Pest Management Regulatory Agency
Agence de réglementation de la lutte antiparasitaire

Dir98-02

Regulatory Directive

Residue Chemistry Guidelines

The Pest Management Regulatory Agency (PMRA) has finalized the *Residue Chemistry Guidelines* (RCGs) as Regulatory Directive Dir98-02. Petitioners are requested to utilize these guidelines in preparing, conducting and reporting experimental studies in support of residue chemistry data requirements. These guidelines are the result of considerable consultation with stakeholders and have been harmonized with the United States Environmental Protection Agency's (EPA) RCGs. Since the PMRA and the EPA RCGs are identical, the preparation of experimental data using either guideline will fulfill the data requirements of both regulatory agencies. The adoption of a common zone map by the PMRA and the EPA maximizes the use of field trial residue data to meet the RCG data requirements. Data waiver requests will always be considered when supported by a scientifically defensible rationale as to why nonconformance to data requirements should be considered as acceptable to the PMRA.

These guidelines were developed to assist the petitioner in submitting a scientific data package that will proceed smoothly and expeditiously through the scientific evaluation/assessment process. The data generated in these studies will allow PMRA scientists to evaluate the validity of each study and to elucidate the nature and magnitude of residues in treated foods. Joint reviews and work sharing will be greatly facilitated with the harmonization of the PMRA's and the EPA's RCGs.

A phase-in period will be permitted to provide petitioners with the time necessary to perform studies as per the RCGs and, hence, move towards more consistent conformance to RCG data requirements. Studies started after December 31, 1998, should be in conformance with these guidelines.

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Table of Contents

PEST MANAGEMENT REGULATORY AGENCY

RESIDUE CHEMISTRY GUIDELINES

SECTION 0

BACKGROUND

Introduction

The fifteen sections contained in this document comprise the Pest Management Regulatory Agency (PMRA) *Residue Chemistry Guidelines* (RCGs). These Guidelines represent a significant achievement in the movement towards harmonizing data requirements related to pesticide regulation. For the first time, zones or regions have been scientifically defined as being unique for the purpose of determining the location(s) of supervised residue field trials within and between two countries. These Guidelines are intended to meet data requirements of both the *Food and Drugs Act and Regulations* (FDAR) and the *Pest Control Products Act and Regulations* (PCPAR).

The PMRA - U.S. Environmental Protection Agency (EPA) Harmonized Residue Chemistry Guidelines

These new Guidelines provide petitioners with specific guidance to allow supervised, crop field, trial data to be gathered from specifically defined regions or zones identified on Geographical Information System (GIS) generated maps of the U.S. and Canada. Where these zones or regions overlap into Canada and the U.S., they are considered equivalent, and residue data generated within these zones/regions are all valid. The zone maps will reduce by a substantial amount the need for industry to provide done-in-Canada data.

As part of the Canada-United States Trade Agreement (CUSTA), and latterly, the North American Free Trade Agreement (NAFTA), the *Residue Chemistry Guidelines* were also vetted through the U.S. Environmental Protection Agency (EPA) Office of Prevention, Pesticides and Toxic Substances (OPPTS) to harmonize data requirements, specific advice, criteria and reporting formats. These harmonization efforts have resulted in an agreement between the U.S. EPA and the Canadian PMRA to consider residue chemistry reviews prepared in one country as acceptable to support import or domestic maximum residue limits (MRLs)/tolerances in the other country, assuming comparable use patterns for each country.

These *Residue Chemistry Guidelines* have been vetted through the Crop Protection Institute (CPI) of Canada and were revised wherever possible to meet the wishes and needs of the Canadian agricultural industry.

The Guidelines

The *Residue Chemistry Guidelines* describe the nature of the scientific data required to support a petition for the registration of an agricultural chemical in Canada. All of these residue chemistry guidelines describe the scientific data requirements of the Food Residue Exposure Assessment Section (FREAS) of the Health Evaluation Division (HED), of the PMRA. These scientific data are necessary to explain the qualitative and quantitative nature of the residue(s) in plant and animal foods. In addition to scientific data requirements, these Guidelines also provide guidance to the petitioner on the criteria and protocols that should be followed for the design, performance, and validation of scientific studies and for the reporting of scientific data.

The scientific studies and information in the *Residue Chemistry Guidelines* are required for the PMRA scientists to evaluate and assess the nature of residues in foods, both plant and animal. In addition, this information is used for dietary risk assessments. Also included in these Guidelines is specific guidance related to supervised crop residue trials conducted in Canada and/or the U.S.

Evolution of the Guidelines

These Guidelines evolved over many years of evaluation by scientists at Health Canada. Prior to formalizing all residue chemistry data requirements, scientists in the Food Residue Exposure Assessment Section of HED consulted the scientific guidance provided by other countries that employ regulatory systems, and by the Food and Agriculture Organization (FAO) of the United Nations.

In particular, the guidance provided by the U.S., Germany, Australia, the United Kingdom (UK) and the FAO was compared and contrasted. All information from each country's regulatory system and the FAO United Nations body was merged and harmonized as appropriate, to provide comprehensive, clear and scientifically defensible data requirements.

The U.S. EPA, *Residue Chemistry Guidelines*, was especially utilized, and attempts were made to harmonize Canada's data requirements for residue chemistry to those of the U.S. EPA.

Data Requirements

Although there are several new and/or extended data requirements that were not previously required or explicitly defined, every effort was made to avoid unnecessary data requirements.

The data requirements of these *Residue Chemistry Guidelines* are considered to be those data necessary to evaluate and assess the nature of the residues that may result from the proposed uses petitioned for, or for support of a MRL/tolerance to cover residues in an imported food.

However, it is important to note that petitioners may still be advised to:

- consult with the PMRA's Health Evaluation Division prior to initiating studies as required, and
- provide a scientifically defensible rationale in support of a data waiver.

Summary

Many scientific documents were consulted in an effort to provide the petitioner with the most comprehensive, clear and scientifically valid guidance possible, while at the same time meeting the scientific needs of the PMRA scientists to evaluate and assess the information submitted. A list of acronyms used throughout the PMRA, *Residue Chemistry Guidelines* is attached to this background section

The following *Residue Chemistry Guidelines* should be utilized as a complete document since individual sections reference other sections for further details and/or comparison. Finally, although many resources

and much effort, consultation and attention to detail were utilized in preparing and realizing this document, the petitioner should understand that revisions to this document will be an ongoing process as scientific knowledge changes, evaluation and assessment tools change, or as risk assessment/management strategies evolve. Such changes, however, will always be made in an effort to strengthen the guidance provided and to remove as much regulatory burden as possible.

A list of acronyms and a bibliography are provided below.

Acronyms

AAFC	Agriculture and Agri-Food Canada
ADI	acceptable daily intake
pADI	provisional acceptable daily intake
ae	acid equivalent
ai	active ingredient
ANSI	American National Standards Institute
AOAC	Association of Official Analytical Chemists
BAI	between application interval
BSI	British Standards Institution
bw	body weight
CA	chemical abstracts
CAS#	chemical abstracts services registry number
CFR	*Code of Federal Regulations*
CLI	Canada Land Inventory
CPI	Crop Protection Institute
CUSTA	Canada-United States Trade Agreement
CV	coefficient of variation
DF	dry flowable
DM	dry matter
EC	emulsifiable concentrate
ECD	electron capture detector
EPA	Environmental Protection Agency (U.S.)
FAO	Food and Agriculture Organization (of the United Nations)
FDA	Food and Drug Administration
FDAR	*Food and Drugs Act* and Regulations
FIC	flowable concentrate
FID	flame ionization detector
FPD	flame photometric detector
FREAS	Food Residue Exposure Assessment Section
FTP	flame thermionic detector
GAP	Good Agricultural Practice (Registered)
gap	good agricultural practice (proposed)

GATT	General Agreement on Tariffs and Trade
GC	gas chromatography
GC/MS	gas chromatography/mass spectrometry
GIS	geographic information system
GLC	gas liquid chromatography
GLP	Good Laboratory Practice
HAFT	highest average field trial
ha	hectare (s)
HED	Health Evaluation Division
HPLC	high performance (or pressure) liquid chromatography
ILV	independent laboratory validation
ISO	International Organization for Standardization
IUPAC	International Union of Pure and Applied Chemistry
JMPR	Joint Meeting of the FAO panel of Experts on Pesticide Residues and the Environment and the World Health Organization (WHO) Expert Group on Pesticide Residues
kg	kilogram (s)
LC	liquid chromatography
LOD	limit of detection
LOQ	limit of quantitation
LSC	liquid scintillation counting
LSS	liquid scintillation spectrometry
mg	milligram (s)
MOR	magnitude of the residue
MRM	multiresidue method
MRL	maximum residue limit
MS	mass spectrometry
NAFTA	North America Free Trade Agreement
NMR	nuclear magnetic resonance
NOAEL	no observable adverse effect level
NOEL	no observable effect level
NPD	nitrogen phosphorous detector
OPP	Office of Pesticide Programs
OPPTS	Office of Prevention, Pesticides and Toxic Substances
PAM	*Pesticide Analytical Manual*
PCPAR	*Pest Control Products Act and Regulations*
PDI	potential daily intake
PHI	preharvest interval
PMRA	Pest Management Regulatory Agency
ppm	parts per million
PSACD	Product Sustainability and Coordination Division
PSI	preslaughter interval
RAC	raw agricultural commodity

Rfd	acute reference dose
rh	relative humidity
ROC	residue of concern
RSD	relative standard deviation
SAGA	spatial analysis and geometrics applications
SC	suspension concentrate (= flowable concentrate)
SL	soluble concentrate
SD	standard deviation
SI	International System of Units
TID	thermionic detector
TLC	thin layer chromatography
TRR	total radioactive residue
TSI	Treatment to Sampling Interval
TTR	Total Terminal Residue
U.S. EPA	United States Environmental Protection Agnecy
WDG	Water Dispersible Granules
WHO	World Health Organization
WG	wettable dispersible granules
WP	wettable powder

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PEST MANAGEMENT REGULATORY AGENCY

RESIDUE CHEMISTRY GUIDELINES

SECTION 1

CHEMICAL IDENTITY

1.1 Preface

This Guideline is intended to meet data requirements of both the *Food and Drugs Act* (FDA) and Regulations and the *Pest Control Products Act* (PCPA) and Regulations. This Pest Management Regulatory Agency (PMRA) Guideline should be used in conjunction with the PMRA Regulatory Directives, Dir98-02, *Chemistry Requirements for the Registration of a Technical Grade of Active Ingredient or an Integrated System Product*, and Dir98-03, *Chemistry Requirements for the Registration of a Manufacturing Concentrate or an End-Use Product Formulated from Registered Technical Grade of Active Ingredients or Integrated System Products* or the appropriate Regulatory Directives.

1.2 Introduction

The Agency needs the information to accurately identify components in the technical mixture; to compare compositions of the test substances, i.e., active ingredients, in all chemistry and toxicology required testing; and to identify compounds, other than the active ingredient, that may need to be regulated, i.e., would require a maximum residue limit (MRL) or an exemption from a MRL.

1.3 Agricultural chemicals, including pest control products

Data requirements for chemical identity are essentially the same as those discussed in Regulatory Directives Dir98-02 and Dir98-03, concerning chemistry data requirements for a technical active ingredient and an end-use product, respectively. In addition to those data required in the *Product Chemistry Guidelines*, the petition should include an assessment of whether any of the impurities will present a residue problem. If an impurity is likely to occur as a significant residue in food/feed, then residue data for the impurity, as described in Sections 2, *Nature of the Residue - Plants, Livestock*, through 10, *Processed Food/Feed*, are required. The determination of whether residue data for an impurity are needed will be based on the impurity's stability, toxicity and detectability.

1.4 Information required for other agrichemicals and adjuvants

Adjuvants of the formulation should be fully described, including the chemical name as well as any trade names. Chemical abstracts services (CAS) registry numbers should be included, if available. The chemical names should be in the same form as those for adjuvants as described in the PMRA Regulatory Directive, Dir93-15, *Registration Requirements for Adjuvant Products.* If only the trade name is known, the petitioner should request that the supplier of the adjuvant furnish the descriptive information, including CAS name, structure and purity, directly to the PMRA. Any adjuvant that has not yet been cleared should be indicated, and a request for clearance initiated as described in Section 11, *Proposed MRLs*.

1.5 References

1. Revised Product Chemistry Regulatory Directives, Dir98-02, *Chemistry Requirements for the Registration of a Technical Grade of Active Ingredient or an Integrated System*

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PEST MANAGEMENT REGULATORY AGENCY

RESIDUE CHEMISTRY GUIDELINES

SECTION 2

NATURE OF THE RESIDUE - PLANTS, LIVESTOCK

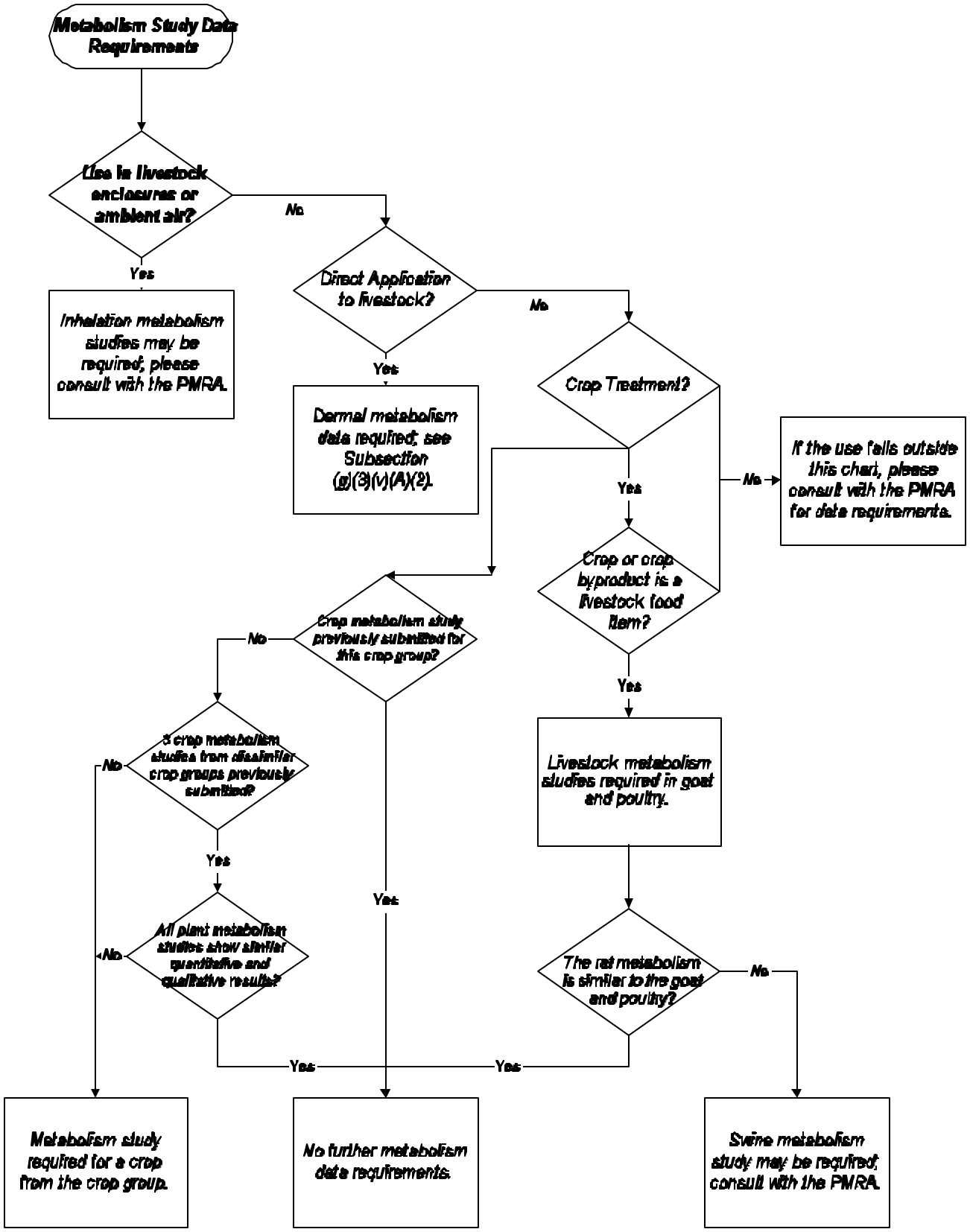
2.1 Preface

This Guideline describes the data requirements of the *Food and Drugs Act* and Regulations (FDAR), the *Pest Control Products Act* (PCPA)and Regulations and the *Feeds Act*.

2.2 Purpose

The purpose of conducting metabolism studies is to determine the qualitative/quantitative metabolic fate, translocation and disposition of the active ingredient, i.e., examine what happens to it when it is applied to a plant or administered to livestock. Many pesticides undergo transformations, i.e., biotic and abiotic metabolism or degradation, during or after application to the soil, water, crop, or livestock. The nature, i.e., composition, of the terminal residue must therefore be determined before complete residue detection methodology and residue quantification data can be developed. To obtain this information, the pesticide is labelled with a radioactive atom(s), to follow the translocation/disposition of the compound to determine the qualitative/quantitative profile of the parent active and its metabolites within a plant or livestock. The determination of whether the residues have been sufficiently characterized/identified is dependent on many factors. Plant metabolism studies are usually required for a minimum of three diverse crops unless the pesticide is to be used on only one or two crops of the same type, i.e., tuber, legume, oilseed, etc. If the metabolism in three diverse crops, i.e., crops whose agronomic characteristics are distinctly different, is similar (metabolic pathways and major metabolites), then the metabolism in other crops is assumed to be similar. If the pesticide is applied to crops used for livestock feed, or if the pesticide is intended for treatment of livestock, then animal metabolism studies are required in addition to plant metabolism data. Animal metabolism studies are generally carried out on ruminants, such as goats, and poultry, such as chickens.

A schematic diagram of the metabolism data requirements for plants and livestock is shown in the figure below:


Metabolism Study Data Requirements
Use in livestock enclosures or ambient air?
Yes
No
Inhalation metabolism studies may be required, please consult with the PMRA.
Direct Application to livestock?
Yes
No
Dermal metabolism data required; see Subsection (g)(3)(v)(A)(2).
Crop Treatment?
Yes
No
If the use falls outside this chart, please consult with the PMRA for data requirements.
Crop or crop byproduct is a livestock food item?
Yes
Livestock metabolism studies required in goat and poultry.
The rat metabolism is similar to the goat and poultry?
No
Swine metabolism study may be required, consult with the PMRA.
Yes
Crop metabolism study previously submitted for this crop group?
No
Yes
3 crop metabolism studies from dissimilar crop groups previously submitted?
No
Yes
All plant metabolism studies show similar quantitative and qualitative results?
No
Yes
Metabolism study required for a crop from the crop group.
Yes
No further metabolism data requirements.

2.3 Introduction

2.3.1 General

i) While in vitro data are useful to show if the pesticide is likely to undergo hydrolysis (acid, base, or enzymatic), oxidation or reduction, photolysis, or other changes, additional data must usually be submitted to show the fate in the plants and animals. These metabolism studies are required whenever a pesticide use is determined to be a food use. Based on the results of the characterization/ identification studies, the chemical definition of the residue of concern (ROC) should be proposed by the petitioner and confirmed by the Agency Metabolism Committee. The term ROC is used to describe the sum of the parent pesticide and its degradation products, metabolites and impurities that are of toxicological concern. All components of the ROC will normally be included in the maximum residue limit (MRL) expression for the pesticide, and residue analytical methods must be developed for all components of the ROC. In addition, if a residue(s) exceeds 0.1 ppm, then under Division 15 of the FDAR (B15.002(1)) said residue(s) must be included in the ROC subject to an exemption under Division 15 (B15.002(2)) of the FDAR. Alternately, an exemption may be possible if the petitioner can, using scientific studies, show that the residue is qualitatively and quantitatively identical to a naturally occurring compound.

ii) The identification of the components of the terminal residue and the definition of the ROC often present complex problems that must be resolved before finalizing the analytical methodology and gathering the quantitative residue data. Thus, the petitioner may wish to consult with the Agency's chemists and toxicologists to determine whether the residue has been sufficiently characterized/identified, which metabolites should be covered by the MRLs, and which components of the residue must be determined by the residue analytical methodology. The determination of whether the residue has been sufficiently characterized/identified will depend on the level of activity remaining unidentified, the importance of the plant or livestock commodity with regards to dietary intake containing the unidentified residue as a food or feed, the chemical structure of the active ingredient and identified metabolites and the toxicity of chemicals similar in structure to potential metabolites.

iii) The petitioner should delineate, preferably in a flowsheet, the routes of degradation or metabolism in plants and animals, and clearly specify the capability of the analytical method(s) to determine the components of the residue, in the raw agricultural commodity (RAC)/sample and in the marc that remains after conventional extraction, i.e., bound fraction. Photographs or autoradiographs of thin layer chromatographic (TLC) plates, paper chromatograms, or radioautographs of plants treated with labelled pesticides should be furnished. Such evidence will contribute significantly to the evaluation of the data. A metabolic profile of chemical structures elucidating transformation pathways should also be provided as hardcopy and WP format; Chemical Abstracts Service (CAS) and International Union of Pure and Applied Chemistry (IUPAC) names should also be provided as a table to structures identified in the profile by Roman numerals. See the Pest Management Regulatory Agency (PMRA)

Regulatory Directives Dir98-02, *Chemistry Requirements for the Registration of a Technical Grade of Active Ingredient or an Integrated System Product*, and Dir98-03, *Chemistry Requirements for the Registration of a Manufacturing Concentrate or an End-Use Product Formulated from Registered Technical Grade of Active Ingredients or Integrated System Products* or the appropriate Regulatory Directives.

iv) The petitioner should always be alert to the possibility of new and unexpected metabolites of the pesticide that may affect future MRL proposals. Where the structure of a metabolite or transformation product is identical to another registered pesticide chemical, the petitioner should state this fact.

2.3.2 Nature of the residue in plants

i) The term, plant metabolism, is used here for convenience to describe the formation of all transformation products of the pesticide in or on plants, regardless of whether they result from plant metabolic processes. Adequate plant metabolism studies fulfil at least four purposes:

A) They provide an estimate of total terminal, radioactive residues in the treated crops.

B) They identify the major components of the terminal residue, thus indicating the components to be looked for in residue quantification studies.

C) They indicate the distribution of residues, e.g., whether the pesticide is absorbed through roots or foliage, whether translocation occurs, or whether the residues are entirely surface residues.

D) They show the efficiency of extraction procedures for various components of the residue.

ii) A metabolism study must be submitted for each type of plant for which use is proposed and should include one of the representative crops of a crop group (Section 15, *Crop Groups*) if the treated crop is listed therein, or if the petitioner anticipates a future need to establish a crop group MRL. For example, metabolism studies in bean plants would be representative of all legumes but would not be translatable to root crops, such as potatoes or carrots. In general, one metabolism study will be required for each of the crop groups defined in Section 15, *Crop Groups*.

iii) If the results of three metabolism studies on dissimilar crops indicate a similar metabolic route in the three crops, then additional metabolism studies will not be required. The petitioner is encouraged to consult the PMRA about which crops metabolism studies should be

conducted on when several commodities may be treated, to ensure that major dietary commodities are included in those studies selected/approved.

2.3.3 Nature of the residue in livestock

i) The purpose of these studies is to identify the nature of the residue in the edible tissues of livestock, milk, and eggs. Animal metabolism studies are required whenever a pesticide is applied directly to livestock or to crops or crop parts used for feed, or when animal premises are to be treated. Information on whether crop byproducts are used for feed can be obtained from Table I of Section 8, *Meat/Milk/Poultry/Eggs*.

ii) Data on the metabolism of a pesticide in laboratory animals that are required in the toxicology section of these Guidelines will generally not substitute for metabolism data on livestock. Laboratory animal metabolism studies should be provided in support of the general metabolic profile in animals. However, they can be duplicated or referenced in the residue chemistry section of a petition to allow for comparisons of the metabolism in several species. In some cases, laboratory animal metabolism data may be used to supplement livestock metabolism studies in which complete characterization/identification of the residue is not attained.

iii) In general, separate metabolism studies are required for ruminants and poultry. The species of choice are usually goats and chickens. Nonruminant (swine) metabolism studies may be required if the rat metabolism is significantly different than the goat or chicken metabolism. Additional animal metabolism studies are required if direct dermal or inhalation application to livestock is proposed. These additional studies should reflect the proposed use so that it can be determined whether dermal or inhalation exposure results in the same metabolic patterns as oral dosing.

iv) The minimum dosage used in livestock metabolism studies should approximate the level of exposure expected from the feeding of MRL level residues on crops with existing, proposed or anticipated MRLs, or the proposed use rate for direct animal treatment. Exaggerated dosages are usually required to obtain sufficient residue in the tissues for characterization/identification. However, dosages that alter the metabolic profile should be avoided. Regardless, for oral studies, livestock must be dosed at least at a level of 10 ppm, i.e., 10 mg per kg feed, in the diet. Sheep, swine, and goats should be dosed daily and orally for at least three days. Poultry should be dosed for at least three days. The dosing material for oral studies should not be a mixture of active ingredient and plant metabolites. In most cases, this study should involve dosing with only the parent pesticide. In those cases where plant and livestock metabolites are found to differ, a separate study in which livestock are dosed with a unique plant metabolite may be required in addition to the study with the parent compound. Direct animal treatment dosing should reflect the proposed use with regard to the dosing material and mode of application.

v) The Agency strongly discourages predosing of livestock. Due to possible changes in the specific activity of the parent and metabolites, predosing may result in low levels of radioactivity in tissues, milk and eggs so as to both mask the degree of residue transfer and preclude the identification of the components of the terminal residue. Also, the resulting differences in specific activities of components of the total radioactive residue may make the comparison of relative amounts of parent and metabolites problematical. However, the acceptability of studies employing predosing will be considered on a case-by-case basis. If the radioactivity levels in such are too low, so as to preclude identification of residues, the study will need to be repeated without predosing the animals.

vi) Animals should be sacrificed within 24 hours of cessation of dosing.

vii) Milk and eggs should be collected twice daily. Tissues to be analyzed should include at least: muscle, liver, kidney (ruminants only), and fat. Characterization of the residue in urine and feces frequently facilitates characterization of the lower levels of residue found in tissue, but is not required.

viii) The 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. The livestock metabolism study should also result in elucidation of the efficiency of extraction of the various components of the residue so that extraction/residue release, i.e., solubilization, procedures can be developed as part of the analytical methods.

2.4 Discussion of test method

2.4.1 Application of radiolabeled pesticide

The first consideration in designing a metabolism study is radiolabeling. The radiolabel should be positioned in the molecule so that potentially significant toxicological moieties or hydrolytic degradation products can be tracked. The study shall be conducted using the radiolabeled analytical grade ingredient. If multiple ring structures or toxicologically significant sidechains are present, separate studies reflecting labeling of each ring or sidechain will normally be required. In choosing the position to be labelled, assurance is required that a labile position is not chosen. This should involve ring labelling (preferred) or even double labels, i.e., molecules containing two rings are labelled in both, or each ring is labelled in separate experiments. Carbon-14 (^{14}C) is the preferred isotope when possible, although isotopes of phosphorus (^{32}P) and sulphur (^{35}S), or other elements may be more appropriate if no carbons or only labile carbon sidechains exist in the molecule. The use of tritium (^{3}H) as a label is strongly discouraged. If a potentially labile sidechain or tritium labelling is chosen, a metabolism study will be considered adequate only if all significant activity in the plant or animal is identified and found to be associated with the pesticide, and not related to loss of the label from the basic structure of the pesticide molecule. Other issues, such as

high specific activity, are desirable and generally part of good experimental design in metabolism studies involving radiolabeled compounds.

Other initial considerations include the method of application and the application rate of radiolabeled pesticide to be used. Since the primary purpose of a metabolism study is to identify the chemical components of the residue, the application rate must be high enough to result in sufficiently high radioactivity levels to allow for characterization/ identification of the residue. A rate of at least 1X the registered application rate should generally be used for plant metabolism or dermal livestock metabolism studies. In the case of oral livestock metabolism studies, the dose should, at a minimum, approximate the maximum anticipated dietary burden, but in no instance should the level be less than 10 ppm in the diet, i.e., 10 mg per kg of feed. However, for certain pesticides/uses it is necessary to apply radioactive material at exaggerated rates. The decision as to what rate to utilize is contingent upon several factors. For example, in the case of herbicides, phytotoxicity that may stress or even kill the plant(s) may limit the exaggerated rate that can be used. For all pesticides, the minimum application rate required to allow adequate characterization/identification of residues, up to a maximum of 10X as discussed further below, must be utilized in plant metabolism studies unless reasons such as phytotoxicity prevent this. Safety concerns when using large amounts of radioactivity must also be considered. In addition, the following should be considered when selecting the dosing material, a method of application, and an application rate or dosage for plant or livestock metabolism studies:

i) The plant should be treated with parent, preferably as the formulated product as applied in the field, i.e., including any on-site tankmixed adjuvant. If parent is applied in a solution (solvent carrier only) then the petitioner should ensure that the solvent or an additive in the solvent is not used if it is a photosensitizer, e.g., acetone, riboflavin.

ii) Livestock metabolism studies should reflect feeding of one compound, usually the parent. If the plant metabolites are also found to be animal metabolites, then additional livestock metabolism experiments that involve dosing with plant metabolites will not generally be required. If a plant metabolite comprises a major portion of the total terminal residue (TTR) on a feed item or is not found to be an animal metabolite, additional livestock metabolism studies involving dosing with the plant metabolite may be required.

iii) The specific activity of the labelled material should be as high as necessary to assure acceptable limits of detection for radioassay of ^{14}C- residues. In cases where there has been little or no characterization/identification of the residue, in crops, milk, eggs, or animal tissues because of low levels of activity, the Agency will make a determination as to the adequacy of efforts that the Petitioner has made to maximize specific activity, such that application rates would yield characterizable/identifiable levels of radioactivity in edible plant parts or livestock commodities.

iv) In cases where low levels of radioactivity are observed even at exaggerated rates, utilization of adjuvants or typical inerts may enhance absorption of the active ingredient into the plant or animal (dermal).

v) Selection of specific crops and use patterns should reflect the situation where the highest amount of radioactivity would be expected in the edible portions of the plant at harvest. If a pesticide has two distinct use patterns that could lead to different metabolic situations, e.g., preplant soil application and a foliar treatment, then two metabolism studies may be required.

vi) If exaggerated application rates of a phytotoxic herbicide are necessary to achieve sufficient radioactivity for characterization/identification of residues, and the required rate causes phytotoxicity in the plant, metabolism information on the sick plant is preferable to having no information due to lack of sufficient radioactive residue. However, use of alternative techniques, such as tissue culture, excised plants, and analysis of immature crop parts, is preferable to using sick plants resulting from treatment at rates that cause phytotoxicity.

2.4.2 Sampling of plant parts

Samples of all RACs, as defined in Table I of Section 8, should be obtained for characterization/identification of residues. In some cases, collection of samples of immature plant parts not in Table I of Section 8 may be considered as an aid to facilitate the characterization/identification of residues when low residue levels are expected in the mature plants. Although collection of immature plant parts not in Table I is not required, it may facilitate characterization/identification of residues in cases where the trigger values as discussed below, subsection 2.4.4, are exceeded, but residues present unusual difficulties in characterization/identification due to low residue levels or the nature of the metabolites. Note that materials such as corn forage are immature plant parts but are considered to be RACs. These data may provide adequate information to allow conclusions to be drawn about the identity of residue in mature parts of the plant. Petitioners may also wish to use mature but inedible crop parts, e.g., apple leaves or potato foliage, to help identify residues on the mature RAC. However, if this information is to be used in support of the study, evidence of similar chromatographic profiles for residues in mature edible and inedible plant portions is preferred.

2.4.3 Analytical phase

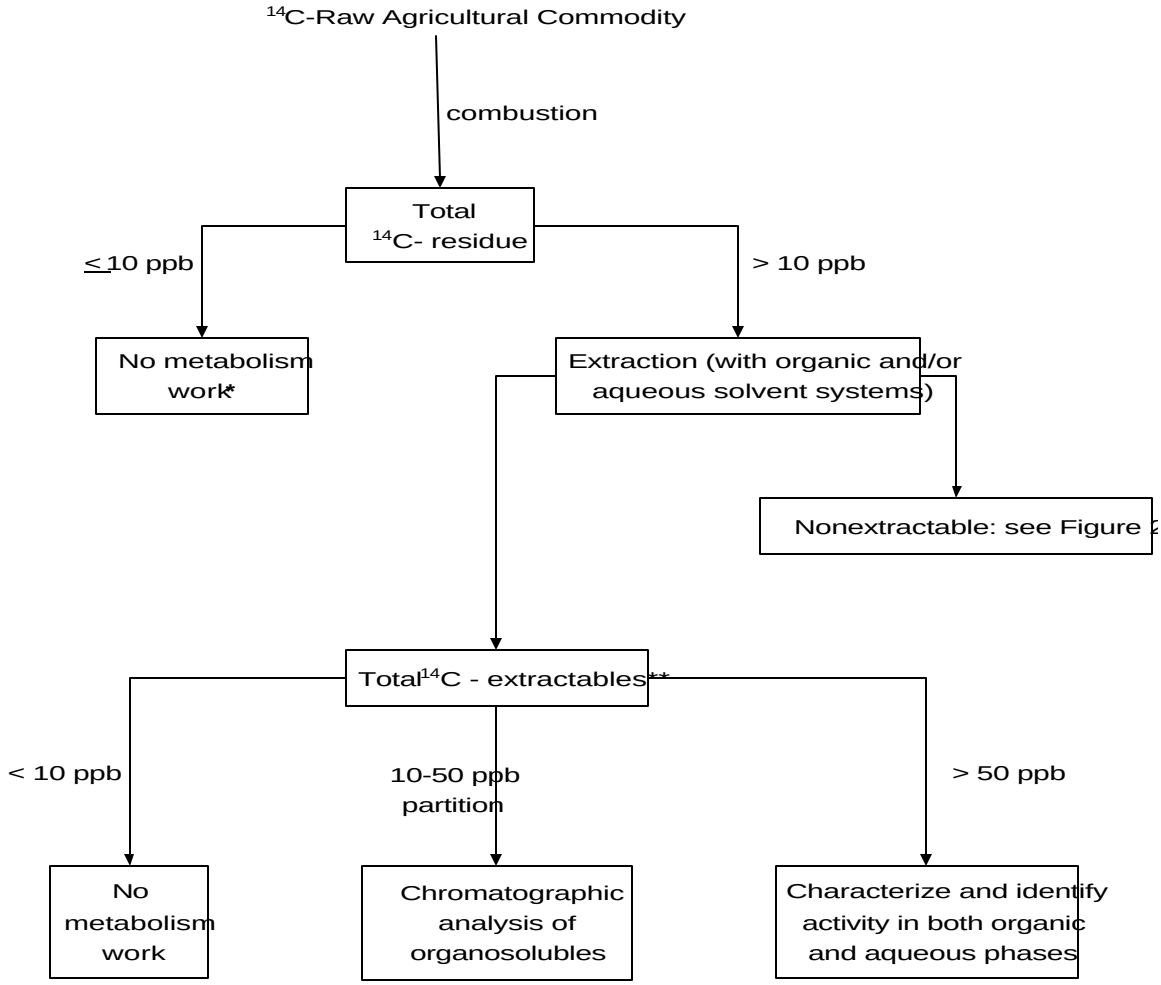
In the analytical phase of a plant/livestock metabolism study, the plant/animal parts to be analyzed are sampled, chopped or homogenized, total radioactivity is determined and the samples are extracted with a series of solvents and/or solvent systems, including aqueous, with various polarities and other characteristics, depending on the nature of the expected residues. These initially obtained residues are defined as extractable residues. The required characterization/identification of extractable residues is summarized in Figure 1. This is a diagram of trigger values described in subsection 2.4.4.

Before discussing Figure 1 in greater detail, the terms characterization and identification of residues will be defined as follows:

i) Identification refers to the exact structural determination of components of the total terminal residue. Typically, this is accomplished by comparing chromatographic behaviour to that of known standards and/or actual spectroscopic analyses, i.e., mass spectrometry (MS), nuclear magnetic resonance (NMR), etc.

ii) Characterization refers to the elucidation of the general nature/characteristics of the radioactive residue short of metabolite identification. Terms used to characterize residues include the following: organosoluble, water or aqueous soluble, neutral, acidic or basic, polar, nonpolar, nonextractable/bound, etc. Characterization may also involve descriptions of chemical moieties known to be present in the molecule, based on conversion to a common structure or due to reactivity with particular reagents. The degree of characterization refers to how close the assignment comes to structural identification. When identification of radioactive residues is not accomplished, the degree of characterization required for a portion of the total radioactivity will depend on several factors, including the amount of residue present, the amount of the total terminal residue already identified, the importance of the crop part as a food or feed, toxicological concern over a class of compounds, the suspected significance of the residue as determined by characterization already performed, and the capability of analytical methods to detect characterized, i.e., by conversion to a common moiety, but unidentified residues. This radiovalidation of the method is important both for future development of enforcement methodology or where a significant amount of radioactivity is observed in a matrix. When the radioactivity consists of a large number of individual moieties at concentrations below trigger values, these may be converted into one or two distinct compounds by procedures such as oxidation or hydrolysis. Therefore, the terms, characterization and identification, clearly have different meanings and should not be used interchangeably.

Figure 1 Strategy for Identification/Characterization in Metabolism Studies of Extractable Residues from Plants and livestock (* = unless toxicological concern, ** = refer to Fig. 2).



Identification of metabolites must be established using two different analytical techniques except when (a) unambiguous identification is made using a spectroscopic method, such as gas chromatography/mass spectrometry (GC/MS), etc., or (b) the metabolite is determined to be of minimal toxicological importance due to its low absolute level (<0.05 ppm) or percentage of the total terminal residue (<10% of TTR). In the case of (b), identification by one technique, such as coelution, with standards will be acceptable. These trigger values are meant as rough guidance and may not apply to situations where a metabolite is suspected to be of toxicological concern, or where <10% of the TTR represents a high absolute residue level. In general, the Agency will not consider chromatographic techniques utilizing the same stationary phase with two different solvent systems to represent a two-method verification of metabolite identity.

2.4.4 Strategy for determining when identification of metabolites is needed

Figure 1 illustrates the strategy for extractable polar and nonpolar residues, developed by Ciba-Geigy, reference subsection 2.8(2), and initially applied primarily to animal metabolism studies. The radioactivity trigger values shown in Figure 1 reflect the characterization/identification required for each RAC. If total radioactivity in a crop/animal part is . 0.01 ppm (10 ppb) or less, no differentiation of the radioactivity would be required unless there are toxicological concerns for residues occurring at lower concentrations. For radioactivity greater than . 0.01 ppm, the sample should be extracted with solvents and/or solvent systems, including aqueous, of various polarities. The levels of extractable and nonextractable activity should then be quantitated to determine the degree of characterization that is needed. If the extractable radioactivity represents . 0.01 ppm or less, it need not be examined further. For extractable radioactivity of . 0.01-0.05 ppm, the partitioning behaviour between aqueous and organic solvents should be determined followed by chromatographic, i.e., TLC, high performance liquid chromatography (HPLC), analysis of the organosoluble activity. The chromatographic behaviour of this activity can be compared to that of the parent pesticide and likely metabolites, i.e., characterization and/or identification. When the extractable activity exceeds . 0.05 ppm, complete characterization and identification should be attempted for both organic and aqueous radioactivity.

It is important that the components of the aqueous soluble portions of the radioactivity be identified since they may contain toxic compounds. For the aqueous soluble portion of the activity, however, the trigger values for characterization and identification would be levels down to 0.05 ppm or 10% of the TTR, whichever is greater. The exception for this would, of course, be toxicology concerns over potential residues that might occur at lower levels. Identities of metabolites should be confirmed with a second technique, i.e., spectroscopic, if possible, as discussed above.

The term, complete characterization and identification, for extractable residues above 0.05 ppm does not necessarily mean that individual components at this level need to be identified. Low level, in terms of both ppm and % of total residue, individual residues do not typically need to be identified if the major components of the residue have been identified. For example, if the total activity in a crop part is 3 ppm and 75% of that has been firmly identified, it is unlikely that

identification of a series of individual residues in the 0.05-0.1 ppm range would be required. On the other hand, extensive efforts toward identification of 0.05-0.1 ppm residues would be expected when the total activity is only 0.3 ppm.

The radioactivity levels shown in Figure 1 apply regardless of the application rate used in plant metabolism studies. However, this is not meant to discourage the use of exaggerated application rates necessary to provide sufficient radioactivity for adequate delineation of the plant metabolism. If application rates are used that are insufficient to provide adequate radioactivity for characterization/identification of residues, additional studies may be required at increased application rates up to the point of unacceptable plant phytotoxicity. The maximum exaggerated rate that will be required for a plant metabolism study is 10X for situations where low residues are present on feed items. It is important to note that plant metabolism studies with little or no identification of residues will not normally be acceptable to support new uses that reflect different kinds of treatments, especially modes of applications that result in higher residues. Supercritical fluid extraction or microwave extraction are recommended as very efficient, nondestructive techniques to solubilize residues.

2.4.5 Release of nonextractable/bound residues

The remainder of this discussion will pertain to nonextractable/bound radioactive residues and will provide guidance on what steps need to be taken to provide enough information to allow the Agency to draw conclusions as to the terminal residue of concern in plants/livestock.

There are three situations in which radioactive residues are observed to be nonextractable in plants/livestock.

i) Incorporation into biomolecules , i.e., amino acids, sugars, etc., that occurs when the test compound is degraded into small, usually one or two carbon units that enter the carbon pool, and that the plant/animal uses to build new compounds.

ii) Chemical reaction with appropriate moieties in biomolecules to form bound residues that can be released via other chemical reactions, e.g., enzymatic or acid/base hydrolysis.

iii) Physical encapsulation or integration of radioactive residues into plant/livestock matrices, such as cellulose and lignin for plants. Release of residues in this situation may require solubilization of the tissue, usually by drastic treatment with base, although use of surfactants or ultrasonication may allow the radioactive residue to be released under less severe conditions.

The following schematic, Figure 2, for dealing with nonextractable/bound residues is intended to provide clarification of Agency policy as well as more specific guidance regarding characterization/identification of these residues.

The extracted solid plant/animal material from Figure 1 should be assayed, and if radioactivity is present down to the trigger values of 0.05 ppm or 10% of the TTR, whichever is greater, release of the activity should be attempted. See Figure 2. It is emphasized that, if toxicology expresses concerns over potential residues at lower levels, the trigger values will not necessarily apply.

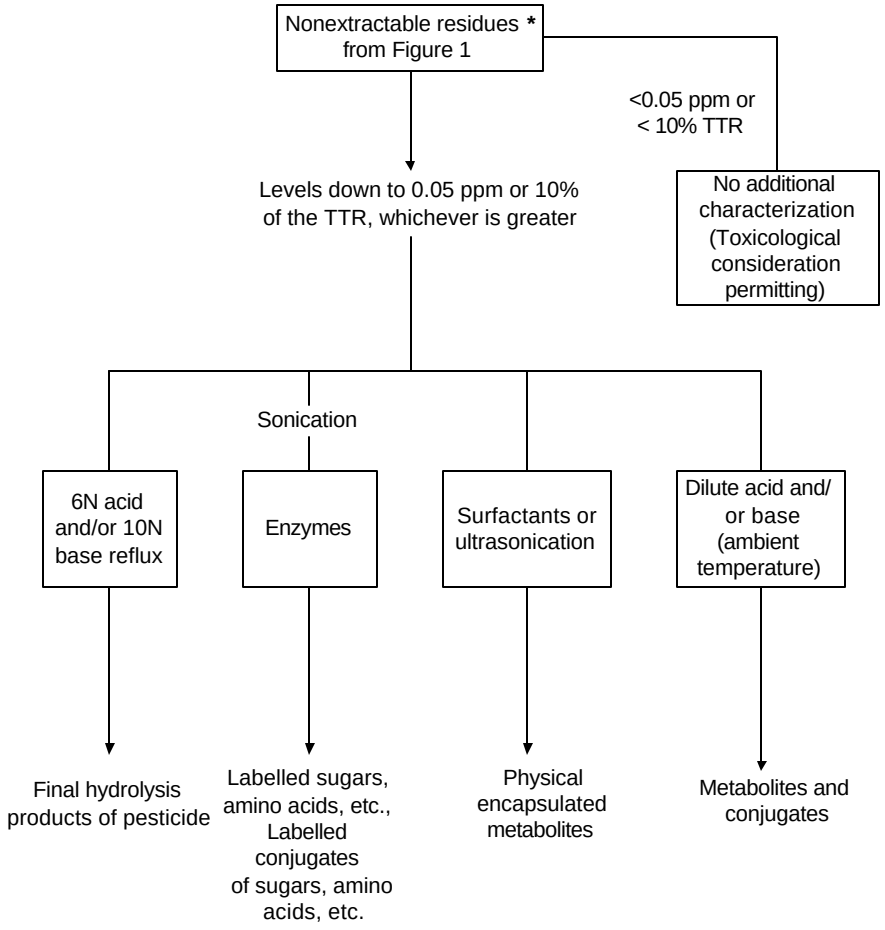
Treatments may be performed on either subsamples or sequentially. The types of treatments include dilute acid and base at ambient temperatures. Note that these procedures should be employed initially for both metabolism and method development considerations, surfactants, enzymes and 6N acid and/or 10N base with reflux. It should be kept in mind that the milder procedures provide more accurate assignments of metabolite structures released, i.e., acid/base reflux would probably release moieties as their final hydrolysis products that could have only a minor relationship to the conjugated form of the radioactivity.

An ambient temperature acid treatment followed by ambient temperature base treatment will provide a mild hydrolysis of conjugated moieties, and again possibly release any biomolecules containing incorporated radioactivity. The use of surfactants may release physically encapsulated or membrane bound residues. Because membrane and/or cell wall disruption may improve substrate accessibility to the enzyme, a sonication step should be employed followed by a carefully chosen enzymatic battery. Note that in each case, the activity of each enzyme utilized should be confirmed using standard substrates and controls. These experiments should be documented. These steps could release chemically bound residues, including any biomolecules containing incorporated radioactivity. The final release steps would involve reflux acid and base hydrolysis that will likely solubilize the plant/animal tissue. Radioactivity released at this time would probably reflect amino acids, sugars and encapsulated or conjugated compounds that may or may not have any relationship to the original bound/encapsulated structures. However, this step does provide evidence that residues of the pesticide can be released, and may provide data on incorporated radioactivity and limited information about the nature of the metabolites. See the discussion above.

In all cases, samples, homogenates and extracts should be buffered and maintained at low temperatures except during hydrolytic steps, in order to reduce degradation/artifact formation. See the discussion in subsection 2.4.7 regarding storage stability in metabolism studies.

Figure 2 provides a visual description of the steps discussed above.

Figure 2: Characterization/Identification of Unextractable/Bound Residues



* Other novel extraction techniques, in addition to those required above, may be used if scientific evidence is provided to support the solubilization of residues as their in situ exocons or conjugates.

Return to Figure 1. at **

Comments on Figures 1 and 2

1. At each step in Figure 2, the radioactivity of the released residues should be quantitated, and if the trigger values shown in Figure 1 for extractable residues are met, the activity should again be partitioned against various solvents/solvent systems and characterized and/or identified as required. With respect to characterization, it should be emphasized that the chromatographic behaviour of the released activity, including water solubles, should be compared to that of the parent and likely metabolites that are close in structure to the parent. This will indicate whether the released activity is chemically different from the parent

molecule. If the remaining unextracted activity after a given procedure is <0.05 ppm or <10% of the TTR, further attempted release of activity is not necessary.

2. The trigger values shown in Figure 1 are meant to negate the need for characterization/identification of metabolites present at very low and insignificant levels. However, in many cases, a potentially important metabolite may partition into multiple fractions because of solubility characteristics, and/or because it is present in both free and conjugated forms. In order for the trigger values to apply, particularly in cases where the TTR is distributed among numerous fractions, it must be demonstrated, e.g., by HPLC analysis of each fraction, that no single metabolite is distributed among the various fractions in such amounts so that the combined level or sum of this component significantly exceeds the trigger value.

3. Identification of specific radiolabeled amino acids, sugars, phenolic compounds, nucleotides, etc., may alleviate the need for further characterization/identification of bound residues in many instances since this usually means that the pesticide has been degraded into small carbon units that have entered the carbon pool. This conclusion does not, however, apply to tritium labeled compounds, or to pesticides in which the ^{14}C label is incorporated at a labile site in the pesticide molecule. This conclusion would also not apply in cases where a single released metabolite, that comprises a significant portion of the total terminal residue (>10% of the TTR or >0.05 ppm), has not been identified.

4. When a fraction, such as lignin, cellulose, or protein, contains radioactivity, the radioactivity does not necessarily consist of radioactive amino acids or sugars. The radioactivity may consist of biological macromolecules having radioactive portions of the pesticide either chemically conjugated onto them, or physically encapsulated within them. This is an important distinction from having the macromolecules constructed from low molecular weight, radiolabeled, building blocks. The petitioner is responsible for providing such determinations in a scientifically supportable manner. The petitioner will make an evaluation of the data and elucidate which of the four conditions exist, i.e., incorporation, conjugation, inclusion or encapsulation.

2.4.6 Further comments

The pathway described above should be viewed as a broad outline of the type of information needed to determine that a plant/livestock metabolism study is acceptable. Different procedures and methodologies may be appropriate in a given circumstance. The basic concepts regarding trigger values for identification of radioactivity, methodologies required for characterization/identification of radioactivity, and steps that should be taken to assure adequate release of nonextractable/bound residues must be observed to assure that the submitted study is adequate.

The following additional comments should be considered in carrying out a plant/livestock metabolism study:

i) For a case where bound residues are present at levels down to 0.05 ppm, or more than 10% of the TTR, whichever is greater, the Agency will require workup and identification where possible.

ii) All unsuccessful attempts at releasing nonextracted radioactivity and characterization and/or identification of the TTR should be fully documented and submitted.

iii) The Agency will not accept situations where the degree of exaggeration of the application rate or livestock dietary burden is used to calculate trigger values. For example, if a crop/animal is treated/dosed with radiolabeled material at an exaggerated rate, e.g., 5X, the resulting radioactivity levels should not be divided by the degree of exaggeration, e.g., 5, to arrive at trigger values. However, when the Agency decides which identified residues are to be of regulatory concern, the degree of exaggeration of relevant metabolism studies will be considered.

iv) Consultation with the Agency prior to initiation and during the metabolism study is appropriate and encouraged.

v) It is the responsibility of the petitioner to utilize state-of-the-art techniques and to provide citations of such techniques when they are used. Flexibility in review is necessary in determining whether a study is adequate for the intended purpose of identifying the nature of the terminal residue to be regulated. Plant/animal metabolism studies will always be examined on a case-by-case basis, and will frequently require scientific judgement to make sound conclusions and recommendations.

vi) A metabolism study should be provided in which $\geq$90% of the ^{14}C-total terminal residues were extracted or solubilized, preferably utilizing extraction techniques employed in analytical methodology for supervised field residue studies or the enforcement method, if different. This criteria should be met for each RAC.

Furthermore, $\geq$90% of the solubilized ^{14}C-total terminal residues should be identified with structure elucidation. The Agency, however, does recognize that in many cases this is not possible, especially when low total levels of ^{14}C-total terminal residues are present and/or when the agrichemical is extensively metabolized to numerous metabolites at low concentrations. In the latter case, it is important for registrants to demonstrate clearly that numerous components are present, and they should attempt to characterize these metabolites by conversion to a common moiety, where feasible.

2.4.7 Storage stability

The issue of storage stability in metabolism studies is generally addressed by elucidating whether sample integrity was maintained during collection, preparation, and storage.

In light of the difficulty of spiking samples before the identity of the residue is known and the length of time needed for metabolism studies, the present Agency position is that storage stability data should not normally be required for samples analyzed within four to six months of collection, provided evidence is given that attempts were made to limit degradation of residues by appropriate storage of matrices and extracts during the analytical portion of the study. The reviewer should be convinced that storage conditions have not invalidated the petitioner's results.

In those cases where a metabolism study can not be completed within four to six months of sample collection, evidence should be provided that the identity of residues did not change during the period between collection and final analysis. This can be done by analyses of representative substrates early in the study and at its completion. Such analyses should show that the basic profile of radiolabeled residues has not changed during that time. If changes are observed, e.g., disappearance of a particular HPLC peak or TLC spot, additional analyses or another metabolism study with a shorter collection to analysis interval may be required. Petitioners are referred to Section 5, *Storage Stability Data*, for further details.

2.5 Clarifications

i) With respect to the determination of total radioactivity in a plant part, it may be difficult to obtain a representative subsample that will give accurate total ^{14}C by combustion for samples where the residue is not evenly distributed or that have a high water content. For these types of samples, it would be acceptable instead to use a combination of extraction and combustion in order to determine the total residue. Since the weighed subsample is extracted by maceration, and the supernatant is separated by centrifugation, there are no losses due to workup. Radioactivity in the liquid extract is determined by liquid scintillation counting (LSC) and radioactivity in the solid residue, which will be much more evenly distributed than in the original sample, and is determined by combustion and LSC.

ii) In chromatography, e.g., HPLC and TLC, of radioactive residues, the polarity of the solvent system should be governed by the polarity of the compounds being analyzed. That is, the solvent polarity should be adjusted to the compounds of interest.

iii) With regard to whether the specific activity should be reported as µCi/mg instead of dpm/g or Curies/mole, any units that would permit calculation of ppm (mg/kg) radioactivity using reported counts (dpm, dpm/g, etc.) are acceptable. Consequently, the registrant must report radioactivity as % TTR and ppm data.

iv) Sufficient information on counts should be provided so that the Agency can verify the ppm reported for crop parts, animal tissues, and the various chromatographic fractions thereof.

Regardless of the unit used, a sample calculation should be submitted showing how the analyst arrived at ppm from the experimental data.

v) Photographs, autoradiograms or other radioassay chromatograms of TLC plates should be provided. If HPLC coupled to a detector capable of measuring radioactivity was employed, then appropriate liquid chromatograms should be submitted. Regardless of the chromatographic technique used, chromatograms showing the behaviour of the analytical standards should also be included in the report.

vi) At a minimum, petitioners should report the total ppm radioactivity, usually in ppm equivalents of parent pesticide, for each crop/livestock part/tissue that could be used for food or feed. For those studies where the activity is measured in all plant/animal parts/tissues, it would be useful to report the % of total plant/animal activity in each part/tissue, but this is not required.

vii) The radiovalidation of analytical methods should be reported in Section 3, *Residue Analytical Method*, or it may stand alone as a report. The cover letter or summary of the full data package should indicate where it has been placed in the submission.

viii) Livestock metabolism studies are now required whenever a pesticide is to be used on a crop having a livestock feed item in Table I of Section 8, *Meat/Milk/Poultry/ Eggs*.

ix) It should be noted that the above part per million trigger values are not absolute requirements, but rough guides as to how much characterization and/or identification is adequate. In the metabolism studies in which highly exaggerated feeding levels are employed and low activity results in tissues, characterization and/or identification requirements should be less stringent than when the expected dietary burdens lead to significant activity in animal products. For example, if the anticipated dietary burden to livestock is about 0.01 ppm, 10 ppm radiolabeled compound is fed (1,000X), and total activity in tissues, milk, or eggs is <0.1 ppm, minimal characterization and/or identification of residues should be adequate, unless residues at this level are of toxicological concern. Such situations often arise with early season herbicides having low application rates.

x) When activities $\geq$0.1 ppm are observed in animal commodities from ingestion of the pesticide at levels expected on feed items, thorough identification of the residues is generally required. This is likely when pesticides are applied to foliage at high rates through the entire growing season.

xi) With respect to the need for conventional feeding studies, such data will not be required when no detectable residues are observed in feed items from crop field trials that reflect the proposed use of the pesticide, i.e., maximum rate and minimum preharvest interval, unless the metabolism study indicates potential for significant bioaccumulation. When trace residues are

detected in the field trials, the Agency will consider the anticipated dietary burdens and the results of the radiolabeled metabolism study when determining whether feeding studies are necessary. In the example cited in subsection 2.5 ix), (0.01 ppm dietary burden, 1,000 X dose leading to <0.1 ppm total activity in meat/milk/eggs), a feeding study would not be necessary as expected residues in animal commodities from ingestion of 0.01 ppm would be on the order of 0.1 ppb, assuming a linear relationship between dose and residues. In this case, the metabolism study also serves as a feeding study, and MRLs would not be needed for meat, milk, poultry, and eggs.

xii) For radioassay procedures where quenching of radioactivity is a problem, quench correction should be explicitly described and methods used to reduce it should be reported.

2.6 Data reporting - plant studies

2.6.1 Purpose

This data reporting guidance is designed to aid petitioners/registrants in the data/ information collection/organization process and thereby facilitate the Agency review process. Petitioners are encouraged to submit complete reports following this guidance for efficient review by the Agency. Additional data reporting guidance is also given in individual sections.

2.6.2 Objective

i) This section gives guidance to pesticide petitioners on the format for their study report so that the Agency can review it efficiently. This section provides an outline for the study report and describes the topics that should be addressed, such as application of radiolabeled materials, identification of residue components, degradation pathways, validation of enforcement methodology, etc., and provides guidance on the presentation of the results of the study.

ii) Petitioners' reports on plant metabolism studies should include all information necessary to provide a complete and accurate description of treatments and procedures. The information submitted in the report should include the following elements:

A) Radiolabeling techniques to include rate, method, and time of radiolabel application in relation to the development and growth cycle of the treated RAC.
B) Extraction, fractionation, and characterization techniques employed for the identification of residue components, whether free or bound, at each sampling interval.

C) Definition of total terminal residues, to include data for all major components of the total terminal residue, reflecting their distribution within the RAC, including processed fractions derived therefrom, expressed as both percentage of the total recovered radioactivity and concentration, in ppm, found at time of harvest and/or when utilized for animal feed.

D) A detailed discussion, preferably accompanied by a flowsheet format, of the possible routes of degradation or pathways of metabolism observed in the subject RAC.

E) When enforcement analytical methodology has been developed, these methods must be validated with radiolabeled samples derived from the plant metabolism study, accompanied by a statement made as to their capability to determine the identified major components of the terminal residue, whether free or bound/conjugated, and all components of the residue of concern, whether free or bound/conjugated in the RAC.

Submitted studies will be screened for completeness before being accepted for evaluation. Study-specific screening forms are available on the PMRA web site or may be obtained upon request from the PMRA.

2.6.3 Format of data report

The following describes the order and format for a study report, item-by-item:

i) Title/Cover Page. Title page and additional documentation requirements, i.e., requirements for data submission and procedures for claims of confidentiality of data, if relevant to the study reported should precede the content of the study formatted below.

ii) Table of Contents. A concise listing, preceding the body of the report, of all essential elements of the study, and the page or table number where the element is located in the report.

iii) Summary/Introduction. Overall, this section should include appropriate background and historical information relative to the study. In addition, the purpose and overall summary of the study, a discussion of the results obtained, and conclusions arrived at regarding the qualitative nature of the total terminal residue in the treated crop should also be included in this section. The following specific topics should be briefly discussed in this section:

A) Registration history and proposed use of the subject chemical.

B) If applicable and/or available via an appropriate citation or reference, compare and contrast observed metabolic routes in the subject RAC to those observed in earlier plant metabolism studies conducted on the subject RAC or on other commodities or to those observed in animal metabolism studies conducted with the subject chemical.

C) The purpose of the study, to include testing strategies employed and the rationale for the selection of these strategies.

D) The overall experimental procedure employed, to include a discussion, if applicable, of unusual experimental problems encountered, attempts made to alleviate these problems that resulted in deviations from the intended test protocol and the effects, if any, of those deviations on the results of the study.

E) The modes and routes of metabolism observed, including a complete description of the identity and quantity, both free and bound, of all major components of the terminal residue and their distribution within the RAC and processed fractions derived therefrom. The foregoing information could be summarized in a narrative with or without tables and/or figures.

F) A conclusion concerning the qualitative nature of the terminal residue in the RAC at time of harvest or when utilized for livestock feed.

G) When enforcement analytical methodology has been developed, these methods must be validated with radiolabeled samples derived from the plant metabolism study, accompanied by a statement made as to their capability to determine the identified major components of the terminal residue, whether free or bound/conjugated, and all components of the ROC, whether free or bound/conjugated in the RAC. The statement should also indicate the detection limits, precision, and accuracy of the methodology employed. Note that if the specified statement/information is provided elsewhere, it need not be reiterated in this section, but should be referenced.

iv) Materials/Methods.

A) Test substance. In this section the following should be included:

1) Identification of the test pesticide active ingredient (ai), including chemical name (CAS and IUPAC), common name (ANSI, BSI, or ISO), company developmental/experimental name, and Chemical Abstracts Service (CAS) number.

2) Chemical structure(s) for the parent compound and metabolites constituting the residue including isomeric ratios.

3) Information on relevant formulation parameters as pertinent, e.g., nature of the solvent, carrier, bait, adjuvant, or other matrix in which the radiolabeled pesticide was applied.

4) For radiolabeled test material, report its purity, specific activity in Curies/mole, disintegrations per minute per gram (dpm/g), nature of the radiolabel and its source,

and the site(s) of labelling in the molecule. The identity of radiolabeled impurities, if any, derived from the test material should also be reported.

5) A rationale provided for selection of radiolabels other than ^{14}C and for site(s) of labelling in the molecule, and where possible, emphasis is placed on labelling the ring position.

6) Any and all additional information petitioners consider appropriate and relevant to provide a complete and thorough description of the test chemical, such as physical/chemical properties, e.g., solubility, etc.

B) Test site. In this section the following should be included:

1) A detailed description of the overall testing environment utilized for the study, i.e., outdoor test plots, greenhouse, or plant growth chambers, including, as appropriate, a record of environmental conditions experienced during the course of the study, i.e., temperature, rainfall and sunlight, and documentation of soil characteristics at the testing site.

2) An explanation or rationale provided by petitioners if the reported testing environment, including testing media, employed in the metabolism study is not representative of or differs significantly from expected cultural practices or environmental conditions under which the test crop would normally be grown.

C) Test crop. In this section the following should be included:

1) Identification of the test crop, including type/variety and crop group classification according to Section 15, *Crop Groups*.

2) A rationale or statement provided by petitioners/registrants for selection of a test crop other than that for which use is proposed.

3) Identification of specific crop part(s) harvested and subjected to radioassay for a determination of the total terminal residue.

4) The developmental stage(s), general condition, i.e., immature/mature, green/ripe, fresh/dry, etc., and size(s) of the test crop at time of pesticide application(s) and at harvest(s).

5) Any and all additional information that petitioners consider appropriate and relevant to provide a complete and thorough description of the test crop.

D) Application of the pesticide. In this section, the following should be included:

1) A detailed description of the type of pesticide application(s) to the test crop, i.e., preplant soil incorporated, over-the-top postemergent foliar application, bait application, etc., including the formulation, i.e., solvent, carrier, bait, adjuvant, or other matrix, in which the radiolabeled pesticide was applied and the method of application, i.e., hand sprayer, topical, soil injection, etc.

2) The actual dosage rate(s), i.e., milligrams per kilogram or parts per million, of plant or soil treated and expressed as kilograms of active ingredient per hectare used in the study.

3) Number and timing of application(s), between-application interval(s), and treatment to sampling interval(s), also known as treatment to sampling interval (TSI), or preharvest interval (PHI).

4) Dates for planting/sowing/transplanting, as applicable; other significant dates in the growing of the crop, e.g., harvesting of immature crop to obtain specific crop part(s) that may be utilized for animal feed; pesticide application(s); harvest of mature crop.

5) An explanation or rationale by petitioners for any significant deviation in either the rate or mode of application to the test crop from the intended use pattern.

E) Sample harvest (collection)

1) Harvest procedures, i.e., method of harvesting or collection, e.g., mechanical/hand, from the plant/ground/flotation, etc.; type of equipment used; number/weight of samples collected per replication and number of replications per treatment level; and sample coding/labelling. These procedures should clearly state the sampling procedure used to obtain representative samples.

2) A detailed description of additional relevant information on the growing of the test crop, application(s) of the pesticide formulated product(s), and harvesting(s) of samples. Refer to the data reporting guidance in Section 9, *Crop Field Trials*, for additional guidance on this subject area.

F) Sample handling and storage stability

1) A detailed description of the handling, preshipping storage, and shipping procedures, as applicable, for harvested, i.e., collected, samples. Refer to the data

reporting in Section 9, *Crop Field Trials*, for additional guidance on this subject area.

2) A detailed description of the conditions and length of storage of harvested, i.e., collected, samples following their receipt in the laboratory. Refer to the data reporting guidance in Section 5, *Storage Stability Data*, for additional guidance on this subject area.

G) Analyses of radioactive residues.

1) Quantitation and distribution of total recovered radioactivity.

2) Total recovered radioactivity remaining on the plant at time of sampling or harvest should be reported.

3) Quantitative radioactivity data reported for all plant parts sampled, including fractions thereof that may be processed into food or feed at the time of normal harvest or at a stage of development when normally utilized for animal feed.

4) A detailed description of sample preparation, i.e., dissection, grinding, lyophilization, etc., prior to oxidative combustion/liquid scintillation analyses.

5) A quantitative accountability for a majority of total radioactivity recovered from the treated crop at times of sampling or harvest as a result of compoite sample analyses.

6) A detailed description provided in narrative, figure, or tabular format of total distribution of radioactivity in the treated crop and processed fractions derived therefrom at the time of sampling or harvest.

7) Details of analytical method parameters, including descriptions of equipment used for determining total radioactivity in each sample, should be furnished.

8) Details of radioactive counting data for selected representative samples to include counting times, total counts recorded, corrected counts, counting efficiencies, ppm equivalents found, sensitivity, and limit of detection, including representative calculations, should be reported.

9) For each sample analyzed, i.e., plant part or fraction, results should be reported as:

I) Total radioactive counts (Bq/g or MBq/g and dpm/g).

II) The percentage that these radioactive counts represent of the total recovered radioactivity in the treated plant at time of sampling or harvest.

III) The ppm equivalents, expressed as parent compound, that these radioactive counts represent of the total recovered radioactivity in the treated plant at the time of sampling or harvest.

H) Extraction and fractionation of radioactivity.

1) A complete description, preferably accompanied by a flowsheet or diagram depicting the overall extraction and fractionation strategies (schema) employed for each sample matrix analyzed.

2) A discussion of and rationale for the selection and extraction sequence for the extracting solvent, i.e., polar vs. nonpolar, used and extraction procedures, i.e., blending, maceration, partitioning, Soxhlet, employed, including use of additional techniques, i.e., decomplexing reagents, ultrasonics, etc., should be provided.

3) A description of conditions employed for the acidic, basic and/or enzymatic hydrolysis of the residue remaining (the filter cake, marc) from previously extracted plant tissue and/or water soluble plant extracts to release conjugated residues from these samples. Specific information on the source, purity, specificity, and activity of all enzymatic preparations utilized for hydrolysis should also be provided.

4) Calculations provided, showing the ratio and/or amounts of total free vs. conjugated parent compound and/or metabolites in each extracted sample matrix.

5) Petitioners should provide a quantitative estimate of residual radioactivity, i.e., unextractable or bound, remaining in the extracted sample matrix following both exhaustive solvent extractions and hydrolytic treatments. The residual radioactivity reported should be expressed as both percentage and ppm, expressed as parent equivalents, of total recovered radioactivity. Attempts at bound residue extraction by exotic or other procedures, or extractions following repeated treatments with concentrated acids and/or bases at elevated temperatures should also be reported by petitioners/ registrants, and a rationale for their use given.

6) Radiochemical extraction efficiencies calculated and reported for all harvested plant tissues.

7) The efficiency of separation and purification for all fractionation and isolation techniques employed in the study, i.e., solvent partitioning, high voltage electrophoresis, ion-exchange, or exclusion column chromatography, HPLC using

gradient elution, and 2-dimensional thin-layer radioautography employing multiple solvent systems, should be reported for a representative sample.

8) Data to account for or track the loss of radioactivity in each subsequent step of the fractionation and isolation procedure should be provided, and attempts made by petitioners to minimize these losses should be discussed.

9) Petitioners should report detailed procedures for the fractionation of unextractable or bound radioactivity in plant tissues into proteins, starch, lignin, cellulose, etc.

10) Following chemical analyses of the fractionated plant tissues described above in subsection 2.6.3 iii) G) 3) for amino acids, glucose, etc., petitioners should then report if significant quantities of the original radioactive residue, characterized as unextractable or bound, have been incorporated into these natural products.

11) The amount of radioactivity in each sample fraction, e.g., water soluble, organosoluble, released by hydrolysis, etc., should be quantified and reported in terms of total radioactive counts, and as both percentage and ppm, expressed as parent equivalents, of total radioactivity recovered in the original sample matrix analyzed.

12) Radioassay methods using quench correction should describe quench correction methodology and methods of decreasing quench reported.

13) A detailed description of the conditions and length of storage of extracts prior to identification of residues should be provided.

I) Characterization/identification of radioactivity.

1) A complete tabular listing and description of all known and suspected metabolites of the parent compound, i.e., model compounds, including their structure and purity, used to facilitate the characterization and/or identification of unknown sample metabolites should be provided.

2) Calculations should be provided and data reported for both sample and reference Rf values on TLC radioautograms and for relative retention times on GC and HPLC columns. Unexpected deviations or variances of observed from expected values, including loss of sample resolution between analytes (samples) in subsequent chromatographic analyses should be reported and steps taken to correct these problems should be discussed.

3) Petitioners should also provide complete details of additional confirmatory analytical procedures used to separate and characterize/identify metabolites, i.e., high voltage electrophoresis, ion-exchange, or exclusion chromatography, derivatization, etc., and determinative methods, i.e., mass spectroscopy in EI and CI modes, used for ultimate identification of metabolite(s).

4) All lost or unaccounted radioactivity in each plant extract or fraction should be explained as fully as possible, and the amount reported should be expressed as both percentage and ppm (expressed as parent equivalents) of total radioactivity recovered from the particular plant part or fraction analyzed and of the total plant at harvest (terminal residue) or when utilized as an animal feed.

5) Individual and/or aggregate quantitative radioactive residue data provided for all nonidentified and/or noncharacterized discrete extractable and resolvable radioactive entities with amounts reported as in subsection 2.6.3 iv) H) 5).

6) Petitioners should report each of the major metabolite components and, if possible, provide information on the chemical nature of discrete (minor) metabolite components. Major metabolite components should be quantified with amounts reported as in subsection 2.6.3 iv) G) 9) III); quantification of minor metabolite components should be attempted and the results reported, if possible.

7) Petitioners should report data/information delineating attempts made to chemically characterize/identify conjugated or complex bound chemical species originating from the parent pesticide in edible plant parts used for food or animal feed.

8) Quantitative data should also be reported by petitioners for each minor metabolite component identified.

9) When enforcement analytical methodology has been developed, petitioners must report the results of analysis by these methods on radiolabeled samples derived from the plant metabolism study. The analysis should specify what percentages of the total radioactivity and of each labeled and identified major component of the terminal residue, whether free or bound/conjugated in/on the treated crop at time of sampling or harvest are accounted for by these methods.

10) A complete description of all instrumentation, equipment, and reagents used, including operating conditions of the instrumentation utilized for the separation, characterization, and identification of radioactive residues should be submitted. Photographs and/or autoradiographs of TLC plates, as well as samples or reproductions of HPLC/GLC chromatograms, including mass spectral scans, etc., should also be submitted.

11) All additional information that petitioners consider appropriate and relevant to provide a complete and thorough description of the conduct of the plant metabolism study and the determination of the total terminal residue.

12) All activity should be reported as either:

a) Free metabolites - normally extractable by organic solvents and do not require chemical treatment to be released.

b) Conjugated metabolites - those that have been metabolized by the animal to form water soluble compounds. Conjugates are made up of two parts, one derived from the pesticide, called the exocon, and one from the plant or animal, called the endocon. The endocon is often a sugar, but there are other possibilities, e.g., sulfates, amino acids, glutathione. Identification of the exocon is not normally possible without cleavage of the conjugate bond. This is normally done by acid, base, or enzymatic hydrolysis. After hydrolysis, the pesticide or pesticide metabolite, free of the conjugating moiety, is usually soluble in organic solvents.

c) Bound metabolites - from pesticide or pesticide metabolites bonding with cellular components to yield products that cannot be removed from the matrix by exhaustive extraction with polar and nonpolar solvents. If these residues are removed chemically, e.g., by acid, base, or enzymatic hydrolysis, a subclass of bound residues must be established.

d) Natural constituent - applies to a pesticide that has been degraded into small fragments that have been channelled into anabolic cycles and is incorporated into normal cell constituents. If soluble, natural constituents may be difficult to distinguish from conjugates and may be misclassified.

13) If the natural constituents are unextractable, they are difficult to distinguish from bound metabolites. This may lead to the misclassification of these residues as bound pesticide residues, when they are not pesticide residues at all. It may be desirable to establish that radioactive residues are natural constituents, particularly if these residues are thought to comprise a large portion of the terminal activity.

v) Results and discussion.

A) Test strategies. This should include a discussion of deviations made from the intended testing protocols or strategies as a result of unusual experimental problems or conditions encountered in growing, treating, or sampling the test crop, to include difficulties in

extraction, fractionation, and characterization of residues and, if applicable, specific extraction and characterization strategies employed for unextractable or bound residues; including a discussion of the impact or effects, if any, of those deviations on the results of the study.

B) Metabolic pathways. If possible, a detailed discussion, preferably accompanied by a flowsheet format, of the routes of degradation or pathways of metabolism observed in the subject RAC should be provided. For discussion purposes, the observed metabolic routes in the subject RAC may be compared and contrasted to known and previously reported metabolic pathways in other RACs or observed in animal metabolism studies conducted with the subject chemical.

C) Characterization/identification and distribution of total terminal residue.

1) In a tabular or graphic format, identification, including name, structure, and quantity, expressed both as percentage and ppm as parent equivalents, of all major components of terminal residue in the RAC, both free and conjugated/bound, and their distribution within the RAC, including plant parts and processed fractions derived therefrom should be reported. All activity should be reported as free, conjugated, or bound metabolites or natural constituents as defined in subsection 2.7.3 v) D) 3) V).

2) If the immature RAC, including plant parts and processed fractions thereof, is normally utilized for animal feed, then identification and quantification of all major components of the residue present at that stage of plant development must also be reported.

3) Petitioners should provide information on any properties and/or characteristics of, their quantities, and distribution within the RAC of all significant unidentifiable and/or uncharacterizable components of the terminal residue.

D) Statistics. If during the course of the plant metabolism study, statistical tests are applied to the raw data obtained during sampling/analyses, then representative examples of these tests should be described.

E) All additional information that petitioners consider appropriate and relevant to provide a complete and thorough description of the plant metabolism study, including quality control measures/precautions taken to ensure validity of all aspects of the study.

vi) Conclusions. Discuss conclusions that may be arrived at as a result of the submitted plant metabolism study, such as:

A) The routes or pathways, mechanisms involved and extent or degree of metabolism observed when the subject RAC is grown to maturity or harvest.

B) The nature, amount, and distribution of the total terminal residue in the RAC at the time of harvest or when normally utilized for animal feed, resulting from the proposed use of the pesticide.

C) Based on the results of validation studies conducted on radiolabeled plant samples, the capability of developed and available enforcement analytical methodology to determine the identified components of the terminal residue, whether free or bound/conjugated, and the capability of the same or modified analytical methodology to determine all components of the ROC, whether free or bound/conjugated in the RAC.

vii) Tables/figures.

A) Tables (for example):

1) Weather and/or environmental data.

2) Distribution and quantity of radioactivity in various harvested plant parts.

3) Name, structure, purity, for all (model compounds) metabolites utilized in study.

4) HPLC/GLC retention times and TLC Rf values for parent compound, metabolites, related compounds and model compounds under different column, solvent (elution) conditions.

5) Name, structure, quantity and location in the RAC of all major identified components of terminal residue.

6) Properties, characteristics, quantities and distribution within the RAC of all significant unidentified components of the terminal residue.

B) Figures (for example):

1) Discussion or diagram of location, topography, and size of outdoor test plot(s).

2) Photographs, figures, or diagram of greenhouse and/or plant growth chamber facilities used in study.

3) Overall extraction and fractionation strategies or schema employed for each sample matrix analyzed.

4) Distribution of radioactivity in various ion exchange (exclusion) or preparative HPLC/GLC fractions.

5) Metabolism flow diagrams or charts.

viii) Certification. A signed and dated certification of authenticity by, and identifying information, i.e., typed name, title, affiliation, address and telephone number, on, the personnel responsible for the various phases of this report, e.g., Study Director, Field Supervisor, and Laboratory Supervisor.

ix) References.

x) Appendix(es).

A) Representative chromatograms, spectra, etc., as applicable.

B) Cite or reference reprints of published and unpublished literature, company reports, letters, analytical methodology, etc., used by petitioners unless physically located elsewhere in the overall data report, in which case, cross-referencing will suffice.

C) Any relevant material not fitting into any of the other sections of this report.

2.7 Data reporting - livestock studies

2.7.1 Purpose

This data reporting guidance is designed to provide a data reporting format for a study of the qualitative nature of residues in food animals.

2.7.2 Objective

i) This section outlines what data are needed to support a livestock metabolism study and in what form those data are to be reported. This guidance will aid the petitioners in the collection and organization of data with the goal of developing complete data packages and facilitating the Agency's review of the study report.

ii) This guidance is designed to aid petitioners in generating reports compatible with the Agency's review process. Data submitters are encouraged to submit complete reports for efficient review by the Agency.

iii) Petitioners' reports on animal metabolism studies should include discussions of the following topics: The test material, the experimental animals, dosing, sample collection, quantitation of

activity, extraction of activity, characterization and identification of activity, conclusions, and raw data. Often data may be more clearly presented in tables or figures, and included in a separate section. As guidance for when this is appropriate, data requirements that are best submitted as a table or figure are identified in the following guideline. A copy of the PMRA initial screening form is provided below as an overview of screening criteria that must be present in the study report.

Submitted studies will be screened for completeness before being accepted for evaluation. Study-specific screening forms are available on the PMRA web site or may be obtained upon request from the PMRA.

2.7.3 Format of the data report.

The following describes the order and format for a study report item-by-item:

i) Title/Cover Page. Title page and additional documentation requirements, i.e., requirements for data submission and statement of data confidentiality claims, if relevant to the study report, should precede the content of the study formatted below.

ii) Table of Contents. The table of contents should provide the page numbers of which pages contain the essential elements of the study, to include the following: Introduction and Summary, Materials, Methods, Results and Discussion, Conclusions, Tables/Figures, Certification, References, and Appendices. The requirements of each of these sections are discussed below.

iii) Introduction and Summary. This section should provide background for the study, and should include the proposed use of the pesticide, the purpose of the study, and a summary of the results. The summary of the experiment should include a discussion of any unusual problems encountered and how these were resolved, a discussion of any deviation from the experiment's protocol and the effect this may have had on the results, and a brief description of the findings of the study, i.e., identity and quantity of significant metabolites in each of the major tissues analyzed, and a proposal as to which metabolites are in need of regulation. A comparison of the results with findings of earlier animal metabolism studies, if any are available, should be included here.

iv) Materials.

A) Test substance.

1) The test pesticide active ingredient should be identified by chemical name (CAS and IUPAC), common name (ANSI, BSI, or ISO), company developmental name or number, and, if available, the CAS number.

2) If the molecule is labeled in a potentially labile portion or a radioactive atom that is subject to exchange reactions is used, a rationale should be provided. Petitioners should explain their choice for the test material.

3) The impurities in the test material and the potential effect of these on the study should be discussed. The purity of the test material should be reported along with its specific activity in Curies per mole (or mCuries per mmole) and disintegrations per minute per gram (dpm/g).

4) Chemical structures should be submitted as figures for parent and metabolites; each should be accompanied by chemical names and, if available, company developmental name or number.

B) Test facilities. The animals' housing should be described. For some pesticides for which volatile metabolites are expected to predominate, it will be necessary to establish that volatilization accounts for a significant amount of activity. It will then be necessary to provide a description of the precautions taken to ensure that this activity is detected.

C) Test animals. A description of the test animals should include their age, gender, weight, health status, and breed. Any health problems or unusual treatment of the animals should be reported; the effect of these on the results of the study should be discussed.

v) Methods.

A) Dosing.

1) For oral metabolism studies, petitioners should describe the preparation of dose, e.g., capsule, with feed, bolus, etc., and the level, timing, and duration of dosing. If the dose is given with feed, the total feed consumed should be reported; the level of pesticide in the feed determined by counting radioactivity should also be reported.

2) For a dermal metabolism study, the number, application level, and type of treatment(s) should be described. A comparison of the treatments to those proposed for use on the animals, with particular attention to and explanation of any differences in the formulation, dosing level, or other experimental parameter, should be provided.

3) Petitioners should describe the precautions taken to assure that dermally applied pesticide is not orally taken up due to grooming; this is particularly important for ruminants.

B) Sample collection.

1) Petitioners should describe the collection of milk and eggs taken, and provide an explanation if this is different from normal practice.

2) The amount of milk and number of eggs, as well as a comparison of these with normal production, should be provided in tabular form.

3) The interval from the last dose to sacrifice should be specified to within one hour. If the animals are sacrificed more than 24 hours after the final dose, an explanation should be provided along with a discussion of the effect of this on the results.

4) A list of the tissues taken and their weights should be provided in a table. If samples are combined from different animals, this should be stated.

5) Tissue samples should be taken immediately after slaughter, and frozen.

C) Sample handling and storage stability. The storage and handling of samples should be described, including the conditions during any shipment and the time in transit. Petitioners should provide evidence that the length or conditions of storage have not significantly affected the results of the study. Additional details are provided in Section 5, *Storage Stability Data.*

D) Analysis of radioactivity.

1) Quantitation and distribution of total recovered radioactivity. In this section, the following should be included:

I) The preparation of the sample prior to counting of activity should be described in detail.

II) The radioactivity recovered in each tissue sampled should be reported in tabular form as total radioactive counts and in ppm expressed as equivalents of parent compound.

III) Counting times, total counts, corrected counts, counting efficiencies, and other raw data, i.e., sample sizes, sensitivity, limit of detection, etc., should be submitted in tabular form. Sample calculations should be reported for representative samples.

2) Extraction and fractionation of radioactivity.

I) The fractionation and extraction strategies for each tissue should be described by way of a flowsheet. The solvents used, the order of their use, the extraction procedures employed, e.g., blending, maceration, Soxhlet, etc., and other extraction techniques should be provided in tabular form.

II) Any efforts to release bound and conjugated residues, i.e., acid, base, or enzyme hydrolysis, exhaustive extraction, etc., should be described. The use of severe conditions, e.g., heat plus strong acid, should be justified, and the possible effect of these treatments on pesticide residues should be discussed.

III) For each tissue, the amount of activity that is water soluble, organosoluble, and unextractable should be reported as a percentage of the total activity in that tissue and in ppm, expressed as parent equivalents.

IV) Detailed description of the conditions and length of storage of extracts prior to identification of residues should be reported.

3) Characterization/identification of radioactivity.

I) A table listing compounds that were synthesized to serve as standards for known and suspected metabolites should be provided. If TLC, GLC, HPLC, or other chromatographic techniques were used to identify metabolites, appropriate retention times should be provided.

II) Any analytical procedures used to identify metabolites should be described in detail.

III) For each tissue, any losses of activity that occur during the various procedures required for characterization and/or identification should be explained as fully as possible. This activity should be reported in ppm, expressed as parent equivalents, and as a percentage of the total radioactive residues. These data requirements are best submitted in tabular form.

IV) For each tissue, milk, or eggs, any discrete, unidentified activity, e.g., an unidentified spot on a TLC plate, should be reported in ppm, expressed as parent equivalents, and as a percentage of the total radioactive residues. For each tissue, milk, or eggs, identified metabolites should be reported in ppm, parent equivalents, and as a percentage of the total radiocative residues. These data requirements are best submitted as figures in tabular form. All data supporting the identification, e.g., reproductions of chromatograms and

spectra, should be provided. Failure to identify a metabolite should be accompanied by an explanation and a description of the attempts that were made to characterize/identify the residue. Any information on the identification and characterization of minor metabolites should be reported.

V) All activity should be reported as either:

a) Free metabolites - normally extractable by organic solvents and do not require chemical treatment to be released.

b) Conjugated metabolites - those that have been metabolized by the animal to form water soluble compounds. Conjugates are made up of two parts, one derived from the pesticide, called the exocon, and one from the plant or animal, called the endocon. The endocon is often a sugar, but there are other possibilities, e.g., sulfates, amino acids, glutathione. Identification of the exocon is not normally possible without cleavage of the conjugate bond. This is normally done by acid, base, or enzymatic hydrolysis. After hydrolysis, the pesticide or pesticide metabolite, free of the conjugating moiety, is usually soluble in organic solvents.

c) Bound metabolites - from pesticide or pesticide metabolites, bonding with cellular components to yield products that cannot be removed from the matrix by exhaustive extraction with polar and nonpolar solvents.

If these residues are removed chemically, e.g., by acid, base, or enzymatic hydrolysis, a subclass of bound residues must be established.

d) Natural constituent - applies to a pesticide that has been degraded into small fragments that have been channelled into anabolic cycles and is incorporated into normal cell constituents. If soluble, natural constituents may be difficult to distinguish from conjugates and may be misclassified.

VI) If the natural constituents are unextractable, they are difficult to distinguish from bound metabolites. This may lead to the misclassification of these residues as bound pesticide residues, when they are not pesticide residues at all. It may be desirable to establish that radioactive residues are natural constituents, particularly if these residues are thought to comprise a large portion of the terminal activity.

vi) Results and discussion.

Residue characterization: For each tissue of concern, i.e., liver, kidney, muscle, fat, milk, and eggs, petitioners should provide a flowsheet depicting the metabolites and how they were uncovered. Petitioners should also provide a discussion of the results, including the significance of activity not fully characterized/identified.

vii) Conclusions. Petitioners should reach a tentative conclusion as to the residue in need of regulation, including a discussion of whether proposed enforcement methodology will determine these compounds.

viii) Tables and figures. This section need only include those tables or figures not included in other sections.

A) The following data should be presented in tabular form:

1) Vital statistics of the test animals, including, as applicable, weight, milk production, egg production, etc.

2) Level of radioactivity, ppm expressed as parent equivalents, in tissues, milk, and eggs.

3) Name, structure, and purity of model compounds used as metabolite standards.

4) Retention times, in the case of gas chromatography (GC) and HPLC data, and Rf values for parent and metabolites under the solvent and stationary phase conditions used.

5) For each tissue of concern (liver, kidney, muscle, and fat) and milk and eggs, the name, structure, and level of all identified metabolites.

B) The following should be presented as figures:

1) Schemes employed for extraction of each tissue.

2) Clear reproductions of TLC plates, GC and HPLC spectra, mass spectra, autoradiograms, and any other graphic data essential to the conclusions of the study.

3) Flowsheets, describing the significant metabolites in each tissue of concern, i.e., liver, kidney, muscle and fat, and how their identity was established.

ix) Certification. Certification of authenticity by the study director, including signature, typed name, title, affiliation, address, telephone number, and date.

x) References. Complete citations to any references cited in the report should be included here.

xi) Appendices. Tables and figures not included elsewhere should be included in the appendices. Reproductions of published reports or other materials that support the submitted study may also be included in this section if, in the registrant's opinion, it will increase the efficiency of the Agency's review of the report.

2.8 References.

The source material for this *Residue Chemistry Guidelines* Section is taken from the following set of documents:

1. U.S. Environmental Protection Agency, *Residue Chemistry Guidelines*, EPA Report No. 7/2-C-96-172 OPPTS 8601300, Nature of the residue-plants, livestock, 1995.

2. *Strategy for Determination of Extent of Metabolism Studies and Development of Residue Methods Based on Trigger Values*, January 27, l988, B. Donzel, Ciba-Geigy Corp.

3. Revised Product Chemistry Regulatory Directives, Dir98-02, *Chemistry Requirements for the Registration of a Technical Grade of Active Ingredient or an Integrated System Product*, and Dir98-03, *Chemistry Requirements for the Registration of a Manufacturing Concentrate or an End-Use Product Formulated from Registered Technical Grade of Active Ingredients or Integrated System Products.*

PEST MANAGEMENT REGULATORY AGENCY

RESIDUE CHEMISTRY GUIDELINES

SECTION 3

RESIDUE ANALYTICAL METHOD

3.1 Preface

This Guideline describes the scientific data requirements of both the *Food and Drugs Act and Regulations* (FDAR) and the *Pest Control Products Act and Regulations* (PCPAR).

3.2 Introduction

Based on plant and animal metabolism study results, the Pest Management Regulatory Agency (PMRA) requires petitioners to develop analytical methods to determine all components of the residue of concern. In some cases, it is not possible to develop a single method that can determine all components of the residue, and several methods are required. Analytical methods, i.e., residue crop/field trials or animal transfer studies and enforcement methodology, are used to obtain residue data on which dietary exposure assessments and maximum residue limits (MRLs) are based, and to enforce the MRL after it is established. Enforcement methods are to be validated by an independent laboratory before submission to the PMRA. This validation may be vetted through the Association of Official Analytical Chemists (AOAC) Peer-Verified Methods Program (reference 7).

The methods for residue analyses serve two functions: (1) they must provide the residue data upon which judgements are made as to the identity and magnitude of residues from the proposed use, and (2) they must provide a means for enforcement of the MRL. The methods described in the Agriculture and Agri-Food Canada (AAFC) guidance document (reference 5), the FDA *Pesticide Analytical Manual* (PAM), Vol. II, and the official methods of analysis of the Association of Official Analytical Chemists (references 2 and 4) can be used as examples of suitable analytical methods.

3.3 Test method

3.3.1 General

The analytical method(s) must be described in a stepwise fashion in sufficient detail to enable competent analysts to apply the method, even though they are unfamiliar with the procedure. Residue analytical methods shall be practical, rapid, and quantitate the residue of concern (ROC) in the MRL expression. The Agency, on a case-by-case basis, may accept the best available methods for the ROC that require state-of-the-art equipment. However, all residue methods must use equipment that is commercially available in Canada and the U.S. Reprints of published methods may be submitted. However, where modifications have been made to adapt a basic method to other crops for which a MRL is proposed, details of the modifications must be described. This includes application to the byproducts, meat, milk, poultry or eggs, if these are a consideration.

The method should not be subject to substrate-related interferences or those arising from reagents. Appropriate cleanup measures should be incorporated to reduce or eliminate spurious responses that might jeopardize the results. For example, in liquid chromatography (LC) and gas-liquid chromatographic (GLC) methods, separation should be sufficiently distinct to yield reasonably

discrete peak(s) for the component(s) of interest rather than a response that appears as a shoulder on an interfering peak.

The Agency encourages submission of more direct and easily performed methods for MRL enforcement. However, the subject methods must meet the Agency's stringent criteria for enforcement procedures See 3.3.5, *Requirements for regulatory methods*, this section. Although methods used solely for data collection do not need to meet all the requirements of enforcement methods, they must be validated in a similar manner to assure the Agency that they are adequate for measuring the ROC.

The use of the FDA multiresidue methods (MRMs) in PAM, Volume I (reference 2) as primary enforcement methods is encouraged. Petitioners are required to submit MRM test data for the parent compound and all regulated metabolites. See Health Evaluation Division (HED) Section 4, *Multiresidue Method*. If one of the MRMs is found to be acceptable as the enforcement method, an independent laboratory validation (ILV) as described in paragraph 3.3.6 will not be required. However, the petitioner will still be expected to provide a single analyte confirmatory method supported by an ILV. The ILV method must be available to all federal, provincial or private laboratories and not be protected as confidential business information for these purposes.

Whenever possible, GLC/high performance liquid chromatography (HPLC) retention times and response values should be reported relative to those of a stable reference compound, particularly when the residue is converted to a derivative prior to GLC/HPLC. GLC/HPLC parameters should be reported in a form such as the Guidelines outlined for the MRM protocol for GLC/HPLC detection found in PAM 1.

3.3.2 Validation of method by petitioner

Methods must be validated by control sample data and recovery data for all components of the ROC for the commodities involved. Control values should be reasonably low, preferably less than 20% of the proposed MRL. Recoveries should be at spiking levels appropriate to the proposed MRL, i.e., limit of quantitation (LOQ), 0.5X, 1X, 2X MRL per analyte. Recoveries should lie between 70% and 120% of the known quantity of the pesticide and its metabolites spiked into the matrix blanks, and should not exceed +/- 20% standard deviation, from sample to sample. Petitioners are to report individual values for recoveries, standard deviations, and confidence limits for the parent pesticide and its metabolites. If 70% recovery is not attainable, the Agency will accept, on a case-by-case basis, methods having lower recoveries for active ingredients that are not acutely toxic, or for minor metabolites. For methods that have a recovery of less than 70%, the petitioner should identify at which steps the loss occurs.

The residues being measured are a major factor in determining the acceptable variability of the method. Appropriate coefficients of variation (CVs) or relative standard deviations (RSDs) as a function of residue level are discussed in Reference 5, cited at the end of this section. The Agency

will consider the variability in recoveries outside the 70-120% range. For example, a method with an average recovery of 65% and a low CV, e.g., 5%, may be viewed more favourably than a method with a average recovery of 95% and a CV of greater than 20%.

The raw agricultural commodity (RAC), processed fraction, tissue, milk, eggs, or a macerate thereof, should be spiked, rather than extracts. The spiked macerate should be mixed and allowed to equilibrate for 30 minutes prior to extraction, or less than 30 minutes if the analyte is unstable or volatile. The portion of the crop to be analysed is specified in the PAM Volume 1 (Reference 2), and in Table I, *Raw Agricultural and Processed Commodities and Livestock Feed Derived from Field Crops*, of Section 8, *Meat/Milk/ Poultry/Eggs*.

The petitioner should state the estimates of the practical limits of detection (LOD) and quantitation (LOQ) as applied to each of the subject crops or tissues or fluids. The estimates of the practical LOD and LOQ should be based on the least concentration of pesticide that can be detected or quantitated with a reasonable degree of assurance, taking into account the size and variation of blanks, i.e., instrument response due to crop extractives and reagents. The petitioner should describe how the values for LOD and LOQ were calculated, show sample calculations and cite any appropriate references.

The analytical method should be validated on each crop for which residue data are generated and a MRL is proposed. In the case of crop group MRLs, e.g., root and tuber vegetables, leafy vegetables (except Brassica vegetables), and cereal grains, the method should be validated on only the representative crops for the group as specified in Section 15, *Crop Groups*, except for control samples for which chromatograms should be submitted for all RACs. The report submitted on the method itself should include recovery data on only a representative number of crops. However, in crop field trial reports, additional validation data should be provided on any crop that was not tested for the analytical method report.

With respect to animal commodities, validation data are required for milk, eggs, and all tissues for which residue data are collected in feeding studies and/or for which MRLs need to be established. The tissues normally include cattle muscle, fat, liver, kidney and poultry muscle, fat and liver. The recovery data for cattle commodities will, in most cases, cover the products of goats, hogs, horses and sheep.

A validated confirmatory method should also be included. Mass spectrometric analysis is preferred, where possible. A full mass spectrum should be provided and/or at least three confirmatory ions identified, if single ion monitoring is employed. Note that for all method validations, at least three samples should be spiked at each level used, to enable a statistical assessment of the method performance.

3.3.3 *Extraction efficiency*

Conventional recovery experiments, as discussed above, do not necessarily reflect the efficiency with which bioincurred residues are extracted from crops. There should be some assurance that aged residues are completely extracted by the analytical procedure.

Analytical methods should be radiovalidated to determine whether the ROC is extracted from plant and animal tissues or fluids containing bioincurred ^{14}C-residues. Radioisotope labeling from the plant and/or livestock metabolism studies provides the best evidence on completeness of extraction, i.e., extraction efficiency should be validated using samples containing ^{14}C-bioincurred residues. Other techniques, such as comparison with exhaustive extraction procedures, may also be used. Samples should undergo the extraction procedure employed in the residue method used for trials, and for the enforcement method, if different from the trials method.

The petitioner needs to demonstrate that the extracted radioactivity accounts for most of the ROC that was identified in the metabolism study. If an analytical method is to be used on both plant and animal commodities, it should be radiovalidated on a plant matrix, an animal tissue, and either eggs or milk. Matrices for which extraction is expected to be most difficult should be used. In the case of plants, this would normally be a dry sample, e.g., straw or fodder, containing ^{14}C-residues that have been on the plant for a relatively long period of time.

Petitioners should provide a rationale for the samples used in the radiovalidation. If the data collection (residue trials) and enforcement methods have significantly different extraction steps, each method should be radiovalidated. Alternatively, analyses of several split samples containing bioincurred/weathered nonradiolabelled residues, showing similar results with the two methods, may be submitted.

Data obtained by surface stripping are not acceptable except for crops where other data on that crop have established that the ROCs are present only as surface residues.

Certain components of the ROC may occur bound with naturally occurring plant constituents, and thus may not be recovered by extraction techniques that are satisfactory for the free components. Such information is evident from metabolism studies. Whenever there are indications of the formation of bound components that may not be recovered by the extracting solvent, modifications should be made in the procedure that will free and recover the liberated components. One such modification would be the initial hydrolysis of the treated crop. These bound components may also be recovered with polar solvents and hydrolyzed under acidic, basic, or enzymatic conditions to free the components. These should not be confused with those fragmentary components that may be so tightly bound or incorporated into the plant's metabolic pool that they are not recoverable by any chemical means. Such components are of interest, but are not usually of toxicological concern. The petitioner should refer also to the discussion on nonextractable (bound) residues in Section 2 *Nature of the Residue - Plants/Livestock*.

3.3.4 Determination of the ROC

The method or methods employed should measure the total terminal residue (TTR) found in the metabolism studies outlined in Section 2, *Nature of the Residue - Plants/Livestock*. Often all components of toxicological concern will contain a common chemical moiety so that the method may be adapted to determine all compounds simultaneously. However, in some cases, it may be necessary to adapt separate extraction-cleanup procedures or even another complete method to measure the TTR or a significant component of the residue. In other cases, one or more components of the residue will be significantly more toxic than other components of the residue and will have to be determined separately.

In some cases, the Agency will accept an enforcement method that measures only a portion (typically the parent compound) of the ROC in order to ease burdens on enforcement agencies and/or to harmonize with international MRLs. This may be referred to as a indicator or marker compound. However, in order to have sufficient data for the dietary risk assessment, a data collection (residue trials) method will normally still be needed to quantitate the ROC. Petitioners contemplating use of an indicator or marker compound in either enforcement or data collection methods are advised to contact the Agency concerning the acceptability of this approach.

The petitioner is encouraged to seek PMRA guidance in establishing the ROC, prior to development of analytical methodology for supervised field trials or for enforcement purposes.

3.3.5 Requirements for regulatory methods

One or more of the methods proposed in the petition must be acceptable to enforce the proposed MRL. Where applicable, use of the AAFC (Reference 5) or the FDA multidetection methodology outlined in Vol. I of the PAM must be evaluated. Also, the enforcement method should be as simple as possible to decrease the cost of monitoring for pesticide residues.

A method that may be valid for gathering residue data is not necessarily suitable for enforcement purposes. In general, an enforcement method should:

i) Not require the use of a sample of the untreated commodity to subtract background interferences, i.e., no background subtraction to quantify the analyte.

ii) Not require exotic equipment, reagents or reagents that are no longer manufactured; for example, immunochemical analysis may be suitable, but availability of antisera, immunogen, monoclonal antibodies (if used), test kits and validation for each batch of antisera must be elucidated.
The petitioner is encouraged to seek PMRA guidance on establishing the suitability of unusual or novel analytical methodology, prior to investing resources in its development.

iii) Be reasonably rapid in execution. In general, residue analytical methods for regulatory purposes should require no more than 24 hours for completion. Methods taking longer than one working day will be considered acceptable on a case-by-case basis. Methods taking less than one working day will be required for acutely toxic residues because of the possibility of enforcement action from accident or misuse situations.

iv) Be sufficiently specific to measure and identify the residue in the presence of residues of other pesticides that could reasonably be expected to be present on the same commodity.

v) Be sufficiently sensitive (slope of calibration curve) in relation to the MRL proposed.

vi) Be practical without the use of extremely hazardous or toxic reagents.

Methods based on cholinesterase inhibition are not regarded as suitable for enforcement purposes. Methods based on paper or thin layer chromatography, and that visually measure the residue, are not adequately quantitative for enforcement purposes. They may be useful, however, as confirmatory methods to help identify the residue.

Although certain gas and liquid chromatographic detection systems possess inherent specificity, methods based on these systems should usually be supplemented by a confirmatory method. In general, confirmation by mass spectrometry is suitable. The specificity may also be enhanced by the use of special extraction-cleanup procedures, derivatization, parallel and/or alternate columns. Provided that a specific confirmatory method is available, the Agency will not require that an interference study be conducted to show whether other pesticides registered on the same commodities interfere with determination of the ROC.

The Agency accepts the use of a common moiety method on a case-by-case basis. Toxicological differences among all metabolites of concern that can be determined by the method are taken into consideration when evaluating the suitability of a common moiety method. In those cases, where a common moiety method is proposed as the primary enforcement method and other regulated pesticides produce the same common moiety, a confirmatory method specific for the ROC should be available to enforcement laboratories. This is especially critical in those instances where two pesticides generating a common moiety are registered on the same crop, but have different MRLs.

The method(s) proposed for enforcement may be subjected to trials in the PMRA laboratories if the pesticide is new, if the analytical method(s) is new and unfamiliar, or if the commodity is known to be difficult to analyse. The burden of proof is on the petitioner, and should the method fail to perform as expected in these trials, the petitioner will be asked to resolve the difficulties. Also, the petitioner will be responsible for improving such a method and furnishing new residue data by the improved method. If the method performs satisfactorily and is acceptable as an enforcement method, it will be made available to interested parties. Thus, a petition must include a copy of the analytical method that is not claimed to be, or stamped as confidential business information.

3.3.6 *Independent laboratory validations (ILV)*

The petitioner must provide adequate residue analytical methods to determine the total toxic residue for pesticides in agricultural commodities and, as appropriate, in processed foods. The ROC includes the parent pesticide and its degradation products, metabolites, either free or bound, and impurities that are of toxicological concern. These methods enable the Agency to establish MRLs after determining the maximum pesticide residues that could be consumed by humans. The analytical methods are subsequently used by federal regulatory laboratories, and provincial laboratories for MRL enforcement and/or pesticide monitoring. The AOAC's Peer-Verified Methods Program may be employed to produce an ILV report acceptable to the PMRA (Reference 7). An enforcement method must be reproducible and suitable for use in federal and provincial laboratories throughout the country. Moreover, sufficient information must be submitted about the analytical method to permit a competent analyst to apply it successfully. This section describes acceptable performance of ILV trials for submission as part of the pesticide petition.

Petitioners are advised to consult with the Agency regarding the need for analytical methods for the quantification of pesticide metabolites.

Independent Laboratory Method Validation

i) ILV trials of analytical methods are required to accompany petitions for a MRL. Results of ILV trials of new analytical methods are required for the parent pesticide, including metabolites of toxicological concern (ROC) and must accompany the following types of petitions:

   A) The first MRL petition for residues of a pesticide in a RAC or processed food/feed.

   B) Any new MRL petition for residues of a pesticide with previously established MRLs if a new method is proposed for enforcement.

   C) Any new MRL for residues of a pesticide with previously established MRLs if the previously approved enforcement method has been significantly modified to accommodate the new commodity. If the petitioner is uncertain whether a method change is significant, the Agency should be contacted.

ii) An ILV trial is also normally not required for confirmatory methods. However, at the discretion of the Agency, an ILV trial may be required for confirmatory methods on a case-by-case basis. One particular instance when the ILV trial is likely to be needed is for a confirmatory method asssociated with a compound whose primary enforcement method is a common moiety procedure that also detects other registered pesticides.

The laboratory personnel, including the study director chosen to conduct the ILV trials, must be unfamiliar with the method, both in its development and in its subsequent use in analyzing field samples. Provided that this criterion is met, and the same equipment, instruments, and supplies are not used, the laboratory chosen to conduct the ILV may be in the petitioner's organization. Other possibilities include laboratories at provincial or state enforcement agencies, laboratories at universities, or private laboratories. The petitioner should apply the same criteria of quality in selecting a laboratory for ILV trials as would be done for any analytical work.

iii) Requirements for ILV trial. A successful ILV trial will require adequate results on at least one set of samples, and the laboratory conducting the ILV trial will be allowed to run up to three sets of samples using the method on a given commodity. A set consists of two control samples, two control samples spiked at the proposed MRL, and two control samples spiked at the LOQ. The method must be run as written with no significant modifications. If the MRL is proposed at the LOQ, the second spiking level should be twice the LOQ.

The laboratory conducting the ILV trial may contact the developers or previous users of the method prior to running the first set of samples, but all communications must be logged and reported to the PMRA. Under no circumstances should anyone from the petitioner, developer, or any previous users visit the laboratory during the ILV trial to observe, offer help, or assist the analysts. If the first or second set is not successful, and the laboratory requires additional contact with the developers or other users of the method, all communication(s) should be recorded. Any subsequent additions or modifications to the original method resulting in improved performance should be incorporated into the method writeup that is sent to the PMRA.

If one method is to be used for several commodities, the ILV trial should be carried out on that commodity that the petitioner has had the most difficulty analysing. If the same method is used for both plant and animal commodities, then separate ILVs should be run on both the most difficult plant and the most difficult animal matrices. The rationale for selection of the commodity should be provided. If, after three sets of samples, the ILV trial has failed to produce adequate results (see below), the petitioner must revise the method and run a second confirmatory trial, using a different laboratory.

For a successful ILV trial, the results on one set of samples, after conducting no more than three sets, must be similar to those achieved by the petitioner. Recovery rates should be 70-120% and interference should be negligible compared to the proposed MRL level.

iv) Information to be reported to the Agency. If the ILV trial is successful, the following should be submitted by the petitioner:

A) Name, address, and telephone number of the study director and other contact person for ILV laboratory;

B) Description of the analytical method;

C) Recovery and control values;

D) Representative chromatograms/spectra of the untreated sample, treated sample and untreated sample spiked at LOQ and MRL level for each analyte in each matrix;

E) Description of the instruments used;

F) Description of any problems encountered and a written description of any changes or modifications that were made during the ILV;

G) Any steps considered critical, i.e., steps where little variation is allowable or directions must be precisely followed;

H) The number of person-hours required to complete one set of samples;

I) Any contact between the ILV laboratory and the method developers or others familiar with the method, including the reasons for the contact, any changes in the method that resulted, and the time of this communication with respect to the progress of the ILV trial, i.e., after the first set, during the second set, etc.; and

J) A statement of adherence to Good Laboratory Practice guidance deemed acceptable by the PMRA as per GLP Regulatory Directive Dir98-01 (Reference 6).

v) A petition that is not accompanied by results of a successful ILV trial will be returned to the petitioner.

3.3.7 Other considerations

The Agency accepts the addition of an internal standard to the final extract just prior to injection to serve as a calibration for retention times and/or peak heights/areas and to improve the precision of quantitation. However, the use of an internal standard throughout the entire procedure to correct for recoveries is not acceptable unless data are available on numerous samples of each matrix to show that the analyte and the internal standard behave identically in each step.

The enforcement method should be validated on each crop for which residue data are generated and a MRL is proposed. In the case of crop group MRLs, e.g., root and tuber vegetables (except Brassica vegetables), and cereal grains, the method needs to be validated on only the representative crops for the group, except for control tissue chromatograms for all RACs. The report submitted with the method should include recovery data from all representative crops for which it was validated. However, in the crop field trial reports, additional validation data should be provided on any crop that was not tested for the enforcement method report.

For chromatographic methods, the peaks for the analyte and internal standard should elute close to one another, but be resolved from each other. As with any other reagent or reference standard used in an enforcement method, the internal standard must be available to enforcement laboratories. If an internal standard is not commercially available, the petitioner must ensure a supply of the chemical to the Agency.

Procedural standards are considered to be standards that are generated by subjecting the reference standard to some or all of the sample preparation procedures specified in the method. The Agency will accept methods using procedural standards generated from a derivatization step under certain conditions. If a procedural standard is used, the petitioner should supply the Agency with not only the pesticide analytical standard, but also the derivatized standard. Availability of the derivatized standard would allow the enforcement laboratory to determine the efficiency of the standard preparation. If the standard is unstable or cannot be provided, the petitioner must provide data to demonstrate the efficiency and reproducibility of the procedure.

3.4 Data reporting format.

Submitted studies will be screened for completeness before being accepted for evaluation. Study-specific screening forms are available on the PMRA web site or may be obtained upon request from the PMRA.

The following format is suggested for the report:

3.4.1 Title/Cover Page.

Title page and additional documentation requirements, i.e., requirements for data submission and procedures for claims of confidentiality of data, if relevant to the study report should precede the content of the study formatted below.

3.4.2 Table of Contents.

The table of contents should provide page numbers on which are found the essential elements of the study, to include the following: Introduction and Summary, Materials and Methods, Results and Discussion, Conclusions, Certification, References, and Appendices. The requirements for each of these sections are discussed below.

3.4.3 Introduction and Summary.

i) Scope (suitable matrices) and source of method, e.g., PAM, company reports, report numbers.

ii) Description of principles for the analytical procedure, including identification of the chemical species determined and the limits of detection and quantitation.

3.4.4 Materials and Methods.

i) Equipment (list and describe);

ii) Reagents and standards (list and describe source and preparation);

iii) Analytical procedure (detail in a stepwise fashion, with special emphasis on reagents or procedural steps requiring special precautions to avoid safety or health hazards);

A) Preparation of sample;

B) Extraction (demonstrate efficiency, if relevant, e.g., dry crop substrates, bound residues, etc.);

C) Spiking, if applicable, i.e., during method validation runs;

D) Cleanup; and

E) Derivatization, if any.

iv) Instrumentation (to include information on):

A) Description, e.g., make/model, type/specificity of detectors, column(s) (packing materials, size), carrier gases, etc.;
B) Operating conditions, e.g., flow rate(s), temperature(s), voltage, etc.; and

C) Calibration procedures.

v) Interference(s) (describe tests):

A) Sample matrices,

B) Other pesticides,

C) Solvents, and

D) Labware.

vi) Confirmatory techniques (describe).

vii) Time required for analysis (to carry a sample/set completely through the analytical procedure, including the determinative step).

viii) Modifications or potential problems, if any, in the analytical method(s) (detail circumstances and corrective action to be taken).

ix) Methods of calculation (describe in a stepwise fashion).

A) Calibration factors, and

B) Analyte in sample.

x) Any and all additional information that the petitioner considers appropriate and relevant to provide a complete and thorough description of residue analytical methodology and the means of calculating the residue results.

3.4.5 Results and discussion. (describe expected performance of method)

i) Accuracy (expected mean and range of recoveries) - include, preferably in tabular format, the individual recovery values, average recoveries, and relative standard deviation thereof for each component of the ROC in each commodity tested during the petitioner's method validation;

ii) Precision (repeatability and reproducibility);

iii) Limits of detection and quantification (provide definition);

iv) Ruggedness testing, if performed;

v) Limitations (critical methodological details which effect accuracy/precision);

vi) Calibration curve (including linear range/sensitivity); and

vii) Specificity.

3.4.6 Conclusions

Discuss applicability of analytical procedure for measuring specific test compound(s) in various test substrate(s), ready availability of equipment, interference(s), etc.

3.4.7 Certification.

Certification of authenticity by the study director (including signature, typed name, title, affiliation, address, telephone number, date).

3.4.8 Tables and figures.

3.4.9 References.

3.4.10 Appendices.

i) *Representative chromatograms*, spectra, etc., as applicable;

ii) Any relevant material not fitting into any of the other sections of this report.

* Chromatograms should be provided for standards and samples from each day of analysis, if applicable.

3.5 References.

1. Horowitz, W. et al, *Quality Assurance in the Analysis of Foods for Trace Constituents*, JAOAC, Vol. 63, No. 6, pp. 1344-1354 (1980).

2. *U.S. Food and Drug Administration, Pesticide Analytical Manual*, Vols. I and II, 1994.

   Available from the National Technical Information Service, Springfield, VA, U.S.

3. U.S. Environmental Protection Agency, PR Notice 96-1, *Tolerance Enforcement Methods - Independent Laboratory Confirmation* by Petitioner, February 7, 1996.

4. *Association of Official Analytical Chemists (AOAC), Official Methods*, latest edition.

   Available from AOAC, 481 North Frederick Ave., Suite 500, Gaithersburg, MD, 20877-2417, U.S., (301-924-7077).

5. AAFC (Agriculture and Agri-FoodCanada): Fillion J. et al, *Multiresidue Determination of Pesticides in Fruit and Vegetables*, by GC-MSD and LC-FD, JAOAC, 78(5), 1252-1266 (1995).

6. PMRA, Regulatory Directive Dir98-01, *Good Laboratory Practice*, October 11, 1996.

7. AOAC International, November 16 1993, *AOAC Peer-Verified Methods, Policies and Procedures*, AOAC International, 481 North Frederick Ave., Suite 500, Gaithersburg, MD, 20877-2417, U.S., (301-9247077).

PEST MANAGEMENT REGULATORY AGENCY

RESIDUE CHEMISTRY GUIDELINES

SECTION 4

MULTIRESIDUE METHOD

4.1 Preface

This Guideline describes the scientific data requirements of the *Food and Drugs Act and Regulations* (FDAR) and the *Pest Control Products Act and Regulations* (PCPAR).

4.2 Introduction

Analytical methods capable of determining many pesticide residues in a single analysis have been developed by the Pest Management Regulatory Agency (PMRA) of Health Canada (HC), the U.S. Food and Drug Administration (FDA), as well as, many other organizations. By using these methods, the data obtained can be used to confirm the presence or absence of many pesticides and their metabolites in commodities. In order to assess the incidence of residues remaining on foods and feeds, the PMRA uses the data compiled by HC and Agriculture and Agri-Food Canada, employing multiresidue methods in their residue monitoring/surveillance programs.

4.3 Method

Specific directions for each multiresidue method used by the FDA are published in that Agency's, *Pesticide Analytical Manual*, Vol. I (PAM I). See subsection 4.4. Compilation of data on the analytical behavior of pesticides and related chemicals is also published in PAM I. The data compiled include the following: relative retention times of the compounds on a variety of gas-liquid chromatographic (GLC) columns; responses of various GLC detectors to the compounds; and recovery of the compound through complete methods and sometimes through important steps within the methods. The large amount of effort spent on the testing of multiresidue methods and compilation of results is justified by the advantages that such compilations offer the analyst. When analytical behavior for numerous compounds through the method in use is known, the analyst is better equipped to recognize the residues that are present in samples of unknown treatment history. In situations where the likelihood of some particular residue is known, the data lists for several methods can be consulted to help choose which method should be used.

An updated compilation of multiresidue methods is provided in Appendix I of PAM I. The PMRA multiresidue methods (MRMs) are referenced in this document (Section 4). All petitioners are expected to provide recovery data for the methods used. Petitioners are expected to follow the directions for the protocols found in PAM I, Appendix II, starting with the *Decision Tree for Multiresidue Methods Testing*, and the accompanying guidance found in the *Suggestions for Producing Quality Data,* i.e., *Decisions on What Protocols to Follow*, and *Proper Application of Methods*. Once the decision tree indicates that recovery is likely, then the petitioner should consult the *Data Development* section of the proper protocol(s) and precisely follow the guidance offered to generate quality data. Alternately, the petitioner may choose to test their analyte(s) using one of the appropriate PMRA MRMs (Reference 3).
It is imperative that all laboratories generating multiresidue methods recovery data follow the directions as written so that the PMRA can determine how a chemical behaves when analyzed according to a precisely defined method. When data have been generated, petitioners are to use the

reporting forms found in PAM I, Appendix II for presenting these data to the Agency. From the completed reports, the appropriate recovery data will be extracted and incorporated for a future update of Appendix I. If the recovery is considered to be complete through any of the protocols, then petitioners are encouraged to use that protocol as their primary enforcement method. However, petitioners need to develop a separate single analyte confirmatory method.

4.4 Study Report

Submitted studies will be screened for completeness before being accepted for evaluation. Study-specific screening forms are available on the PMRA web site or may be obtained upon request from the PMRA.

4.5 References

1. *U.S. Environmental Protection Agency, Residue Chemistry Test Guidelines*, OPPTS860. EPA Report No.7/2-C-96-169, August, 1996.

   Available from the National Technical Information Service, Springfield, VA

2. *Pesticide Analytical Manual* (PAM), Vol. I and II, 1994, Food and Drug Administration, Washington, D.C.

   Available from Field Operations Division (7506C), Office of Pesticide Programs, Environmental Protection Agency, 401 M St., S.W., Washington, DC, 20460, U.S.; or electronically for E-Mail: Guidelines@epamail.epa.gov ; or for Internet site: www.epa.gov.

3. *PMRA Multiresidue Methods*, Compliance Laboratory Services and Regional Operation Division, Laboratory Services Subdivision, Building 22 Central Experimental Farm, Ottawa, ON, K1A 0C6.

3.1 Method Number P-RE-023-96 (7.1)-FV, *Multiresidue Method for the Determination of Pesticides in Fruits and Vegetables by GC/MSD and HPLC Fluorescence Detection.*

3.2 Method Number P-RE-025-97(4) - Dairy/Meat, *The Determination of Organochlorines in Dairy Products (Butter and Cheese) and Meat by GC/ECD.*

3.3 Method Number P-RE-039-97(3) - Feed, *Multiresidue Method for the Determination of Pesticides in Animal Feeds by GC-MSD and HPLC Fluorescence Detection.*

3.4 Method Number P-054-97(2) - Egg, *Multiresidue Method for the Determination for Organochlorines in Eggs by GC-ECD.*

PEST MANAGEMENT REGULATORY AGENCY

RESIDUE CHEMISTRY GUIDELINES

SECTION 5

STORAGE STABILITY DATA

5.1 Preface

This Guideline describes the scientific data requirements of the *Food and Drugs Act and Regulations* (FDAR) and the *Pest Control Products Act and Regulations* (PCPAR).

5.2 Introduction

Storage stablity data are required to validate the stability or rate of decomposition of the residue of concern (ROC) in or on the raw agricultural commodity (RAC) or processed commodity between the time of harvest or sample collection and the final analysis of the residue.

5.3 General

In most instances, samples collected for determining the magnitude of the residue (MOR) and the nature of the residue, i.e., metabolism, are stored for a period of time prior to their analysis. During this storage period, residues of the pesticide and/or its metabolites may be lost by processes, such as volatilization or reaction with enzymes. Therefore, in order to be certain that the nature and level of residues that were present on samples at the time of their collection are the same at the time of analysis, controlled studies are needed to assess the effect that sample storage has on the ROCs, i.e., total toxic residue. In other words, registrants need to show that pesticide residues are stable during storage of analytical samples, or they need to show the degree to which residues are lost or changed in that time.

The term storage stability in this document does not address (1) manufacturing-use product or end-use product storage stability data required under the product chemistry Regulatory Directives Dir98-02, *Chemistry Requirements for the Registration of a Technical Grade of Active Ingredient or an Integrated System Product*, and Dir98-03, *Chemistry Requirements for the Registration of a Manufacturing Concentrate or an End-Use Product Formulated from Registered Technical Grade of Active Ingredients or Integrated System Products*, (Reference 4) or (2) the storage of food commodities under typical commercial conditions, e.g., during the storage and transport of produce prior to its reaching the consumer. Studies addressing the latter are examples of reduction of the residue or anticipated residue studies that are occasionally required to obtain a more realistic estimate of residues in food at the time of consumption. The purpose of the present document is to address storage of analytical samples, in most cases under frozen conditions.

Storage stability data will be required in conjunction with most MOR studies, e.g., crop field trials, processing studies, livestock feeding studies, and for primary standards, stock solutions and working solutions of standards. The Agency will make the following exception: unless a pesticide/ROC is otherwise known to be volatile or labile, storage stability data will not be needed for samples stored frozen for <30 days. The judgment as to what constitutes volatile or labile will be based on information, such as basic physical properties and the results of metabolism studies.

Storage stability requirements for the nature of the residue or metabolism studies are discussed later in section 5.5 of this document.

5.3.1 Need for concurrent studies.

Ideally, storage stability data should be obtained as part of a MOR study, not independent from it. Placing samples with known residue levels into storage along with the treated commodity samples represents quality assurance similar to, for example, verifying the identity of test material. If the treated samples were subjected to erratic storage conditions due to loss of electrical power, the samples with known residue levels could be used as a direct measure of any effects that temperature fluctuations might have on residues. Thus, use of concurrent storage stability samples represents simple, good, analytical practice.

Thus, the Agency prefers that storage stability studies be conducted concurrently with the corresponding MOR study when possible. While this may not be possible for data needed to support completed field trials used for reregistration purposes, it should be possible in conjunction with new MOR studies being initiated in support of registration or reregistration. Concurrent storage stability studies will not be required in many cases. Provided that the pesticide residues are found to be stable in the matrices of interest, a storage stability study run in a separate freezer at a different time period will be acceptable if the storage conditions, especially temperature, are the same as those in the corresponding MOR study. However, for pesticides whose residues are known or suspected to be unstable or volatile, concurrent studies may be needed. In fact, for such pesticides, it is advisable to run a storage stability study in advance of the MOR studies to determine proper storage conditions and maximum storage times before treated samples are placed into storage.

5.3.2 Representative commodities to be analyzed.

Use of crop grouping is acceptable as listed in Section 15, *Crop Groups*. If residues are shown to be stable in a given commodity, the residues in other crops of the same group would be assumed to be stable for the same time period under the same experimental conditions.

With regard to how many representative crops need to be analyzed with residues shown to be stable before the assumption can be made that residues are stable in all crops, the Agency believes that at least five diverse crops need to be tested. If a pesticide is to be applied to all types of crops, suggested crops for a storage stability study are (1) an oilseed, soybean or nut, (2) a nonoily grain, (3) a leafy vegetable, (4) a root crop, and (5) a fruit or fruiting vegetable. The fruit/fruiting vegetable should be an acidic commodity, such as citrus or tomatoes. Field corn grain is to be considered a nonoily grain as opposed to an oilseed. The crop parts to be examined in these studies are those used for food and feed; in other words, those on which residue data are generated and maximum residue limits (MRLs) established, e.g., wheat grain, or the magnitude of feed residues are assessed, e.g.,wheat forage and wheat straw.

The above guidance on representative crops is directed toward a pesticide that will be applied to all crop groups. Many pesticides are applied to only a portion of these groups. Therefore, the five crop types listed above will not always be the most appropriate ones. Since the Agency can not provide guidance for all the possible combinations of crops that might be treated, petitioners will need to use judgment as to which representative commodities they should use for storage stability studies. One example will be presented here. Suppose that a pesticide is to be applied to only cucurbit vegetables and stone fruit. In this case, storage stability data should be provided on one crop from each of these groups. Petitioners may contact the Agency if questions arise as to which commodities should be tested for a particular combination of treated crops.

If residues are found to be unstable in any representative commodity, additional storage stability studies will normally be required on additional commodities of that group if MRLs are being sought on such crops. Under these circumstances, the concept of combining crop groups in Section 15, *Crop Groups,* may no longer be applicable.

There are three major types of crops for which the Agency receives MOR data for processed commodities: oilseeds, grains, and fruits/fruiting vegetables that are mainly citrus, apples and tomatoes. Since some of the processed commodities, e.g., oils, juices and soapstocks, have matrices quite different from the starting RAC, storage stability data are required to support processing studies. If the ROCs of a particular pesticide have been shown to be stable in the processed commodities from one each of the three types of crops cited above, additional storage stability data will generally not be required on other processed commodities provided, of course, that the storage conditions are similar and samples are not stored longer than those of the representative processed commodities.

As with crops, this guidance on processed commodities is directed toward pesticides applied to all types of crops that have processed commodities in which residues may occur or concentrate. For pesticides that are not applied to all such crops, storage stability data may be needed on processed commodities other than the three types mentioned above. For example, if a pesticide is to be used on only root crops, storage stability data should be generated on the processed fractions of potatoes or sugar beets.

With respect to animal commodities, storage stability data are normally required to support livestock feeding or dermal treatment studies. The representative commodities to be examined should include muscle from cattle or poultry, liver from cattle or poultry, milk, and eggs. If residues are stable in these matrices, analyses of other tissues, such as fat and kidney, will not be needed.

5.4 Storage stability requirements for magnitude of residue studies

5.4.1 General.

Storage stability data normally are required for each component of the ROC that is measured in the MOR studies. In most cases, this means all components included in the MRL expression. On a

case-by-case basis, the Agency will allow representative components of the residue to be employed when numerous compounds are included in the MRL.

Petitioners are advised to contact the Agency when questions arise in this regard.

5.4.2 Storage Stability of Standard Solutions.

It is important that the stability of a standard working solution be demonstrated to ensure that absorption, adsorption and degradation of the standard in solution were not significant during the period required to analyze samples for residues.

The stability of working solutions must be explicitly defined to show the relationship between detector response at a given concentration as a function of time stored in the refrigerator or freezer and as a function of time during the day of analysis when the standard solution(s) is maintained at room temperature during determination of the analyte(s).

The petitioner is referred to the Pest Management Regulatory Agency (PMRA) Regulatory Directive Dir98-01, *Good Laboratory Practice,* (Reference 3) for regulatory requirements for GLP, concerning stability of test and reference standards and working solutions.

A graph should be provided for each analyte working solution showing detector response (area units) as a function of time, covering the period required for a given day and over the period of several days, e.g., four to five days, during which time analyses were conducted. These data may be extracted from the standard injections that would be part of the determination data collected for residue studies and method validation.

If the solvent used for preparation of the stock or working solution is changed, then new graphs illustrating the stability of each analyte in the new solvent must be provided, as discussed above.

Detector response factors, i.e., area units/ug or ng at a given attenuation, should be reported for all new working solutions over the period required for analysis of all residue analyses for metabolism, residue, crop rotation and feeding studies.

i) **Test compounds and analytical methods**. The samples could either be from crops or animals that have been treated with pesticides in the field, or from the spiking of control, i.e., untreated, samples with known amounts of each analyte. In all cases, the storage stability samples should be analyzed using the same analytical procedure that was employed in the corresponding MOR studies. If not, data will be needed to show that the method gives results equivalent to those obtained by the method used in the MOR studies.

The samples used in the storage stability study could also be those obtained from metabolism studies using radiolabeled material. If these are to be used, the residues should be measured using

the cold analytical method that was employed in the MOR studies or another method validated for quantitating the ROC. In other words, the storage stability data should not be based on simply counting total radioactivity. Note that the discussion in this paragraph is not referring to the storage stability data needed to support a metabolism study. The latter involves examining the chromatographic profile of all radiolabeled residues as described in section 5.5 of this document.

In those instances where no detectable residues, or low levels of residues close to the analytical method's limit of quantitation are found in field treated commodities, the Agency advises that spiked control samples be employed in the storage stability studies. Related to this point, it is suggested that the MRL to be used in storage stability studies be 10X the method's limit of quantitation (LOQ) with the minimum to be 0.1 parts per million (ppm). This will make it less likely that the stability of the residues can not be ascertained due to highly variable recoveries. If typical residues observed in the MOR studies are much higher than the minimum level suggested above, it is preferable, although not required, for the storage stability study to employ comparable residue levels.

Analytical methods yielding low and variable recoveries should be avoided when conducting storage stability studies as well as MOR studies. Regardless of the method used, freshly spiked samples should be analyzed at each time point when storage stability samples are removed from storage for analysis. This will allow for correction of observed residue values for the stored samples if recoveries are significantly higher or lower than 100% for the freshly spiked samples.

In those instances where the ROC consists of more than one component, i.e., parent compound plus metabolite(s), the storage stability samples may be spiked with the mixture if the analytical method is capable of measuring each component of the residue separately. In those cases where the method converts all residues to a common moiety, spiking with mixtures or using field treated/weathered residues is discouraged. The type of chemical and toxicity involved would determine the acceptability of spiking with a mixture or using field treated samples when a common moiety method is employed. For example, with pesticides where similar chronic toxicity concerns exist over numerous components of the residue, spiking with a mixture followed by use of a common moiety method is probably acceptable. On the other hand, it would not be acceptable to use a common moiety method for cholinesterase inhibitors where significant differences in toxicity may occur as the parent compound oxidizes to assorted metabolites. In other words, in the latter case the method would need to detect each of the metabolites separately.

5.4.3 Sample form.

Ideally, the form of the commodity, e.g., homogenate, coarse chop, whole commodity, or extract, in a storage stability study should be the same as that in the corresponding MOR study. In some cases, the storage stability study may need to reflect storage of more than one of the above forms. For example, if crop field trial samples are stored as homogenates for several months, extracted,

and the extracts stored for several weeks prior to final analysis, the storage stability samples should be handled in the same manner.

If a storage stability study does not reflect the storage of extracts prior to final analysis, the whole study need not be repeated. It would be acceptable to spike extracts of untreated samples, hold them in storage for the same time and under the same conditions as the corresponding extracts in the MOR samples, and then analyze them to determine the stability of residues in the extract. To avoid this additional study, registrants are advised to routinely include the storage of extracts in their storage stability studies unless their standard laboratory practice is to analyze extracts on the same day as they are obtained.

The Agency has recently learned that some petitioners have been storing MOR samples in a whole state, while the storage stability samples are kept as homogenates. The latter is necessary to ensure that the sample can be spiked uniformly. Provided that the residues are found to be stable, the Agency will normally accept such studies since the use of an homogenate in the storage stability study is likely to represent a worse case versus the use of a whole commodity. The homogenization process can release enzymes, acids, and other chemicals that react with the pesticide or its metabolites. If residues are unstable in the homogenate, the Agency will decide on a case-by-case basis whether to correct for loss of residues in the stored, whole commodities based on the results of the homogenate, or the Agency may take another course of action, e.g., require field trials to be repeated with the samples stored in a different form and/or analyzed closer to the time of collection. The factors to be considered in making this decision include the degree of loss observed in the homogenized samples and a risk assessment of the pesticide.

The Food and Agriculture Organization (FAO) Guidelines (Reference 1) state the following: *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 'bound' in the tissue*.

While the PMRA does not believe that this procedure should be the preferred method of storing samples, it is an acceptable alternative to storing whole samples or homogenates, provided that the storage stability samples are handled in the same manner.

5.4.4 Sample container.

As with most parameters in a storage stability study, the sample container ideally should be the same as that used for the MOR samples. However, the Agency has recently learned that petitioners commonly store MOR samples in plastic bags for ease of handling, while storing large samples, that may not be homogenized, as well as stability samples in glass jars. The last involves smaller, usually homogenized, samples that need to be spiked with the ROC in most cases. The Agency has reservations about this practice since the containers may differ in their air tightness and the pesticide

might adsorb differently to the two materials. However, as long as the pesticide is not volatile, studies will not be rejected solely due to the use of different containers.

5.4.5 Storage conditions.

The Agency recognizes that MOR samples almost always require transport from the site of treatment to the laboratory prior to placement into storage, until residue analysis can be performed. Efforts should be made to keep samples cold during transport, e.g., packed with dry ice with the transport period as short as possible. The storage stability study should then simulate the conditions, i.e., temperature, humidity, and light, used in the laboratory for storage of MOR samples prior to their analysis. With equipment that is available today, storage temperatures preferably should be -20°C or lower. For classes of pesticides with known instability, petitioners should consider using even lower temperatures to avoid, or at least reduce, loss of residues in storage. Samples should also be kept in the dark to eliminate the possibility of photochemical reactions. While the focus of the present document is on the storage stability study, the Agency wishes to emphasize that efforts should always be made to assure the integrity of MOR samples from the time of their collection until being placed into storage in the laboratory. MOR study reports should detail how samples are handled and stored prior to receipt by the laboratory.

For reregistration, older MOR studies may be submitted/reviewed for which the exact storage temperature is not known although samples were kept in a freezer. If such studies are to be used in support of reregistration, the Agency requires that storage stability studies be conducted at two temperatures, e.g., -5°C and -20°C, to address the uncertainty regarding storage temperature of the older samples. Samples stored at the higher temperature should be analyzed first. If residues are stable at that temperature, the samples stored at the lower temperature do not need to be analyzed.

5.4.6 Frequency of sampling.

The Agency has no strict requirements on the number of sampling intervals that should be examined in a storage stability study. There need to be a sufficient number of time points to establish that the residues are stable throughout the maximum storage period used for MOR samples or to show how much of the residue is lost at various time points, if it becomes necessary to correct for such losses. In all cases, the sampling points should include zero time to establish the residue levels present at the time that samples are placed into storage. The minimum number of sampling times will vary depending upon the stability of the residues and the maximum length of the storage period for the MOR samples. For example, if the storage period is only a few months, it may be sufficient to examine samples stored that amount of time and some intermediate time, in addition to the zero time sample, if residues are stable. On the other hand, more time points would be necessary if the samples are stored several years or if residues are observed to decline significantly during the several months of storage.

The following represent intervals suggested in the FAO Guidelines. See Reference 2. These are not intended to be Agency requirements, but possibilities to be considered by petitioners. If relatively rapid degradation of residues is likely, sampling intervals, such as 0, 14, 28, 56 and 112 days could

be chosen. For longer storage periods involving stable residues, intervals of 0, 1, 3, 6 and 12 months are suggested. In any case, the longest storage interval in the MOR study needs to be included as discussed in the next section of this document.

The storage intervals observed in a MOR study typically will encompass a wide range. The corresponding storage stability study does not have to include each and every sampling time from the MOR study. The Agency will usually interpolate results when corrections for loss are necessary, and the intervals from the two studies do not match.

The Agency also has no strict requirements with regard to the minimum number of samples per time point for each analyte. Although one stored sample, in addition to the freshly spiked sample(s), may suffice in many cases, the Agency strongly encourages registrants to have reserve samples in case problems are encountered, e.g., poor recoveries observed in freshly spiked samples or an apparently aberrant result, i.e., the availability of additional samples may provide justification for discarding such a value. Reserve storage stability samples are also useful if treated samples end up being stored longer than anticipated or additional analyses of treated samples already in storage are requested by the Agency.

5.4.7 Length of storage period.

The duration of a storage stability study should normally be equal to or longer than the maximum storage period for the corresponding samples in the MOR study. However, for cases in which samples from storage stability studies were stored for shorter intervals than samples from the corresponding MOR studies, extrapolation of the storage stability data to longer intervals will be considered on a case-by-case basis when minimal losses have been observed at the shorter storage intervals. Such extrapolation will be considered only in cases where the storage stability data are available for at least six months and reflect at least three time points in addition to the time zero point.

Under some circumstances, the Agency may also accept the analyses of retained split samples from field trials as an alternative to the extrapolation described above. In some cases, the treated samples from field trials or other MOR studies are split into several portions, with one portion analyzed quickly, i.e., within 30 days of harvest, and the other portion(s) placed in frozen storage. If analysis of the stored portion(s) after an extended period in the freezer shows the same residue level as the portion analyzed within 30 days of harvest, the Agency will consider using such analyses to support MOR studies.

It should be noted that the extrapolation process and use of split samples discussed in the previous two paragraphs will normally not be applicable when residues of a pesticide have been found to be unstable in any commodity. In other words, the available data on other crops need to show that residues are stable for the Agency to consider these alternatives in support of field trials on a particular crop.

During evaluation, questions may arise with respect to the need for conducting new crop field trials versus conducting storage stability studies to support old field trials. The decision as to which studies should be conducted will normally be based on which can be completed in a shorter time frame. For example, field trials may be available for a given crop, but the samples were stored for four years, and no storage stability data are available. In this case, in order to expedite reregistration, the Agency would want new crop field trials to be carried out since they could be completed in a much shorter time than a four-year, storage stability study.

5.4.8 Use of storage stability results.

If a storage stability study shows limited decline of residues during the storage period observed for the corresponding MOR study, correction factors will generally be used to determine the residue levels that were present at the time of sample collection in the MOR study. However, if extensive dissipation of residues has occurred during storage, the MOR study may need to be repeated with samples analyzed closer to their time of collection. Correction factors will be applied to losses in storage up to 30%. Beyond that point, the Agency will consider corrections on a case-by-case basis, taking into account factors such as the absolute (ppm) and relative (%ROC) residue levels of the component that is unstable in storage.

The degree of loss will normally be adjusted or corrected for analytical method recoveries before applying the 30% rule of thumb. In other words, the apparent residue level of an analyte after storage should be divided by the analytical method recoveries obtained for freshly spiked samples analyzed at the same time. For example, suppose that a storage stability sample was originally prepared by spiking at 1.0 ppm with the level confirmed by zero day analysis after correcting for method recovery of 75% on a freshly spiked sample. After a given period of storage, a portion of the sample is analyzed and found to contain only 0.63 ppm, an apparent loss of 37%. If the method recoveries for freshly spiked samples analyzed at the same time are 70%, the corrected residue level in the stored sample is 0.63 ppm/0.70 = 0.90 ppm. Thus, the corrected degree of loss in storage is 10%, or corrected recovery of 90% for the stored sample.

Petitioners should report uncorrected and corrected data for both storage losses and analytical method recoveries for all MOR studies. Petitioners should provide the equation for the storage stability curve and the correction factor(s) used.

5.5 Storage stability requirements for metabolism studies

The Agency needs to determine if sample integrity was maintained during collection, preparation and storage of samples in plant and animal metabolism studies. In light of the difficulty of spiking samples before the identity of the residue is known and the length of time needed for metabolism studies, the present Agency position is that storage stability data should not normally be required for samples analyzed within four to six months of collection, provided evidence is given that attempts were made to limit degradation of residues by appropriate storage of matrices and extracts during

the analytical portion of the study. In other words, the reviewer should be convinced that storage conditions have not invalidated the petitioner's results.

In those cases where a metabolism study can not be completed within four to six months of sample collection, evidence should be provided that the identity of residues did not change during the period between collection and final analysis. This can be done by analyses of representative substrates early in the study and at its completion. Such analyses should show that the basic profile of radiolabeled residues has not changed during that time. If changes are observed, e.g., disappearance of a particular high performance liquid chromatography (HPLC) peak or thin layer chromatography (TLC) spot, additional analyses or another metabolism study with a shorter collection to analysis interval may be required.

5.6 Data Reporting

Reports on storage stability studies should include a detailed description of the commodities that were stored, whether raw or processed; the test compound(s); the experimental design and storage conditions, e.g., freezer temperature, length of storage, type of containers, etc.; residue method(s) and instrumentation; storage stability results and reporting of the data; statistical analysis; and quality control measures/precautions taken to ensure the validity of these operations, including the dates for each step above. In light of some of the earlier discussion in this document, it is especially important for petitioners to describe how samples are prepared, e.g., coarsely chopped or homogenized, and the containers in which they are placed. Differences between these and the sample preparation/containers used in the corresponding MOR studies should be pointed out, and data or a rationale provided as to why they should not invalidate the studies. If known, the study number of the corresponding MOR studies should be provided.

The values for individual samples as opposed to just reporting a mean should be reported in all cases where multiple samples have been analyzed at a given time point. A suggested tabular format for reporting the results that incorporates corrections for recoveries in freshly spiked samples follows.

Commodity	Analyte	Residue Level	Storage Period	Fresh Spike Recovery	Apparent Recovery in Stored Sample	Corrected Recovery in Stored Sample

The values in the second column from the right represent the apparent recovery in the stored samples. These can be divided by the recoveries obtained in the freshly spiked samples to determine the corrected recovery, i.e., the measure of the stability of the residue in storage as discussed in the previous section of this document.

Submitted studies will be screened for completeness before being accepted for evaluation. Study-specific screening forms are available on the PMRA web site or may be obtained upon request from the PMRA.

5.7 Data reporting format

The following describes the order and format for a study, item-by-item.

5.7.1 Title/Cover Page.

Title page and additional documentation requirements, i.e., requirements for data submission and procedures for claims of confidentiality of data, if relevant to the study report, should precede the content of the study formatted below.

5.7.2 Table of Contents.

5.7.3 Summary/Introduction.

This section should include the following: Purpose, Introduction (include summary table of storage validation data), Sample Preparation and Spiking, Storage and Sampling Procedures, Analytical Procedures, and Methods of Calculation.

5.7.4 Materials.

i) Test substance.

A) If spiking is used, describe the test substance(s), i.e., chemical/ common/experimental/chemical abstracts service (CAS) name(s), including the determination/ check of the purity of the test compound(s), i.e., parent plus any metabolites(s) of special concern, and preparation of standard solutions.

B) If weathered residue samples are used, identify the nature and amount of test substance(s) therein at zero time, defined as the beginning of the storage stability testing.

C) Any and all additional information that the petitioner considers appropriate and relevant to provide a complete and thorough description and identification of the test substance(s) used in storage stability validation testing.

ii) Test commodity.

A) Identification of the RAC(s) (crop/type/variety/botanical name) and the specific crop part(s) or processed commodity to be used in storage stability testing.

B) The development stages(s), general condition, e.g., immature/ mature, green/ripe, fresh/dry, etc., and size(s) of the RAC samples used in storage stability testing.

C) Treatment/preparation of RAC or processed commodity sample(s) prior to storage stability testing, e.g., trimming, cleaning, or other means of residue removal, compositing, subsampling, chopping, extraction, etc. Refer to the U.S. FDA, PAM, Vol. I, sections 141-142 for recommended procedures; see Reference 5.

D) Sample identification number. Source of sample(s), field trial identification number, control or weathered residue sample, coding and labeling information that should be the same as, or cross-referenced to, the sample coding/labeling assigned at harvest.

E) Other. Any and all additional information that the petitioner considers appropriate and relevant to provide a complete and thorough description of the RAC(s).

5.7.5 Methods.

i) Experimental design, e.g., number of test commodities, number of test substances, number and magnitude of test levels, number of replicate samples per test compound per test level, number of sampling intervals, representativeness of test commodities to the matrices of concern, etc.

ii) Test procedures.

A) Spiking procedure, if used. Detail the manner in which the test compound(s) was/were introduced to the test substrate(s).

B) Storage conditions. Temperature, humidity, lighting, container type(s)/size, crop form, i.e., extract/macerate/etc., sample size(s)/weight(s), duration, etc. should be provided.

C) Sampling. Describe the sampling procedure at zero time and at regular intervals thereafter. The duration of study should correspond to the length of storage of the field trial samples collected for residue analysis.

D) Dates of sample preparation. Maceration/extraction/etc., spiking or determining the type/amount of weathered residue (zero time), periodic sampling intervals, end of storage, and residue analyses should be provided.

E) Methods of residue analysis.

1) Title/designation/date and source, e.g., PAM, Vol. II; scientific literature; company reports, etc., or cross-reference the *Analytical Method Section* of submission if same method(s) used should be submitted.

2) Discuss any deviations in reagents, procedures, instrumentation, operating parameters, etc., from the Analytical Method(s) used for residue analysis of field trial samples or processed commodities if same method(s) is/are used.

3) Detail the principles and stepwise procedures, i.e., extraction/clean-up, derivatization, and determination, including any modification(s) made, chemical species determined, confirmatory techniques used, if any, etc., and extraction efficiency, if pertinent.

4) Instrumentation and operating parameters. Make/model, type/specificity of detector(s), column(s) (packing materials, size), carrier gas(es), flow rate(s), temperature(s), voltage, limit of detection and sensitivity, calibration procedures, etc. should be provided.

5) Reagents or procedural steps requiring special precautions to avoid safety or health hazards should be explained.

6) Time required for analysis to carry a sample/set completely through the analytical procedure, including the determinative step should be submitted.

7) Procedure(s) for calculating residue level(s) and percent recoveries, i.e., detail, should be reported.

8) Any other additional information that the petitioner considers appropriate and relevant to provide a thorough description of the analytical methodology and the means of calculating the residue results must be provided.

5.7.6 Results/Discussion

i) Residue results. Raw data, dilution factors(s), peak heights/areas, method correction factor(s) applied, formula(e)/standard curve(s) used, ppm theoretical/found, recovery levels (range), percent recovery versus length of storage (dissipation data), appropriateness of length of storage study, etc. should be provided.

ii) Statistical treatment(s). Describe test(s) applied to the raw data.

iii) Quality control. Report the control measures/precautions followed to ensure the fidelity of storage stability validation(s).

iv) Any additional information that the petitioner considers appropriate and relevant to provide a complete and thorough description of storage stability validation results should be provided.

5.7.7 Conclusion.

Discuss conclusions that may be drawn regarding the stability of the test compound(s) in the test matrices as a function of storage time.

5.7.8 Certification.

Certification of authenticity by the study director, including signature, typed name, title, affiliation, address, telephone number and date should be provided.

5.7.9 Tables/Figures.

i) Tables(s) of raw data from storage stability validation testing and a summary table of residue levels in stored samples as a function of commodity and storage time should be submitted.

ii) Graphs, figures, flowcharts, etc., as relevant, should be included.

iii) The equation for the storage stability curve should be provided.

5.7.10 References.

5.7.11 Appendix(es).

i) Representative chromatograms, spectra, etc. should be provided.

ii) Reprints of methods and other studies cited unless physically located elsewhere in the overall data submission, in which case cross-referencing will suffice, should be submitted.

iii) Include any relevant material not fitting into any of the other sections of this report.

5.8 References

1. United Nations Food and Agricultural Organization (FAO), Stability of Pesticide Residues in Stored Analytical Samples, 1994 draft prepared by Codex Committee on Pesticide Residues Working Group on Methods of Analysis and Sampling.

2. United Nations Food and Agricultural Organization (FAO), *Guidelines on Pesticide Residue Trials to Provide Data for the Registration of Pesticides and the Establishment of Maximum Residue Limits - Part 1 - Plants and Plant Products,* 1986.

3. The PMRA Regulatory Directive Dir98-01, *Good Laboratory Practice,* October 11, 1996.

4. Revised Product Chemistry Regulatory Directives Dir98-02, *Chemistry Requirements for the Registration of a Technical Grade of Active Ingredient or an Integrated System Product*; Dir98-03, *Chemistry Requirements for the Registration of a Manufacturing Concentrate or an End-Use Product Formulated from Registered Technical Grade of Active Ingredients or Integrated System Products*; and the PMRA Regulatory Directive Dir93-13, *Registration Requirements for Adjuvant Products*, (October 28, 1993).

5. *Pesticide Analytical Manual* (PAM), Vol. I and II, 1994, Food and Drug Administration, Washington, D.C.

Available from Field Operations Division (7506C), Office of Pesticide Programs, Environmental Protection Agency, 401 M St., S.W., Washington, DC, 20460, U.S., or electronically: Guidelines@epamail.epa.gov for E-Mail or www.epa.gov for Internet site.

PEST MANAGEMENT REGULATORY AGENCY

RESIDUE CHEMISTRY GUIDELINES

SECTION 6

WATER AND FISH

6.1 Preface

This Guideline describes the scientific data requirements of the *Food and Drugs Act and Regulations* (FDAR) and the *Pest Control Products Act and Regulations* (PCPAR). The quality guidelines for drinking water are established by Environment Canada, Ecosystems Conservation Directorate. Maximum Residue Limits (MRLs) may be established for pesticides applied on or near bodies of water that are, or are intended for, human consumption.

6.2 Introduction

These studies are used by the Pest Management Regulatory Agency (PMRA) to determine the levels of pesticide residues in water, fish, and irrigated crops when agricultural chemicals are applied directly to water. The data are used in dietary risk assessments and, in the case of fish and irrigated crops, to establish MRLs for enforcement purposes. Chemigation labeling and residue data requirements are covered under the PMRA Regulatory Directive 93-13, *Chemigation*. See Reference 3.

6.3 General

1. Pesticides may be used in or near aquatic sites, including, ponds, lakes, impoundments, dugouts, and fields. Fields that are typically flooded and drained as a part of normal agricultural practice, whether before, during, or after treatment with pesticides, and other use sites, may lead to residues in water, fish, shellfish and irrigated crops, as well as in meat, milk, poultry and eggs. For each of these commodities, adequate data are needed to demonstrate both the nature of the residue and the level of residues resulting from the maximum proposed use. Because of the nature of aquatic uses, emphasis must be placed on the employment of practical use restrictions that will be followed by the applicator.

2. The design of field studies to demonstrate the fate of the pesticide in the aquatic environment must be directly related to the typical use pattern and restrictions imposed on the use. In the case of fields treated either before or after flooding, the timing, volume, and release of the flood water as dictated by normal agricultural practice must be considered in the field study design. As another example, use in impounded bodies that are completely under the control of the user may be subject to practical label restrictions that would preclude livestock watering, fishing, or use for drinking or irrigation for a specified time period after treatment. On the other hand, such restrictions would not be practical for the use of a pesticide in a river system. In this type of use, restrictions against treatment within a given distance of irrigation or domestic water intakes may be practical.

3. In general, separate and distinct protocols will be required for still waters, i.e., lakes and ponds, flowing water, irrigation conveyance systems, fields that are flooded and drained, and tidal estuaries. The fate of the compound must be demonstrated with respect to rate of

dispersion downstream, degradation, volatilization, or sorption by plants or hydrosoil. Degradation products in water should be identified and may need to be quantified.

6.4 Water

Residue data are required for any water, as described above, in the various aquatic systems that either are directly or may be inadvertently impacted by a pesticide use, i.e., pond, field, drainage canal, river, or estuary. The data collected must show the highest level likely to occur in water. If a monitoring scheme is used, it should include samples taken prior to treatment with pesticides and then periodically to show the decline of the pesticide residues.

Unless covered off by environmental fate studies, residue data should be provided for treated water at or near the point of application as a function of time posttreatment, until a decline (three data points or decline curve) in the residue concentrations in water is observed.

6.5 Fish

1. A fish metabolism study on a predator, such as bass, or bottom feeder, such as catfish, is required when fish may be exposed to the pesticide or its degradation products. If no radioactivity is detected in fish in a static metabolism study, then the following fish residue studies are not required. However, shellfish residue studies will still be required.

2. The fish and shellfish residue studies may be of various types, depending on the aquatic system involved. Controlled exposure for appropriate time intervals may be carried out under static or dynamic conditions in aquaria, or the specimens may be exposed in natural sites if the treated area can be isolated, for example, by cages. Field studies under natural conditions are preferred. Samples for analyses should reflect the fish commodity definition in the *Pesticide Analytical Manual* (PAM), Volume I. See Reference 2. The proposal for MRLs in fish should be expressed on the basis of the edible portion. For fish, residue data are needed for both bottom feeders, such as catfish, and predators, such as bass. For shellfish, data are needed for both molluscs, e.g., clams and oysters, and crustaceans, e.g., shrimp and crabs. If use in estuarine areas is planned, data on whole fish protein concentrate, and smoked, canned, or other processed fish products may be needed.

6.6 Irrigated crops

Experiments to show possible residues in crops that have been irrigated with treated water may utilize the crop grouping scheme as described in Section 15, *Crop Groups*. Residue data for representative crops in each crop group are normally required. If it has been determined that residues are likely to occur in water when the water could be ingested by livestock, then animal metabolism and possibly feeding studies must be carried out as described in Sections 2, *Nature of the Residue - Plants*, *Livestock*, and 8, *Meat/Milk/Poultry/Eggs*, respectively.

6.7 References

1. *Pesticide Analytical Manual*, Vols. I and II, (1994), Food and Drug Administration, Washington, D.C.

   Available from the National Technical Information Service, Springfield, VA 22161.

2. U.S. Environmental Protection Agency, *Residue Chemistry Test Guidelines*, OPPTS860. EPA Report No.7/2-C-96-169, August, 1996.

   Available from the National Technical Information Service, Springfield, VA.

3. The PMRA Regulatory Directive 93-13, *Chemigation*,(1993).

PEST MANAGEMENT REGULATORY AGENCY

RESIDUE CHEMISTRY GUIDELINES

SECTION 7

FOOD HANDLING

7.1 Preface

This Guideline describes the data requirements of the *Food and Drugs Act and Regulations* (FDAR) and the *Pest Control Products Act and Regulations* (PCPAR).

7.2 Introduction

Studies must be conducted to determine the residues in food or feed, resulting from the treatment of food/feed handling establishments with pesticides.

7.3 Definitions

i) A food handling establishment is an area or place in which food is held, processed, prepared and/or served.

A) Nonfood areas of food handling establishments include garbage rooms, lavatories, floor drains to sewers, entries and vestibules, offices, locker rooms, machine rooms, boiler rooms, garages, mop closets, and storage areas for canned, bottled or packaged products.

B) Food areas of food handling establishments include areas for receiving; serving; storing of dry, cold, frozen or raw foods; packaging, such as canning, bottling, wrapping and boxing; preparing, such as cleaning, slicing, cooking and grinding; edible waste storage; and enclosed processing systems, such as mills and dairies, or those systems used to produce edible oils and syrups.

ii) The modes of application of pesticides in food handling establishments are defined in the following manner:

A) Space treatment is the dispersal of pesticides into the air by foggers, misters, aerosol devices or vapor dispensers for the control of flying pests.

B) General treatment is the application to broad expanses of surfaces, such as walls, floors, and ceilings, or the application as an outside treatment.

C) Spot treatment is the application to limited areas where pests are likely to occur, but in areas that will not be in contact with food or utensils, and that will not ordinarily be contacted by workers. Those areas may occur on floors, walls, and bases or undersides of equipment. For this purpose, a spot will not exceed 0.186 m^2.

D) Crack and crevice treatment is the application of small amounts of pesticides into cracks and crevices in which pests hide or through which they may enter the building. Such openings commonly occur at expansion joints, between different elements of

construction, and between equipment and floors. These openings may lead to voids, such as hollow walls, equipment legs and bases, conduits, motor housings and junction or switch boxes.

7.4 Procedure

i) Establishments to be treated will be typical commercial operations that are selected from among the various types as listed under each of the categories shown in Table 1.

TABLE I

Categories and Representative Types of Food Handling Establishments

Category	Representative Types
Food Services[1]	restaurants, cafeterias, taverns, delicatessens, mess halls, school and institutional dining areas, hospitals, mobile canteens, vending machines, groceries and markets.
Manufacturing Establishments[2]	candy plants, ice cream plants, spaghetti or macaroni plants, food mix plants, breakfast cereal plants, bakeries, breweries, wineries, soft drinks bottling plants, pizza plants.
Processing Establishments[3]	meats, poultry, and seafood slaughtering and/or packing plants, spice plants, edible fats and oils plants, fruit and vegetable canneries, pickle factories, beverage, e.g., coffee or tea, plants, frozen fresh food plants, grain mills, dairies.

1 Any food handling establishment whose principal business involves the sale of food directly to the consuming public. The manufacture and/or processing of food by such an establishment is only incidental to achieving its principal business objective.

2 Any food handling establishment whose principal business involves the production and/or packaging of man-made foods that are normally intended for sale through or by food service establishments. Such foods are generally composed of two or more ingredients that have been altered in such a manner as to change their basic identity.

3 Any food handling establishment whose principal business involves the upgrading and/or preservation of raw agricultural commodities in such a manner as to maintain their essential identity. Such establishments may sell their product directly to the consuming public and/or food service or food handling establishments.

Data obtained from tests conducted in two different types of establishments in each category will normally be adequate for clearance of the pesticide for use in all types of establishments as defined by the category of which the test establishment is a part. Careful judgment will have to be applied in selecting the types of establishments to be tested as well as in choosing the number of tests necessary in order to ensure adequate representation of that category. More than two types of establishments may require testing as the individual case indicates. Existing sanitation programs and practices as well as the type of building construction, e.g., wood, cement block, etc., at a plant site are important factors that should be considered. Usage will normally involve the application of the pesticide as a space, general, spot, or crack and crevice treatment, and will include both nonfood and food areas of the establishment that is used as the test site. Acceptable results from a test of the most rigorous type of treatment (space > general > spot > crack and crevice) will preclude the need for residue tests involving less rigorous treatments, and will allow registration of the pesticide for use by the less rigorous method(s). In fact, in many cases, one thorough study representing a worst-case-scenario for residues, will suffice to cover use in all types of establishments. Petitioners are advised to submit a protocol before initiating a residue study that is intended to support use in food handling establishments. The treatment of establishments for the purposes of this test should be performed in accordance with the proposed labelling.

ii) The experiment should be designed to reflect all possible avenues of contamination, taking into account the physical and chemical properties of the pesticide, the proximity of foods and the protective barriers, as may be specified in the regulation, the mode of application, and the use restrictions.

iii) Considerations should be given to at least the following residue transfer routes, where applicable:

A) Direct deposition of spray droplets on foods and direct absorption of fumigant or airborne dust particles.

B) Volatilization of residual deposits and the subsequent absorption into foods.

C) Direct transfer of residues from treated spaces, e.g., countertops, cupboards, utensils, packaging materials, etc.

D) Volatilization with condensation on surfaces where food is subsequently placed.

E) Leakage or weeping of the chemical from devices or impregnated materials that are hung in food establishments for pest control.

F) Transfer of the pesticide through pesticide barriers, e.g., from impregnated shelf papers to packaged food.

G) Tracking of residues from bait stations or sprayed areas to foods or food contact surfaces by pests, or contamination from fallen insects.

H) Deposition of solid or crystalline chemicals from repeated sprays on ceilings over food handling areas.

I) Distribution of vapors, droplets or particulate matter through forced ventilation systems, e.g., central air conditioning or duct heating systems.

J) Distribution of residues in continuous process food operations from the treatment of ends and tailings, conveyor lines, boats, etc., when the operation is shut down, e.g., flour mills.

iv) Many sources of contamination may be eliminated or greatly diminished as practical sources of contamination through restrictions, variations in the mode of application, the type of establishment treated or the nature of the product or formulation. Data should be submitted to establish the relative importance of these factors on the levels of residue that may be expected to result from the pesticidal application. Experiments should be conducted by the analyses of representative foods that are subjected to exposure by any of the above routes that are potential avenues of contamination.

v) The selection of samples for analyses in the more specialized uses, e.g., flour mills, would be apparent. In the more generalized exposure situations, e.g., grocery stores, it is suggested that the selection of samples for analyses represents a range of foods, such as an oily food, e.g., butter; baked cereal products, e.g., bread; beverages, e.g., milk; raw and processed meats; and fresh fruits and vegetables, e.g., lettuce.

vi) In order to demonstrate the residues resulting from the wide variation of conditions anticipated in actual situations, and to gauge the potential for misuse, the experiment should include some exaggerated exposure. This might include spraying at a 2X rate, exposure of foods for longer periods than might normally be expected, or even exposure of some foods when there is a restriction to cover foods when treating.

7.5 References

1. U.S. Environmental Protection Agency, *Residue Chemistry Test Guidelines*, OPPTS 860. EPA Report No.7/2-C-96-169, August, 1996.

Available from the National Technical Information Service, Springfield, VA.

PEST MANAGEMENT REGULATORY AGENCY

RESIDUE CHEMISTRY GUIDELINES

SECTION 8

MEAT/MILK/POULTRY/EGGS

8.1 Preface

This Guideline describes the scientific data requirements of the *Food and Drugs Act and Regulations* (FDAR) and the *Pest Control Products Act and Regulations* (PCPAR).

8.2 Introduction

Whenever pesticide residues are detected in feed items, data on the transfer of residues to meat, milk, poultry, and eggs are required. Residue studies are also required if a pesticide is to be applied directly to animals. Data from these studies are used to determine which components of the residue of concern (ROC) are present and at what concentrations secondary residues could result in meat, milk, poultry and eggs, in order to set appropriate maximum residue limits (MRLs).

8.3 Data requirements

Data must be submitted to show the level of residues that will result in ruminant meat (muscle), meat byproducts (liver, kidney) and fat, poultry (muscle, fat, liver), eggs, or milk. These data are needed whenever a pesticide is to be applied directly to livestock or whenever residues occur on a livestock feed. Since MRLs for residues in animal products may be required, the animal feeding studies must not only show whether residues transfer, but may also need to serve as a basis for setting appropriate MRLs for the animal products.

8.4 Conduct of studies

8.4.1 Feeding studies

i) In most cases, only the parent pesticide should be fed to livestock. However, in those cases where the parent compound comprises only a minor proportion of the ROC, it may be acceptable to feed livestock a mixture of parent and plant metabolites. In cases where a unique plant metabolite exists, i.e., one that is not formed in livestock, a separate feeding study may be required, dosing with that metabolite. Any petitioner considering dosing with a mixture or a unique plant metabolite should contact the Pest Management Regulatory Agency (PMRA) prior to the initiation of such a study. The feeding study should include the level of intake expected (1X); see the next paragraph (ii); it should also include two exaggerated levels of 3X and l0X. The 1X level should represent the worst case estimate of the potential livestock exposure, based on the assumption of all components of the feed having residues. The exaggerated levels are especially important to cover possible future uses for the pesticide on additional feed items and to allow estimation of whether residue levels in tissues vary linearly with the level in the feed. The dosage levels should be expressed in terms of concentration, i.e., parts per million (ppm), in the total ration calculated on a dry weight basis, so that the Agency can relate the dosage to that expected from the proposed use. The feeding level should be expressed in terms of milligrams per kilogram body weight.

ii) In selecting the dosage levels based on total rations, the petitioner should take into account the proportion in the diet of the feed item bearing the residue and, in the case of ruminants,

the percentage of dry matter (DM) in the feed. Table I of this Section 8, *Meat/Milk/Poultry/Eggs*, should be used as a guide in determining the proportion of the diet of the various food items. The correction for percentage of DM is explained in more detail in subsection 8.5 of this Guideline. For example, field corn stover, i.e., fodder, with 83 percent DM content, may in some circumstances comprise up to 25 percent of the total ration, calculated on a dry weight basis for beef cattle. If a residue level of 5 ppm of a given pesticide was expected on field corn stover, the total diet, calculated on a dry weight basis, should be spiked at the 1.5 ppm level, i.e., [5.0 ppm/0.83]x[0.25], to reflect the expected level of intake (1X). If other feed items containing residues could also be fed in combination with field corn stover, the contribution from these feed items should also be added into the calculation. As noted, two dosages at exaggerated levels are also required, preferably threefold and tenfold or higher where not precluded by the toxicity of the pesticide.

iii) Separate feeding studies are required for a ruminant and poultry whenever residues occur on the feeds of these classes of livestock, or direct animal treatment is proposed. The species of choice for these feeding studies are the cow and chicken. In most cases the results of the cattle feeding study will be used to establish MRLs on goats, hogs, horses and sheep. Data will not be translated from other meat animals to poultry. However, within the poultry group, data on chickens will usually be accepted in lieu of data on turkeys. Data on residues in milk from dairy cows will usually apply as well to dairy goats.

iv) In addition to establishing a baseline or blank in a predosing period, control animals should be carried through the experiment with treated animals. This is highly desirable, since values for control animals have been observed to change during feeding studies. The number of animals carried at each treatment level and as controls will vary with the circumstances, but as a general rule, each group in a cattle feeding study should be comprised of a minimum of three animals. For chicken feeding studies, a minimum of ten birds per group should be used. It is often advisable to have additional animals on test that can be used to determine the rate of decline of residues on the cessation of dosing, so that if residues above the MRL are found, data on the time necessary for residues to fall to the MRL are available.

v) Livestock should be dosed daily for a minimum of 28 days, or until residues plateau in milk or eggs if they have not done so in 28 days. A withdrawal period after feeding, usually seven days, using one extra animal at the highest dose level, would be desirable.

vi) If a feed-through-formulation is specifically designed to change absorption characteristics within the digestive system, this formulation should be employed in the feeding study.

8.4.2 Direct animal treatment

i) When a pesticide is proposed for direct use on food animals, data are required to show the extent of residues incurred by the use. The experimental treatment should reflect as closely as

possible, the conditions under which the pesticide will be used commercially. Control animals should be carried along with treated animals. Factors, such as whether sheep passing through a dip tank were freshly shorn or unshorn, should be considered. Generally, separate studies should be carried out for each species of livestock to be treated.

ii) When a pesticide may be applied in more than one type of formulation or by more than one mode of treatment, separate studies reflecting the usage or combination of usages proposed are required. However, data from dips or high pressure wetting sprays on cattle may be accepted in lieu of data from dust treatments, but not vice versa. When the use of devices that permit unlimited access, e.g., backrubbers, are proposed, the experiment should be designed to assure the maximum exposure of the animal to the pesticide. Data reflecting exaggerated treatments are desirable.

iii) If livestock are exposed to the pesticide both in feed and as a direct treatment, the magnitude of the residue study should reflect the level of residues to be expected from the combined exposure scenarios. If separate feeding and direct treatment studies have been conducted, it is normally acceptable to add the residues from these studies to determine the appropriate MRLs. However, this may result in higher than necessary MRLs for animal commodities.

8.4.3 Agricultural premise use studies

When the use of pesticides in agricultural buildings are such that restrictions cannot preclude the possibility of residues in meat, milk, poultry or eggs, residue studies should be carried out reflecting the maximum conditions of exposure. Separate studies are required for ruminants (cattle), nonruminants (swine) and poultry (chicken). The studies should reflect all possible residue transfer routes such as:

i) Direct absorption, i.e., dermal or inhalation, from sprays, mists, or fogs with animals present.

ii) Direct consumption, e.g., by the animal licking surfaces treated with sugar base baits, the pick up of bait granules by poultry, or the contamination of feed, feed troughs, or water troughs.

iii) Direct contamination of milk from deposition on milking equipment, treatment of milk rooms, etc.

8.4.4 Meat, milk, poultry and egg sampling

i) Milk and egg samples should be taken twice daily. For sample size, refer to Codex Alimentarius recommendations, found in Attachment I of Section 9, *Crop Field Trials*, entitled, *Guidelines on Minimum Sample Sizes for Agricultural Commodities from Supervised Field Trials for Residue Analysis*. Eggs from birds within a dosage group may be pooled, if necessary, so that adequate sample weight is available for analysis and retained

samples. Milk from animals within a dosage group should not be pooled, so that data for individual animals are available. However, compositing the a.m. and p.m. milk from each individual cow in the ratio of production is acceptable. Enough of the pooled daily milk and egg samples should be analyzed, preferably at least twice weekly, to allow for a determination of trends in storage of residues with time. Three unique samples of milk and eggs should be analyzed at each time point for each feeding level. Petitioners are advised to analyze first the samples from the highest feeding level. If no quantifiable residues are observed in all such samples, those from the lower feeding levels do not need to be analyzed.

ii) If detectable residues occur in whole milk at any dosing level, analyses of four samples of milk fat are required once residues have plateaued to show how residues partition into that commodity. This information can be used to determine if a specific MRL value should be specified for milk fat and to calculate dietary risk more accurately. If the metabolism study indicated that there were no detectable ^{14}C residues in the milk or that residues did not partition or concentrate into milk fat, then the above data requirement may be waived.

iii) Analysis of eggs. The analysis should be conducted on the egg yolk and white combined in one sample. They may also be analyzed separately provided that the weights of each are known so that the residue can be calculated on a whole egg basis.

iv) Animals should be slaughtered within 24 hours of the last dosing, and tissue samples should be taken and frozen as soon as possible, unless an animal is held for a withdrawal period after feeding. Tissue residue level results from animals slaughtered long after cessation of dosing are not usable in estimating MRLs, and thus, if only such samples are analyzed, the feeding study will have to be repeated. The commodities to be analyzed in a feeding study include the following tissues that are used as human food: muscle, fat, liver and, in the case of cattle only, kidney. For dermal uses on poultry or swine, skin should also be analyzed. As noted above for milk and eggs, three unique samples, using the same tissue type from three different animals, of edible tissues should be analyzed at each dose level to show the variability of residues among different animals. In the case of cattle, this usually means one sample per animal since three cows are generally dosed at each level. For poultry, tissue samples from three to four birds may be composited to generate the three unique samples for each dosage group. If no quantifiable residues of a pesticide are observed in a tissue at the highest dose level, no further analyses of that tissue at lower feeding levels are required.

v) Dermal treatment of livestock. Animals should be sacrificed within the preslaughter interval (PSI) prescribed on the product label. However, PSIs longer than three days are not considered to be practical by the Agency in most cases. Since it has been observed that residues may not peak in tissues until a week or so after application, additional data reflecting longer PSIs should be obtained to establish the maximum levels for MRLs.

vi) The components of the residue to be analyzed in tissues, milk and eggs should be those found to constitute the ROCs in the animal products as determined in the livestock metabolism study described in Section 2, *Nature of the Residue - Plants, Livestock*. The analytical method should be described in detail or referenced. Spiked samples should be run concurrently with those from the feeding study to validate the method. The required limit of quantitation (LOQ) for the animal products will be related to the toxicity of the compound but should generally be on the order of 0.01-0.05 ppm or less. Requirements for analytical methods are described in detail in Section 3, *Residue Analytical Method*.

8.4.5 Storage stability data

Appropriate storage stability data are required on representative livestock commodities as outlined in Section 5, *Storage Stability Data.*

8.4.6 Waiver of livestock feeding studies

When low residues are present in feed items, petitioners should refer to Section 2, *Nature of the Residue - Plant, Livestock*, subsection 2.5 ix); xi), for a possible waiver of conventional livestock feeding studies. In some cases, the livestock metabolism study will indicate that a feeding study, and meat and milk MRLs are not necessary.

8.5 Guidance procedure for calculating livestock dietary exposure

The feed percentages listed for ruminants, i.e., beef and dairy cattle, in Table I, are on a DM basis, while residues for these feed items are calculated on an as-fed basis. Percentages for ruminants in the *Guide For Estimating Toxic Residues in Animal Feeds or Diets*, authored by Dr. L. Harris, 1975, and commonly known as the Harris Guide, (see Reference 2), and the *Update of Livestock Feed Consumption*, (Animal Nutrition, Inc., 1993), referred to in this document as the ANI Report, are also listed on a DM basis. See Reference 3. Therefore, the correct calculation of ruminant dietary burden includes the conversion of the feed to a DM basis in the diet.

Percentages of the diet for poultry and swine feeds in the Harris Guide are on a DM basis. However, poultry and swine listings in the updated Table I of Section 8, *Meat/Milk/ Poultry/Eggs,* and the ANI Report are on an as-fed basis since almost all feeds for poultry and swine are in the dry category. Therefore, the dietary burden calculation for poultry and swine, using the updated Table I, does not require conversion of the feed to a DM basis.

The dietary burden calculation must also handle the situation that arises when the feed item(s) on which there is (are) residue(s) for a given chemical do not comprise a complete diet for the animal. For example, pesticide A has residues on alfalfa hay (70% of beef cattle diet) and alfalfa meal (25%), but on no other feed items. In this case, there is no information on the feed item(s) that would be used to round out the animal's diet. If those additional feed items are wet, the residues on

an as-fed basis will be diluted more than they would be if the feed items were dry. Errors in the estimate of the dietary burden to the animal could result.

These problems can be avoided, however, if the burden is calculated in terms of the weight, as opposed to the concentration, of the pesticide consumed by the animal, and that amount is compared with a standard amount of feed consumed by the animal. Using this approach, the following equation, Equation A, is derived for such calculations.

For ruminants, where feed percentages are expressed on a DM basis, equation A should be used to calculate the total dietary burden.

$$\left(\begin{matrix}dietary\\burden\ [DM]\end{matrix}\right)(ppm) = \sum_i \frac{(\%diet\ [DM])_i}{(\%DM)_i} \times (tolerance)_i \left(\frac{mg}{kg}\right) \qquad \textbf{(A)}$$

i = commodity type

(dietary burden [DM]) (ppm) = estimation of total exposure of a pesticide through feeds on a dry-matter (DM) basis, expressed in ppm (mg pesticide per kg feed)

$(\%\ diet\ [DM])_i$ = percentage in the animal diet of commodity i expressed on a dry-matter basis

$(\%\ [DM])_i$ = dry-matter percentage in feed commodity "i"

$(residue)_i$ (mg/kg) = existing maximum residue in feed expressed in mg/kg, i.e., parts per million (ppm)

The burden thus calculated is on a DM basis. Therefore, ruminant feeding and metabolism studies submitted to the Agency must have their feeding levels calculated on a DM basis. For feeding studies in which the pesticide has been introduced via capsule, the petitioner should report the feed items and intake of each animal so that the dietary burden can be calculated on a DM basis.

The feed percentages for poultry and swine in Table 1, are on an as-fed basis. In that case, no correction will have to be made for percent moisture; the dietary burden for poultry and swine will be simply calculated by Equation B as follows:

$$\left(\begin{matrix}dietary\\burden\end{matrix}\right)(ppm) = \sum_i (\%diet)_i \times (residue)_i \left(\frac{mg}{kg}\right) \qquad \textbf{(B)}$$

The dietary burden in this case will be on an as-fed basis.

The following sample calculations, using both Equations A and B, show how DM correction(s) can alter the estimated dietary burden.

Scenario l. All feed items in the selected diet have residues, and all feed items have low moisture content.

For example, consider the burden for beef cattle to Pesticide B that are fed the following diet (percentages from Table 1). The dietary burdens are calculated with and without correcting for moisture content, using the feed items chosen for the animal's diet that have relatively low moisture contents.

corn grain	80% of diet	88% DM	0.1 ppm residue
corn fodder	20% of diet	83% DM	10.0 ppm residue

Calculation of the burden by Equation B, i.e., without conversion to a DM basis, would give the following:

$$(0.80)\ x\ (0.1\ ppm)\ \%\ (0.20)\ x\ (10.0\ ppm)\ '\ 2.1\ ppm$$

When the adjustment for moisture content is made, a difference of 0.4 ppm is observed using Equation A:

$$\frac{(0.80)}{(0.88)}\ x\ (0.1\ ppm)\ \%\ \frac{(0.20)}{(0.83)}\ x\ (10.0\ ppm)\ '\ 2.5\ ppm$$

Scenario 2. All feed items in the selected diet have residues, and some, or all feed items have a high moisture content.
If wet items are included in the diet, e.g., forages, substantial errors in the estimated ruminant dietary burden could result if the calculations are not corrected for the moisture content. For example, if corn fodder in the above diet is replaced with corn forage,

corn forage	20% of diet	25% DM	10.0 ppm residue

without correcting for moisture, the same 2.1 ppm burden would be calculated by Equation B. However, correcting for moisture, the burden calculated by Equation A would be:

$$\frac{(0.80)}{(0.88)}\ x\ (0.1\ ppm)\ \%\ \frac{(.20)}{(0.25)}\ x\ (10.0\ ppm)\ '\ 8.1\ ppm$$

Thus, using only Equation B, the dietary burden for beef cattle would be seriously underestimated.

Scenario 3. Not all feed items in the selected diet have residues.

Similar underestimation of an animal's dietary burden can occur if the available feed items do not comprise a complete diet. Using the example of Pesticide A for beef cattle,

alfalfa forage	50% of diet	35% DM	2.0 ppm residue
alfalfa hay	25% of diet	89% DM	8.0 ppm residue

the dietary burden, if calculated using Equation B without conversion to a DM basis, follows:

$$(0.50)\ x\ (2.0\ ppm)\ \%\ (0.25)\ x\ (8.0\ ppm)\ '\ 3.0\ ppm$$

Using Equation A, the dietary burden is calculated as follows:

$$\frac{(0.50)}{(0.35)}\ x\ (2.0\ ppm)\ \%\ \frac{(0.25)}{(0.89)}\ X\ (8.0\ ppm)\ '\ 5.1\ ppm$$

This latter number represents a worst-case scenario; thus, the burden cannot be more than 5.1 ppm.

8.6 Data reporting format

Submitted studies will be screened for completeness before being accepted for evaluation. Study-specific screening forms are available on the PMRA web site or may be obtained upon request from the PMRA.

The following format is suggested for the report:

8.6.1 Cover Page

Title page and additional documentation requirements, i.e., requirements for data submission and statement of data confidentiality claims, if relevant to the study report, should precede the content of the study that is formatted below.

8.6.2 Table of Contents

The table of contents should provide page numbers on which are found the essential elements of the study, to include the following: Introduction and Summary, Materials, Methods, Results and Discussion, Conclusions, Tables/Figures, i.e., flowsheets, etc., Certification, References, and Appendices. The requirements of each of these sections are discussed below.

8.6.3 Introduction and Summary

i) This section should provide background and historical perspective for the study. It should include the following:

A) the registration history,

B) the proposed use of the pesticide,

C) the purpose of the study, and

D) a summary of the results.

ii) The summary of the experiment should include the following:

A) a discussion of any unusual problems encountered, and how these were resolved;

B) a discussion of any deviation from the experiment's protocol, and the effect that this may have had on the results; and

C) a brief description of the study's findings addressing such questions as those listed below.

1) Do residues transfer?

2) Is there preferential accumulation in certain organs?

3) What are the highest residues?

4) When did residues plateau?

iii) A comparison of the results to those of the animal metabolism studies would also be useful.

8.6.4 Materials

i) Test substance

A) The pesticidal active ingredient and/or its metabolites that are fed should be identified by:

1) chemical name,

2) common name (ANSI, BSI, IS0),

3) company developmental name/number, and

4) International Union of Pure and Applied Chemistry (IUPAC) and Chemical Abstracts Service (CAS) names and CAS number.

B) The source and purity of each compound should be specified.

C) Chemical structures of these compounds are also required.

D) The rationale for feeding compounds other than parent pesticide should be given.

ii) Test facilities

A) The animals' housing should be described. Factors to consider include the following:

1) size of enclosure(s),

2) individual versus group housing,

3) food and water containers,
4) temperature,

5) lighting, and

6) waste handling.

iii) Test animals

A) A description of the test animals should include the following:

1) species,

2) breed,

3) age,

4) weight,

5) health status, and

6) gender.

B) The number of animals per feeding level must be specified.

C) The mode of identification should be noted, e.g., ear tags.

D) Body weights and egg/milk production should be reported for both the acclimation and the dosing periods.

E) Any health problems, abnormal behavior, or unusual treatment of the animals should be reported, and the effect of these items on study results should be discussed.

iv) Feed

A) The animals' diet during acclimation and the dosing period should be described in regards to both of the following:

1) the types of feed, e.g., corn grain, layers mash or alfalfa pellets, and liquids; and

2) the quantities provided, i.e., specific amounts or ad libitum.

B) Feed consumption, i.e., dry weight, should be reported on an individual or treatment group basis throughout the study.

8.6.5 Methods

i) Dosing

A) The preparation of the dose should be described, e.g., mixing with feed or concentrate ration, gelatin capsule, bolus, etc. The ppm (mg/kg feed) level of the test material in the total diet on a dry weight basis is required. The recommended doses are 1X, 3X and 1OX the anticipated dietary intakes from proposed usages of the pesticide. The calculation of these dietary burdens based on Table I, and the procedure in paragraph (e) of this Guideline should be explained. The petitioner should consider possible future uses of the pesticide when determining the dosages to be fed. Dosing schemes other than 1X, 3X, and 1OX are acceptable provided that a satisfactory rationale is given.

B) The date of dose preparation should be specified along with the storage conditions prior to its administration.

C) A brief description of the method used to analyze spiked feeds and the results of such analyses should be presented. These analyses should demonstrate that the pesticide was stable in the feed or dosing material throughout its entire storage period.

D) The frequency of dosing should be reported if the test material is not incorporated into the total diet or feed.

E) The dates of the initial and final doses, or the total length of the dosing period, should be indicated.

ii) Sample collection

A) The collection of milk and eggs should be described with any differences from normal practice explained. Any compositing or pooling of samples should be noted, although milk from animals within a dosage group should not be pooled. Compositing the a.m. and p.m. milk from each individual cow in the ratio of production is acceptable.

B) The collection dates for those samples that are analyzed for the ROC should be reported.

C) The mode of sacrifice and the time interval in hours between the sacrifice and the administration of the last dose should be specified. An explanation of intervals longer than 24 hours should be presented along with a discussion of their effect on residues.

D) The tissues taken after sacrifice, their type, e.g., thigh muscle, omental fat, etc., and their weights should be listed. The combining of samples from different animals should be noted; this is usually acceptable for poultry, but not for ruminants.

iii) Sample handling and storage stability

A) The storage and handling of tissues, eggs and milk between sample collection and analysis should be described. Factors to consider are these:

1) sample preparation, e.g., chopping, prior to storage;

2) containers;

3) how quickly the samples are put into storage;

4) storage temperature;

5) length of storage, i.e., dates of collection, shipping, analysis, etc.; and

6) mode of shipping, if applicable.

B) Evidence should be presented, showing that the storage did not affect the results of the study. Preferably, this is obtained by concurrently spiking control samples and storing them under the same conditions as samples from treated animals. For guidance in this area refer to Section 5, *Storage Stability Data.* If such information is provided in another section of the overall data package, the study may be referenced.

iv) Analysis of samples

A) A detailed description of the analytical method employed to measure residues should be provided along with a statement as to which chemical species were measured, i.e., parent pesticide or metabolites. When the method has been submitted as a separate report in the total data package, as is often the case, it may simply be referenced. See Section 3, *Residue Analytical Method*, for assistance on how to describe the methodology.

B) Recovery data should be obtained concurrently with the residue analyses to validate the method and establish its sensitivity, i.e., lowest, reliable LOQ. The experimental design of these validation studies should be described including the following:

1) the identity of test compounds and substrates, e.g., tissues, milk and eggs;

2) the magnitudes of spiking levels; and

3) the number of replicates per test compound per level, etc.;

C) The dates of sample spiking, extraction, and analysis of extracts should be listed. If extracts are not analyzed on the day of preparation, storage conditions should be described.

D) Raw data, such as sample weights, final volumes of extracts, and peak heights/areas should be furnished for control, spiked (including those for storage stability data) and treated samples to support reported residue values and recoveries. Analytical responses of standards (calibration curves) are also needed.

E) Representative chromatograms should be supplied for control, spiked, and treated samples of each matrix, i.e., milk, eggs, each edible tissue, etc., along with a few sample calculations of residue levels and percent recoveries using the raw data.

8.6.6 Results and Discussion

i) Recovery percentages, including all values and not just averages or ranges, for the pesticide and/or its metabolites should be reported for tissues, milk, and eggs that are fortified with these compounds.

ii) Storage stability data showing the behavior of residues as a function of time in tissues, milk and eggs should be submitted or referenced. Storage duration and temperature of these samples should be specified.

iii) Levels of the ROC should be reported for each tissue for each feeding level, including control, i.e., untreated, samples. The tissues recommended for analysis include muscle, fat, liver and kidney; the last is not required for poultry. The individual values should be listed for all samples and not merely averages or ranges. It should be clearly indicated whether or not residues have been corrected for recoveries. If the parent pesticide and its metabolites are measured separately, the residues of each should be reported.

iv) Residues in milk and eggs should be listed for each feeding level, including controls, along with the dates of the sample collections. As with tissue residues, the values for each sample should be reported and not just ranges or means.

v) Discussion should be presented as to whether the data indicate that residues of the pesticide transfer to tissues, milk and eggs. If so, when did residues plateau in milk and eggs? Do residues preferentially accumulate in certain tissues? Are the results consistent with the radiolabeled pesticide metabolism studies?

8.6.7 Conclusions

A conclusion must be reached as to whether residues of the pesticide transfer from feed items to meat, milk, poultry and eggs. If so, the extent of transfer should be discussed. The results can be summarized by a table, showing either the ranges or maximum residues in each type of sample for each feeding level. Such a table could then be used to determine appropriate residue levels each time that additional feed items are registered.

8.6.8 Tables and Figures

Note that this section need only include those tables or figures not included in subsections 8.6.4 through 8.6.7.

i) The following data should be presented in tabular form:

A) Vital statistics of the test animals throughout the study, including body weights, egg or milk production, and feed consumption.

B) Dates of sample collection, spiking, extraction, and analysis.

C) Raw data, such as responses of standards, sample weights, final volumes of extract, volumes of aliquots injected, and peak heights/areas for all control, spiked, (including storage stability), and treated samples.

D) Recoveries of parent compound and/or its metabolites from tissues, milk and eggs.

E) Residues of parent pesticide and/or its metabolites in storage stability samples as a function of time.

F) Levels of the ROC in tissues, milk and eggs from both treated and untreated, i.e., control, animals.

ii) The following should be presented as figures:

A) chemical structures and names of compounds that are fed to test animals and of those that are measured in tissues, milk and eggs; and

B) reproductions of representative chromatograms, e.g., gas liquid chromatography (GLC), high performance liquid chromatography (HPLC), thin layer chromatography (TLC), etc., for control, spiked and treated samples and of any other graphic data, e.g., mass spectra, calibration curves, plot of egg/milk residues as a function of time, plot of residues versus time for storage stability samples, etc., that are essential to the study.

8.6.9 Certification

Certification of authenticity by the study director, including signature, typed name, title, affiliation, address, telephone number and date must be included.

8.6.10 References

Any references that are cited in the report should be included here.

8.6.11 Appendices

Reproductions of published reports that support the submitted study may also be included here if, in the registrant's opinion, they will increase the efficiency of the study's review by the Agency.

8.7 References

1. U.S. Environmental Protection Agency, *Residue Chemistry Test Guidelines*, OPPTS860. EPA Report No.7/2-C-96-169, August, 1996.

Available from the National Technical Information Service, Springfield, VA, U.S.

2. Harris, L., *Guide for Estimating Toxic Residues in Animal Feeds or Diets,* 1975.

3. *Update of Livestock Feed Consumption,* Animal Nutrition, Inc., 1993.

Available the from National Technical Information Service, Springfield, VA, U.S.

APPENDIX A

PESTICIDE ASSESSMENT GUIDELINES

RESIDUE CHEMISTRY

TABLE I

RAW AGRICULTURAL AND PROCESSED COMMODITIES

AND

LIVESTOCK FEEDS DERIVED FROM FIELD CROPS

A blank space or unnamed fraction in the processed commodity, feedstuff, or percent of livestock diet columns of Table I for a specific crop, does not necessarily mean that such items are not produced from this crop, and/or used as human foods or feedstuffs. The Agency may add and update the table during ongoing assessments of other/novel food and/or feed fractions.

RAW AGRICULTURAL AND PROCESSED COMMODITIES AND LIVESTOCK FEEDS DERIVED FROM FIELD CROPS								
CROP	**RAC**	**PROCESSED COMMODITY**	**FEED**		**PERCENT OF LIVESTOCK DIET** (1,2)			
			FEEDSTUFF	**% DM** (3)	**BEEF CATTLE**	**DAIRY CATTLE**	**POULTRY**	**SWINE**
Alfalfa (4)	forage hay seed (5)		forage	35	70	60	NU(6)	NU
			hay	89	70	60	NU	NU
			meal (7)	89	25	50	10	10
			silage (8)	40	70	60	NU	NU
Almond	nutmeat hulls		hulls	90	10	10	NU	NU
Apple	fruit	pomace, wet juice	pomace, wet	40	40	20	NU	NU
Apricot	fruit (9)							
Artichoke, Globe	flower head							
Asparagus	spears (stems)							
Avocado	fruit (9)							
Banana (10)	whole fruit							
Barley (11)	grain (12) hay straw	pearled barley flour bran	grain (12)	88	50	40	75	80
			hay	88	25	60	NU	NU
			straw	89	10	10	NU	NU
Bean (13)	bean, succulent seed							
Beet, garden	root tops (leaves)							
Beet, sugar	root tops (leaves)	sugar, refined (14) pulp, dried molasses	tops (leaves)	23	20	10	NU	NU
			pulp, dried	88	20	20	NU	NU
			molasses	75	10	10	NU	NU
Blackberry (15)	berry							
Blueberry	berry							
Broccoli	flower head and stem							
Brussels sprouts	leaf sprouts							

RAW AGRICULTURAL AND PROCESSED COMMODITIES AND LIVESTOCK FEEDS DERIVED FROM FIELD CROPS								
CROP	**RAC**	**PROCESSED COMMODITY**	**FEED**		**PERCENT OF LIVESTOCK DIET** (1,2)			
			FEEDSTUFF	**% DM** (3)	**BEEF CATTLE**	**DAIRY CATTLE**	**POULTRY**	**SWINE**
Buckwheat	grain (16)	flour						
Cabbage	fresh, w/wrapper leaves (17)							
Cacao bean	bean	roasted bean cocoa powder chocolate						
Canola	seed	meal oil, refined	meal	88	15	15	15	15
Carob bean	bean							
Carrot	root		culls (18)	12	25	25	NU	10
Cauliflower	flower head and stem							
Celery	untrimmed leaf stalk (petiole)							
Cherry, sweet	fruit (9)							
Cherry, tart (sour)	fruit (9)							
Chicory	root tops (leaves)							
Citrus	fruit, whole	pulp, dried oil juice	pulp, dried	91	25	20	NU	NU
Clover (19)	forage hay		forage	30	30	60	NU	NU
			hay	89	30	60	NU	NU
			silage (20)	30	30	60	NU	NU
Coconut	coconut (meat and liquid combined)	copra (dried meat) oil						
Coffee (21)	bean, green	bean, roasted instant						
Collards	greens							

RAW AGRICULTURAL AND PROCESSED COMMODITIES AND LIVESTOCK FEEDS DERIVED FROM FIELD CROPS								
CROP	**RAC**	**PROCESSED COMMODITY**	**FEED**		**PERCENT OF LIVESTOCK DIET** (1,2)			
			FEEDSTUFF	**% DM** (3)	**BEEF CATTLE**	**DAIRY CATTLE**	**POULTRY**	**SWINE**
Corn, field	grain starch (25) forage (22) stover (23) grits flour aspirated grain fractions (24)	wet milling: oil, refined dry milling: meal oil, refined	grain	88	80	40	80	80
			forage (22)	40	40	50	NU	NU
			stover (23)	83	25	15	NU	NU
			aspirated grain fractions (24)	85	20	20	NU	20
			milled bypdts (26)	85	50	25	60	75
Corn, pop	grain stover (23)		grain	88	80	40	80	80
			stover (23)	85	25	15	NU	NU
Corn, sweet (27)	sweet corn (K+CWHR) (28) forage (29) stover (23)		forage (29)	48	40	50	NU	NU
			stover (23)	83	25	15	NU	NU
			cannery waste (30)	30	35	20	NU	NU
Cotton	undelinted seed cotton gin bypdts (31)	meal hulls oil, refined	undelinted seed	88	25	25	NU	NU
			cotton gin bypdts (31)	90	20	20	NU	NU
			meals	89	15	15	20	15
			hulls	90	20	15	NU	NU
Cowpea (32)	seed hay forage		seed	88	20	20	10	50
			hay	86	40	40	NU	NU
			forage	30	40	40	NU	15
Crabapple	fruit							
Cranberry	berry							
Crownvetch (33)	forage hay		forage	30	20	60	NU	NU
			hay	90	20	60	NU	NU
Cucumber	fruit							
Currant	fruit							

RAW AGRICULTURAL AND PROCESSED COMMODITIES AND LIVESTOCK FEEDS DERIVED FROM FIELD CROPS								
CROP	**RAC**	**PROCESSED COMMODITY**	**FEED**		**PERCENT OF LIVESTOCK DIET** (1,2)			
			FEEDSTUFF	**% DM** (3)	**BEEF CATTLE**	**DAIRY CATTLE**	**POULTRY**	**SWINE**
Date	fruit, dried (9)							
Dewberry	berry							
Eggplant	fruit							
Elderberry	berry							
Endive/ Escarole	leaves							
Fig	fruit	dried						
Flax	seed	meal	meal	88	10	10	30	10
Garlic	bulb							
Ginseng	root, dried							
Gooseberry	berry							
Grape	fruit	raisin juice						
Grass (pasture & rangeland) (34)	forage hay		forage	25	60	60	NU	NU
			hay	88	60	60	NU	NU
			silage (35)	40	60	60	NU	NU
Herbs (36)	fresh	dried						
Hops	hops cones, dried (37)							
Horseradish	root							
Huckleberry	berry							
Jerusalem artichoke	tuber							
Kale	leaves							
Kiwifruit	fruit							
Kohlrabi	bulbous stem and leaves							
Kumquat	fruit							
Leek	whole plant							
Lentil	seed							

RAW AGRICULTURAL AND PROCESSED COMMODITIES AND LIVESTOCK FEEDS DERIVED FROM FIELD CROPS								
CROP	**RAC**	**PROCESSED COMMODITY**	**FEED**		**PERCENT OF LIVESTOCK DIET** (1,2)			
			FEEDSTUFF	**% DM** (3)	**BEEF CATTLE**	**DAIRY CATTLE**	**POULTRY**	**SWINE**
Lespedeza (38)	forage		forage	22	20	60	NU	NU
	hay		hay	88	20	60	NU	NU
Lettuce, head	fresh, w/wrapper leaves (39)							
Lettuce, leaf	leaves (40)							
Loganberry	berry							
Lupin	seed		seed	88	20	20	15	20
Mango	fruit (9)							

RAW AGRICULTURAL AND PROCESSED COMMODITIES AND LIVESTOCK FEEDS DERIVED FROM FIELD CROPS								
CROP	**RAC**	**PROCESSED COMMODITY**	**FEED**		**PERCENT OF LIVESTOCK DIET** (1,2)			
			FEEDSTUFF	**% DM** (3)	**BEEF CATTLE**	**DAIRY CATTLE**	**POULTRY**	**SWINE**
Millet (41)	grain (42) forage hay straw (43)	flour (44)	grain (42)	88	50	40	70	75
			forage	30	25	60	NU	NU
			hay	85	25	60	NU	NU
			straw (43)	90	10	10	NU	NU
Mung bean	bean bean sprouts (45)							
Mushroom	cap and stem							
Muskmelon (46)	fruit							
Mustard greens	greens (leaves)							
Nectarine	fruit (9)							
Nuts (47)	nutmeat							
Oats (48)	grain (12) forage hay straw	flour groats/rolled oats	grain (12)	89	50	40	80	80
			forage	30	25	60	NU	NU
			hay	90	25	60	NU	NU
			straw	90	10	10	NU	NU
Okra	fruit (pods)							
Olives	fruit (9)	oil						
Onion, bulb	bulb							
Onion, green	whole plant,w/o roots							
Papaya	fruit							
Parsley (49)	leaves, fresh	dried						
Parsnip	root							
Passion fruit	fruit							
Pawpaw	fruit							
Pea (50)	pea, succulent (51) seed (52)							

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CROP	**RAC**	**PROCESSED COMMODITY**	**FEED**		**PERCENT OF LIVESTOCK DIET** (1,2)			
			FEEDSTUFF	**% DM** (3)	**BEEF CATTLE**	**DAIRY CATTLE**	**POULTRY**	**SWINE**
Pea, field (53)	seed vines hay		seed	90	20	20	20	20
			vines	25	25	50	NU	NU
			hay	88	25	50	NU	NU
			silage (54)	40	25	50	NU	NU
Peach	fruit (9)							
Peanut	nutmeat hay (55)	meal oil, refined	meal	85	15	15	25	15
			hay (55) (R) (56)	85	25	50	NU	NU
Pear	fruit							
Pepper, bell and nonbell (57)	fruit							
Peppermint	tops (leaves and stems)	oil						
Pimento (58)	fruit							
Pineapple	fruit	process residue (59) juice	process residue (59)	25	30	20	NU	NU
Plantain (60)	whole fruit							
Plum	fruit (9)	prune						
Potato	tuber	granules/flakes (61) chips peel, wet	culls	20	75	40	NU	50
			processed potato waste (62)	15	75	40	NU	NU
Pumpkin	fruit							
Quince	fruit							
Radicchio (red chicory)	leaves, fresh							
Radish	root tops (leaves)							
Rape	seed forage	meal (63)	meal	88	15	15	15	15

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CROP	**RAC**	**PROCESSED COMMODITY**	**FEED**		**PERCENT OF LIVESTOCK DIET** (1,2)			
			FEEDSTUFF	**% DM** (3)	**BEEF CATTLE**	**DAIRY CATTLE**	**POULTRY**	**SWINE**
			forage	30	30	30	NU	NU
Rape greens (64)	greens (leaves)							
Raspberry, black and red	berry							
Rhubarb	petioles							
Rice (65)	grain (12) straw	polished rice hulls bran	grain (12)	88	40	40	60	65
			straw	90	10	10	NU	NU
			hulls	90	10	10	15	NU
			bran	90	15	15	25	15
Rutabaga	root							
Rye (66)	grain (67) forage straw	flour bran	grain (67)	88	40	40	50	50
			forage	30	25	60	NU	NU
			straw	88	10	10	NU	NU
Safflower	seed	meal oil, refined	meal	91	10	15	25	25
Salsify	root tops (leaves)							
Sesame	seed	oil						
Shallot	bulb							
Sorghum, grain	grain forage (22) stover (23) aspirated grain fractions (24)	flour (68)	grain	86	40	40	80	90
			forage (22)	35	40	50	NU	NU
			stover (23)	88	25	15	NU	NU
			aspirated grain fractions (24)	85	20	20	NU	20
Sorghum, sweet (69)	stalk	syrup						
Sorghum forages, Sudan grass	(See Grass)							

RAW AGRICULTURAL AND PROCESSED COMMODITIES AND LIVESTOCK FEEDS DERIVED FROM FIELD CROPS								
CROP	**RAC**	**PROCESSED COMMODITY**	**FEED**		**PERCENT OF LIVESTOCK DIET** (1,2)			
			FEEDSTUFF	**% DM** (3)	**BEEF CATTLE**	**DAIRY CATTLE**	**POULTRY**	**SWINE**
Soybean (70)	seed forage hay aspirated grain fractions (24)	meal hulls oil, refined	seed	89	15	15	20	25
			forage (R) (56)	35	30	30	NU	NU
			hay (R) (56)	85	30	30	NU	NU
			aspirated grain fractions (24)	85	20	20	NU	20
			meal	92	15	15	40	25
			hulls	90	20	20	20	NU
			silage (71)	30	30	30	NU	NU
Spearmint	tops (leaves and stems)	oil						
Spices (72)	fresh	dried						
Spinach	leaves							
Squash	fruit							
Strawberry	berry							
Sugarcane (73)	cane	molasses (74) sugar, refined (14)	molasses (74)	75	10	10	NU	NU
Sunflower	seed	meal oil, refined	meal	92	15	15	30	20
Sweet potato	root							
Swiss chard	petioles							
Taro	corm foliage							
Tea (75)	plucked leaves	dried instant						
Tomato	fruit	paste (76) puree						
Trefoil (77)	forage hay		forage	30	20	60	NU	10
			hay	85	20	60	NU	NU
Turnip	root tops (leaves)		root	15	75	20	NU	40
			tops (leaves)	30	50	30	NU	NU

RAW AGRICULTURAL AND PROCESSED COMMODITIES AND LIVESTOCK FEEDS DERIVED FROM FIELD CROPS								
CROP	**RAC**	**PROCESSED COMMODITY**	**FEED**		**PERCENT OF LIVESTOCK DIET** (1,2)			
			FEEDSTUFF	**% DM** (3)	**BEEF CATTLE**	**DAIRY CATTLE**	**POULTRY**	**SWINE**
Vetch (78)	forage hay		forage	30	20	60	NU	NU
			hay	85	20	60	NU	NU
Watercress	leaves and stems							
Watermelon	fruit							
Wheat (79) (80)	grain (67) forage hay straw aspirated grain fractions (24)	bran flour middlings shorts germ	grain (67)	89	50	40	80	80
			forage	25	25	60	NU	NU
			hay	88	25	60	NU	NU
			straw	88	10	10	NU	NU
			aspirated grain fractions (24)	85	20	20	NU	NU
			milled byproducts (81)	88	40	50	50	50
Yam	tuber							

Table notes. The following notes are referenced in the table.

1) **Percent of Livestock Diet**. Percentages of feedstuffs in livestock diets other than those listed here can be found in the complete contract (#68-DO-0107) report dated May 17, 1993, that was prepared by Animal Nutrition, Inc., Breese, IL, under the technical guidance of the Chemistry/Tolerance Support, HED, OPP, OPPTS. A copy of this report is available from the National Technical Information Service, 5285 Port Royal Road, Springfield, VA 22161 (#PB-94-107877).

2) **Percent of Livestock Diet.** Maximum percent diet on a dry weight basis for finishing beef and lactating dairy cattle, and on an as-fed basis for poultry and finishing swine (hogs).

3) **% DM (percent dry matter)** For beef and dairy feedstuffs, the percent moisture should be reported for representative samples of raw agricultural and processed commodities.

4) **Alfalfa.** Residue data are needed from a minimum of three cuttings unless climatic conditions restrict the number of cuttings. Cut sample at late bud to early bloom stage (first cut), and/or at early (one tenth) bloom stage (later cuts).

5) **Alfalfa seed.** For registered uses on alfalfa grown for seed, residue data should be provided on seed, and hay; for all other uses, data should only be provided on forage and hay.

6) **NU.** Not used or a minor feedstuff, i.e., less than 10 percent of livestock diet.

7) **Alfalfa meal.** Residue data are not needed for meal; however, the meal should be included in the livestock diet, using the hay tolerance level. Hay should be field dried to a moisture content of 10 to 20 percent.

8) **Alfalfa silage.** Residue data on silage are optimal, but are desirable for assessment of dietary exposure. Cut at late bud to one-tenth bloom stage for alfalfa, allow to wilt to approximately 60 percent moisture, then chop fine, pack tight, and allow to ferment for three weeks maximum in an airtight environment until it reaches pH 4. This applies to both silage and haylage. In the absence of silage data, residues in forage will be used for silage, with correction for DM.

9) **Fruit.** Fruit should be analysed after removing and discarding the stem, and stone or pit.

10) **Banana**. Field residue data on both bagged and unbagged bananas should be provided. The required number of field trials may be split between bagged and unbagged bananas. Alternatively, one sample each of bagged and unbagged bananas may be taken from each site. Data are required on the whole commodity, including peel after removing and discarding the crown tissue and stalk, for establishing tolerances. At the petitioner's discretion, residue data on just the banana pulp may be provided for purposes of dietary risk assessment.

11) **Barley hay.** Cut when the grain is in the milk to soft dough stage. Hay should be field dried to a moisture content of 10 to 20 percent. **Barley straw.** Plant residue, i.e., dried stalks or stems with leaves, left after the grain has been harvested, i.e., threshed.

12) **Barley grain, oat grain, or rice grain.** Kernel (caryopsis) plus hull (lemma and palea).

13) **Bean.** See Crop Group 6: *Legume Vegetables*, under Section 15 (U.S. EPA - 40 CFR 180.41) for cultivars of beans. **Bean seed.** Dried seed for uses on dried shelled beans; succulent seed without pod for uses on succulent shelled beans, e.g., lima beans; succulent seed with pod for edible-podded beans, e.g., snap beans. Cowpea is the only bean crop considered for livestock feeding. See cowpea. Residue data for forage and hay are required only for cowpea.

14) **Beat, sugar.** Residue data may be supplied for raw sugar or refined sugar, or both raw and refined. **Sugarcane.** Residue data may be supplied in the same manner.

15) **Blackberry.** See Crop Group 13: *Berries*, under Section 15 (U.S. EPA- 40 CFR 180.41) for cultivars of blackberries.

16) **Buckwheat grain.** Seed (achene) plus hull.

17) **Cabbage fresh, with wrapper leaves.** Entire cabbage head with obviously decomposed or withered leaves removed. In addition, residue data on cabbage head, without wrapper leaves, are desirable particularly when a more accurate assessment of dietary exposure is necessary.

18) **Carrot culls.** Data for raw agricultural commodities will cover residues on culls.

19) **Clover forage.** Cut sample at the four to eight inch to prebloom stage, at approximately 30 percent DM. **Clover hay.** Cut at early to full bloom stage. Hay should be field dried to a moisture content of 10 to 20 percent. Residue data for clover seeds are not needed.

20) **Clover silage.** Residue data on silage are optional, but are desirable for assessment of dietary exposure. Cut sample at early to one-fourth bloom for clover, allow to wilt to approximately 60 percent moisture, then chop fine, pack tight, and allow to ferment for three weeks maximum in an airtight environment until it reaches pH 4. This applies to both silage and haylage. In the absence of silage data, residues in forage will be used for silage, with correction for DM.

21) **Coffee.** Residue data are required on the green bean, the roasted bean, and on instant coffee. Maximum residue limits (MRLs) on the roasted bean and instant coffee will be established under Division 15 Table II of the FDAR, if residues exceed those on the green bean. The green bean is the dried seed of the coffee bean.

22) **Field corn forage.** Cut sample, i.e., whole aerial portion of the plant, at late dough/early dent stage (black ring/layer stage for corn only). **Sorghum forage.** Cut sample, i.e., whole aerial portion of the plant, at soft dough to hard dough stage. Forage samples should be analyzed as is, or may be analyzed after ensiling for three weeks maximum, and reaching pH 5 or less, with correction for DM.

23) **Corn stover.** Mature dried stalks from which the grain or whole ear, i.e., cob + grain, has been removed; containing 80 to 85 percent DM. **Sorghum stover.** Mature dried stalks from which the grain has been removed; containing approximately 85 percent DM.

24) **Aspirated grain fractions** , previously called **grain dust**. Dust collected at grain elevators for environmental and safety reasons. Residue data should be provided for any postharvest use on corn, sorghum, soybeans, or wheat. For a preharvest use after the reproduction stage begins and seed heads are formed, data are needed unless residues in the grain are less than the limit of quantitation (LOQ) of the analytical method. For a preharvest use during the vegetative stage, i.e., before the reproduction stage begins, data will not normally be needed unless the plant metabolism or processing study shows a concentration of residues of regulatory concern in an outer seed coat, e.g., wheat bran or soybean hulls.

25) **Corn starch.** Residue data from starch will be used for corn syrup. Petitioners may also provide data on syrup for a more accurate assessment of dietary exposure.

26) **Corn milled byproducts.** Use residue data for corn dry-milled processed commodities having the highest residues, excluding oils.

27) **Sweet corn.** Residue data on early sampled field corn should suffice to provide residue data on sweet corn, provided that the residue data are generated at the milk stage on kernel plus cob with husk removed, and there are adequate numbers of trials and geographical representation from the sweet corn growing regions.

28) **Sweet corn (K + CWHR)**. Kernels plus cob with husks removed.

29) **Sweet corn forage.** Samples should be taken when sweet corn is normally harvested for fresh market, and may or may not include the ears. Petitioners may analyze the freshly cut samples, or may analyze the ensiled samples after ensiling for three weeks maximum, and reaching pH 5 or less, with correction for percent DM.

30) **Sweet corn cannery waste.** Includes husks, leaves, cobs, and kernels. Residue data for forage will be used for sweet corn cannery waste.

31) **Cotton gin byproducts,** commonly called **gin trash**. Includes the plant residues from ginning cotton, and consists of burrs, leaves, stems, lint, immature seeds, and sand and/or dirt. Cotton must be harvested by commercial equipment, i.e., stripper and mechanical picker, to provide an adequate representation of plant residue for the ginning process. At least three field trials for each type of harvesting , i.e., stripper and picker, are needed, for a total of six field trials.

32) **Cowpea forage.** Cut forage at six inch to prebloom stage, at approximately 30 percent DM. **Cowpea hay.** Cut when pods are one-half to fully mature. Hay should be field dried to a moisture content of 10 to 20 percent.

33) **Crownvetch forage.** Cut sample at six inch to prebloom stage, at approximately 30 percent DM. **Crown vetch hay.** Cut at full bloom stage. Hay should be field dried to a moisture content of 10 to 20 percent.

34) **Grass.** Zero day crop field residue data for grasses cut for forage should be provided unless it is not feasible, e.g., preplant/preemergent pesticide uses. A reasonable interval before cutting for hay is allowed. **Grass forage.** Cut sample at six to eight inch to boot stage, at approximately 25 percent DM. **Grass hay.** Cut in boot to early head stage. Hay should be field dried to a moisture content of 10 to 20 percent. Grasses include barnyardgrass, bentgrass, Bermudagrass, Kentucky bluegrass, big bluestem, smooth bromegrass, buffalograss, reed canarygrass, crabgrass, cupgrass, dallisgrass, sand dropseed, meadow foxtail, eastern gramagrass, side-oats grama, guineagrass, Indiangrass, Johnsongrass, lovegrass, napiergrass, oatgrass, orchardgrass, pangolagrass, redtop, Italian ryegrass, sprangletop, squirreltailgrass, stargrass, swithgrass, timothy, crested wheatgrass, and wildryegrass. Also included are sudangrass and sorghum forages and their hybrids. For grass grown for seed only, PGIs (pregrazing intervals) and PHIs (preharvest intervals) are acceptable. Residue data may be based on the regrowth after harvesting the seed.

35) **Grass silage.** Residue data on silage are optional, but are desirable for the assessment of dietary exposure. Cut sample at boot to early head stage, allow to wilt to 55 to 65 percent moisture, then chop fine, pack tight, and allow to ferment for three weeks maximum in an airtight environment until it reaches pH 4. In the absence of silage data, residues in forage will be used for silage, with correction for DM.

36) **Herbs.** Consist primarily of leaves, stems, and flowers and are marketed fresh, i.e., succulent, or dried. See Crop Subgroup 19-A under Section 15 (U.S. EPA- 40 CFR 180.41) for a listing of herbs.

37) **Hops, cones, dried.** According to PR Notice 93-012 (December 23, 1993), dried hops will be considered as a raw agricultural commodity for regulatory purposes. Residue data are needed for dried hops only.

38) **Lespedeza forage.** Cut sample at four to six inch to prebloom stage, at 20 to 25 percent DM. **Lespedeza hay: Annual/Korean.** Cut at early blossom to full bloom stage. **Sericea.** Cut when 12 to 15 inches tall. Hay should be field dried to a moisture content of 10 to 20 percent.

39) **Lettuce, fresh, with wrapper leaves.** Entire lettuce head, with the obviously decomposed or withered leaves removed. In addition, residue data on lettuce head, without wrapper leaves, are desirable, particularly when more accurate assessment of dietary exposure is necessary.

40) **Lettuce, leaf.** Residue data should be on samples with the obviously decomposed or withered leaves removed.

41) **Millet forage.** Cut sample at ten inches to early boot stage, at approximately 30 percent DM. **Millet hay.** Cut at early boot stage or approximately 40 inches tall, whichever is reached first. Hay should be field dried to a moisture content of 10 to 20 percent. Millet, including pearl millet.

42) **Millet grain.** Kernel plus hull (lemma and palea). **Pearl millet grain.** Kernel with hull (lemma and palea) removed.

43) **Millet straw.** Data are required for proso millet only. **Proso millet staw.** Plant residue, i.e., dried stalks or stems with leaves, left after the grain has been harvested.

44) **Millet flour.** Not produced significantly in the United States for human consumption. Residue data are not needed at this time.

45) **Mung bean**. Data on mung bean covers sprouts except when the pesticide is used on the sprouts per se.

46) **Muskmelon.** Includes cantaloupe, casaba, crenshaw, etc. See Crop Group 9: *Cucurbit Vegetables* Section 15 *Crop Groups* (U.S. EPA 40 CFR) for other cultivars of muskmelons.

47) **Nuts.** Includes Croup Group 14: *Tree Nuts*, Section 15, *Crop Groups*, (U.S. EPA 40 CFR 180.41), except for almonds. Pistachio is under consideration to be added to Crop Group 14. Residue data for tree nuts may be used to support uses on pistachio. See Crop Group 14 for a listing of nuts. Also see almonds. Almond hulls are considered a significant feedstuff. Hulls from other tree nuts are not considered significant feedstuffs.

48) **Oats forage.** Cut sample between tillering to stem elongation (jointing) stage. **Oats hay.** Cut sample from early flower to soft dough stage. Hay should be field dried to a moisture content of 10 to 20 percent. **Oats straw.** Cut plant residue, i.e., dried stalks or stems with leaves, left after the grain has been harvested, i.e., threshed.

49) **Parsley.** Fresh parsley is included in Crop Group 4: *Leafy Vegetables*, under 40 CFR 180.41. Dried parsley is included in Crop Subgroup 19A: *Herbs,* under Section 15 (U.S. EPA-0 CFR 180.41).

50) **Pea.** Residue data for forage and hay are required for cowpea. See cowpea. Residue data for vines and hay are required for field peas only. See pea, field.

51) **Pea, succulent.** Succulent seed with pod for edible-podded peas, e.g., snow peas; succulent seed without pod for uses on succulent shelled peas, e.g., English peas.

52) **Pea seed.** Mature dried seed for uses on dried, shelled peas.

53) **Pea, field.** Does not include the canning field pea cultivars used for human food. Includes cultivars grown for livestock feeding only, such as **Australian winter pea. Field pea vines.** Cut sample anytime after pods begin to form, at approximately 25 percent DM. **Field pea hay.** Succulent plant cut from full bloom through pod formation. Hay should be field dried to a moisture content of 10 to 20 percent.

54) **Pea, field, silage.** Use field pea vine residue data for field pea silage with correction for DM.

55) **Peanut hay.** Peanut hay consists of the dried vines and leaves left after the mechanical harvesting of peanuts from vines that have been sun-dried to a moisture content of 10 to 20 percent.

56) **(R):** Label restrictions against feeding may be allowed, e.g., *Do not feed green immature growing plants to livestock,* or, Do not harvest for livestock feed.

57) **Pepper.** Nonbell pepper includes chili pepper.

58) **Pimento.** The official name adopted by the Georgia Pimento Growers Association.

59) **Pineapple process residue ,** also known as **wet bran**. A wet waste byproduct from the fresh-cut product line that includes pineapple tops (minus crown), bottoms, peels, any trimmings with peel cut up, and the pulp that is left after squeezing for juice; it can include culls.

60) **Plantain.** Banana MRL will cover plantain.

61) **Potato granules/flakes.** Residue data may be provided for either.

62) **Processed potato waste.** MRLs for wet peel should be used for dietary burden calculations. Residue data may be provided from actual processed potato waste generated, using a pilot or commercial scale process that gives the highest percentage of wet peel in the waste.

63) **Rapeseed meal.** Residue data are not needed for rapeseed oil since it is produced for industrial uses and is not an edible oil. The edible oil is only produced from canola. See canola.

64) **Rape greens.** A commodity listed in Crop Group 5: *Brassica (Cole) Leafy Vegetable Group*, under Section 15 (U.S. EPA- 40 CFR 180.41).

65) **Rice straw.** Stubble, i.e., basal portion of the stems, left standing after harvesting the grain.

66) **Rye forage.** Cut sample at six to eight inch stage to stem elongation (jointing) stage, at approximately 30 percent DM. **Rye straw.** Cut plant residue, i.e., dried stalks or stems with leaves, left after the grain has been harvested, i.e., threshed.

67) **Rye grain or wheat grain.** Kernel (caryopsis) with hull (lemma and palea) removed.

68) **Sorghum flour.** Residue data are not needed at this time since sorghum flour is used exclusively in the United States as a component for drywall, and not as either a human food or a feedstuff. However, because 50 percent of the worldwide sorghum production goes toward human consumption, data may be needed at a later date.

69) **Sorghum, sweet.** Sweet sorghum commodities, i.e., seed and forage, will be covered by the sorghum grain tolerances.

70) **Soybean forage.** Cut samples at six to eight inches tall (sixth node) to beginning pod formation, at approximately 35 percent DM. **Soybean hay.** Cut samples at mid-to-full bloom stage and before bottom leaves begin to fall, or when pods are approximately 50 percent developed. Hay should be field dried to a moisture content of 10 to 20 percent.

71) **Soybean silage.** Residue data on silage are optional. Harvest sample when pods are one-half to fully mature, i.e., full pod, stage. In the absence of silage data, residues in forage will be used for silage, with correction for DM.

72) **Spices.** Include aromatic seeds, buds, bark, berries, pods, and roots consumed and marketed primarily in their dried form. See Crop Subgroup 19-B under Section 15 (U.S. EPA- 40 CFR 180.41) for a listing of spices.

73) **Sugarcane bagasse.** Information indicates that sugercane bagasse is mainly used for fuel. Residue data will not be needed at this time, but may be needed at a later date.

74) **Sugarcane molasses.** Residue data are needed for blackstrap molasses.

75) **Tea.** Residue data are required on plucked or freshly picked leaves, dried tea, and instant tea.

76) **Tomato paste.** Residue data on tomato paste cover tomato processed products, e.g., sauce, juice and catsup, except tomato puree, which covers canned tomatoes.

77) **Trefoil forage.** Cut sample at five to ten inch or early bloom stage, at approximately 30 percent DM. **Trefoil hay.** Cut at first flower to full bloom. Hay should be field dried to a moisture content of 10 to 20 percent.

78) **Vetch forage.** Cut sample at six inch to prebloom stage, at approximately 30 percent DM. **Vetch hay.** Cut at early bloom stage to when seeds in the lower half of the plant are approximately 50 percent developed. Hay should be field dried to a moisture content of 10 to 20 percent. Vetch does not include crownvetch.

79) **Wheat forage.** Cut sample at six to eight inch stage to stem elongation (jointing) stage, at approximately 25 percent DM. **Wheat hay.** Cut samples at early flower (boot) to soft dough stage. Hay should be field dried to a moisture content of 10 to 20 percent. **Wheat straw.** Cut plant residue, i.e., dried stalks or stems with leaves, left after the grain has been harvested, i.e., threshed.

80) **Wheat.** Includes emmer wheat and triticale. No processing study is needed for a specific MRL on emmer wheat.

81) **Wheat milled byproducts.** Use highest value for wheat middlings, bran, and shorts.