GUIDELINES ON

PESTICIDE RESIDUE TRIALS TO PROVIDE DATA FOR THE REGISTRATION OF PESTICIDES AND THE ESTABLISHMENT OF MAXIMUM RESIDUE

FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS

Guidelines on Pesticide Residue Trials to provide Data for the Registration of Pesticides and the Establishment of Maximum Residue Limits

FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS
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FORWARD

Adequate data from properly conducted trials involving agricultural plants or farm animals intended for the production of food are needed to establish the parameters of "good agricultural practices in the use of pesticides". The levels of residues of pesticides remaining unavoidably in food products entering trade following such practices, form the basis for setting maximum residue limits. It is important that the results of residue analysis in supervised trials relate to those chemical species in the residue which are relevant for the purpose of setting maximum residue limits. It is equally important that the food products examined, as well as portions thereof examined, be relevant to the commodity moving in trade. Other aspects should also be borne in mind during pesticide residue trials, which have a bearing on the usefulness of the residue data used in setting maximum residue limits.

The Guidelines contained in this publication were elaborated by the Codex Committee on Pesticide Residues through its Working Group on Development of Residues Data and Sampling. Part I of these Guidelines was part of a more comprehensive document "FAO Guidelines on Crop Residue Data for Pesticides" presented to the Fifth Meeting of the Group on Pesticide Registration Requirements of the FAO Panel of Experts on Pesticide Specifications, Registration Requirements and Application Standards (Rome, December 1984). The FAO Guidelines were largely based on material developed by the IUPAC Commission of Pesticide Chemistry. Part II of these Guidelines was elaborated by the CCPR's Working Group at its 1986 Session. The valuable contribution of the Chairman of the Working Group is gratefully acknowledged.

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PART I - PLANTS AND PLANT PRODUCTS

I. INTRODUCTION

The use of a pesticide on crops or commodities for human or animal consumption can lead to, and occasionally aims at, a residue remaining at harvest or other appropriate stage. Additionally a pesticide may move from the site of application and remain for a time elsewhere in the environment.

The ability of a pesticide to persist for a certain length of time can be desirable and has been recognized as important in some situations for successful control of pests and diseases. Thus a knowledge of residues of a pesticide, or arising from the use of pesticide, is useful in establishing its efficacy. However, the assessment of the human hazards arising from very small quantities of a pesticide in food and the environment has become an important part of the overall risk/benefit evaluation and is essential before a pesticide can be introduced.

One of the basic prerequisites of such assessment is the availability of reliable data on pesticide residues in food, feed and the environment so that a realistic estimate can be made of the human exposure. The increasing demands of national registration and health authorities include residue data on treated crops and commodities and additionally in water, soil, air and wildlife. These authorities will only reach conclusions and make decisions if they are satisfied that the data are reliable.

However, variations in methods and techniques used in obtaining these data, including the selection, preparation and analysis of samples, have made it difficult to compare results and decide if the results are valid. Secondly the validity of a set of results depends primarily on an adequate design of the trial. These variations have made it difficult to compare information from different sources and have contributed to differences in the regulations adopted in different countries.

These difficulties are most apparent when considering the conclusions reached by national authorities during the registration of pesticides and the use of residue data to set and enforce legal maximum residue limits for pesticides in food and feed. These limits have become important in the movement of food and feed commodities in international trade and the harmonization of the methods used in the production of residue data. A more uniform approach to evaluating the data is also urgently needed.

Guidance on the many aspects of producing and evaluating residue data is desirable. It will be of particular value to those countries still in the process of initiating procedures for the official control of pesticides. The need for guidance has been recognized by a number of national and international organizations and committees and several are already making contributions.

These guidelines have been developed because of an urgent need to improve and harmonize the procedures for obtaining residue data for proposing and enforcing maximum residue limits of pesticides in food. Much of the advice, however, is relevant to, and may be adapted for, other types of residue data including environmental samples. Care must be taken to ensure that adaptation is done carefully and selectively since objectives will be different in certain situations. In monitoring environmental samples, for example, the scope and aims of the monitoring, the levels investigated and need for confirmation of "detected" pesticides can introduce other, overriding considerations.

Metabolism of the pesticide in the plant or soil has a major influence on the identity of the residues which need to be measured. Metabolism studies are important pre-requisites of supervised field trials. The term "metabolite" is not a suitable description for all the compounds or degradation products resulting from the parent pesticide but is often used. Consideration must be given to which of these need to be determined by the analytical procedure and which then should be included in the "total residue". A special problem in metabolic studies and in the measurement of pesticide residues is created by "bound" and "conjugated" residues. Any statement to the effect that residues are "bound" (in a substrate or matrix) must necessarily be a function of the effort taken to "free" them, that is the efficacy of the extraction procedure in an analytical context. Thus the term "non-extractable residue" is preferred to "bound residue".

The initial reason for carrying out supervised residue studies is to assist the evaluation, during registration, of the safety and efficacy of the product. In some countries another important reason for carrying out these studies is to obtain the data for establishing maximum limits for residues of the pesticide in food or agricultural commodities. Usually the same data serve for registration purposes and for the estimation of maximum residue levels on which the legal limits are based.

In designing residue trials, early consideration must be given to the intended use of the residue data and to the sampling programme required. If the data are to support registrations or establishment of a maximum residue limit through registration procedures, results from a number of replicated experiments in several soil and climatic conditions are normally required. Major trials should only be done with commercial formulations and equipment in a manner similar to that used by farmers. Treatments should be made at the rate recommended or likely to be recommended for the commercial product. A treatment at twice or three times the recommended rate should be included. Data from such a treatment would indicate what might happen if users deliberately or accidentally apply quantities greater than those recommended. Since supervised residue trials provide the basis for legal maximum residue limits in some countries, the design of the experiment should include the determination and evaluation of the conditions and factors which lead to the highest residue levels following recommended use patterns.

Residue data from supervised trials carried out in conformity with registered or approved use patterns, defined as "good agricultural practice", are the main source of data.

The uses of any compound for pest control on a particular crop vary considerably from region to region, owing to difference in ecology, climate and cultural practices: consequently residue levels at harvest will vary. As far as possible, agricultural practices in all regions from which data are received and the pesticide residues likely to result from these practices are considered when estimating maximum residue levels. These estimates are based on normal agricultural practices in the regions where there is a need to use the pesticide. If the requirements of certain regions justify multiple applications or applications shortly before harvest, consideration should be given to these needs and recommended levels should not necessarily interfere with pest control practices.

The importance of careful sampling in the field by trained personnel cannot be over-emphasized. The best approach for any given situation can be best determined by someone who is capable of recognizing and interpreting the importance and usefulness of the results.

It is necessary to take samples which, when reduced and analysed, will give residue results which will both represent the average residue levels of the entire crop in the plot and indicate the range of residues found. The field sample must be representative of the plot and the individual units

comprising it must be typical of those taken in a commercial harvest. It is very important that residue data obtained from field samples should be comparable with data obtained following sampling procedures used in enforcing maximum residue limits.

Adequate sampling of the untreated (control) plot is also important, especially if the residue level of the treated crop is expected to be low. Arrangements need to be made in advance if the samples have to be stored for any length of time, or as frequently happens a sample is transported elsewhere for analysis.

Residues remaining on or in the crop commodity depend on many interacting influences of varying importance including growth dilution, ratio of crop surface to mass, volatility of deposit and degree of absorption on to and absorption into surface layers. The residue resulting from a given method, timing and dosage of pesticide application will also vary with site and climate and the limits of such variation are important to the assessment of safety and particularly to the establishment of maximum residue limits. In order to obtain the necessary data to estimate a maximum level, crop commodities should be analysed from crops with known pesticide treatment, reflecting good agricultural practice and grown under a representative range of agricultural and climatic conditions. Factors which may influence the disappearance of residues should be recorded. Thus, the procedures outlined in these guidelines refer to "supervised trials" which have served for many years as the primary source of residue information for the registration of pesticides and for setting maximum residue limits.

Because of legal and commercial implications such trials must be carefully planned, conscientiously executed, carefully evaluated and intelligently interpreted to ensure that the decisions taken are meaningful and that they reflect the practical situation resulting from approved uses of the chemical.

Cooperation between scientists of several disciplines is usually necessary to achieve the desired result and careful consideration must be given to all the factors and their variability. For example, if the crop sample is not truly representative of the material from which it is obtained, all the careful and costly work put into the subsequent analysis will be wasted. An erroneous result is worse than none at all. The analyst's residue data may be precisely determined but the results can be inaccurate because of inadequate field sampling.

Variations in residue trial techniques have contributed to the difficulties in evaluating data relating to the occurrence, disappearance and fate of residues in or on crops and often make it difficult or impossible to compare information from different sources.

Thus there is an urgent need for internationally accepted guidelines on the experimental design, procedures and reporting of supervised trials and the purpose of these Guidelines is:

- to indicate the techniques which should be followed in order to secure valid experimental data appropriate to the above objectives; and

- to promote the establishment of harmonized procedures to facilitate international acceptance of the data obtained.

They refer to the use of pesticides on crops and stored products intended for food for humans or animals. Part 2 covers trials when treated crops are fed to animals or when the pesticide is applied directly to the animal.

2. DESIGN OF RESIDUE TRIALS

In designing a residue trial, early consideration must be given to the intended use of the residue data to be obtained and to the sampling programme and analytical work that this entails. If data are sought to support petitions for establishing a maximum residue limit, results from a number of experiments in several geographical areas or during typical periods of the year and farming practices are often required. When a product is applied to a crop near maturity, studies on residue disappearance with time are usually needed to determine acceptable pre-harvest intervals. Such considerations markedly influence the location of the test plots. The size and number of samples that must be taken from each plot determines the size of the experimental plots.

Major trials should only be done with proposed commercial formulations. It is meaningless to carry out this work with laboratory preparations since the fate of the residue may be influenced by the nature of the formulation. It is preferable to make the application with commercial equipment in a manner analogous to that used by farmers, but the greatest care should be exercised to see that the application is uniform and thorough.

Trials should be designed to cover a range of representative field conditions, typical periods of the year, cropping and farming practices which are commonly encountered. Since climatic conditions have an important influence on the persistence and performance of a chemical, trials should be carried out in those areas where the product is to be finally used.

Whenever possible, and certainly whenever it is considered likely to influence residue levels, trials should be repeated on different varieties, different stages of growth at typical periods of the year and under different agricultural regimes to determine residue levels under various conditions.

Since one of the objectives of residue studies is to provide the basis for the estimation of maximum residue levels, the design of the experiments should be directed to the determination and evaluation of the conditions and factors which lead to the highest residue levels following recommended use patterns. If it is anticipated that the interactions of various factors could produce widely varying residue levels, experiments should be designed to demonstrate the effect of such interactions on residue levels.

Residue trials have to be especially designed in most cases and the presence of a target organism is not necessary. Trials intended for biological evaluation may be suitable for obtaining residue samples if the full range of the recommendations is reflected and if the plot size is large enough to obtain adequately representative samples.

Where the product is applied to the growing plant, the prime objective should be to obtain data on the residue remaining in or on the crop at the time of harvest. If significant residues are expected at the time of harvest, it will be necessary to obtain information on the effects of storage and processing on the residue subsequent to harvesting, as this will provide a basis for assessing the likely intake by consumers. After post-harvest treatments, commodities should also be sampled when they leave the store.

When the product is applied to the harvesting crop, information should be obtained on the alteration of the amounts and nature of the residues during the normal course of storage and handling of the crop after treatment. It is desirable to know, in the case of a fumigant, for example, how much

is taken up by a foodstuff during treatment, and whether and to what extent the pesticide disappears or reacts with particular food constituents.

Residue data will normally not be required for a crop which is not used for human or animal consumption. Examples are: flower bulbs, ornamental shrubs, etc.

However, the possible persistence of pesticides in the soil and their subsequent uptake by edible crops should not be overlooked. Where the use of a pesticide is likely to result in soil residues after harvest of the treated crop or residues in water used for irrigation purposes, residue data in edible parts of subsequent crops should be obtained.

Because of the large variety of crops and commodities on which a pesticide may be used, it may not always be necessary to carry out trials on all crops/species/commodities. The Codex Committee on Pesticide Residues has recently adopted a Classification of Foods and Animal Feedstuffs (Ref. CAC/PR4-1986) in which assignment of a commodity to a group involved considerations such as botanical family, use of different parts of the commodity, potential for residues and agricultural practices.

Although residue data will normally be required for most major commodities in a group, a study of this classification will suggest circumstances when the results of trials on one or more major commodity may be regarded as applicable to others in the group provided the rates and methods of application of the pesticide and cultural conditions are similar. However, care must be exercised in the extrapolation of the results from one commodity to another.

2.1 Trials Lay-Out

2.1.1 Selection of sites

Trials should be carried out in major areas of cultivation or production and should be sited to cover the range of relevant representative conditions (climatic, seasonal, soil, cropping system, farming, etc.) likely to be met for the intended use of the pesticide. Areas or sites where atypical conditions occur and which are not representative should be avoided unless it is expected that use under these conditions can result in higher

2.1.2 Number of sites

The number of sites needed depends upon the range of conditions to be covered, the uniformity of crops and agricultural practice, and the data already available. Whilst it may not be necessary to require that trials be repeated for all regions with different ecological and climatic conditions in which the use of the product is intended or all seasons with widely varying climatic conditions, sufficient data must be available to confirm that patterns determined hold for all regions and the total range of conditions including those which are likely to give rise to the highest residues. Trials in at least two growing seasons are normally needed.

2.1.3 Replication

Since the variations in residue levels between replicates at individual sites are small compared with those found in data from different sites, it is usually not necessary to replicate treatments at individual sites. However it is useful to have three or four replicates at one site to study experimental

uniformity and determine the within-site variations. In glasshouses or stores, the use of products with a high vapour pressure, (fumigants, aerosols, smokes or fogs) will generally not allow for true replicates at one site. If an efficacy trial with replicated plots has to be sampled, then samples taken from plots receiving "identical" treatments should be analysed separately to provide an indication of the within-site variations.

2.1.4 Plots

Residue data should not be generated from plots which are too small to be representative. The size of the individual plots will vary from crop to crop but should be large enough:

(1) to apply the pesticide in an accurate and realistic manner, preferably under the same conditions as in normal local commercial practice; and

(2) to provide representative crop samples **(see 3. SAMPLING PESTICIDE RESIDUE TRIALS)**.

A control plot for the supply of untreated samples is necessary for the reasons indicated in 2.2.3. The control plot should be large enough to satisfy these requirements and should be located close enough to secure identical growing and climatic conditions. However, it has to be sufficiently separated to exclude any contamination from the treated plots (drift, volatilization, leaching, etc.). For products with a high vapour pressure, fumigants, aerosols, smokes or fogs used in glasshouses or in stores, provision should be made for control samples from untreated crops or stored products - e.g. in separate glasshouses/stores or separate compartments, grown/kept under almost the same conditions.

A sufficient buffer zone (lanes, guard rows, etc.) should be left between plots to prevent cross-contamination. In general, close proximity of a high dose level treatment and control plot should be avoided and untreated plots should be placed upwind from the treated plots.

2.1.5 Type/variety of crop/commodity/cropping system

The type or variety of a crop and the way in which it is grown may influence the residue pattern. In these circumstances, data should be available on the most commonly used type or variety or cropping system and on the factor or combination of factors most likely to result in the highest residue levels.

2.2 Application of the Pesticide

2.2.1 Formulation

The formulation to be marketed (or one of similar type and composition) should be used in the residue trials. Prior to the introduction of other formulations, a limited amount of information from comparative trials should be obtained to check that the residue levels will not be significantly affected by changes in formulation.

2.2.2 Methods of application

The method of application should reflect the intended recommendation. As far as possible, applications should be made with equipment similar to that used in local commercial practice. Experimental plot applicators are convenient and readily calibrated and can be used in residue trials as

an alternative method of application provided they are compatible with normal practice. Care should be taken to ensure uniformity of application and to avoid contamination of neighbouring plots, either during or after application. In glasshouses, using products with a high vapour pressure,(fumigants, aerosols, smokes or fogs), the whole glasshouse/store or compartment has to be treated. It will not be possible, in general, to have replicated plots, other dosage rates and untreated control in the same glasshouse/store or compartment. With fumigants, aerosols, smokes and fogs, special attention should be paid to equal and uniform distribution and a preliminary check on this particular aspect may be required. Furthermore, recommended procedures in the glasshouse/store during and after the application (e.g. doors/windows shut/open) should be carefully followed.

2.2.3 Dosage rates

At least two dosage rates should be included in a residue trial: the maximum rate which is likely to be recommended and another rate, preferably double the recommended rate, if considerations of phytotoxicity allow. This will give guidance on likely residue levels should dosage rates exceed recommendations and allow some assessment of the relationship between dosage and residue levels.

When sprays are used the volume per unit area should reflect practical conditions and be the same for all sites in the region and the volume applied recorded if relevant. The concentration of pesticides should be expressed as units of active ingredient per unit area recorded in international (SI) units. In glasshouses/stores, for products with a high vapour pressure, (fumigants, aerosols, smokes or fogs) dosage rates should be expressed both per unit area and per unit volume.

In addition to the two treatment rates mentioned, a control plot should always be included in any residue experiments carried out to provide the analyst with a sample known to be free from residues of the pesticide under investigation.

Control samples are needed:

(a) to ascertain that no artefact in the crop derived from local conditions could give rise to interference in the analysis;

(b) to establish the recovery level of the pesticide from the crop or soil by the analytical method;

(c) in the case of a new crop or pesticide, to investigate the storage stability of any residue.

When two or more dosage rates are included, particular care should be taken to avoid cross-contamination. In glasshouses or stores, the use of products with a high vapour pressure, (fumigants, aerosols, smokes or fogs) will not allow in general for more than one dosage rate per glasshouse/store or compartment nor for untreated control. Provision has to be made in order to obtain samples from untreated crops/commodities and from treatments at another dosage rate - e.g. from separate glasshouse/stores or separate compartments, grown/kept under as near the same conditions as possible.

2.2.4 Number and Timing of Applications

The presence of a target organism is not essential, and regardless of occurrence or level of infestation in the residue trial, the number of treatments and the intervals between applications should reflect the latest and maximum use of the product to be recommended.

2.2.5 Additional pesticides

Unless unavoidable, no pesticide in addition to that to be analysed should be applied to control or test plots before or during the same period. However, since it is of primary importance that both the untreated and treated plants be healthy, the use of other pesticides may be necessary. In this case only those pesticides that will not interfere with the analysis of the residues of the test compound may be used. The pesticides used should be noted; where possible the advice of the analyst should be obtained. It is important that control and test plot receive the same treatment.

2.3 Degradation Studies

Residue trials are sometimes used to obtain information which, although supplementary to the main purpose of the trial, is extremely valuable in studying the properties of the compound under test and in enabling a fuller safety assessment to be made. The trial may be used, for example, for studies on the metabolism and degradation of a pesticide under field conditions. Such requirements should be given early consideration in the planning of the trial.

2.4 Residue Disappearance Studies and Safety Intervals

The disappearance of a pesticide deposit may be due to one or more of factors, principally:

1. Physical removal, e.g. by washing or volatilization
2. Chemical degradation or metabolism in/on the plant
3. Apparent disappearance due to crop growth dilution.

Disappearance studies are of particular value in understanding the significance of these factors, especially when at the moment of application a considerable amount of the future consumable part is already developed or when soil-applied, volatile or systemic pesticides are used.

Samples should be taken as soon as the spray has dried; (care should be taken if a risk to people handling treated plants is anticipated) one to three days later and at intervals thereafter); the intervals will vary from one trial to another and will depend on the persistence of the chemical and on the .anticipated waiting period between treatment and harvest. If multiple applications are anticipated, a sample taken just prior to the final application may be of value. Sampling on at least four occasions, up to and including harvest, is recommended and it is important that the plot size is large enough to allow for valid sampling after each interval. More than one replicate should be sampled and analysed separately.

The range of residue levels at sampling times is much more important than the average levels particularly just before and at harvest. Residue disappearance curves may be plotted using maximum values as well as average levels.

The weather conditions and age and growth of the crop during this type of experiment are particularly important and should be carefully recorded.

3. SAMPLING PESTICIDE RESIDUE TRIALS

In most cases, it is not practical or feasible to collect all of the crop from a trials plot and it is generally necessary to devise a means of taking a sample referred to as the field sample, which, when reduced and analysed, will demonstrate a residue that, for all practical purposes, will represent the maximum residue level of the crop in the plot.

It has always been recognized that it is extremely difficult to obtain uniform application of a pesticide in the field and deposit data following careful application have demonstrated up to 10-fold differences in deposits; thus in taking a sample for residue analysis, it is necessary to approach the task in an intelligent, realistic manner if the results of analysis are to be valid or useful for estimating maximum residue levels.

Generally, the selection of the portions that make up the field sample is done randomly, systematically, or selectively from pre-determined "stations", depending upon the circumstances. The best approach for any given plot can only be determined by a fully qualified person who is capable of recognizing and interpreting the importance and usefulness of the residue data sought. In setting up sampling stations and/or the sampling methods, it is necessary to give consideration to all factors that control the residue distribution over the entire experimental plot. In certain cases where there is likely to be considerable within-plot variation, such as orchard and glasshouse trials, there should be at least three sample replicates per plot at or near harvest and the sample integrity should be maintained through to separate analyses to determine the within-plot variation and collect information on the performance of the treated plot and the individual units comprising it must be typical of those taken in a commercial harvest. The units of the field sample should be identical with the normal harvested product as regards any trimming or cleaning. Separate guidance is available on the recommended portion of the field sample to be prepared for the determination of pesticide residues. (See CAC/PR 6-1984. Portion of Commodities to which Codex Maximum Residue Limits apply and which is analysed; also CAC/PR 4-1986. Classification of Foods and Animal Feedstuffs).

Adequate sampling of the untreated crop is an important consideration - especially if the residue level in the treated crop is expected to be low. While it is not so important to select control crop samples with the care needed for treated samples, it is important to have an abundant amount of such samples.

3.1 Representative Field Samples

Representative samples of the crop in each plot must be taken by a recognized procedure. Although each plant or fruit should normally have an equal chance of being chosen, emphasis should be directed towards identifying the highest residue levels.

Consider the following points:

(a) when taking a sample at harvest, avoid taking diseased or under-sized crop parts or commodities at a stage when they would not normally be harvested;

(b) sample the parts of the crop that normally constitute the commercial commodity;

(c) take samples in such a way as to be reasonably representative of typical harvesting practice;

(d) take care not to remove surface residues during handling, packing or preparation; and

(e) take and bag the required weight of samples in the field and do not sub-sample.

The weight of the sample suggested in paragraph 4.4.4, is the minimum that experience has shown is needed to give a valid sample. Detailed procedures, in specific cases, are given for guidance; in other cases special protocols may be required. The guidance is specifically on taking a field sample; advice on sample packing and sample storage is given in Section 8.

3.2 Contamination

It is vital to avoid contamination of the field sample with the pesticide under study during sampling, transportation or subsequent operations. Pay special attention to the following:

(a) be certain tools are clean;

(b) use new storage bags of suitable type and adequate strength;

(c) avoid contamination of the sample by hands and clothes which may have been in contact with pesticides.

(d) do not transport field crop samples for analysis in vehicles carrying pesticide formulations, and

(e) avoid any damage or deterioration of the sample which might affect residue levels.

3.3 Control Samples

Always take control samples. These are as important as samples from test plots. Control samples should be of similar quality to that of the test samples and may be from plots treated with another pesticide providing these are specified in the trial details. Control samples should be taken before the treated samples, so as to avoid the possibility of contamination from handling. For control samples, using products with a high vapour pressure,(fumigants, aerosols, smoke or fogs) in glasshouses or stores, see Section 2.2.3.

3.4 Sampling Procedures for Field Crops

The amounts of different commodities required to constitute a satisfactory sample obviously vary according to the commodity. The amounts indicated below are given as minima. The recommended size of the field samples may differ from those recommended for the enforcement of maximum residue limits because field samples are often required to satisfy other needs such as research programmes.

Crops quoted are meant as examples and are grouped as far as possible according to the Classification of Foods and Animal Feedstuffs developed by the Codex Committee on Pesticide Residues (CAC/PR 4-1986).

3.4.1 Vegetables

Root, tuber and bulb vegetables

Take samples from all over the plot. Remove as much adhering soil as possible from crops but do not wash. (Note: In some cases, where leaf parts are used as feed, they may need to be sampled separately).

Quantity

(a) Root crops (large) - 5 kg samples (not less than 5 items)
Beet (red, sugar, fodder), onions, parsnips, potatoes, sweet potatoes, turnips.

(b) Root crops (small) - 2 kg samples
Carrots, radish, spring onions.

Leafy, stem, fruiting and legume vegetables

Take the sample from all parts of the plot. Sample items of crops such as fruiting vegetables, peas or beans from those protected from the spray by foliage as well as from those exposed to the spray. Remove as much soil as possible from crops such as celery.

Quantity

(a) Leafy or stem vegetables (large) - 5 kg samples (not less than 5 items)
Brassicases (cabbage, cauliflower, broccoli, kohlrabi, curly kale).

(b) Leafy or stem vegetables (small) - 2 kg samples
Asparagus, brussel sprouts, celery, chicory, lettuce, spinach, turnip tops.

(c) Fruiting vegetables (large) - 5 kg samples (not less than 5 items)
Cucumber, melon, squashes, eggplant (aubergines).

(d) Fruiting vegetables (small) - 2 kg samples.
Peppers, tomatoes, gherkins.

(e) Legume vegetables - 2 kg samples
Beans, peas etc., (with pods)

3.4.2 Fruits

All tree and bush fruit, including vines, small and other fruits

Select fruit from all parts of the tree/bush, high and low, and from both sides of the row, and select fruits according to abundance whether in each segment or the whole tree/bush. More fruit will therefore be selected from the more densely laden parts of the crop. Sample fruits exposed to the spray and also those apparently protected by foliage. Take large and small fruits, perfect or slightly blemished, but not so small or blemished that they would not normally be saleable.

Quantity

(a) Tree fruit (large) - 5 kg samples Apples, citrus, palm fruits (coconut and oil palm), peaches, pears.

(b) Tree fruit (small) - 2 kg samples Cherries, dates, nuts, olives, plums.

(c) Small fruits, berries, and vines - 2 kg samples Bush fruit (all types), grapes, strawberries.

(d) Miscellaneous (large items) - 5 kg samples (not less than 5 items) Bananas (take four fruits from each bunch), papaws, pineapples.

3.4.3 Grasses

Cereal grains

Cut not less than ten small areas (approximately 0.1 m2) chosen randomly from all over the plot. Cut stalks about 10 cm above the ground. Remove the grain from the straw. If an experimental mechanical harvester is available the whole plot may be harvested but residue samples should not include material from the first few metres of a plot in order to avoid contamination from the previously harvested plot. Take not less than ten grab samples of grain and/or straw from the harvester, uniformly spacing them over the entire plot. (Note: Care should be taken to avoid contamination when mechanical methods are used to separate the parts of the crop).

Quantity

(a) Maize (grain and cobs) - 2 kg samples

(b) Small grains - 1 kg samples

Fodder and straws

Harvest the crop in a way to simulate cutting practice. Record height of cutting and avoid soil contamination. Samples should be taken from not less than ten points (approximately 0.1 m2) in each plot.

Quantity

(a) Grass and forage (smaller leaves) - 1 kg samples Clover, grass

(b) Forage (large leaves) - 2 kg samples Alfalfa, beet tops, etc.

(c) Straw (all cereals except maize) - 1 kg samples Maize silage (green plant at various stages of growth) and stover (dry remains of plants after grain harvesting): five plants (excluding roots).

(d) Other animal feed items. Samples of 1-2 kg are normally sufficient depending on nature of the material.

3.4.4 Nuts and seeds

Oil Seeds

(a) Cotton

Pick the cotton at the normal stage of harvesting and remove as much fibre from the seeds as convenient.

Quantity

1 kg of delinted seed (or 2 kg with fibre)

(b) Sesame, rape, soyabeans: Collect the heads when they have reached the stage of maturity at which they are normally harvested and if convenient thresh to remove the seeds.

Quantity

1 kg of seeds

(c) Sunflower: Select ripe heads randomly over the plot and remove the seeds by shaking.

Quantity

1 kg of seeds

d) Groundnuts: 1 kg (or 2 kg in fibre)

Coffee, Cocoa

Samples representative of each treated plot should be taken in the field in a manner reflecting common practice and should then be processed through to the dried state using the locally typical process. Normally, the freshly harvested product is not required.

Quantity

Cocoa, coffee - 2 kg

3.4.5 Herbs, spices and tea leaves

Sampling representative of each treated plot should be taken in the field in a manner reflecting common practice and should then be processed if appropriate through to the dried state using the locally typical process. Normally the freshly harvested product is not required for tea although herbs such as parsley and chives should be sampled fresh.

Quantity

tea - 1 kg

3.4.6 Other products not classified

Sugar-cane

Take short sections (about 20 cm) from various portions of the length of the canes, and from all parts of the plot.

Quantity

5 kg samples

Juice: Care is necessary due to the rapid changes which normally occur in cane juices. If required, samples (1 litre) should be taken and frozen immediately and sent in cans.

4. SAMPLING OF PROCESSED COMMODITIES

Where a commodity is normally processed between harvest and marketing, such as by milling, pressing, fermentation, drying or extraction, data may be required on the processed crop or its products. Details of the processing method should be supplied with the samples along with storage and handling histories. In such cases, the trials should be planned to provide samples with appropriate residue levels so that the fate of residues can be studied during the processing. Sample separately any cleanings, husks or by-products which could be used for animal feed.

5. SAMPLING OF STORED COMMODITIES

Supervised trials with stored products/post harvest treatments should be carried out over a wide range of storage facilities and the sampling technique must be carefully chosen if a valid sample from most commodities in storage units is well established. Such procedures are acceptable in sampling for pesticide residue analysis and may be used if adequate references are given.

The sampling procedures are usually designed for three kinds of storage conditions.

5.1 Sampling From Bulk

Obtaining a representative sample from a (large) bulk container (e.g. cereal grains) is difficult and, if possible, the sample should be taken at frequent intervals from the stream during a transfer into another container. A probe sample is not representative but may be acceptable if:

- it is possible to reach every part of the storage container.
- a large number of individual samples are taken before mixing and reducing to get a final sample.

Pesticide residues are normally higher in the dust fraction and this should be recognized in the sampling procedure.

5.2 Sampling Bagged Commodities

Sampling of the commodity within a bag must be random and a representative sample from a large stack of bags can be obtained only if every bag is -accessible. This is not always possible in practice and the alternative is to obtain a sample from a number of randomly chosen bags by probing. Since pesticide treatments are often directed to the surface of the bag, then selective sampling to show the effect of the position of the bag in the stack and the penetration of the pesticide into the bag may be necessary.

5.3 Sampling Fruit and Vegetables in Packing Houses

Where post-harvest treatments are applied to fruit and vegetables in packing houses, an adequate number of samples must be taken to determine the range of residue levels resulting from variations in the treatment process. The effects of concentration, temperature, duration of treatment, drying (of dip treatments) and subsequent handling on residue levels may need to be considered.

6. SOIL SAMPLING

During the course of obtaining information on residues in a crop, useful information may be obtained, if needed, on the degradation of the pesticide in soil under local conditions. It will then be necessary to take samples at intervals, possibly over a period of at least one season. The first sample should be taken immediately after the last application to be made compound. Samples taken at the time of harvest and at the beginning of the following season are particularly important if there is a possibility of carry-over into a subsequent crop. There are special problems inherent in soil sampling and further guidance should be obtained before planning soil studies.

7. SAMPLE SIZE REDUCTION

Ideally, the field sample should be submitted intact for analysis, although the requirements of the analyst should not influence the sampler to take a smaller sample than is necessary for a valid field sample. In practice, a valid field sample is often much larger than the sample needed by the analyst and cannot be handled economically, especially if freezing and long transport are involved. In such cases, a reduction in the size of the field sample is desirable.

For samples consisting of small units, such as cereal grains or even small fruit, there is little difficulty in valid sample reduction and the normal procedure of mixing, quartering and rejection of opposite quarters until the desired reduction is achieved is satisfactory. With samples of medium sized products such as apples, potatoes, beans and peas in the pod and citrus, there is an increased risk of losing sample validity by sample reduction. However, the random selection of the required number of units to make up the laboratory sample from a well-mixed field sample is probably the most satisfactory procedure.

Since it is unacceptable to cut or divide sample units, the problem is greatest with large fruit and vegetables such as cabbage or melons. In these situations, there is little alternative to shipment of the whole field sample to the laboratory, particularly since the number of items required for an acceptable laboratory sample is often the same as that required for the field sample.

8. SAMPLE PACKING AND STORAGE

Once packed and labelled, samples may be stored or immediately sent to the Residue Laboratory according to the nature of the sample, the stability of the residue and the kind of study undertaken.

It is important that the packing and shipment be done so that the samples arrive as soon as possible (normally within 24-36 hours) after being taken and without change of any kind, e.g. deterioration, physical damage, contamination, loss of residue, or change in moisture content.

8.1 Packing

8.1.1 Containers

Individual samples should be placed in suitable containers, e.g. heavy polyethylene bags and then put inside additional heavy paper bags and, where necessary, frozen or refrigerated as soon as possible after sampling, according to the nature of the chemical involved. Polyethylene bags alone may become brittle in contact with dry ice and therefore risk breakage and subsequent loss of sample.

Avoid other plastic containers, or plastic-lined caps, unless made of Teflon or other inert plastic which does not interfere in the analytical method; laboratories frequently have experienced such interferences and PVC bags should be avoided. If cans are used, they should first be checked to demonstrate the absence of materials such as oil films, lacquers, resin from soldered joints, etc., that could interfere with analyses.

Glass containers should be used for water or liquid samples and should be thoroughly cleaned and rinsed with one or more suitable pesticide free solvents such as acetone, isopropanol, or hexane, and dried before use. Pesticides can migrate to the walls of a container and be absorbed; hence even a glass container, after the water sample is poured out, should be rinsed with solvent if the extraction is not made in the container itself.

In summary, any type of container or wrapping material should be checked before use for possible interferences in the analytical method and at the limit of detection employed in the analysis. Fasten boxes securely with strong twine, rope or tape.

8.1.2 Shipment of samples

Non-perishable commodities containing residues that are known to be stable over the period required to reach the laboratory can be shipped in a non-frozen state but samples should be protected against any effects which might cause degradation or contamination.

Where samples need to be frozen, use shipping containers of polystyrene foam, if available, as they are excellent for this purpose. If not available, use two cardboard boxes of slightly different size with insulation in between. Proper insulation is essential to ensure samples arriving at the residue laboratory still frozen. Sufficient dry ice must be used so that some will still remain when received at the residue laboratory. This usually requires a minimum of one kg of dry ice per kg of sample. For journeys lasting more than two days, two kg of dry ice or more per kg of sample may be required. Poorly insulated containers require more dry ice. Use caution in handling dry ice (gloves and ventilated work area). Packages must of course comply with current transport regulations.

Frozen samples must never be allowed to thaw, either before or during shipment. They must be shipped under conditions that permit their arrival at the residue laboratory still solidly frozen.

Advise consignee by telegram or telex full details of shipment of samples, including shipping document numbers, flight numbers, etc., so that delay in delivery to the laboratory is avoided.

When samples have to be shipped across national boundaries, quarantine regulations must be observed and appropriate permits obtained well in advance of despatching samples.

8.2 Labels and Records

Label each sample with the appropriate sample identification. The label and ink should be such that the writing cannot become illegible if it becomes wet. Attach the label securely so that it cannot become loose during shipment and place the label so that it will not become wet from condensation.

Complete the residue data sheets clearly and accurately with the requested trial details. Failure to do this may mean that data will not be acceptable. The completed sheets should be protected by enclosing them in protective polythene bags and they should be sent with the sample. Duplicate sheets should be kept by the sender.

Use a label on the outside of the shipping container showing the following: "Perishable Goods": "Deliver immediately upon arrival" and "This material is not fit for human consumption".

8.3 Sample Reception and Handling

Immediately upon arrival of the samples, the Residue Laboratory personnel should:

8.3.1 Verify that the copy of the residue form is included with the samples.

Check and report on the condition of the samples.

Check to see that the samples match the details of the residue form.

Check the residue form for accuracy (especially the rate and interval data) and to verify that the information is complete.

Check the residue form to determine if any special treatment or testing is indicated.

8.3.2 If there are any deviations of any consequence or the residue form is not received, or is incomplete tin such a way that a proper comparison is not possible), then the samples should be preserved in the simplest form that will preserve the residue and the crop. The trial organizer should then be immediately contacted to determine how to proceed.

8.3.3 Note: It is dangerous to put packages containing dry ice into deep freeze.

8.4 Storage of Samples

Samples should be analysed as quickly as possible after collection before physical and chemical changes occur. If prolonged storage is unavoidable, it is usually preferable to extract the sample, remove most or all of the solvent and store the extracts at a low temperature, preferably at or below -20□C. This removes the residue from contact with enzymes which might degrade the pesticide and also prevents further possibility of residues being wound" in the tissue. Do not store homogenized samples for analysis unless an adequate check has been made on the stability of the residue.

Studies of the stability of residues in samples of extracts, with time at temperature of storage should be carried out with representative pesticides and substrates. When there is doubt about the stability of residues in storage, spiked control samples should be held under the same conditions as the samples or extracts.

Light degrades many pesticides, therefore it is advisable to protect the sample and any solutions or extracts from needless exposure. Samples other than water should ordinarily be stored in a freezer, preferably at -20□C or below. Even then, physical and chemical changes may occur in either the sample or in the residues sought. Extended storage in freezers can cause moisture to migrate to the surface of the sample then to the freezer coils, slowly desiccating the sample. This effect may be of importance if water content affects the subsequent analysis and can affect the calculated residue concentration. Water samples should be stored slightly above freezing to avoid rupture of the container as a result of freezing.

9. REPORTING ON RESIDUE TRIALS

All the data relating to the treatment and history of the residue trials should be recorded. It is usually convenient to record these data in standard form and essential items for specific trials may be drawn from the following list. These refer to the supervised trial, field sampling and shipment of sample to the laboratory. Further data on the chemical analyses will be provided by the analyst. Model report forms are attached.
(See Appendix l).

9.1 General Information on the Supervised Trial

- Pesticide (active ingredient and trade name)
- Formulation
- Trial number and type (field/glasshouse/other)
- Commodity (l)
- Variety
- Test locations (country and site)
- Soil characteristics, pH, physical and chemical properties
- Names (and signature) of the person(s) responsible for the trial and for collecting the sample.

9.2 Application Data for Field Trials

- Crop planting or sowing date
- Description of plot plan/crop layout/cropping system
- Plot size or number of plants per plot/unit area
- Number of plots per treatment

- Target pest or disease (if any)
- Method of application and equipment
- Number of applications and application date(s)
- Application details (overall, banded, etc.)
- Dose rate - active ingredient/ha
  - weight/volume of formulation/ha
  - applied dilution
- Climatic conditions during and after applications, preferably for the whole period of the trial
- Other pesticides applied to trial plot with relevant details as above
- Cultural treatments before, during and after application - include irrigation and fertilizer information
- Growth stage at (last) treatment.

In glasshouse/stores for the application of fumigants, aerosols, smokes or fogs, the procedure of the application and the disposition of fixed equipment/generators should be described. Any anomaly occurring during the application or during the post-application period (e.g. doors or windows opened) should be reported. Dosage rates should be expressed both per unit and per unit volume.

9.3 Application Data for Stored Products/Post-Harvest Trials

- district, number, volume and area of the trials site;
- description of the store including total capacity at time of trials, type of ventilation and state of hygiene;
- details, if available, of other recent pesticide treatments in store;
- description and quantities of products and details of packaging conditions (whether in sacks, boxes, bales, tins or in bulk);
- formulation(s) used;
- rates, methods and dates of application;
- temperatures and humidity in the storage area during and shortly after applications of pesticide and the mean temperature and moisture content within the stored product between time of treatment and sampling.

(l) See Codex Classification of Food and Animal Feedstuffs (Ref. CAC/PR4-1986)

9.4 Sampling Data

- Growth stage at sampling - normal harvest date.
- Method of sampling.
- Sampled part(s). Number of units in sample, if relevant, (e.g. lettuce, pomefruit).
- Sample weight and preparation (trimming/washing/other if common practice in preparing the commodity).(2)
- Control treated.
- Date of sampling with time interval between last application and sampling.
- Storage conditions before shipment.
- Date shipped.
- Method of packaging.

10. ANALYTICAL REQUIREMENTS

The results from supervised trials are only relevant if an analytical method is available which will determine the components of the residue as described in **1. INTRODUCTION.** Until the composition of the residue is known, its toxicological relevance cannot be estimated.

Before an appropriate analytical method can be designed, the components of the residue must be identified. Furthermore, it is some help to elucidate their behaviour with respect to translocation, volatilization and binding to our conjugation with plant constituents. The latter is particularly related to the bioavailability of the residue and the ease with which it can be extracted for determination.

The study of possible translocation is important to determine whether or not residues can occur in the crop at harvest. For example, a post-emergence herbicide in cereals may not be translocated in the crop either as active ingredient or a metabolite. Therefore a residue cannot be expected at harvest and unnecessary analytical effort can be avoided. A clarification of such properties is valuable before residue field trials are planned. The necessary experiments may be carried out in the laboratory, outdoors or in simulated outdoor conditions using the active ingredient with or without radio-labelling.

In practice, it is more convenient to use radio-labelled material to obtain the following information:

(l) the behaviour of the residue from the time of application until harvest - distribution in the plant, kinetics of disappearance, binding to plant constituents, etc.;

(2) the possible formation and identity of metabolites;

(3) the changes in composition of the residue including metabolites with time; and

(4) an overall material balance for the applied active ingredient.

It is recommended that the pesticide, formulated in an intended commercial formulation should be applied at twice the proposed rate to one or two of the relevant major crops. The treatments should be at the time(s) required by good agricultural practice and relevant climatic conditions should be simulated as far as possible. The conditions for the experiments should be chosen so that the behaviour of the active ingredient can be investigated in both the target crop and in soil which may receive part of the applied dose. After harvest the test system should be kept for a possible study of the uptake of residues from soil by subsequent rotational crops.

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(2) See Portion of Commodities to which Codex Maximum Residues apply and which are analyzed (Ref. CAC/PR6-1984)

Most pesticides, however, leave very low residues at harvest and the identification of metabolites at this stage is often difficult. To identify metabolites it is advisable to carry out a duplicate experiment but sample the crop at a time when the total residue and metabolites are present in relatively high amounts. It is necessary to produce enough metabolites for isolation and comparison of chemical and physicochemical properties (e.g. mass spectra, infrared and ultra violet spectra, chromatographic characteristics, etc.) with compounds synthesized with a theoretical knowledge of possible structures formed by metabolism.

10.1 Metabolites as Components of the Total Residue

There are two general considerations which are basic to the decision as to whether or not specific metabolites/degradation products should be included in the definition and expression of a residue:

(l) their basic toxicology; and

(2) their presence in significant amounts.

A number of principles and subsequent specific options may be used in deciding which metabolites/degradation products to include in definition of residue and the expression of the residue, namely:

A. Residues may be expressed as parent compound if:

(1) there are no metabolites;

(2) metabolites are known to be insignificant and can be ignored;

(3) metabolites are known to be of toxicological concern but are not present in significant amounts;

(4) metabolites of toxicological concern are known to be present in significant amounts and the analytical method measures the total residue as a single compound which may be numerically expressed as parent compound. In this case the metabolites included in the residue are listed; and

(5) metabolites of toxicological concern are known to be present in significant amounts and the analytical method measures parent compound and metabolites separately. In such cases the compounds of the total residue may be expressed additively as parent compound, with recalculation for differences in molecular weight, only when the differences are substantial (e.g. greater than 20%).

B. Residues may be expressed as a single metabolite or alteration product if :

(1) parent is quantitatively converted to another chemical entity - e.g. aluminium phosphide to phosphine;

(2) metabolites of toxicological concern are known to be present in significant amounts and the analytical method measures the total residue as a single metabolite. The results may be expressed as that metabolite but the compounds included in the residue should be listed; and

(3) metabolites of toxicological concern are known to be present in significant amounts and the analytical method measures residue components, including parent compound if present, separately. The result may be expressed additively in terms of metabolite with recalculation for differences in molecular weight only when differences are substantial (e.g. greater than 20%).

C. Residues maybe expressed as parent and metabolites separately if:

Metabolites are known to be present in significant amounts and the analytical method measures each component of the total residue separately.

All metabolites/degradation products included in the definition of residue should be listed regardless of the method of determination. Highly toxic impurities in pesticides must be treated separately.

10.2 Non-extracted or "Bound" Residues

A special problem in both metabolic studies and description of residues is created by conjugates and bound residues. Any statement to the effect that residues are "bound" (to a substrate, or matrix) must necessarily be a function of the effort undertaken to "free" them, e.g. of the extraction procedure used, and remains meaningless unless the conditions under which such bound residues were found to be unextractable are specified. It is preferable, therefore, not to use the term "bound residue" in an analytical context but to substitute it by "not extracted residue", each individual case being supported by a statement of the method of investigation.

Only in such instances where residues are found to be unextractable under usual chemical laboratory conditions,- e.g. without running the risk of changing them by applying very rigorous extraction methods, should they be termed "bound" or "chemically unavailable". The question then remains whether such residues will be available to biological systems, e.g. to micro-organisms, invertebrates, or following crops (when persisting in the soil), or to the digestive tract of ruminants or other warm-blooded animals, including man (when persisting in certain constituents of feed or food). If chemical and biological unavailability or residues can be demonstrated, such residues should be considered negligible.

"Conjugated" residues or metabolites may be subject to hydrolysis in biological systems different from those in which they were shown to have formed. If such a process can be demonstrated or regarded as very likely to occur, the respective conjugate found in an edible plant should be considered as a residue only if it can be demonstrated or it is considered highly likely that the non-physiological portion of the conjugated molecule may become physiologically available to an animal via its digestive tract.

These considerations, for both unextractable and conjugated residues, will apply only in such instances where the compound in question (e.g. the "bioavailable" substance, or the aglycone) satisfies the agreed definition of a "residue". When it does so, the respective substance should be included in the residue analytical procedure.

10.3 Analytical Methods

Only when a decision has been made on whether or not specific metabolites/degradation products should be included in the definition and expression of the residue from a particular pesticide can the residue analytical procedure(s) be established. The design of suitable analytical procedures and criteria for their applicability and performance are outside the scope of this Section but it is advantageous if the parent compound, metabolites and conjugates can be artificially degraded to a single common moeity. Such a "total residue" approach reduces the number of chemical entities to be determined and improves the efficiency and sensitivity of the analysis. This is of particular importance for an analytical method required for enforcement purposes but not so for methods used in the development of a pesticide when measurements of specific metabolites may be required.

Other characteristics of the active ingredient such as:

- solubility in water
- vapour pressure
- partition coefficient in water/n-octanol
- hydrolysis rate at pH 5, 7 and 9
- rate of photolysis

may also be of help in designing the analytical method as well as predicting the behaviour of the compound in the crop or in the environment.

Codex guidelines on Good Analytical Practice in Pesticide Residue Analysis (Guide to Codex Recommendations Concerning Pesticide Residues, Part 7) gives guidance on various aspects of residue analysis. The Codex Recommendations for Methods of Analysis of Pesticide Residues (Guide to Codex Recommendations Concerning Pesticide Residues, Part 8) provides a series of recommendations for methods suitable for the determination of residences of a wide range of pesticides in a variety of substrates.

11. REPORTING RESULTS

This aspect of pesticide residue analysis depends very much on the requirements of the organization demanding the analytical information and it is difficult to lay down strict rules of reporting, or even on the accuracy

required. It is recommended that both analyst and user of the information fully appreciate the capability of the methods used and the interpretation to be placed upon data produced before the work is started.

Valid interpretation of residue data depends on a knowledge of how the various factors contribute to the variability in results. Thus a sufficient number of analyses must be carried out to show the extent of errors involved and the standard deviation should be calculated.

All the analytical data obtained from the analysis of samples, including where relevant the parent component and the main metabolites should be provided, and not just a summary or an average figure. It should be clearly stated how the residues are calculated and expressed.

If necessary, explanatory notes for erratic results should be given.

For most commodities the residues of the pesticide and its metabolites will be expressed on the basis of the whole product as it moves in commerce or is prepared for marketing, e.g. certain vegetables without outer leaves, root vegetables after removing aerial parts, etc.

Residue data should be supported by:

1. a full description of, or adequate reference to the analytical method used, including apparatus and reagents;

2. data on the specificity of the method used;

3. data on the limits of determination of the analytical method on the commodity in question.

4. adequate recovery data at levels (which should be stated) corresponding to those found in practice;

5. the value of the untreated control and its standard deviation, including the number of observations upon which the standard deviation is based;

6. a statement on whether or not the results presented have been corrected for blanks (untreated controls) or recoveries, or for both;

7. an indication on whether a pre-treatment of the sample, e.g.- washing, peeling, making soil free or any other methods of preparing has occurred before analysis. This should be stated in expressing the amount of residue found. An example of the type of form that might be used for reporting analytical results is to be found in Appendix 2.

12. FURTHER READING

Burke, J., and McMahon, B. "Analysis of Food for Residues of Pesticides", FDA By-lines, No. 4, January 1977

Cochane, W.P., Whitney, W. The Canadian Check Sample Programme on Pesticide Residue Analysis: Reliability and Performance. Pesticide Residues, 1979, Pergamon Press

Car, M. Internal Laboratory Quality Control in the Routine Determination of Chlorinated Pesticide Residues. Pesticide Residues, 1979, Pergamo Press.

Telling, G.M. Good Analytical Practice in Pesticide Residue Analysis. Proc. Analyt. Div. Chem. Soc. Jan. 1979

"Guidelines on Analytical Methodology for Pesticide Residues Monitoring", Federal Working Group on Pest Management, Washington, D.C. 20460, June 1975.

Sherma, J. "Manual of Quality Control for Pesticides and Related Compounds in Human and Environmental Samples", USA Environmental Protection Agency, EPA 600/1 76 017. February 1976.

"Pesticide Analytical Manual", Volume le US Department of Health, Education and Welfare, Food and Dr
Administratic

APPENDIX 1

REPORT ON PESTICIDE TRIAL. - PART A. FIELD REPORT
(Please type or use block capitals)

1. RESPONSIBILITY

1 Year		3 Company or organization Name and address	
2 Trial identity or number			
4 person (s) responsible for: (included signature)		a. Trial design..... b. Application..... c. Sample ..... d. Analysis.....	

2. IDENTITY OF TRIAL

5 Active (common name)	6 Class of pesticide or	7 Trade name(s) or code number(s)	8 Formulation		
			Type	Concentration in SI units	Comm/ Exper.
....					

Crop/commodity

9 Type	
10 Variety/ cultivar	
11Codex commodity	

12 Country/ region	
13 site or map ref. (Include address)	

classification			

14. Pests/disease	

3. GENERAL INFORMATION ON THE TRIAL

15 Crop production system or lay out (e.g. commercial orchard/ glasshouse: crop planting date: age of crop; guard rows soil type)	

Plot data

16 Plot dimension in International Units		19 crop spacing	
17 Number of plots per treatment (replicates)		20 Number of plants per plot	
18 Number of control plots		21 Number of rows per plot (if relevant)	

22 Previous year's pesticide treatment	
23 Other pesticides applied to the plot (rates and times) during trial	
24 Cultural treatments (e.g., irrigation, fertilizers)	
25 Summary of climatic conditions. (eg,temperature (□C) rainfall, wind, Sunlight: attach details if available)	1. Before application (96 hours) 2. During application 3. After application (up to sampling)

4. APPLICATION DATA

26	

Method/equipment Type of application (e.g. spray to run-off band, overall volume applied)	
27 Dose rate a.i. (g/ha)	
28 Dilution or spray concentration in Sl unlts	
29 Numbers of applications	
30 Dates of applications	
31 Growth stage at last treatment (internationally recognized scales if available)	

5. SAMPLING

32 Control/Treated (delete as applicable)			
33 Sampled part of crop		34 Growth stage at sampling	
35 Method of sampling			
36 No. of samples per plot		38 Sample weight and treatment	
37 No. of units in primary sample			

39 Dates 40 Intervals (days)

sampling				
Freezing				
Receipt in laboratory				

Last treatment/sampling				
Sampling/freezing				
Sampling/receipt in laboratory				

APPENDIX 2

REPORT ON PESTICIDE RESIDUE TRIAL - PART B. ANALYTICAL REPORT

(Please type or use block capitals)
Person (s) responsible for the analysis

IDENTITY OF SAMPLE

crop Commodity		Sample identity or number	
Pesticide (s) used on samples (s)			

CONDITION AND TREATMENT OF SAMPLES (S)

Date(s) of receipt in laboratory		Date (s) of analysis	
Method of storage and condition of sample (s)			
Portion of sample (s) to be analysed			

ANALYSIS

Method of analysis (or reference) and/or modifications Extraction: Clean up Method of determination and expression of residue Recoveries Limit of determination	

RESULTS

Dosage Rate	____	____	____	____	____	____
Interval (treatment to sampling)	____	____	____	____	____	____
Residue* (Not corrected for recovery or control)	____	____	____	____	____	____
Control (Including standard deviation)						
	_____	_____	_____	___	___	____

Other information/ e.g. stability of residues under storage conditions:

* give mean value, range and number of analysis

PART 2 - FOODS OF ANIMAL ORIGIN

1. INTRODUCTION

Uptake of pesticides by animals, leading to residues in foods of animal origin, can occur following either direct application of the pesticide to the animal or ingestion of feed containing residues of pesticides. In the first of these situations the application of the pesticide is deliberate and can therefore be controlled by the farmer. In recognition of the fact that residues in foods of animal origin can result from residues in feed, and that such animal feeds enter into commerce, the Codex Committee on Pesticide Residues (CCPR) has recommended maximum residue limits (MRLs) on agricultural commodities specifically grown for, or occasionally used as, animal feed. The residues in food of animal origin (meat, milk, eggs) resulting both from the ingestion of feeds containing pesticide residues and from direct application of the pesticide to the animal, have also been evaluated by the Joint FAO/WHO Meeting on Pesticide Residues (JMPR) and MRLs have been recommended where appropriate.

In order to obtain the necessary data to estimate maximum residue levels, studies are needed where pesticide residues are present in the feed of farm animals, or when pesticides are applied directly to farm animals.

There is a need for internationally accepted guidelines on the experimental design, procedures and reporting of such studies. The purpose of these Guidelines is:

(1) to indicate acceptable techniques which may be followed in order to secure valid experimental data appropriate to the above objectives; and

(2) to promote the establishment of harmonized procedures to facilitate international acceptance of the data obtained.

Initial studies using radiolabelled chemicals are essential to identify the metabolites present in animal products intended for human consumption and hence to define the nature of the residue. If residues are found, it may be necessary to follow the radiolabelled study with a "cold" feeding study to quantify the level of residues involved.

These studies must be carefully planned, conscientiously executed, carefully evaluated and intelligently interpreted to ensure that the decisions taken are meaningful and that they reflect the practical situation resulting from approved uses of the chemical. Where appropriate, the studies should be carried out according to Good Laboratory Practice Standards (GLP). It may be helpful to consult registration authorities regarding specific details of protocols.

2. DESIGN OF STUDIES

It is customary to use radiolabelled compounds in metabolism studies on animals to identify the nature of residues in animal products. Quantitative measurements of these residues are then made using non-radiolabelled compounds.

Usually the most important studies are those involving ruminants and poultry. Normally lactating cows are used but, in the interests of economy, a goat is an acceptable alternative.

In the case of poultry, chickens are the animals of choice. Except in special cases, it is usually not necessary to carry out metabolism studies with pigs since information on metabolism in a monogastric animal is available from studies with rats. If metabolism in the rat is different from that in the cow, goat and chicken, pig metabolism studies may be necessary.

Where feeding studies are required, they are usually carried out using the parent pesticide which is normally the residue of greatest toxicological significance on the treated crop. In cases where this is not so, the animal transfer studies may be carried out using a metabolite or a realistic crop residue mixture. Discussion with Registration Authorities before the studies are started is advisable.

Where external treatment of animals is needed, this is best carried out with the formulated product in a manner which reflects the proposed use.

2.1 Radiolabelled Studies (Metabolism Studies)

The purpose of these studies is to identify the nature of the residue in the edible tissue of livestock, milk and eggs. Animal metabolism studies are required whenever a pesticide is applied directly to livestock, animal premises are to be treated, or residues occur in crops or crop parts used for feed.

A livestock metabolism study should primarily identify the compounds for which analytical methods and residue data must be generated. It should also indicate the distribution of residues in tissues, eggs and milk, and whether residues are stored and accumulated. A livestock metabolism study should also lead to the elucidation of the efficiency of extraction of the various components of the residue so that extraction/residue release procedures can be developed as part of the analytical methods.

For these studies, the position of radiolabelling must be chosen so that the label is not easily lost by metabolic transformation. For example, ring labelling is preferred for aromatic and cyclic compounds. Where the molecule is complex and more than one large fragment may be formed in animals, it may b e important to carry out more than one study with samples labelled in different parts of the molecule. Alternatively a chemical labelled in more than one position may. However, this may mean that results are difficult to interpret and this approach should only be adopted after careful consideration of such problems.

For each position of radiolabel chosen, at least one ruminant (cow or goat) and/or three chickens should be dosed or treated with the chemical.

2.1.1 Animal feeding studies

In the case of feeding studies, the dosage should be at least equivalent to the daily intake estimated from feed containing residues at the level of existing or proposed MRLs. However, if the estimated intake is low and the identification of metabolites consequently difficult, then a higher standardized daily dose is recommended. In this case, a dose equivalent to 10 mg kg -1 in the total feed ration is usually sufficient. Ruminants can be conveniently dosed twice a day at each milking whereas with chickens it may be better to dose only once daily. The dose should be administered to the animals in the least stressful manner. Successful procedures for administration are outlined in Appendix 1.

During the study, the animals under test should be suitably housedcows maintained indoors in a stall, goats in a metabolism crate and chickens in battery cages. The animals should be housed in this way for a few days before dosing commences. This allows an acclimatization period to overcome any effect on milk yield or egg production as a result of moving the animals. During the acclimatization period, samples of milk and eggs can be taken to establish background levels of radioactivity.

When animals are acclimatized, dosing should be started. Ruminants are preferably dosed for three days, chickens for 14 days and in no case should either be less than three days. This normally allows total radiochemial levels in milk and eggs to reach or be close to plateau levels.

Establishing a plateau level in milk and eggs is an indication that levels in tissues may also be close to a plateau level.

2.1.1.1 Sampling

Samples of excreta and milk or eggs should be collected for analysis during the dosing period. The animals should be sacrificed 24 hours after the final dose and samples of tissues taken for analysis.

The following tissues are essential:

Ruminants: Meat (leg and forequarter) fat (renal and subcutaneous) liver and kidneys.

Chickens: Meat (leg and breast) with overlaying skin and any associated fat, liver, abdominal fat.

2.1.1.2 Analysis

The following analyses of the samples are usually required.

(1) Levels of radioactivity in excreta. It is difficult and unnecessary to make this fully quantitative but total recoveries in the range of 60-80% of the applied dose indicate that the intended dose has been administered and that the chemical is readily excreted.

(2) It may also be useful to identify metabolites in urine and/or faeces as these will usually indicate metabolites which may be present in tissues, milk and eggs.

(3) Levels of radioactivity in tissues, milk and eggs.

(4) Identification of residues in tissues, milk and eggs. Identification or characterization of residues present at greater than 0.05 mq kg -1 should normally be attempted. Only occasionally will identification below this level be needed for example, if a low dose rate has been selected.

2.1.2 Animal treatment studies (Dermal application)

If a previous oral dosing study has been carried out, dermal treatment using radiolabelled chemical may not be necessary. This is because ingestion of a dermally applied chemical, as a result of the animal grooming itself, is a major route of uptake and metabolites may be satisfactorily

characterized in the oral study. However, if the chemical is to be applied dermally and no oral studies have been done, treatment studies are usually needed. When treatment is needed, this should simulate the proposed use patten of the chemical and the following ways of applying formulated radiochemical have been found useful.

An area equivalent to 25% of the surface area of a cow can be painted with the formulation at four times the normal application rate. Similarly, formulated product can be applied with a brush to the skin and base of the feathers on the breast, abdominal area and upper leg of a chicken.

Following treatment, the animal should be allowed to lick itself or groom normally. This is important because much of the residue found in normal use may well be derived from ingestion rather than dermal penetration.

(In special cases, e.g. treatment along the backbone of an animal, grooming may not result in a major oral uptake).

Samples of milk and eggs can be collected for a period until sacrifice. At this stage, tissues should be taken from below the treated area of the animal as well as those listed under animal feeding studies (2.1.1.1). In this case, analysis of tissues, milk and eggs are required to determine levels of radioactivity and identity of residues see 2.1.1.2).

2.2 Non-Radiolabelled "Cold" Animal Studies

If residues have been identified in animal products in the radiochemical studies, and if the crop on which it is proposed to use the pesticides is an important part of the animal diet, feeding studies should be carried out using normal technical grade pesticide. Similarly, treatment studies using commercial formulations will be necessary where a pesticide is to be applied to the skin of an animal. These studies will provide the quantitative data on which a maximum residue limit (MRL) can be set for animal products.

2.2.1 Animal feeding studies

Separate feeding studies are required for a ruminant and poultry whenever residues occur in items of feed for these animals. Lactating cows and laying chickens are normally the animals of choice. Pig feeding studies may also be required if accumulation of residues in tissues is likely, if residues occur at a significant level in major components of pig feed, or if metabolism of the pesticide in pigs is significantly different from that in the cow and poultry.

Often the parent pesticide is the residue of concern and it should be incorporated into the diet to simulate, as far as practical, a normal residue. In other cases, a metabolite or metabolite mixture may be needed for the study. For a definition of the residue of concern, see FAO Plant Production and Protection Paper No. 20 - Pesticide Residues in Food, 1979.

The feeding study should include a control group, a group dosed with the expected level of intake (lx) and one group dosed with an exaggerated level (3-5x occasionally a higher level (lOx) may be needed). The latter allows an estimate of what will happen if the normal level is exceeded, will indicate whether residues are proportional to intake and will provide additional data in the event that new uses of the product are introduced.

The calculation of the ingestion level (lx) should take into account all the residues on various feed materials which may form part of the animal's diet. The composition of animal diets will vary from country to country but the only information readily available pertains to the USA and is given in the current EPA Guidelines (subdivision 0: Residue Chemistry Oct. 1982, pp 40-57).

As an example, in choosing the dosage level based on total rations, the proportions in the diet of the feed item bearing the residue must be considered. For example, dried citrus pulp may in some circumstances comprise up to 20% of the total ration (dry weight basis) of dairy cows. If a residue of 5 ppm of a given pesticide were likely on dried citrus pulp, the total diet (dry weight basis) should be fortified at the 1 ppm level to reflect the expected level of intake (lx). If additional residue occurs on other feed items which could also be fed in combination with citrus pulp, the contribution from these feed items should also be added in.

However, there are still problems in selecting the dose level (ix). The components of the diet will never all contain residues at the maximum residue level. Nor in fact will each component always be treated material. In addition, if a wide range of produce is treated, the sum of each component will give a total well above 100% of an animal's diet. As an example from the EPA table, if alfalfa, almonds and apples are treated, the resulting hay, hulls and pomace could amount to 130% of an animal's feed intake.

Clearly a sensible judgement of the expected ingestion level (lx) must be made and it is suggested that the probable contribution from the typical residue level on a range of feed components should be calculated.

For these feeding studies, the size of groups treated with each dose level should be a minimum of 3 for larger animals (cows) and 5-10 for poultry (laying chickens), although the control groups may be smaller. Cows should be in mid-lactation, producing an average milk yield and chickens should be in full egg production before dosing is started. If it is considered desirable to study the decline of residues after cessation of dosing, additional animals should also be treated.

To administer the required dose, a portion of the animal's feed (normally the concentrate ration) may be treated with the chemical in a suitable solvent (e.g. corn oil) and the required amount fed to individual animals.

For chickens, it may be more convenient to fortify the total feed at the required level and allow the birds to eat ad libitum. It is not recommended that animals are dosed daily with capsules containing the required dose.

Animals are normally dosed for 28 days. Before starting the experiment, analytical checks should be made to ensure the consistency of level and stability of the chemical in the feed over this length of time.

2.2.1.1 Sampling

It is useful to analyse milk and eggs on two occasions before dosing to determine background levels. Samples should also be analysed at least twice weekly during the feeding period. This enables the development and establishment of plateau levels to be checked. Milk samples from individual animals should be analysed separately.

Animals should be sacrificed within 24 hours of the end of the feeding period and the following samples should be taken for analysis:

Cows and/or pigs	Meat (fore and hindquarter and diaphragm muscle) 1 (kg) Fat (subcutaneous and renal) (1 kg) Liver and kidney (entire organ or 1 kg)
Chickens	Meat within overlaying skin and any associated fat (composite sample of leg and breast) (0.5 kg) Liver (entire organ) and all abdominal fat.

All samples from individual animals should be analysed for the dosed chemical (normally parent pesticide) and any major metabolites with toxicological significance (see FAO Plant Production and Protection Paper No. 20). Analytical methods should be sensitive to residues at levels of 0.01-0.05 mg kg -1 but lower limits of determination may be needed for whole milk. For fat-soluble compounds, where residues can also be expressed on a fat basis, it is desirable to determine the fat content of the milk.

2.2.2 Animal dermal treatment studies

Animal dermal treatment studies, which are required for cows, pigs, chickens and other species when direct treatment is proposed, often present special problems.

The most common methods of application are by dipping or spraying. The formulated product should be applied in the recommended manner or used in a close simulation of this. Residue data reflecting exaggerated rates of use (x 2) are desirable provided such application rates do not cause ill effects in the animals. The concentration of pesticide in the working solution is the primary consideration in dips and sprays. When dips are used, instructions are needed for recharging and maintaining a constant strength in the dip tank, and for the disposal of spent dip solutions. Factor which may affect the deposition of residues on the animals should be taken into account in planning the study, e.g. - state of the animals' coat, the maximum number of retreatments, duration of the animal in the dip tank, nozzle type, pressure, or delivery rate of sprays, and the amount of solution to be applied per animal for "pour-on" or other specialized treatments.

When application of pesticides is made by automatic devices (i.e. photoelectric or treadle actuated sprays) or backrubbers, they should be used to give maximum treatment.

Samples of milk and eggs should again be taken at intervals after treatment. At the anticipated withholding interval, the animals should be sacrificed and samples taken for analysis as described under Section 2.2.11.

Extreme care should be taken in collecting these samples to ensure that residues on the skin of the animals are not transferred to the meat. If the withholding interval, between treatment and when animals may be slaughtered in normal practice, has not been defined, consideration should be given for the inclusion of additional animals to monitor the residues present at different intervals after treatment. Where treatments will be repeated, the possibility of stepwise accumulation should be studied.

3. STORAGE OF SAMPLES

When samples are taken, these should be labelled unambiguously in waterproof ink and frozen within a few hours of collection. The samples should be stored in airtight containers at or below -20□C. Samples should be analysed as quickly as possible after collection, before physical and chemical changes take place. If prolonged storage of residue samples is unavoidable, it is essential to carry out a separate study to confirm the stability of the residue over the storage period.

Extended storage in freezers can cause moisture to migrate to the surface of a sample and then to the freezer coils, thus slowly desiccating the sample. This effect may result in an increase in the calculated residue level.

Milk samples should be stored in partially filled glass, plastic or aluminium bottles. Glass bottles, although fragile, may have advantages where the residue may be absorbed onto a plastic surface. Prolonged storage of milk is highly undesirable as the emulsion becomes broken on thawing. It is then virtually impossible to obtain a representative sample.

4. REPORTING OF RESIDUE TRIALS

Before commencing these studies, a protocol should be drawn up and agreed by all parties contributing to the study.

At the completion of a study, a full report should be produced to include the following:

4.1 In-Life Part of the Study

This should give details of:

- the type (age, breed, strain), number, housing and weight of the animals used.
- the preparation of the dose and the timing and means of administration or application.
- the feeding of the animals and any unusual behaviour or health effects noted.
- the collection of milk and eggs during the study. Also urine and faeces in the radiochemical study.
- the slaughter and sampling of the animals at the end of the feeding period.
- the labelling of the samples.
- the names of the personnel involved in this phase of the study.

In the planning stage of a study, it is useful to consult Sections 8 and 9 of Part I of these Guidelines for further information on sampling and reporting.

4.2 The Analysis of the Samples

This should report on:

- purity of the test chemical and specific radio-activity (if appropriate).
- stability checks and analysis of levels of chemical in the doses or application. Also analysis to check the uniformity of feeding levels where appropriate.

- stability of the chemical in the samples or in appropriately "spiked" samples and also the extractability from samples.
- receipt, storage, preparation and extraction of the samples.
- analyses performed and the results of these analyses on both identification of products (where appropriate) and levels of residue found.*
- names of personnel involved in this phase of the study.

*It is useful to include examples of key spectra and charts from chromatographs.

APPENDIX 1

SUGGESTED PROCEDURES FOR DOSING ANIMALS WITH RADIOLABELLED CHEMICALS

Goats

Goats are very sensitive and can easily cease lactation. Milk supply is often better in the summer months. The doses of radiochemical can be administered as follows:

A small quantity of powdered food material (e.g. 100-200 mg of cow concentrate pellet) is inserted into a hard gelatin capsule. A solution of the radiochemical in a volatile solvent (50% of a daily dose in 50-100 ul of solvent) is added and the solvent allowed to evaporate. The lid of the capsule is sealed in place. The capsule is wrapped in a leaf which it is known that the goat likes, and this is offered to the goat. Additional leaves are then offered to ensure total ingestion of the sample. This procedure is carried out at the morning and afternoon milking.

Cows

If cows are used, an alternative to capsule dosing as described for goats is to apply a solution of the radiochemical in a suitable solvent to a sample (500 g) of the feed concentrate pellets contained in a plastic bucket. Additional untreated pellets (200-500 g) are mixed with the material in the bucket and the cow is allowed to eat this.

It is convenient to apply half the required daily dose to the pellets and administer this at the morning milking. A second dose is given at the afternoon milking. Before starting the study, a check should be made to see that the cow will readily eat the concentrate pellets.

Chickens

A total daily dose is inserted into a gelatin capsule filled with powdered chicken food as described under goats. After sealing, one capsule is administered each day to the chicken by force feeding.

Pigs

(a) A capsule containing the dose is prepared as under goats. This is mixed with a small amount of the pigs food. The total is readily eaten by the animal and extra food is given to ensure proper swallowing of the dose.

(b) Some experts prefer to hold the pig securely, open its mouth and insert a stomach tube. The radiochemical is fed through the tube in solution and the dose is washed down with additional amounts of water or oil.

COLLECTION OF EXCRETA

Cows

The cow is fitted with a harness leading to a device for separating urine and faeces. The faeces is collected in a bag suspended from the harness and the urine is led away through a tube to a suitable collecting vessel.

Alternatively, the animal can be catheterized. This is a convenient way of collecting urine but may lead to an infection which can require antibiotic treatment. Such treatment will affect the gut microflora and could invalidate study.

Goats

A goat is kept in a metabolism crate fitted with a strong, wire mesh floor.

The urine and faeces fall through the mesh on to fine, sloping mesh. The urine drains through this and is run away into a suitable container. The faecal pellets roll down the mesh and can be collected in a separate container. This system does not give total separation but separation is adequate for these studies.

Chickens

Chickens are maintained in individual battery cages. The eggs roll from the front of the cage and the excreta falls through the wire mesh floor on to a collection tray.

Pigs

The pig is kept in a metabolism crate. The faeces falls through the floor and can be collected. The urine is collected by catheterizing the animal.