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RECOMMENDED APPROACH TO THE EVALUATION OF THE ENVIRONMENTAL BEHAVIOUR OF PESTICIDES

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Recommended approach to the evaluation of the environmental behaviour of pesticides (IUPAC reports on pesticides, no. 24)

Abstract - Concepts and experimental approaches are proposed which lead to a rational testing of the fate of pesticides in soil, water, air, plants and animals. The stepwise test programme presented is flexible, cost effective and ensures that all aspects of a pesticide's environmental fate are studied in sufficient detail. It also indicates, at different stages of testing, the results which are of relevance to the estimation of exposure to this pesticide, an essential element of the hazard assessment process concerning producers/applicators, consumers and the environment.

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1. INTRODUCTION AND OBJECTIVES

1.1 Current approaches to testing environmental behaviour

In recent years comprehensive schemes for the registration of pesticides have been elaborated by a number of national registration authorities so that considerable experience is now available. It is now timely to evaluate the usefulness of these schemes for making an assessment of the behaviour of a pesticide in the environment. Some aspects of these schemes may be modified to make a more effective use of the resources available to assess environmental impact.

There has also been considerable activity in moving towards testing schemes for notification or registration of general chemicals. Schemes developed by groups such as OECD have included tier and stepwise approaches.

Extensive studies of the environmental behaviour of pesticides, using radio-labelled samples, have been carried out for many years. There is therefore now considerable prior art in the testing of pesticides and much of the existing data can be used in a predictive manner. With this background, a stepwise testing scheme for evaluating the environmental behaviour of pesticides has many attractions.

The FAO and the USA Environmental Protection Agency have done much to update approaches to evaluating the environmental fate of pesticides and to list experimental methods available. However, care must be taken to avoid a fixed data requirements package which, in turn, gives rise to a checklist approach to registration. Otherwise, considerable effort may be expended on aspects of the behaviour of a chemical where there is not likely to be an environmental hazard. Conversely, in areas where there may be real environmental concern, work may be too limited.

1.2 Alternative approach

A more rational approach to the preparation of data packages would be to use a stepwise testing scheme. This would be more flexible and call for further testing only in an area or areas where basic data are insufficient to allow assessment of environmental impact. The scheme should start with a basic set of experiments which should be applied to all new pesticides. Additional data would be required only in areas where further information was indicated by the results of the basic experiments. This system would generate a scientifically valid data package and would allow resources to be used economically and effectively.

The first step in this approach should be an analysis of likely exposure to the pesticide based on its use pattern. This theme should be developed further by including data generated by measurement of physico-chemical properties and from the results of simple tests or calculations on compartmental partitioning and degradation. That part of the package which is concerned with the environmental mobility/distribution of the test chemical would be supported by reference to chemicals with similar use patterns and properties which could serve as relevant "bench marks". The applicability of the benchmark approach to testing for degradation should be restricted to structurally closely related compounds. The nature and extent of the studies that should follow will depend upon the results obtained up to this stage in the environmental studies and from ecotoxicity testing. The intermediate results of the individual studies should be carefully assessed with regard to their wider implications and their impact on the design of subsequent studies. In this way, an overall environmental exposure analysis can be built up.

A number of groups are at present involved in devising models, both experimental and mathematical, to predict the environmental behaviour of pesticides. Both approaches are interesting. However, at present, information to predict environmental behaviour may be obtained more easily and reliably from basic experiments than from complex systems. Natural ecosystems such as small ponds(rather than artificially composed laboratory ecosystems) may be appropriate in the later stages of testing to confirm trends observed in laboratory studies and to help elaborate any particular problem area.

Mathematical models are still very much in the process of development and encouragement should be given to further work in this area. Mostly, they are concerned with the mobility of a compound in defined environmental scenarios based on the compound's physico-chemical properties and, in some models, on the rate of its degradation. At present, some of these models are being tested with regard to the reliability of their prediction potential. They should only be used for hazard assessment when fully validated.

The objective of the present recommendation is to outline a stepwise approach which also has a valid scientific basis. Inevitably, aspects of toxicology and ecotoxicology are closely enmeshed with environmental behaviour. It is, therefore, necessary to refer to biological effects of pesticides in a number of areas but this report does not discuss in detail the direct impact of pesticides on man or on other organisms. It is also obvious that close attention to existing requirements of regulatory authorities of individual countries including GLP principles has to be paid in this approach.

2. FACTORS INFLUENCING ENVIRONMENTAL BEHAVIOUR

When designing schemes to evaluate the fate of a pesticide in the environment it is important to take all the following factors into account. They provide a basis for an assessment of the expected environmental concentration of a pesticide in individual situations, and therefore the extent of testing needed.

2.1 Amount of chemical used

At a given time, the environmental residues of parent pesticides will be directly proportional to application levels. If of biological relevance, the residue behaviour of major persistent metabolites needs to be assessed separately.

2.2 Routes and rate of entry into the environment

The actual use and application of the chemical will affect the environmental behaviour and hence the type of data which should be obtained. The following should be considered:

- whether the use is localised or widespread,
- whether the pesticide will be applied by conventional means, by aerial or low volume uses or as a seed dressing, etc.,
- what types of formulations will be used, and
- the application method (e.g., pre- or post-emergence spray), schedule (e.g., number and season of applications), and rate (e.g., quantity of active ingredient per hectare).

2.3 Distribution and transformation

The physical and chemical properties of a given pesticide as well as the environmental situation when it is applied (e.g., climatic factors, soil characteristics, etc.) influence the nature and the extent of the dispersion and degradation of the pesticide in the environment.

2.3.1 Distribution/Accumulation of a Pesticide after Use

Solubility in water, n-octanol/water partition coefficient, vapour pressure, and soil adsorption/desorption constants can be used to estimate the potential of a chemical to partition into the various environmental compartments, water, soil/sediment and air (see also 3.2).

These properties can also be used to predict whether a chemical possesses a significant potential for bioaccumulation. However, if the chemical is degraded readily to polar products, bioaccumulation is not likely to be of concern. Transformation to polar products may be achieved by only one reaction step (e.g., hydrolysis) or by more complex reactions which transform an originally lipophilic pesticide into a polar degradation product.

2.3.2 Degradation

It is important that pesticides have reasonable stability and bioavailability to give the desired degree of control of their target organism(s). However, excessive stability is undesirable and it is important that chemicals degrade in or can be removed from the environment. Degradation may be chemical or biological (biodegradation) but it should not be necessary to distinguish between these two processes for regulatory purposes.

Simple balance type tests should be carried out to assess hydrolysis, photolysis and biodegradation in plants and soil. If, after these tests, the compound appears to have undue stability, more elaborate studies may be needed if these are likely to give a more realistic picture of the environmental behaviour of the chemical.

When a pesticide is metabolised or degraded, it is necessary to understand the nature of the products formed. Identification of major metabolites and characterisation of minor metabolites provide the foundation for residue method development and contribute to the molecular basis for the interpretation of toxicity.

3. EXPERIMENTAL CONCEPTS

3.1 Test material

In order to carry out kinetic and metabolic studies to answer the question about the nature and behaviour of degradation products, it is normally necessary to use a radiolabelled sample of the pesticide. Carbon-14 is normally the isotope of choice but studies using other isotopes may be appropriate. The position of the carbon-14 label should be chosen so that it is not likely to be easily lost during initial metabolic conversion of the chemical.

When the test substance has a complex chemical structure, it may be necessary to label the molecule in more than one position to elucidate fully the metabolic fate of the chemical. In these cases, two or more radiolabelled samples should be prepared. Tests are then normally carried out separately with each sample. Use of stable isotopes such as ^{13}C, ^{15}N and ^{2}H can also provide very useful structural information for metabolites, especially when applied in combination with ^{14}C-labels. They facilitate spectral identification of metabolites by nuclear magnetic resonance (^{13}C) and mass spectrometric (^{13}C, ^{15}N, ^{2}H) methods.

The test substance for metabolism studies should be as pure as possible (both chemically and radiochemically) and for many tests can be used unformulated. Formulation is normally only required when, in larger scale tests, it makes application of the radiolabelled chemical easier and more realistic (e.g., spraying large plants or experimental farm plots).

Some of the tests to assess environmental behaviour may be field studies (e.g., in-use, soil-dissipation studies). In these cases, use of a radiochemical may be inappropriate or unnecessary. The test material of choice will be a formulated technical grade product, the fate of which is followed by specific residue analytical procedures. These procedures should also enable biologically significant metabolites/degradation products to be monitored.

3.2 Determination of properties with environmental relevance

3.2.1 Properties with Relevance to Distribution/Accumulation

A small number of physico-chemical properties can be used to predict a chemical's mobility within and between environmental compartments.

The most important properties are:

- physical state at ambient temperature (melting and boiling point or range, mp and bp)
- solubility in water, Sw
- vapour pressure (at 20 - 25 °C), p
- partition coefficient (n-octanol/water), Pow
- adsorption/desorption (adsorption coefficient favourably based on organic carbon content of soil or sediment, Koc; not applicable to cationics)
- dissociation constant, pK (if applicable)
- density, d
- relative molecular mass (volume), M

Standard methods for determining these properties have been published by OECD. The accuracy required for these measurements need only be enough to permit optimal interpretation of experiments and model calculations. In extreme cases, such as very low water solubility or vapour pressure, precise measurement of Sw below 0.01 ppm and of p below 10^{-7} Pa is only exceptionally necessary. In these cases, data may be expressed by orders of magnitude. The n-octanol/water partition coefficient deserves special attention in this respect as its determination is strongly influenced by the purity of the test compound and the solvents used. When the Pow greatly exceeds 10^{4}, HPLC methods should be used. These give good estimations of the Pow's of parent and impurities.

The Pow of strongly ionized chemicals need not be determined as they are

ionized over the whole pH range. The Pow of a weakly ionized chemical at environmental pH's depends on its pK and may be determined (by the shake-flask method) at conditions of pH and salt concentration which prevail in the environmental compartment of interest. Methods to calculate Pow are available and represent a useful tool for predictions, estimating extreme cases or checking experimental values.

A few highly ionizable, cationic pesticides adsorb strongly to mineral components in soils, in contrast to lipophilic pesticides which partition into the organic soil component. As the kinetics of adsorption and desorption of cationic compounds may vary drastically, a normal adsorption/desorption coefficient may not be determinable.

The sensitivity of the experimental data to impurities should be kept in mind, especially when using technical grade products and solvents.

Several of these properties are interrelated, e.g., Sw, Pow and Koc. If certain properties cannot be determined with reasonable accuracy (impurities, mixtures, etc.) one property may be used to estimate the others. An extensive literature exists on these relationships.

The properties listed above govern a number of phenomena such as volatility, leaching, run-off and bioaccumulation which are of practical importance in determining environmental distributions. The potential behaviour of a chemical in a given situation can often be estimated from one or two of these properties, e.g., volatility from water may be calculated from Sw and p (combined in Henry's law constant, H). Similarly, bioaccumulation and leaching are largely determined by Pow and Koc. The degradability of the chemical should be considered to assess whether the potential is likely to be realised in practice.

Unequivocal data may obviate further study of these phenomena. For example, experience shows that a significant bioaccumulation potential (expressed by the bioaccumulation factor, BF, i.e., ratio of concentration of a chemical in organism to concentration in immediate environment at "steady-state") can be expected when Pow values are >1000 (BF values begin to exceed 100). Thus, chemicals with Pow below this value (or with a Sw >1 g/l) need no bioaccumulation testing. Similarly, the known relationship between Koc and leaching can be used to conclude that, for example, chemicals with high Koc values (e.g., $>10^4$ or, if not determinable, Sw $<10^{-4}$ g/l or Pow $>10^5$) or which do not move on soil thin layer plates have no significant potential for leaching and require no further testing of this property.

Further refinement of the knowledge about compartmental distribution may be achieved by using models which consider the relevant properties for a given phenomenon such as volatility from soil or defined environmental scenarios such as an oligotrophic lake or a surface water. Often, these current models assume thermodynamic equilibrium conditions in defined two- or multiple-compartmental systems, with some of them taking degradation rates into account. Though modeling is still in its infancy it is expected to improve in the future to better simulate the multi-variate situations involving pesticides in the environment. For current models, a substantial amount of validation and field verification studies still remain to be accomplished before significant reliance can be placed on modeling results and their predictive power.

Assessment of the extent to which a given chemical exhibits predicted potentials in practice requires consideration of all relevant properties, including those on degradability discussed in chapter 3.2.2. The assessment should proceed stepwise as outlined in chapter 4.

3.2.2 Properties with Relevance to Transformation

Physico-chemical properties of chemicals (dealt with in the preceeding section 3.2.1) which determine distribution and accumulation, are also important for transformations. Among these properties, those governing adsorption are particularly important. If a compound is adsorbed to surfaces, its bio-availability to micro-organisms which may break down the chemical is diminished. Moreover, penetration into plant and animal cells, where metabolic conversion takes place, is also largely determined by the

physico-chemical properties of a given chemical and partly by the metabolic capacity of those cells through which the chemical has to pass first. Examples have been published showing that uptake of chemicals by plant roots correlates with their octanol-water partition coefficients.

Among purely chemical transformation processes, hydrolysis and photolysis are the most important ones. Rates of hydrolysis depend on pH and, of course, on temperature. It is advisable to investigate the rate of hydrolysis at several realistic values of pH (e.g., 5, 7 and 9) and one appropriate temperature. Hydrolysis tests need not be run with compounds which do not contain a hydrolysable group.

Photochemical degradation experiments with pesticides have been performed so far mainly in aqueous solution, and to a lesser degree on soil surfaces. Simulated sunlight was generally used in these studies, sometimes natural sunlight if local conditions were appropriate. UV light with λ <290 nm is not present in sunlight and therefore irrelevant for photochemical studies. The absorption spectrum of a compound should be known since this gives information on the direct absorption of photons by the chemical. Photodegradation tests with a suitable photosensitizer may be useful, because solar energy can be transfered to chemicals which do not absorb sunlight. Several reviews discuss the design of such photodegradation experiments, including those on vapour-phase photolysis of highly volatile chemicals. Useful protocols for photodegradation in water are now in use but there is still no internationally accepted and validated protocol for vapour-phase photolysis.

Early approaches to estimate structure-degradability correlations for chemicals in soil have tried to relate bioavailability in soil water (estimated from Koc or Pow) with degradability (e.g., assessment of degradation of the most labile functional group of a molecule; degradation rate constants for phosphorous esters). Further attempts in the field of structure-activity relationships (SAR) to correlate physico-chemical properties and other structural parameters as well as (eco)toxic properties with (so far mostly microbial) degradability of chemicals indicate the lines on which future structure-degradability correlations may be developed further.

The reactivity of certain functional groups in the environment can sometimes be predicted. For example, aromatic nitro groups can be converted in the environment to amino groups and may contribute, for example, to the formation of non-extractable residues in soil and sediment by reaction with humic materials. A careful inspection of the molecular structure combined with experience may yield insight into the rate and route of degradation. For example, increasing numbers of chlorine atoms in aromatics enhance their environmental stability, whereas chemically active monochloro aliphatic substituents can increase degradability.

3.3 Determination of environmental behaviour

3.3.1 Behaviour in Soils and the Aquatic Environment

A. Introduction

The evaluation of the potential impact of a pesticide must consider its distribution and fate in the soil and aquatic environment.

It is first of all important to consider the proposed use pattern of the pesticide. Where the use is local, e.g., in greenhouses and around buildings, there will be only a limited environmental contamination, if at all. Reduced environmental testing may be appropriate under these situations. Where the use is widespread, i.e., normal field use of pesticides, an understanding of the environmental fate of the chemical is needed.

Where studies are needed, it is essential to consider the environmental distribution of the pesticide and its degradation products. Most pesticides are either applied directly to the soil or will reach the soil in spray drift or run off during the spraying of crops. Studies of the fate in soil are therefore normally essential. In addition, where the pesticide is applied directly to water or when, via leaching or run off, significant amounts of the chemical or its toxicologically relevant degradation products may reach aquatic systems, studies of the fate of the chemical in soil/water or sediment/water systems should be undertaken.

These studies should supply information about

- rates of degradation of the chemical in soil
- nature and likely persistence of the major degradation products
- distribution of the pesticide and its degradation products via leaching, run-off and volatilisation
- possible contamination of aquatic systems
- fate of the chemical and/or its degradation products in aquatic systems (when needed)

This information is vital for the prediction of the environmental fate of a pesticide. In chapters 4.2.1 through 4.2.3 it is recommended how this information may be generated in a stepwise, scientifically satisfactory and economically feasible way.

The choice of soil types and the selection of the types of studies must take into account the properties of the chemical and its proposed uses. Soil properties with the greatest influence on degradation which have to be considered are pH, organic matter content, particle size distribution and the content of viable microflora (biomass).

Experience shows that it is essential to use fresh agricultural soils in the degradation studies as a rapid loss of part of their degradative capacity generally occurs upon drying and/or storage. The living biomass of soil should be used as a measure of this capacity (e.g., by measuring respiration after addition of glucose). Also, in sediment/water studies, fresh samples of representative sediment (or, as a second alternative, agricultural soil) are best obtained locally and should be fully characterized.

B. Distribution via leaching, run-off and volatilisation

Leaching studies are needed to understand the likely movement of the pesticide and its degradation products through the soil. The potential contribution of run-off should also be estimated. When determining the mobility of a pesticide, it is advantageous to use a reference compound with a known environmental behaviour. This can validate experimental methodology and facilitate the interpretation of results. Highly sorptive pesticides (e.g., Koc > 10^4 or Pow > 10^5; see 3.2.1) generally need testing according to B.2 and B.3 of this section whereas with pesticides of a certain leaching potential testing should start according to B.1.

B.1 Leaching of parent pesticide

This study should cover a range of typical soils on which the chemical may be used. Movement is best measured relative to a chosen standard chemical. The test can be simply carried out by studying the movement of a radiolabelled sample of pesticide on thick layers of soil eluted with water. Considering the known reverse correlation between adsorption/desorption and leaching, the adsorption constant (e.g., Freundlich) can also be used as a predictive measure.

Where low mobility is noted, no further studies of the leaching of the parent chemical are needed. Where the chemical is found to be mobile through the soil, the significance of this result should be assessed in relation to the rate of degradation of the pesticide in soil.

B.2 Leaching of parent pesticide and its degradation products (aged-residue leaching)

This study is needed to provide information on the likely movement, through the soil profile, of the degradation products formed during aging of a pesticide. The radio-labelled pesticide is incubated with a soil, known to degrade the chemical (see chapter C), and the incubated soil sample is applied to columns of a range of typical soils on which the pesticide will be used. The columns are then leached with water and radiochemical eluting through the column is measured. When necessary, the column may be sectioned and the distribution of the chemical down the soil profile determined.

Where low mobility is noted, no further studies of the leaching of the degradation products are needed. When leaching is observed, its significance

should be assessed in relation to the rate of degradation of the pesticide and its primary degradation products in soil. Further leaching studies in larger lysimeters or under field conditions may be considered. In field studies of leaching, seasonal fluctuations of water table and soil water content can move pesticide and metabolites upwards as well as downwards thus increasing the time span available for their biodegradation. This is not considered in several current leaching models.

B.3 Run-off of parent pesticide

In the case of strongly adsorbed pesticides and under special local conditions, run-off may contribute to the mobility of a given pesticide. Model calculations may be used to estimate the extent of this contribution. However, for adsorbed chemicals, run-off is related more closely to local prevailing conditions rather than to the individual chemical. Since run-off will occur in certain situations, the most important study will be the fate of the chemical when adsorbed to soil in aquatic systems.

B.4 Volatility from water and soil

Model calculations based upon the solubility in water, vapour pressure and adsorption coefficient of the chemical generally permit reasonable estimates to be made.

C. Soil degradation studies

Laboratory incubation studies with ^{14}C-labelled pesticide are required to assess the likely rate of degradation of parent pesticide in soil and to provide information on the structure and likely degradability of metabolites. The studies should also assess the rate of mineralisation of the pesticide by monitoring the rate of formation of $^{14}CO_2$. Traps should also be included to retain any other volatile compounds released from the soil. The ^{14}C-mass balance resulting from these studies is an important element of the hazard assessment process.

In contrast to studies on the physical distribution of a pesticide in which the use of a small number of soil types is appropriate to assess its range of mobility, it is generally sufficient to study degradation in one type of soil which is typical of a major use area of a given pesticide. Variation of degradation found in different soils generally concerns rates but not pathways, and residue analyses can cover this aspect satisfactorily.

Degradation under aerobic conditions is of primary interest. Testing under anaerobic conditions may be important in certain cases to observe reductive reactions, e.g., with pesticides which penetrate deeply into the soil profile or which are used in paddy rice. Incubation with sterilized soil, sometimes carried out to determine the extent to which chemical mechanisms participate in the degradation of a pesticide, is of academic interest only.

When the chemical is readily degraded to natural products or is largely mineralized in laboratory studies, only minimal field dissipation studies are necessary.

When the half-life of the pesticide is expected to be longer (i.e., between 1 and 6 months) and mineralisation takes place only slowly, it is likely that residues will persist for sometime in the soil and could be taken up by subsequent crops. In this case, a crop rotation study may be appropriate (see subchapter B of 3.3.3). When the half-life of the chemical found in the laboratory is greater than 6 months and especially when the rate of mineralisation is insignificant, the rotational crop study becomes essential and an assessment of the persistence of the chemical in the field becomes important (see following subchapter D).

Pesticides may give rise to non-extractable residues in soil. Their contribution to crop residues is included in the rotational crop studies.

Another possible route of loss of adsorptive, slowly biodegradable chemicals in soil is by photodegradation on the soil surface. This can be estimated by using a thin layer of a sandy soil treated with the radiolabelled chemical which is exposed to sunlight or to an appropriate artifi-

cial light source for up to 30 days. Analysis at intervals will reveal whether the pesticide is photodegraded and whether volatile fragments are formed.

The results of the above degradation studies should be assessed in conjunction with the results of the distribution studies described in preceding subchapter B. This information allows the principal fate of the chemical in soil to be predicted. It can also help in the design of field experiments (see following subchapter D).

D. Soil residue studies

These studies are needed to determine the persistence of the parent pesticide and significant degradation products in typical soils after recommended field use.

Considerable effort is needed to develop analytical methods which are specific, sensitive and reproducible. Only degradation products, shown to still possess biological activity (as the parent pesticide) and to be bioavailable to following crops or to have significant ecotoxic potential, need be studied.

The persistence and mobility anticipated from the laboratory studies as well as the use pattern and geographical distribution will determine the design of these studies. They are best performed in combination with crop residue studies (see subchapter C of 3.3.3) to take advantage of common experiments.

Where long persistence is anticipated, provision should be made in the planning of field studies for continuing the assessment of the fate of the chemical for more than one season. As plants may contribute substantially to the degradation of a pesticide via their rhizosphere microflora, bare soil studies mostly yield degradation rates which are too low.

Laboratory mobility (especially leaching) studies may show results which need substantiation under more practical situations to allow an assessment of their significance to be made. In field studies of this type, care must be taken in selecting appropriate sites. Soil type, undisturbed soil profile, water-table, time of the year for application, etc., should be considered to avoid unrealistic assessment of the leaching potential of a given pesticide. When determining the frequency of sampling, the rate of biodegradability must also be taken into account in order to obtain reliable decline curves for half-life estimations.

E. Degradation in the aquatic environment

Studies of the degradation and distribution of the chemical in aquatic systems are important when the chemical is applied directly to water. Some studies may also be required if the chemical has significant ecotoxic potential and is used close to water so that drift, run-off or leaching may cause significant residues to appear in the water.

Preliminary simple studies and/or estimates such as described in the preceding subchapters will give information of whether the chemical may be hydrolysed, photodegraded or biodegraded in water. Some indication of the adsorption of the chemical to soil and of the ecotoxic potential towards aquatic organisms will also be available from preliminary studies.

Where further information is needed on the behaviour of the chemical in water, a laboratory study with radiolabelled chemicals should be carried out using a flooded sediment or soil system prepared from fresh representative field samples.

This study should substantiate the Koc based prediction of the partitioning of the pesticide between water and sediment. It should also indicate whether the pesticide is degraded in the water or on the sediment and allow the distribution of the degradation products between the phases to be monitored.

When the results of the laboratory or other small scale studies indicate that the pesticide remains in the aqueous phase or is only slowly degraded,

trials in outdoor pond systems are indicated especially if the chemical possesses a significant ecotoxic potential. The design of these studies requires special consideration to cover the likely level of residues which may result in practice and to ensure that this residue is applied to the system in a realistic form, e.g., as a spray or adsorbed on soil particles. The possibility of volatilization and photodegradation should also be considered. These trials should assess both the fate of the chemical in the system as well as the effects of residues of the pesticide and its degradation products on a range of organisms within the system.

The results of these studies are important in assessing, on a more realistic scale, the fate of the chemical in aquatic systems as well as providing a basis for assessing possible ecotoxic effects.

3.3.2 Behaviour in Air

Having considered the behaviour of pesticides in soil and water, for completeness it is also necessary to consider their behaviour in air. In making a decision as to whether studies are needed the following parameters should be considered on a case by case basis:

1. Volatility of the chemical
2. Method of application
3. Knowledge of the chemical reactivity
4. Expected tonnage of chemical to be used

The atmosphere is so vast that the transfer of a pesticide into it, especially for chemicals of low volatility, is unlikely to result in significant concentrations. In addition, the adsorption of a chemical to soil or plant surfaces may also act to reduce its apparent volatility. Assessment of the volatility of a chemical can be made by considering the factors and procedures discussed in chapter 3.3.1.

Pesticides can enter the atmosphere by two routes:

a) spray drift at application
b) volatilisation from plant surfaces or from soil

They may be present, therefore, as an aerosol or as true vapour. In addition, some may be present adsorbed to solid particles following wind erosion of soil. As soil particles are relatively large, wind erosion does not contribute significantly to long range pesticide dispersion.

Before elimination of aerosols or vapours from the atmosphere by rainfall, by adsorption to soil or plants or by solution in water, small amounts may be transported for considerable distances.

When pesticides are applied as coarse droplets close to the soil surface, there will be only small losses to the atmosphere at the application stage. In the case of pesticides, such as fungicides and insecticides, which are applied through mist blowers or by aircraft, attention must be paid to spray drift losses and distribution profiles away from the application site.

The primary factors influencing the behaviour of the pesticides in air are the reactivity of the chemical and the ability of the atmosphere to degrade most chemicals. Two types of degradation are most important:

a) direct photolysis in the vapour phase or as an aerosol (including the effect of sensitizers)

b) reaction with active species such as hydroxyl radicals and ozone

Experimental problems are considerable in studying these routes of degradation and there is no agreed feasible methodology. However, an understanding of the general reactivity of the chemical is normally sufficient to make some judgement. If there are structural indications of potential photoreactivity, e.g., UV absorption above 290 nm, or reactivity with $OH^{\bullet}$ and O_3 indicate degradation, the need for additional studies in air may be un-

necessary. If additional information is needed, this may be obtained by simple photodegradation tests in solution or on surface. Generally, there is no need for a detailed study of the degradation of pesticides in air.

3.3.3 Behaviour in Plants

A. Introduction

Knowledge of the behaviour of pesticides in plants is a major factor in their safety evaluation. Studies are generally directed towards identifying and measuring residues with significance to consumers and/or farm animals such as ruminants and poultry. Accordingly, metabolism studies have to follow the qualitative aspect of pesticide uptake, translocation and metabolic conversion. Residue studies have to provide a quantitative picture of residual parent pesticide and relevant metabolites in the raw agricultural commodity after recommended field use of the pesticide.

Pesticides enter plants following direct application and by uptake from soil. The choice of the test plant species and application procedure is determined by the intended use of the pesticide. If more than one crop is to be treated by a given pesticide, an economically important target crop must be selected for the principal metabolism studies. Such studies have to be designed to provide sufficiently detailed information on the uptake, translocation and metabolic conversion of the test pesticide and the identity of its major metabolites. This may be followed by the study of the other species in model systems such as plant cell cultures. The latter normally provide sufficient information to decide which compound should be confirmed by residue analysis (see following subchapter B).

Pesticide residues may remain in the soil and be available for uptake by following crops. Studies to determine the fate of these residues may also be required (rotational crop studies).

Although plants constitute by far the highest proportion of the living biomass, the transfer of pesticides from the atmosphere into plants is of minor importance.

The results obtained in studies on the fate of a pesticide in plants provide a basis for the development of a comprehensive method for residue analysis and are a prerequisite to an assessment of the potential toxicological significance of the residue. They allow a comparison of the plant metabolite pattern with that found in the rat metabolism studies. The organisation of these studies in a stepwise approach is presented in chapters 4.2.5 and 4.2.6.

B. Metabolism studies

In order to provide qualitative information on the fate of a pesticide in and on a plant, metabolism studies are carried out using radiolabelled chemicals and should follow proposed use patterns as closely as possible. Thus the chemical should be applied:

- as formulated material (especially when used in field studies)
- at a realistic, (if possible, recommended) rate
- in the recommended way
- to the crop(s) on which the chemical will finally be used
- at relevant growth stages and relevant intervals

The studies may be performed under controlled conditions (greenhouse) or under open field conditions, if appropriate.

Harvesting of the treated crop is carried out at times matching the expected harvest interval(s) to be used in practice. In certain cases, the harvested crop undergoes processing, e.g., oil seeds to meal and oil, and the distribution of significant radioactivity in these components can be compared to the radioactivity levels in the raw agricultural commodity to determine whether or not residue concentration has occurred in the processed component.

Often a pesticide is used to protect several crops. If the crops are closely related, e.g., are representatives of cereals or leguminous plants, only

one major crop may be used to establish the principal metabolite pattern. Residue analysis is then adequate for other members of the crop group. If the crops are not related, other approaches such as cell cultures, stem cuts, floating leaf discs and others may be used to substantiate that the pesticide is metabolised in a similar way in different crops.

If these approaches are not used, one representative of each crop group to which the pesticide is to be applied may be used to elucidate the metabolism pathways (studying a maximum of 3 groups is generally sufficient).

The number of metabolites which should be identified must be determined on a case-by-case basis. In view of the objectives, namely to serve as the basis for residue analysis methodology and to allow comparison with the animal metabolite pattern, the major pathways should be defined. Complex structures may degrade into many transformation products. In this case, it may be impossible to identify a large percentage of the metabolites. Then a "common moiety" approach should be considered (see following subchapter C).

Metabolism studies are essentially qualitative studies and therefore actual concentrations of the metabolites found in such studies may have little quantitative significance. They should only be used in residue data evaluations with special care.

Crop rotation studies, when needed, may be performed in the greenhouse or in the field using a few crops which are representative for a given target crop situation (e.g., a root crop, a cereal and a leguminous crop). If significant radioactive soil residues are taken up by immature plants, the nature of the finite residues in the edible parts of the rotational crops should be determined.

The variable nature and use patterns of individual pesticides require considerable flexibility in the organisation of plant metabolism studies. This can best be met by a stepwise procedure. Such an approach is presented in chapters 4.2.4 and 4.2.5. In the preliminary study, the following information is obtained from the major target crop (immature, if appropriate):

- uptake and translocation pattern
- loss by volatility, if any
- rate of metabolism of parent pesticide
- number and possible importance of individual metabolites ("finger-print" or metabolite profile)

In the main study, the state of metabolism in edible parts of the major target crop at harvest (raw agricultural commodity) is established:

- percentage of extractable and non-extractable radioactivity
- number and importance of metabolites
- identity of major metabolites (metabolite pattern)

As mentioned above, certain special studies may be required to provide additional information, e.g., on:

- metabolite pattern in non-related target crops
- residue pattern in edible parts of rotational crops
- behaviour of residues during food processing.

C. Crop residue studies

Residue studies are needed to quantify the actual residues of parent and metabolites under recommended use conditions. They provide the quantitative data package which is used to set MRL's (Maximum Residue Limits) and which, together with toxicological data, is used to assess the safety of residues to consumers. They also provide an understanding of the behaviour of pesticides in plants under field conditions. Generally, the residue method used is specific for the parent pesticide. If metabolites still retain significant biological activity or are of toxicological significance, they should also be covered by the same or a separate method. In certain cases, e.g., if a metabolite pattern is composed of a multitude of minor metabolites, it may be appropriate to convert the majority of these metabolites into a common which can then be analyzed. However, it should not be necessary to analyse for all minor metabolites by a residue method.

Guidelines for these studies exist in the registration procedures for several countries and international organisations. However, it may be helpful to highlight a few essential comments on the design of these studies. Residue trials should cover a range of climatic/geographical regions in which the product will be sold. These do not necessarily conform to national boundaries and it is desirable that residue data generated in one country can be used in another country with a similar climate when the agronomic practices are the same. This requires flexibility in how registration guidelines are written and interpreted in different countries by both regulatory authorities and registrants.

The trials should relate closely to the actual use proposed for a product and the results should demonstrate typical residues and also residues resulting from realistic "worst case" situations. It is therefore advisable to carry out trials over at least two seasons in order to examine, in detail, situations giving rise to variations in residue levels, e.g., various soil types, crop varieties, timing of sprays.

Residue trials should be carried out by staff specially trained in the conduct of such trials, and it is essential that the trials are properly sampled. The details of the sampling and climatic conditions must be clearly recorded. The necessary data requirements have already been reported. The samples should be shipped and stored prior to analysis under conditions which have been shown to maintain stability of the residue. Storage stability testing is an essential requirement.

The samples should be prepared and analysed for residues of parent pesticide and metabolites of toxicological significance by methods which have been fully validated. Checks should be made on the sample preparation techniques, extractability of the residue and the recovery of residue during the method. Finally, the data obtained during this process must be thoroughly checked and interpreted carefully by an experienced professional.

By following this procedure meticulously and by detailed advanced planning of a trials programme, a reliable package of data on which to quantitatively assess the behaviour of pesticides in plants under various climatic conditions can be generated. Careful documentation of all aspects of residue projects according to GLP principles will assure the quality of the data generated.

3.3.4 Behaviour in Animals

A. Introduction

Man may come into contact with pesticides either directly during manufacture, application and use of these chemicals or indirectly via food and water containing pesticide residues. Farm animals such as ruminants and poultry may also be exposed directly through pesticide treatment or indirectly if their feed contains pesticide residues. Wildlife may come into contact with pesticides mainly via residues in their diet. It is therefore imperative to study the fate of pesticides in animals, the latter representing either models for man or direct target organisms.

Pesticide metabolism studies in animals should supply information about:

- rates and routes of uptake, distribution and excretion
- biodegradation and metabolic pathways
- retention in specific organs and tissues
- variation of metabolic behaviour depending on the sex of the test animals, dose and (if necessary) route of pesticide administration (oral or dermal) and, in certain cases, with species

This information is needed:

- to contribute to the interpretation of the nature and selectivity of observed toxic effects with regard to
    - pharmacokinetic behaviour (origin, duration, possible accumulation of effects)
    - molecular basis of the toxic effects observed (comparison with chemicals of similar structure/behaviour/effects)

  - transferability of data between animal species and possibly from animals to man

- to assess the toxicological potential of plant metabolites;
- to provide, if needed, the basis for monitoring exposure to pesticides by establishing a relationship between the amount of specific metabolites excreted and the dose or exposure.

Generally, the rat is the primary test animal. The selection of other test species and the type of studies to be performed depend on the nature and extent of exposure to the pesticide, e.g., whether it is via feed or directly as in case of ecto- and endoparasiticides, whether it is oral or dermal and whether it is over short or longer periods.

Some information should be available on the dependence of the kinetic and metabolic behaviour of a pesticide on the dose administered. Performing such studies in animals which are in a state of poisoning by massive overdosing of the pesticide, should be avoided. Unlike in toxicology, where biological effects on the animals are observed, pharmaco-kinetic and metabolic studies focus upon the fate of the chemical itself. Application of statistical considerations in kinetic studies of pesticides is irrelevant because the major phenomena (e.g., degree of absorption, rate and route of excretion; extent of retention in specific organs, etc.) can be recognized reliably with a small number of animals. In addition, the excellent sensitivity levels inherent in radiolabelled studies provide adequate information needed with regard to accuracy of metabolic pathway definition and variations among individual animals.

A stepwise approach is presented in detail in chapter 4.2.6.

B. Kinetic investigations

These investigations provide information on the absorption, distribution, excretion and retention of the pesticide in test animal species. They must be conducted in such a way that they provide an accurate total balance for the radioactivity administered.

B.1 Single dose balance study

In this study, the excretion of radioactivity after a single oral dose of labelled test pesticide to rats and the retention of radioactivity in their organs at the end of the excretion phase are determined. Other routes of application, e.g., dermal, and other test animal species, e.g., farm animals, may be required if the use pattern or the result of toxicity studies indicate a need. In studies with rats, generally male and female animals are exposed to two dose levels. One dose level should be in the order of potential residues, e.g., 0.5 mg/kg body weight corresponding to about 5 ppm in food. The other dose level should be significantly higher (e.g., if possible 50 mg/kg body weight but not exceeding $^1/_4$ of the LD_{50}) to investigate a potential dependency of the metabolism pathways on dose level.

B.2 Multiple dose balance study

This study is needed when single-dose experiments show that significant retention of radioactivity takes place in certain organs, and possibly, when there are concerns about cumulative toxicological effects. Information is developed on the concentration of radioactivity in organs and tissues when the pesticide is dosed, generally orally, over a certain period of time, and on the depletion of radioactivity from the body after termination of dosing. The test species generally used is the rat. If farm animals may be exposed to the pesticide via their feed, goats (as models of milk producing ruminants) and poultry may be used. In case of direct exposure (e.g., to ecto- or endoparaciticides), the target animals (e.g., sheep and cows) should be used.

The results provide information about the rate of build-up and the magnitude of "steady-state" (plateau) concentrations of total radioactivity and parent pesticide in:

- major organs and tissues
- meat and milk or eggs if farm animals are investigated

The results will also indicate the rate of depletion of radioactivity from organs and tissues and measure any organ specific retention of radioactivity.

This type of study is also useful when the significance of unidentified radiolabelled residues in plants of relevance to human or animal nutrition is to be assessed. In these "transfer studies", the labelled pesticide-treated plant material is administered to farm animals such as goats or poultry in a suitable form as part of their daily ration over a given period of time. This is followed by an adequate depletion time. Principally, the same type of results are obtained as described above for parent pesticides. Where plant metabolites are identified and are different from those found in animals, synthetic samples of labelled metabolites can be used in "transfer studies".

B.3 Special kinetic studies

The necessity for and the design of these experiments are usually determined by the nature of the additional information required to interprete special behaviour/effects observed in the preceding kinetic studies and/or toxicity testing such as substantially prolonged excretion half-life; significant interspecies variations of toxic effects; residence time of veterinary drugs in blood/organs; etc.

The studies may provide information on special kinetic aspects of absorption and excretion such as blood levels, biliary excretion, enterohepatic circulation and the like.

C. Metabolic pathways

C.1 Identification of metabolites

These studies provide information on the structural transformation and degradation of the test pesticide in a given test species. Generally the rat is the animal of choice and the major metabolites excreted in urine, feces or expired air are identified. Kinetic studies may require elucidation of significant organ residues. Other animals such as farm animals may have to be used if they are exposed to the pesticide and prior kinetic studies indicate significant radioactive residues in milk, meat and eggs or individual organs. In the absence of the latter residues, a comparison by fingerprinting of the metabolite pattern in the excreta of these animals and the rat supported by co-chromatography with references may give sufficient information.

The results of these studies enable comparison of the pattern of metabolism between species to be made, especially between those species used in toxicity assessment and between the laboratory species and farm animals. This may help to interpret toxicity differences between species. The studies may also indicate possible reactive metabolic intermediates which again may help to interpret toxicological results. Finally, the results will show whether the metabolites found in plants and animal products are the same as or similar to those found in the animal species used in toxicological evaluation studies. Plant metabolites which occur in these animal species need no further toxicity testing.

C.2 Special metabolism studies

These studies, which will only be needed in special cases, should be specifically designed to provide information on special metabolic reactivity of a test pesticide if indicated in C.1 and/or in Toxicity Studies.

The studies may be required to explain

- the nature of significant (non-extractable) radioactivity retained in specific tissue(s) (binding or incorporation of pesticide fragments)
- toxic effects at high application rates, e.g., by over-loading of metabolic capacity
- differences in toxic response in different species (e.g., comparative metabolism in rat and mouse)
- metabolic basis of certain toxic effects (e.g., genotoxic/non-genotoxic mechanisms: reactive intermediates, binding to DNA and other biopolymers; mechanisms of detoxification)

The results are valuable in assisting, at the molecular level, the interpretation of the potential toxicological consequences of a given pesticide for man and terrestrial animals.

D. Residues in food of animal origin

If direct application (e.g., of ecto- and endo-parasiticides) or expected intake (e.g., of feed containing pesticide residues) indicate that residues of a pesticide may occur in farm animals, metabolism data should be complemented by residue data from the species concerned (e.g., cow, sheep, poultry). The pesticide should be administered as recommended or at rates that reflect the residue situation in the animal feed crop(s) occurring after recommended pesticide use. The residue method used should be validated and cover parent pesticide and, if necessary, toxicologically significant metabolites in meat, milk and eggs. Generally, the limit of determination of these methods is in the order of 0.025 ppm.

The residue data obtained in these studies, together with the residue data in the major food crop(s), represent the exposure element needed in the frame of Hazard Assessment to set Acceptable Daily Intakes and other regulatory limits.

4. RECOMMENDED APPROACH

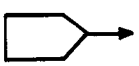
4.1 Introduction

In the previous chapters, the rationale and the available experimental systems for assessing the environmental behaviour of pesticides have been described. The following sequential test approach is based upon this. It provides, at different levels, information which can be used directly to satisfy specific regulatory requirements and as elements needed in integrated hazard assessments. As hazard is defined as a function of exposure and effect, this information is primarily related to the exposure part. In combination with the use pattern of a given pesticide, it allows an assessment to be made of the distribution and degradation, i.e., finally the concentration, of this pesticide and its significant degradation products in the relevant compartments and organisms of the environment. With respect to animals this information obviously is also of direct relevance to the toxicological assessment.

It is recommended that Good Laboratory Practice (GLP) principles should be applied when conducting the studies described in this test approach in order to assure the quality of all data used in registration procedures.

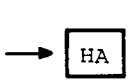
4.2 Test approach

In the following subchapters, the recommended test approach is presented in form of flow-schemes which are supplemented by brief explanations. Each scheme has to be handled with flexibility: Sound professional judgement, considering relevant information obtained from other schemes, should be made at each decision point. A number of symbols is used to present the information in an easy-to-read form:


: Input information prerequisite to design/performance of test/assessment

: Description of test or calculation/assessment to be performed

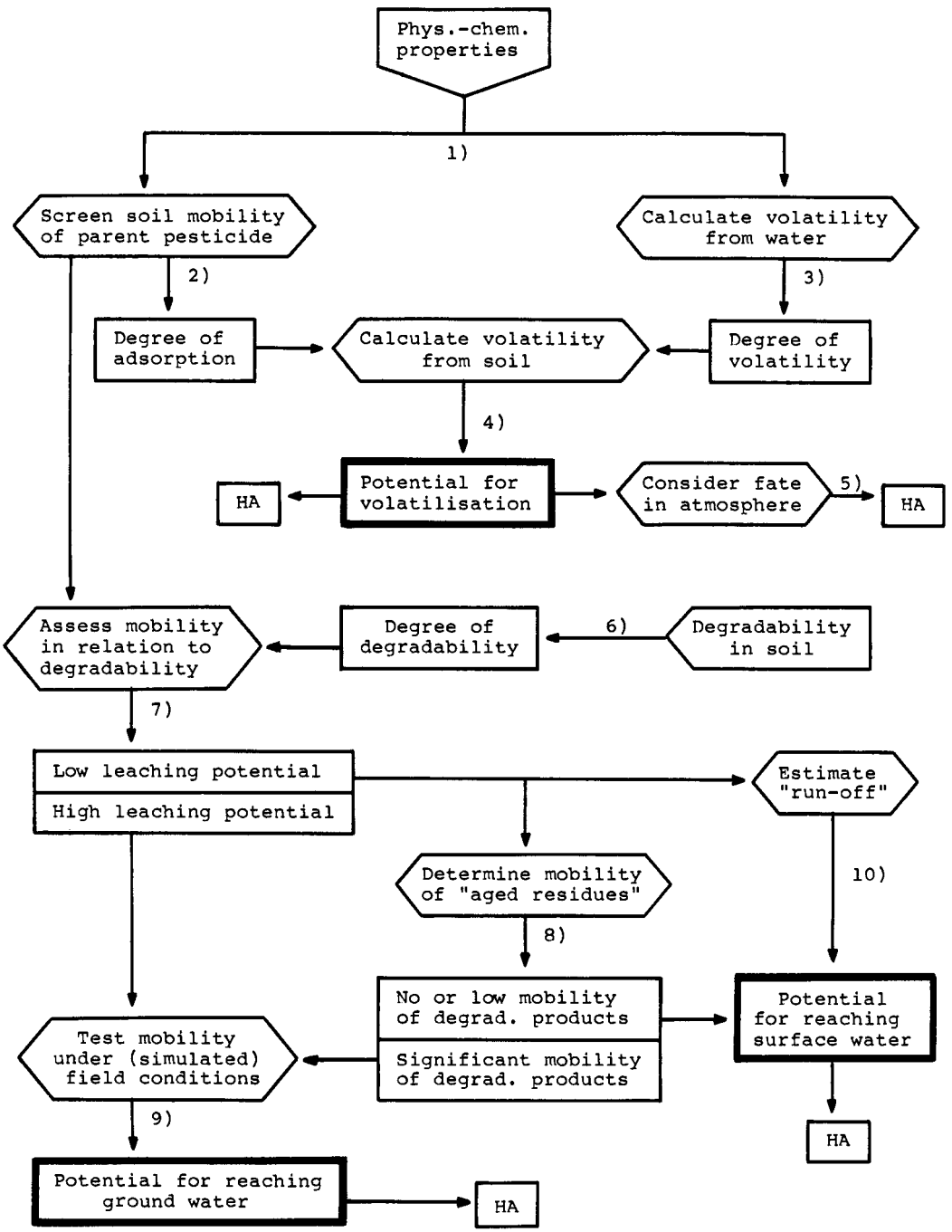
: Description of result of test/assessment; frequently divided in two parts if one result leads out of the test scheme or the two possible results require different further testing



: Output information for use as an element of a hazard assessment either directly or after its further verification/refinement in other test schemes.

The explanatory sheets added to each test scheme give, in a very abbreviated form, some additional details on the performance of the tests and calculations/assessments as well as the interpretation of their results. The corresponding subchapters of chapters 2 and 3 which are pertinent to the individual property under study and offer a broader reasoning concerning test design and result interpretation should be consulted.

4.2.1 Testing environmental mobility of pesticides



Commentary to Test Scheme 4.2.1

Consider potential impact of results from schemes 4.2.2 and 4.2.3 on mobility testing. Whenever possible, compare behaviour of test pesticide with that of (related) "benchmark" chemicals of known environmental behaviour.

1) Relevant parameters: Sw, Pow, p and Koc; in case of non-charged chemicals Koc may be calculated from Pow.

2) Use calculated Koc as first estimate; in case of medium sorptivity substantiate by TLC on soil plates (3 soil types with variable organic carbon content). In case of non-charged test pesticides with high lipophilicity, e.g., with Pow >4, determination of the adsorption coefficient (e.g. Freundlich) may replace the soil TLC approach.

3) Calculate Henry's constant as a first approximation

4) Use estimated (in case of high adsorptivity) or measured soil adsorption coefficient (preferably Koc) and Henry's constant for calculation of volatilisation.

5) Estimate photoreactivity and use, if possible, data on photodegradability in water and/or surfaces to assess fate of a volatile pesticide in the atmosphere.

6) Use laboratory degradation data (^{14}C-balance). Ready degradability: soil $\tau/2$ < 1 month; moderate degradability: $\tau/2$ 1 - 6 months; low degradability: $\tau/2$ > 6 months (see 4.2.2, comment 3).

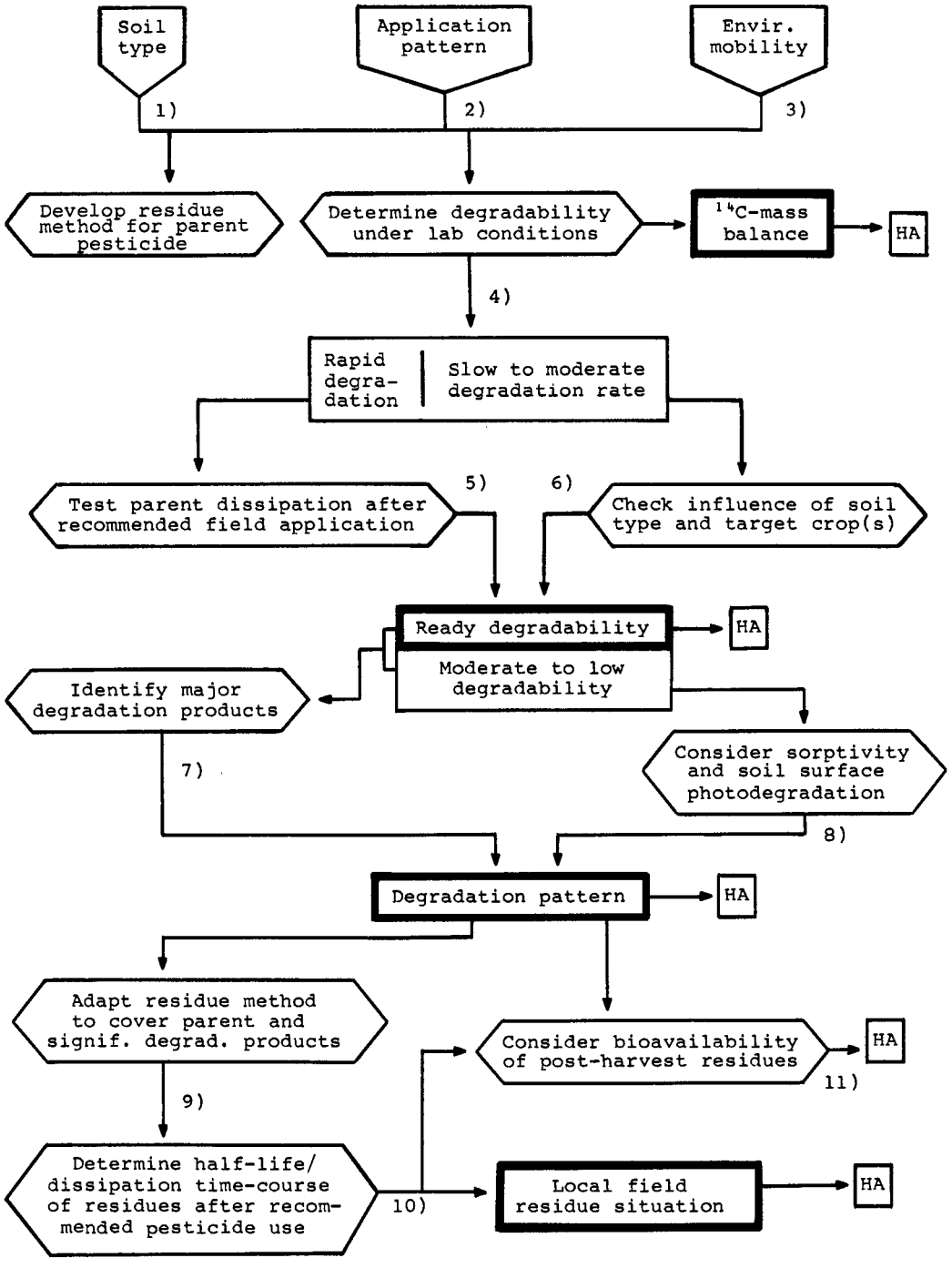
7) Include use and application pattern in assessment; higher degradation rates may lower leaching potential of test pesticide or in certain cases obviate further testing. Estimate potential of test chemical on the basis of Koc values of suitable "benchmark" chemicals.

8) Use biologically active fresh soil (preferably from the degradation study) for aging of parent pesticide (up to one half-life period); add aged residues on top of soil column and apply realistic watering regime.

9) Larger lysimeter systems containing soil columns from an undisturbed soil profile or field tests in areas carefully selected with regard to topography, soil composition, drainage, watertable, etc. may be used to assess the practical relevance of the leaching potential of a pesticide determined in the laboratory.

10) Estimate or use model calculations under critical consideration of use and application pattern; consider that ready degradability will reduce potential for "run-off" significantly. If estimate shows medium to high "run-off", substantiation of this potential under field conditions is indicated. In the case of aquatic use, e.g., rice, see scheme 4.2.3).

4.2.2 Testing behaviour of pesticides in soil



Commentary to Test Scheme 4.2.2

1) Use fresh samples of one well-characterized soil typical for growth of major target crop.

2) Treat the soil at the likely field concentration resulting from the maximum application rate in a 5 - 10 cm soil layer; allow for crop interception and avoid exaggerated test concentrations.

3) Consider volatility and sorptivity of test chemical (see 4.2.1) in experimental approach to obtain mass-balance and to decide on need for anaerobic incubation.

4) Incubate under aerobic conditions at realistic water holding capacity (e.g. > 40 %) and temperature (e.g. 15 - 25 °C); test under anaerobic conditions if test chemical has moderate to low sorptivity (e.g. Koc < 1000); incubate for up to 6 months; the degradative capacity of the soil microflora under laboratory conditions decreases drastically after this period. Use ^{14}C-mass balance for estimating the persistence potential in the early phase of HA.

   High aerobic degradation potential (i.e. ready degradability with parent half-life $\tau/2 \leq 1$ month; especially if simultaneous mineralization to $^{14}CO_2$ is observed) needs no further laboratory testing but confirmation in field dissipation studies using parent residue method.

5) Check typical field situations to recognize conditions which affect ready biodegradability.

6) Moderate ($\tau/2$ 1 - 6 months) and slow ($\tau/2$ > 6 months) degradation in 4) after aerobic and anaerobic incubation may be checked under cropped soil conditions.

7) Use soil of laboratory study 4) and of studies 5) and 6), if suitable, for isolation and identification of major extractable metabolites (e.g. > 5 % of initial concentration at the end of the incubation period). Generally, available methodology and justifiable economic effort do not allow identification of individual extractable metabolites below 0.025 ppm. Use the pattern of degradation products as an element of HA, e.g., for ecotoxicity.

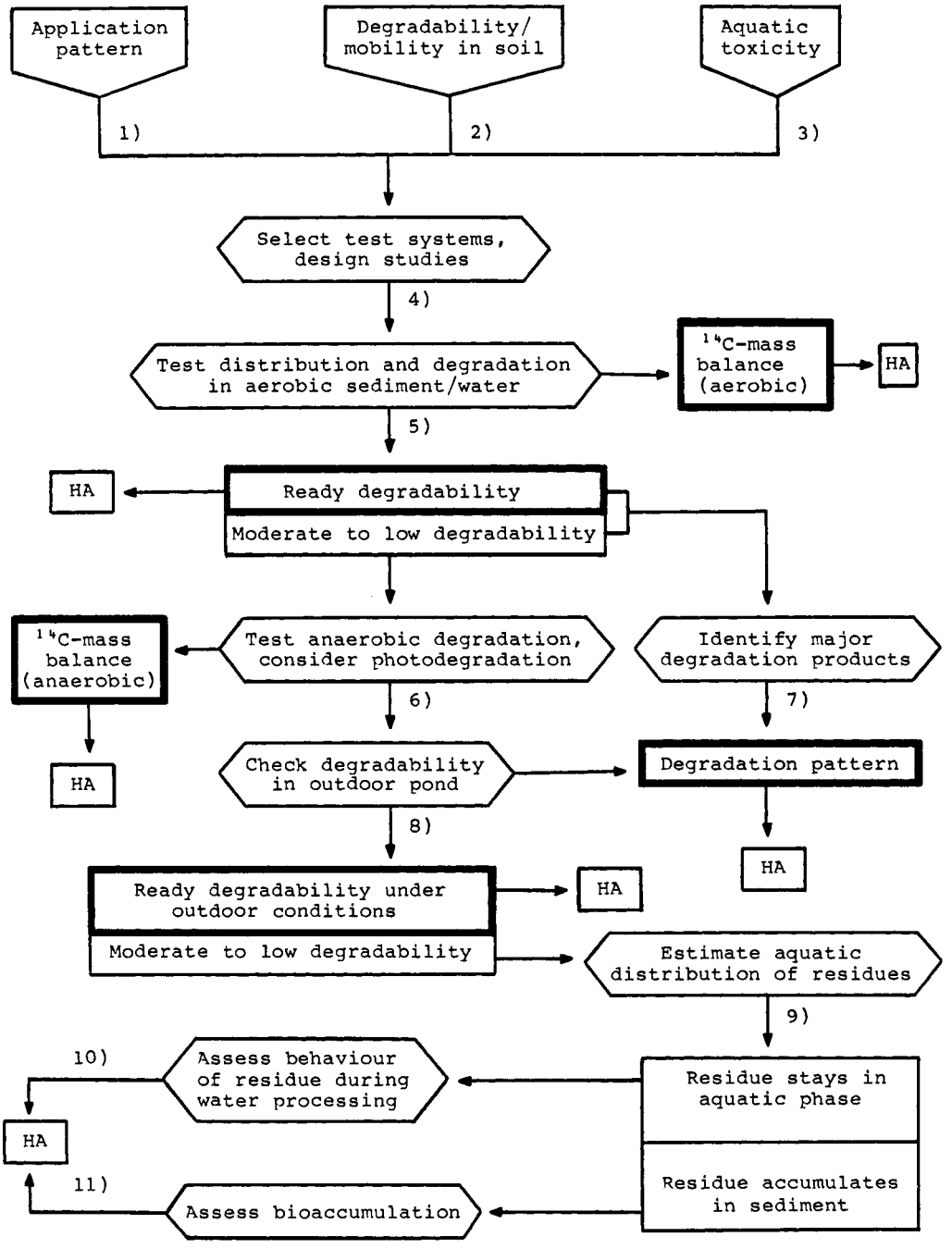
8) In case of highly sorptive pesticides with low to moderate degradability check influence of soil surface photodegradation.

9) A validated residue method should cover the parent pesticide and major metabolites retaining biological activity. With readily degradable pesticides, the residue method for parent pesticide is generally sufficient. In case of complex pattern, analysis of a fragment of the pesticide structure common to the majority of degradation products is the method of choice ("common moiety" approach).

10) Take geographical distribution, major uses, recommended application and major types of formulation into consideration when establishing typical local field residue situations.

11) Significant non-extractable residues and moderate to low degradability recognized along routes 7) and 8) as well as significant soil residues observed after harvest of the target crop(s) require bioavailability testing in rotational crops according to 4.2.5.

4.2.3 Testing pesticide behaviour in aquatic systems



Commentary to Test Scheme 4.2.3

1) Consider whether test pesticide enters water directly (e.g. treatment of paddy rice, water pest control, spray drift) or indirectly by run-off or leaching; in the latter case use run-off or leaching potential estimated in 4.2.1 in defining the test approach; consider impact of special types of application (e.g. pre-plant incorporation, aerial spraying, seed dressing, minimum tilling) in final assessment of potential pesticide concentration in water.

2) Hydrolytic stability and degradability in soil are important background data for estimating length of study and sampling regimen. Soil adsorption data will indicate the distribution of the pesticide in the test system.

3) Aquatic toxicity (e.g. LC_{50} for fish, crustacea, microorganisms) of parent pesticide and, if necessary, of certain degradation products is the basic reference against which the significance of a given aquatic residue (concentration) should be assessed.

4) Use fresh samples of natural sediment and water (e.g. pond or river); if volatility of pesticide is significant, use closed aerated system to obtain ^{14}C-mass balance; generally, incubation intervals of 3 months should not be exceeded; avoid exaggerated test pesticide concentrations.

5) Ready degradability ($\tau/2 \leq 1$ month) needs no further testing; in case of significant ecotoxicity potential of parent assure that degradation is accompanied by detoxification. Moderate ($\tau/2$ 1 to 6 months) and low ($\tau/2 > 6$ months) degradability indicate testing under anaerobic conditions and consideration of potential contribution of photodegradation; if possible, give anaerobic ^{14}C-mass balance.

6) If test chemical shows moderate to low degradability also under anaerobic conditions, check its degradability in outdoor pond considering comments 1) and 5) for setting pesticide concentration.

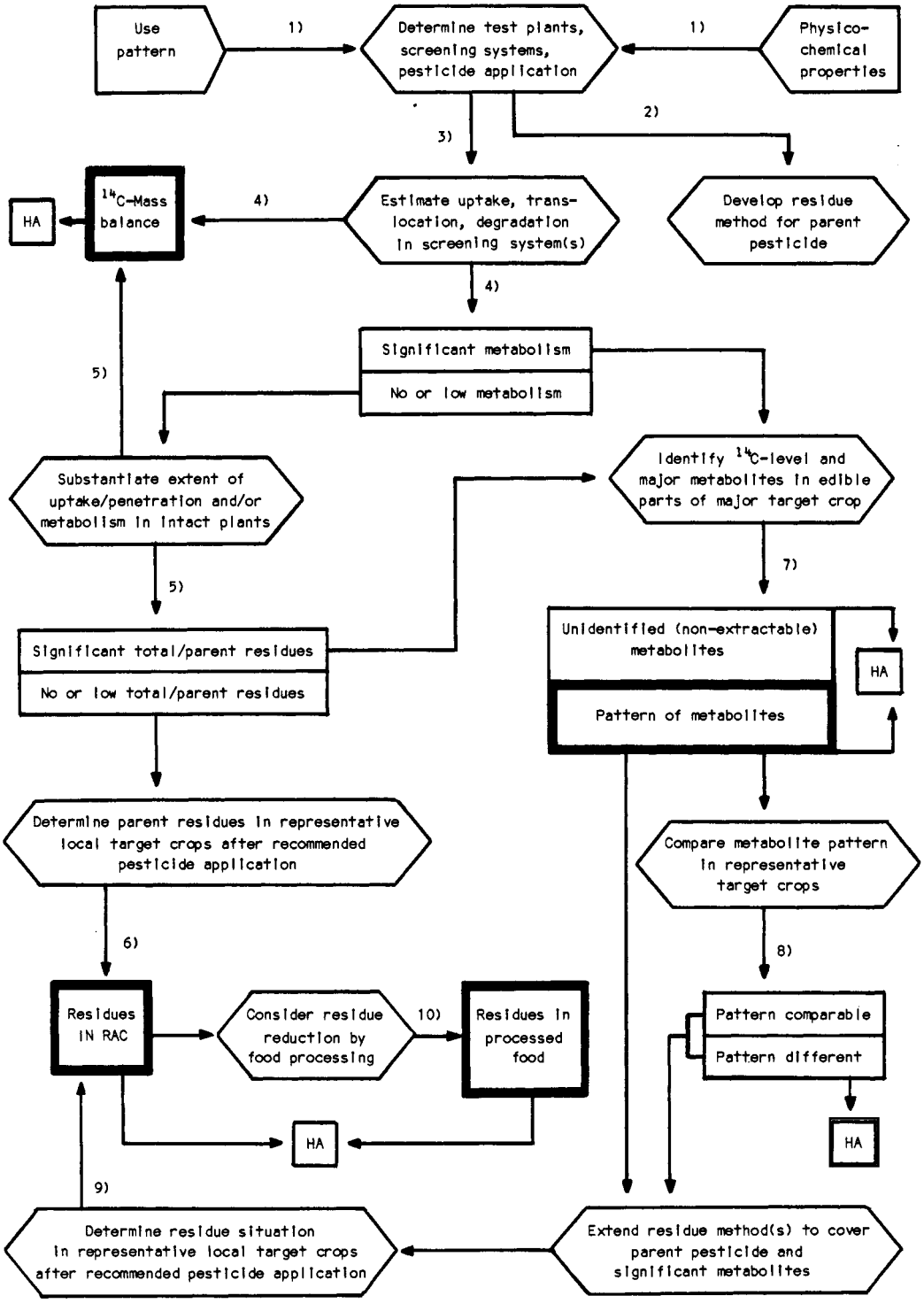
7) Identify major (extractable) metabolites, e.g., > 0.025 ppm or > 5 % of initial concentration at the end of incubation; consider results from outdoor pond study when describing the degradation pattern. Assess (see comment 3)) whether regular aquatic toxicity testing of parent pesticide covers toxic potential of degradation products (e.g. degradation $\tau/2$ versus length of ecotoxicity testing).

8) Pesticides proved to be readily degradable under outdoor conditions need no further testing; with chemicals of moderate to low degradability, in conjunction with significant sorptivity (see comment 9)), bioaccumulation testing should be considered.

9) If a significant portion of the residue remains in the aqueous phase, its behaviour during water processing should be assessed; if residues accumulate in sediment, aquatic toxicity testing (see comment 7)) and bioaccumulation potential in aquatic organisms (e.g. fish) should be considered.

10)/ 11) Use results obtained in toxicity and ecotoxicity considerations of HA.

4.2.4 Testing behaviour of pesticides in crop plants



Commentary to Test Scheme 4.2.4

1) Consider use pattern. Select one representative crop for each major crop group and the pertinent application procedure if pesticide finds broad use. Give attention to those physico-chemical properties which have impact on uptake and translocation such as Sw, Pow and pK.

2) Develop specific analytical method for parent pesticide; validate it with those crops for which tolerances are to be set.

3) Use small screening tests such as immature plants, stem injection, excised leaves and cell cultures of major target crop for this estimate. If relevant, consider influence of volatility and photodegradation on parent dissipation.

4) If intact plants (in closed systems) are used, a ^{14}C-mass balance can be obtained which may allow assessment of the extent of bioavailability and sources of loss of pesticide during and after its application. If no or slow metabolism is observed in plant model systems, check in intact target plants for uptake/penetration/metabolism (greenhouse, field). In case of significant metabolism, identify major metabolites produced in the model system to define primary pathways and to facilitate identification of significant residues (e.g., >0.025 ppm extractable ^{14}C) in edible parts of the main target plant which had been treated with the pesticide as recommended (greenhouse or field).

5) If not obtained in the screening system and uptake/penetration behaviour indicates a need, a ^{14}C-mass balance can be established in this study. In case of significant parent (and few metabolite) residues follow as indicated in 4) Significant Metabolism. A low parent residue situation needs confirmation in representative target field crops by residue analysis.

6) Determine parent residue levels in the "raw agricultural commodity" (RAC) of individual target crops from a restricted number of representative locations (including re-entry situations, if appropriate) using residue method as specified in comment 2). Use data for the tolerance setting process in HA.

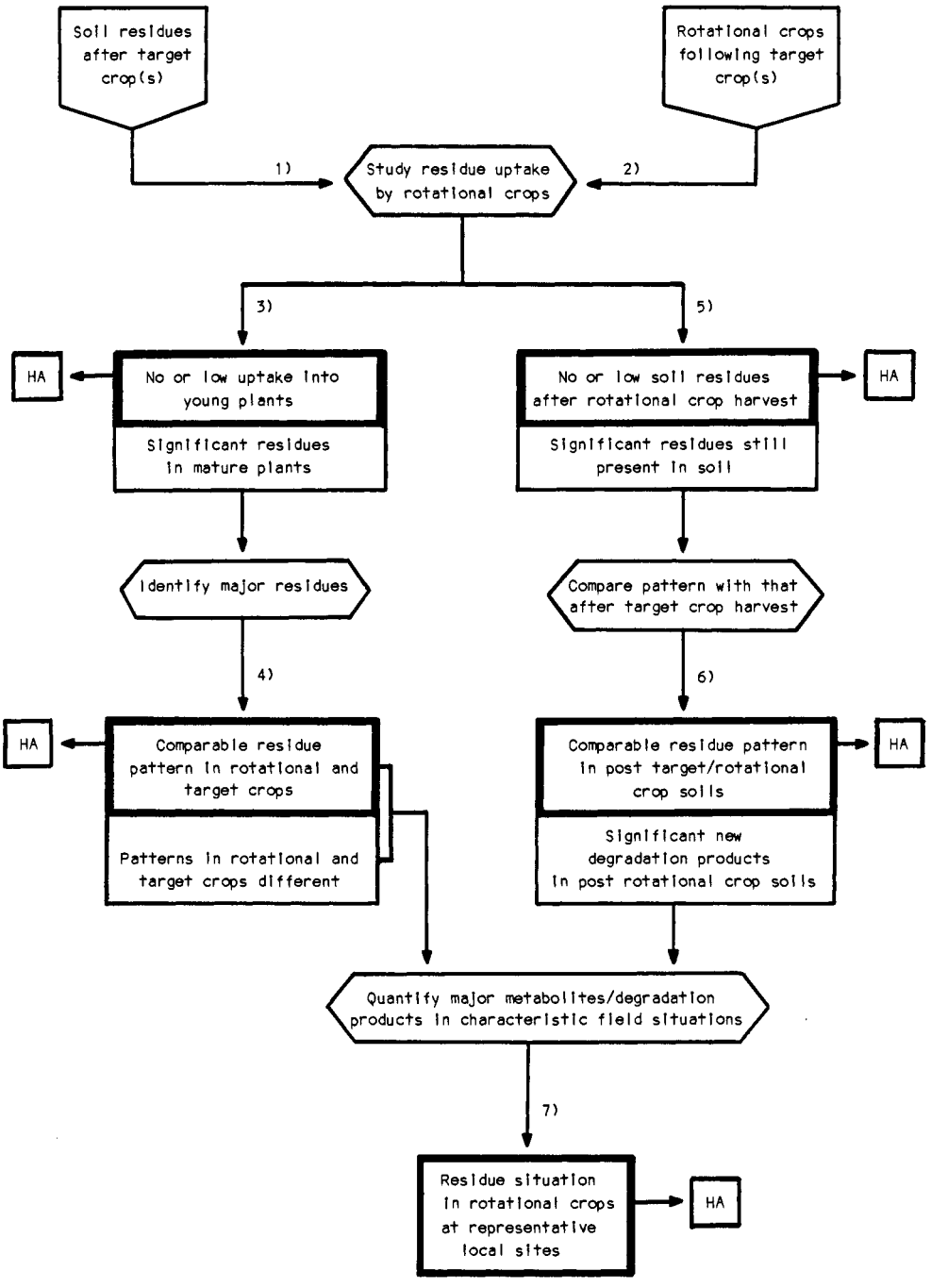
7) Evaluate toxicological relevance of metabolites in HA by comparing the metabolite pattern in edible plant parts with that found in the animal species used in the toxicity studies, preferably the rat. Major plant metabolites not found in this animal species may require metabolism testing in animals. Bioavailability of radiolabelled unidentified/non-extractable metabolites may be checked in "transfer studies" by direct feeding (see 4.2.5). Consider major metabolites of biological or toxicological significance in recommended residue method(s); if pattern is highly complex development of a "common moiety" approach is indicated.

8) If the pesticide is applied to several non-related target crops, comparison of the metabolite pattern from (maximum 3) species is indicated; if possible, use screening systems of 3). If patterns contain (structurally) different major metabolite(s) of biological or toxicological significance, adapt residue method(s) accordingly; follow tolerance setting considerations individually in these target crops.

9) Determine residue levels of parent pesticide plus relevant metabolites in the RAC of individual target crops from a representative number of typical locations (including re-entry situations, if appropriate) for use in the tolerance setting process in HA.

10) Check reduction of significant residues in RAC (e.g., >0.025 ppm ^{14}C in form of parent and/or metabolites) by food processing, if appropriate; use residue level in processed food as a factor in HA.

4.2.5 Testing bioavailability of pesticide soil residues to non-target plants



Commentary to Test Scheme 4.2.5

1) Soil residue situations to be considered before setting up rotation crop studies:

   a) residues in the upper 7.5 cm layer are > 0.05 ppm extractable ^{14}C or > 0.2 ppm total ^{14}C including non-extractables

   b) residues lower than those specified in a) which still show pesticidal activity (follow until maximal 3 half-lives of parent pesticide have elapsed)

2) Set up study in the greenhouse or in the field using the soil in which the target crop had been treated with test pesticide at the maximum rate recommended and grown to maturity. Select set of generally 3 rotation crops typically following the target crop. Choose from corn, leafy vegetable, root crop, cereal or leguminous plant (situation a)).

3) Determine uptake of ^{14}C into immature rotational crops. Discontinue study if no or low uptake of ^{14}C is observed in young plants (e.g. <0.025 ppm in 4 weeks); if higher values are obtained or uptake is continuous over longer growth periods of individual rotation plant(s), identification of major finite residues in edible parts of these mature crops is indicated.

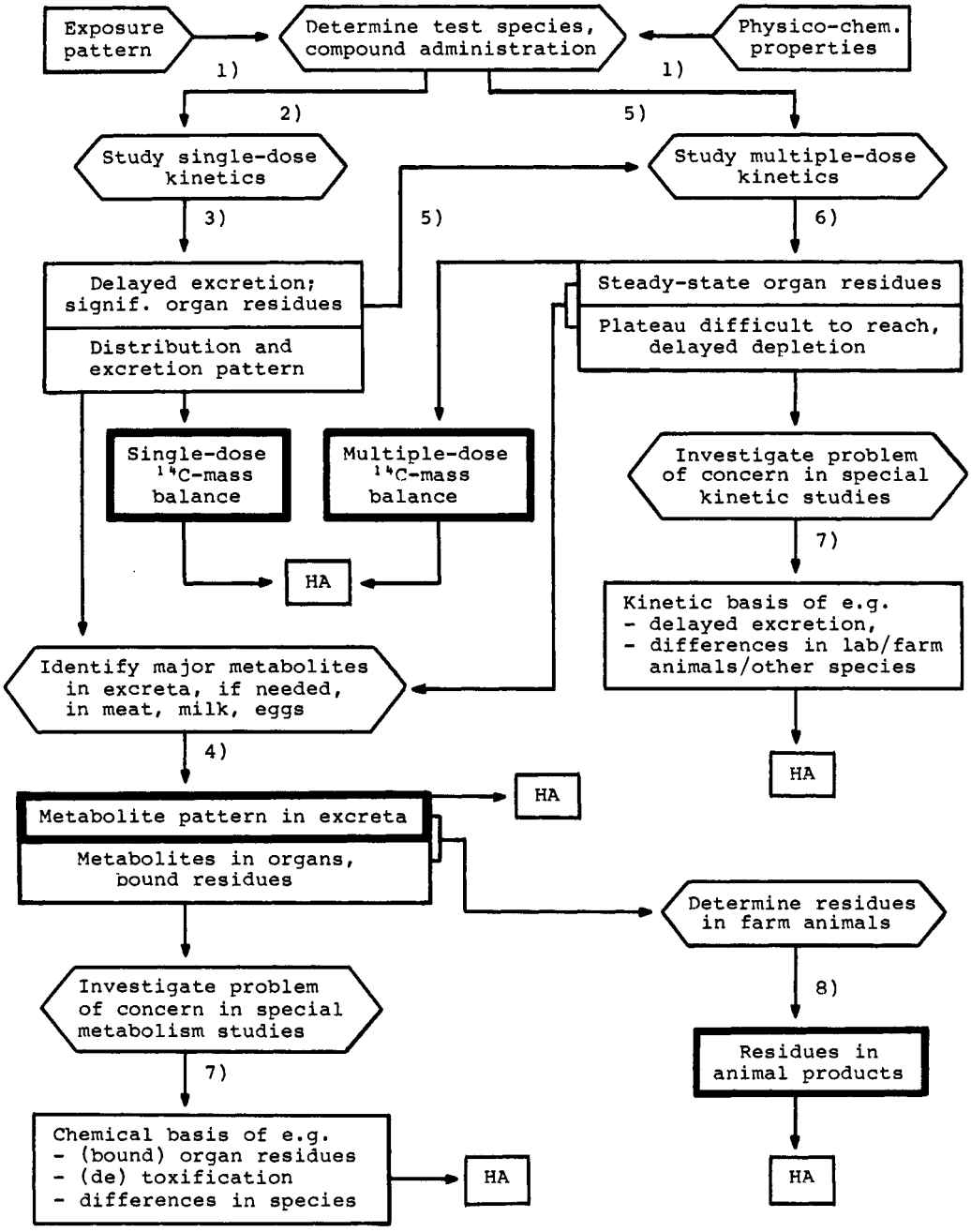
4) If residue pattern in rotation crops is comparable to that of target crops, use residue analytical method recommended for target crops to check residue in rotation crops significantly exposed; use information accordingly in the tolerance setting process in HA. If patterns are different, identify major new metabolites (e.g., >0.025 ppm); determine the residues of these metabolites occurring in rotational crops grown in the field after regularly treated target crop(s).

5) Discontinue study if no or low soil residues remain after harvest of rotational crop(s) (e.g., <0.025 ppm extractable ^{14}C in the upper 15 cm layer). Should significant extractable residues still be present in soil, compare pattern with that existing after target crop harvest.

6) If the residue patterns in post target/post rotational crop soils are comparable, apply dissipation characteristics obtained with the parent pesticide to estimate half-life of these metabolites. Occurrence of significant new type(s) of metabolite requires their identification and adaptation of residue method to quantify the major residues.

7) Use information on residue situation in typical rotational crops/soils after recommended use of a parent pesticide at characteristic local sites in environmental impact statements of HA.

4.2.6 Testing pesticide behaviour in animals



Commentary to Test Scheme 4.2.6

1) Consider in study design possible exposure situations of applicators of pesticide and consumers of food with potential pesticide residues, if appropriate also of farm animals to be treated with pesticide; relevant physico-chemical properties such as Sw, Pow and p should be considered in product formulation and administration, especially in case of farm animals.

2) Start animal testing with this study involving parent pesticide and any major non-animal metabolite occurring in the RAC of the target crop(s); generally, small numbers of male and female rats are used and two dose levels orally administered (see chapter 3.3.4, D). If monitoring of worker exposure (mainly by the dermal route) is required, an additional single-dose ^{14}C-balance study after dermal application of the pesticide is indicated to relate quantitatively major urinary metabolites with the dose actually absorbed.

3) Establish material balance of absorption, distribution and excretion. If excretion via urine is very low and essentially via faeces, special kinetic studies may be considered to estimate extent of pesticide absorption (e.g., use of a bile fistula may be more appropriate than comparing excretion after oral and intravenous application). In case of rapid and complete excretion, use result "Single-dose ^{14}C-mass balance" as an element in HA.

4) Define metabolic pathways from metabolites identified in excreta (if possible > 50 % of excreted radioactivity or at least the primary metabolic reactions) and major metabolites in meat, milk, eggs. Compare in HA metabolic pathways with those identified in plants to assess toxicological relevance of important plant metabolites. If significant radioactivity is accumulated and/or bound in individual organs, run Special Metabolism Studies to assess nature of these metabolites.

5) Investigate multiple-dose kinetics if result of single-dose study indicates (e.g., significant residues in individual organs at end of ^{14}C-excretion phase) or if farm animals such as ruminants or poultry are administered directly more than one dose of a pesticide (e.g., ecto- or endoparasiticides) or if there is a likely intake of parent pesticide or unknown plant residues via the food of animals or man (e.g., transfer studies).

6) If radioactivity reaches steady-state in major organs and/or milk and eggs reasonably fast and shows complete depletion, no further kinetic studies are generally needed; use result "Multiple dose ^{14}C-mass balance" in HA. If plateau of radiolabel is not reached within reasonable time (e.g., within 1 to 2 weeks) or delayed depletion of significant radiolabel accumulated in individual organ(s) is observed, special studies should be designed to estimate the kinetic basis of the phenomena involved.

7) Special kinetic and/or metabolism studies are also indicated if the basis of toxification or detoxification must be known or when toxicological differences in various species (e.g., rat vs. mouse, lab vs. farm animals) have to be evaluated. A careful multidisciplinary approach is required to define objectives and design of experiments in these studies.

8) In case of farm animals, check level of residues in meat, milk and eggs after realistic exposure situations using validated residue analytical methods; consider results in exposure considerations of HA.

5. CONCLUSIONS

This paper recommends concepts and experimental approaches, which lead to a rational, stepwise programme for testing the environmental fate of pesticides. The programme should be followed in a logical way so that resources are not wasted in areas where there are no problems. As much resource as possible should be channelled into aspects which require "in depth" study to fully understand the environmental fate of a pesticide. This is the prerequisite to a reliable assessment of the potential exposure of man and the environment to this pesticide.

Generally, the potential hazard of a pesticide for workers/applicators handling the product, consumers of treated produce and for the general environment must be assessed. Each of these areas requires an individual exposure analysis. A stepwise evaluation which progresses from an exploratory level (confined and representative/model exposure situations) over a verification level (expected range of local exposure situations), and, if necessary, to a surveillance level (control of local exposure situations) should be followed. The following Scheme summarizes this approach by briefly outlining the test systems needed to provide the information and the criteria used to judge its relevance:

EXPOSURE ANALYSIS

Test Level	Worker/Applicator	Consumer	Environment
I EXPLORATION			
Test systems	Laboratory animals		Soil/lab, field
		Target plants	Aquatic milieu/lab
		Target animals	Models
Criteria	Exposure potential	Range of residues in edible parts	Distribution and dissipation profile
	Degree of absorption		
	Degradation and distribution profile		
II VERIFICATION			
Test systems	Workers in normal job situations	Target organisms	Local fields
		Non-target organisms: rotational crops, farm animals	Confined aquatic systems (e.g., ponds)
			Aquatic organisms
Criteria	Highest-likely level and duration of exposure	Maximum residue levels	Highest-likely levels and kinetics of residues
		Contribution of crops and animal produce to total diet	
III SURVEILLANCE			
Test systems	Biological monitoring	Food basket	Surface/ground water
		Drinking water	Problem soils
Criteria	Accepted standard levels of residues in urine (blood)	Regulatory residue tolerance levels	Long-term behaviour of residues
			Regulatory tolerance

At the exploratory level, simple experiments should be carried out under controlled conditions. These should simulate the potential contact with the pesticide following its recommended use. They should explore the possible range of exposure, the resulting residues, and the distribution and degradation profile. Individual testing in the 3 assessment areas involves

- worker/applicator: animal models and physical measurements to estimate the potential for exposure, absorption, degradation and excretion

- consumer: laboratory and small-scale field metabolism and residue studies to determine the nature and range of residues in the edible parts of treated crops and treated animals (meat, milk, eggs)

- the environment: model calculations and laboratory and small-scale field tests to assess the rate of dissipation and the distribution of the pesticide from the area(s) of use into other environmental compartments.

At the verification level, small scale field studies using the expected ranges of exposure and use conditions are needed to verify exploratory data and to expand the metabolism/residue information to organisms which may indirectly come into contact with the pesticide. Individual testing in the 3 assessment areas involves

- worker/applicator: analysis of normal work-place situations to determine the highest-likely exposure; if required, elaboration of methods in animal models suitable for human monitoring

- consumer: analysis of parent and relevant metabolite residues in the target crops and animals treated according to good agricultural practice considering the expected range of local use situations; metabolism/residue studies with non-target organisms such as rotational crops and farm animals such as ruminants and poultry if exposed via treated feed. Evaluation of the relevance of the maximum residue levels found in these studies should consider the contribution of the crops and animal produce to the total diet

- the environment: determination of parent and relevant metabolite residues in typical local field soils and of the fate of the pesticide in confined aquatic systems such as ponds if transfer from soil is likely; verification of significant bioaccumulation potential, e.g., in fish. The highest-likely level of the residues and their distribution kinetics are major criteria to evaluate the relevance of the ecotoxic potential of the pesticide.

At the surveillance level, defined programmes should monitor the (longer-term) variability of individual exposure situations in practice if certain aspects of the behaviour of the pesticide observed during "verification" require more in-depth information. Individual testing in the 3 assessment areas involves

- worker/applicator: biological monitoring of the exposure at selected work-places by determining parent pesticide or suitable metabolites in urine (or measuring reaction of suitable blood parameters) to assure that internally and, if established, internationally accepted standards are observed

- consumer: monitoring of suitable residues in those components of food baskets (in certain cases in drinking water), which originate from raw agricultural commodities of crops or animal produce treated with the pesticide, to assure that established tolerance levels are met

- the environment: monitoring of the level and long-term behaviour of residues in surface and/or ground water and in problem soils to detect undesirable existence and cumulation of residues and to assure observation of any regulatory tolerance levels

At each stage of these exposure analyses, the results should be considered alongside a hazard evaluation (toxicological and ecotoxicological test results) to allow assessments of hazard and, finally, risk to man and his environment. It is outside the scope of this paper to deal with either hazard evaluation or risk assessment. These require a separate discussion which also considers existing guidelines of national and international regulatory agencies.

Our intention was to advocate a more rational programme for testing the environmental fate of pesticides in a stepwise procedure. This will be the most cost effective approach and should ensure that all aspects of a pesticide's behaviour are studied in sufficient detail to give a clear picture of its environmental fate.