Revised guidelines on environmental criteria for the registration of pesticides

Food and Agriculture Organization of the United Nations

REVISED
GUIDELINES ON ENVIRONMENTAL CRITERIA FOR THE REGISTRATION OF PESTICIDES

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FAO: ENVIRONMENTAL CRITERIA FOR REGISTRATION OF PESTICIDES

GLOSSARY OF ABBREVIATIONS AND ACRONYMS

PART 1 - PRINCIPLES

1.1 INTRODUCTION

Assessment of effects on the environment is an integral part of the process of pesticide development and registration. This assessment should be designed to identify potential hazards, and thus enable 8 risks of adverse effects on the environment to be quantified and evaluated in relation to benefits, as illustrated in Figure 1.

The nature and amount of data required for pesticide registration depends on the properties and use of each substance. Research resources should be focused on the identification and evaluation of major risks, and data requirements which are excessive and stifle innovation must be avoided. A stepwise sequence allows an efficient selection of tests essential to each individual risk analysis.

Following each step, a preliminary assessment of risks and benefits allows decisions to be made on the need for further testing. Tests closer to practical use conditions may be required if there are doubts that benefits clearly outweigh risks.

The steps are:

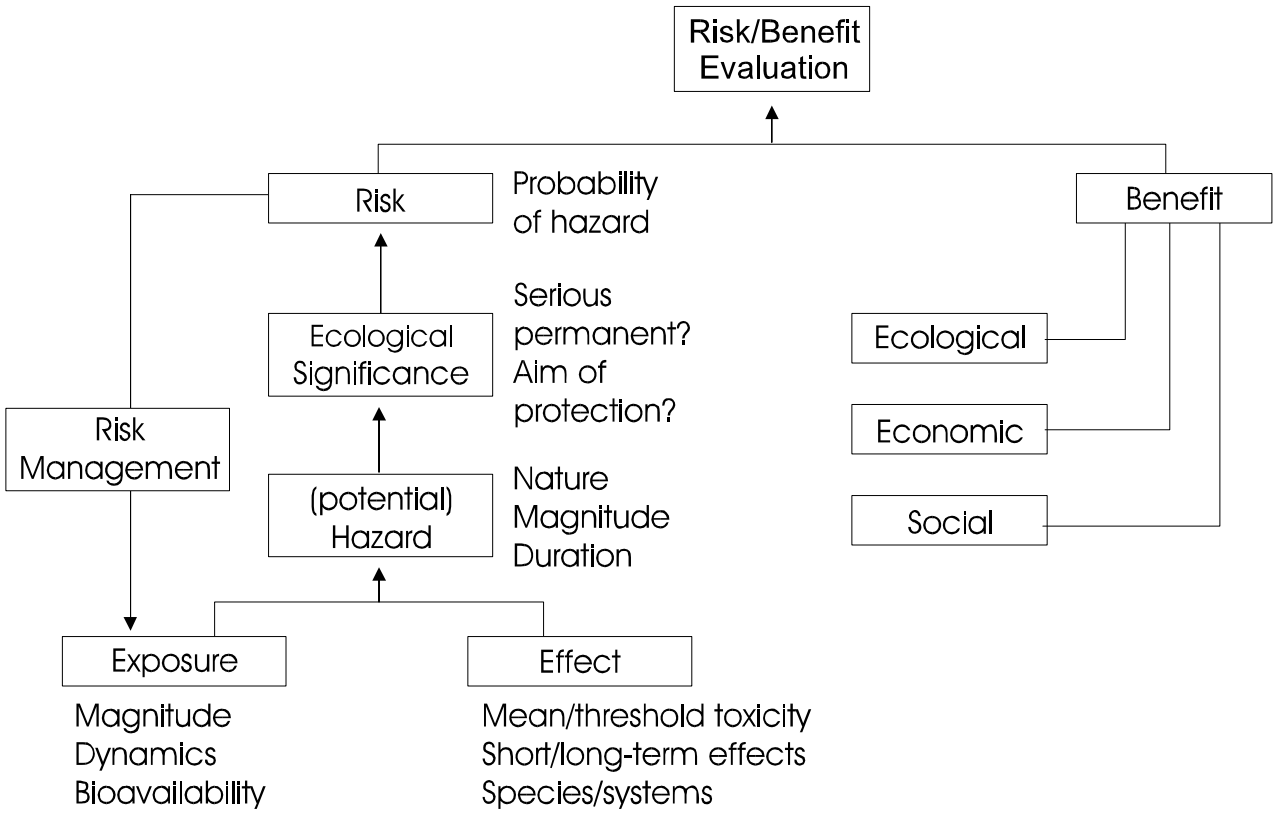
Step 1: Standard laboratory tests on physical and chemical properties, primary fate of the compound and acute or short term biological effects - generally necessary for all products.

Step 2: Supplementary laboratory studies on environmental distribution and degradation and additional toxicity tests including sublethal and chronic effects. The choice will be determined by the individual properties and uses of a substance.

Step 3: Simulated field and field studies, in case a product's hazard cannot sufficiently be assessed from laboratory studies (Steps 1 and 2) and experience.

Step 4: Post-registration monitoring, designed programmes and/or incident investigations during commercial use.

Figure 1
Environmental risk assessment as part
of the pesticide development and registration process



1.2 EXPOSURE

The exposure of an organism to a pesticide depends primarily on:

- concentration of chemical in relevant environmental compartment
- biological availability of the chemical
- biology of the organism (including location, season and feeding habits).

1.2.1 Environmental Concentrations

The highest concentrations usually occur during and immediately after application. Typical values are given in Tables 1 and 2. The great diversity of values emphasizes the importance of obtaining realistic estimates of environmental concentrations. Their order of magnitude can often be estimated by simple calculations (Ref. 1).

Following application, the concentration of residues declines due to:

- degradation
- movement into other environmental compartments
- dilution (e.g. during growth of treated leaf)

Degradation

Pesticides are degraded by a range of mechanisms including:

- chemical (e.g. hydrolysis)
- photochemical (e.g. on plant surfaces)
- metabolism (e.g. in plants or by microorganisms in soil or water)

The rates of degradation should normally be studied and identification made of major breakdown products in plants, soil, water and mammals using radio-labelled chemicals under controlled conditions.

Movement

Following application, a chemical moves between different parts of the environment mainly by mass flow in water, and diffusion\mass flow in the gaseous phase. This reduces the concentration in the treated compartment but transports residues to untreated compartments, e.g. from plant surface to soil or soil to water.

To estimate the potential of a chemical to move, it is necessary to know how a chemical is distributed between the three phases.

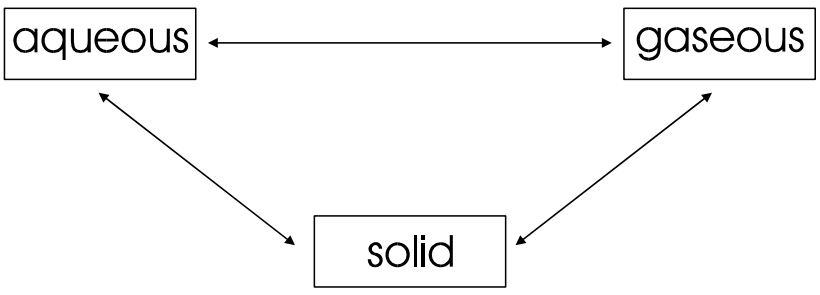


Table 1

Typical Concentrations of Pesticides
(actual values depend on individual circumstances)

Material	Concentration (mg a.i. /kg)
Product (50% w/w active ingredient)	500,000
Granules (5% w/w active ingredient)	50,000
Spray solution 0.5% of product)	2,500
Plants*	100
Seeds exposed to spray*	5
Soil (upper 5cm)**	0.5

* Immediately after spraying with 0.5 kg a.i./ha (adapted from Ref. 1).
** Uniform distribution of 0.5 kg a.i. /ha

Table 2

Possible environmental concentrations of pesticides
immediately after application in treated and adjacent areas
(assuming 1% of application rate as deposition rate from drift following application by ground sprayer)

Application rate (g a.i. /ha)	Material	Environmental Conc. (mg a.i. /kg)	
		Treated Area	Adjacent Area*
1000	Plants	200	2
	Seeds (on plants)	10	0.1
	Soil (upper 5 cm)	1	0.01
100	Plants	20	0.2
	Seeds (on plants)	1	0.01
	Soil (upper 5 cm)	0.1	0.001
10	Plants	2	0.02
	Seeds (on plants)	0.1	0.001
	Soil (upper 5 cm)	0.01	0.0001

* Level of possible contamination rapidly decreasing with distance.

Partially adapted from Hoerger and Kenaga (Ref. 1).

This distribution can be estimated either from the physicochemical properties of the chemical or measured.

1.2.2 Bioavailability

The availability of a chemical to organisms is primarily related to its concentration in the aqueous and gaseous phases. Thus, adsorption can have a major effect on the availability of the chemical.

When strongly adsorbed on sediment, the availability of a chemical to aquatic organisms such as fish can be several orders of magnitude less than if the chemical was in solution. Similarly adsorption by soil can greatly reduce a chemical's availability to plants and other soil organisms such as earthworms. Sorption onto plant material can reduce its availability to organisms living or feeding on the plant.

1.2.3 Biology of Organisms

Exposure assessment requires a knowledge of the location and feeding habits of the organism. For example:

- will it be living in an area when/where the pesticide is used?
- will it consume granules, treated seed or plants?
- will it be physically protected from higher concentrations of chemical, e.g. by living within the soil.

If the initial hazard assessment indicates a marginal safety level then further exposure assessments might be necessary, for example:

- palatability of granules and treated seed to birds including repellency effects.
- degradation and movement of the chemical under field conditions.

1.2.4 Stepwise Sequence of Data Production

Step 1 : Standard Data/Laboratory Tests

Identity of Active Ingredient

- a common name proposed or accepted by ISO
- structural formula
- chemical name (IUPAC nomenclature)

Physical-Chemical Properties of Active Ingredient

- melting/boiling point
- density
- physical state
- absorption spectra - ultra violet, visible and infra-red
- vapour pressure (preferably in temperature range 20-25^{0}C)
- solubility in water (preferably in temperature range 20-25^{0}C)

- partition coefficient between water and an appropriate non-miscible solvent (e.g. n-octanol)
- acid/base dissociation constants (when appropriate)

Composition of Technical Grade Material

- concentration of active ingredient
- the nature and amount of significant impurities

Properties of Formulated Product

- type of formulation (e.g. water dispersible powder, emulsifiable concentrate, powder, granule, seed-dressing, aerosol)
- content of active ingredient(s)
- content and nature of components of the formulation, including adjuvants
- storage stability (in respect to composition and physical properties related to use)
- physical characteristics (e.g. density, particle size distribution)
- acidity/alkalinity

Mobility of Active Ingredient

- adsorption/desorption and/or leaching in soil

Fate of Active Ingredient

- hydrolysis
- photolysis in water
- rate of degradation and identification of major breakdown products in mammals, plants and soil
- method of analysis

Step 2 : Supplementary Laboratory Studies

These depend on the properties of the product and on the outcome of the initial hazard assessment. They are not standard requirements

- degradation in water/sediment
- leaching of major degradation products
- photolysis on soil surface
- method of analysis for relevant degradation products bioaccumulation in fish (if octanol/water partition coefficient is greater than 1000)
- estimation of volatility (e.g. from soil).

Step 3 : Simulated Field and Field Trials

If following additional laboratory studies and further hazard assessment there are still doubts about the environmental acceptability of a chemical then further field studies might be necessary, particularly for chemicals which are relatively persistent or exhibit high mobility.

Examples of the types of studies which might be helpful are:

- rates of degradation in soils following recommended application
- leaching
- volatilization

Some field studies on environmental fate are best combined with biological assessments (see Section 1.3, Step 3).

Step 4 : Post-Registration Monitoring

Monitoring of residues in the environment during normal commercial use of a pesticide (e.g. soil, water and wildlife). This can be combined with biological assessments (see Section 1.3, Step 4).

Examples of suitable methods for the above tests are given in Part 2.

(NOTE: Public disclosure of data should include safeguards that will serve to preclude unauthorized competitive use of such data (Refs. 2 and 3).

1.3 EFFECTS

Measurement of toxicity should be carried out using a step-wise procedure. After each step, a hazard assessment is made to decide if further testing is necessary.

Step 1 and 2 tests are normally carried out with the active ingredient, but a formulation is sometimes necessary to apply compounds with low water solubility, e.g. in tests for aquatic organisms and bees. Testing of a typical formulation on mammals, honey bees and fish might also be necessary if the formulation is expected to increase the toxicity to levels of environmental concern.

Laboratory tests are designed to maximize the availability of the pesticide and thus reveal maximum hazard. For example, aquatic studies are carried out in clean water to minimize adsorption of the chemical. Soils which might have a relatively high adsorption capacity such as soils with a high organic matter content should be avoided.

Step 1 : Standard Laboratory Tests

These tests are normally required for all substances unless the organism is not exposed.

Mammals

There is an abundance of data on mammals as a result of the work done to assess risk to humans. Acute tests include oral and dermal toxicity to rat; inhalation (rat); skin irritation (rat or rabbit); skin sensitization (guinea pig); eye irritation (rabbit).

Sub-chronic and chronic (three months - two years) feeding studies in rat, dog and mouse; rat and rabbit teratology; rat reproduction.

Other studies include several mutagenicity tests.

Birds

Acute LD_{50} or dietary LC_{50} toxicity to one or two species (e.g. quail and mallard).

Honey bees

Acute oral and contact toxicity.

Fish

Acute toxicity (96 h) to one species (e.g. trout).

Daphnia

Acute toxicity (48 h) to first instars.

Examples of suitable methods for the above tests are given in Section 2.3.2.

Step 2 : Supplementary Laboratory Tests

These depend on the outcome of the initial hazard assessment and are not a standard requirement.

Additional studies which might be useful include:

- tests on soil organisms, such as earthworms, and the functions of soil microorganisms in situations where substantial residues reach the soil and give cause for concern.
- further aquatic tests, if there is likely to be significant contamination of water; e.g. freshwater algae, or estuarine and marine organisms if appreciable residues could reach estuarine and marine environments.
- effects on relevant predators and/or parasites if the pesticide is to be recommended for use in integrated control programmes.
- longer-term toxicity studies, e.g. bird reproduction, Daphnia reproduction, fish long-term or early-life stage test, if organisms are exposed to the pesticide for long periods, e.g. if the pesticide has a relatively long persistence or is applied many times in a season.
- data on toxicity to plants are normally available from screening and efficacy studies.

Step 3 : Simulated Field or Field Trials

If, following additional laboratory studies and a further hazard assessment there are still doubts about the environmental acceptability of a product, then these may be resolved using simulated field or field trials. Such studies are extremely demanding on resources, and must be individually designed to answer specific questions; otherwise trials effort may be wasted and/or the trial yield inconclusive results. Scientific experience is often insufficient to fully evaluate current approaches of field test design and data interpretation.

Sometimes relatively simple simulated field or field trials, such as determining the palatability of pesticide granules/baits, can provide sufficient information for assessment.

In other cases, it might be necessary to combine a range of methods such as:

- assessing the utilization of treated field by wildlife, i.e. potential exposure
- determining the animal's main diet
- residue analysis of the animal diet searching for dead animals and investigating cause of death
- residue analysis of dead or live caught birds and mammals
- non-destructive biochemical studies on the organisms.

The range of available methods is discussed in more detail in Section 2.3. However, a particular technique should be used only if it helps to answer specific questions.

Step 4 : Post Registration Monitoring

Monitoring studies can extend the conditions in which potential hazards are surveyed and provide a check on the accuracy of conclusions drawn from studies in earlier Steps.

Many countries consider monitoring to be an essential responsibility of authorities or public institutions.

Monitoring can be carried out in several ways. One is to design a monitoring programme which involves investigations on chosen species.

General monitoring of wildlife population levels (e.g. by national surveys) can also provide useful information, but this is limited to identifying trends, and is difficult to use for demonstrating causes and effects.

Another approach is the investigation of wildlife mortality. The work chiefly involves autopsies and residue analysis of animals found dead, and enquiries into the circumstances that caused death.

The monitoring approach has several advantages:

a. It offers an opportunity to detect previously unpredicted hazards.

b. It covers all situations in which pesticides are used under practical conditions.

c. The misuse of pesticides, by deliberate action or by carelessness, can be identified.

1.4 HAZARD

Hazard is a function of exposure and effect. Hazard assessment can be used to either refute or quantify potentially harmful effects, in regard to their nature, their magnitude and their duration.

"worst-case" approach

In hazard evaluation, the so-called "worst-case" approach is frequently used by combining the highest exposure values with the highest toxicity levels. This approach is a useful tool for detecting potential hazards and defining the need for more detailed considerations. However, if the principle is over-stressed, the hazard can be greatly over-estimated while the actual probability of the hazard occurring, i.e. the risk, may be minimal (see Section 1.6 - Risk).

Pre-assessment classification

Experience has shown that, with typical pesticide use rates in normal spray application, compounds with toxicity values in the range or in excess of those in Table 3 can be considered to be practically non-hazardous. These pesticides generally do not require further evaluation.

TABLE 3

Organism, test	Range of toxicity values, taken from Refs. 4 to 15, within or above which no hazard is expected in typical practical use
Birds, acute LD_{50}	100 - 500 mg/kg body weight
Birds, 5-day dietary LC_{50}	500 - 1000 mg/kg food
Wild mammals, rat LD_{50}	100 - 500 mg/kg body weight
Fish, acute LC_{50} (96 h)	5 - 10 mg/l water
Aquatic invertebrates, daphnia EC_{50} (48 h)	5 - 10 mg/l water
Bees, acute oral LD_{50}	50 - 100 ug/bee

Regarding the values and organisms listed in Table 3, a lower LD, LC or EC_{50} value does not a priori indicate a specific hazard. It only means that a subsequent hazard assessment is recommended.

Hazard assessment

In hazard assessment, the effect values (mostly mean toxicity, e.g. LD/LC_{50}, or limit values, e.g. LOEC and NOEC for the lowest and no-effect level) for different organisms are compared with the estimated (predicted) environmental concentration (EEC or PEC) in the relevant compartment or food element. For example, for aquatic organisms, LC_{50} values are compared with expected concentrations in the aqueous phase over a comparable time, taking account of losses in natural aquatic ecosystems due to e.g. sorption and degradation. For birds, oral LD_{50} and dietary LC_{50} values are compared with expected concentrations in diet, taking into account relevant data concerning feeding behaviour and food consumption.

Comparison of EEC values with acute LD/LC_{50} values is appropriate if exposure to the compound is short. However, if the "safety factor" is small and there is potential for prolonged exposure, then the assessment should be based on the results of longer-term tests (e.g. LOEC/NOEC values from reproduction studies) and, if necessary, on results from simulated field and field studies. The use of stepwise testing procedures during the hazard evaluation process is described in greater detail in Section 2.3.

If the exposure concentration is 5-10 times less than the LD_{50}/EC_{50} or less than the NOEC in the corresponding time period then a practical hazard is unlikely. If these criteria are not fulfilled then further evaluations to quantify the risk are appropriate. Risk management measures such as label warnings or restrictions, and special application methods may be necessary. (See Section 1.6 - Risk).

There are uncertainties in extrapolation of effects, e.g. from species to species, laboratory to field, individual to population. However, the magnitudes of differences and extrapolation factors can be estimated from numerous studies and experience. Laboratory tests are run on sensitive species, with test conditions simulating "worst-case" situations, i.e. maximum bioavailability. Increasingly, field studies show that extrapolation from laboratory to the "in field effects" is possible within practical limits (See Section 1.5 - Ecological Significance).

The effect of a combination of substances are usually additive (Refs. 16 and 17) and can be calculated from Finney's harmonic-mean formula:

$$\frac{C_A}{T_A} + \frac{C_B}{T_B} + \ldots + \frac{C_Z}{T_Z} = \frac{100}{T_M}$$

where C = % concentration of active ingredients A, B ... Z, T = the toxicity(LD/LC_{50} values) of A, B ... Z, and T_M = the resulting toxicity value of the mixture (Ref. 17).

Example: A formulation shall contain 10% of compound A with an LC_{50} of 6 mg/l for trout and 30% of compound B with an LC_{50} of 20 mg/l.

$$\frac{10}{6} + \frac{30}{20} = \frac{100}{LC}50$$ of these ingredients in mixture.

i.e. for this formulation, an LC_{50} of 32 mg/l.

Such "procedure" applies in principle to compounds with a similar mode of action, but gives generally good results with other compounds as well. Cases of unpredictable synergisms are extremely rare, limited in magnitude and restricted to the level of concentration where effects occur. They are expected to be detected in efficacy trials and mammalian toxicology tests of such combinations. (Ref. 16).

1.5 ECOLOGICAL SIGNIFICANCE

The aim of environmental risk assessment is the protection and conservation of the environment. However, hazard predictions are rarely based on ecosystem studies because such studies are complex and difficult to clearly interpret.

The ecological approach requires an adequate knowledge of the presence and fate of the pesticide in the environment, a sound and professional biological background, which includes experience from ecological field work and agricultural practice, and a clear definition of which type of environment and which biological community should be protected. Environments where agricultural pesticides are applied are sites of more or less intensive agricultural activity. It should be remembered that human activity, in particular agriculture, has in some instances created types of environment which are now considered worthy of protection.

Ecological evaluation has to differentiate between transient effects, which have no significant ecological consequences, and long-term adverse effects which may not be acceptable. Attention should be given to effects that may occur outside the agricultural vicinity, e.g. when pesticides move outside the treated area. The mobility of wild-life species must also be considered.

The ecological importance of environmental effects caused by pesticides has to be evaluated in comparison to:

- natural abiotic variations: drought, freezing, flooding, temperature changes often have drastic effects on organisms;

- biotic fluctuations: the abundance of food will determine the population size of feeders;

- the "repopulation potential": organisms with a high intrinsic rate natural increase and dispersal may quickly compensate occasional depressions; however, care should be taken if organisms with a low repopulation capacity are affected;

- the rarity of the species;

- the ecological impact of other human activities including agriculture;

- the definition of the aims of protection and conservation.

The aims vary widely and different answers will be appropriate in different cases and countries. However, the final aim of the environmental hazard evaluation is adequate protection and conservation of the environment.

1.6 RISK

Hazard indicates the potential for damage to the environment. Risk is the probability of a defined hazard occurring. For example, if a worst-case-exposure-scenario predicts possible harmful contaminations of water bodies following heavy rain, a risk evaluation would have to consider the frequency of such events at the time and place of application.

A relatively low hazard with a high frequency may result in higher risk than a greater hazard with a lower frequency. The more extreme a "worst-case" scenario is, the more it must be considered to be rare and limited.

Quantification of probability factors will often be difficult. Possible approaches include:

- frequency of certain environmental conditions, e.g. heavy rain, drought, etc. (from meteorological records);

- frequency of biological risk conditions, e.g. times of abnormal hunger or thirst for wildlife (from game conservancy reports and meteorology),

- frequency of observed incidents attributable to the pesticides (field reports and toxicological investigations).

The quality of data and reports varies between countries. However, most pesticides are widely used and experiences from other countries can greatly help to identify possible hazards under comparable conditions. In any case, a separate evaluation is recommended to confirm or refute specific risks for a particular country or region.

Risk management

The environmental risk can be greatly reduced by limiting the exposure by, for example:

- modified formulations;

- change in application method; timing of application;

- practical recommendations and indication of potential hazards to be communicated by producers and salesmen;

- increased responsibility of applicators and training for correct use;

- minimization of wildlife exposure through buffer zones; care in cleaning the application devices;

- suitable disposal of empty containers.

REFERENCES

Hoerger, F.D., and Kenaga, E.E. (1972). Pesticide Residues on Plants. Correlation of representative data as a basis for estimation of their magnitude in the environment. Environmental Quality. Academic Press, New York, 1: 9-28. (Ref. 1).

Second Government Consultation on International Harmonization of Pesticide Registration Requirements (Rome, 11-15 October 1982. (Ref. 2).

Industrial Property Rights Essential to the Development of Chemical Technology for Agriculture (GIFAP/IPROE, July 1986). (Ref. 3).

US EPA Hazard Evaluation Division, Standard Evaluation Procedure: Avian single-dose oral LD_{50} test. EPA-540/9-85-007 (1985). (Ref. 4).

US EPA Hazard Evaluation Division, Standard Evaluation Procedure: Avian dietary LC_{50} test. EPA-540/9-5-008 (1985). (Ref. 5).

US EPA Hazard Evaluation Division, Standard Evaluation Procedure: Ecological risk assessment. EPA-540/9-85-001 (1986). (Ref. 6).

Kenaga, E.E. Factors to be considered in the evaluation of the toxicity of pesticides to birds in their environment. Environ. Qual. Safety 2, 166-181 (1973). (Ref. 7).

US EPA Hazard Evaluation Division, Standard Evaluation Procedure: Wild mammal toxicity test. EPA-540/9-85-004 (1985). (Ref. 8).

Bathe, R., Ullmann, L., Sachsse, K.: Toxizitätsbestimmung von Pflanzenschutznitteln an Fischen. Schr. Reihe Ver. Wass.-Boden-Lufthyg. Berlin-Dahlem, H. 37, Stuttgart: 241-256 (1972). (Ref. 9).

US EPA Hazard Evaluation Division, Standard Evaluation Procedure: Acute toxicity to freshwater fish. EPA-540/9-85-006 (1985). (Ref. 10).

Heidler, G. Die Betrachtung von Pflanzenschutzmitteln hinsichtlich ihrer Wirkung auf das aquatische Ökosystem. Mitt. Biol. Bundesanst. Land- u. Forstwirtsch. H. 232: 298-299 (1986). (Ref. 11).

Nishiuchi, Y. Testing methods for the toxicity of agricultural chemicals to aquatic organisms. Jap. Pet. Inf. No. 19 (April 1974). (Ref. 12).

US EPA Hazard Evaluation Division, Standard Evaluation Procedure: Acute toxicity test for freshwater invertebrates. EPA-540/9-85-005 (1985). (Ref. 13).

Industrieverband Agrar (IVA). Classification scheme and toxicity thresholds. Frankfurt/M. (1988). (Unpublished document available on request). (Ref. 14).

Felton, J.C., Oomen, P.A., Stevenson, J.H. Toxicity and hazard of pesticides to honey bees: harmonization of test methods. Bee World 67: 114-124 (1986). (Ref. 15).

GIFAP Position Paper on Toxicology of Crop Protection Products in Combination. Brussels (1988). (Ref. 16).

World Health Organization. The WHO recommended classification of pesticides by hazard and guidelines to classification 1986-87. WH0-VBC/86.1, p. 5 (1986). (Ref. 17).

PART 2 - GUIDELINES FOR APPROPRIATE TEST PROCEDURES

The concept of pre-registration hazard evaluation and prediction from primary and additional data has been described in Part 1 of this report. Part 2 lists test procedures considered to be appropriate in providing data for the assessment of the potential environmental effects presented by a pesticide.

The test procedures and sequences described originate from a variety of sources. Many are well established methods that have been used successfully many years in research as well as in connection with pesticide registration. Many others have been devised specifically for regulatory purposes by individual governments and international organizations. For those topics for which testing has only recently been required appropriate test procedures are still in a state of development.

The test procedures and sequences referred to in Part 2 fulfil common criteria, the most important of these being that they are widely applicable, and can be used to produce reliable, accurate and reproducible results.

References to some guidelines, particularly from national agencies and international organizations refer to current guidelines. In view of the continuous procedures for updating guidelines, it should always be ascertained if the latest version varies from the reference quoted.

2.1 PHYSICAL-CHEMICAL PROPERTIES

The physical-chemical properties usually provided for regulatory purposes and listed in Part 1 are necessary to identify and describe the active ingredient and the product. Vapour pressure, water solubility, and octanol/water partition coefficient have particular value in the prediction of the environmental behaviour of a pesticide. The following test procedures are recommended.

2.1.1 Vapour pressure

The vapour pressure of a pesticide is important in assessing its distribution in the environment. It is the major property which can be used to calculate the volatility of a substance and to predict whether or not a pesticide is likely to enter the atmosphere in significant concentrations. Conversely, the distribution between air and other compartments can be estimated.

Definition

Vapour pressure is defined as the saturation pressure above a solid or liquid substance at a particular temperature. The S.I. unit for pressure is the Pascal (Newton/m^2).

Criteria and test sequence

Determination of vapour pressure should not be required for pesticides with standard boiling point of less than 30^{o}C. The vapour pressure at a given temperature is measured in the laboratory at that temperature or calculated from an experimentally derived vapour pressure curve. If calculated from the vapour pressure curve, it must be ascertained that a change of

state or a transition point or decomposition does not occur within the temperature range under consideration.

Test requirements

The use of analytically pure active ingredient is preferred; however, technical material can also be used for the static and gas-saturation systems.

Appropriate test procedures

Several methods are reported in the literature (Refs. 1 to 5) which cover a range of vapour pressures from less than 10^{-3} to 10^{5} Pa, but no single method covers the entire range of vapour pressures. Five methods are recommended, each covering a definite range of vapour pressure (Ref. 3). Estimation of ambient vapour pressures of pesticides can be made from gas chromatographic retention data (Ref. 6).

1. Dynamic method (10^{3} up to 10^{5} Pa, between 20 and 100□C). In the dynamic method, the boiling temperature at a specified pressure is measured.

2. Static method (10 up to 10^{5} Pa, between 0 and 100□C). In the static process, at thermodynamic equilibrium the vapour pressure established in a closed system is determined at a specified temperature. This method is suitable for one component and multi-component solids and liquids.

3. Isoteniscope (from 10^{2} to 10^{5} Pa, between 0 and l00°C). This standardization method is also a static method but is usually not suitable for multi-component systems. Additional information is available from Ref. 2.

4. Vapour pressure balance (10^{-3} to 1 Pa, between 0 and 100°C). The quantity of substance leaving a cell per unit time through an opening of known size is determined under vacuum conditions such that return of substance into the cell is negligible (e.g. by measurement of the pulse generated on a sensitive balance by a vapour jet or by measuring the weight loss).

5. Gas saturation method (up to 1 Pa). A stream of inert carrier gas is passed over the substance in such a way that it becomes saturated with its vapour and the vapour is then collected in a suitable trap. Measurement of the amount of material transported by a known amount of carrier gas is used to calculate the vapour pressure at a given temperature.

REFERENCES

Thompson, G.W. and Douslin, D.R., (1971). Vapour pressure in physical methods of chemistry, Vol. 1, Part V, Chap. II. Ed. Weissberger, A. and Rossiter, B.W.. Wiley Interscience. (Ref. 1).

ASTM D 2879-75. Standard Test Method for Vapour Pressure. (Ref. 2).

OECD Test Guidelines No. 104. Vapour pressure curve. OECD, Paris (1981). (Ref. 3)

US Environmental Protection Agency, Pesticide Assessment Guidelines, Subdivision D, Product Chemistry, 9 September 1983, 71: 8136 (Ref. 4).

Spencer, W.F. and Cliath, M.M. (1983). Measurement of pesticide vapor pressures. Residue Rev., Vol. 85, 57-71. (Ref. 5).

Seiber, J.N., Woodrow, J.E., and Sanders, P.F. (1981). Estimation of ambient vapor pressures of pesticides from gas chromatographic retention data. Abstract, 1982nd Amer. Chem. Soc. Meeting, New York. (Ref. 6).

2.1.2 Water Solubility

The water solubility of a chemical is an important parameter determining its environmental transport and distribution. In addition, water solubility can affect adsorption and desorption on soils and volatility from aquatic systems, as well as possible transformation by hydrolysis, photolysis, oxidation, reduction and biodegradation in water.

Definition

Water solubility of a pesticide is defined as its saturation concentration in pure water at a given temperature. Water solubility is generally expressed in g/l or mg/l, though the S.I. unit is kg m^{-3}.

Criteria and test sequence

Evaluation of water solubility should be carried out for every pesticide which is stable in water during the required test period. A preliminary test to establish the approximate value of solubility is useful for the choice of the final method of measurement.

Test requirements

The purity of the test substance should be specified. Very pure water (double distilled) has to be used.

Temperature control is needed and the limits within which the temperature is maintained should be given. Tests are frequently carried out at 20^{o}C. If climatic conditions make it relevant and an environmental gradient is expected, tests should be carried out at two temperatures. Care has to be taken to avoid errors arising from the aggregation of finely dispersed particles of hydrophobic compounds.

Appropriate test procedures

Several methods are reported in the literature (Refs. 1, 2, 3 and 4) but no single method covers the entire range of water solubility. At least two methods are therefore needed; the first is applicable to substances of low solubility (less than 10 mg/l) and the second to substances of higher solubility (more than 10 mg/l). Estimations of water solubility of pesticides can also be made by chromatographic methods, such as high performance liquid chromatography. (Ref. 5).

1. Test for solubilities lower than about 10 mg/l (Ref. 4). The method is based on the elution of the pesticide with water from a microcolumn which is charged with an inert carrier material, such as glass beads, silica gel, sand, or cromosorb and an excess of test substance. The water solubility is determined when the mass concentration of the eluate is constant, tested at different flow rates. It must be ascertained that the identity of the pesticide is not changed during the procedure.

2. Test for solubilities higher than about 10 mg/l (Ref. 4). The substance (pulverized if solid) is dissolved in water at a temperature slightly higher than the test temperature. When saturation is achieved, the mixture is cooled and kept at the test temperature under stirring as long as necessary to reach equilibrium. Undissolved particles are removed from the solution and the dissolved pesticide is determined by a suitable specific analytical method.

3. Test for solubilities of gaseous and volatile compounds. No appropriate methodology can presently be proposed.

REFERENCES

Mader, W.J., and Grady, L.T., (1971). Determination of solubility. Physical Methods of Chemistry. Vol. 1 - Part V - Chapter V. Ed. Weissberger, A., and Rossiter, B.W. Wiley Interscience. (Ref. 1).

May, W.E., Wasik, S.P., and Freeman, D.H., (1978). Determination of solubility behaviour of some polycyclic aromatic hydrocarbons in water. Anal. Chem. 50 (7), 997. (Ref. 2).

US Environmental Protection Agency. Pesticide Assessment Guidelines, Subdivision D, Product Chemistry, 9 September 1983, 71: 8136. (Ref. 3).

OECD Test Guidelines No.105. Water solubility. OECD, Paris (1981). (Ref. 4).

Whitehouse, B.G., and Cooke, R.C. (1982). Estimating the aqueous solubility of aromatic hydrocarbons by high performance liquid chromatography. Chemosphere, Vol. 11: 689-699. (Ref. 5).

2.1.3 Octanol/Water Partition Coefficient

The octanol/water partition coefficient (P) is used as an indicator of bioaccumulation potential in living organisms.

Accumulation and transport of a chemical substance in living organisms are governed by polarity, water solubility, affinity for fatty tissues, and the nature of potential binding to biological receptors. The octanol/water partition is a measure for the distribution of a substance between the lipophilic and water phases of the test system. It therefore serves as an indicator of bioaccumulation potential in fatty tissues. Accumulation potential is an important factor in hazard assessment. In conjunction with data on degradation, accumulation potential may be used to identify chemicals which may be transported via food chains.

Definition

The partition coefficient of a chemical between two largely immiscible solvents is defined as the equilibrium ratio of the molar concentrations (c) of the substance dissolved in the two-phase system. For a dilute solution of a pesticide in the octanol/water system, it is:

$$P = \frac{c(octanol)}{c(water)} \qquad (1)$$

The partition coefficient, P, is a constant for a given pesticide at a given temperature, and it is usually expressed in the form of its logarithm to base ten (log P).

If the chemical does not dissociate or associate in water and octanol, then equation (1) applies, provided that the concentration in either phase is no more than 0.01 mol/l. Deviations from equation (1) are indicated by the fact that the partition coefficient becomes dependent upon the concentration of the solutions.

Criteria and test sequence

The determination of octanol/water partition coefficient should not be required for solid and liquid substances whose water solubility is less than about 10 ug/l, or greater than 2 g/l.

Substances which dissociate or associate in water and/or octanol will not show a constant value for P with varying concentrations due to speciation effects.

Because of the multiple equilibria involved, the recommended method should not be applied to compounds which reversibly ionize or protonate and the use of buffer solutions in place of water should be considered for such compounds.

A preliminary estimate of the partition coefficient can be done by calculation (Refs. 1 and 2) or by use of the solubilities of the test substance in the pure water (Sw) and octanol (So). In this latter case the estimated value of P is given by the ratio So/Sw.

Test requirements

A comparison between calculated and experimentally determined P values (Ref. 2) is recommended.

Very pure solvents and active ingredient should be used.

Appropriate test procedures

Several methods can be used to measure the partition coefficient in the laboratory. A commonly used method (Refs. 3 and 4) is to introduce the pesticide into octanol and water, to mix the biphasic system thoroughly to assure equilibrium and measure the concentration of the chemical in each of the two phases. However, application of this technique becomes increasingly difficult at higher values of lipophilicity (log P more than 4).

Chromatographic methods based on high pressure liquid chromatography (Refs. 5 and 8) and reversed phase thin-layer chromatography (Refs. 6 and 7) are available from the technical

literature. The calculation methods of Hansch and Rekker and the HPLC method have been proposed to be included in the OECD test guideline No.107 (Ref. 9).

REFERENCES

Leo, A., Hansch, C., and Elkins, D. (1971). partition coefficients and their uses. Chem. Rev., 71: 525. (Ref. 1).

Rekker, R.F. The hydrophobic fragmental constant. Elsevier, Amsterdam 1977. (Ref. 2).

US Environmental Protection Agency, Pesticide Assessment Guidelines, Subdivision D, Product Chemistry, 9 September 1983, 71: 8136. (Ref. 3).

OECD Test Guidelines No.107. Partition coefficient (n-octanol/water) OECD, Paris (1981). (Ref. 4).

Veith, G.D., and Morris, R.T. (1978). A rapid method for estimating log P for organic chemicals EPA - 600/3 - 78 - 049. NTIS PB - 284386. (Ref. 5).

Hulshoff, A., and Perrin, J.H. (1976). A reversed-phase thin-layer chromatographic method for the determination of relative partition coefficient of very lipophilic compounds. Journal of Chromatography, 120: 65. (Ref. 6).

Biagi, G.L., Barbaro, A.M., Guerra, M.C., Hakim, C., Solaini, G.C., and Borea, P.A. (1979). R values of naphtols and acetophenones in structure activity studies. Journal of Chromatography, 177, 34-49. (Ref. 7).

Eadsforth, Ch. V. (1986). Application of reverse-phase HPLC for the determination of partition coefficients. Pest. Sci. 17: 311-325. (Ref. 8),

Klein, W., Koerdel, W., Weiss, M., and Poremaki, H.J. (1988). Updating of the OECD test guideline 107, "Partition coefficient n-octanol/water": OECD laboratory intercomparison test on the HPLC method. Chemosphere, Vol. 17: 361-386. (Ref. 9).

2.2 FATE AND MOBILITY IN THE ENVIRONMENT

2.2.1 Fate in the environment

2.2.1.1 Degradation in mammals and plants

These are studied in great detail during the work done to assess human safety and are not discussed in detail in this section. (Refs. 1, 2 and 3).

REFERENCES

EPA Guidelines, Pesticide Assessment Guidelines, Subdivision F: Hazard Evaluation: Human and Domestic Animals, Rat: Part 86-1 (1982). (Ref. 1).

OECD Guideline for Testing of Chemicals, No.417. "Toxicokinetics" (Rat) (1984). (Ref.2).

EPA Guidelines, Pesticide Assessment Guidelines, Subdivision O: Residue Chemistry, Plants: Part 171-4 (1982). (Ref. 3).

2.2.1.2 Degradation in soil

Studies on the degradation of pesticides in soil will determine the rate and products of transformation in soil that may be caused by microbiological, chemical and photochemical processes. Despite the fact that microbial degradation is the most important cause of pesticide breakdown, a considerable amount of work has been done to distinguish between these three causes of pesticide degradation in soil. However, information as to whether a pesticide is degraded in particular by microbial, chemical or photochemical mechanisms can be considered of limited relevance from the point of view of environmental safety.

Available information indicates that photochemical degradation is not an important mechanism in the breakdown of pesticides in soil. Furthermore, laboratory experiments have shown that products identical with those resulting from microbial and chemical reactions are often formed.

Criteria and test sequence

Degradation studies in soil should be required only for pesticides likely to reach the soil when used according to recommendations.

Soil degradation tests should be carried out in the laboratory. If the compound is shown to be persistent in these and laboratory soil photolysis studies, simulated field or field tests should be carried out.

Test requirements

Pure pesticides or technical material may be used. However, for studies on the products of degradation, the use of radiolabelled material is recommended. For field studies, the formulated material is preferred.

The amount of pesticide tested either in laboratory or under field conditions should be in line with use recommendations. The soil used should be freshly sampled agricultural soil, e.g. sandy loam, preferably prepared according to procedures described in Ref. 8. Soil characteristics including pH, organic carbon, caution exchange capacity, particle size distribution and water holding capacity should be recorded. An estimation of microbial activity at initiation of the study is desirable. The use of stored standard soil (Ref. 7) should be avoided. Rate studies should be carried out in a minimum of two soils, whereas sufficient information on major degradation products can be obtained from experiments in one soil only (Ref. 3).

The incubation temperature and the soil moisture should be specified. Studies should normally be carried out under aerobic conditions. However, when the pesticide is recommended for use in flooded soils (e.g. paddy-rice culture) laboratory studies should simulate these conditions.

Appropriate test procedures

Laboratory tests

A summary and critical evaluation of experimental models of degradation can be found in a series of pertinent reviews (Refs. 1 to 4). Additional information can be obtained from Refs. 5 and 6.

Field tests

Field test guidelines should follow the recommendations for field residue trials (Ref. 9). Acceptable procedures can be found in Refs. 2 to 6.

Soil photolysis tests

Laboratory systems presently available have been recently reviewed (Refs. 3 and 4). A more recent model system is described (Ref. 10). Methodology is still under development.

REFERENCES

Kaufmann, D.D. Approaches to investigating soil degradation and dissipation of pesticides. Terrestrial Microcosms and Environmental Chemistry (J.M. Witt and J.W. Gillett, Eds.), 41-53. National Science Foundation/RA 79-0026, Washington (1977). (Ref. 1).

Hill, I.R., and Arnold, D.J. Transformations of pesticides in the environment - The experimental approach. In Pesticide Microbiology. Microbiological aspects of pesticide behaviour in the environment. (I.R. Hill and S.J.L. Wright, Eds.), 203-345, Academic Press. London and New York (Ref. 2).

Guth, J.A. The study of transformation. The Interactions between Herbicides and the Soil (R.J. Hance, Ed.), 133-157, Academic Press, London and New York (1980). (Ref. 3).

Guth, J.A. Experimental approaches to studying the fate of pesticides in soil. In Progress in Pesticide Biochemistry (D.H. Hutson and T.R. Roberts, Eds.) Vol. I, 85-114. John Wiley & Sons, Ltd. (1981). (Ref. 4).

US Environmental Protection Agency - Registration of pesticides in the United States, CFR 40, Subdiv. N, 162-1 (NTIS-PB83-153973) (1982). (Ref. 5).

US Environmental Protection Agency - Registration of pesticides in the United States, CFR 40, Subdiv. N, 171-4 (NTIS-PB83-153973) (1982). (Ref. 6).

Schinkel, K., Nolting, H.-G., Lundehn, J.-R. (1986); Verblieb von Pflanzenschutznitteln im Boden-Abbau, Umwandlung und Metabolismus-Richtlinien fur die amtliche Proofing von Pflanzenschutzmitteln Teil IV, 4-1, BBA, FRG. (Ref. 7).

Anderson, J.P.E. (1987); Handling and Storage of Soils for Pesticide Experiments in Pesticide Effects on Soil Microflora. Edited by L. Somerville and M.P. Greaves Taylor & Francis, pages 45-60. (Ref. 8).

FAO Guideline on Residue Field Trials Methodology (1980). (Ref. 9).

Leahey, J. et al. Development of a method to investigate the photodegradation of pesticides. Brighton Crop Protection Conference, Pests and Diseases (1988). 7c-2, 663-668. (Ref. 10).

2.2.1.3 Degradation in the aquatic environment

Studies on the degradation of pesticides in water give information on the rate and products of transformations by hydrolysis, photolysis and biodegradation. When studying aquatic systems, it should be recognized that the bottom and suspended sediments of ponds, rivers, streams, etc., in general play an important role in determining the fate of pesticides. Since it is impossible to maintain the environmental quality of natural waters under laboratory conditions, the considerations of water/sediment systems may be more useful.

A short, simple evaluation of hydrolytic potential of the compound is all that is generally necessary as a first stage. The available data (Refs. 2 to 4) indicate that H+ and OH- contribute significantly to catalyzed hydrolysis in natural freshwaters. Hence laboratory studies in buffered water at a range of pH are likely to yield those compounds which are also formed in natural environments. Laboratory studies of the degradation of pesticides in water/sediment systems do not normally take account of natural photodegradation which is complicated by the presence of light absorbers or photosensitizers (e.g. humic and fulvic acids, and riboflavin).

Numerous laboratory methods for determining the fate of chemicals in aquatic environments are quoted in the literature (Ref. 1). However, for the incubation of natural water/sediment systems under laboratory conditions few generally accepted test procedures are available. Investigations of the fate of pesticides in such systems together with studies of hydrolysis and photolysis will, however, be of value in understanding of their behaviour in aquatic environments.

Criteria and test sequence

Studies are required for all pesticides applied directly to water bodies or those for which their use pattern is likely to result in water contamination. Tests on biodegradation, hydrolysis and photolysis should generally be carried out under laboratory conditions to determine rates and products of degradation. If laboratory tests indicate that compounds may persist in the aquatic environment at concentrations which could put aquatic organisms at risk, simulated field or field tests should be considered.

Test requirements

1. Hydrolysis.

The analytically pure active ingredient in sterile buffered water should be used and the use of radio-labelled compounds is valuable whenever possible.

A single concentration should be tested at a level approximating the likely environmental concentration but not exceeding 50% of water solubility. Where lack of solubility is a limiting factor, the addition of organic solvents (e.g. acetonitrile) at low concentration (> 1%) may be necessary. Alcohols should not be used.

Sealed, sterile containers are required to maintain sterility and minimize volatilization. The test should be carried out in darkness at pH values within the ranges: acidic, pH 3-5.5, neutral, pH 5.5-8, basic, pH 8-10 and each value separated by at least two pH units.

2. Aqueous photolysis.

Analytically pure active ingredient in sterile distilled water should be used. The use of a commercial formulation should be avoided as the constituents may cause indirect photochemical reactions. For ease of analysis, radio-labelled chemical should be used wherever possible.

A single concentration should be tested at a level approximating the likely environmental concentration but not exceeding 50% of water solubility.

Whilst this is necessary to minimize reactions which would not occur at likely environmental concentration, higher rates may be required to facilitate identification of photoproducts. Where solubility is a limiting factor, a suitable non-photosensitizing cosolvent (> 1%) (e.g. acetonitrile - Ref. 4) may be used. Alcohols should not be used.

The test should be carried out within the pH range 5-8 (preferably near neutrality) in air-saturated distilled water. Studies at more than one pH value may be necessary for compounds that ionize or protonate.

Sterilized glass containers (capable of transmitting 290 nm and above) are required such that biodegradation is eliminated and volatilization minimized.

Artificial light sources are recommended and should resemble sunlight in wavelength distribution, especially in the UV region. Xenon arc lamps are preferred. Mercury lamps can also be used. Filters should be included to eliminate wavelengths below 290 nm. The manufacturer's specification for the lamp(s) and filters must be quoted. However, consideration should be give to the effect of ageing on the relative intensity of different wavelengths. Chemical actinometers could also be valuable in such studies. Samples should be exposed to the artificial light source for a sufficient period of time to allow for the estimation of the half-life. Controls should be kept in the dark, all conditions being comparable, to quantify the chemical contribution to any transformation processes.

Although the rates of photochemical reactions are not appreciably influenced by temperature the test should preferably be carried out at constant temperature.

3. Biodegradation studies

The sediment used should be analyzed for its pH (beginning and end of study), organic carbon, cation exchange capacity, particle size distribution and microbial biomass. The water pH should also be measured.

Pure or technical material may be used and the use of radio-labelled pesticide is recommended for studies on the products of degradation. Formulated material is preferred for field studies. A pesticide should be tested at levels approximating those likely to occur in bodies of water when used as recommended.

Test duration should not exceed three months.

In laboratory studies the incubation temperature should be kept constant.

Appropriate test procedures

1. Hydrolysis

If less than 10% degradation is observed in preliminary hydrolysis studies at 50°C over a period of five days the chemical can be considered hydrolytically stable and will require no further testing. If more than 10% degradation is observed, a further study should be carried out at a lower temperature. If the results then indicate the need for further work, a number of test procedures of varying degrees of complexity are available (Refs. 7 to 14).

2. Aqueous photolysis

A number of procedures can be found in Refs. 8 and 9, many of which are of greater complexity than are generally required. The procedure outlined above in the test requirements should be adequate in most circumstances.

3. Biodegradation studies

In the absence of generally accepted test procedures the following laboratory method is suggested (Refs. 5 and 6). Water/sediment systems representative of likely environmental application or contamination sites (e.g. ditches, rivers, etc.) should be used. The water and sediment should be collected from such environments either separately, to be mixed in the laboratory, or as water/sediment cores. Where anoxic conditions are required in the sediments, the water/sediment system should be preincubated prior to application of the pesticide and it may also be necessary to measure Redox potential (Eh). Wherever possible, biometer flasks or incubation systems with an air flow-through should be used such that degradation products evolved into the air may be collected. Procedures for field studies are currently being developed (Ref. 8).

REFERENCES

Howard, P., Saxena, T., Durking, P.R., and Ou, L.T. Review and evaluation of available techniques for determining persistence and routes of degradation of chemical substances in the environment. EPA Office of Toxic Substances, Final Report. NTIS PB-243825 (1975). (Ref. 1).

Mill, T. Chemical and Photo-oxidation. In the Handbook of Environmental Chemistry, Vol. 2, Part A. Reactions and Processes, p. 77. Ed. Hutzinger O., Berlin, Springer Verlag (1980). (Ref. 2).

Mabey, W.R., and Mill, T. Critical review of hydrolysis of organic compounds in water under environmental conditions. J. Phys. Chem. Ref. Data, 7, 383 (1978). (Ref. 3).

Smith, J.H., Mabet, W.R., Bohonos, N., Holt, B.R., Lee, S.S., Chou, T.W., Bomberger, D.C., and Mill, T. Environmental pathways of selected chemicals in freshwater systems. Part I - Background and experimental approach. EPA-600/7-77-113 (1977). (Ref. 4).

Guth, J.A. The study of transformation. The Interactions between Herbicides and the Soil (R.J. Hance, Ed.), 133-157, Academic Press, London and New York (1980). (Ref. 5).

Guth, J.A. Experimental approaches to studying the fate of pesticides in soil. In Progress in Pesticide Biochemistry (D.H. Hutson and T.R. Roberts, Eds.) Vol. I, 85-114. John Wiley & Sons, Ltd. (1981). (Ref. 6).

Smith, J.H. et al. Environmental pathways of selected chemicals in freshwater systems. Part II. Laboratory studies. EPA-600/7-78-074 (1978). (Ref. 7)

Crossland, N.O., Wolff, C.J.M. Outdoor ponds: their construction, management and use in environmental toxicology. The Handbook of Environmental Chemistry, Vol. 2, Part D. Hutzinger, O. (Ed), SpringerVerlag (1988). (Ref. 8).

FAO Guidelines for the Registration and Control of Pesticides (Rome, 1985). (Ref. 9).

US Environmental Protection Agency - Registration of pesticides in the United States. 40 CFR Subdiv. N, 161-1 (NTIS-PB83-153973) (1982). (NTIS-PB88-159892) (1987). (Ref. 10).

US Environmental Protection Agency. Toxic Substances Control. Discussion of premanufacture testing policy and technical issues; request for comment. Federal Register, 44, (53), 16 March, Part IV, 16268 (1979). (Ref. 11).

Gomma, H.M., Suffet, I.H., and Faust, S.D., Kinetics of hydrolysis of diazinon and diazoxon. Residue Reviews. 29, 171 (1969). (Ref. 12).

FRG BBA Verhalten von Pflanzenbehandlungsmittel im Wasser. Teil 1. Hydrolyse und Verdampfung. Merkblatt 55 (1980). (Ref. 13).

OECD Test Guideline No.111. Hydrolysis as a function of pH. OECD, Paris (1981). (Ref. 14).

2.2.2 Mobility in the Environment

The mobility of a pesticide is its ability to move within the environment and it is therefore important to consider it as a determinant for the transport of the pesticide and its degradation products. A number of characteristics, mostly influenced by physical-chemical properties, are significant for the prediction of mobility thus permitting estimates to be made on the distribution between environmental compartments. Volatility, absorption/desorption and leaching are the most important of these.

2.2.2.1 Adsorption/desorption

Adsorption and desorption characteristics of a pesticide in soil contribute to the prediction of the environmental distribution of a chemical. Leaching in soil (e.g. Ref. 6 of section 2.2.2.2)

and volatilization from wet soil surfaces are directly influenced by the adsorption/ desorption equilibrium in the soil/water system as this can define the extent to which a chemical is available for degradation.

Criteria and test sequence

The adsorption/desorption characteristics should be measured for each pesticide likely to reach the soil or aquatic environment.

The stability should be known before carrying out soil adsorption tests. If degradation is rapid it is not necessary to measure adsorption/desorption of the active ingredient. To be able to define the range of pesticide concentrations to be tested, the water solubility of the substance should be known.

Test requirements

The test should be conducted using the active ingredient, preferably radiolabelled.

For the determination of the adsorption isotherm for a given pesticide in a given soil, the study should be carried out with at least four pesticide concentrations.

Studies in at least two soil types are recommended. The characteristics of the soil used (soil pH, organic carbon, cation exchange capacity and particle size distribution) should be reported.

Appropriate test procedures

The most widely-used method for carrying out soil-pesticide adsorption/desorption studies is the slurry procedure where soil is shaken with aqueous pesticide solutions of known pesticide concentrations until an equilibrium is reached. The aqueous suspensions are then separated by centrifugation or filtration and the equilibrium concentrations in the water and if necessary in the soil are determined either by direct analysis or after extraction with an organic solvent.

Other methods used for soil adsorption include continuous flow equilibrium (Ref. 1) and dialysis (Ref. 2) techniques. Although other mathematical models are known, the Freundlich equation is most frequently used to describe adsorption in soil. Test methods for determining adsorption/desorption have been recommended (Refs. 3 and 4).

REFERENCES

Bunhill, S., Cardew, M.H., Hayes, M.H.B., and Smedley, R.J. Continuous flow methods for studying adsorption of herbicides by soil dispersions and soil columns. Proc. Eur. Weed Res. Counc. Symp. Herbicides - Soil, p. 70-79 (1973). (Ref. 1).

Hayes, M.H.B., Stacey, M., and Thompson, J.M. Adsorption of a s-triazine herbicides by soil organic matter preparations isotopes and radiation in soil organic matter studies, p. 75, Internat. Atomic Energy Agency, Vienna (1968). (Ref. 2).

US Environmental Protection Agency. Registration of pesticides in the United States, CFR 40, Subdiv. N, 163-1 (NTIS-PB83-153973, PB88-161195) (1982/87). (Ref. 3).

OECD Test Guidelines No.106 - Adsorption/Desorption. OECD Paris (1981). (Ref. 4).

2.2.2.2 Leaching

Data on the leaching behaviour of pesticides are required for all compounds intended for agricultural use, although the need for additional studies may be reduced when measurement of adsorption/desorption coefficients on various soils are available (Refs. 1 to 3). It has been shown that there is a correlation between adsorption and leaching and thus the adsorption constants can be used to estimate the mobility of pesticides.

Criteria and test sequence

Soil plate or soil column chromatography can be used to evaluate leaching of the parent compound. The column model is most appropriate to show the leaching properties of the degradation products of a pesticide after applying the parent compound. Substances which are mobile and persistent may require additional testing under field conditions, e.g. lysimeter studies with undisturbed soil monoliths or field studies with/without suction-lysimeters.

Test requirements

When plate or column leaching studies with the parent compound are necessary either the active ingredient or formulated material may be used. In field tests the formulations should be used.

The pesticide concentrations tested in the laboratory should have a realistic relationship to recommended doses, and field leaching studies should be carried out at the recommended rate for the pesticide.

Soil from the same sample as used for the degradation Study should be used to study the leaching of pesticide degradation compounds in a soil column model. The need for such a study will depend on the amounts and on the stability of the degradation compounds. Characteristics of the soil used in laboratory studies (pH, organic carbon, cation exchange capacity, particle size distribution, water holding capacity) should be recorded.

Test locations for field leaching studies should be selected according to the recommended use of the pesticide. Meteorological measurements, such as temperatures, rainfall and cropping should be recorded as well as soil characteristics.

Appropriate test procedures

Several published methods covering a range from simple laboratory tests to field studies simulate the leaching behaviour of pesticide chemicals in soil.

Soil layer chromatography

Soil thin and thick layer chromatography are simple and valuable techniques to indicate the mobility of a pesticide in a soil (Refs. 4, 5 and 6). The soil to be tested is used as adsorbent and distilled water (on aqueous 0.01 M $CaCl_2$ or $Ca(NO_3)_2$ solutions) as the mobile phase.

Soil columns

A review of appropriate soil column studies is given in Ref. 7.

Lysimeters

Lysimeters with undisturbed soil monoliths can be useful models to study the fate of pesticides in soil/plant systems under outdoor conditions. Standardized model lysimeters are described in Refs. 8, 9 and 10.

Field leaching

The key problem in the investigation of field leaching is the appropriate method of sampling in order to avoid contamination of the lower soil layers by soil particles from the upper layers. Sampling is best accomplished with special drilling devices to obtain undisturbed soil cores (Refs. 11 and 12). Sampling of soil water by suction probes is a further technique used to study the leaching of a chemical under field conditions (Ref. 13).

REFERENCES

Guth, J.A. Adsorptions und Einwaschverhalten von Pflanzenschutznitteln in Boden Schr. Reihe Ver. Wass.- Boden- Lufthyg. Berlin Dahlem, Heft 37: 143-154 (1972). (Ref. 1).

Leistra, M. Computation models for the transport of pesticides in soil. Residue Rev., 49: 87-130 (1973). (Ref. 2).

Leistra, M., Smelt, J.H., Verlaat, J.G. and Zandvoort, Z. Measured and computed concentration patterns of propryzamide in field soils. Weed Res., 14: 87-95 (1974). (Ref. 3).

Helling, C.S. and Turner, B.C. Pesticide mobility: determination by soil thin-layer chromatography. Science, 162: 526-563 (1968). (Ref. 4).

Helling, C.S. Pesticide mobility in soils I. Parameters of soil thin-layer chromatography. Soil Sci. Soc. Amer. Proc., 35: 732-737. II. Application of soil thin-layer chromatography. Soil Sci. Soc. Amer. Proc., 35: 737-743 (1971). (Ref. 5).

Gerber, H.R., Ziegler, T. and Dubach P. Leaching as a tool in the evaluation of herbicides. Proc. 10th Brit. Weed Control Conf. 118-125 (1970). (Ref. 6).

US Environmental Protection Agency. Registration of pesticides in the United States, CFR 40, Subdiv. N, 163-1 (NTIS-PB83-153973 NTIS-PB86-129384) (1982). (Ref. 7).

Fhür, F., Cheng, H.H. and Mittelstaedt, W. Pesticide, balance and metabolism studies with standardized lysimeters. Landwirtschaftliche Forschung 32/I Sonderheft: 271-275 (1975). (Ref. 8).

Roland Kubiak, Fritz Fuhr, Werner Mittelstaedt, Manfred Hansper, and Wilhelm Steffens. Transferability of lysimeter results to actual field situations. Weed Science 1988, Volume 36: 514-518 (1988). (Ref. 9).

K□rdel, W., Herrchen, M., Klein. M., Klein, W., Hamm, R.T. Lysimeter Experiments and simulation Models to Evaluate the Potential of Pesticides to Leach into Groundwater. Brighton Crop Protection Conference, Pests and Diseases (1988). (Ref. 10).

Hormann, W.D., Earhuber, B., Ramsteiner, K.A. and Eberle, D.O. Soil sampling for residue analysis. Proc. Eur. Weed Res. Coun. Symp. Herbicides - Soil, 129-140 (1973). (Ref. 11).

Hayden, C.W., and Robbins, C.W. Mechanical snake river undisturbed soil core sampler. Soil Science, 120: 153-155 (1975). (Ref. 12).

Reeve, R.C., and Doering, E.J. Sampling the soil solution for salinity appraisal. Soil Science, 99: 339-344 (1965). (Ref. 13).

2.2.2.3 Volatility

Volatilization can be a way of redistribution of pesticides between air, soil and water. When the vapour pressure, the water solubility and the soil adsorption/desorption characteristics are known, an estimate can be made whether volatilization is likely to occur under practical conditions.

Criteria and test sequence

Evaluation of volatilization should be made for all compounds likely to enter the soil or aquatic environment. Volatility tests should not be necessary for compounds which can be considered non-volatile from soil and water as predicted from soil/air and water/air distribution coefficients which can be calculated by using water solubility, vapour pressure and soil adsorption (Refs. 1 to 3). Information on the volatility of pesticides and degradation products can also be obtained from degradation studies in air flow-through systems. Field studies should only be required where indicated by prediction or results from laboratory studies.

Test requirements

Actual volatility in a field situation will depend upon a variety of factors (e.g. soil characteristics, meteorological data), therefore careful recording and reporting of the experimental conditions will be necessary. Field studies should be conducted with the formulated products at the recommended dosage.

Appropriate test procedures

Procedures for measuring volatilization have been published for laboratory studies (Refs. 1 to 3) and field studies (Refs. 4 to 7).

REFERENCES

Burkhard, N., and Guth, J.A. Rate of volatilization of pesticides from soil surfaces. Comparison of calculated results with those determined a laboratory model system. Pestic. Sci., 12: 37-44 (1981). (Ref. 1).

US Environmental Protection Agency. Registration of Pesticides in the United States, CFR 40, Subdiv. N, 163-2 (NTIS PB83-153973) (1982). (Ref. 2).

FRG BBA. Verhalten von Pflanzenbehandlungsmittel in Wasser. Teil 1, Hydrolyse und Verdampfung. Merkblatt 55 (1980). (Ref. 3).

Parmele, L.H., Lemon, E.R., and Taylor, A.W. Micrometeorological measurement of pesticide vapour flux from bare soil and corn under field conditions. Water, Air and Soil Pollut., 1: 533-541 (1972). (Ref. 4).

Harper, L.A., White, A.W., Bruce, R.R., Thomas, A.W., and Leonard, R.A. Soil and microclimate effects of trifluralin volatilization. J. Environ. Qual., 5: 263-342 (1976). (Ref. 5).

Cliath, M.M., Spencer, W.F., Farmer, W.J., Shoup, T.D., and Grover, R. Volatilization of S-ethyl-N, N-dipropylthiocarbamate from water and wet soil during and after flood irrigation of an alfalfa field. J. Agric. Food Chem., 28: 610-613 (1980). (Ref. 6).

Hill, I.R., and Arnold, D.J. Transformations of pesticides in the environment. The experimental approach in pesticide microbiology. Microbiological aspects of pesticide behaviour in the environment. (I.R. Hill and S.J.L. Wright Eds.), 203-245. Academic Press, London and New York (1978). (Ref. 7).

2.3 EFFECTS ON THE ENVIRONMENT

2.3.1 Vertebrate wildlife - mammals and birds

Data generated for all pesticides during the mammalian toxicology and metabolism programme will indicate the general toxicological properties of the compound, its mode of action as well as the target organs and functions. Such information is useful in identifying hazards to other groups of organisms.

For birds, particular attention should be paid to situations where the use of the compound could result in significant exposure, such as use in seed dressings and formulation as baits and granules.

Criteria and test sequence

Basic data should be generated for all pesticides intended for outdoor use.

Results from the mammalian toxicology programme can be used to better indicate any potential hazards to wild mammals.

For birds, the test sequence should begin with an acute oral toxicity (LD_{50}) study for one species. If the use of the compound is likely to result in considerable exposure to birds and/or the results of toxicity studies with mammals indicate cumulative action, a five-day dietary exposure study should be carried out with one species of bird. Additional species should, however, be tested if the use of the compound could result in hazardous exposure, or if the results of mammalian toxicity studies Show; significant interspecific variation in susceptibility.

Where cumulative effects or the potential for accumulation are indicated together with prolonged and significant exposure, longer-term laboratory studies should be considered, including an investigation of possible effects on reproduction. If accumulation occurs, it may be desirable to characterize and quantify residues in appropriate organs, so that the toxicological significance of residues found under conditions of commercial use of the compound can be assessed.

Field cage tests may be required if the oral toxicity of a compound, when considered in terms of likely exposure levels, indicate that birds may be at risk. Cage tests should be undertaken with the formulated product based on the recommended application rate. The test should simulate as closely as possible application methods recommended for commercial use.

If after laboratory and field cage studies doubts remain whether the level of safety is sufficiently high, field trials should be carried with the formulated product. The use of the compound in such studies (i.e. formulation type, application method and dosage rate) should accurately reflect recommendations for commercial use.

Test requirements

There should be no significant mortality among controls in laboratory tests. Compounds can be tested as either technical material or formulated products.

Suitable bird species include quails and the mallard duck. The domestic hen is not considered to be an appropriate species for use in such tests. All birds should be pen reared. Specimens collected from the wild are not suitable since their previous history and age are not known.

Appropriate test procedures

Laboratory tests

Avian single dose LD_{50} test

Appropriate test procedures are given in Refs. 1 and 2. The compound is dispersed in water or another inert carrier and administered either by intubation or by inserting a gelatin capsule containing the test compound into the proventriculus or crop.

Suitably spaced dosage levels should enable an LD_{50} to be calculated. For compounds with low toxicity, an approximate LD_{50} or a threshold value is generally acceptable.

Avian dietary LC_{50}

Appropriate test procedures are described in Ref. 3. The compound is dispersed in a standard bird diet and presented to the test animals as the only feed for a period of five days, followed by a period of at least three days when the birds are maintained on untreated diet.

Suitably spaced dosage levels should enable an LC_{50} to be calculated. For compounds of low toxicity, an approximate LC_{50} value or a threshold value may suffice.

Avian reproduction studies

For certain compounds, it may be necessary to investigate possible effects on reproductive parameters. Appropriate guidelines are given in Ref. 4.

Field studies - birds and mammals

Useful protocols for carrying out such studies are given in Ref. 5. Further recommendations for field studies are given in Refs. 6, 8 and 9. A protocol for large-scale field studies has also been developed by the United States Environmental Protection Agency (Ref. 7). It should be noted that field studies with mammals and birds are difficult to carry out and the results may be difficult to interpret. The objectives of such a study should therefore be very clearly defined at the planning stage. More generally, methodology in this area is still being developed.

REFERENCES

US EPA Pesticide Assessment Guidelines, Subdivision E, Hazard Evaluation: Wildlife and Aquatic Organisms, Series 71 - Avian and Mammalian Testing. 71-1 Avian single-dose oral LD_{50} test, pp 33-37 (1982). (Ref. 1).

UK Data Requirements for Approval of Pesticides under the Control of Pesticides Regulations 1986. Working Document 7/5. Evaluating the acute oral and short-term cumulative oral toxicity of pesticides to birds. (Ref. 2).

OECD Test Guideline 205: Avian Dietary Toxicity Test. OECD, Paris, 1984. (Ref. 3)

OECD Test Guideline 206: Avian Reproduction Test. OECD, Paris, 1984. (Ref. 4).

UK Data Requirements for Approval of Pesticides under the Control of Pesticides Regulations 1986. Working Document 7/1. Assessing the short-term risks to terrestrial vertebrate wildlife. (Ref. 5).

Pesticides and Wildlife - Field Testing. Recommendations of an International Workshop on Terrestrial Field Testing of Pesticides, 12-15 September 1988, Cambridge. In Prep. (Ref. 6).

US EPA Guideline Document for Conducting Terrestrial Field Studies. (Ref. 7).

Edwards, P.J., Breww, S.M., Fletcher, M.R., Stanley, P.I. The use of a bird territory mapping method for detecting mortality following pesticide application. Agriculture, Ecosystems and Environment 5, pp 271-282 (1984). (Ref. 8).

Greaves, M.P., Smith, B.D., Greig-Smith, P.W. (Eds.) Field methods for the study of environmental effects of pesticides. British Crop Protection Council Monograph No.40, British Crop Protection Council, Thornton Heath, U.K. (1988). (Ref. 9).

2.3.2 Non-target aquatic organisms

Assessment of hazard to non-target aquatic organisms is based on the results of toxicity tests with fish, aquatic invertebrates and phytoplankton. Interpretation of the results of such tests should take full account of physico-chemical processes which influence exposure in natural waters, such as solubility, sorption and degradation. Also, populations of aquatic invertebrates and phytoplankton are generally characterized by high intrinsic rates of population growth, and affected populations can recover rapidly following termination of exposure. Effects on such groups are therefore particularly dependent on the persistence of the compound in the aqueous phase. If persistence is short and exposure levels are low compared with the LC_{50} values from toxicity tests, then it is likely that any effects on these groups will be of little ecological significance.

Criteria and test sequence

The toxicity of a pesticide to aquatic organisms should be investigated if it is intended for outdoor use.

The test sequence should commence with laboratory tests for acute toxicity. Longer-term toxicity studies can be indicated for compounds applied directly to water bodies. Bioaccumulation studies with fish can be required for compounds with sufficient stability in water if the octanol/water partition coefficient is greater than 1000 (log Pow >3) and the water solubility less than 1.0 mg/l. Such studies should be designed to measure rates of both uptake and elimination from tissues.

If the results of laboratory studies do not allow an adequate hazard assessment to be made, then simulated field and field studies may be helpful. The aims of field experiments should be clearly defined at the planning stage. In general, such studies should include investigation of the fate and effects of the compound in the aquatic ecosystem, and enable observations to be carried out on recovery of affected populations. In some cases, it may also be possible to investigate indirect effects on the various species in the system (e.g. effects on fish growth, possible induction of algal blooms).

Test requirements

There should be no significant mortality in the controls in laboratory tests.

Pesticides may be tested as either technical grade materials or formulated products. Solvents and dispersants may be necessary to prepare the test solutions. Such materials must be of proven low toxicity to the test organism, and solvent/dispersant controls should be included in the test system.

The water used to prepare test solutions should be of known quality or reconstituted to fulfil established criteria, as outlined in the relevant guidelines.

Methods for fish toxicity and bioaccumulation studies can utilize static, semi-static or continuous flow procedures. The method used should be selected according to prevalent circumstances, taking special account of the physical properties of the compound and its stability in water.

Concentrations of the test material in water should be determined at intervals (determined by the stability of the compound in water) to quantify exposure concentrations. Any disparity between measured and nominal concentrations should be noted and taken into account when analyzing the data and interpreting results.

Appropriate test procedures

Acute toxicity to fish

Several comparable procedures (Refs. 1, 2, 3 and 4) are well established and widely accepted. Compounds should be tested at suitably spaced concentrations to enable LC_{50} values to be calculated. LC_{50} values should be calculated for the total exposure period (generally of 96 h), and intermediate mortality figures should also be provided.

Prolonged exposure to fish

If it is anticipated that fish will be exposed to chemicals for extended periods of time, or if the results of acute toxicity tests indicate that the mortality rate or morbidity are still substantially increasing during the later part of the test, then it may be appropriate to carry out a prolonged toxicity test. A suitable procedure is described in Ref. 5. Studies assessing effects on early life stages of fish may be useful substitutes for whole life cycle studies. Appropriate guidelines are given in Ref. 6.

Bioaccumulation studies with fish

Suitable procedures are described in Refs. 7 and 8. Concentrations of the test compound in water should be sufficiently low to prevent any mortality. Such studies should seek to measure the magnitude, rate of uptake and elimination of the compound, and results should be interpreted in relation to expected environmental concentrations.

Toxicity to other aquatic organisms

The water flea, Daphnia magna, is widely used as an appropriate test species to indicate possible effects on aquatic Crustacea, which are generally considered to be highly susceptible to pesticides. These organisms are important as fish food, and may also be ecologically significant components of aquatic invertebrate communities. Methods for acute toxicity tests with daphnids are given in Refs. 9, 10 and 11. Ref. 9 includes a Daphnia reproduction test.

In some circumstances, it may be considered appropriate to investigate toxicity to aquatic algae, which fulfil the role of primary producers in the aquatic food chain. A suitable experimental procedure is described in Ref. 12.

Field studies

Recent years have seen significant advances in the development of methodology for simulated field studies to investigate the fate and effects of pesticides in aquatic systems. Such experiments may be based on the use of whole experimental ponds or the use of enclosures within water bodies. Experiments should be designed to investigate both the fate and effects of the compound in the aquatic environment, and may include provision for assessing possible indirect effects on specific taxa. The design of such studies should take full account of the physico-chemical properties of the test compound. Method development work is ongoing in this area and readers are referred to Refs. 13 and 14 for relevant information concerning the design and execution of such studies.

REFERENCES

US EPA Environmental Effects Test Guidelines EG9. Fish acute toxicity test. United States Environmental Protection Agency, Office of Pesticides and Toxic Substances, Washington. Doc. EPA 560/6-82-002 (1982). (Ref. 1).

OECD Test Guideline 203. Fish, acute toxicity test. OECD, Paris (1981). (Ref. 2).

EEC Directive 79/831, Annex V, ENV/286/80 Rev. 2, Part C: Methods for the determination of Ecotoxicity 5.1.1. Acute toxicity for fish. (Ref. 3).

International Organization for Standardization (ISO). Determination of acute lethal toxicity of substances to a freshwater fish (Brachydaniorerio) Hamilton-Buchanan; Teleostei, Cyprinidae) using a static (I)/semistatic (II)/flow-through (III) procedure. ISO DP 7346/I to III. (Ref. 4).

OECD Test Guideline 204. Fish prolonged toxicity test: 14-day study. OECD, Paris (1984). (Ref. 5).

US EPA Environmental Effects Test Guidelines EG11. Fish early life stage toxicity test. United States Environmental Protection Agency, Office of Pesticides and Toxic Substances, Washington. Doc. EPA 560/6-82-002 (1982). (Ref. 6).

OECD Test Guideline 305 (A-E). Bioaccumulation. OECD, Paris (1981). (Ref. 7)

US EPA Environmental Effects Test Guidelines EG10. Fish bioconcentration test. United States Environmental Protection Agency, Office of Pesticides and Toxic Substances, Washington. Doc. EPA 560/6-82-002 (1982). (Ref. 8).

OECD Test Guideline 202. Daphnia Sp., acute immobilization test and reproduction test. OECD, Paris (1981). (Ref. 9).

EEC Directive 79/831, Annex V, ENV/920/80 Rev. 1, Part C. Methods for determination of ecotoxicity 5.1.2. Acute toxicity to Daphnia. (Ref. 10).

US EPA Environmental Effects Test Guideline EG1. Daphnid acute toxicity test. United States Environmental Protection Agency, Office of Pesticides and Toxic Substances, Washington. Doc. EPA 560/6-82-002 (1982). (Ref. 11).

OECD Test Guideline 201. Alga: growth inhibition test. OECD, Paris (1981). (Ref. 12).

Crossland, N.O., Wolff, C.J.M. Outdoor ponds: their construction, management, and use in environmental toxicology. In: The Handbook of Environmental Chemistry, Vol. 2, Part D. Hutzinger, O. (Ed.), SpringerVerlag (1988). (Ref. 13).

Solomon, K.R., Liber, K. Fate of pesticides in aquatic mesocosm studies an overview of methodology. Proceedings of the 1988 British Crop Protection Conference, Pests and Diseases. Vol. 1: 139-148 (1988). (Ref. 14).

2.3.3 Soil non-target micro-organisms and earthworms

Soil micro-organisms are very resilient to perturbation and major changes in micro-organism populations can occur under natural conditions without adverse effects on soil fertility. Thus, major changes in one component of the flora can be compensated for by other components of the flora so that overall functions are not substantially disturbed.

It is therefore generally accepted that studies of pesticide effects on soil micro-organisms should be directed towards investigating possible effects on soil functions rather than on specific organisms. While information concerning pesticide effects on these functions can sometimes be useful in predicting possible effects on soil fertility, data should not be over-interpreted. Both the magnitude and duration of any effects observed should be considered.

Criteria and test sequence

When data concerning relevant physical and chemical properties, spectrum of activity, environmental fate and use pattern indicate the possibility of adverse effects on soil micro-organisms, studies should be selected from the following test sequence.

Studies of pesticide effects on soil micro-organisms are required in a few countries. They are usually performed as laboratory tests to investigate effects on overall function as indicated by respiration rates (C-cycle) and or on nitrogen transformations (N-cycle).

Some authorities require data concerning effects on earthworms. The test sequence should begin with a simple laboratory toxicity test. If the results of this test indicate significant toxicity, field experiments can be carried out to assess the hazard on the population level. Studies on methodology and significance of results are still under development.

Test requirements

Soil microflora

Pesticides can be tested as either technical grade materials or formulated products. Dosages tested should have a realistic relationship with recommendations for use.

One or two typical agricultural soils (e.g. a sandy loam and a loam with different organic carbon content) should be used in these studies. It is essential that the handling and storage of samples to be used in laboratory experiments should follow recognized procedures, as discussed in Ref. 1. Soil characteristics such as pH, organic carbon content, cation exchange capacity, particle size distribution and water holding capacity should be recorded.

Earthworms

There should be no significant mortality in controls in laboratory tests. Compounds can be tested in the laboratory as either technical grade material or formulated products.

Field experiments should be performed on plots (e.g. on grassland) containing a sufficient number of earthworms to allow statistical comparisons with untreated controls. Dosages used in field studies should reflect those recommended for commercial use, and experiments should be designed to ensure that they give a reliable indication of major effects which could occur under conditions of commercial use.

Appropriate test procedures

Soil microflora

As noted above, there is general agreement that a "functional" approach should be adopted when investigating pesticide effects on soil microorganisms. Following several international symposia and workshops held between 1973 and 1985, "Recommended Laboratory Tests for Assessing the Side Effects of Pesticides on Soil Microflora" have been published (Ref. 2), including proposals concerning interpretation of results. This reference should be considered as a key source document by those concerned with assessing pesticide effects on soil micro-organisms.

Earthworms

The toxicity of pesticides to earthworms can be investigated in the laboratory using the method described in Ref. 3. A suitable species which can be reared in the laboratory is Eisenia foetida.

Ref. 4 provides an appropriate experimental design for assessing effects on earthworm populations in the field. Earthworm populations are generally sampled using the method described in Ref. 5, although alternative techniques are available (Ref. 6).

REFERENCES

Anderson, J.P.E. Handling and storage of soils for pesticide experiments. In: Somerville and Greaves (below). (Ref. 1).

Somerville, L. and Greaves, M.P. (Eds.) Pesticide Effects on Soil Microflora. Taylor and Francis, London, New York, Philadelphia (1987). (Ref. 2).

OECD Guideline 207. Earthworm, Acute Toxicity Tests. OECD Guidelines for Testing of Chemicals, OECD, Paris (1981). (Ref. 3).

UK Data requirements for approval under the control of pesticide regulations 1986. Working document 7.6. Laboratory and field testing of pesticide products for effects on soil macro-organisms. (Ref. 4).

Raw, F. Estimating earthworm populations by using formalin. Nature, Lond. 184: 1661-1662 (1959). (Ref. 5).

Southwood, T.R.E. Ecological Methods. 2nd Edition. Chapman and Hall (1978). (Ref. 6).

2.3.4 Honey Bees

Bees may be at risk from pesticide applications to crops in flower, crops containing weeds in flower and crops infested with aphids which produce honeydew, which can be attractive to bees. Exposure can occur by direct contact with spray droplets, by contact with residues on plant material (including pollen, both during foraging and following transfer of pollen to the hives), and by drinking spray solution.

Criteria and test sequence

Data should only be required for compounds with a use pattern which may put bees at risk, e.g. not for pre-emergence herbicides, seed dressings, granules or stored products.

The test sequence should take account of the pesticide type and its use pattern, including laboratory tests to assess the oral and contact toxicity of the compound, with provision for further testing in cases where hazard cannot be reliably assessed on the basis of laboratory data alone.

A useful tool in guiding the decision-making process at the laboratory stage is the hazard ratio (Ref. 1): dose/ha (highest recommended dose in g a.i./ha)/LD_{50} (lower value from oral and contact LD_{50} tests in ug/bee). This function relates the results of laboratory toxicity tests to projected commercial dose rates and can thus provide an indication of hazard under field conditions.

Test requirements

Laboratory studies can be carried out using either technical grade materials or formulated products. There should be no significant mortality in the controls in laboratory tests. Appropriate reference compounds should be included in acute toxicity tests. Rates used in laboratory experiments other than acute toxicity tests should be within the range of possible exposure resulting from the commercial use of the compound.

The design of simulated field and field experiments should reflect the use recommendations of the compound if these are likely to put bees at risk. Appropriate reference compounds should be included in field experiments. These experiments should include assessments of both short-term effects (e.g. mortality and behaviour) and longer-term effects (e.g. monitoring colony development). It may also be considered appropriate to investigate the fate of residues in pollen, honey and wax.

Appropriate test procedures

A series of symposia organized by the International Commission for Bee Botany has resulted in recommendations for the harmonization of appropriate laboratory, simulated field and field methods for assessing the hazard of pesticides to bees. These methods are summarized in Ref. 2. Other relevant documents are Refs. 3, 4 and 5. Requirements and methods in different countries vary widely.

Laboratory tests

Oral toxicity

The test compound should be dispersed in sugar solution or honey water and presented to individual or small groups of worker bees at a range of dosages such that LD_{50} values can be calculated. Relevant test procedures are described in Ref. 2. Where necessary, the compound can be solubilized using acetone.

Contact toxicity

The compound should be dispersed in acetone and topically applied to individual worker honey bees at a range of doses such that an LD_{50} value can be calculated. Suitable test procedures are described in Ref. 2.

Residual toxicity

Laboratory methods have been developed for assessing the toxicity of pesticide residues to honey bees (e.g. Ref. 6). Such tests can be useful for identifying compounds and formulations which are repellent to bees and for investigating possible effects of ageing of residues on their toxicity. The nature of the substrate tested must be documented. Tests with plant material will provide the most meaningful data.

Field experiments

Simulated field tests

In many cases, much of the information from a full-scale field trial (see below) can be obtained using the simulated field techniques of small-scale field cages and "tunnel" tests. Appropriate procedures are described in Refs. 2 and 5.

Full or open field tests

If the hazard presented by a compound to bees cannot be determined using the laboratory or simulated field tests, it may be necessary to undertake a large scale field study. The results have to be compared with an untreated plot. Suitable procedures are described in Refs. 2, 4 and 7. Observations on the general condition and development of the experimental hives should be carried out for an extended period of time following initial exposure to investigate possible longer term effects on the colonies. Biological observations should be complemented by a programme of residue analysis to investigate possible transfer of residues to hives and to determine the fate of residues in the colonies.

REFERENCES

Smart, L.E., Stevenson, J.H. Laboratory estimation of toxicity of pyrethroid insecticides to honey bees: relevance to hazard in the field. Bee World 63: 150-152 (1982). (Ref. 1).

Felton, J.C., Oomen, P.A., Stevenson, J.H. Toxicity and hazard of pesticides to honey bees: harmonization of test methods. Bee World 67: 114-124 (1986). (Ref. 2).

UK Data Requirements for Approval of Pesticides under the Control of Pesticides Regulations 1986. Working Document 7/3. Laboratory testing for toxicity to honey bees. (Ref. 3).

UK Data Requirements for Approval of Pesticides under the Control of Pesticides Regulations 1986. Working Document 7/4. Testing for toxicity to foraging bees under field conditions. (Ref. 4).

Wilkinson, W., Gough, H.J., Collins, I.G. PP321. Safety to honey bees (Apis mellifera) when used in cereals. Proceedings of the 1986 British Crop Protection Conference, Pests and Diseases. Vol. 3: 1085-1092 (1986). (Ref. 5).

Murray, A. Acute and residual toxicity of a new pyrethroid insecticide, WL85871, to honey bees. Bull. Environ. Contam. Toxicol. 34: 560-564 (1985). (Ref. 6).

Shires, S.W., LeBlanc, J., Murray, A., Forbes, S., Debray, P. A field trial to assess the effects of a new pyrethroid insecticide, WL85871, on foraging honey bees in oilseed rape. J. Apic. Res. 23: 217-226 (1984). (Ref. 7).

2.3.5 Predatory and parasitic arthropods

Effects of pesticides on predatory and parasitic arthropods are important where integrated pest management practices are implemented (Refs. 1 and 2). However, it should be noted that assessment of the effects of pesticides on predatory and parasitic groups comprises only one component of the development of integrated pest management programmes.

Criteria and test sequence

Data should only be required for compounds intended for use in integrated pest management programmes.

The stepwise test sequence may include laboratory toxicity tests, but greatest weight should be placed on data from simulated field and field studies where predator/pest and parasite/host interactions are well understood. Where a claim is made for selectivity or for use in integrated pest management programmes, observations from actual use programmes should be required.

Test requirements

The importance of particular test organisms as natural pest control agents should be well established prior to studies commencing. Methods for assessing the relative importance of different taxa are described in Ref. 3.

Laboratory experiments should be designed to generate data from which a conclusion can be drawn concerning relative toxicity to predator/parasite and prey/host.

The aim of simulated field and field studies should be clearly defined prior to drawing up experimental protocols and the design of such studies should reflect the recommended use pattern of the compound. As with the laboratory studies, the experiment should be designed to generate data concerning effects both on predator/parasite and prey/host groups. Field experiments should be of sufficient duration to identify possible effects such as pest "resurgence". It is important that results from field trials should be interpreted in relation to function (i.e. ecological significance).

For example, any changes in the abundance of a particular predator/parasite should be considered in relation to overall effects on the prey/host relation and to species succession.

Appropriate test procedures

Methodology in this area is still under development.

The International Organization for Biological Control working group "Pesticides and Beneficial Organisms" has published a series of guidelines (Ref. 4) for methods for assessing the effects of pesticides on a range of entomophagous groups, including entomopathogenic fungi.

These guidelines should be regarded as illustrative of an experimental approach, rather than providing definitive methodology. Other approaches are described in Ref. 5.

Refs. 6, 7 and 8 are recommended to give background information concerning the application of data concerning pesticide effects on entomophagous groups to the development of integrated pest management programmes.

REFERENCES

Solomon, M.G. Fruit and Hops. In: Integrated Pest Management. Burn, A.J., Coaker, T.H., Jepson, P.C. (Eds.) Academic Press (1987). (Ref. 1).

Gould, H.J. Protected Crops. In: Integrated pest Management. Burn, A.J., Coaker, T.H., Jepson, P.C. (Eds.) Academic Press (1987). (Ref. 2).

Wratten, S.D. The Effectiveness of Native Natural Enemies. In: Integrated Pest Management. Burn, A.J., Coaker, T.H., Jepson, P.C. (Eds.) Academic Press (1987). (Ref. 3).

Hassan, S.A. Standard methods to test the side-effects of pesticides on natural enemies of insects and mites developed by the IOBC/WPRS Working Group "Pesticides and Beneficial Organisms". EPPO Bulletin 15: 214-255 (1985). (Ref. 4).

Greaves, M.P., Smith, B.D., Greig-Smith, P.W. (Eds.) Field methods for the study of environmental effects of pesticides. British Crop Protection Monograph No.40, British Crop Protection Council, Thornton Heath, U.K. (1988). (Ref. 5).

Cranham, J.E., Tardivel, G.M., Kapetanakis, E.G. Orchard trials to assess the effects of pesticides on Typhlodromus pyri and its prey Panonychus ulmi. Acarology VI: 680-685 (1984). (Ref. 6).

Croft, B.A. Use of crop protection chemicals for integrated pest control. Phil. Trans. R. Soc. Lond. B295: 125-141 (1981). (Ref. 7).

Gruys, P. Hits and misses. The ecological approach to pest control in orchards. Entomol. Exp. Appl. 31: 70-87 (1982). (Ref. 8).

2.3.6 Plants

Effects on non-target plants can be extrapolated from data generated in screening tests and subsequent field testing. Additional testing for phytotoxicity is therefore not necessary.

GLOSSARY OF ABBREVIATIONS AND ACRONYMS

a.i.	:	Active Ingredient
EEC	:	Expected Environmental Concentration (= PEC)
g	:	Gram(s)
ha	:	Hectare
HPLC	:	High Performance Liquid Chromatography
ISO	:	International Standardization Organization
IUPAC	:	International Union of Pure and Applied Chemistry
kg	:	Kilogram(s)
L	:	Litre(s)
LC_{50}	:	The concentration required to kill 50% of test organisms
LD_{50}	:	The dose required to kill 50% of test organisms
LOEC	:	Lowest Observed Effect Concentration
mg	:	Milligram(s)
nm	:	Nanometre(s)
NOEC	:	No Observed Effect Concentration
Pa	:	Pascal
PEC	:	Predicted Environmental Concentration (= EEC)
SI	:	Système International (Units)
w/w	:	Proportion by weight
ug	:	Microgram (s)