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APPRAISAL OF THE FATE OF AGROCHEMICALS IN PLANTS AND SOIL: A COST-EFFECTIVE INTEGRATED APPROACH

(Technical Report)

Prepared for publication by

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ABSTRACT

Radiolabelled confined field studies performed with formulated test materials at the recommended use rates and timing can be designed to generate integrated data on soil/plant metabolism, soil dissipation and bioavailability of soil residues to rotational crops. They offer the possibility to investigate the fate of the degradates in each compartment at an early stage of the development program. Due to the quantitative nature of the results they represent an adequate source of field residue data to detect a "negligible total residue situation" in raw agricultural commodities which eliminates the need for lengthy development of specific analytical residue methods on degradates. As a consequence they represent a substantial rationalization of the overall environmental testing program.

CONTENTS

1. INTRODUCTION

Various international organizations and governmental regulatory agencies are involved in evaluating or updating concepts and methods used to assess the environmental fate of agrochemicals. They have generally stressed the necessity to avoid rigid investigation schemes which might result in wasted effort where there is no environmental concern, or insufficient effort in cases where further investigative research is appropriate (ref. 1). It is certainly to the advantage of the industry and regulatory agencies to have avail-

able a data package to highlight critical environmental issues as early as possible in the review process in order to perform a sound risk assessment of the experimental agrochemical. Such an assessment has to be concerned with the behaviour of the parent molecule *and* all its toxicologically relevant metabolites. It is therefore essential to apply stepwise testing schemes which include data on degradates as early as possible in the development program of a product.

The present paper proposes a strategy for testing the fate of agrochemicals in soil and in plants using radiolabelled confined field studies as a means of generating integrated field data on soil dissipation, soil/plant metabolism and plant bioavailability of soil residues. These data may be used for the validation of laboratory results (first experimental tier) and to decide whether or not long term field studies should be initiated for a candidate agrochemical (last experimental tier) within the environmental testing program. They represent the first ^{14}C-data produced under practical field conditions according to recommended use patterns and integrate all the processes occurring in the plant /soil system.

2. CONCEPT AND OBJECTIVES

Assessment of the environmental impact of an agrochemical is not only concerned with the dissipation of the parent molecule in the various environmental compartments but also with the fate of its metabolites. The designation "readily degradable" should therefore be interpreted as efficiently transformed into CO_2 or into natural constituents. Experience has shown that mineralization often occurs slowly compared to the rate of disappearance of the parent compound itself and consequently that the contribution of the degradates to the overall environmental impact of the individual agrochemical may be relevant, in any case has to be evaluated. Furthermore, chemical structures of agrochemicals are becoming more and more complex, often representing multiple ring systems. The fate of each part of the molecule has to be followed, a task which often reveals complex patterns of metabolites.

The stepwise approaches proposed, so far, often concentrate at the beginning of the testing scheme, on the environmental fate of the parent molecule alone, for which the major physico-chemical properties needed for model simulations (usually first testing steps) are known or readily determinable. Only at a later stage, i.e. once metabolic pathways are known, can a similar program be initiated for selected metabolites.

Considering the complexity of metabolite patterns often observed in the various environmental compartments, the overall testing programs for new agrochemicals are becoming protracted. There is therefore a need for a test protocol which allows one to monitor simultaneously the behaviour of all relevant constituents at an early stage of the testing program.

Extensive programs for characterizing the fate of agrochemicals in plants, soil, water, and air are being applied to fulfill registration requirements. Those studies are usually performed for each compartment separately. However, the fate of a compound in one compartment is often drastically influenced by its fate in the other compartments. Typical examples are: the dependence of the nature of residues of a pesticide in plants upon its fate in soil or its photolytic and hydrolytic stabilities on the leaf surface; influence of plant rhizosphere microflora on the degradation in soil, etc. Such specific interactions often explain qualitative or quantitative differences observed between laboratory and field experiments.

It is therefore of interest to generate environmental fate data which take into account these interactive effects.

The present proposal to perform integrated soil/plant metabolism studies as radiolabelled confined field experiments under practical conditions aims at satisfying those needs and represents the most efficient approach to generate data on:

- the fate of a test substance in target crops
- the bioavailability of the test substance and its metabolites in representative rotational crops under good agricultural practices
- the fate of the same chemical in unperturbed soil with respect to persistence, degradation and mobility of parent compound and metabolites.

Furthermore, this data package should suffice to decide whether multisite field accumulation studies on rotational crops or long term field dissipation studies have to be initiated. The limitations of the proposed testing program as well as possible alternatives are discussed.

3. EXPERIMENTAL REQUIREMENTS

3.1. Fate in the plant

3.1.1. Target crops The environmental impact of an agrochemical after its application on plants is primarily determined by two parameters: its dissipation from the leaf surface by volatilization, chemical degradation or run-off and its penetration into plant tissues. Factors influencing the dissipation are related to the intrinsic physico-chemical characteristics of the molecule i.e. vapour pressure, hydrolytic and photolytic stability, water solubility etc. The overall behaviour, however, can be drastically influenced by the type of formulation used. Formulations are usually developed to minimize dissipation and abiotic degradation and to optimize penetration. Realistic data on dissipation and penetration should therefore be generated by using formulated test materials.

The fate of the active ingredient once penetrated into the plant/soil system is the major concern of the metabolism chemist. It involves evaluation of the rate of degradation as well as transport of the parent compound and its metabolites within the test system. For this purpose, the nature and amount of radiolabelled residues in various plant parts and soil are determined at various time intervals.

Nature and amount of residues in raw agricultural commodities at plant maturity allow an evaluation of the dietary exposure of consumers to the particular agrochemical. Metabolism studies with radiolabelled materials are principally concerned with the qualitative aspect of the analyses, i.e. the determination of the structure of all relevant metabolites, namely those which are addressed as the total toxic residue. Quantification of the total toxic residue is then performed on a broad scale through supervised residue trials under various climatic conditions using optimized and validated residue methods. Radiolabelled metabolism studies, however, when designed as confined field studies, can fulfil most criteria of a residue trial, provided that the radiolabelled test chemical is applied as formulated material at the recommended use rate, and that the analytical results obtained are statistically sound. Under these circumstances realistic values for total residues in raw agricultural commodities are obtained. These results can well serve as trigger to define a negligible residue situation, preventing the development of sophisticated, time-consuming residue methods (cf. Fig. 2) and consequently substantially reducing the "residue analysis" program.

Radiolabelled plant metabolism studies performed in the field according to recommended use pattern produce, at least in the case of new, highly active agrochemicals, low levels of residues in plants, often below 200 ppb (Note a). Such quantities limit the ability to identify the constituents, especially in cases of complex metabolite patterns, and necessitate the use of stem injection techniques or indirect methods like excised leaves, leaf discs or tissue culture techniques (ref. 2). Such studies can be initiated as needed in the course of the metabolism program and are usually efficient at producing sufficient amounts of ^{14}C-metabolites. They can only be used, however, for qualitative purposes, as they do not reflect the metabolite distribution in raw agricultural commodities of whole plants, due in part to the lack of compartmentalization. Determination of the quantitative metabolite pattern therefore has to be performed on whole plant samples obtained preferentially from trials performed with formulated ^{14}C-test material at the recommended use rate, which avoids any significant phytotoxic effect on the target crop.

3.1.2. Rotational crops The presence of pesticide residues in rotational crops represent another potential source of exposure for consumers. Therefore, the bioavailability of pesticide soil residues to selected crops has to be assessed. In choosing a testing scheme, two facts must be considered:

a) The level and nature of residues in rotational crops often depend upon the aging period of the pesticide in soil, due to the fact that the soil metabolites formed possess their own properties with regard to bioavailability and fate in the plant. Experience has shown that the nature of residues in rotational crops at maturity might be quite different compared to target crops, due to selective root uptake of soil metabolites. Typical examples are the fungicides of the triazole family (propiconazole, penconazole, triadimenol, triadimefon, etc.) which are degraded by soil microorganisms with the concomitant release of the systemic metabolite triazole; similarly various acetamide type fungicides which undergo N-monodealkylation preferentially in soil are further degraded to the corresponding aniline derivatives in the plant (ref. 3, 4).

Note a: ppb: part per billion (10^{-9}) as mass fraction
ppm: part per million (10^{-6}) as mass fraction

b) The amount and nature of residues in rotational crops might be species dependent. Such differences reflect the biological and/or biochemical crop selectivities with respect to root uptake, transport and metabolism.

As a consequence reliable quantitative and qualitative data on rotational crop residues can only be obtained by respecting normal agricultural practices, i.e. representative aging periods and choice of crops. Accumulation factors can not be adequately predicted by short term laboratory experiments (ref. 5)

Choice of crops: In agricultural practice, the choice of the crop succession is influenced by many factors, among them: economical importance of the crop and its yield, the structure of the farm (the presence of farm animals predestine feed crops to be incorporated in the rotation), kind of predators and weeds to control, type of soil, erosion problems and weather patterns. Crop rotation programs, therefore, are country or even region specific.

However, in the case of confined rotational crop field studies with radiolabelled test material, a standardization of the rotational schedule is desired. To acquire some worldwide representativity, it should comprise one major crop of at least three crop groupings (ref. 6): root crop, small grain and leafy or legume vegetables. Planting time of the rotational crops should approximate the anticipated agricultural practice, i.e. harvest year of the target crop for leafy vegetables and winter cereals, the following year for root crops, corn or soybeans. Representative post-application intervals would be 120 days and 1 year, respectively. An additional interval of 30 days may be included in order to assess circumstances of crop failure.

3.2. Fate in soil

The environmental impact of pesticide residues in soil depends on their bioavailability to plants and soil microorganisms, on their rate of dissipation into the air and ground water and as a consequence on their bioavailability to aquatic organisms. Model calculations and laboratory experiments permit first estimates of leaching potential, volatilization, abiotic degradation and bioavailability of a given agrochemical. These estimations are very useful as they allow a comparison of the behaviour of various pesticides under standard conditions to be made. The validation of those results under field conditions is needed in order to take into account interdependent effects like seasonal fluctuations (light intensity, temperature profiles, water movement), time and rate of application, formulation, rate of degradation, microbial activity, etc. It is often observed that the disappearance rate of an agrochemical in soil is higher in the field compared to the laboratory experiment. It can be due to faster dissipation from soil surface (volatility, photodegradation) or faster biodegradation (regular supply with energy and nutrients). The most striking discrepancies between laboratory and field experiments were observed in the case of photodegradable test substances (ref. 7).

Model calculations based on physico-chemical properties of the chemical are limited to known structures, i.e. to the parent molecule, exclusively, before soil metabolism data are available. Experimental data on the environmental behaviour of metabolites are available only if test programs allow monitoring of all relevant parameters (leaching, volatility, rate of degradation) from the time of application. This is possible by using ^{14}C-confined field studies to generate relevant soil dissipation data.

Soil represents the environmental compartment where most pesticide long term behaviour (most often related to persistence and ground-water contamination) originates. To evaluate the relevance of such effects, regulatory agencies require field dissipation studies to be performed at different testing sites, using unlabelled, typical end use products. Parameters to be evaluated from these studies are mobility, degradation and dissipation of residues under practical field conditions. It seems obvious that radiolabelled confined field studies can achieve these objectives, provided that a sound statistical analysis of the residue data can be done. The presence of the radiolabel permits the behaviour of all major constituents to be monitored. Furthermore, the studies can be prolonged, or used for repeated applications if required. In any case they represent a solid basis for triggering field accumulation studies or long term dissipation studies with unlabelled products.

4. EXPERIMENTAL DESIGN

4.1. Field plot

4.1.1. Location and safety measures Field studies, as compared to indoor experiments, bear additional risks inherent to the field situation. Continuous surveillance of the plots is certainly of first importance in order to initiate prompt corrective action against extreme weather conditions (hail, frost, storm or

drought), predators (surrounding the plot with a fence and installing a net against birds) and, when necessary, diseases and insects (monitoring the first symptoms followed by careful treatments).

Radiolabelled field studies must be performed in full compliance with the local governmental regulations. These usually require a balance of the radioactivity applied over the year including total activity in the harvested samples, total activity remaining in the field, total activity lost by drift (difference) and in the drainage water. Furthermore the trials must be performed in restricted area open only to appointed personal. For security reasons, the restricted area is best located at a company or governmental research station. The size of the area should be large enough to rotate the plots over a suitable period (four to six years) to allow the soil radioactivity to reach background. In the meantime the plots can be used for biological trials. In any case they should be cultivated to favour soil decontamination. In certain cases (high persistence of the test chemical and high rate of applications) it may be necessary to remove the contaminated upper soil layer and dispose of it according to local regulations.

4.1.2. Plot arrangement The size of a treatment plot is limited due to the use of radiolabelled material. It will depend on the number of sampling intervals anticipated and on the crops to be grown. Crop yields should cover the needs for the metabolism study *and* for validation of the residue analysis method(s). The number of soil cores which can be taken at each sampling interval is restricted compared to typical residue trials. However they must be sufficient to allow generation of statistically valid residue data.

As a consequence optimal application conditions must be met to achieve even distribution of the test chemical. This is most easily obtained by bare ground application. Plot arrangement will therefore vary, whether the radiolabelled material is applied preemergent or postemergent. Possible plot arrangements are shown in Fig. 1. Plot sizes of 4-6 m^2 for the target crop and 4-6 m^2 for rotational crop and soil dissipation studies were used successfully. By preemergent application, the target plot is divided after harvest into 4 sections, each one being used to grow one rotational crop. Soil samples can be taken over the target plot (version A) or from treated zones around the target plot (version B). In the case of postemergent spray applications, a separate plot is best reserved for the rotational crop/field dissipation study. Two different geometrical arrangements (version A and B) are shown in Fig. 1. To maintain optimal rhizosphere microflora activity it is advisable to cultivate the rotational plot during the aging time of the soil applied pesticide.

For standard field dissipation studies, regulatory agencies usually require locations which are representative of the area where the pesticide is intended to be used. In the case of radiolabelled confined studies, the choice of the site is relatively limited as the trials have to be performed in a controlled, restricted area. An average sandy loam is best chosen as a representative soil type.

4.2. Treatment

The analytically pure radiolabelled test substance is applied as formulated material at the recommended use rate and timing. Final formulations should be used when available. If not, representative EC or WP formulations may be used for spray treatments.

The use of radiolabelled test materials restrict the number of application in the case of multiple application programs to no more than 3-4 treatments. Timing for the first and last treatments should preferentially correspond to the recommended schedule and accommodate the proposed pre-harvest interval. The total rate of application (g a.i./ha) should equal the anticipated dose.

Target plot and rotational plot are treated simultaneously. Before application it is advisable to till and level the upper (3-5 cm) soil layer to favour uniform distribution of the test compound. In the case of preemergent applications, the same plot can be used for the target and rotational crop trials.

Rotational crops are planted on bare ground treated soil according to the schedule dictated by normal agricultural practices. The maximum recommended use rate should be applied even in the case of postemergent target application simulating complete transfer of the agrochemical to the ground.

A substantial portion of the radiolabelled compound may be lost by drift during spray application. It is essential to prevent contamination of the surroundings by erecting plastic walls around the treatment area and by choosing windless conditions. Whenever possible, the application should be performed during relatively stable weather conditions to avoid rainfall during the first 12-24 hours after spray treatment and consequently rinse of the deposit.

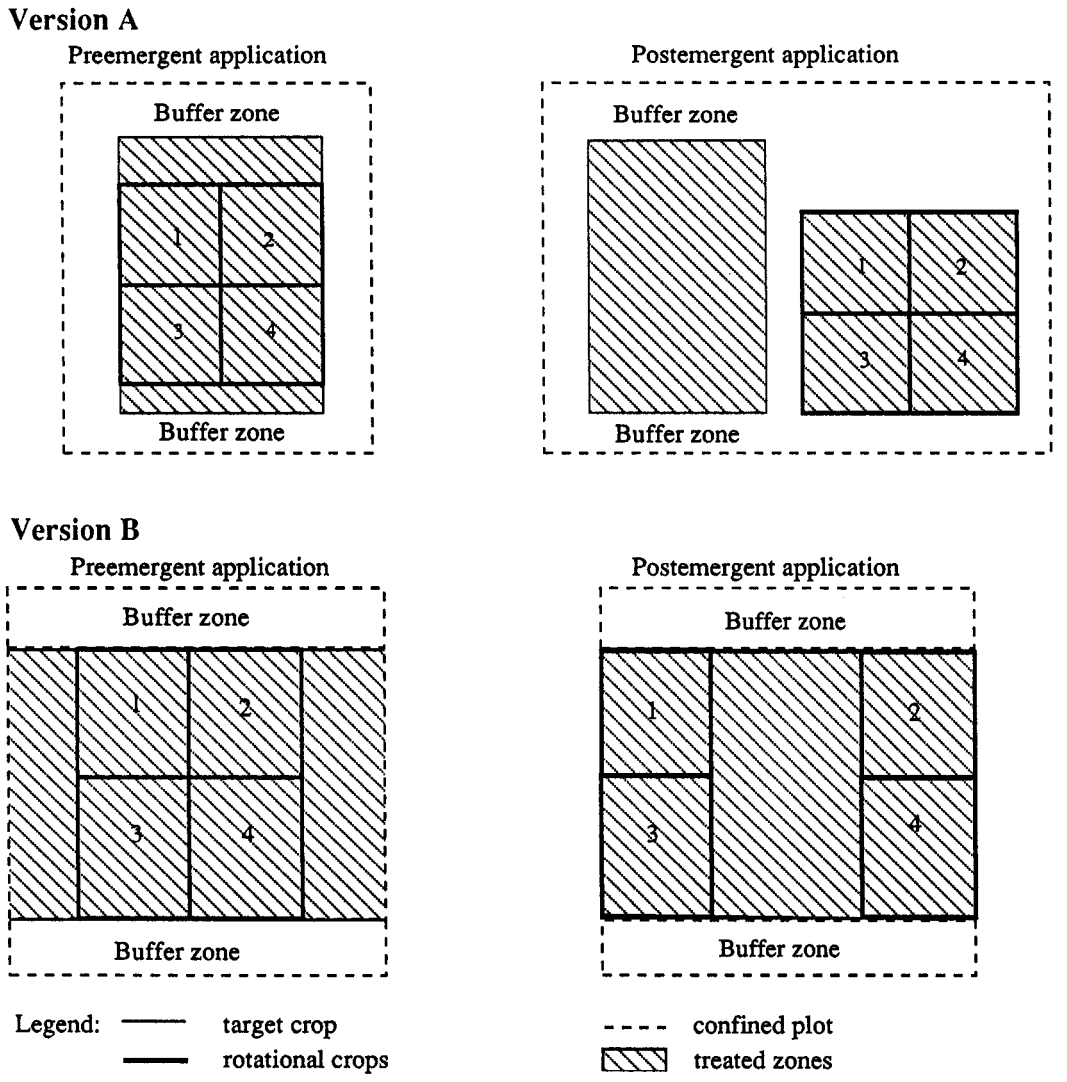


Fig. 1. Possible plot arrangements

4.3. Sampling

4.3.1. Plant samples The minimum number of sampling intervals is dictated for each crop by the normal agricultural practices, i.e. each raw agricultural commodity (food or feed) has to be harvested (Ref. 12). Additional samples are taken just after application in order to determine the initial residue concentration in plant and soil. If the experiment is used to determine the short term behaviour of the formulated ^{14}C-material (root uptake, leaf penetration, surface dissipation, systemicity) additional samples are taken as needed.

Sampling of the rotational crops is usually performed at regular time intervals up to maturity (2 for lettuce to maximum 4 for cereals) for adequate monitoring of uptake and selective transport of aged residues into grains or tubers.

4.3.2. Soil samples In general, soil samples are taken at each crop sampling. Furthermore probes are taken prior to the application (controls), just after application (initial ^{14}C-concentration) and at addional intervals as needed for estimation of the rate of dissipation of the test substance. Typical sampling schedule for field dissipation studies is 0, 24 hours (for photolytically and hydrolytically unstable compounds), 7 days, 30 days, 60 days, 90 days, 180 days, 12 months and 18 months. A minimum of 5 soil cores (ca. 5 cm of diameter) should be taken at each interval to allow valid statistical analysis and reject outlying results if necessary. Soil probes should be taken to a depth sufficient to determine the extent of leaching, i.e. the last layer analysed should be free of ^{14}C-residues.

4.4. Analysis and evaluation of data

Plant samples (divided into representative plant parts) and soil samples (divided into various layers: typically 0-5 cm, 5-10 cm, 10-20 cm, 20-30 cm,tc.) are analysed for their total ^{14}C-residues, quantified as parent compound, metabolites and non-extractables. The level of parent compound and the metabolite profile in plant and soil extracts are analytically compared at each sampling interval to detect significant migration of individual soil metabolites into the plant or adjacent soil layers. Characterization/identification work is undertaken indifferently in plant or soil extracts, according to the amount and concentration of the ^{14}C-constituents available. The same laboratory samples can be used for validation of the residue analysis methods.

The statistical significance of the ^{14}C-balance data strongly depends on the pesticide distribution reached during application. It is therefore important to evaluate the initial ^{14}C-content over the whole area treated by sampling the soil surface just after application. Sampling and analytical techniques should be designed to allow estimation of the variance of the concentration data. The major limitation of radiolabelled confined field studies compared to supervised residue trials results in the reduced field sample size. However, the distribution of the test material during application can be better controlled compared to larger field trials by using a rigid frame to support the spraying device over the whole plot. Sampling is best performed systematically at regular intervals in the spraying direction, avoiding the extremities of the plot.

It is worthwhile to note that in the case of ^{14}C-confined studies representative laboratory samples are obtained by randomization of the whole field sample, thus avoiding any loss of sample validity as a result of sample reduction.

5. ALTERNATIVES

5.1. Introduction

Radiolabelled confined field studies, as described, are performed under conditions which are representative of agricultural practice, i.e.:

- plants and soil are subjected to typical weather conditions and seasonal fluctuations;
- plants can develop their root system freely, according to water and nutrients needs;
- in a typical field arrangement the spaces between the confined plots are composed of the same soil type as the plots themselves, and therefore no "edge effects" are to be expected. This is quite important with respect to water movements and consequently soil mobility of the test substance and its metabolites. Also the root system is not perturbed by physical obstacles.
- Treatment of a relatively large surface under field conditions with commercially used nozzles using recommended spraying volumes allows a reliable estimation of the per hectare rate of application.

Moreover the size of the test plot can be chosen according to the specific needs of the study.

Field trials with radiolabelled test substances are not allowed, however, in every country. Therefore alternative solutions must be found which comply with the legislation prevailing in those countries.

5.2. Lysimeter trials

Lysimeters (ref. 8, 9) are designed to monitor the leaching characteristics of a test substance and its metabolites and to evaluate the probability of groundwater contamination. Lysimeters are cylinders or polygons of concrete, between 60 and 100 cm wide and usually 100 cm deep, filled with selected soil monoliths. They are placed in a pit in such a way that the surface of the soil is at the same level as the surrounding ground. A drainage system at the bottom allows collection of the leachate. To maintain the soil as close as possible to field conditions, plants are usually grown during the testing period. Such an arrangement is similar to a miniaturized confined plot.

The major limitations of lysimeters in the generation of plant/soil metabolism data are:

- Size: compared to a typical field plot the treated surface is about 4-6 times smaller. In order to produce enough raw agricultural commodities to study metabolism and validate the analytical residue methods, several lysimeters, in most cases, would have to be treated with the radiolabelled test substance.
- Edge effects which are more pronounced in lysimeters of small size (<100 cm wide) can influence root growth and water movement.

- Difficulties in extrapolating data to a per-hectare basis.
- A rational use of lysimeters necessitates replacement of the soil for each experiment.
- High investment costs.

Those limitations show that lysimeters are not well suited to accomplish the full integrated field program described for the confined field plots.

5.3. Container trials

A similar approach to lysimeter studies is the use of plant containers located in a vegetation hall under outdoor conditions. This test design represents a situation intermediary between typical field and laboratory conditions. Container trials are generally used to achieve the following objectives:

- Determine the level and nature of residues in intact plants grown under outdoor climatic conditions until maturity.
- Measure the translocation processes within the plant.
- Monitor the uptake of aged residues from soil in rotational crops.
- Evaluate the volatilization of the active ingredient from leaf surfaces.
- Production of terminal residues in plant parts which can be used to validate the routine residue analytical methods.
- Follow the degradation kinetics of the active ingredient and its metabolites in plant and soil under outdoor climatic conditions.

Stainless steel containers with the dimensions 0.7 m x 1 m and 0.5 m depth were used for this purpose. A testing area smaller than 0.25 m^2 was found to have influence on the plant growth behaviour and uptake of soil residues into plants. The container is equipped with a 10 cm broad subirrigation and leachate sampling chamber formed by a double wall on one side of the container which is in contact with the gravel layer at the bottom. The container is filled first with a 5 cm gravel layer, then with a coarsely sieved soil to a hight of 5 cm below the upper container edge. The soil is usually aged for approximately 2 months. It is to note that the resulting soil structure does not represent an undisturbed soil core taken from an intact field and *cannot* be used to generate valid leaching data.

The crops under investigation are cultivated in the containers. After treatment of the plants or bare soil with the radiolabelled material the containers are moved to a vegetation hall. This is an area completely fenced in with a small meshed wire fence, in which outdoor conditions prevail.

Part of the vegetation hall is covered by a glass roof. Moving the containers into this area simulates outdoor conditions under rain, hail or snow protection. In order to overcome extreme winter climatic conditions the use of a heating jacket may be necessary.

After harvest of the mature plants and completion of the trials the containers are emptied, decontaminated and the soil subjected to controlled waste management.

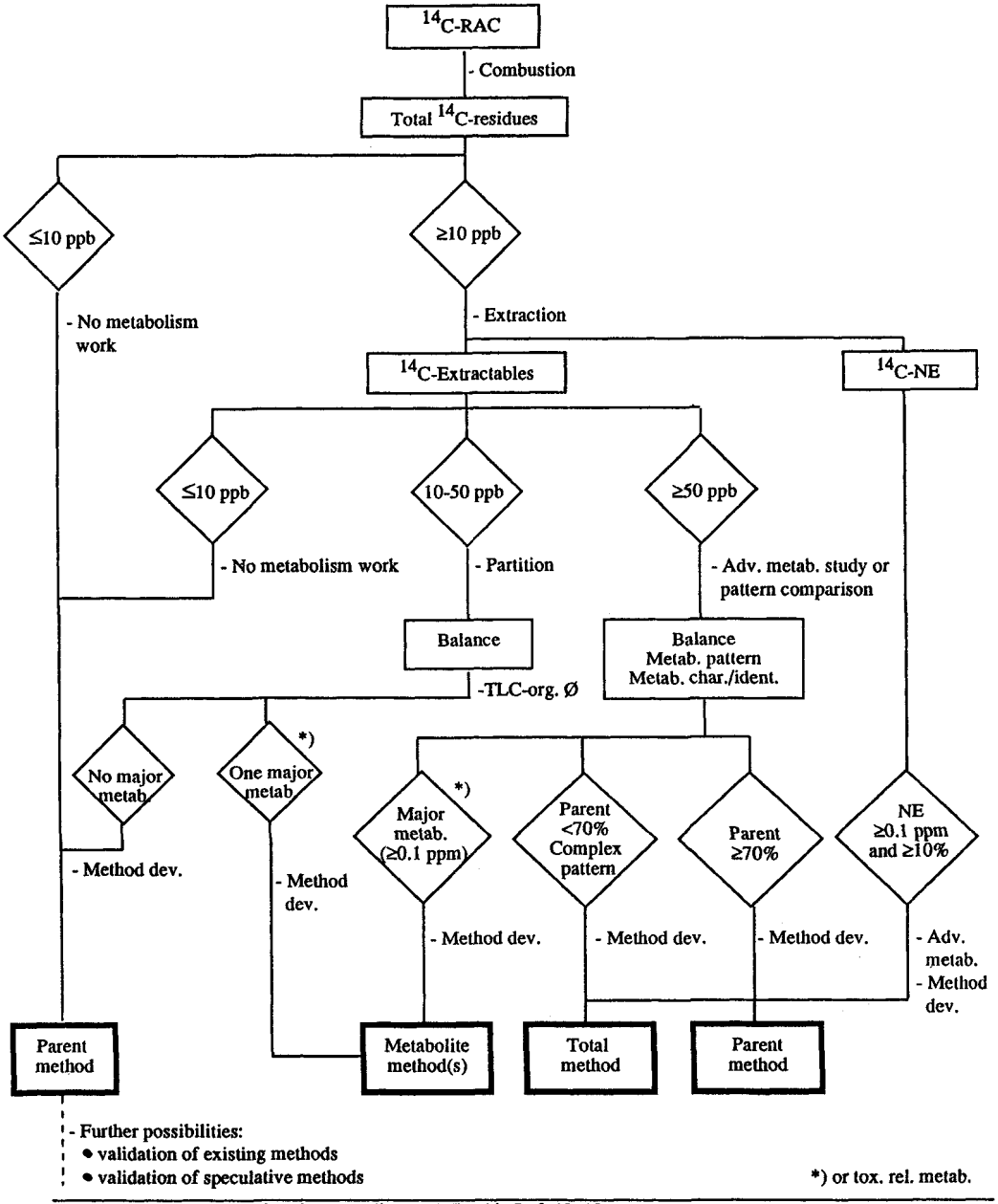
The container trials when compared with radiolabelled confined field studies, show some advantages with respect to reproducibility, flexibility, and adaptability of the test conditions. They do not, however, reproduce true field conditions and have limitations concerning sample size. As a consequence container trials can not be used to generate valid data on the dissipation of pesticide residues in soil.

6. EXTENT OF METABOLISM STUDIES

6.1. Strategy

Critical factors in evaluating the extent of metabolism studies are the limit of detection of the analytical methods (^{14}C-measurements) and the minimum amounts of a single metabolite required for uniquoval spectroscopic analysis. Performances of analytical and spectroscopic methods for characterization and structure determination of metabolites improve with time, i.e. significant advances were achieved over the last decade. Experience shows that at present a minimum residue level of about 50 ppb extractable radioactivity in the raw agricultural commodity (RAC) is necessary in order to obtain resolved metabolite patterns with a 1% limit of detection. This estimation is based on a specific activity of the applied agrochemical of about 50 µCi/mg. For isolation and structure determination (i.e. 10 metabolites, 20-50 µg each) a minimum concentration of about 0.5-1 ppm extractable residues is required. Below this range, indirect methods like stem injection, cell tissue cultures or excised leaf techniques are recommended. It

should be noted that the results obtained with these indirect methods do not reflect the normal distribution of metabolite in RAC's, and consequently are unsuitable for validation of residue methods. Therefore, below the threshold value for the resolution of metabolite pattern (set at 50 ppb), the amount of information which will be gained for the design of a residue method will be limited. A global characterization of the total ^{14}C-residues still remains possible in general: it consists of a quantification of the extractable and non-extractable radioactivity, determination of the partitioning behaviour of the extractable portion and TLC analysis of the organic phase. A domain between 10 ppb and 50 ppb residues in RAC is proposed to trigger this analytical program. Below 10 ppb no differentiation of the radioactivity is attempted in standard cases. A schematic representation of this strategy and its consequences with respect to the development of residue analysis methods is shown in Fig. 2. The scheme is valid only for studies performed under practical application conditions which reflect good agricultural practice.



RAC: raw agricultural commodity, as defined in Ref. 10, 12
NE: non-extractable residues
ppb: part per billion (10^{-9}) as mass fraction
ppm: part per million (10^{-6}) as mass fraction

Fig. 2. Decision tree for the determination of extent of metabolism studies and development of residue methods

This evaluation should be performed for each individual RAC (Ref. 10) of a particular crop. If a "negligible residue" situation is found in wheat grains, for instance, information on metabolism pathways in wheat will be usually available from straw and forage analysis. Exaggerated rates might be used to obtain information on the pattern of metabolites. Application rates, however, can only be chosen in the non-phytotoxic range of the particular compound.

Radiolabelled residue levels are mostly dependent upon the position of the label. The "negligible residue" definition (10 ppb) only holds for cases where the label adequately allows the detection of terminal residues (label positions at the most stable part of the molecule). If necessary, more than one labelled form has to be used.

The accountability of a total residue method is often lowered by the presence of non-extractable residues. In the most favorable case they can be characterized as natural compounds and be substracted from the total toxic residues, or they are found to have structures or be transformed into structures already identified in the extracts. A threshold of 0.1 ppm (or 10% of total ^{14}C-residues) has been proposed by regulatory agencies (Ref. 11) to trigger characterization of non-extractable residues.

After having properly identified the individual metabolites the total toxic residue has to be defined.

6.2. Choice of residue method

According to the trigger values discussed in the previous chapter, three different residue situations are defined, namely: ≥50 ppb (a), 10-50 ppb (b), and ≤10 ppb (c) (cf. Fig. 2).

In situation (a) it is expected that the required quantitative and qualitative metabolism data will be available. The choice will then depend essentially upon the nature and complexity of the metabolite pattern and relative abundance of its components. A parent method is developed practically in every case, as it is needed for the control of good agricultural practices, i.e. to detect misuses. A total method is usually developed for a complex pattern of about equal distribution of metabolites. Metabolite methods are being increasingly required by the registration authorities and should be developed for each metabolite present at 0.1 ppm level or having toxicologal significance.

In situations (b) and (c), the parent method and/or the residue method developed for other RAC's may be appropriate. If during characterization of the extractable radioactivity (case (b)) one major metabolite is singled out, a metabolite method should be developed. Accountabilities will in any case be clearly established by method validation using radioactive aged residues.

The use of trigger values to define the extent of metabolism studies does allow a significant reduction of the work load, especially in the case of low residue situations. In order to realize those benefits, however, this strategy should be generally applied to all cases where no critical toxicological issue is expected. These trigger values were set according to the actual limitations of the methodologies in use and in view of existing national registration requirements.

7. CONCLUSIONS AND RECOMMENDATIONS

Metabolism studies with agrochemicals aim at providing reliable evaluation of their environmental fate. No single experimental design will provide all the information needed. Field experiments with radiolabelled materials, for instance, do not deliver a total balance sheet of radioactivity and consequently are inappropriate to determine the nature of volatile components and often not designed to quantify short term effects like leaf penetration, root uptake and translocation. Those parameters are usually determined in complementary indoor systems (greenhouses, growth chambers and closed laboratory system) specifically conceived to delineate single effects (Ref. 2). Comparative studies between plant species, soil types or environmental parameters, for instance, which require controlled experimental conditions are of course best performed indoor. Moreover, residues in field samples are often too low to be isolated and spectroscopically identified. For this purpose indirect *in vitro* methods are available (Ref. 13, 14).

Field studies conducted in accordance with normal agricultural practice using formulated test substances at recommended use rates can best produce data with practical relevance on the amounts and distribution of metabolites within the plant/soil system and when necessary over a long period of time. They confer to the metabolism studies (which principally are concerned with the nature of residues) their quantitative significance. As a consequence they allow the chemist to choose the most adequate analytical residue methods and to identify negligible residue situations.

Radiolabelled confined field studies designed to generate integrated data on soil/plant metabolism, dissipation and bioavailability of agrochemicals, represent a significant reduction of work load and overall testing time, compared to individual field studies concerned with the dissipation of the test substance in soil, in the target crop or its uptake in rotational crops.

Moreover radiolabelled confined field studies offer an optimal source of samples to validate laboratory data. Especially in the case of soil dissipation, adsorption/desorption and leaching data the use of the same soil for model and field experiments may eliminate various sources of discrepancy between the two data sets.

In summary, the full data package obtainable from an approx. 18 months lasting radiolabelled confined field study describes

- the parent and metabolite profiles in the target crop at representative growth stages up to maturity
- the carry-over factors (bioavailability) of the parent compound and relevant soil metabolites in 3-4 different rotational crops at representative aging times and growth stages
- the identity of the pesticide residues in the target crop, rotational crops and in soil leading to a comprehensive description of the degradation pathways in plant and soil
- the dissipation of the parent compound and selected metabolites in soil (DT_{50}, DT_{90})
- the downward migration of the parent and its metabolites in the test soil.

One such radiolabelled confined field study, therefore, should substitute one standard field dissipation study and one field accumulation study on rotational crops if it is carried out under conditions, relevant for the intended use.

8. REFERENCES

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