylphenyl)-N-(phenylacetyl) methyl ester] is a systemic fungicide effective on a wide range of diseases in grapes, potatoes, tobacco and other crops induced by phycomycetes.

The aim of this paper is to determine the physico-chemical properties of Benalaxyl, and correlate them with the behaviour of such fungicide in air, water and soil, as well as to investigate its toxicity on wildlife, bees, earthworms and aquatic organisms.

Materials and Methods

Physical and chemical properties such as water solubility, vapour pressure, octanol-water partition coefficient, soil-water adsorption coefficient, hydrolytic and photo-stability were obtained according to International Agencies guidelines. Both wet soil-air and water-air ratios were calculated from equations proposed by EPA guidelines. Degradation in the soil was performed under aerobic conditions with ^{14}C-Benalaxyl according to EPA guidelines. Toxicological studies were designed so as to meet the requirements of EPA and OECD guidelines.

Results and Conclusions

The data obtained from laboratory measurements show that Benalaxyl is stable to hydrolysis and sunlight and poorly volatile from water and wet soil.

It is highly adsorbed in the soil organic matter and scarcely leached.

In the soil the degradation of Benalaxyl appears to be essentially dependent on the presence of micro-organisms. If the soil is autoclaved, degradation becomes very slow in agreement with the high hydrolytic stability. In natural soils half-life ranges from 30 to 100 days, depending on soil and dose. Methyl N-(2,6-xylyl)-N- malonyl-D, L-alanine, N-(2,6-xylyl)-N-malonyl are the main metabolic products identified in the soil.

The results of toxicological studies carried out on Benalaxyl indicate that such fungicide is safe against wildlife, bees, earthworms and aquatic organisms. Benalaxyl in a soil-water system is highly adsorbed in the upper layer of soil and does not leach through it. Its soil degradation products could be leached, but they are harmless to fish (LC_{50}>100 ppm).

4B—R26

RESIDUAL TOXICITY OF SOME HERBICIDES

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Objectives

A large number of chemicals with herbicidal properties are used in weed control. A considerable portion of these herbicides applied to crops end up in soil where they undergo physical and chemical changes. As a result of these, the residual toxicity of herbicides tend to decrease although residues of some herbicides have shown to exert a greater toxicity than the original compound (Andus, 1952). Studies reported in this paper were carried out to examine the residual toxicity of some commonly used herbicides in Sri Lanka.

Materials and Methods

The commercially available compounds of

(1) Sodium 2,2-dichloropropionate (Dalapon)
(2) 3-(3,4-dichlorophenyl)-1, 1-dimethyl urea (Diuron)
(3) Pentachlorophenol (PCP)

were incorporated separately with four types of soils viz. Reddish Brown Earth, Reddish Brown Latasolic, Immature Brown Loam and Low - humic gley. The final concentration of the 3 compounds Dalapon, Diuron and PCP in the soils were 15, 2 and 20 ppm respectively. The soil-herbicide mixtures were incubated at 50% field capacity and under flooded conditions at room temperature. At fortnightly intervals the residual toxicity of the soil - herbicide mixtures were determined by bio-assay tests (Andus, 1951) over a period of 13 weeks. Residual concentrations of the 3 herbicides in sterile soils (sterilized by heating to 150°C for 48 hours) incubated at 50% field capacity at room temperature were also determined as in the previous experiments.

Results

The residual concentration of the 3 herbicides in the soils examined dropped rapidly during the first week probably due to adsorption. By the end of the subsequent 12 week period the concentration of Dalapon decreased down to 4 ppm except in LHG soil where the value was 2 ppm. Diuron and PCP decomposed in all 4 soils. At the end of the experimental period Diuron concentration was 0.8, 1.2, 0.6 and 0.5 ppm respectively. The corresponding values for PCP were 5, 5, 10 and 5 ppm. Under flooded conditions, there was slow decomposition of all 3 herbicides. The final concentration of Dalapon in the 4 soils were 8, 8, 8 and 6 ppm respectively. Values for Diuron were 1.5, 1.5, 1.5 and 1.0 ppm and for PCP all soils had a residual concentration of 10 ppm.

In sterile soils, the residual concentration of the 3 herbicides did not decrease by any appreciable amounts indicating that soil microorganisms were responsible for the decrease in the residual concentration of the herbicides.

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PESTICIDE REGISTRATION AND PLANT PROTECTION IN THE UNITED STATES

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The Federal Insecticide, Fungicide, and Rodenticide Act authorizes The U.S. Environmental Protection Agency (U.S. EPA) to register pesticides that do not cause unreasonable adverse effects on the environment, including adverse effects on plants, animals, and humans. The protection of plants extends to agronomic crops, ornamentals, turf, trees, and other plants that are intentionally sprayed or otherwise treated, and to plants outside of the area of intended application which include food and cover vegetation for animals, food, fiber, fuel, and ornamental plants for man, and endangered and threatened plants.

The U.S. EPA has written non-regulatory guidelines to provide direction on the methodologies for assessing phytotoxicity. The guidelines are divided into several sections. The first portion contains an introduction and a discussion of general issues that have been raised during the development of the guidelines. Within the guidelines, general methods and standards and reporting procedures for the required tests are provided. Another portion deals with target area phytotoxicity testing, which is used to evaluate pesticide toxicity to those plants that would experience intentional application.

The largest portion of the guidelines consists of the tier testing sequences (three tiers) employed to study and report on pesticide toxicity to non-target area plants. The effects of the pesticides are determined through a series of tests as dictated by specific requirements of each test and tier and results of the previous tier studies. The tests are designed to provide guidance for gathering pesticide effects information on terrestial and aquatic plant growth and development through laboratory, greenhouse, and field studies. The influences of geographical, seasonal, and species variation are also addressed. Detailed protocols for the laboratory and greenhouse non-target area tests are included.

The phytotoxicity data submitted along with data on environmental fate and efficacy are used to assess the potential hazard of pesticides on non-target plants. Where a potential hazard exists, precautionary labeling, other statements, or other actions are instituted to minimize the potential adverse effects to non-target plants.

At present the U.S. EPA is not requiring the submission of the phytotoxicity data to the Agency except in special instances as determined on a case-by-case basis. These instances may include where endangered or threatened plants may be affected. The Agency believes that, in bringing their products to market, the pesticide registrants will adequately test for phytotoxic effects for both target and non-target area plants and will have instituted practices to greatly reduce these adverse effects.

4B—R28

DISTRIBUTION AND FATE OF TWO CARBAMATE INSECTICIDES IN PADDY RICE ECOSYSTEM

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The distribution and fate of the carbamates isoprocarb and BPMC, two insecticides that are used extensively in paddy rice protection, the physical components of a simulated paddy rice environment such as water and muds and the biotic components rice, kangkong (*Ipomoea aquatica*), *Tilapia* (*Tilapia nilotica*), and snails (*Pila luzonica*) were studied.

The residues of isoprocarb in paddy water declined following approximately first-order kinetics with a half-life of 1.2 days. Under flooded conditions, the residues in soil had a half-life of 6.4 compared with 38.5 days in unflooded conditions. Two metabolites were found in the soil and one was found in the water. Isoprocarb was taken up into the tissues of rice, kangkong, *Tilapia*, and snails.

The BPMC residues in rice leaves were higher than in the stems and grains. Almost 90% of these residues was lost one day after spray application. This insecticide was distributed more on the stems of kangkong than on the leaves. Residues of BPMC in *Tilapia* were low.

Data on the uptake, bioconcentration and occurrence of bound residues are presented and discussed.